SYNTHESIS AND REACTIONS OF PYRAZOLOQUINOXALINES

Thesis submitted to
the Cochin University of Science and Technology
in partial fulfilment of the requirements
of the degree of
DOCTOR OF PHILOSOPHY
in the Faculty of Science

By

SANJEEV BHAT V.

DEPARTMENT OF APPLIED CHEMISTRY
COCHIN UNIVERSITY OF SCIENCE AND TECHNOLOGY
COCHIN - 682 022

1989

CERTIFICATE

Certified that this thesis is based on the work done by Mr.V.Sanjeev Bhat under my guidance in the Department of Applied Chemistry, Cochin University of Science and Technology and no part of this has been presented by him for any other degree.

P. Madhavan Pillai

Dr.P.Madhavan Pillai
(Supervising Teacher)
Professor, Department of
Applied Chemistry
Cochin University of
Science & Technology

Cochin 682 022
4 December 1989

DECLARATION

Certified that the work presented in this thesis is based on the original work done by me under the guidance of Dr.P.Madhavan Pillai, Professor, Department of Applied Chemistry, Cochin University of Science and Technology, and has not been included in any other thesis submitted for the award of any degree.

V.Sanjeev Bhat

Cochin 682 022
4 December 1989

ACKNOWLEDGEMENT

The author records his deep sense of indebtedness and sincere appreciation to his supervising teacher, Dr.P.Madhavan Pillai, Professor, Department of Applied Chemistry, Cochin University of Science and Technology, for his stimulating guidance and encouragement throughout this work. The author is also thankful to Professor Paul A.Vatakencherry, Head, Department of Applied Chemistry, Cochin University of Science and Technology.

The author also wishes to express his thanks to Kerala State Drugs and Pharmaceuticals Limited, Alleppey; Vikram Sarabhai Space Centre, Trivandrum; Indian Institute of Science, Bangalore and National Chemical Laboratory, Pune for providing spectral data and elemental analysis. Financial supports from Cochin University of Science and Technology and University Grants Commission are gratefully acknowledged.

I offer my thanks to Mr.Sasi and Mr.Siby for their assistance in typing this thesis. Finally, I wish to express my appreciation to my colleagues and friends who rendered untiring help and extended co-operation throughout my work.

CONTENTS

CHAPTER I

INTRODUCTION

1H-Pyrazolo[3,4-b]quinoxaline derivatives were first prepared by Ohle and co-workers in 1941 by the condensation of glucose with o-phenylene diamine and phenylhydrazine in the presence of acetic acid. The compounds containing this new ring system were called flavazoles because of their yellow colour. Many derivatives of this heterocyclic system were later found to possess important biological activities such as diuretic, anti-inflammatory, analgesic, antileukemic, tuberculostatic and immunochemical properties and their application as potential agricultural chemicals are also worth exploring. The synthesis and screening of this class of compounds have therefore gained added significance recently.

Earlier work in this area was confined mainly to the synthesis of 1-phenyl substituted pyrazolo[3,4-b]-quinoxalines. The present studies, however, has worked out methods for the synthesis of 3-substituted pyrazoloquinoxalines with position 1 free or protected as the acetyl derivative. The synthesis of 1-acetylpyrazolo[3,4-b]-quinoxalines substituted at position 3 with chloro, amino, hydroxy and methoxy groups were achieved starting from the known ethyl 2-hydroxyquinoxaline-3-carboxylate. Reactions 1-acetyl-3-chloropyrazolo[3,4-b]quinoxaline with ammonia,

several secondary amines and sodium carbonate and methanol have been studied and the products have been identified. In addition to the substitution products, compounds formed as a result of the pyrazole ring opening have also been separated and characterised. The hydrolysis of the 1-acetyl group without rupturing the heterocyclic ring system has been accomplished in some cases under very mild reaction conditions. Mechanisms for ring opening reactions have been proposed.

The use of thionyl chloride as a synthetic reagent for chlorination of heterocyclic compounds such as anilino-quinoxaline and pyrazoloquinoxaline have been reported for the first time. The experimental conditions for chlorinations have been worked out and the chlorinated derivatives have been fully characterised. Treatment of the chlorinated pyrazoloquinoxalines with sodium borohydride led to a different type of ring opening. Mechanisms for chlorination using thionyl chloride and for the ring opening reactions have been suggested which account for all the products in these reactions.

The present work also reports the synthesis of 2-aryl-3-oxo-3-pyrazolino[3,4-b]quinoxalines for the first

time. These compounds have been prepared by the reaction of ethyl 2-chloroquinoxaline-3-carboxylate with different phenylhydrazines. 2-Aryl-3-oxo-3-pyrazolino[3,4-b]quinoxalines are generally light yellow in either neutral or acid solutions but changed the colour to deep violet or green in basic media. The change in colour appears to be sharp and therefore these compounds may be used as acid base indicators. Their UV absorption maxima under acidic and basic media are also very different. However, the actual conditions under which these compounds may be used as indicators have not been worked out.

The synthesis and reactions of a new heterocyclic system, 1H-1,5-benzodiazepino[2,3-b]quinoxaline is also reported here. This novel nitrogen heterocycle was prepared by the condensation of ethyl 2-chloroquinoxaline-3-carboxylate with o-phenylene diamine and subsequent manipulations to give the parent compound. Several derivatives which are expected to have valuable biological properties have also been reported.

The structures of all new compounds have been established by elemental analysis and also by analysing their spectral data such as ultraviolet, infrared, nuclear

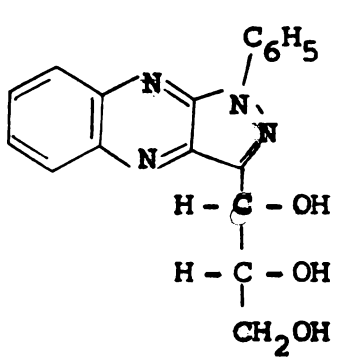
magnetic resonance and mass spectrometry. Compounds obtained from this work will be submitted for screening their biological properties.

CHAPTER II

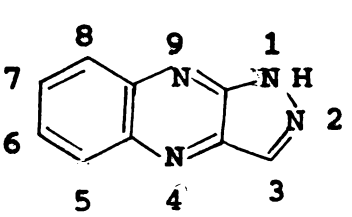
HISTORICAL REVIEW

2.1 Introduction

The first derivative (1) of 1H-pyrazolo[3,4-b]-quinoxaline system (2) was prepared by the reaction of glucose with o-phenylene diamine and phenylhydrazine in the presence of an acid[2]. All reducing sugars which are not substituted at positions 2 and 3 were found to give this reaction and the pyrazolo[3,4-b]quinoxalines formed were highly coloured which gave the name flavazole to this new class of compounds. The formation of 1H-pyrazolo[3,4-b]-quinoxalines was used for the characterisation of the sugars[3,4] because they are crystallised readily and could be identified by means of their melting points or powder x-ray diffraction patterns.



1

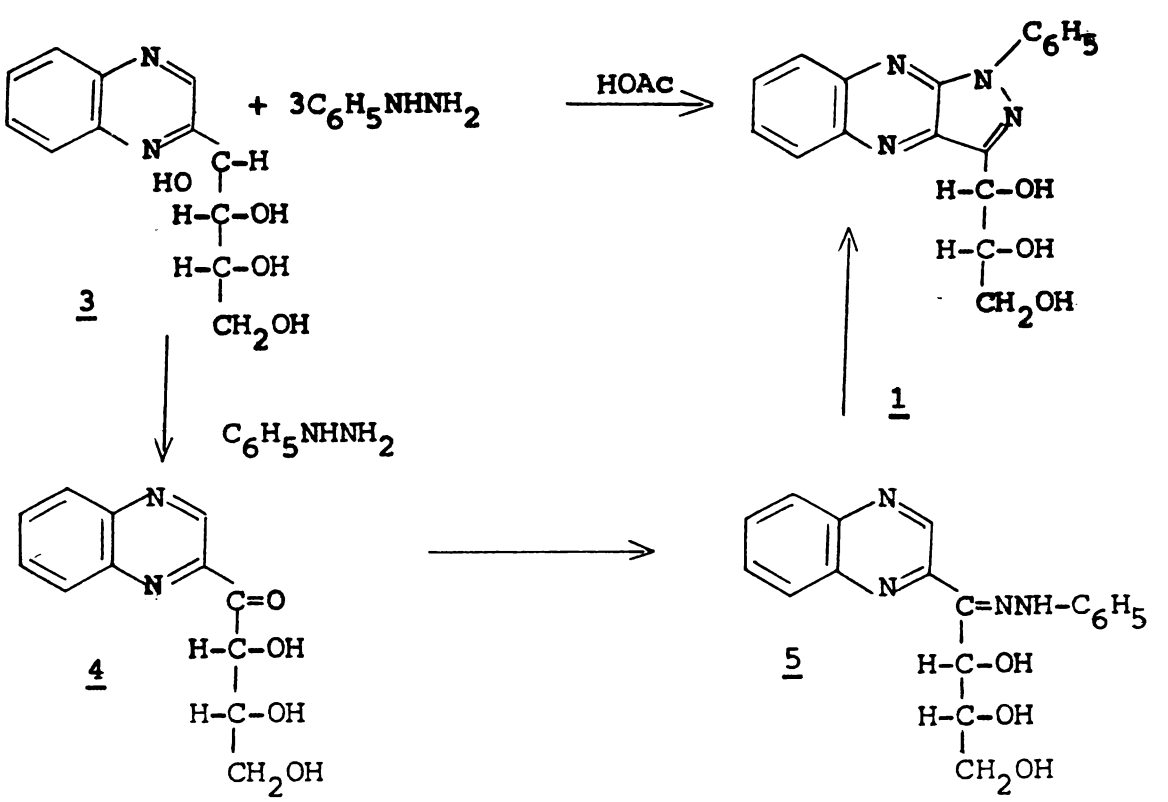


2

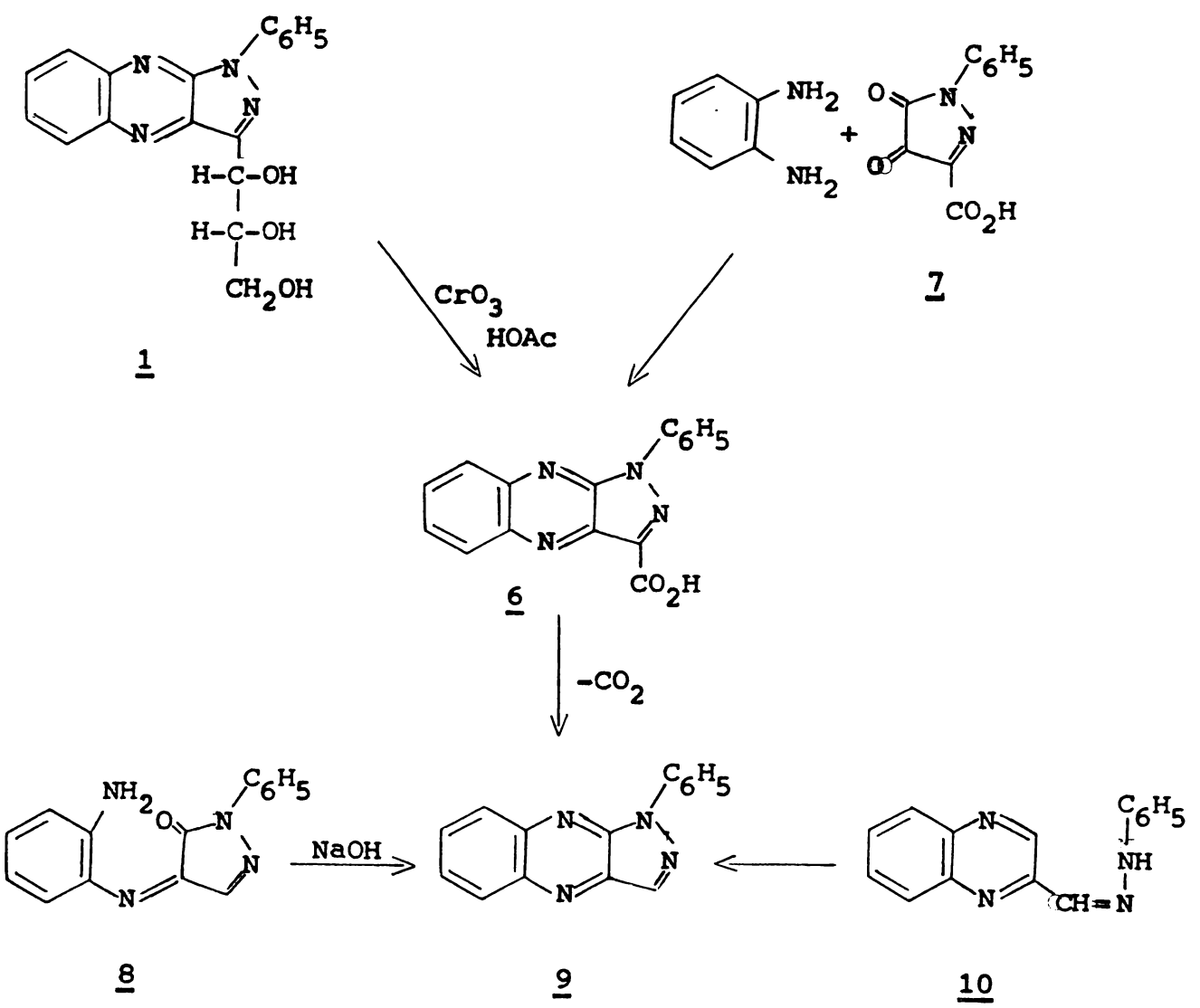
2.2 Preparation of pyrazoloquinoxalines

2.2.1 Preparation of 1-Phenyl-1H-pyrazolo[3,4-b]quinoxalines

The 1H-pyrazolo[3,4-b]quinoxaline ring system was first prepared by Ohle and co-workers[1,2] in 1941 by the treatment of 3-(D-arabino-tetrahydroxybutyl)quinoxaline (3) with phenylhydrazine in acetic acid when 1-phenyl-3-(D-erythro-1,2,3-trihydroxypropyl)-1H-pyrazolo[3,4-b]quinoxaline (1) was obtained in 97% yield. It was suggested that one molecule of phenylhydrazine first dehydrogenated 3 to the keto-derivative, 4 which then condensed with another molecule of phenylhydrazine to form a phenylhydrazone, 5. Phenylhydrazone, 5 lastly undergoes oxidative cyclisation to give the 1H-pyrazolo[3,4-b]quinoxaline derivative, 1, consuming a third molecule of phenylhydrazine. When phenylhydrazine in water was used in the absence of acetic acid, 3 gave 9% of 5[1] alongwith ammonia (18%) and aniline (11%), and no cyclised product was obtained.

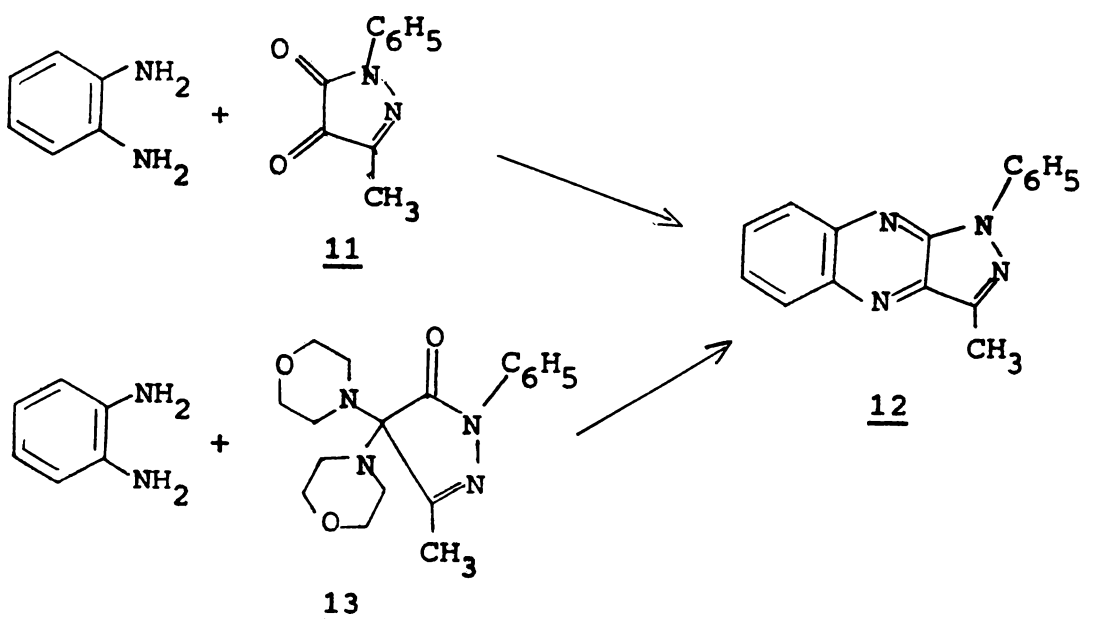


Ohle and co-workers also elucidated the structure of 1 including the position of the sugar residue in 1941. They oxidised 1 with chromic anhydride in acetic acid to 1-phenyl-1H-pyrazolo[3,4-b]quinoxaline-3-carboxylic acid (6) which was synthesised independently by the condensation of o-phenylenediamine with 1-phenyl-4,5-dioxo-2-pyrazoline-3-carboxylic acid (7). When 6 was heated above its melting point, it readily lost carbon dioxide and provided 1-phenyl-1H-pyrazolo[3,4-b]quinoxaline (9) which was also obtained by the cyclisation of 8 in the presence of sodium hydroxide

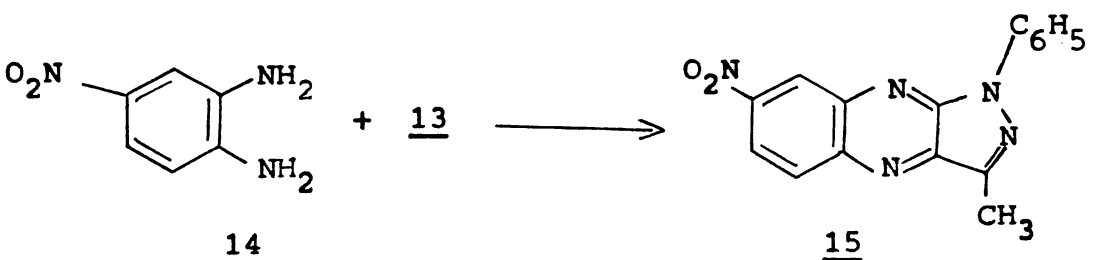


solution[5]. 1-Phenyl-1H-pyrazolo[3,4-b]quinoxaline (9) was later prepared by Henseke and co-workers from quinoxaline-2-carboxaldehyde phenylhydrazone (10), by oxidative cyclisation using phenylhydrazine in acetic acid solution[6].

3-Methyl-1-phenyl-4,5-dioxo-2-pyrazoline (11) was condensed with o-phenylene diamine[5] to give 3-methyl-1-phenyl-1H-pyrazolo[3,4-b]quinoxaline. The reaction of the aminal, 13 with o-phenylene diamine also provided 12.[7] The methyl group in 12 was found to be inactive towards oxidising agents and benzaldehyde[5].

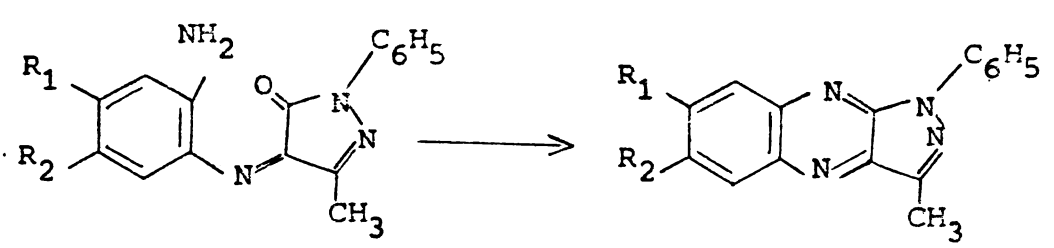


Klicnar reported the preparation of 3-methyl-7-nitro-1-phenyl-1H-pyrazolo[3,4-b]quinoxaline (15) by the condensation of 13 with 4-nitro-o-phenylenediamine (14)[8].



14 15

Hydrazine in the presence of Raney Nickel reduced 15 and gave the amino derivative 17. 7-Amino-3-methyl-1-phenyl-1H-pyrazolo[3,4-b]quinoxaline (17) and two of its analogs 19 and 21 had already been prepared by Vanicela by the cyclisation of the indoaniline dyes 16, 18 and 20 by heating them in acetic acid solutions[9].



16, $R_1 = NH_2$, $R_2 = H$ 17, $R_1 = NH_2$, $R_2 = H$

18, $R_1 = NH_2$, $R_2 = CH_3$ 19, $R_1 = NH_2$, $R_2 = CH_3$

20, $R_1 = N(CH_3)_2$, $R_2 = H$ 21, $R_1 = N(CH_3)_2$, $R_2 = H$

Ohle and Liebig who were the first to synthesise the 1H-pyrazolo[3,4-b]quinoxaline ring system proposed two routes for its preparation starting from carbohydrates[10]. In method one, an aqueous solution of the sugar and o-phenylene diamine was heated in the presence of hydrazine hydrate, boric acid and acetic acid under carbon dioxide atmosphere to give a dark brown solution of quinoxaline derivative. To this quinoxaline derivative was then added phenylhydrazine, acetic acid and hydrochloric acid and the mixture then heated under a stream of carbon dioxide when the 1H-pyrazolo[3,4-b]-quinoxaline derivative separated out as a solid. In the second method, a mixture of the sugar solution in water, o-phenylene diamine and phenylhydrazine was heated in the presence of acetic acid and hydrochloric acid for 20 to 24 hours under a stream of carbon dioxide. A comparison in the yields of the products formed from a few sugars by both methods is tabulated in Table I.

Aldoses provide the osazone derivatives as well as 1H-pyrazolo[3,4-b]quinoxaline derivatives, whereas the osazone formation of the aldoses involve only one assymetric carbon atom, namely C-2, the 1H-pyrazolo[3,4-b]quinoxaline formation involves C-2 and C-3. Ohle and Liebig therefore suggested that if two sugars give different osazones but

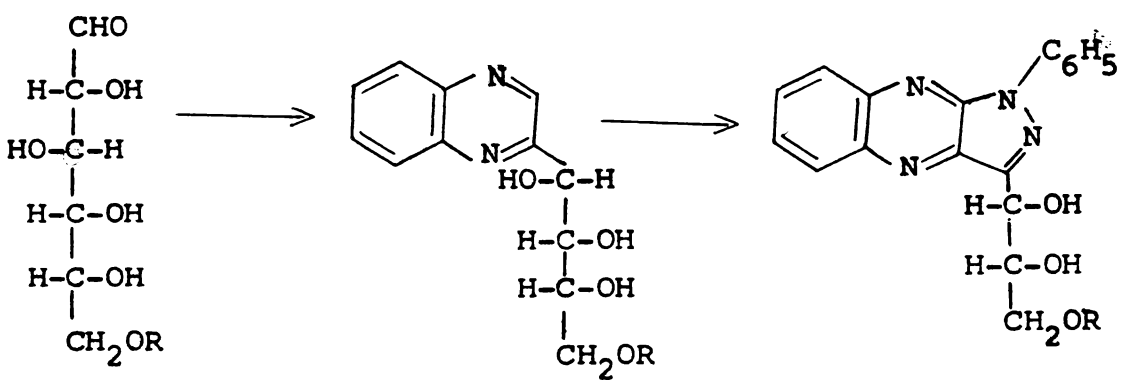
Table I

Percentage yields of 1-phenyl pyrazoloquinoxaline formation by two different methods[13]

Name of sugar	Name of product	Percentage yield	
		Method I	Method II
D-Galactose	1-Phenyl-3-(D-threo-trihydroxypropyl) pyrazoloquinoxaline	10	33
L-Sorbose	1-Phenyl-3-(L-threo-trihydroxypropyl) pyrazoloquinoxaline	3.3	12
D-Xylose	1-Phenyl-3-(D-dihydroxy-ethyl)pyrazoloquinoxaline	12	11.4
L-Arabinose	1-Phenyl-3-(L-dihydroxy-ethyl)pyrazoloquinoxaline	10	10
L-Rhamnose	1-Phenyl-3-(L-erythro-dihydroxypropyl) pyrazoloquinoxaline	25	14

the same 1H-pyrazolo[3,4-b]quinoxaline, they have opposite configuration at C-3 but have the same stereochemistry for the remaining carbon atoms[10].

Ohle and Kryff[11] worked out a general procedure for the characterisation of sugars and sugar derivatives as 1H-pyrazolo[3,4-b]quinoxalines. Carbohydrate was first converted into the quinoxaline derivative by treatment with o-phenylene diamine, hydrazine hydrate and acetic acid in pyridine. Quinoxaline derivative in solution was subsequently reacted with phenylhydrazine, after the separation of the byproduct azine, to give the 1H-pyrazolo[3,4-b]quinoxaline derivative. 6-Substituted glucoses, 6-O-methyl-D-glucose (22) and 6-O-phenyl-D-glucose (23) were converted into the

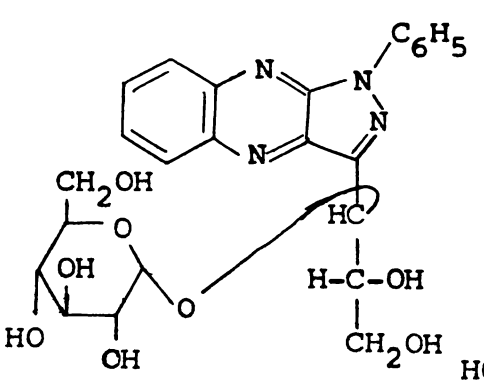


22, R = CH_3 24, R = CH_3 26, R = CH_3

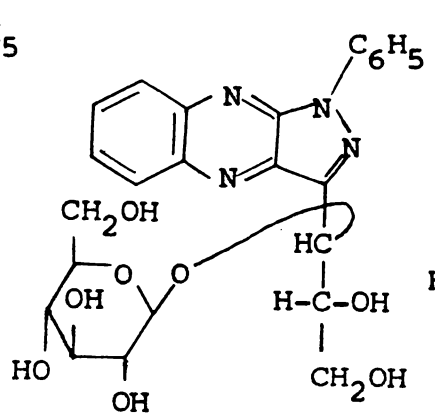
23, R = C_6H_5 25, R = C_6H_5 27, R = C_6H_5

1H-pyrazolo[3,4-b]quinoxaline derivatives, 1-phenyl-3-(3-O-methyl-D-erythro-trihydroxypropyl)-1H-pyrazolo[3,4-b]-quinoxaline (26) and 1-phenyl-3-(3-O-phenyl-D-erythro-trihydroxypropyl)-1H-pyrazolo[3,4-b]quinoxaline (27) through the corresponding quinoxaline derivatives, 24 and 25 using the above procedure.

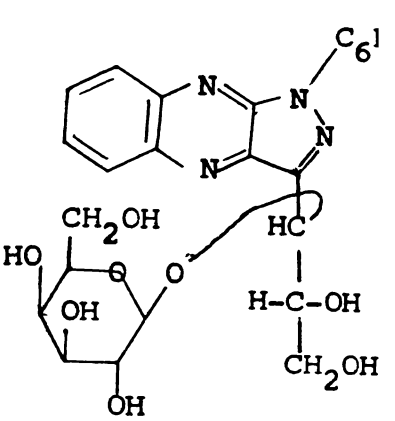
Newmuller prepared 1H-pyrazolo[3,4-b]quinoxaline derivatives 28, 29 and 30 of the disaccharides, maltose, cellobiose and lactose respectively and were characterised[12]. He also converted a trisaccharide derivative obtained by the action of malt on starch into the 1H-pyrazolo[3,4-b]-quinoxaline derivative to study the application of this method in the determination of the structure of oligosaccharides obtained by enzymic action[12]. Periodic acid in the presence



28

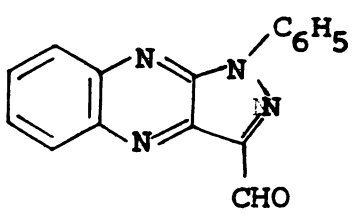


29



30

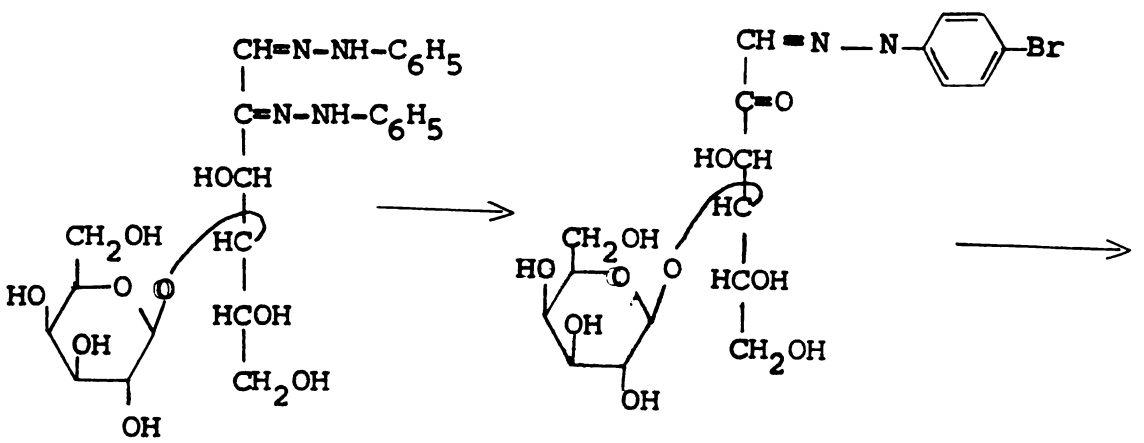
of acetic acid oxidised the 1H-pyrazolo[3,4-b]quinoxalines 28, 29 and 30, consuming 4 molecules each of periodic acid. Products obtained thus underwent further oxidation but very slowly. Pyrazolo[3,4-b]quinoxaline obtained from the starch dextrin also underwent oxidation consuming 5 molecules of periodic acid. As the product of the oxidation could not be isolated[12] the structure was not proved conclusively. However, the structure of the fermentation product obtained from barley with malt amylase was shown to be an isomaltose containing trisaccharide from the fact that it consumed 5 molecules of peridodic acid and the yield of the product did not correspond to 1-phenyl-1H-pyrazolo[3,4-b]quinoxaline-3-carboxaldehyde (31) but to a material having a 1,6-glycoside linkage[13].



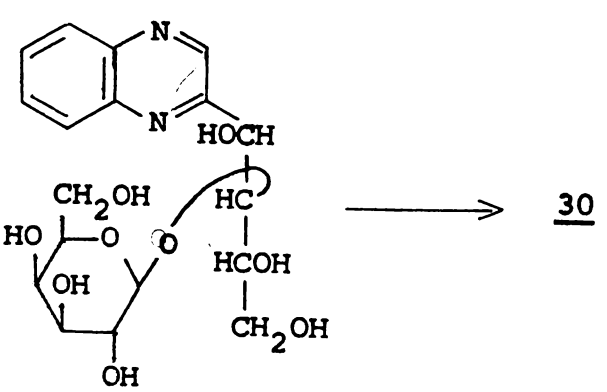
31

3-(1- β -D-Galactosido-D-erythro-trihydroxypropyl)-1-phenyl-1H-pyrazolo[3,4-b]quinoxaline (30) was also obtained from lactose phenylosazone[14] (32). Conversion of 32 into

lactosone-1-α-methyl-p-bromophenylhydrazone (33) and subsequent reaction with o-phenylene diamine gave 2-(1-β-D-galactosido-D-arabino-trihydroxybutyl)quinoxaline (34) which when treated with phenylhydrazine yielded 30.

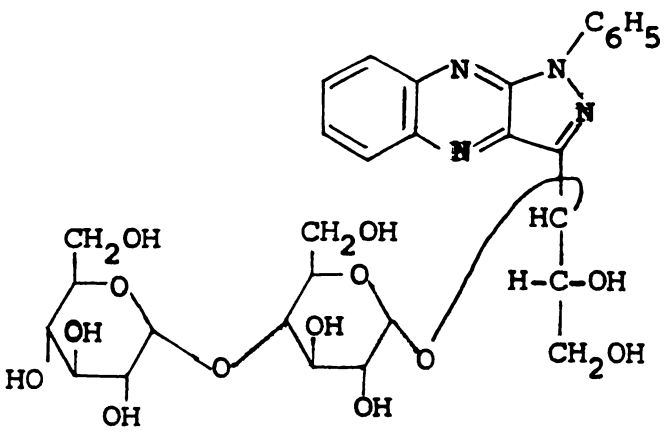


32 33



34 30

The trisaccharide, maltotriose was converted into 3-[1-(O-α-D-glucopyranosyl(1 ⟶ 4)O-α-D-glucopyranosyl)-D-erythro-trihydroxypropyl]-1-phenyl-1H-pyrazolo[3,4-b]quinoxaline (35) in 12% yield by a one step reaction involving o-phenylene diamine and phenylhydrazine[15].

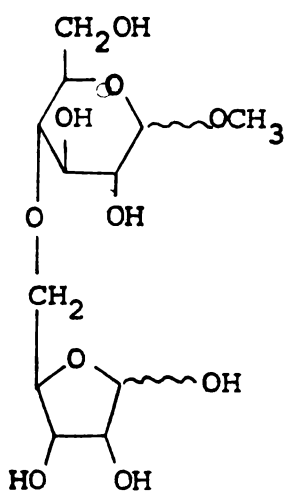


35

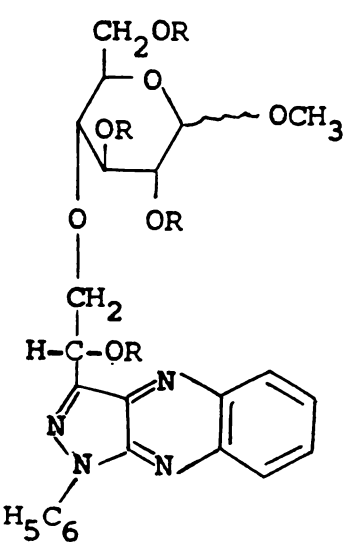
Courtois and Ariyoshi prepared and characterised the 1H-pyrazolo[3,4-b]quinoxaline derivatives of a number of sugars[16] (see Table II) as a means of their identification according to the method of French and co-workers who had prepared the 1H-pyrazolo[3,4-b]quinoxaline derivative of mannitotriose[17].

Nordin and French prepared the 1H-pyrazolo[3,4-b]-quinoxaline derivatives of the singly branched dextrines containing 4 to 7 glucose units obtained from waxy corn starch by the action of salivary amylase. The individual dextrin pyrazoloquinoxalines reacted with amyloglucosidase to yield a single branched pyrazoloquinoxaline derivative containing 4 glucose units[18]. An anomeric mixture of the methylglycoside, 36 of the disaccharide obtained by the hydrolysis of exotoxin[19] was converted into a mixture of

the 1-phenyl pyrazoloquinoxalines, 37. An nmr spectral analysis of the tetraacetate, 38 showed that the mixture contained 80% of the β-anomer.



36



37, R = H

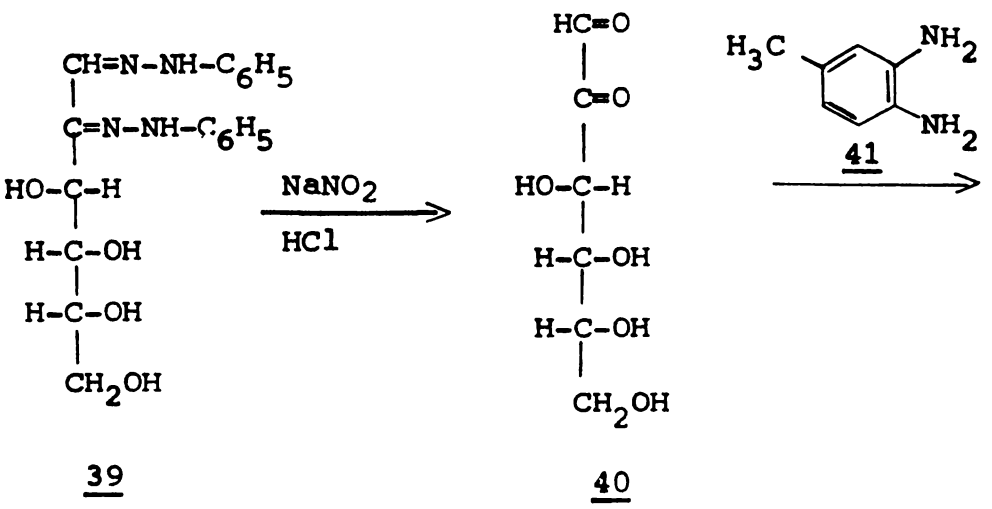
38, R = Ac

Henseke and Bahner[20] prepared pyrazoloquinoxaline substituted with a methyl group at position 6 (7) starting from D-fructose phenylosazone (39). Osone 40 prepared from 39 was treated with 3,4-diaminotoluene (41) to give 42 which on condensation with phenylhydrazine gave 6(D)-methyl-1-phenyl-3-(D)-erythro-trihydroxypropyl)pyrazoloquinoxaline[20] (43). The exact position of the methyl group was not established.

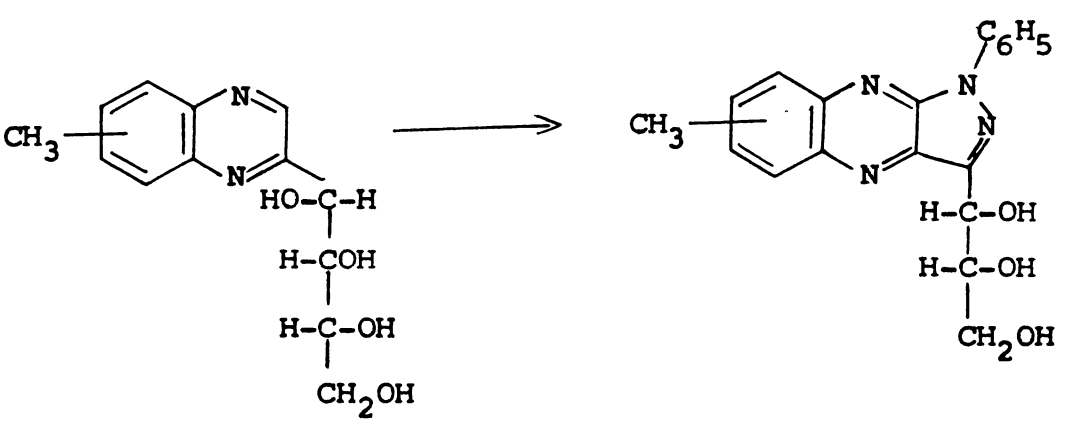
Table II

Physical constants of 1-phenyl pyrazoloquinoxaline derivatives of some sugars

Name of sugar	Yield (%) of 1-phenyl pyrazoloquinoxaline derivative	Solvent of crystallisation	Melting point	$[\alpha]_D^{20}$ c=1 (pyridine)
L-Arabinose	21.5	25% EtOH	215°	-6.9°
L-Rhamnose	25.6	MeOH	214°	+43.8°
D-Glucose	25.5	95% EtOH	218°	-20°
D-Galactose	23.5	95% EtOH	194-5°	-49.3°
Maltose	14.4	1:9-C_5H_5N-EtOH	265°	+53.50°
Gentiobiose	32.3	95% EtOH	245-7°	-43°
Melibiose	34.5	1:9-C_5H_5N-EtOH	218-21°	+45°
Lactose	24.1	1:9-C_5H_5-N-EtOH	272°	-88°
Vicianose	23.3	MeOH	216-20°	--
Manninotriose	--	EtOH	236-8°	+60°
Trigalactosidoglucose	34.0	MeOH and MeCOEt	257-62°	+80°



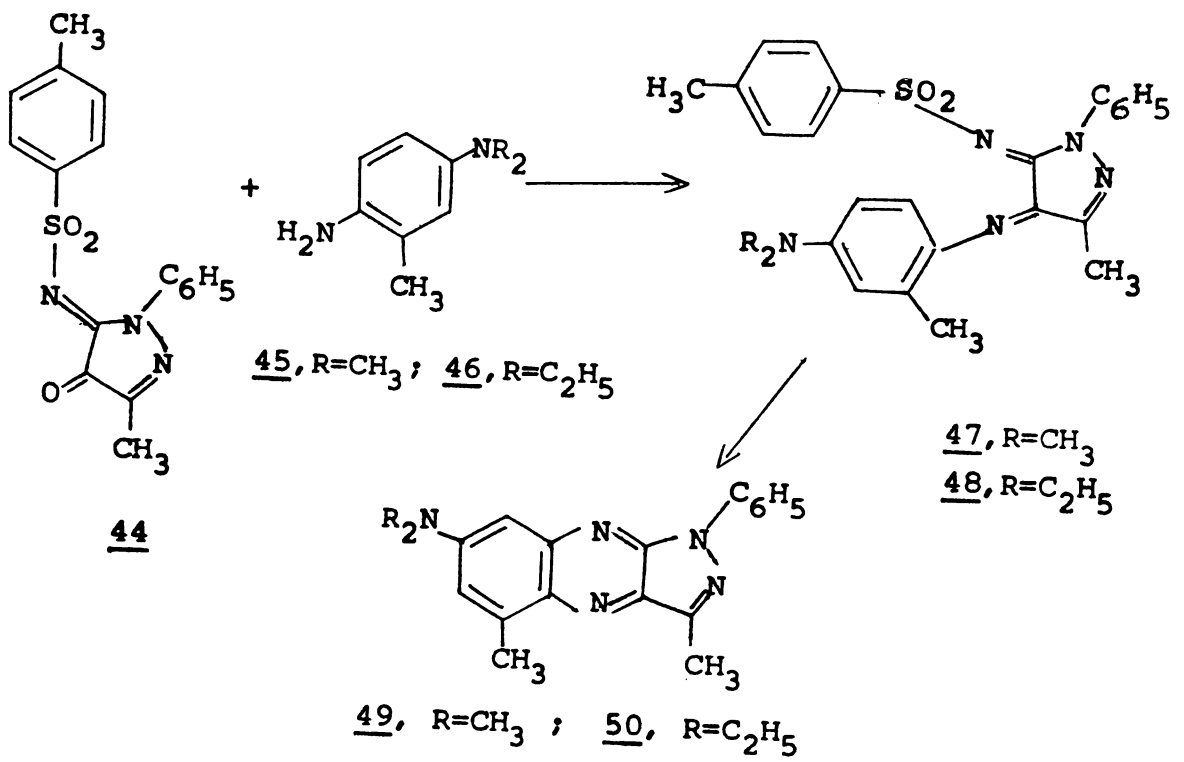
39 40 41

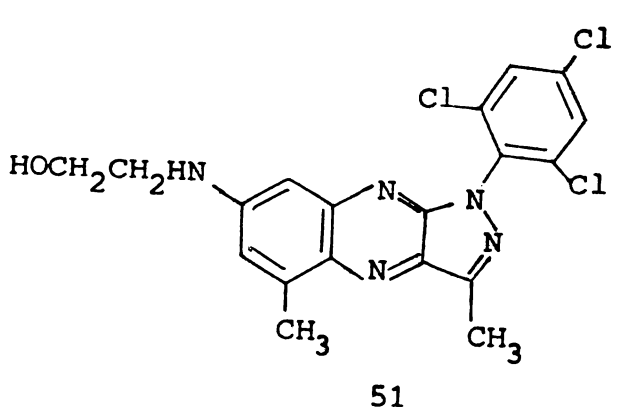


42 43

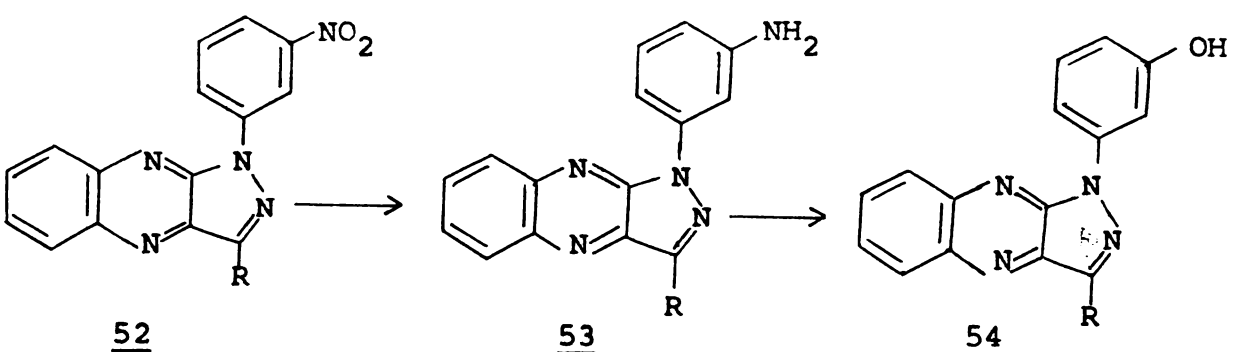
Credner has reported the preparation of pyrazoloquinoxalines with fluorescence[21,22] which he prepared from azomethine dyes. By coupling 5-(p-toluene sulphonimido)-3-methyl-1-phenyl-4-pyrazolone (44) with 2-amino-5-dialkylaminotoluenes, 45 and 46 in the presence of potassium persulphate or silver bromide gave the corresponding unstable

azomethine dyes, 47 and 48 which on heating itself or by treatment with hydrochloric acid yielded the fluorescent 7-alkylamino-3,5-dimethyl-1-phenylpyrazoloquinoxalines 49 and 50. Credner and Pueschel have also reported the preparation of a similar compound, 51 with a trichlorophenyl group at position 1[22].





B.Teichmann and co-workers has reported the preparation of pyrazoloquinoxaline derivatives of oligosaccharides of isomaltose, maltose and cellobiose series[23]. The oligosaccharide was treated with o-phenylene diamine and m-nitrophenylhydrazine to give the corresponding 1-(m-nitrophenyl)pyrazoloquinoxaline (52). Nitroderivatives of twentyone such oligosaccharides were prepared which were converted into the corresponding amino derivatives (53) by catalytic hydrogenation and further converted to the hydroxy derivatives (54) by diazotisation[25].

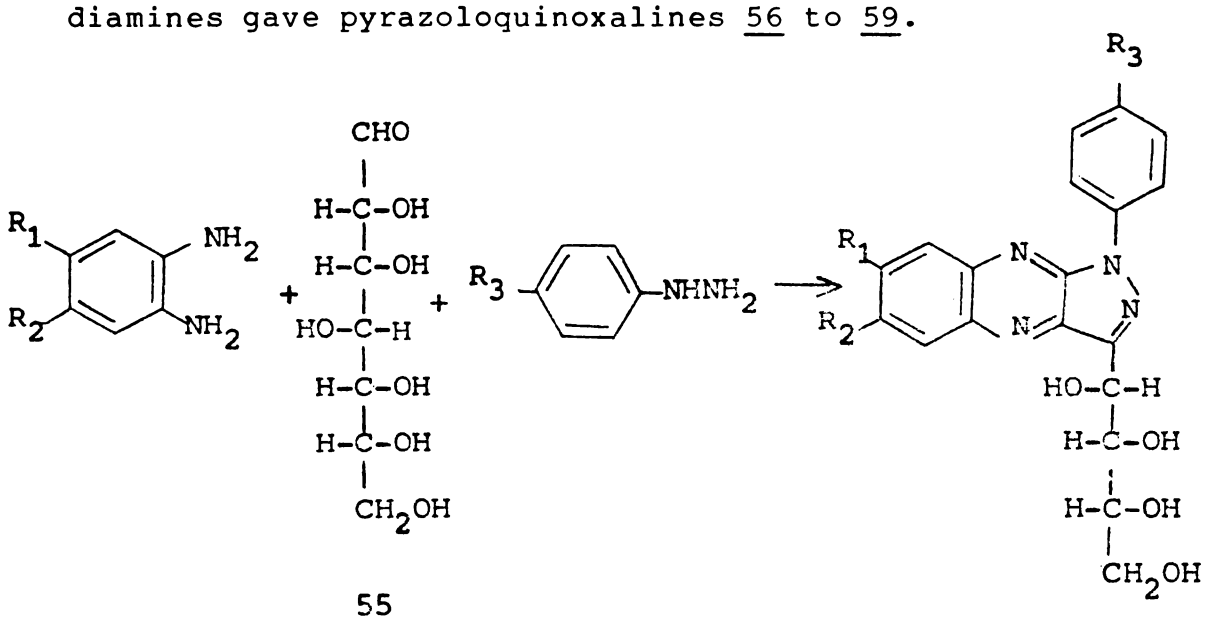


52 53 54

R = Sugar residue

Heptose sugars also undergo condensation with o-phenylene diamine and phenylhydrazine to give the pyrazoloquinoxaline[26-28]. D-glycero-D-guloheptose (55), phenyl-

hydrazine or p-fluorophenylhydrazine and substituted o-phenylene diamines gave pyrazoloquinoxalines 56 to 59.



55

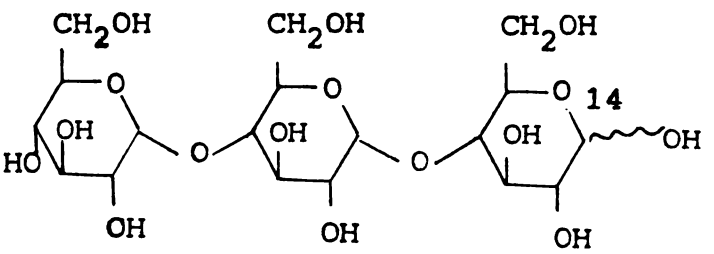
56, $R_1 = R_2 = R_3 = H$

57, $R_1 = R_3 = H$, $R_2 = Cl$

58, $R_1 = R_2 = CH_3$, $R_3 = H$

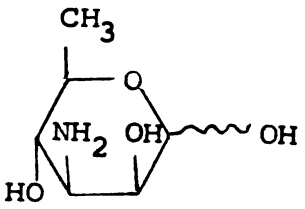
59, $R_1 = R_2 = H$, $R_3 = F$

J.H.Pazur used the method of preparing pyrazoloquinoxaline derivative of the sugars for confirming the structure of the trisaccharide amylotriose[29]. 1-^{14}C-Amylotriose was converted to the pyrazoloquinoxaline derivative and its X-ray diffraction data was studied, which confirmed its structure as O- α -D-glucopyranosyl(1→4)-O- α -D-glucopyranosyl(1→4)-D-1-^{14}C-glucose (60).

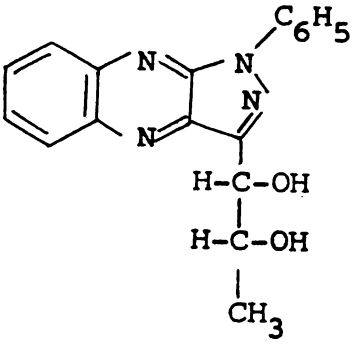


60

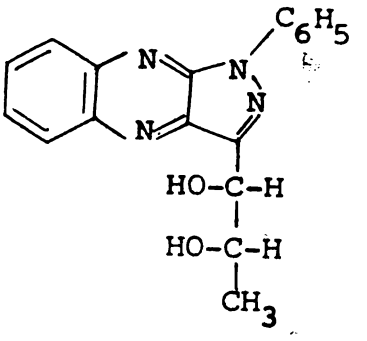
The 3-amino-3,6-dideoxy-D-aldohexose, mycosamine (61) was converted to its phenyl pyrazoloquinoxaline (62). The configuration at C_4 of 61 was established from the fact that 62 was the enantiomorph of the 1-phenylpyrazoloquinoxaline (63) formed from L-rhamnose[30].



61

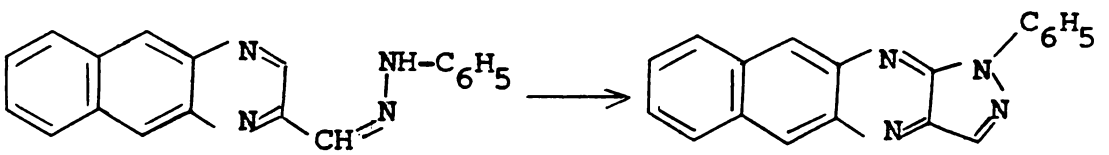


62



63

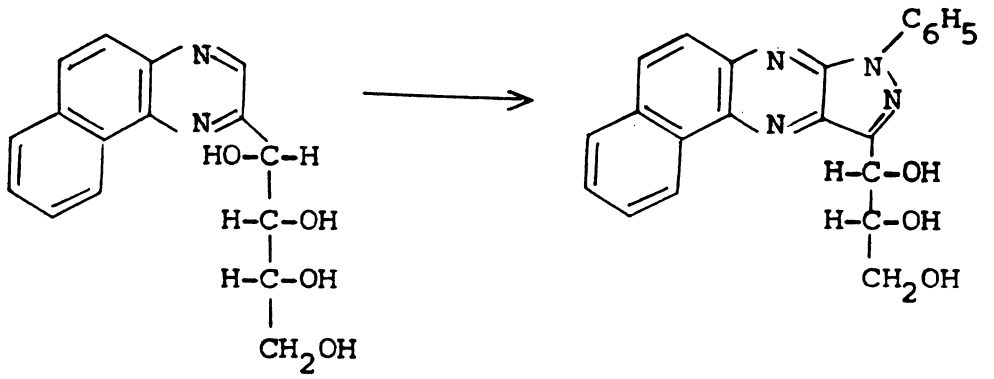
Amylose gave the corresponding 1-phenylpyrazoloquinoxaline on condensation with o-phenylene diamine and phenylhydrazine. Henseke and co-workers[6] prepared the higher condensed pyrazoloquinoxalines. On oxidative cyclisation of 6,7-benzoquinoxaline-2-carboxaldehyde phenylhydrazone (64) with phenylhydrazine in acetic acid they obtained 1-phenyl-6,7-benzopyrazoloquinoxaline (65).



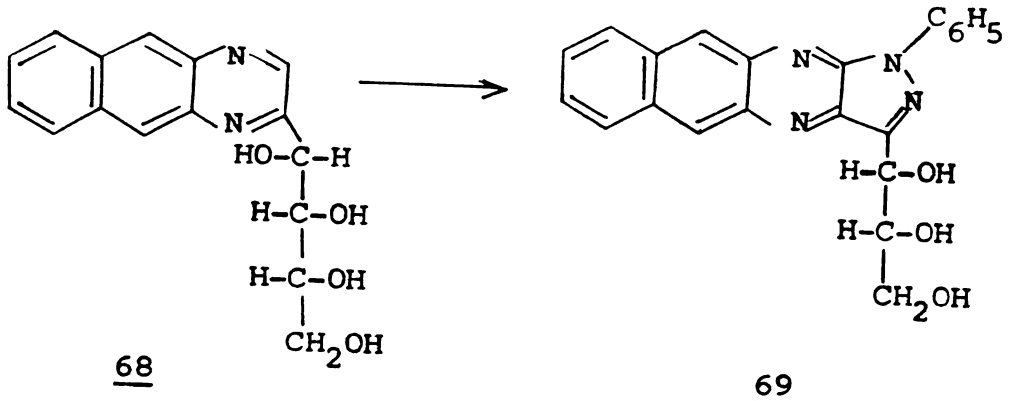
64 65

D-Fructosone-1-methylphenylhydrazone[32] on condensation with 1,2-diaminonaphthalene gave 3-(D-arabino-tetrahydroxybutyl)-5,6-benzoquinoxaline (66) which on treatment with phenylhydrazine under acidic conditions[33] gave 3-(D-erythro-trihydroxypropyl)-1-phenyl-5,6-benzopyrazoloquinoxaline (67). Similarly, D-fructosone-1-methylphenylhydrazone condensed with 2,3-diaminonaphthalene to give 3-(D-arabino-tetrahydroxybutyl)-6,7-benzoquinoxaline (68), which was

converted into the pyrazoloquinoxaline, 69 by treatment with phenylhydrazine.



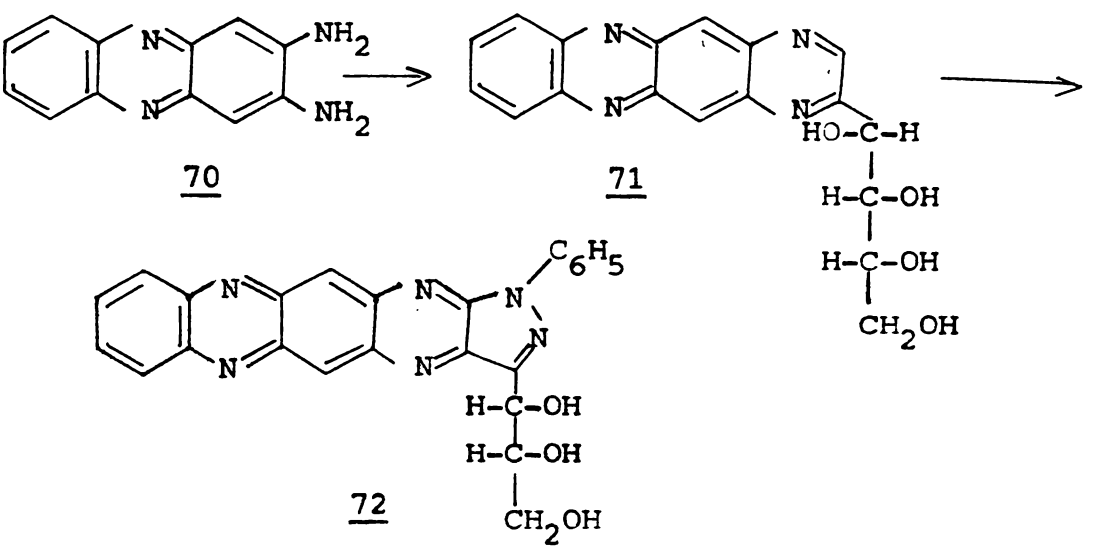
66 67



68 69

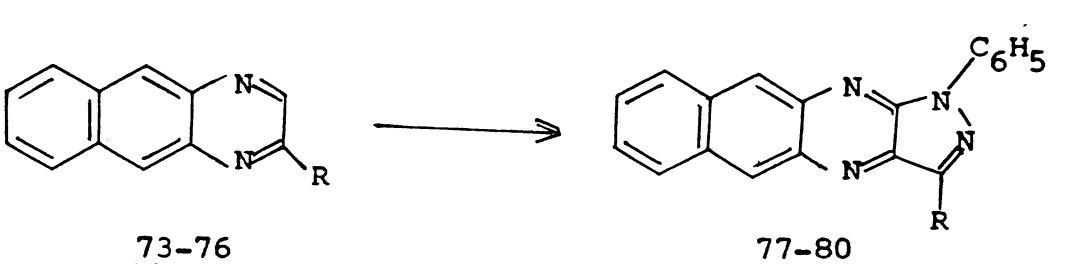
Henseke and co-workers have reported a still higher condensed pyrazoloquinoxaline, 72, which was prepared by the condensation of phenylhydrazine with 2-(D-arabino-tetrahydroxybutyl)quinoxalino[6,7-b]quinoxaline (71) which in

turn was obtained by the reaction of 2,3-diaminophenazine (70) with D-fructose-1-methylphenylhydrazone[33].



70 71 72

Preparation of pyrazolobenzoquinoxalines 77-80 has also been reported by Henseke and Brauer, which were obtained by the reaction of the corresponding benzoquinoxalines 73-76 with phenylhydrazine in the presence of acid[34].



73-76 77-80

73, R = D-lyxo-tetrahydroxybutyl

74, R = L-xylo-tetrahydroxybutyl

75, R = D-threo-trihydroxypropyl

76, R = L-erythro-trihydroxypropyl

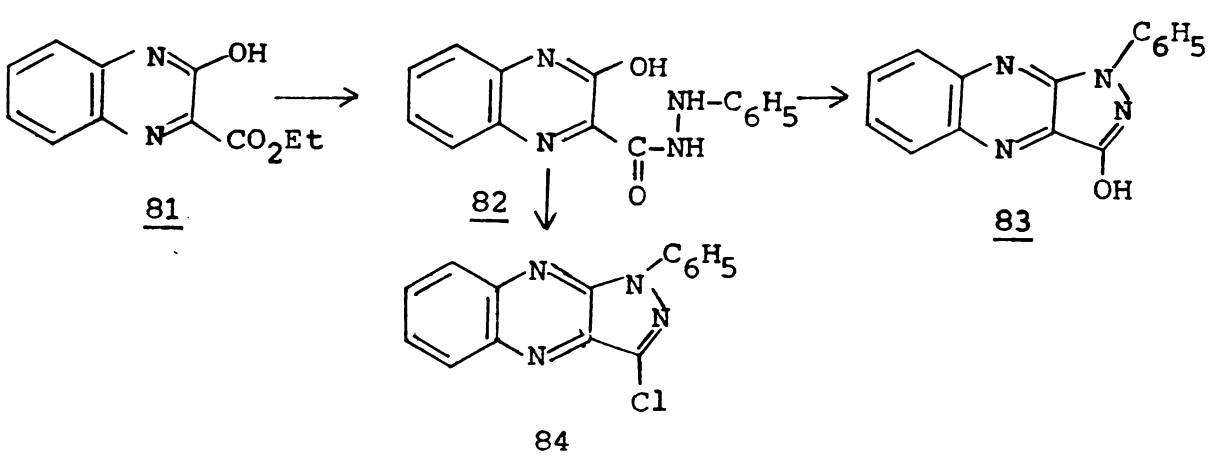
77, R = D-threo-trihydroxypropyl

78, R = L-threo-trihydroxypropyl

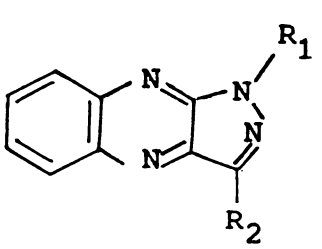
79, R = D-dihydroxyethyl

80, R = L-dihydroxyethyl

Pyrazoloquinoxalines with various groups such as chloro, amino, hydroxy, chloromethyl, carboxamido, trichloromethyl, N-pyrrolidyl and N-pyrrolidylmethyl at position 3 have been reported by P.M.Pillai and P.Ramabhadran[35]. Ethyl-2-hydroxyquinoxaline-3-carboxylate (81) was condensed with phenylhydrazine to give the hydrazide (82) which when cyclised using p-toluenesulphonic acid gave 3-hydroxy-1-phenylpyrazoloquinoxaline (83) and when $POCl_3$ is used for cyclisation the product was 3-chloro-1-phenylpyrazoloquinoxaline (84) and a small quantity of 83[36].



They have also reported new methods for the oxidative cyclisation of the phenylhydrazones[36]. In one of the methods they have reported the use of azobenzene as the dehydrogenating agent, which gave excellent yield of pyrazoloquinoxaline. In a second method they have achieved the cyclisation of phenylhydrazone just by heating it above its melting point in an atmosphere containing oxygen. In a third method they have reported the use of air oxidised phenylhydrazine as the oxidising agent when an unusual phenylation reaction was found to take place[37]. Quinoxaline-2-carboxaldehyde phenylhydrazone when treated with air oxidised phenylhydrazine they obtained 1,3-diphenyl pyrazoloquinoxaline. By extending this method they have prepared 1,3-diphenyl, 1-p-tolyl-3-phenyl, 1-p-chlorophenyl-3-phenyl, 1-p-bromophenyl-3-phenyl, and 1-phenyl-3-ptolyl-pyrazoloquinoxalines 85 to 89. Preparation of 1-phenyl-3-trichloromethyl-1H-pyrazoloquinoxaline (90) has also been reported by the same authors.



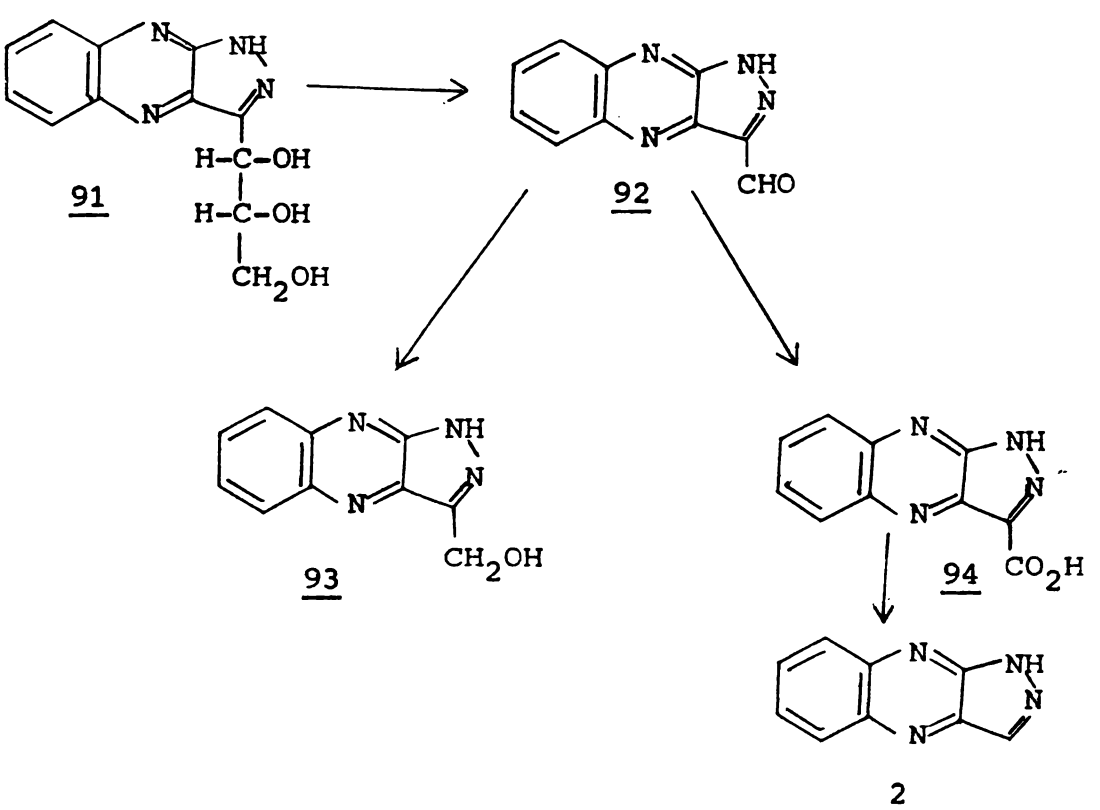
85, $R_1 = C_6H_5$, $R_2 = C_6H_5$ 86, $R_1 = p\text{-}C_6H_4\text{-}CH_3$, $R_2 = C_6H_5$

87, $R_1 = p\text{-}C_6H_4Cl$, $R_2 = C_6H_5$ 88, $R_1 = p\text{-}BrC_6H_4$, $R_2 = C_6H_5$

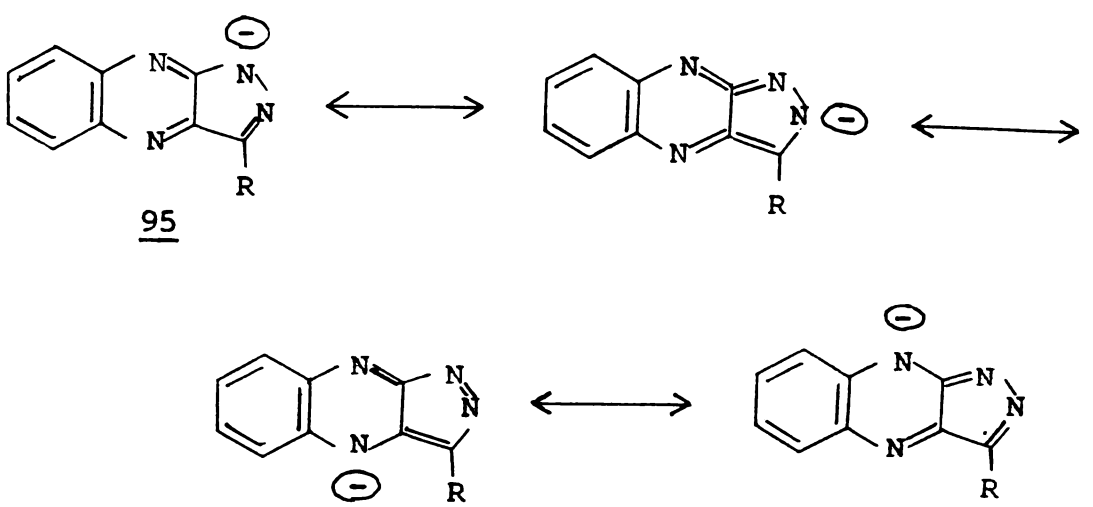
89, $R_1 = C_6H_5$, $R_2 = p\text{-}C_6H_4CH_3$ 90, $R_1 = C_6H_5$, $R_2 = CCl_3$

2.2.2 Pyrazoloquinoxalines unsubstituted at position 1

Ohle and Iltgen were the first to prepare the parent, unsubstituted 1H-pyrazolo[3,4-b]quinoxaline[38] (2). 2-(D-arabino-tetrahydroxybutyl)quinoxaline[2] (3) was condensed with hydrazine to give 40% of 3-(D-erythro-trihydroxypropyl) pyrazoloquinoxaline (91) under optimum conditions. The sugar residue of 91 was oxidised using lead tetraacetate or periodic acid, which gave the carboxaldehyde 92, peridodic acid being the better reagent. 92 underwent the Cannizzaro's reaction with hot concentrated alkali and gave a mixture of the primary alcohol, 93 and carboxylic acid, 94. Acid 94 was also

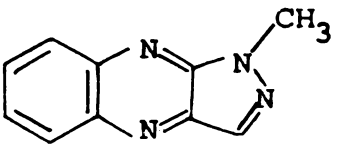


obtained by the oxidation of carboxaldehyde, 92 with chromic anhydride in 50% sulphuric acid. Carboxylic acid 94 when sublimed under atmospheric pressure provided the pyrazoloquinoxaline 2. It was observed that all pyrazoloquinoxalines with a free 1-position form orange to yellow metal salts, the colour being ascribed to the flavozole anion, 95 which, may have a number of resonance forms as shown below.

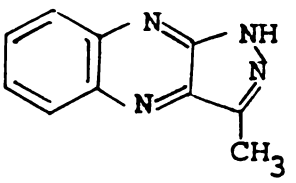


95

The same authors have reported the preparation of 1-methyl pyrazoloquinoxaline 96 starting from methylhydrazine instead of hydrazine in the reaction with 3 and that of 3-methylpyrazoloquinoxaline (97) by the reduction of 92 with hydrazine[38].

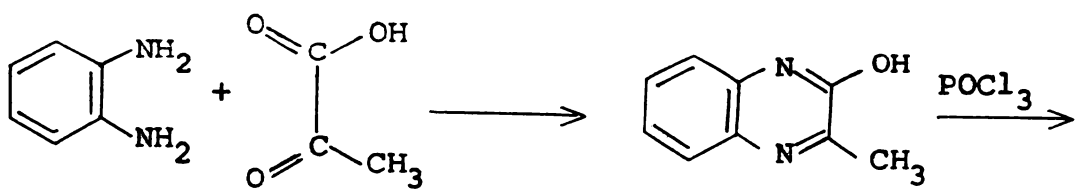


96

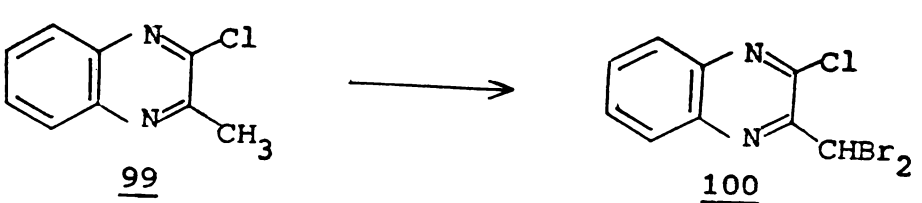


97

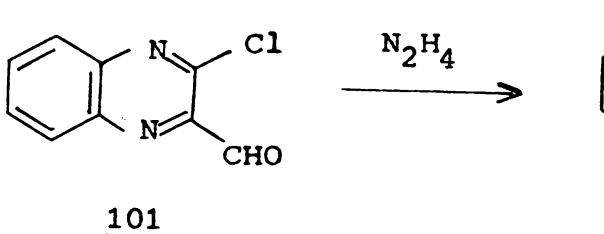
Pyrazoloquinoxaline, 2 was also prepared starting from pyruvic acid[39]. 2-Hydroxy-3-methylquinoxaline (98), prepared by the condensation of o-phenylene diamine with pyruvic acid on reaction with $POCl_3$ gave 2-chloro-3-methylquinoxaline (99). Bromination of the methyl group of 99 gave the dibromo derivative, 100 which on treatment with hydrazine hydrate provided the unsubstituted pyrazoloquinoxaline, 2. Romenenko and Burnistrov



98



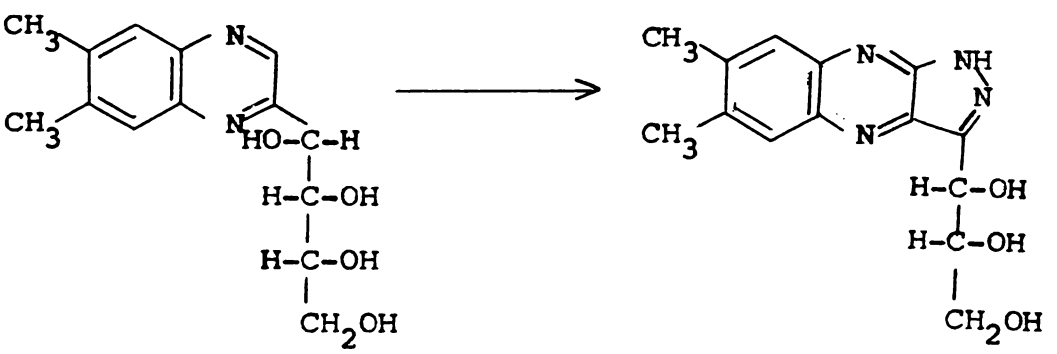
99 100



101 2

also prepared 2 in 71% yield by the reaction of 2-chloroquinoxaline-3-carboxaldehyde (101) with hydrazine[40].

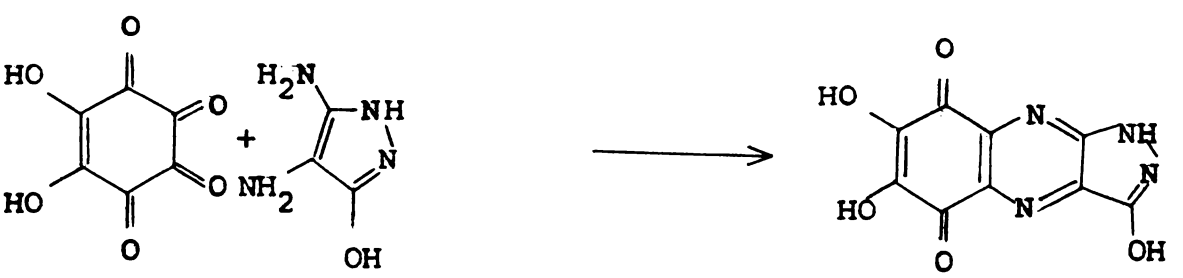
In order to study the pharmacological activity 7,8-dimethyl pyrazoloquinoxalines with a sugar residue at position 3 were synthesised by N.P.Bu Hoi and co-workers[41]. 2-(D-arabino-tetrahydroxybutyl)-6-7-dimethylquinoxaline (102) was treated with hydrazine hydrate in acetic acid and copper power to give 3-(D-erythro-trihydroxy propyl)-6,7-dimethyl pyrazoloquinoxaline (103).



102

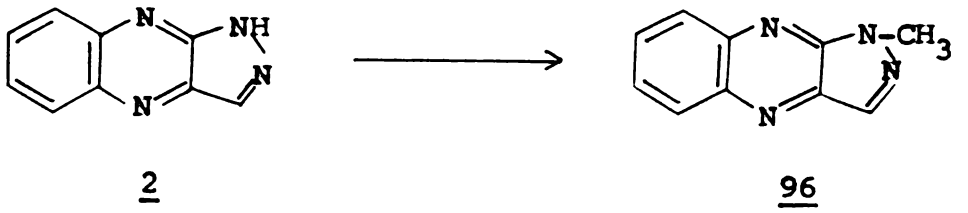
103

A highly oxidised pyrazoloquinoxaline derivative, 3,6,7-trihydroxy pyrazoloquinoxaline-5,8-dione (106) was prepared by the treatment of a solution of dipotassium rhodizonate 104 with 3,4-diamino-5-hydroxypyrazole, 105 in the presence of sulphuric acid[42].

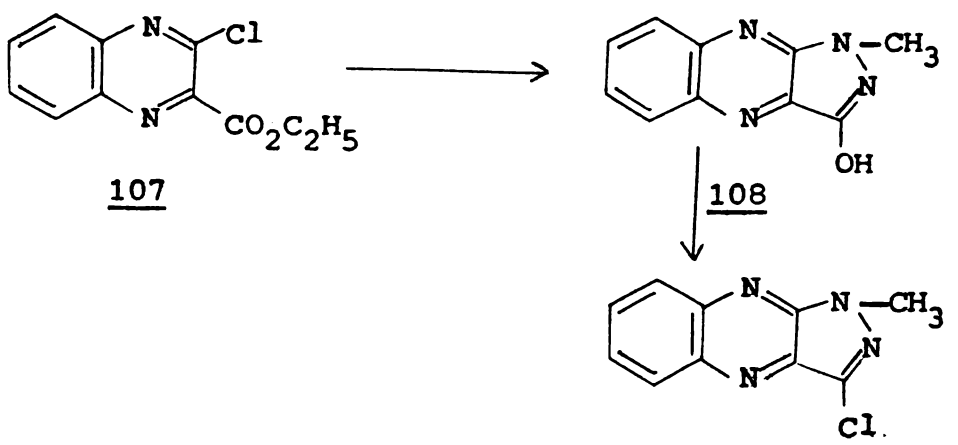


104 105 106

1-Methyl pyrazoloquinoxaline (96) has been prepared by the alkylation of 1H-pyrazoloquinoxaline (2) using alkyl halide and potassium carbonate in dimethyl formamide[43]. Hans Bayer has also reported the preparation of 3-hydroxy-1-methyl-pyrazoloquinoxaline (108) and 3-chloro-1-methyl pyrazolo-quinoxaline (109) starting from ethyl-2-chloroquinoxaline-3-carboxylate (107). Chloro ester 107 on condensation with methylhydrazine gave 108 which was converted to 3-chloro-1-methyl pyrazoloquinoxaline by treatment with $POCl_3$.

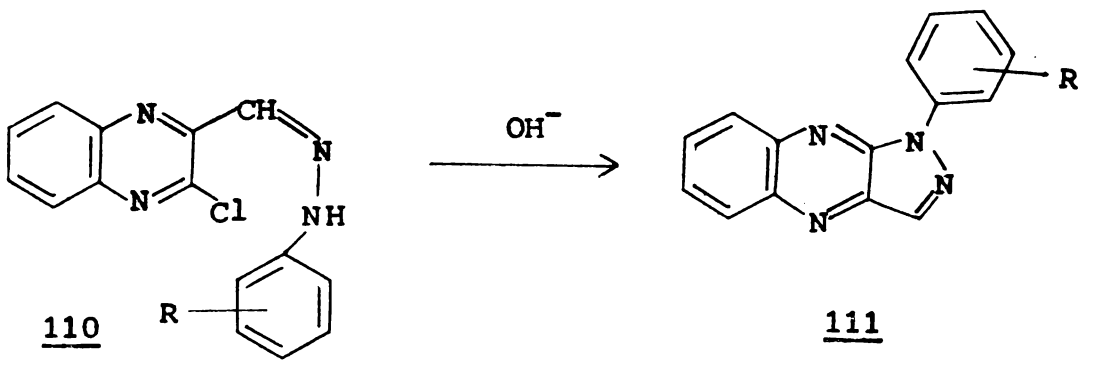


2 96



107 108 109

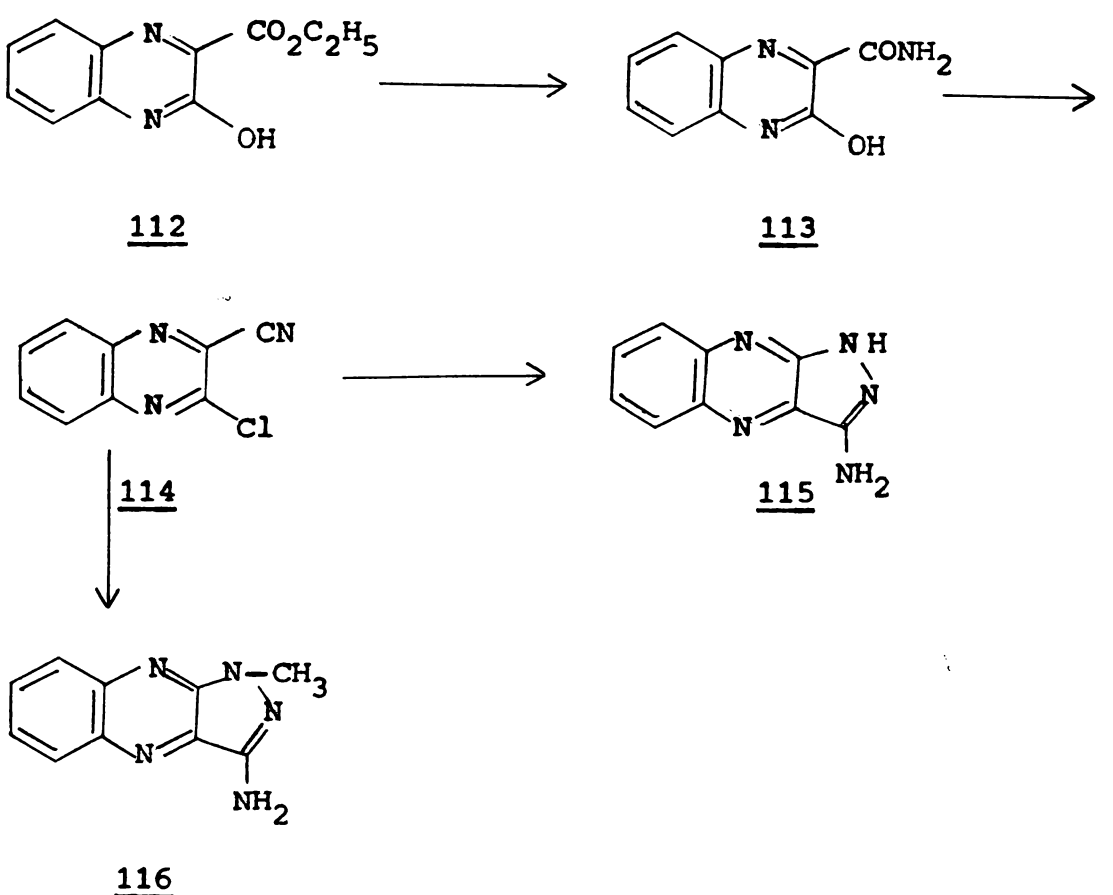
The same author has obtained 1-phenylpyrazolo----quinoxalines with substituents such as Cl, NO_2, 2,4 nitro, groups on the 1-phenyl ring by the cyclisation of the corresponding phenylhydrazones of 3-chloroquinoxaline-2-carboxaldehyde in an alkaline solution[43].



110 111

R = p-Cl, p-NO_2, m-NO_2, 2,4-dinitro

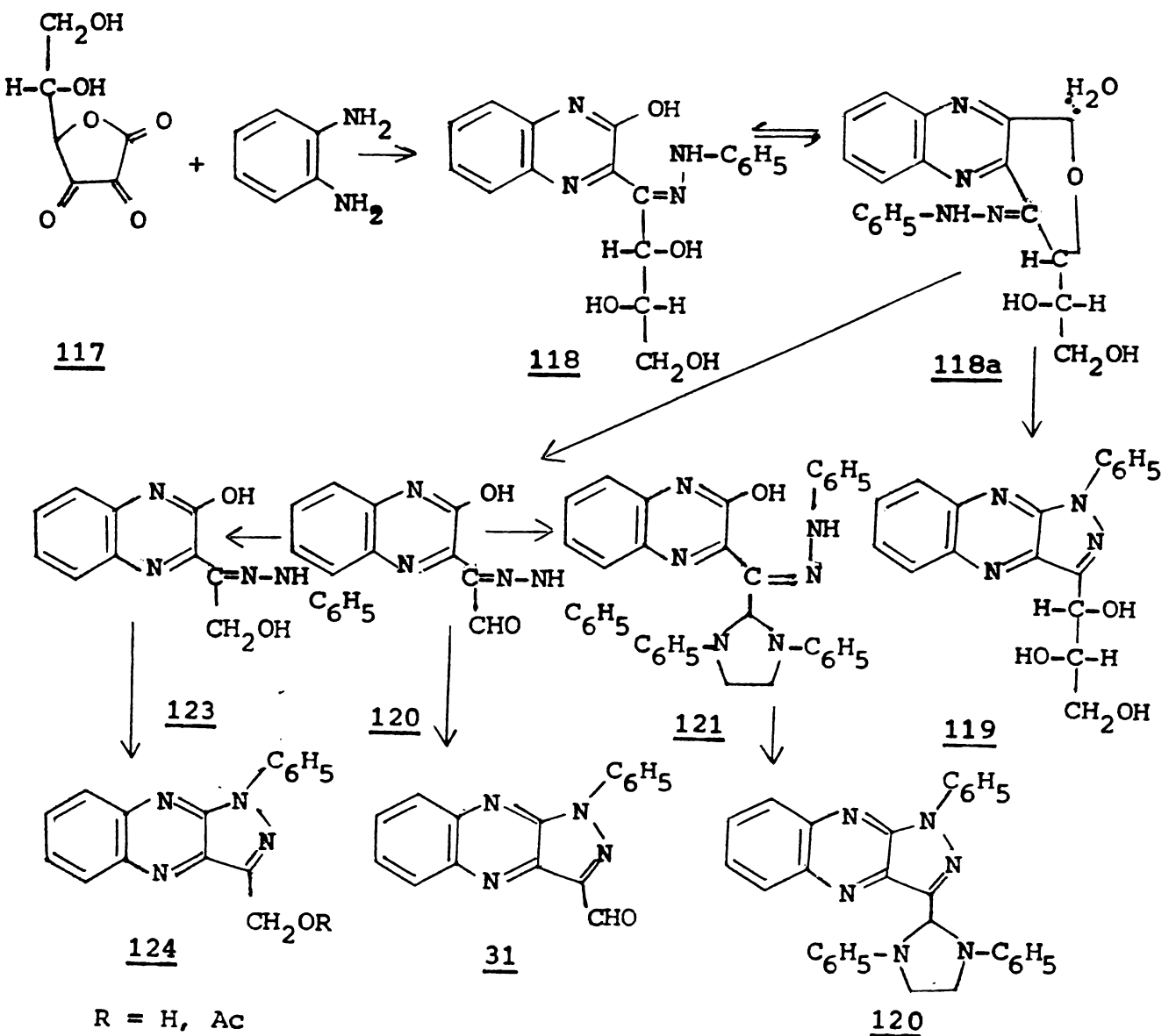
Ke Yoshida and Hirotaka Otomasu has reported the preparation of 3-aminopyrazoloquinoxaline[44] (115). Ethyl 2-hydroxyquinoxaline-3-carboxylate (112) was treated with ammonium carbonate to give the amide (113) which on treatment with $POCl_3/PCl_5$ gave 2-chloroquinoxaline-3-carbonitrile (114) in 85% yield. Refluxing an ethanolic solution of 114 with hydrazine hydrate gave 3-aminopyrazoloquinoxaline (115). Similarly treatment of 114 with methylhydrazine gave 3-amino-1-methyl pyrazoloquinoxaline (116).



112 113 114 115 116

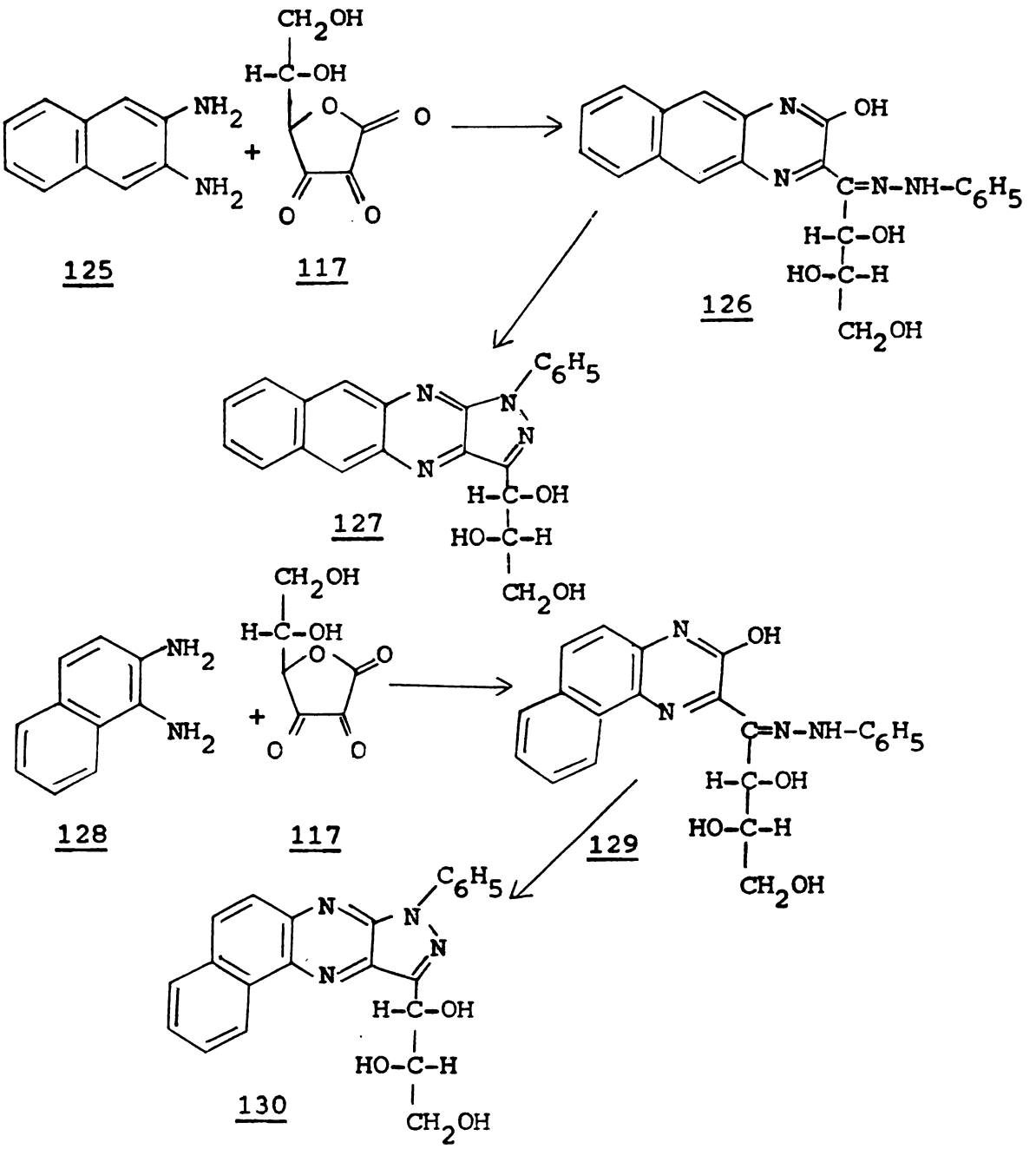
2.2.3 Pyrazoloquinoxalines from dehydro-L-ascorbic acid and related compounds

Dehydro-L-ascorbic acid (117) prepared by the oxidation of L-ascorbic acid with p-benzoquinone, when treated with o-phenylene diamine and phenylhydrazine gave 2-hydroxy-3-(1-phenylhydrazone-L-threo-2,3,4-trihydroxybutyl)quinoxaline[45] (118) which was first believed to have the cyclic structure, 118a.



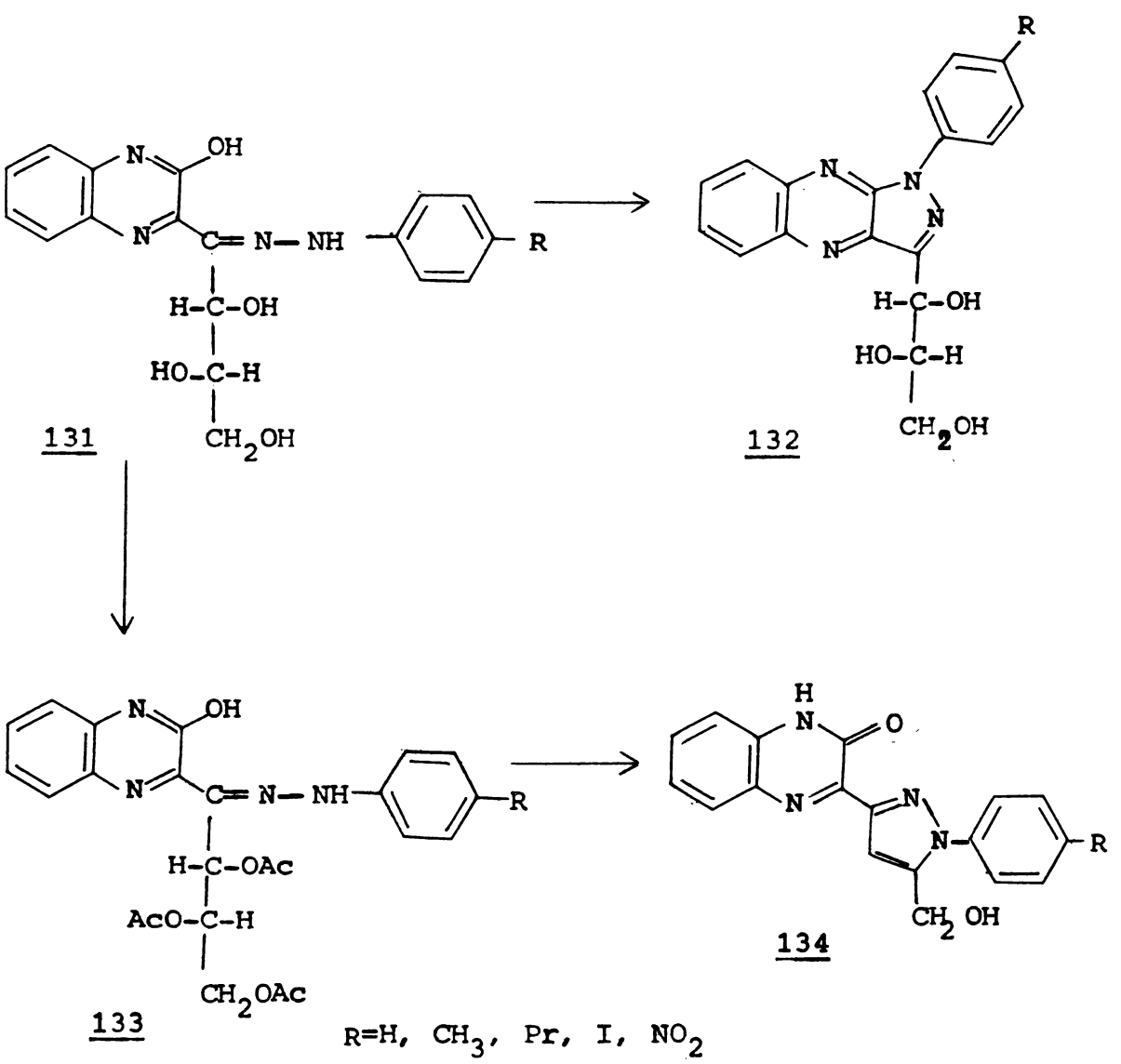
1-phenyl-3-(L-threo-trihydroxypropyl)pyrazoloquinoxaline was obtained in excellent yield when 118 was heated with sodium hydroxide solution[45]. Also oxidation of 118 with periodic acid gave the carboxaldehyde, 120 which when treated with 1,2-dianilinoethane gave 121 and subsequent cyclisation in the presence of methanolic sodium hydroxide provided 1-phenyl-3-(1,3-diphenyl-2-imidazolinyl)pyrazoloquinoxaline[46] (122). 1-[2-Hydroxyquinoxalyl-3-]glyoxal-1-phenylhydrazone (120) itself cyclised to give 1-phenylpyrazoloquinoxaline-3-carboxaldehyde (31) in the presence of alkali. Compound 31 was also converted into 122 by treatment with 1,2-dianilinoethane[46]. The aldehyde 120 when reduced with sodium borohydride gave the alcohol, 123 which on treatment with a base or acetic anhydride provided the pyrazoloquinoxaline, 124 with the hydroxyl group either free or acetylated[47].

L-Dehydroascorbic acid on treatment with 2,3-diamino-naphthalene (125) and phenylhydrazine gave 2-hydroxy-3-(1-phenylhydrazono-L-threo-trihydroxybutyl)-6,7-benzoquinoxaline (126), which on cyclisation with sodium hydroxide in aqueous n-butanol provided 1-phenyl-3-(L-threo-trihydroxypropyl)-6,7-benbzopyrazoloquinoxaline[46] (78). When 1,2-diaminonaphthalene was used instead of 2,3-diaminonaphthalene in the above reaction, 2-hydroxy-3-(1-phenylhydrazono-L-threo-trihydroxybutyl)-5,6-benzoquinoxaline (129) was obtained, which when cyclised gave the pyrazoloquinoxaline[46], 130.

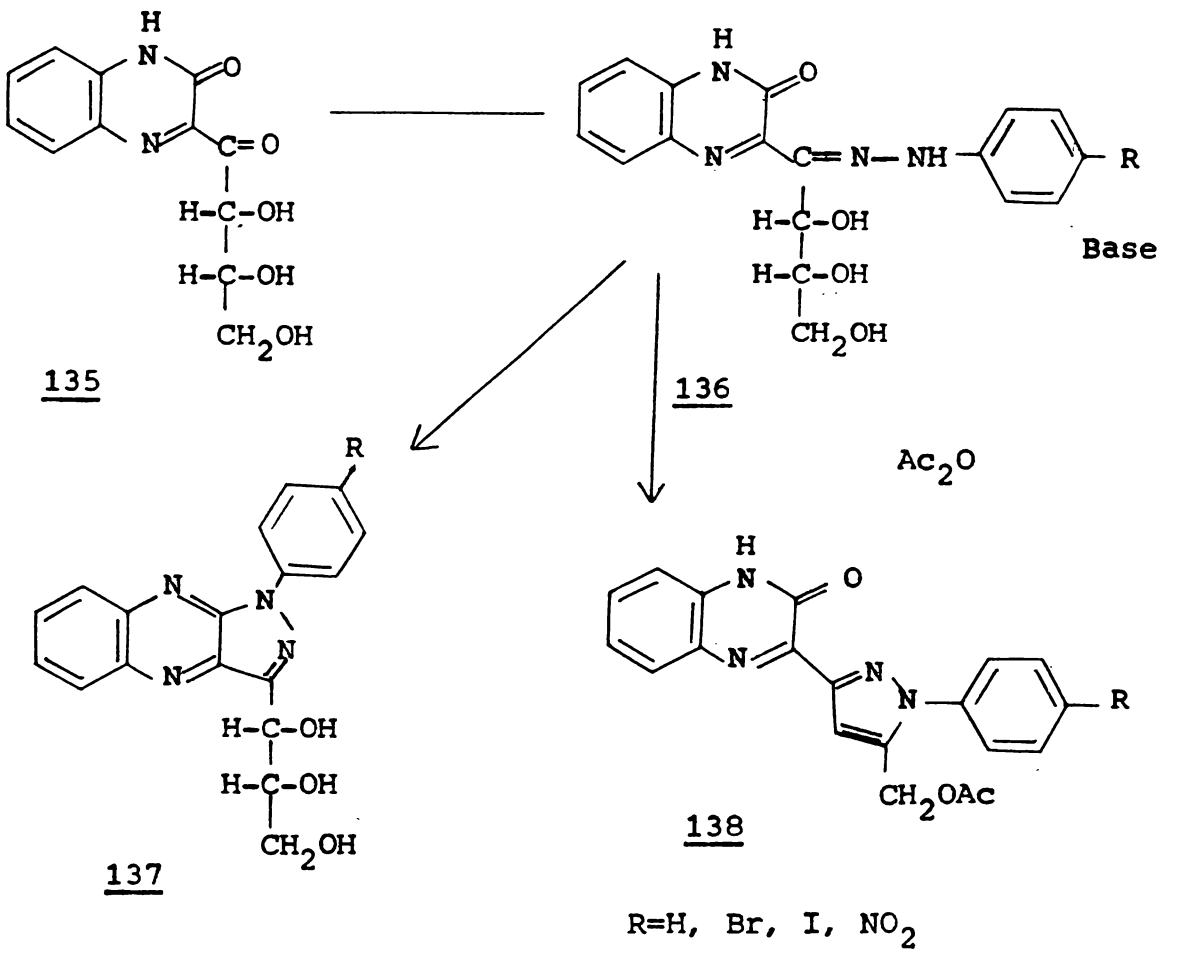


El Ashry and co-workers prepared a number of para-substituted 1-phenyl-3-(L-threo-trihydroxypropyl)pyrazolo-quinoxaline (132) by the simultaneous treatment of dehydro-L-ascorbic acid with o-phenylene diamine and para-substituted

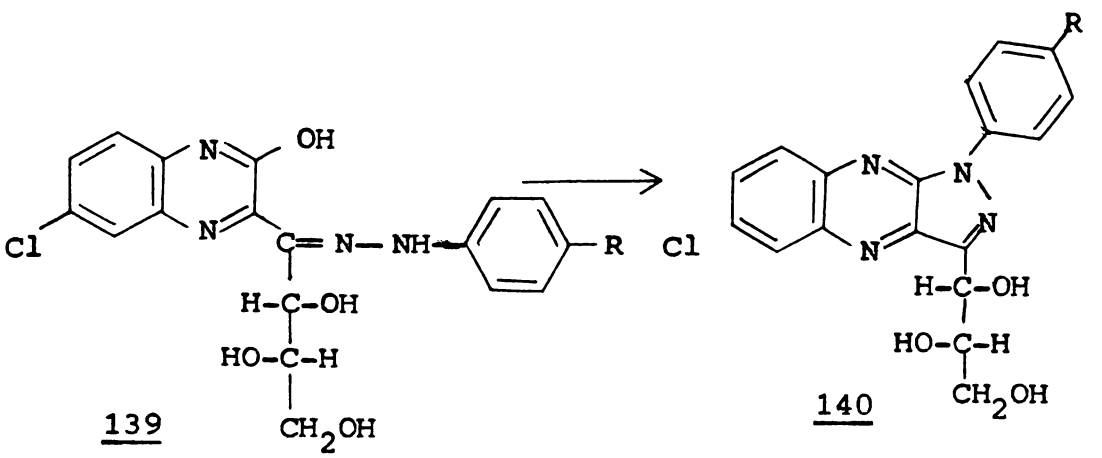
phenylhydrazine and subsequent cyclisation of the quinoxaline derivatives (131) in the presence of alkali[48]. However the corresponding acetylated quinoxalines (133) on treatment with alkali underwent deacetylation and dehydration to 3-(1-aryl-5-hydroxymethylpyrazolol-3-yl)-2-quinoxalines[48] (134).



D-Dehydroascorbic acid also reacted in the same manner producing the corresponding derivatives, 135, 136 and 137 other than the cyclisation of phenylhydrazones using acetic anhydride in which case product obtained was 138[50].

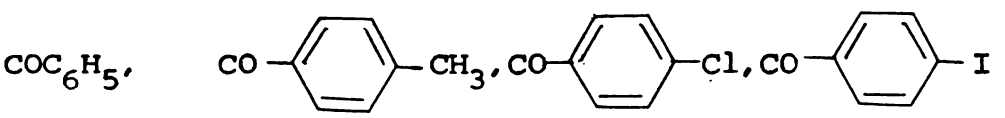


Reaction of dehydro-L-ascorbic acid with 4-chloro-o-phenylene diamine followed by treatment with para-substituted phenylhydrazines gave 6-chloro-3-(1-arylhydrazono-L-threo-2,3,4-trihydroxybutyl)-2-quinoxalinones[50] (139), which on cyclisation gave the corresponding pyrazoloquinoxalines (140).

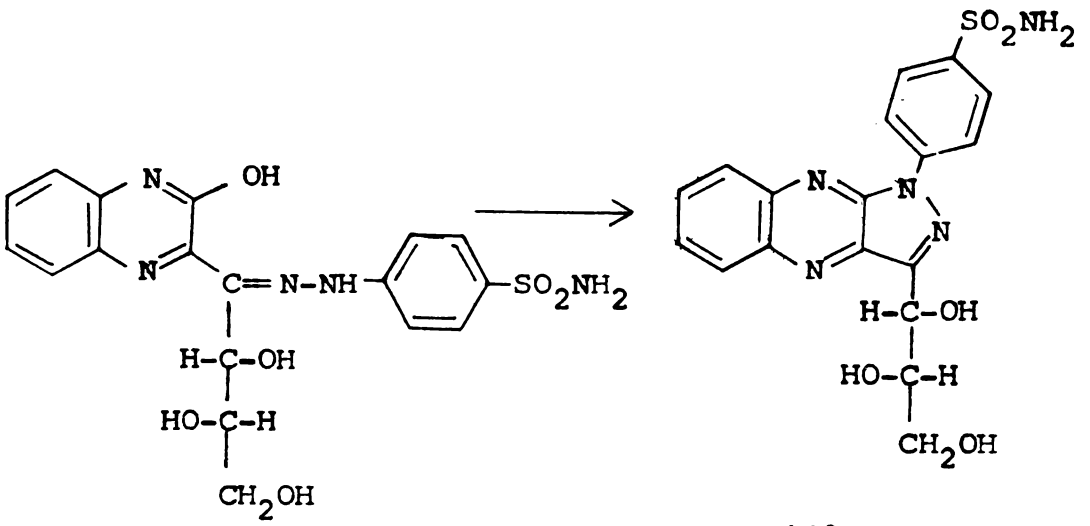


139 140

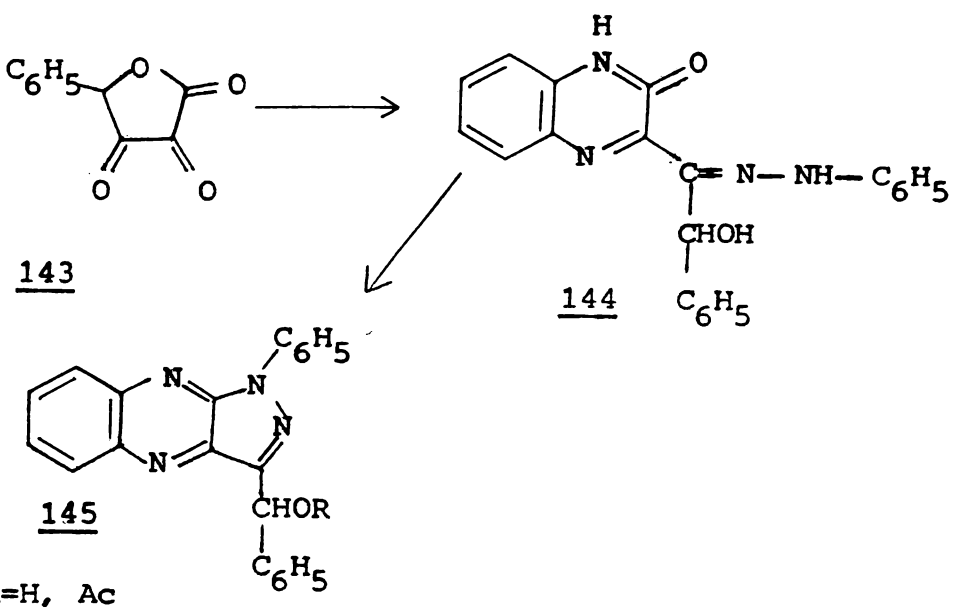
R=CH_3, OCH_3, Br, I, NO_2, SO_2NH_2,



3-(L-Threo-trihydroxypropyl)-1-(p-sulphynyl-phenyl) pyrazoloquinoxaline (142) was similarly prepared[51] by the cyclisation of the corresponding quinoxaline derivative, 141. 4-Phenyl-2,3-dibutanol-1,4-lactone (143) also reacted with o-phenylene diamine and phenylhydrazine in an analogous manner producing 3-(2-aryl-1-phenylhydrazono-2-hydroxyethyl)-2-quinoxaline[49] (144), which was cyclised to 3-(∝-hydroxybenzyl)-1-phenyl-pyrazoloquinoxaline[49] (145).



141 142

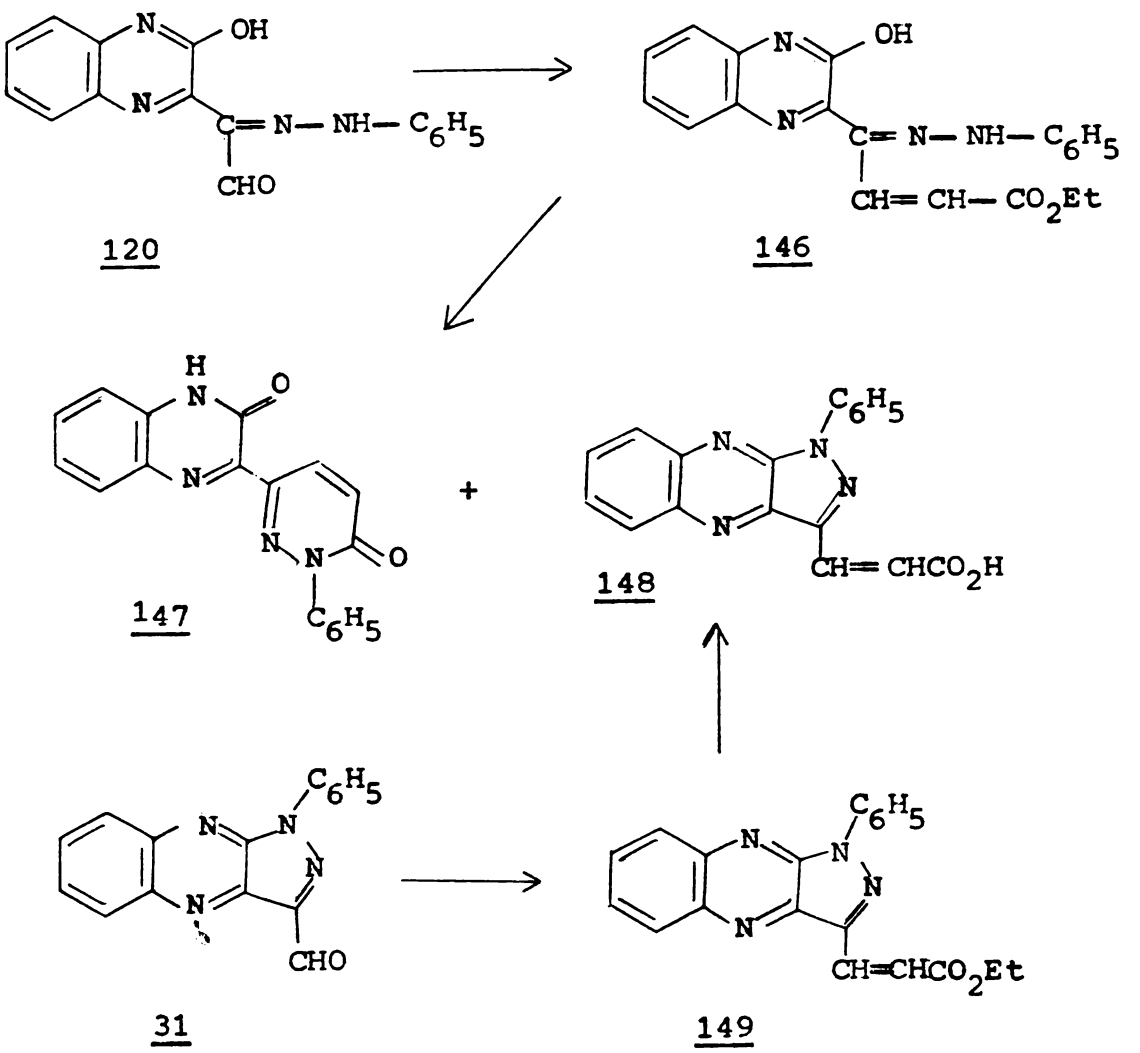


143 144 145

R=H, Ac

A witting reaction of the carboxaldehyde[46], 120 with carboethoxymethylidene triphenylphosphorane gave the condensation product[52], 146. Cyclisation of 146 with alkali gave two products, the pyridazinone, 147 and the pyrazoloquinoxaline,

148. The formation of the free carboxylic acid, 148 may be explained as the hydrolysis product of the ester 149 which was also obtained by a Wittig reaction of 1-phenyl pyrazolo-quinoxaline-3-carboxaldehyde, 31.

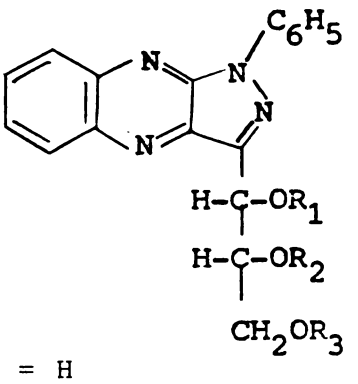


2.3 Reactions of pyrazoloquinoxalines

A large number of the chemical reactions of pyrazoloquinoxalines have been studied as summarised below.

2.3.1 Reactions involving the sugar residue at position 3

Pyrazoloquinoxalines were prepared mainly from carbohydrates and hence had a sugar residue at position 3. The number and nature of these hydroxyl groups were studied by preparing their derivatives[31]. Thus 1 gave the tri-o-acetyl derivative, 150 on treatment with acetic anhydride and pyridine[1], and monomethylether, 151 on treatment with triphenylmethylchloride, which on acetylation yielded the di-o-acetate, 152. Reaction of 1 with acetone gave a mixture of isopropylidene derivatives 153 and 154, benzoylation of which gave a mixture of 155 and 156 respectively. Alkali hydrolysis of 155 gave back 153, while a mild acid hydrolysis yielded the monobenzoate, 157. 1 on treatment with one mole of benzoylchloride in pyridine at 20°C for 15 hours provided the monobenzoate, 159 while with excess benzoylchloride in pyridine at 100° gave the tribenzoate, 158. With acetone and a catalytic amount of sulphuric acid, 159 yielded the isopropylidene derivative, 156.



1, $R_1 = R_2 = R_3 = H$

150, $R_1 = R_2 = R^3 = Ac$

151, $R_1 = R_2 = H$, $R_3 = -C9C_6H_5)_3$

152, $R_1 = R_2 = Ac$, $R_3 = -C(C_6H_5)_3$

153, $R_1 = H$, R_2, $R_3 = C(CH_3)_2$

154 R_1, $R_2 = C(CH_3)_2$, $R_3 = H$

155, $R_1 = COC_6H_5$, R_2, $R_3 = C(CH_3)_2$

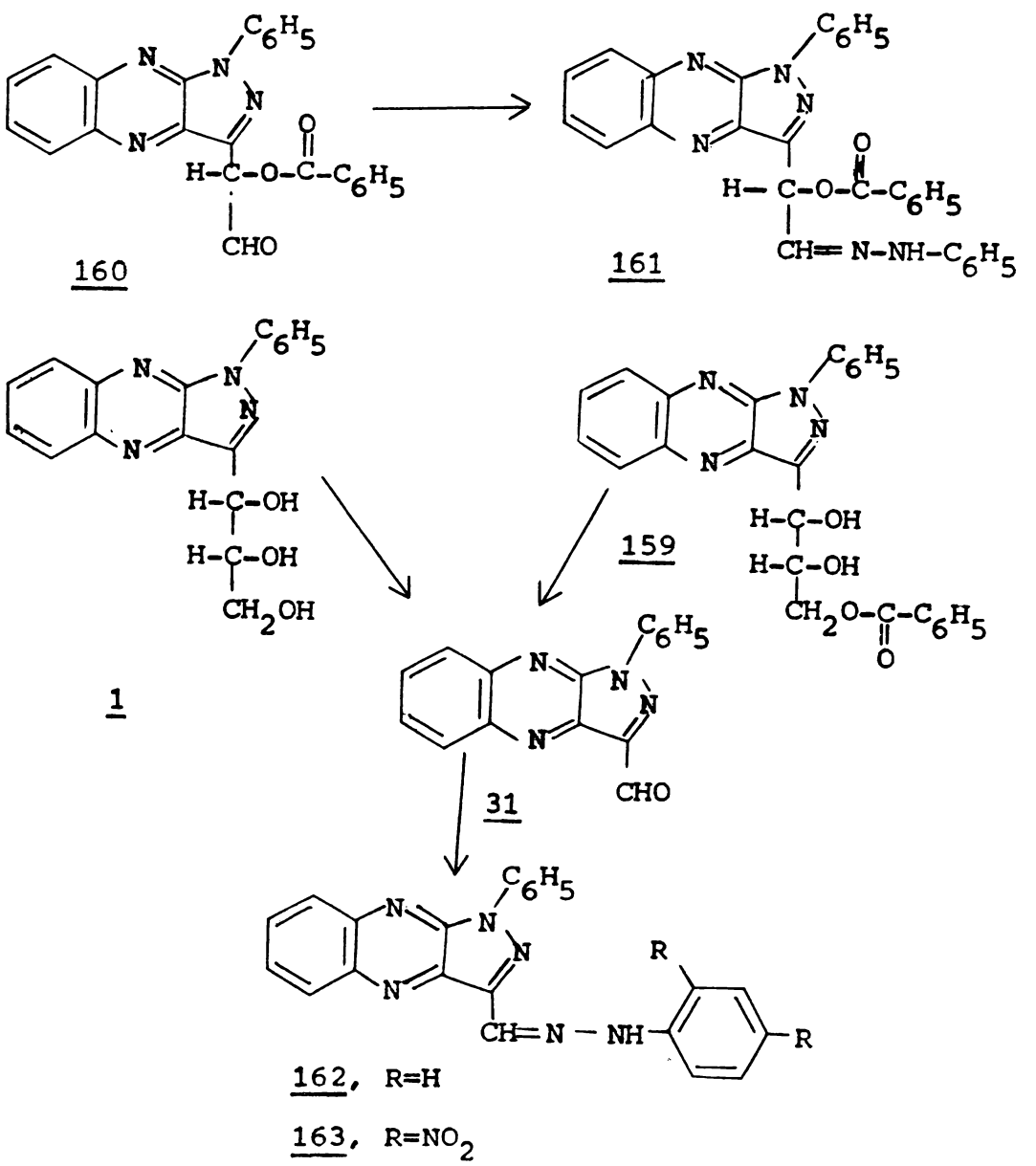
156, R_1, $R_2 = C(CH_3)_2$, $R_3 = COC_6H_5$

157, $R_1 = COC_6H_5$, $R_2 = R_3 = H$

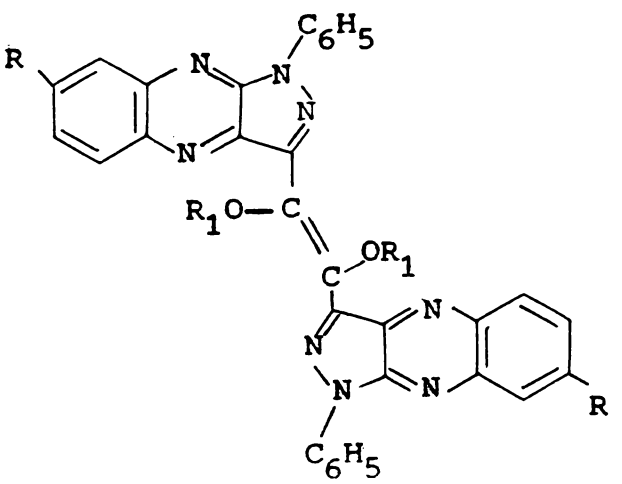
158, $R_1 = R_2 = R_3 = COC_6H_5$

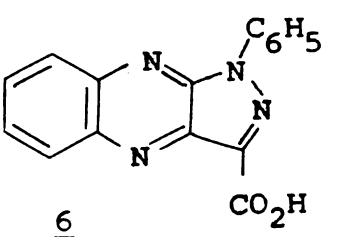
159, $R_1 = R_2 = H$, $R_3 = COC_6H_5$

Cleavage of 157 with lead tetraacetate in benzene yielded (1-phenyl-3-pyrazoloquinoxaloyl)-o-benzoylglyoxal, 160 which formed a phenylhydrazone, 161. Oxidation of 159 with lead tetraacetate in benzene gave the 1-phenylpyrazoloquinoxaline-3-carboxaldehyde 31 which was also obtained by the direct oxidation of 1 with lead tetraacetate. The phenylhydrazone, 162 and 2,4-dinitrophenylhydrazone, 163 of 31 were also reported[2].



Acyloin reaction of pyrazoloquinoxaline-3-carboxaldehydes using aqueous potassium cyanide have been reported[46]. So the carboxaldehyde, 31 gave 164 and 7-chloro-1-phenylpyrazoloquinoxaline-3-carboxaldehyde gave 165, which was also characterised as its diacetate, 166 and dibenzoate[54], 167.





6

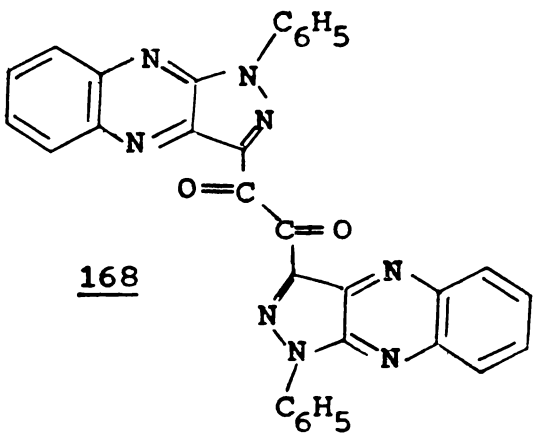
164, R = R_1 = H

165, R = Cl, R_1 = H

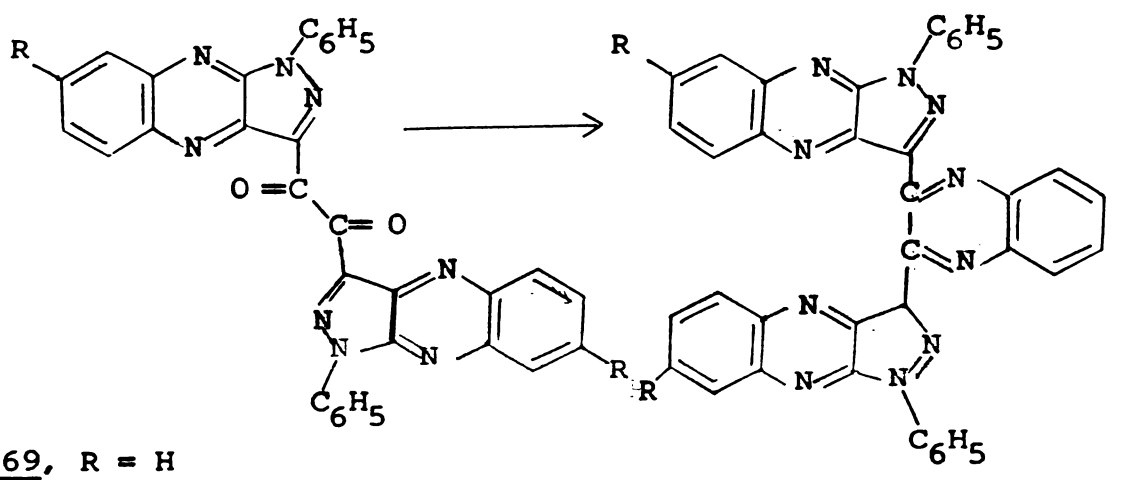
166, R = Cl, R_1 = Ac

167, R = Cl, R_1 = C_6H_5CO

Preparation of pyrazoloquinoxalils (168) have been reported by Henseke and co-workers[54]. The pyrazoloquinoxaloins 164 and 165 were easily oxidised by air to the corresponding pyrazoloquinoxalils, 169 and 170. Treatment of the pyrazoloquinoxalil 169 with o-phenylene diamine provided 2,3-bis(1-phenylpyrazoloquinoxalol-3-yl)quinoxaline[54] (171).



168

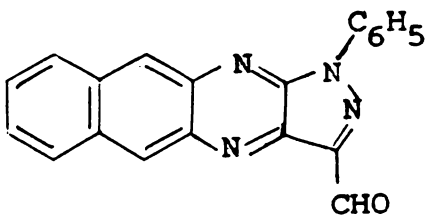


169, R = H

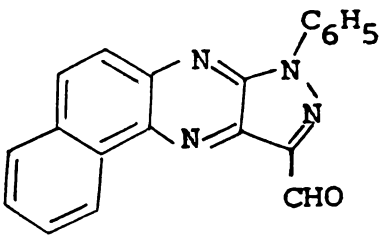
170, R = Cl

171, R = H

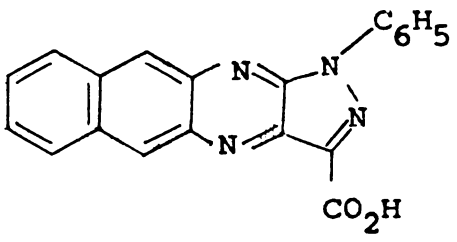
Oxidation of the sugar residue in higher condensed pyrazoloquinoxalines by lead tetraacetate or periodic acid provided the corresponding 3-formyl pyrazoloquinoxalines. Thus compounds 172[31,33] and 173[46] were obtained from the corresponding starting compounds. Aldehyde 172 was further converted to the carboxylic acid, 174 and the phenylhydrazone, 175.



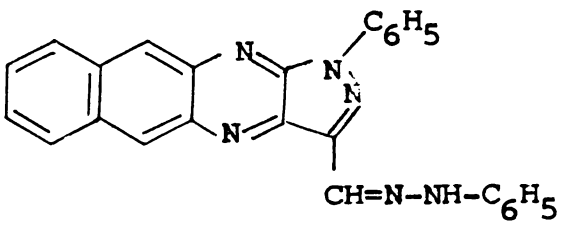
172



173

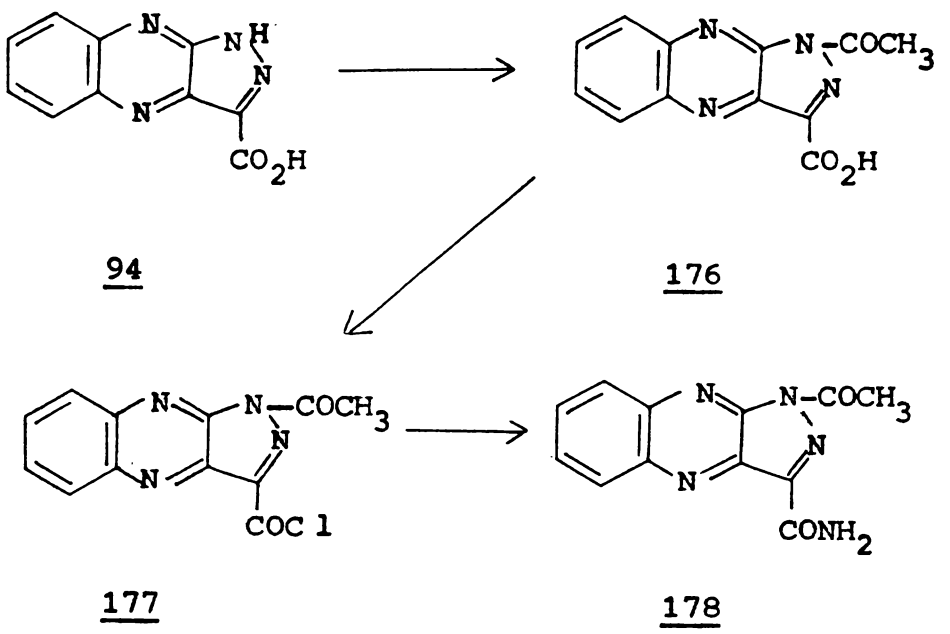


174



175

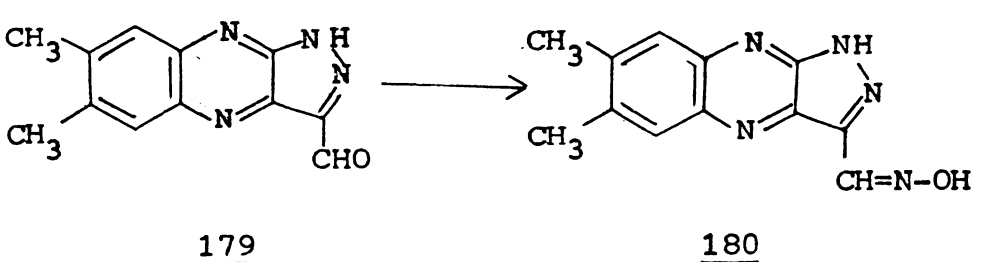
Pyrazoloquinoxaline-3-carboxylic acid (94) obtained from 3-(D-erythro-trihydroxypropyl)pyrazoloquinoxaline (91) on acetylation gave 176. 176 on treatment with thionyl chloride gave the acid chloride, 177 which when reacted with methanolic ammonia yielded the carboxamide[38], 178.



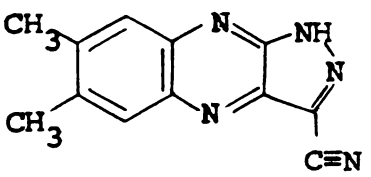
94 176

177 178

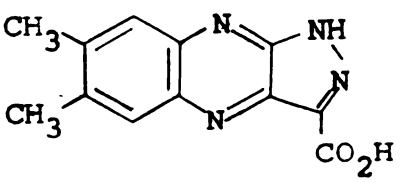
3-(D-Erythro-trihydroxypropyl)-6,7-dimethyl pyrazoloquinoxaline (103) was also oxidised to the 3-formyl derivative, 179[41]. The conversion of 179 to the oxime, 180, followed by dehydration provided the 3-cyano-6,7-dimethyl pyrazoloquinoxaline (181) which was hydrolysed to the carboxylic acid, 182.



179 180

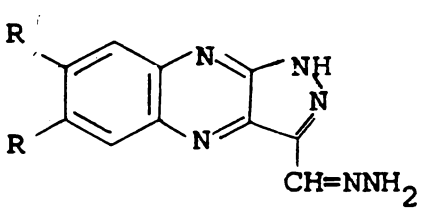


181

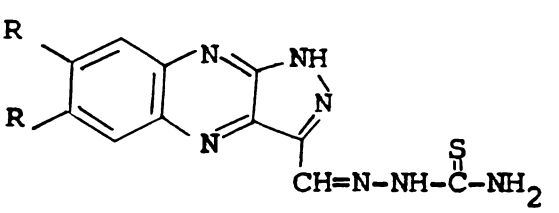


182

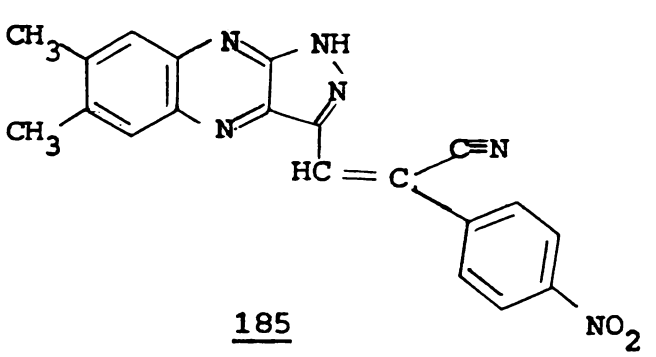
The following derivatives, 183-187 of 3-formyl pyrazoloquinoxaline were also prepared in order to study their tuberculostatic activity.[55]



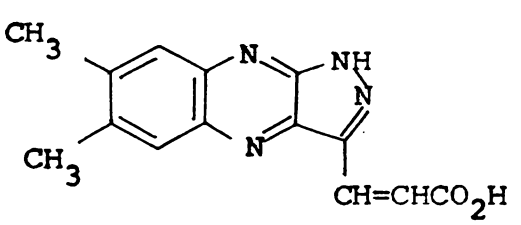
183, R=H, CH_3



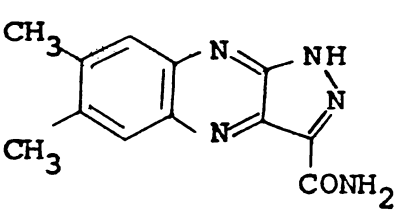
184, R=H, CH_3



185



186



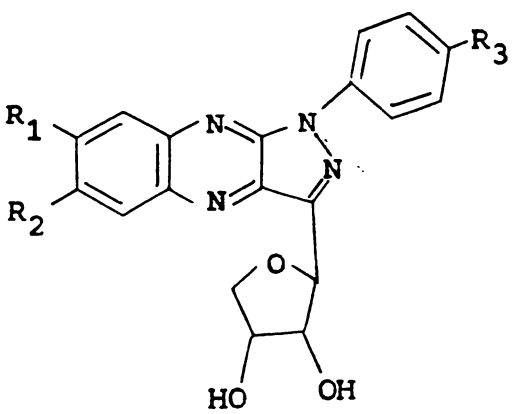
187

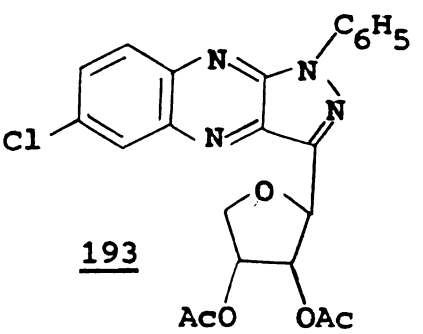
J.N.Bemiller and co-workers have studied the kinetics of hydrolysis of glycoside linkage in the pyrazoloquinoxalines[56]. Hydrolysis of 1-phenylpyrazoloquinoxaline of maltose (28) showed a linear relation between log k and Hammett acidity function (Ho) suggesting unimolecular decomposition of the conjugate acid of 28 without participation of water and absence of intramolecular catalysis. The kinetics of hydrolysis of the 1-phenylpyrazoloquinoxaline of cellobiose (29) also indicate hydrolysis by the same mechanism. There was no evidence to indicate reverse anomeric effect influencing the hydrolytic behaviour of 28.

2.3.2 Formation of C-nucleosides incorporating pyrazoloquinoxalines

As C-nucleosides are gaining importance recently because of their pharmacological properties[57], attempts have been made to prepare new C-nucleosides incorporating pyrazoloquinoxalines as the nitrogen heterocyclic system. The first such C-nucleoside 3-(β -D-erythro-furanosyl)-1-phenyl pyrazoloquinoxaline (188) was prepared by Sallam[26] by the dehydration of the 1-phenyl-3-(D-arabino-tetrahydroxybutyl) pyrazoloquinoxaline (56). The structure of the compound and configuration of the anomeric carbon atom were elucidated by periodic oxidation, CD, NMR and mass spectrometry. Sallam and co-workers have also prepared similar nucleosides 189-192 with substituents at different positions[27,28]. The

di-o-acetate, 193 and an isopropylidene derivative, 194 of the C-nucleoside, 190 have also been prepared[27].





193

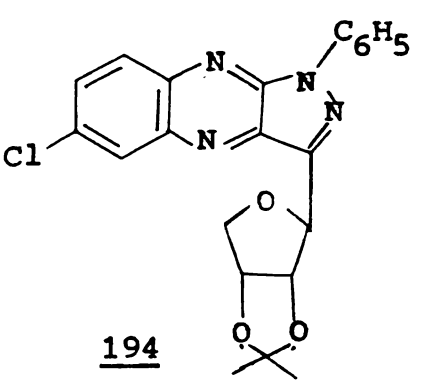
188, $R_1 = R_2 = R_3 = H$

189, $R_1 = R_2 = H$, $R_3 = CH_3$

190, $R_1 = H$, $R_2 = Cl$, $R_3 = H$

191, $R_1 = R_2 = CH_3$, $R_3 = H$

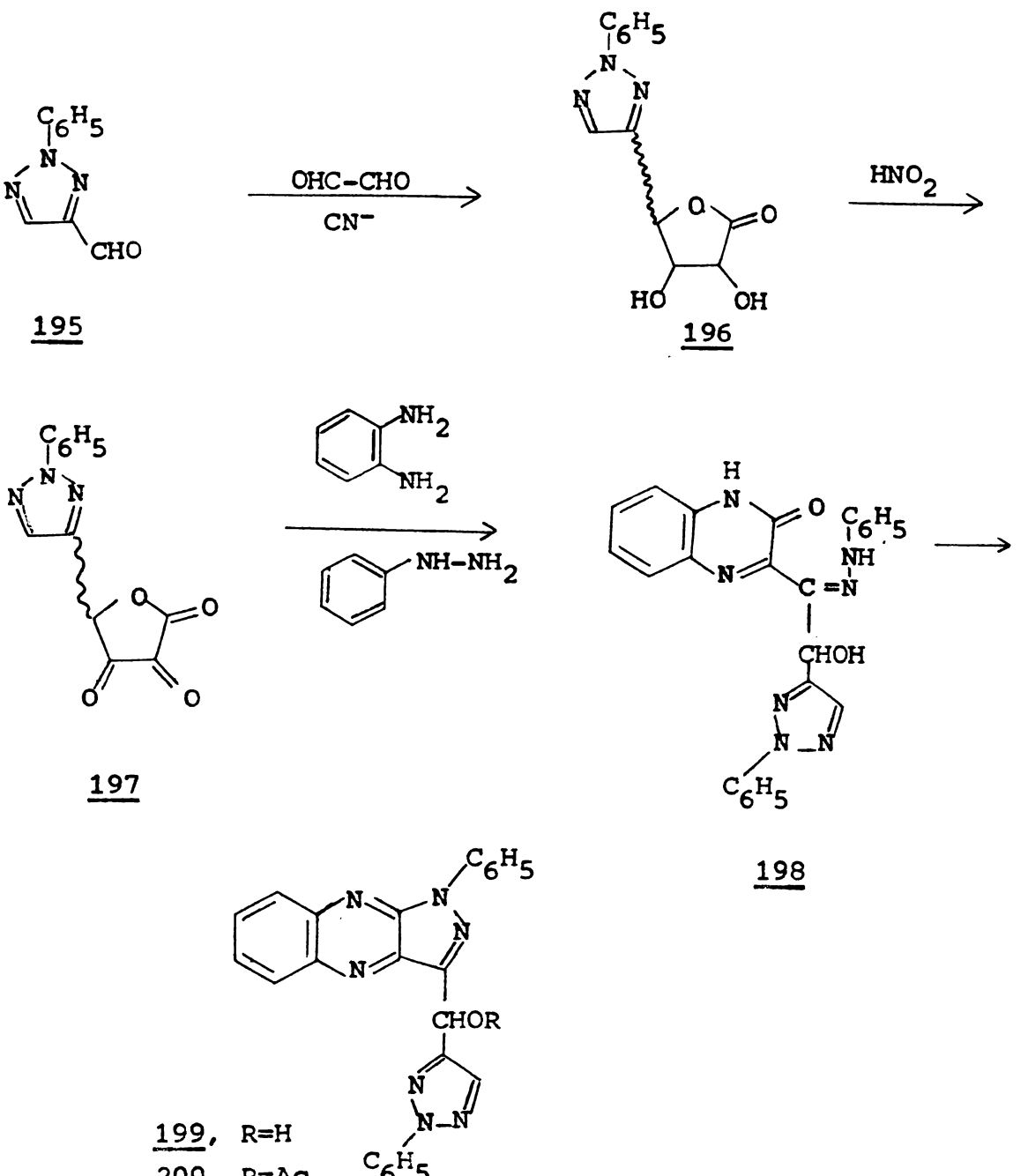
192, $R_1 = R_2 = H$, $R_3 = F$



194

El Ashry and co-workers has prepared a triazolyl C-nucleoside analog of dehydro-L-ascorbic acid which was converted into a pyrazoloquinoxaline derivative[58,59]. 4-Formyl-2-phenyl-1,2,3-triazole (195) was treated with glyoxal in the presence of potassium cyanide to give the triazolylfuranone, 196 which on reaction with nitrous acid gave 197, a triazolyl C-nucleoside of dehydroascorbic acid.

Treatment of 197 with o-phenylene diamine and phenylhydrazine gave the quinoxaline derivative, 198 which was converted into the pyrazoloquinoxaline, 199 in the usual way by treatment with alkali. The compound 199 was further characterised as the o-acetate, 200.

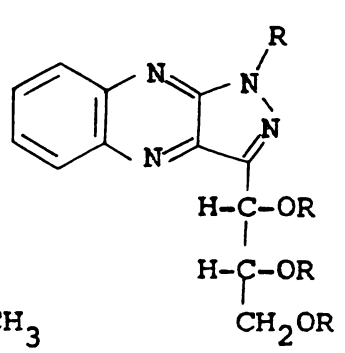


195 196 197 198

199, R=H
200, R=Ac

2.3.3 Substitution reactions

Pyrazoloquinoxaline ring system undergo substitution reactions at various positions. Acylation reactions take place very easily in the pyrazoloquinoxalines with position 1 unsubstituted. 3-(D-Erythro-trihydroxypropyl)-pyrazoloquinoxaline (91) underwent acetylation and benzoylation to give the tetraacylated products, 201 and 202 respectively[38]. Similarly 1-acetylpyrazoloquinoxaline-3-carboxylic acid (176) was obtained from pyrazoloquinoxaline-3-carboxylic acid (93) on treatment with acetic anhydride and pyridine[38]. Deacetylation takes place under mild conditions at position 1. 1-Acetylpyrazoloquinoxaline-3-carbonyl-chloride (177) on treatment with methanolic ammonia gave pyrazoloquinoxaline-3-carboxamide (178)[38].



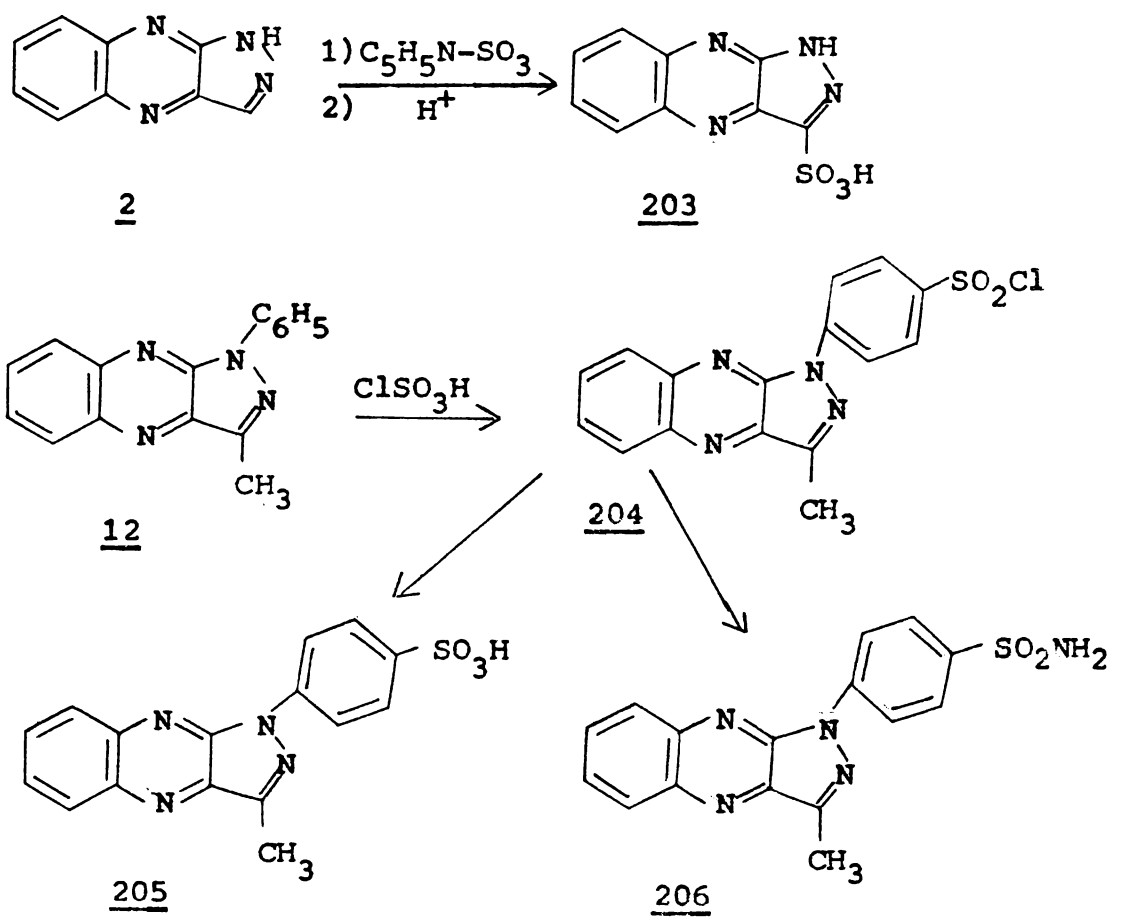
91, R = H

201, R = $COCH_3$

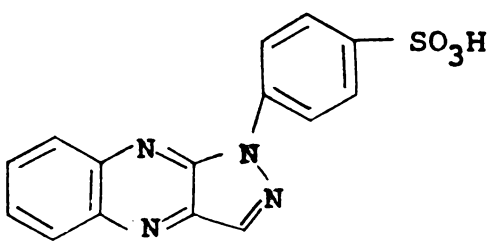
202, R = COC_6H_5

Sulphonation of pyrazoloquinoxaline 2 with pyridine-sulphur trioxide adduct at 170° followed by treatment of the pyridinium salt with sodium hydroxide and subsequent

liberation of the free acid by ion exchange gave pyrazoloquinoxaline-3-carboxylic acid[60], (203). 1-Phenyl-3-methyl pyrazoloquinoxaline (12) on treatment with chlorosulphonic acid gave a single product 204 which was converted into the free sulphonic acid, 205 and the sulphonamide[60], 206. Pyrazoloquinoxaline 2 did not react with methyliodide even at 100° in sealed tubes in 20 hours or with diazomethane in ether at 20°[38].

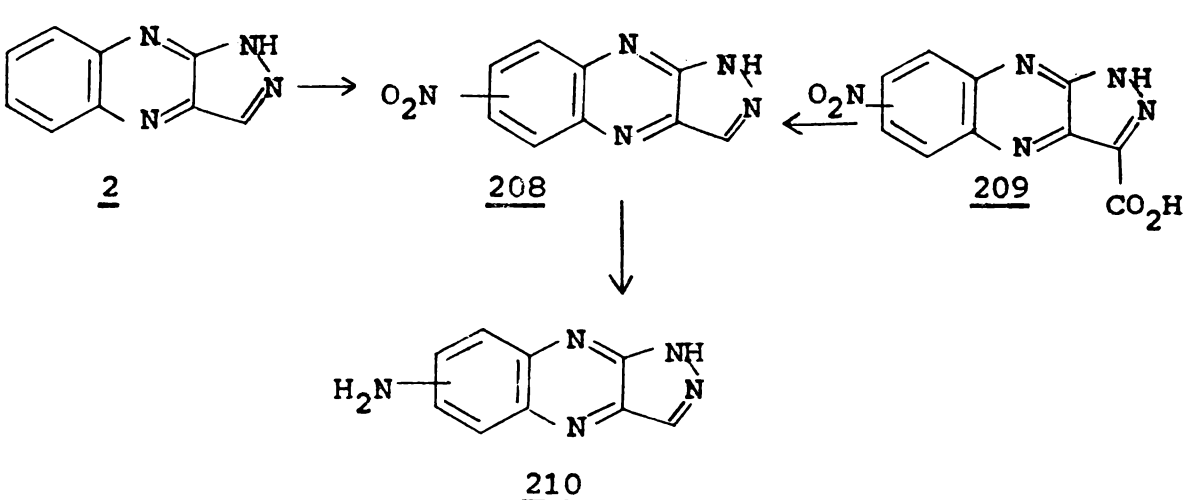


Sulphonation of 1-phenylpyrazoloquinoxaline, (9) took place only when boiled with chlorosulphonic acid and a mixture of products was obtained in which 207 was found to be the major isomer.



207

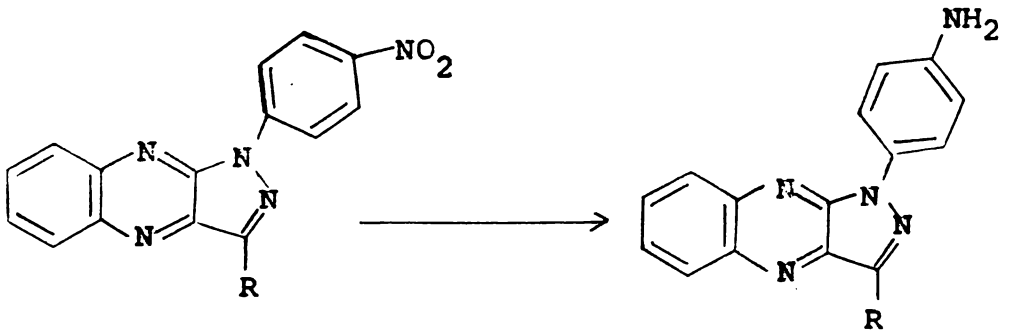
Pyrazoloquinoxaline (2) on nitration gave an isomeric mixture of mononitro derivatives in the ratio 1:10 which were also obtained by the decarboxylation of the nitro substituted 3-carboxylic acids[61], 209. NMR spectra showed that the nitro group of the major components was located at position 6 or 7 while that of the minor component was located at position 5 or 8. The exact position of the nitro group was not determined[61]. Reduction of the nitro group produced the corresponding amino-pyrazoloquinoxalines (210).



2 208 209

210

Nitration of 1-phenylpyrazoloquinoxaline (9) and 3-methyl-1-phenylpyrazoloquinoxaline (12) gave a mixture of isomers with the p-nitro derivatives 211 and 212, predominating[62]. The p-amino derivatives 213 and 214 were obtained by reduction of 211 and 212 respectively. These amino compounds were characterised as their azo dyes and also as their p-acetyl aminosulphonamides. Nitration of pyrazoloquinoxalines with a sugar residue having free hydroxyl groups at position 3 proceeded with two side reactions: oxidation and esterification of the sugar residue[62].



211, R = H

212, R = CH_3

213, R = H

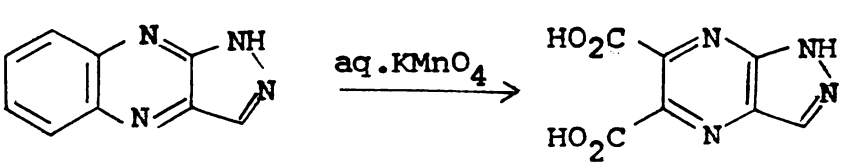
214, R = CH_3

An unusual phenylation reaction of phenyl pyrazoloquinoxaline has been reported by P.M.Pillai and P.Ramabhadran[37]. When 1-phenylpyrazoloquinoxaline was treated with air oxidised

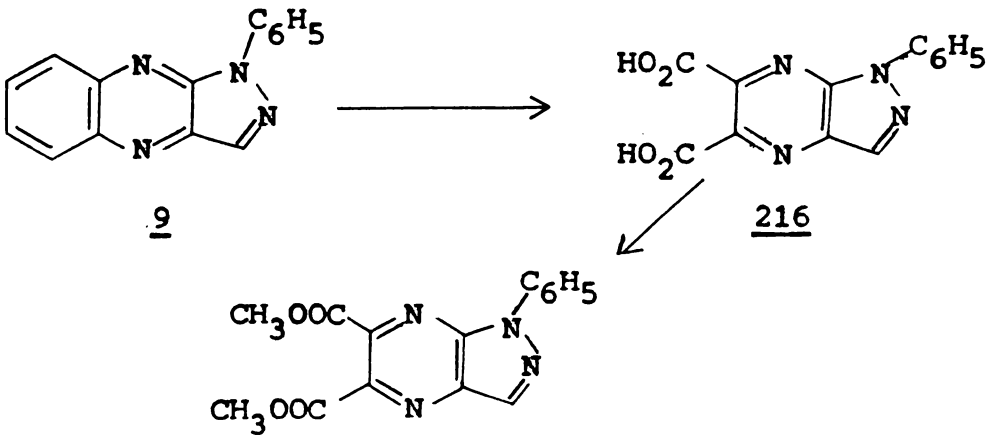
phenylhydrazine,1,3-diphenylpyrazoloquinoxaline was obtained. The same product was obtained when 1-phenylpyrazoloquinoxaline was treated with an excess of benzoyl peroxide in aqueous propanol and acetic acid[37]. But benzoyl peroxide in $CHCl_3$ gave a different compound, 1-phenyl-3-trichloromethyl-1H-pyrazoloquinoxaline. Bromination of 1-phenyl pyrazoloquinoxaline gave a single product 1-p-bromophenyl pyrazoloquinoxaline. Similarly 1,3-diphenylpyrazoloquinoxaline gave 1-p-bromophenyl-3-phenylpyrazoloquinoxaline[63].

2.3.4 Oxidation reactions

Oxidation of pyrazoloquinoxaline (2) with aqueous potassium permanganate gave pyrazolo[3,4-b]pyrazine-5,6-dicarboxalic acid[36] (215). Similarly oxidation of 1-phenylpyrazoloquinoxaline with a neutral solution of potassium permanganate at 120° gave 1-phenylpyrazolo[3,4-b]pyrazine-5,6-dicarboxylic acid 216 which was also characterised as its dimethyl ester 217, by treatment with diazomethane.[63]



2 215

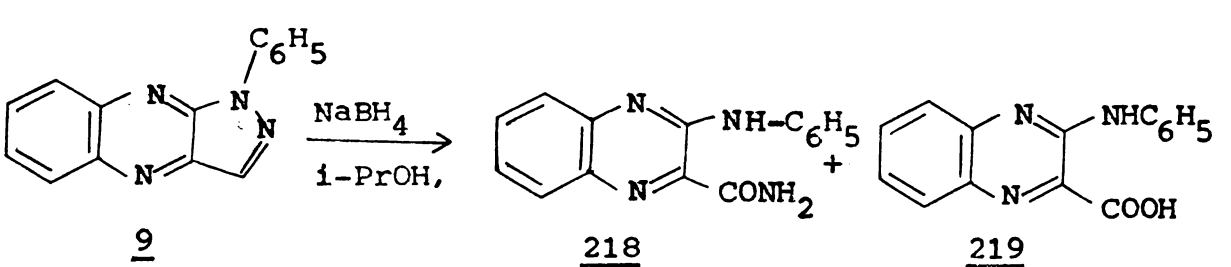


9 216

217

2.3.5 Ring opening reactions

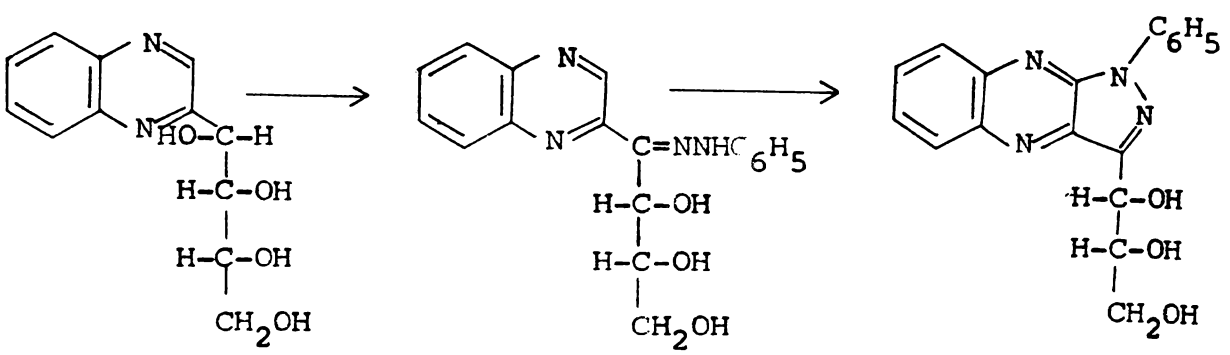
Being a stable aromatic system attempts to reduce 1-phenylpyrazoloquinoxaline (9) with $NaBH_4$ in methanol and LAH in ether at room temperature were reported to be unsuccessful. However treatment of 9 with $NaBH_4$ in boiling isopropanol gave 2-anilinoquinoxaline-3-carboxamide (218) and 2-anilinoquinoxaline-3-carboxylic acid (219). Similarly treatment of 9 with 10% sodium hydroxide solution resulted in the hydrolysis of 9 producing the carboxylic acid[63], 219.



9 218 219

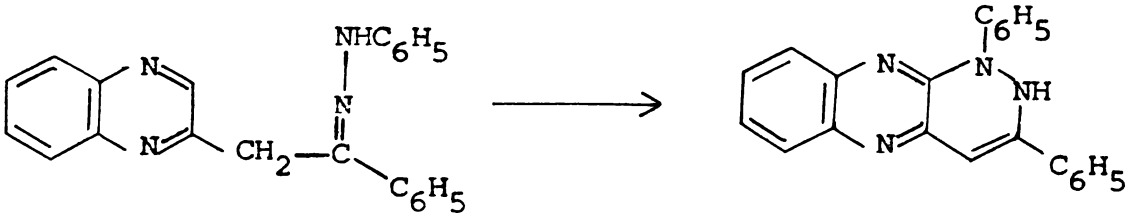
2.4 Mechanism of formation of pyrazoloquinoxalines

Formation of 1-phenylpyrazoloquinoxaline from 2-tetrahydroxybutyl quinoxaline, 3 by the reaction of phenylhydrazines may be considered to take place in two steps. In the first step C-1 of the side chain reacts with the hydrazine, in a way resembling the reactions that occur during osazone formation[64] giving the phenylhydrazone, 5. Ohle and Heilscher[1] has provided some experimental evidence for this concept by the isolation of aniline (11%) and ammonia (18%) in the reaction of 3 with 5 mole equivalents of phenylhydrazine in neutral medium producing 9% of 5. Second step is an oxidative cyclisation of 5 to the pyrazoloquinoxaline, 1 where phenylhydrazine itself is used for the oxidation. Other oxidising agents such as copper sulphate, hydrazine and copper powder in acetic acid have also been successfully employed.

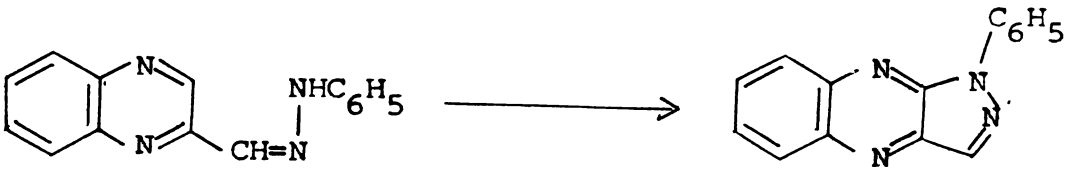


3 5 1

Dahn and Fumeause have studied the cyclisations of 2-phenylquinoxaline phenylhydrazone (220) and quinoxaline-2-carboxaldehyde phenylhydrazone (10) to 1,3-diphenyl-2H-pyridazino[3,4-b]quinoxaline (221) and 1-phenylpyrazolo-quinoxaline (9) respectively in hot hydrochloric acid in the presence of excess phenylhydrazine[65].

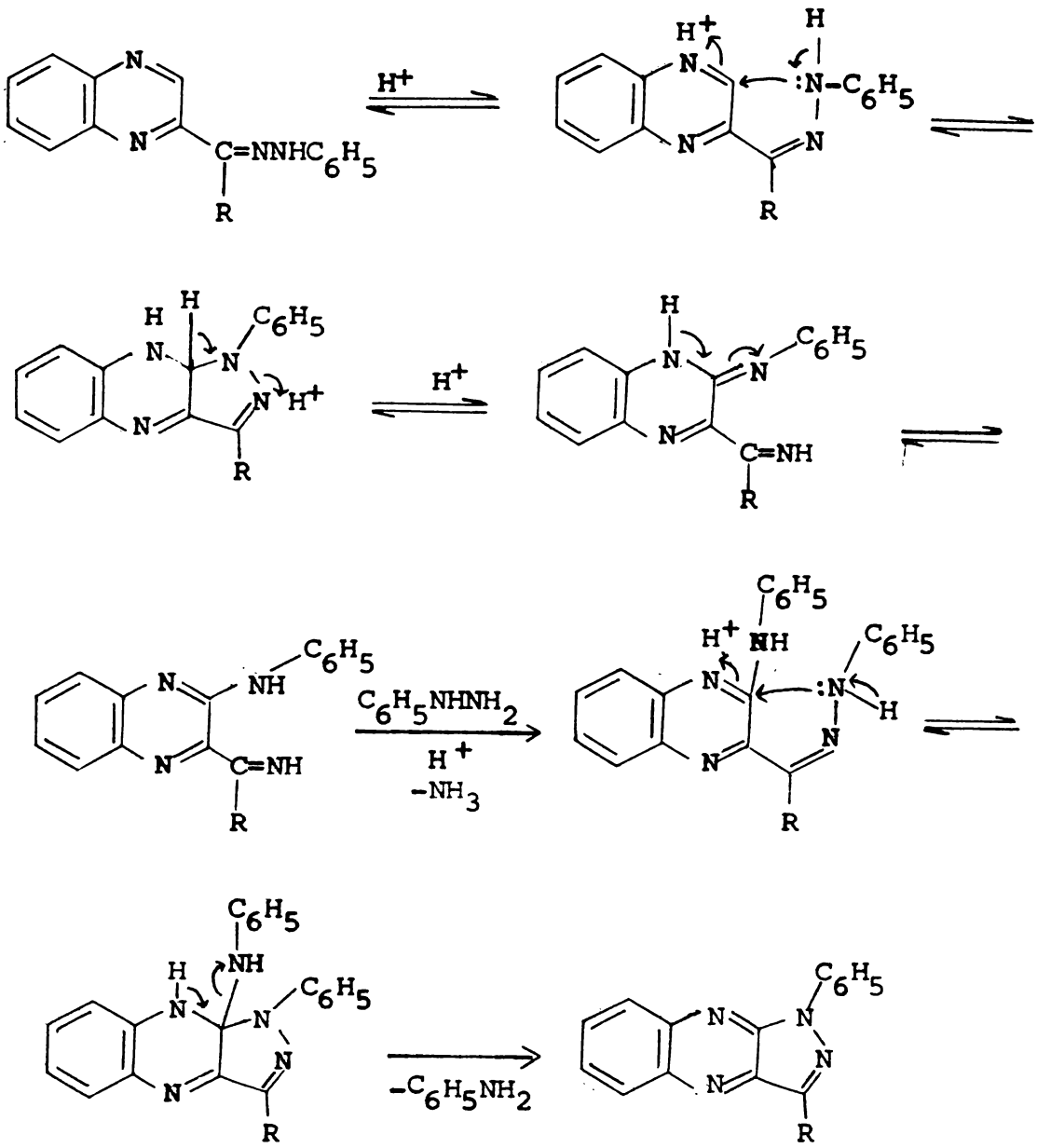


220 221



10 9

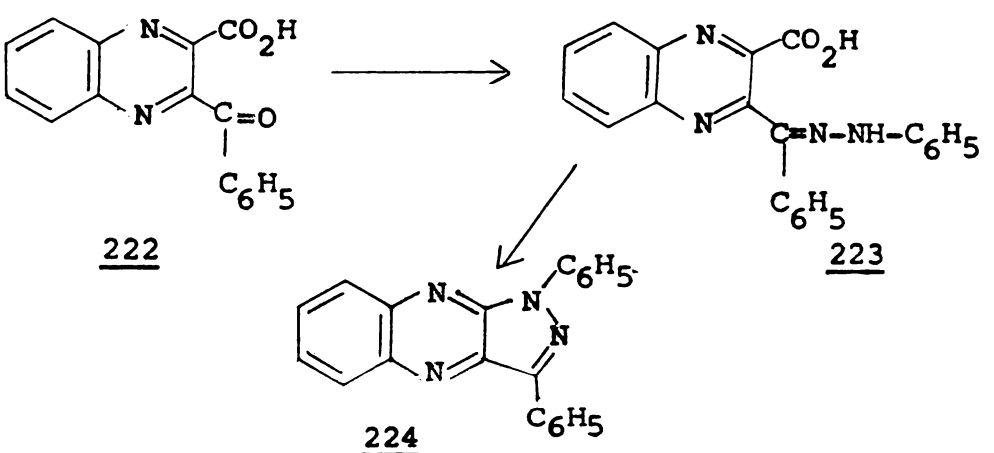
They have proposed a mechanism for this cyclisation similar to the formation of osazones[65-68].



R = H, alkyl, aryl or hydroxyalkyl

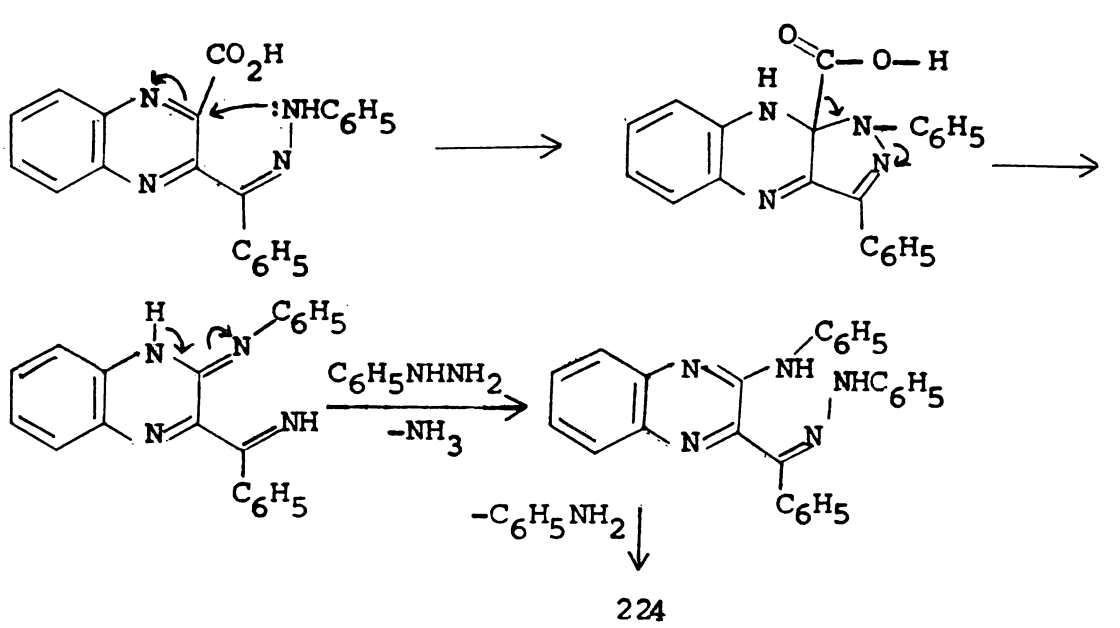
A similar mechanism was suggested[65] for the formation of 221 from 220.

3-Benzoylquinoxaline-2-carboxylic acid (222) and its amide, (225) when heated with phenylhydrazine gave 1,3-diphenylpyrazoloquinoxaline (224), showing that the COOH and $CONH_2$ group at position 2 can act as leaving groups[67] as in the case of the benzidine rearrangements[69]. The phenylhydrazones, 223 and 226 are intermediates in these reactions.



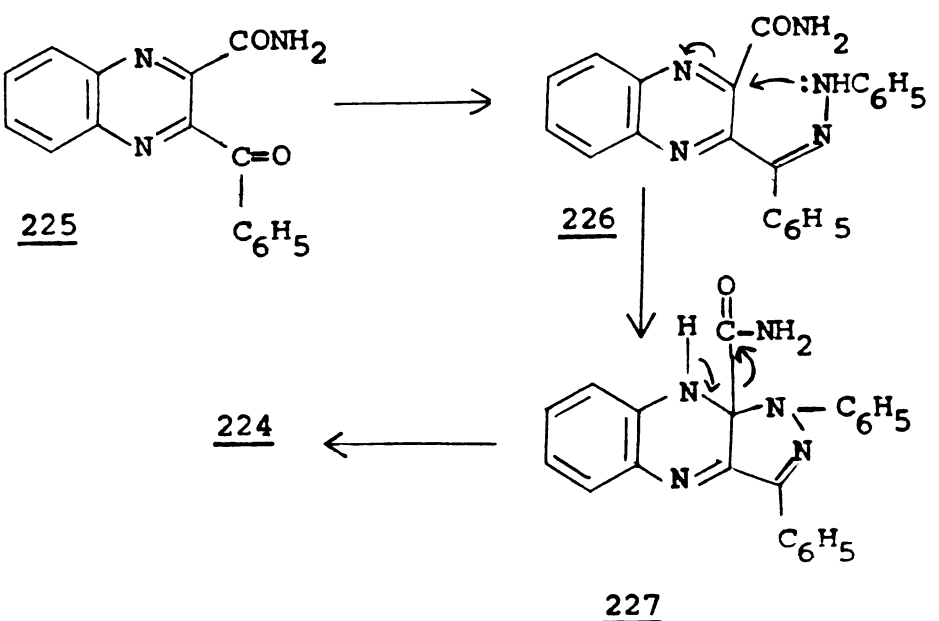
222 223 224

The carboxylic acid group can easily be eliminated as carbon dioxide during cyclisation as shown below.

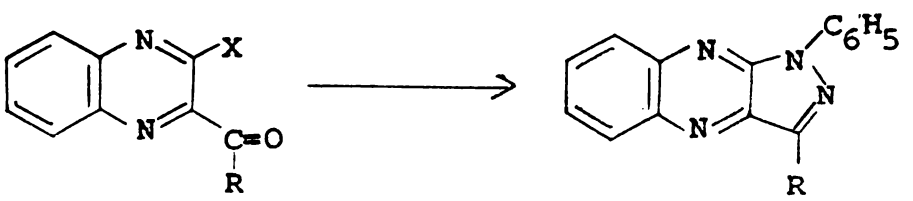


224

A different mechanism was proposed by Dahn and Moll for the formation of a pyrazoloquinoxaline ring with the loss of a carboxamide group at position 2 of the quinoxaline ring[67].

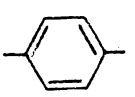


Dahn and Nussbaun have studied the formation of pyrazoloquinoxalines from quinoxalines substituted with different groups at positions 2 and 3. The studies showed that H, OH, Cl, CN, CO_2H, $CONH_2$, $-CH_2C_6H_5$ and COC_6H_5 act as leaving groups when a quinoxaline substituted with these groups at position 2 and a benzoyl or a p-methoxybenzoyl group substituted at position 3 were treated with phenylhydrazine[68].



228 229

X = H, OH, Cl, CN, CO_2H, $CONH_2$, $-CH_2C_6H_5$, COC_6H_5

R = C_6H_5 or CH_3O- 

Pyrazoloquinoxalines were not formed when x = CH_3 or C_6H_5 under the same experimental conditions. It is possisble that groups such as OH, Cl and CN depart as anions as in the case of the amide (see 227), whereas, $-CH_2C_6H_5$ and $-COC_6H_5$ may be eliminated as cation as in the case of hydrogen and carboxylic acid lost as carbon dioxide. However no experimental evidence can be cited in support this view.

The fact that 3-acylquinoxalines substituted with CH_3 and C_6H_5 at position 2 do not yield pyrazoloquinoxalines when treated with phenylhydrazine may be because they are not good leaving groups either as anions or as cations.

2.5 Physical methods of characterisation

Pyrazoloquinoxalines are generally highly coloured crystalline compounds with definite melting points. They often show an intense green fluorescence especially in non-polar solvents[21,22]. Pyrazoloquinoxalines may be identified by their melting points and X-ray diffraction patterns[3,4]. Among spectrometric methods, nuclear magnetic resonance has been extensively used for their characterisation, especially to understand the substitution pattern of the aromatic system[60,61] and to determine the nature and configuration of the carbohydrate part[19,27].

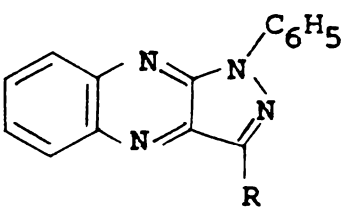
Mass spectral analysis of pyrazoloquinoxalines have also been useful in their structural determination[27,51]. A detailed study of the mass spectra of the 1-phenyl-1H-pyrazolo[3,4-b]quinoxaline derivatives of monosaccharides showed structurally characteristic and easily interpretable fragmentation patterns[70]. Also the position of the deoxy or methoxy grouping on the sugar residue may be conveniently determined using mass spectrometry[70].

The ultraviolet absorption spectra of pyrazoloquinoxaline derivatives are quite characteristic. The λ_{max}^{iPrOH} of glucose 1-phenylpyrazoloquinoxaline (1) were found

to be at 276 nm (ϵ 3.98×10^4), 355 nm (ϵ 1.01×10^4) and 410 nm (ϵ 3.7×10^3). The peak at 410 nm obeyed Beer's law and is useful for the colorimetric or spectrophotometric determination of the pyrazoloquinoxaline[71]. In water this absorption was shifted to 405 nm. Maltose 1-phenylpyrazoloquinoxaline (30) and lactose 1-phenylpyrazoloquinoxaline (30) also had $\lambda_{max}^{i\text{-}PrOH}$ at 410 nm (ϵ 3.7×10^3)[71]. The uv spectrum of the 1-phenylpyrazoloquinoxaline of amylose showed λ_{max}^{DMSO} at 323 nm, 336 nm and 407 nm[31].

The optical rotation of pyrazoloquinoxalines of sugars depend on the nature of the carbohydrate residue at position 3 (see table II) although there is no direct correlation between the optical rotation of the pyrazoloquinoxaline and absolute configuration of the sugars. The circular dichroism of a number of 3-polyhydroxyalkyl-1-phenylpyrazoloquinoxalines (230) was studied by Sallam[72].

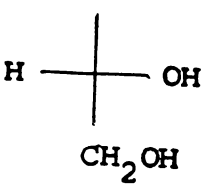
In dioxane solutions they all showed multiple cotton effects and a direct correlation was observed between the sign of the cotton effect at the long wavelength absorption (442-450 nm) and the absolute configuration of C-1. Thus compounds which have R chirality at C-1 (C-4 of the original sugar) in the Fischer projection formula showed positive



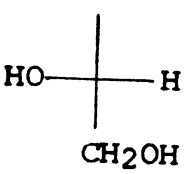
230

R

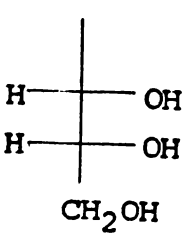
(a)



(b)

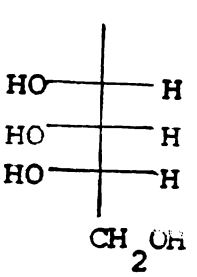


(c)

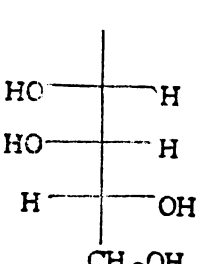


1

(d)

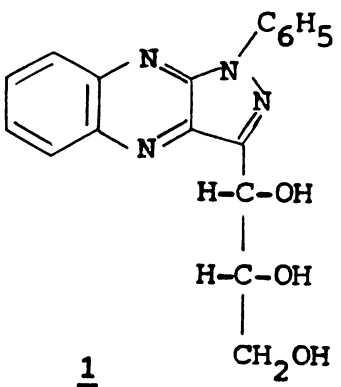


(e)

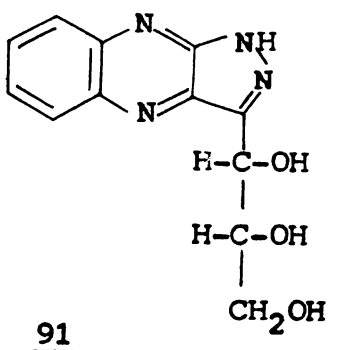


cotton effect. Cetres of asymmetry other than at C-1 e only on the intensity of the band[72]. Chilton and Kra also reported that configuration at C-4 of sugar the pyrazoloquinoxaline) may be studied from optical rotatory

dispersion curves of their 1-phenylpyrazoloquinoxaline or its derivatives[73]. Thus in the case of glucose, compounds 1 and 91 exhibit negative cotton effects centred at 410 and 390 nm respectively.



1



91

Kobayashi and co-workers[74] have reported the preparation and paper chromatographic separation of the 1-phenylpyrazoloquinoxaline derivatives of glucose and a few other di and oligosaccharides. A solvent system consisting of butanol : ethanol : water : ammonia :: 40 : 10 : 49 : 1 (upper layer) gave the best results for separation by paper chromatography. The R_f values of the 1-phenylpyrazoloquinoxaline derivatives of the different sugars were as follows: glucose 0.89, isomaltose 0.77, maltose 0.73, isomaltotriose 0.56,

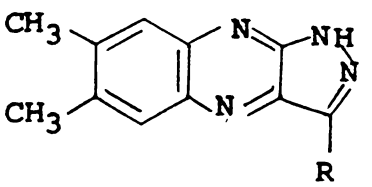
a tetrose isolated from dextrin hydrolysate 0.44 and a pentose isolated from the same hydrolysate 0.34.

2.6 Biological studies

May be because of the limited solubility of this class of compounds, biological studies on pyrazoloquinoxalines have been rather limited. However, more soluble derivatives have been prepared recently by increasing the hydroxyl groups on the heterocyclic system[42], and by prepar ng polyhydrogen sulphate salts on the hydroxyl groups of the sugar residue[75,76].

One of the first biological screening experiment for which a pyrazoloquinoxaline was submitted was the study of the inhibition of multiplication of staphylococcus K phage by a number of compounds including the 1-phenylpyrazoloquinoxaline of glucose, 1. These studies were carried out with mass lysis and one step growth curve techniques. The growth rate of staphylococcus aureus was inhibited 5% to 100% when added to growth cultures at concentrations necessary to double lysis time[77]. Similarly 1-phenylpyrazoloquinoxaline was found to be active against Clostridium septicum infections in mice[78].

Buu-Hoi and co-workers[41] studied the antibacterial, antiinflammatory and analgesic properties of pyrazoloquinoxalines, 179 to 182. Also the tuberculostatic activity of the hydrazone 183 and semicarbazone, 184 were reported by the same authors[55]. They showed invitro activity as tuberculostatics at 10 to 70 mg/kg in middlebrook media.

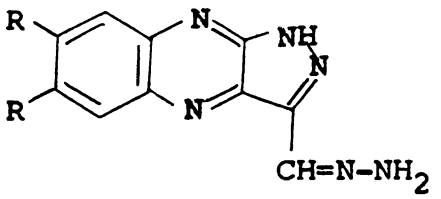


179, R = CHO

180, R = CH=N-OH

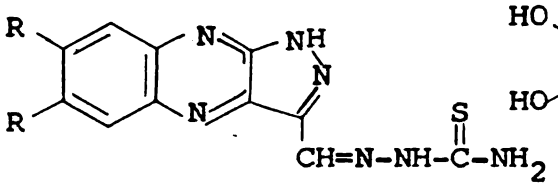
181, R = CN

182, R = CO_2H



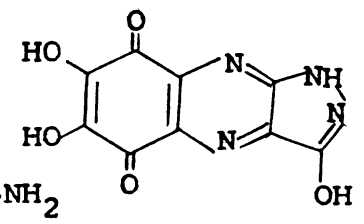
183

R = H or CH_3



184

R = H or CH_3



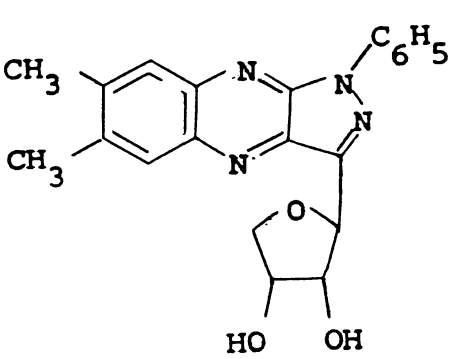
106

As other related heterocyclic systems did not show any activity, it was suggested that the tuberculostatic activity is intrinsic to the pyrazoloquinoxaline system[55].

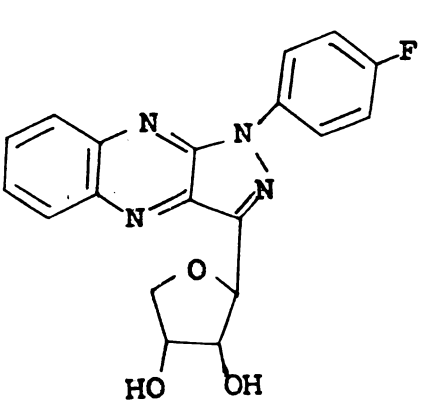
The biological activity of 3,6,7-trihydroxypyrazoloquinoxaline-5,8-dione (106) and its Na, K, Li, ammonium, methylammonium, ethylammonium, n-hexylammonium and benzylammonium salts was reported by Wendt, and Ludig in a U.S. Patent[42]. Compound 106 when given 10 mg/kg showed diuretic activity and at 50 mg/kg caused 49% inhibition of edema.

Nair and Benrstein prepared the polytrimethylammonium sulphate polysalts of the 1-phenylpyrazoloquinoxalines obtained from cellobiose, 29 and maltotriose, 35. While the salt of cellobiose showed *in vitro* complement inhibiting activity[76], that of maltotriose exhibited *in vivo* (guinea pig) and *in vitro* complement inhibiting activity[75].

Sallam and co-workers[27,28] have shown that C-nucleoside 192 exhibited *in vitro* cytotoxic activity against K_B cells (a human epidermoid carcinoma of the nasopherynx) whereas 191 was inactive. Also cyclisation of the polyhydroxy chain in 58 and 59 to give the nucleosides 191 and 192 increased the antileukaemic activity.



191



192

Moreno and co-workers has carried out a number of immunological studies using pyrazoloquinoxalines. 1-Phenylpyrazoloquinoxaline of isomaltohexose coupled to chicken gamma globullin induced T-cell dependent anti- (1→6)dextron specific IgM and IgG responses in CBA, BALB/C and A Strain mice[79]. The IgG responses were of restricted heterogeneity and belonged mostly to the IgG1 subclass with a minor I_gG3 component in the case of BALB/C and CBA mice. All 4 subclasses of I_gG were produced in a strain mice[79].

Tiechmann and co-workers[23] prepared 1-(m-nitrophenyl)-pyrazoloquinoxalines from oligosaccharides of isomaltose, maltose and cellobiose series and were used as model compounds for immunochemical studies. Their m-aminophenyl derivatives had similar properties as the nitro derivative and were useful for the manufacture of immunogen and antigen models and immunoadsorbents with oligosaccharide specific determinant group[80]. Immunogens with oligosaccharide determinant groups were prepared by azo coupling of the 1-(m-aminophenyl)pyrazoloquinoxalines (prepared from oligosaccharides) to proteins[81]. It was seen that unsubstituted hydroxyl groups on position 2 and 3 adjacent to the reducing end of the sugar were required and the method appeared especially suited for oligosaccharides having a polymerisation degree of 3 to 8. Oligosaccharides pyrazoloquinoxaline-azoedestin conjugates were

tested for immunogeneity in rabbits and specific antioligosaccharide antibodies were formed in all cases. High titers of dextron specific antibodies were obtained upon immunisation with an isomaltoheptose-pyrazoloquinoxalines-azo-edestin conjugate[81].

The 1-(m-nitrophenyl)pyrazoloquinoxalines of the isomaltose oligosaccharides were used to study their interaction with human antidextran by the quantitative hepten inhibition and fluorescence quenching techniques[82]. It was found that the m-nitrophenylpyrazoloquinoxaline heptens inhibited in the dextran-antidextran system in whole series in the same order as did the isomaltose oligosaccharides. Thus the m-nitrophenylpyrazoloquinoxaline of isomaltoheptose was the best inhibitor and the inhibitory potency decreased progressively to the m-nitrophenylpyrazoloquinoxaline of isomaltotetrose[82].

The preparation of immuno adsorbents based on cellulose derivatives with the specificities of pyrazoloquinoxalines of the sugars of the maltose and isomaltose series was reported by Teichmann[83]. Cellulose-m-aminobenzyloxymethylether and p-aminobenzylcellulose were diazotised and coupled with

1-(m-hydroxylphenyl)pyrazoloquinoxaline and 1-(m-aminophenyl)-pyrazoloquinoxaline of sugars of the maltose and isomaltose series to obtain sugar specific immuno adsorbants for isolation of anti-oligosaccharide antibodies from antiserums[83].

CHAPTER III

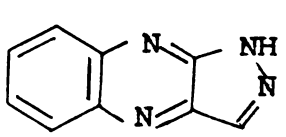
DISCUSSION OF EXPERIMENTAL RESULTS

3.1 Synthesis of 1H-pyrazolo[3,4-b]quinoxalines substituted at position 3

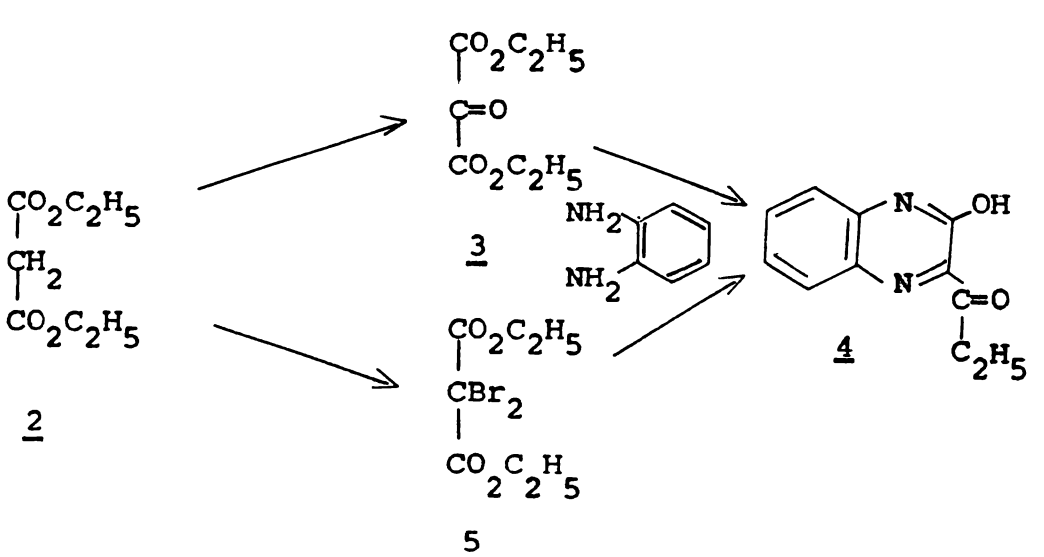
The synthesis of unsubstituted 1H-pyrazolo[3,4-b]-quinoxaline (1) has been reported by Ohle and Iltegen by a series of low yield reactions starting from D-glucose, o-phenylene diamine and hydrazine[38]. We are now reporting a new method for the synthesis of this novel heterocyclic system substituted at 3 position as substituents such as OH, Cl and NH_2 groups are known to increase the biological activity of similar compounds[42].

Oxidation of diethyl malonate (2) with selenium dioxide at 120-130° by a known procedure gave ethyl mesoxalate (3) in 32% yield[84]. Condensation of ethyl meso-xalate (3) with o-phenylene diamine provided ethyl 2-hydroxy-quinoxaline-3-carboxylate[85] (4) in 93% yield. Quinoxaline derivative 4 was also obtained by a new route starting from diethyl malonate (2). Bromination of 2 with Br_2 to the dibromo derivative[86] 5 followed by treatment with o-phenylene diamine gave 4 in 40% yield. Treatment of the ester 4 with hydrazine hydrate in ethyl alcohol at room temperature yielded 85% of the expected 2-hydroxyquinoxaline-3-carbonylhydrazide (6). The idea was to cyclise this hydrazide to the pyrazoloquinoxaline system using various

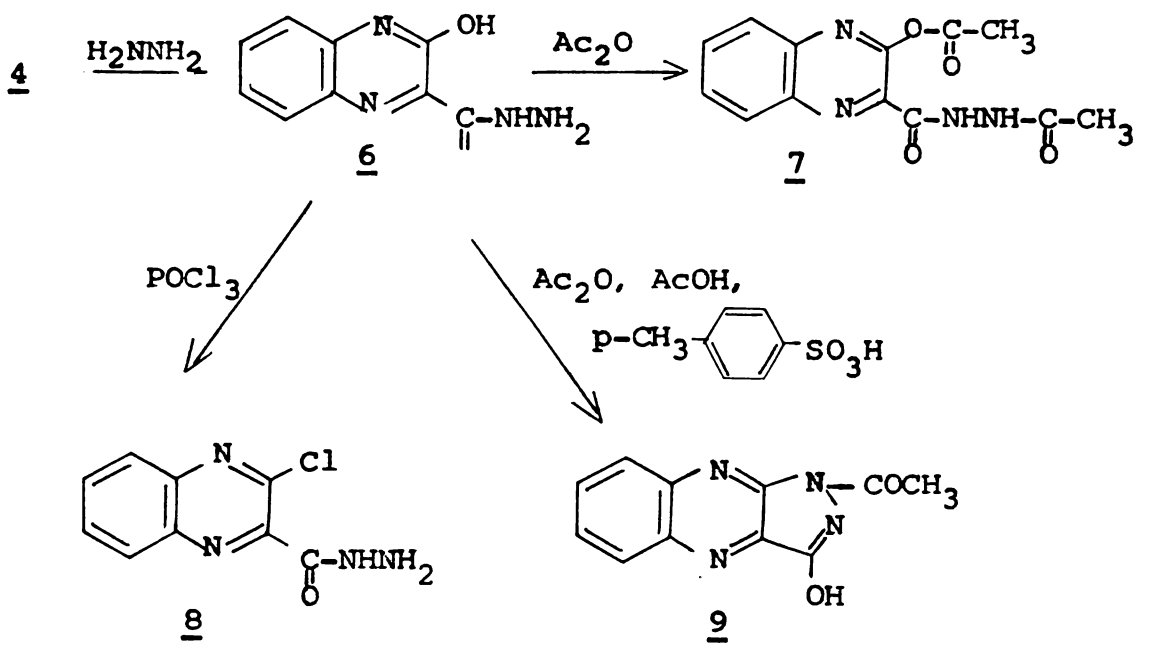
reagents. However, attempted cyclisation of 6 using acetic anhydride did not yield any cyclised product. The only product isolated from this reaction was the diacetyl derivative, 7. Similarly heating a mixture of 6 with phosphorous oxychloride, a method which was successful in the cyclisation of 2-hydroxyquinoxaline-3-carbonylphenylhydrazide to 1-phenyl-3-chloro-1H-pyrazolo[3,4-b]quinoxaline[35] also failed as a method for cyclisation as the product obtained by this process was 2-chloroquinoxaline-3-carbonylhydrazide (8). Treatment of 6 with acetic anhydride and acetic acid in the presence of p-toluene sulphonic acid as catalyst on a boiling water bath yielded the cyclised product 1-acetyl-3-hydroxy-1H-pyrazolo[3,4-b]quinoxaline (9) in 50% yield. The structure of 9 was established by its elemental analysis and spectral data. The IR spectrum of 9 showed absorption for C=O (1678 cm^{-1}) and OH (3556 cm^{-1}).



1



2 3 4 5

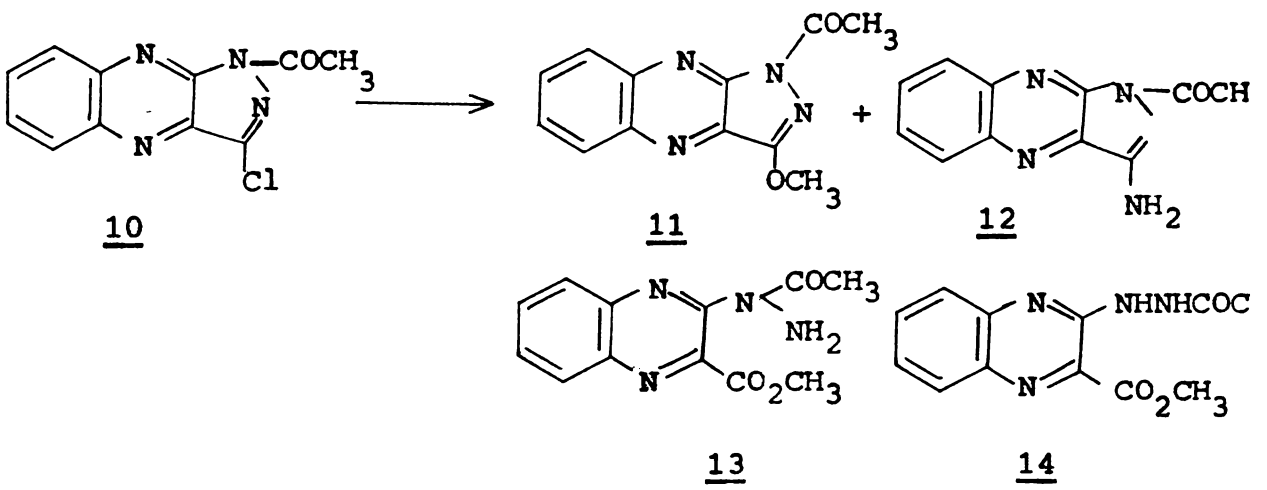


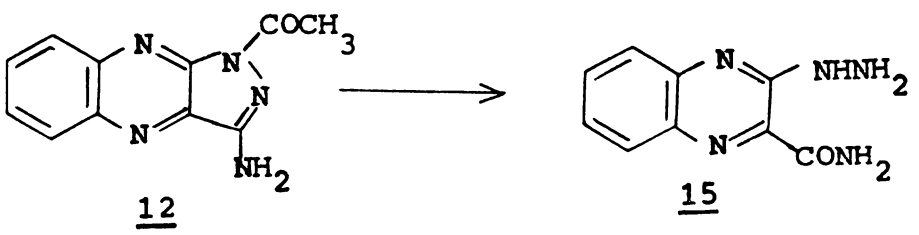
The mass spectrum provided the molecular ion peak at m/z 228 and a $COCH_3$ group was indicated by a peak at m/z 43. The NMR spectrum indicated the presence of a $COCH_3$ group by absorption at δ 2.8, -OH group δ 4.6 and the 4 aromatic protons appeared at δ 7.7-8.2.

Having thus achieved the cyclisation to a pyrazolo-quinoxaline system, attempts were made to further derivatise at position 3 and also to remove the acetyl group from position 1. Thus treatment of 9 with phosphorous oxychloride

on a boiling water bath for 2 hours provided 1-acetyl-3-chloro-1H-pyrazolo[3,4-b]quinoxaline (10) in about 66% yield. The mass spectrum of 10 showed characteristic m/z peaks for a monochloro derivative in that the $[M]^{+\cdot}$ appeared at m/z 246 and an M+2 peak of about 1/3 intensity at m/z 248. The other spectral data were also in complete agreement with the structure as given in the experimental section.

Treatment of the chloro derivative 10 with 30% of liquor ammonia in methanol at room temperature in an attempt to remove the N-acetyl group at position 1, not only did not yield the desired result but provided a mixture consisting of 1-acetyl-3-methoxy-1H-pyrazolo[3,4-b]quinoxaline (11), 1-acetyl-3-amino-1H-pyrazolo[3,4-b]quinoxaline (12) and a ring opened product which has a structure of either 13 or 14. These compounds were separated by column

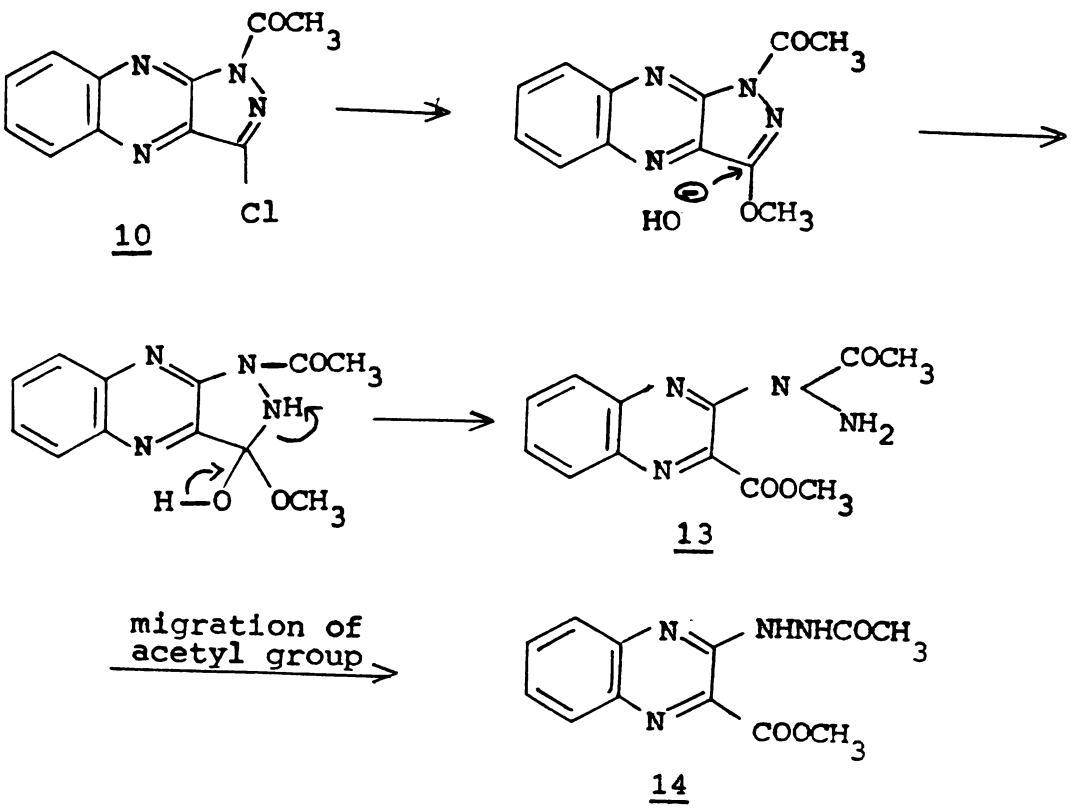




12 15

chromatography on alumina. The structure of 11 was established by NMR in addition to mass spectrum and elemental analysis. In the NMR, it showed peaks at δ 2.7 and δ 4.3 for the $COCH_3$ and OCH_3 hydrogens.

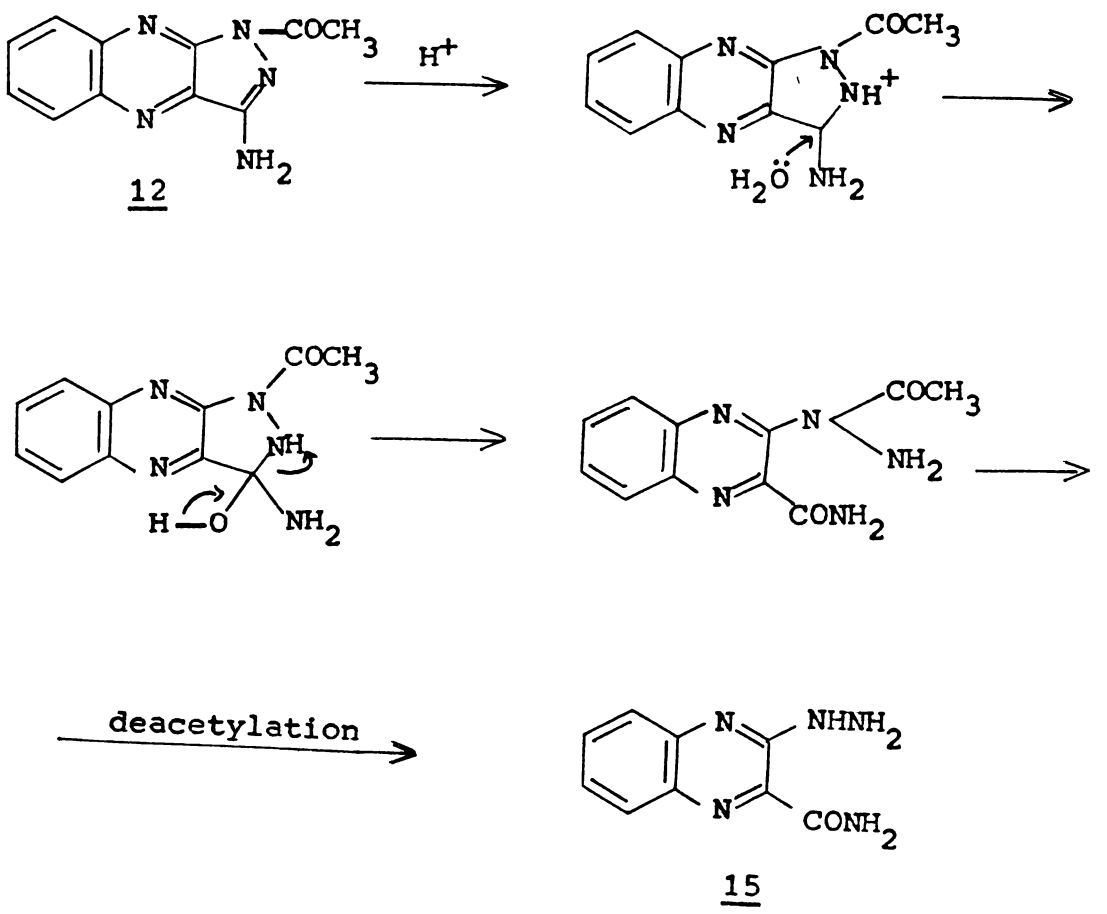
The mass spectrum of 12 showed the $[M]^+$ at m/z 227 and the NMR spectrum exhibited peaks for NH_2 (δ 1.7), $COCH_3$ (δ 2.7) and a multiplet for aromatic protons at δ 7.7. The third compound formed in this reaction also showed $NCOCH_3$ and $COOCH_3$ absorptions (IR and NMR) and the mass spectrum and analysis indicated a molecular formula $C_{12}H_{12}N_4O_3$ (see experimental section for details). However it was not clear whether the N-acetyl group remained with the same nitrogen after ring opening as in 13 or migrated to the end nitrogen atom as shown in 14. As this compound did not appear to be of any practical importance at the moment, its structure was not further elucidated. The mechanism of its formation from 10 may be indicated as follows:



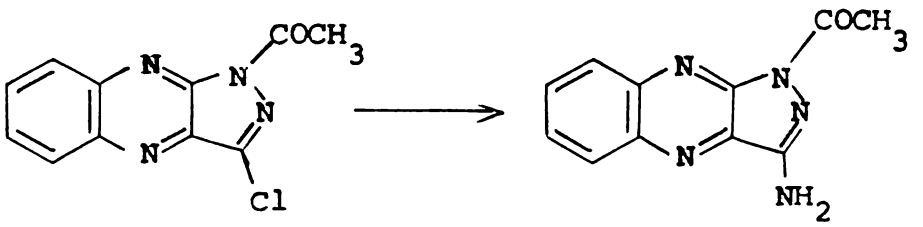
Although opening of the pyrazole ring in pyrazoloquinoxalines have been reported earlier, with breakage of the N, N bond[63] opening with breakage of the C=N bond has been observed for the first time.

Another method of attempted hydrolysis using 2N hydrochloric acid at 100°C also resulted in the opening of the pyrazole ring system. In this case 1-acetyl-3-amino-1H-pyrazolo[3,4-b]quinoxaline (12) was used as the substrate.

The product obtained was 2-hydrazinoquinoxaline-3-carboxamide (15) which may be formed as follows:

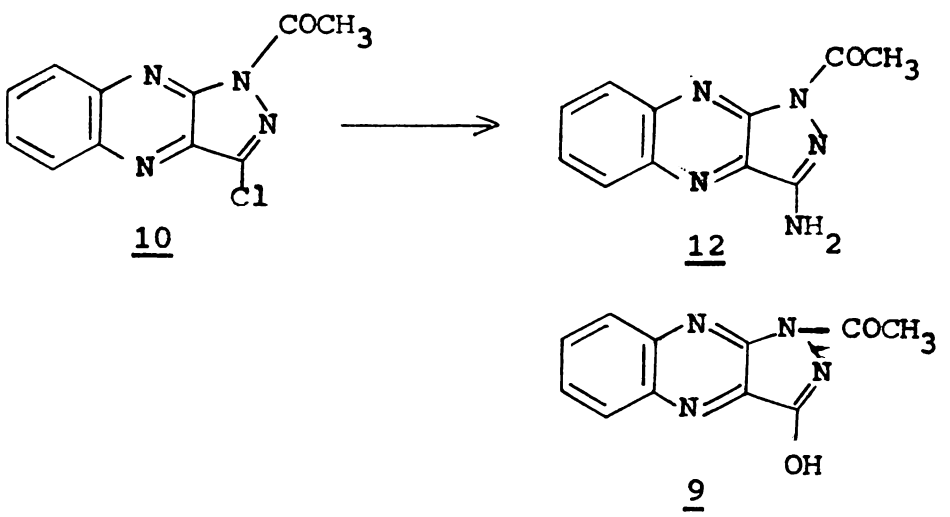


Displacement of the chlorine in 10 with amino group without the formation of byproducts was achieved by the treatment of 10 with urea at 130°C. The 1-Acetyl-3-amino pyrazoloquinoxaline (12) was obtained in 80% yield which was identical with the samples of 12 obtained earlier.



10 12

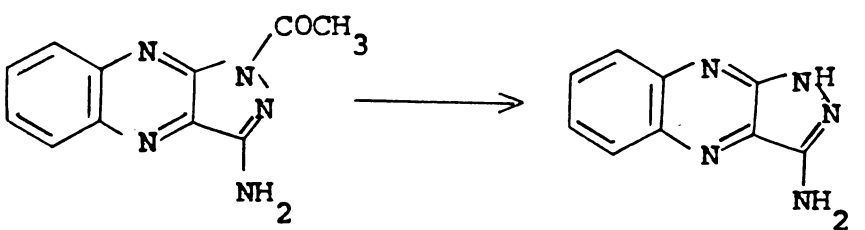
Treatment of the chloroderivative 10 with liquor ammonia at room temperature also failed to remove the N-acetyl group. However the chlorine in 10 was replaced by both amino and hydroxyl groups giving a mixture of 12 and 9. The mixture was separated by column chromatography



10 12 9

on silica gel and the identities of the products were established by comparison with authentic samples prepared and characterised earlier.

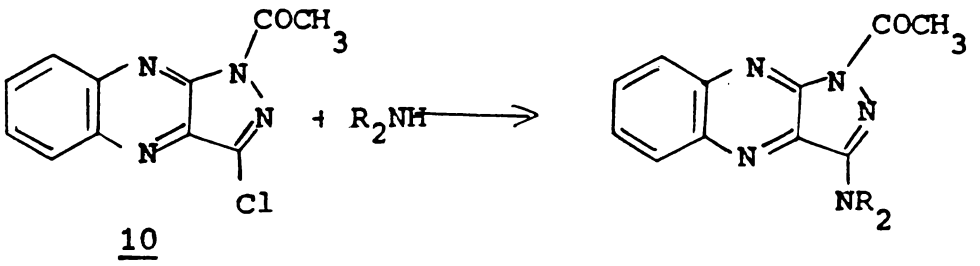
Finally, treatment of the amino derivative 12 with sodium carbonate in methanol under reflux conditions provided the deacetylated product, 3-amino-1H-pyrazolo[3,4-b]-quinoxaline in 65% yield. This method appears to be the only successful method under mild reaction condition to bring about deacetylation from position 1. The structure



12 16

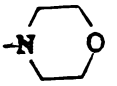
of 16 was established by NMR spectrometry which did not show a $COCH_3$ group in the molecule. Mass spectral and elemental analysis also support this finding.

Treatment of the chloroderivative 10 with several secondary amines such as piperidine, morpholine, pyrrolidine and diethylamine have provided the corresponding amino derivatives 17, 18, 19 and 20 respectively. However



10

17, NR_2 = 

18, NR_2 = 

19, NR_2 = 

20, $NR_2 = -N(C_2H_5)_2$

surprisingly acetyl group at position 1 remained intact under these reaction conditions. These compounds are of interest because of their potential biological activity. The structures of compounds 17-20 were also established conclusively by NMR, mass spectrometry and elemental analysis.

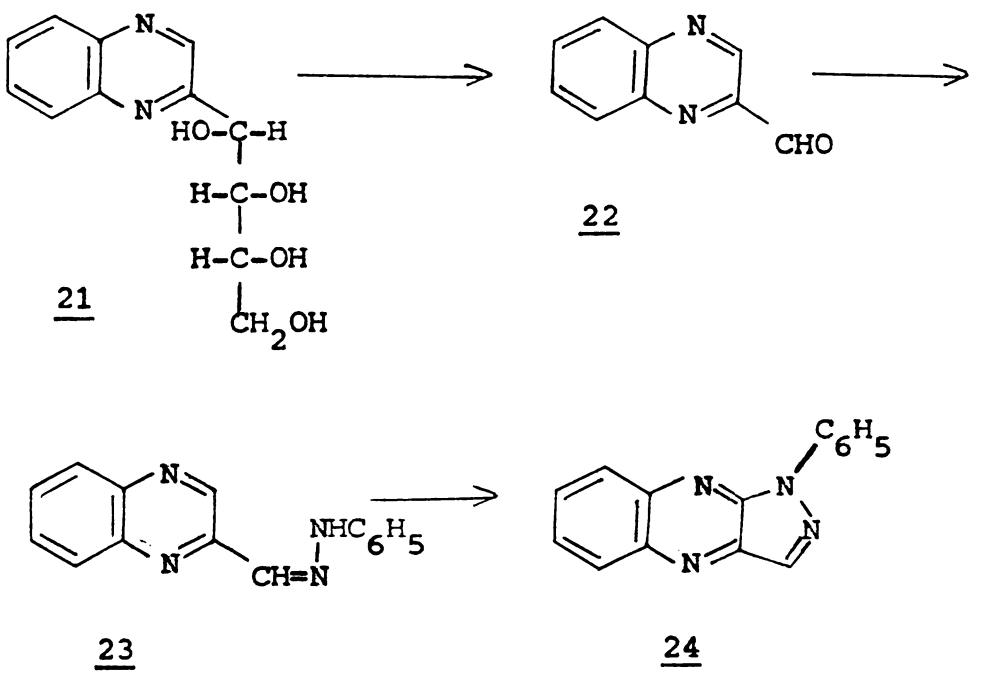
3.2 Chlorination of pyrazoloquinoxalines and related compounds using thionyl chloride

The major use of thionyl chloride as a reagent in organic synthesis has been for the replacement of hydroxyl groups with chlorine atoms in carboxylic acids and alcohols to give the corresponding acid chlorides or chloroderivatives[87]. However, a few reactions in which

thionyl chloride has acted as a chlorinating agent have also been reported[88,89]. In this work we have specifically employed thionyl chloride to chlorinate 1-phenyl-1H-pyrazolo-[3,4-b]quinoxalines and anilinoquinoxaline derivatives in order to prepare the chlorinated derivatives in reasonable yields. As it has already been pointed out, pyrazoloquinoxalines possess valuable biological activity[41,90], and chlorination is known to enhance biological properties of many classes of compounds[91], the preparation and characterisation of these classes of compounds are of considerable interest.

1-Phenyl-1H-pyrazolo[3,4-b]quinoxaline (24) was synthesised for studying its chlorination with thionyl chloride, as follows. Treatment of D-glucose with o-phenylene diamine in the presence of hydrazine hydrate on a boiling water bath provided the known 2-(D-arabino-tetrahydroxybutyl)quinoxaline[1] (21) in 34% yield. Oxidation of 21 with sodium metaperiodate in water in the presence of acetic acid cleaved the side chain and provided 63% of quinoxaline-2-carboxaldehyde[92] (22). This aldehyde 22 was converted into its phenylhydrazone 23 by treatment with phenylhydrazine in methanol at room temperature[93]. Oxidative cyclisation of quinoxaline-2-carboxaldehyde phenylhydrazone

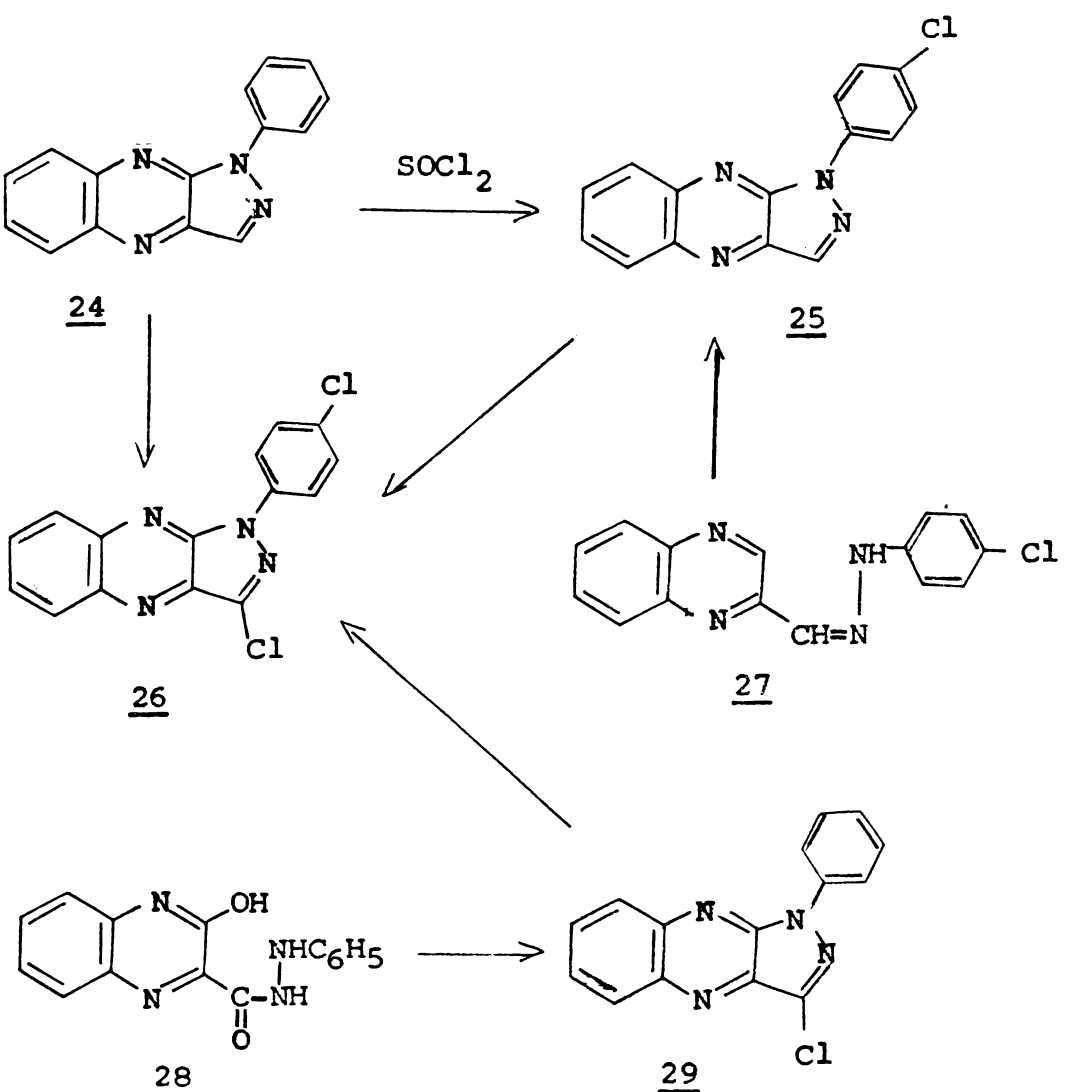
(23) may be brought about by a number of methods[33,36]. Treatment of 23 with azobenzene in 60% aqueous n-propanol in the presence of acetic and hydrochloric acids provided 93% of 1-phenyl-1H-pyrazolo[3,4-b]quinoxaline (24).



21 22 23 24

Two products may be obtained by the chlorination of 24 with thionyl chloride. Treatment of 24 with thionyl chloride at room temperature for about 120 hours caused monochlorination, the product being 1-p-chlorophenyl-1H-pyrazolo[3,4-b]quinoxaline (25) isolated in 85% yield. Reaction of 24 with refluxing thionyl chloride for abo 80 hours led to a mixture of 25 and the dichlorinate

product 1-p-chlorophenyl-3-chloro-1H-pyrazolo[3,4-b]quinoxaline (26) which were separated by column chromatography on silica gel. That the dichlorinated product 26 was also obtained by the continued chlorination of either 25 or 3-chloro-1-phenyl-1H-pyrazolo[3,4-b]quinoxaline (29) with refluxing thionyl chloride established that this product was formed in two steps from 24.



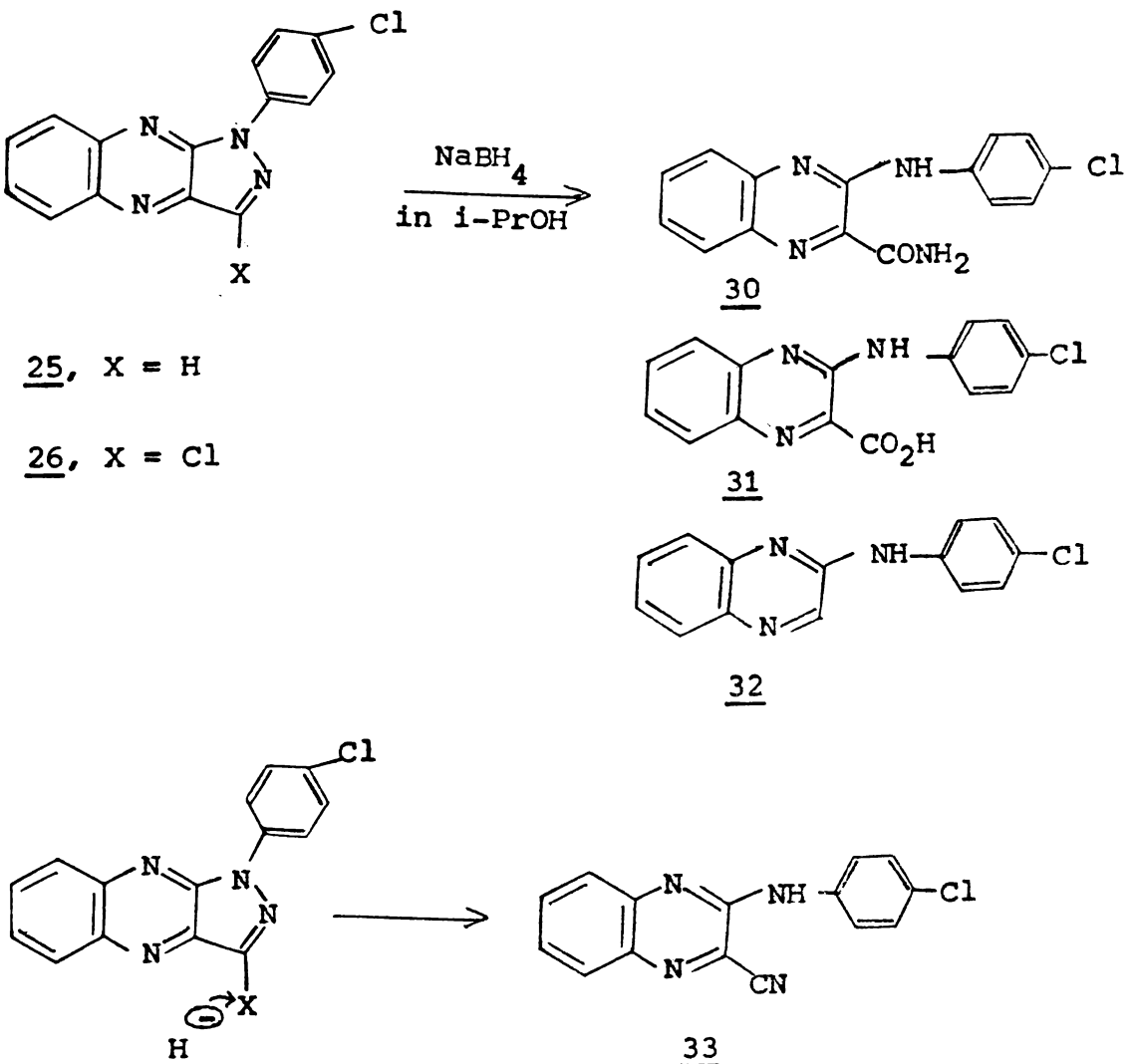
24 25 26 27 28 29

The structure of 25 was established by its direct comparison with an authentic sample prepared by the oxidative cyclisation of quinoxaline-2-carboxaldehyde-p-chlorophenyl-hydrazone[37] (27) using azobenzene.

The structure of the dichlorinated product 26 was established by its analytical and spectral data and also by its formation by chlorination of both 25 and 29. The 3-chloroderivative 29 was prepared starting from ethyl 2-hydroxyquinoxaline-3-carboxylate[85] (4). Condensation of 4 with phenylhydrazine on a boiling water bath gave 82% of 2-hydroxyquinoxaline-3-carbonylphenylhydrazide[35] (28) which on treatment with phosphorous oxychloride on a steam bath provided the known 3-chloro-1-phenyl-1H-pyrazolo[3,4-b]quinoxaline[35] (29).

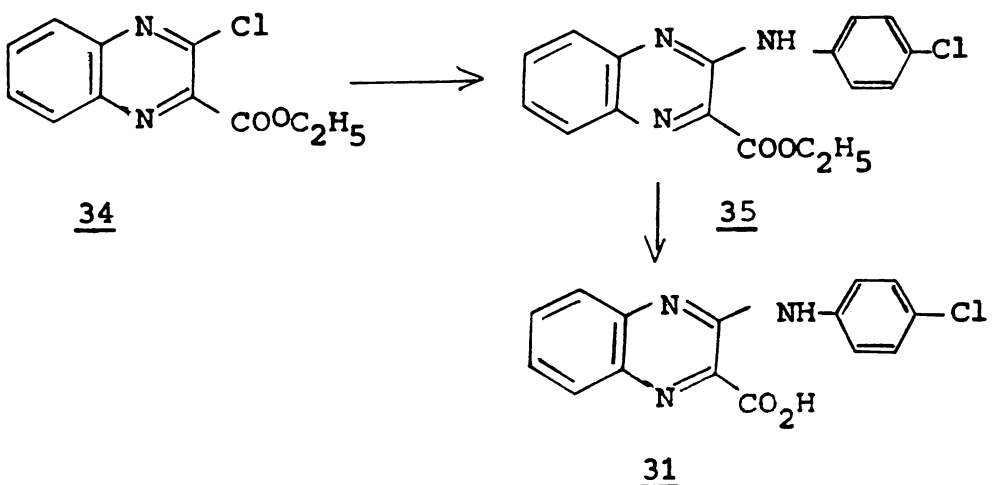
Attempted dechlorination of 25 and 26 using sodium borohydride in methanol at room temperature was unsuccessful as there was no reaction with sodium borohydride under these conditions. However treatment of both 25 and 26 with sodium borohydride in boiling isopropanol provided three new compounds, 3-p-chloroanilinoquinoxaline-2-carboxamide (30) 3-p-chloroanilinoquinoxaline-2-carboxylic acid (31) and 3-p-chloroanilinoquinoxaline (32). The formation

of these compounds may be easily explained by proposing a base induced ring opening of 25 or 26 to give the nitrile, 33 as an intermediate which undergoes partial hydrolysis



to give the carboxamide, 30 and further hydrolysis producing the carboxylic acid 31 and subsequent decarboxylation leading to 32. This pathway finds support from the fact that alkaline hydrolysis of 30 leads to 31 and decarboxylation

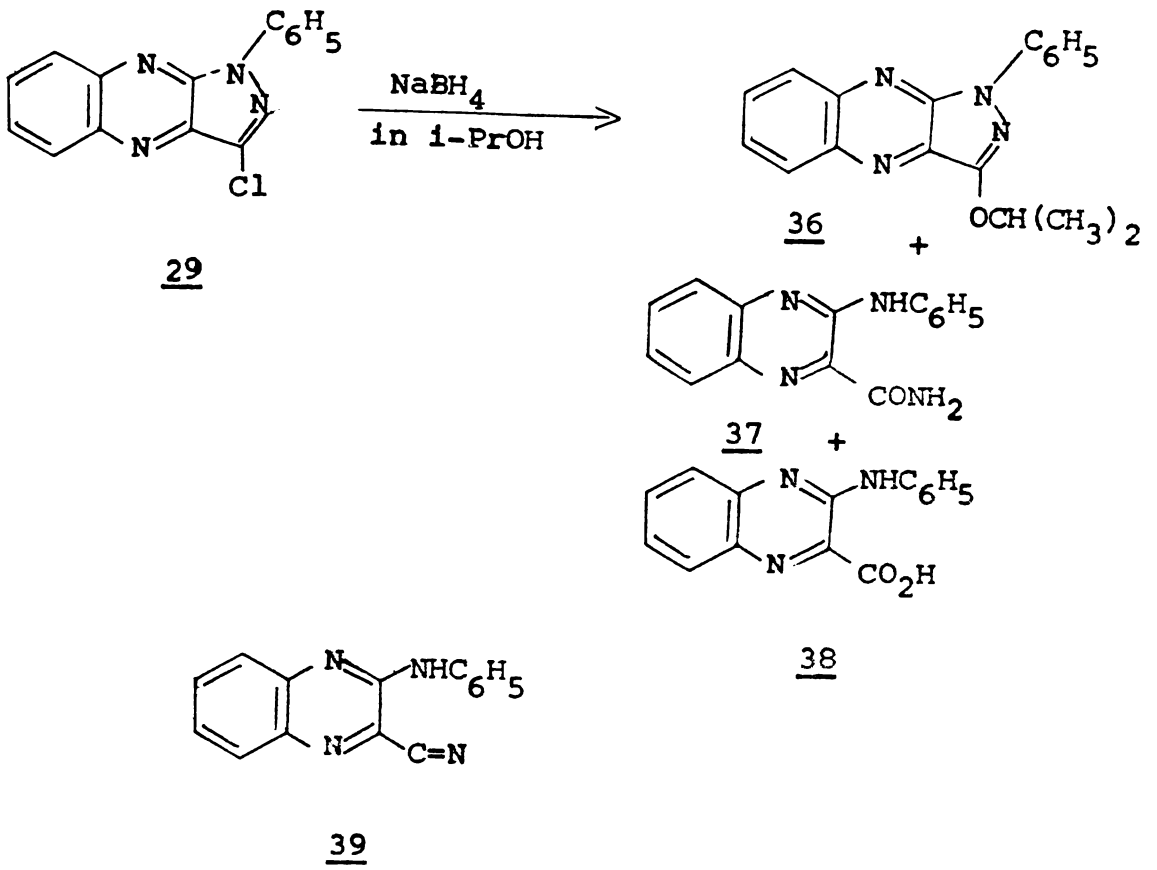
of 31 leads to 32 in excellent yields. The structure of the carboxylic acid 31 was established by an unambiguous method of preparation as follows. Treatment of ethyl 2-hydroxyquinoxaline-3-carboxylate (4) with phosphorous oxychloride on a boiling water bath provided ethyl 2-chloroquinoxaline-3-carboxylate (34) which when condensed with



p-chloroaniline gave ethyl 2-p-chloroanilinoquinoxaline-3-carboxylate (35). Mild alkaline hydrolysis of 35 using aqueous sodium hydroxide provided the carboxylic acid 31 which was identical with the sample prepared previously.

Treatment of 3-chloro-1-phenyl-1H-pyrazolo[3,4-b]-quinoxaline (29) with sodium borohydride in boiling isopropanol gave a mixture of the direct substitution product, 3-isopropyloxy-1-phenyl-1H-pyrazolo[3,4-b]quinoxaline (36),

the ring opened product, 2-anilinoquinoxaline-3-carboxamide (37) and the hydrolysis product 2-anilinoquinoxaline-3-carboxylic acid (38). Although the formation of a nitrile intermediate (39) would easily explain how 37 and 38 are formed in this reaction, neither 33 nor 39 could be isolated from the reaction mixtures.



29

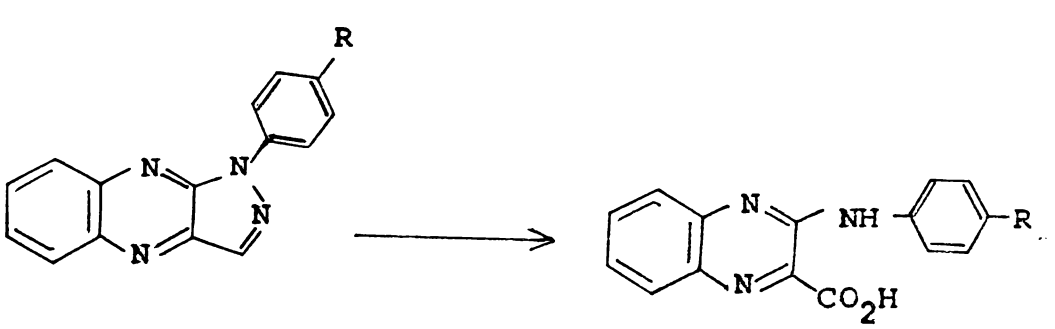
36 + 37 + 38

39

The structure of 36 was clearly established from its NMR spectrum which showed clear doublet at 1.6 for the methyl

groups and a seplat at δ 5.5 for the CH hydrogen. The mass spectral and other analytical data were also in agreement with the structure. Compounds 37 and 38 were previously reported from this laboratory and were identical with samples earlier prepared.

While sodium borohydride in refluxing isopropanol would open the pyrazole ring of the pyrazoloquinoxaline systems of both the 3-chlorosubstituted 26 and 29 and the 3-unsubstituted 24 and 25, only the unsubstituted 24 and 25 were found to be hydrolysed by refluxing with a 10% solution of aqueous sodium hydroxide. This may be because, while -OH can easily pull off a proton from position 3 of 24 and 25 it is not strong enough to remove a chlorine from the same position of 26 and 29. These compounds (26 and 29) were not reactive towards sodium hydroxide under these conditions.

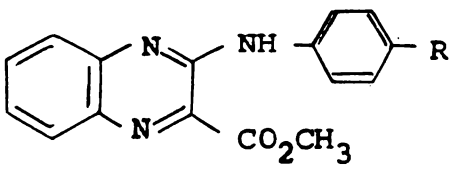


24, R = H

25, R = Cl

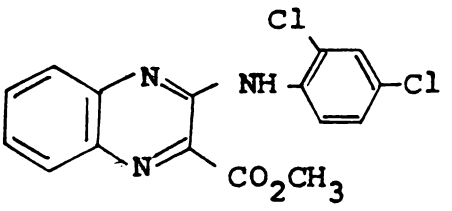
38, R = H

31, R = Cl

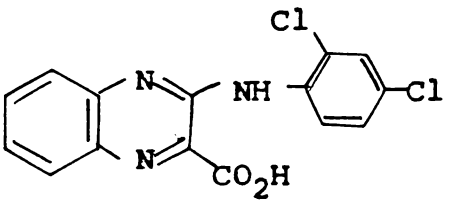


40, R = H

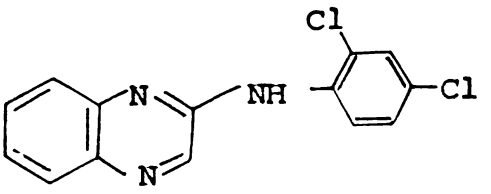
41, R = Cl



42



43

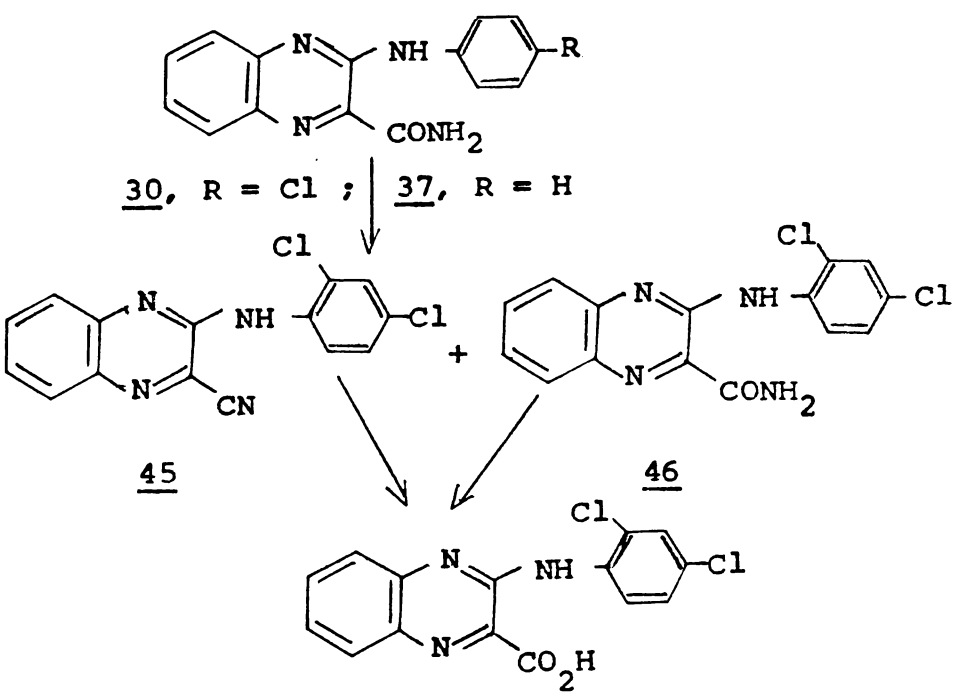


44

Hydrolysis of 24 and 25 with 10% aqueous sodium hydroxide under refluxing conditions followed by acidification provided the previously described carboxylic acids 38 and 31 respectively. In order to study the chlorination reactions with thionyl chloride, the carboxylic acids 31 and 38 were esterified using diazomethane and the methyl esters 40 and 41 were isolated and characterised. Treatment of both 40 and 41 with refluxing thionyl chloride gave the same methyl 2-(2,4-dichloroanilino)quinoxaline-3-carboxylate (42) in good yields. Alkaline hydrolysis of the methyl ester gave the carboxylic acid 43 which was decarboxylated easily by heating at its melting point to give 2-(2,4-dichloroanilino)quinoxaline (44). This compound was also

obtained in good yield by the direct chlorination of 2-p-chloroanilinoquinoxaline (32). Chlorinated anilinoquinoxalines have been reported to be useful agricultural chemicals recently.[94]

In order to study the reaction of thionyl chloride with a carboxamide, 2-anilinoquinoxaline-3-carboxamide (37) was treated with thionyl chloride under refluxing conditions for 80 hours when a mixture of two products was formed. These two compounds were separated by column chromatography on silica gel to give 2-cyano-3(2,4-dichloroanilino)quinoxaline (45) and 2-(2,4-dichloroanilino)-3-carboxamide, (46). A similar mixture of products was also

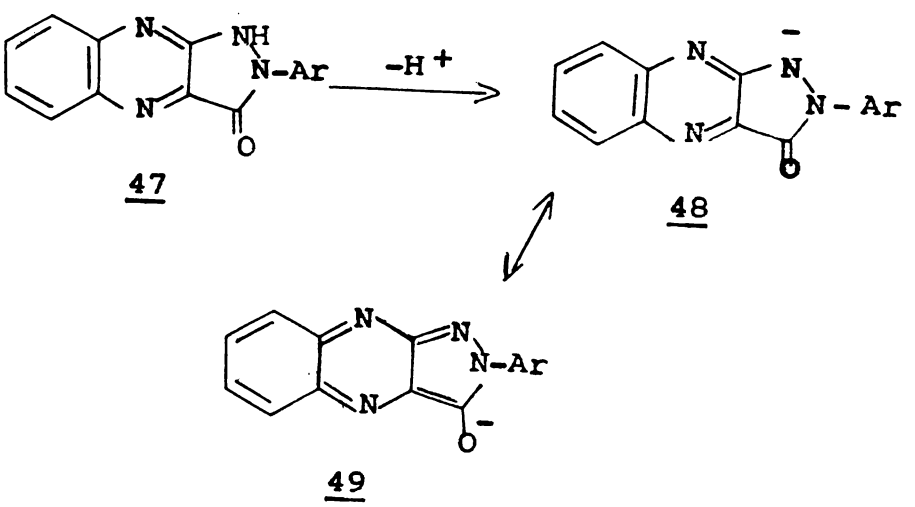


obtained by the chlorination of 2-p-chloroanilinoquinoxaline-3-carboxamide (30). This mixture was also separated by column chromatography on silica gel. The structure of these compounds were established by their spectral data and also by their hydrolysis to the previously characterised 2-(2,4-dichloroanilino)quinoxaline-3-carboxylic acid (43).

The mechanism by which thionyl chloride acts as a chlorinating agent has not been fully understood. As chlorination generally takes place at the electron rich positions (ortho and para positions of the aniline moiety of anilinoquinoxalines) the chlorination must be taking place either by an electrophilic or by a free radical mechanism. Since an electrophilic mechanism for chlorination is very difficult to be conceived using thionyl chloride under these conditions, a free radical mechanism involving chlorine radicals is a possibility. Another possible explanation is that thionyl chloride undergoes oxidation to sulfuryl chloride in the presence of atmospheric oxygen and it is the sulphuryl chloride which is a well known chlorinating agent for many organic substrates, that brings about this unusual reaction. However, more work has to be carried out for a clearer picture of the mechanism of chlorination using thionyl chloride.

3.3 Synthesis of 2-aryl-3-oxo-3-pyrazolino[3,4-b]-quinoxalines

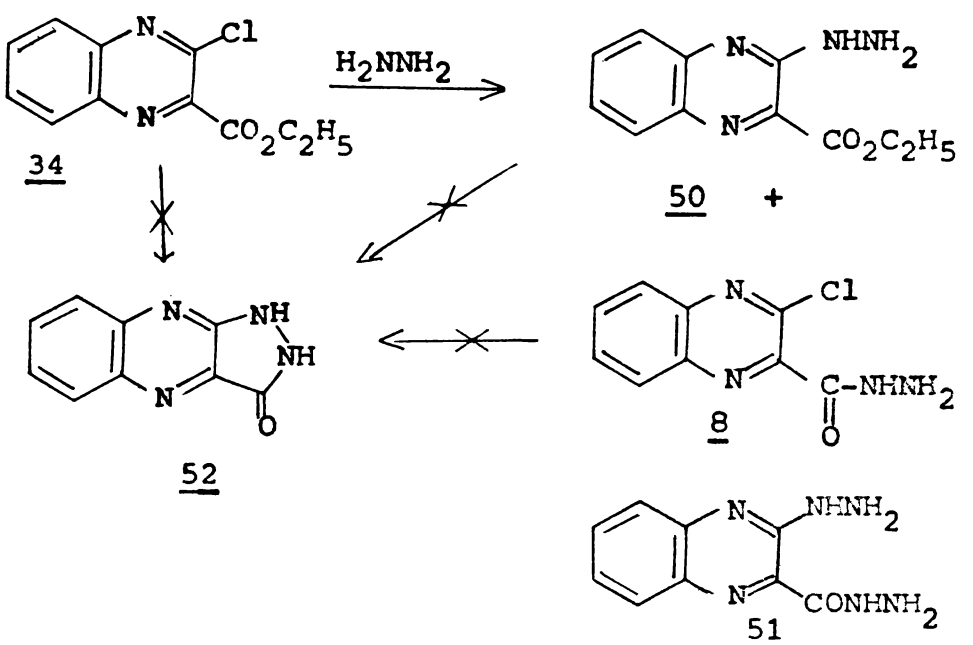
2-Aryl substituted 3-oxo-3-pyrazolino[3,4-b]-quinoxalines (47) give rise to different colours under acid and basic medium and therefore may be used as acid-base indicators. The apparent sharp change in colour may be due to the formation of a stable anion which has different resonance forms, 48 and 49.



47 48 49

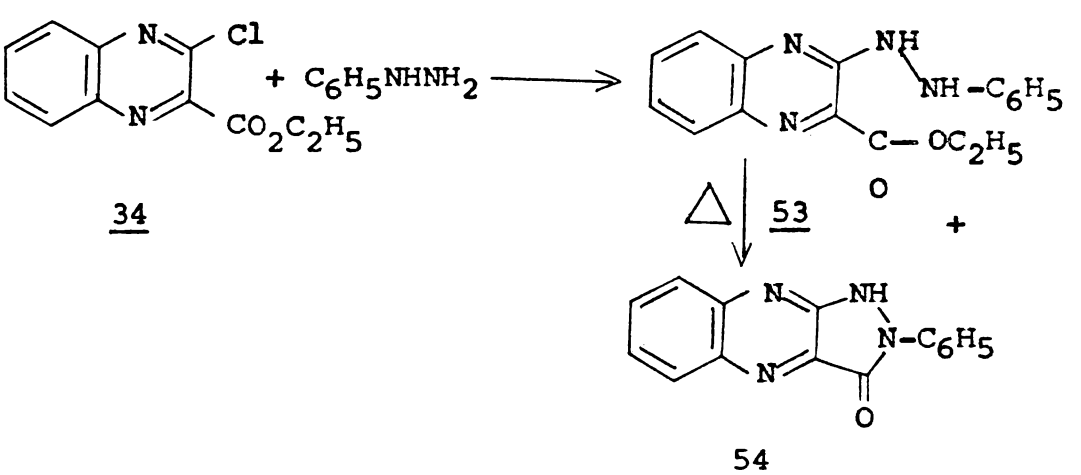
For the preparation of 3-oxo-3-pyrazolino-[3,4-b]quinoxaline and its various derivatives, ethyl 2-chloroquinoxaline-3-carboxylate (34), the preparation

of which has already been described in the previous section was considered to be a good starting material. Condensation of 34 with different hydrazines resulted in the formation of various types of products. Thus when hydrazine hydrate was treated with the ester 34 in methanol at room temperature, gave a mixture of two products which were separated by column chromatography on silica gel to give ethyl 2-hydrazinoquinoxaline-3-carboxylate (50) and 2-chloroquinoxaline-3-carbonylhydrazide (8). Addition of 34 to an excess of hydrazine hydrate resulted in the formation of 2-hydrazinoquinoxaline-3-carbonylhydrazide (51).

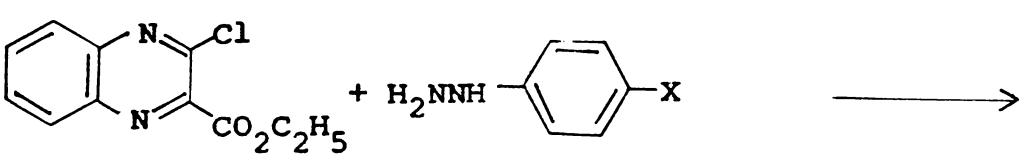


The expected product, 3-oxo-3-pyrazolino[3,4-b]quinoxaline (52) was not obtained. Also attempts to cyclise 50 or 8 by a number of methods were not successful.

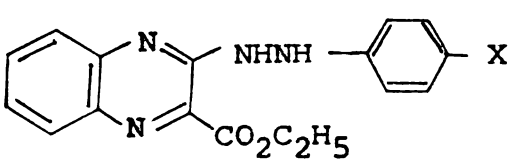
Condensation of the ester 34 with phenylhydrazine took place both at room temperature as well as at 100° giving the same mixture of products, the reaction being slow at room temperature. A mixture of phenylhydrazine and the chloroester 34 on heating on a boiling water bath for 2 hours, gave a mixture of products which were separated on a column of silica gel to give ethyl 2-phenylhydrazino-quinoxaline-3-carboxylate (53) and 2-phenyl-3-oxo-3'-pyrazolino-[3,4-b]quinoxaline (54). The infrared spectrum of 54 showed a C=O group at 1675 cm^{-1}. The mass spectrum of 54 was also characteristic giving the $[M]^+$ peak at m/z 262 and peaks at m/z 234 (M^+ - CO) and m/z 77 (C_6H_5). The phenyl-hydrazino ester 53 was also converted into 54 by heating 53 at its melting point.



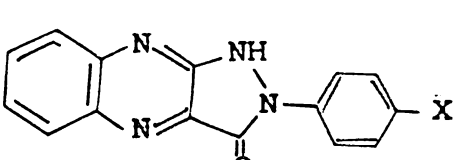
Similarly p-tolylhydrazine condensed with the chloroester 34 giving a mixture of products, ethyl 2-tolylhydrazinoquinoxaline-3-carboxylate (55) and 2-tolyl-3-oxo-3-pyrazolino-[3,4-b]quinoxaline (56) which were separated by column chromatography on silica gel. The spectral data of 56 and 54 were very similar and 55 was converted into 56 by heating at its melting point. p-Bromophenylhydrazine also condensed with the chloroester 34 to give similar results: Ethyl 2-p-Bromophenylhydrazinoquinoxaline-3-carboxylate (57) and 2-p-bromophenyl-3-oxo-3-pyrazolino[3,4-b]quinoxaline (58) were obtained which were separated by column chromatography and characterised by spectral and analytical data.



34

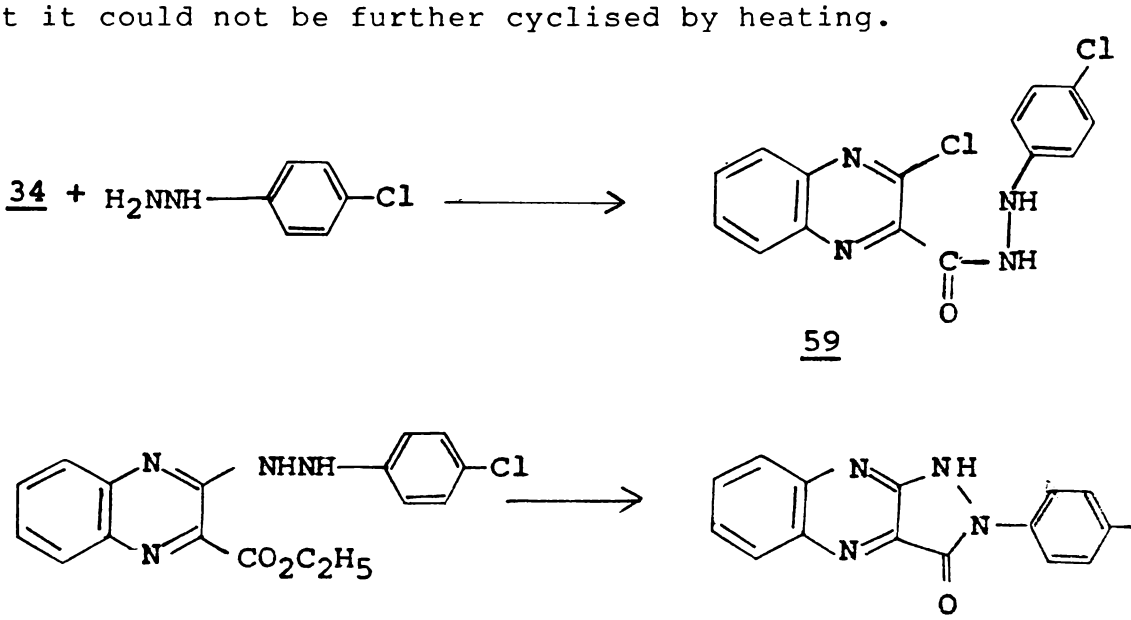


55, X = CH_3 ; 57, X = Br



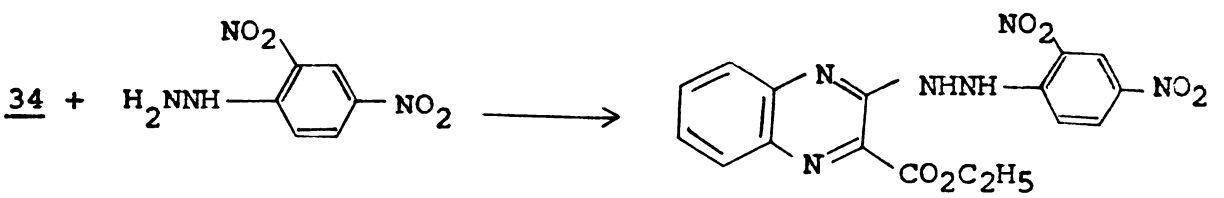
56, X = CH_3 ; 58, X = Br

Treatment of p-chlorophenylhydrazine with ethyl 2-chloroquinoxaline-3-carboxylate gave slightly different results. The mixture of products when separated by column chromatography gave ethyl 2-p-chlorophenylhydrazinoquinoxaline-3-carboxylate (60) and 2-chloroquinoxaline-3-carbonyl-p-chlorophenylhydrazide (59). Cyclisation of 60 by heating at its melting point gave the 3-oxopyrazolinoquinoxaline 61. Condensation of the chloroester 34 with 2,4-dinitrophenylhydrazine gave the chlorine replaced product (62) but it could not be further cyclised by heating.



59

60 61



62

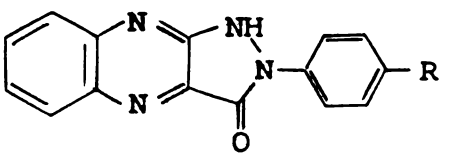
The 3-oxo-3-pyrazolino[3,4-b]quinoxalines are generally light yellow in either neutral or acid solutions but changed the colour to deep violet or green in basic media. The change in colour appears to be sharp and therefore these compounds may be used as acid base indicators. Their UV absorption maxima under neutral and basic conditions are also very different (see Table 3). The exact pH at which colour change takes place and other conditions for using these as indicators have not been investigated.

3.4 Synthesis and reactions of 1H-1,5-benzodiazepino-[2,3-b]quinoxaline, a new heterocyclic system

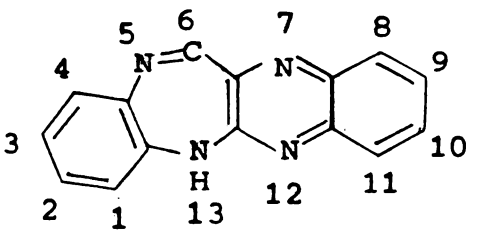
Both benzodiazepines[95] and quinoxalines[96,97] are heterocyclic systems of useful biological activity and therefore it must be interesting to find out the pharmacological properties of benzodiazepines fused with quinoxalines. Although such system do not come under the title, pyrazoloquinoxalines, we have undertaken the synthesis of 1H-1,5-benzodiazepino[2,3-b]quinoxaline (63) derivatives as they can be easily obtained from ethyl 2-chloroquinoxaline-3-carboxylate (34) and o-phenylene diamine. Such a heterocyclic system has not been described in the literature previously.

Table III

UV-Visible absorption maxima for 2-aryl-3-oxo-3-pyrazolino-[3,4-b]quinoxalines under neutral and alkaline media

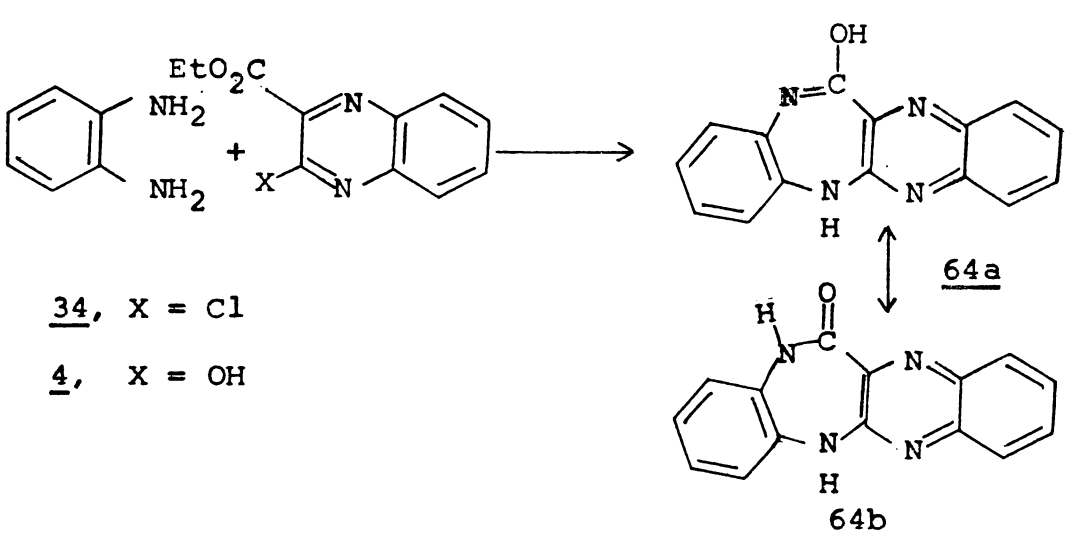


Sl.No.	Compound No.	R	MeOH λ_{max} nm	
			Neutral	Alkaline
1.	54	H	206.4, 283, 359.2, 544	213.6, 285.4, 360, 536
2.	56	CH_3	207, 284, 352, 547	216, 286.8, 366.6, 543
3.	58	Br	207, 287, 363, 551	213, 277, 363.4, 448, 476.8, 510
4.	61	Cl	205, 288.2, 351.6, 549	214, 289, 357



63

Treatment of the chloroester 34 with o-phenylene diamine at 120-130° gave 6-hydroxy-1H-1,5-benzodiazepino-[2,3-b]quinoxaline (64a) which may also exist as its tautomer (64b). Compound 64 was also obtained by the reaction of ethyl 2-hydroxyquinoxaline-3-carboxylate (4) with o-phenylene diamine at 170° in 80% yield. The IR spectra of 64 showed NH absorption at 3260 cm^{-1}, C=O at 1700 cm^{-1} and a weak broad absorption at 3480 cm^{-1} for -OH. The mass spectrum gave a molecular ion peak at m/z



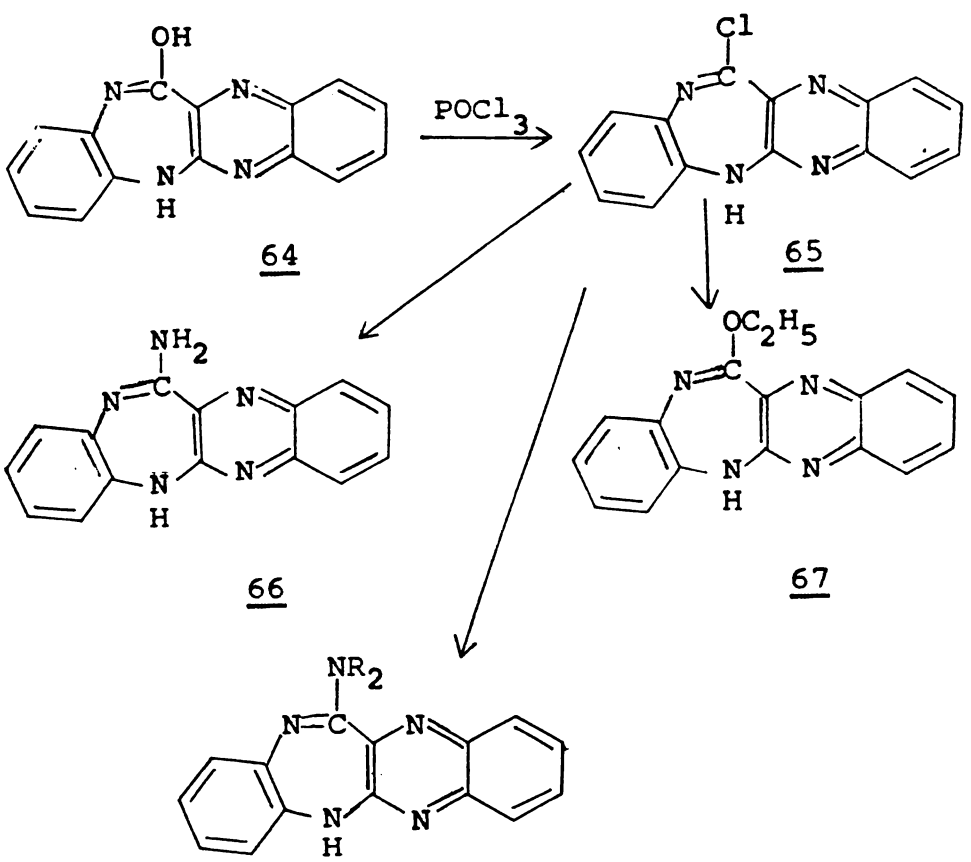
34, X = Cl

4, X = OH

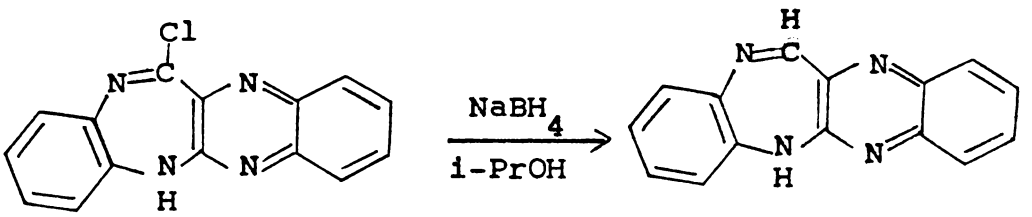
64a

64b

Treatment of 64 with phosphorous oxychloride on a boiling water bath provided the 6-chloroderivative, 65. This compound is a useful intermediate as it was converted into different derivatives by displacement of the chlorine atom with various nucleophiles. Thus, treatment of 65 with urea at 130° gave the 6-amino derivative 66, while reaction with ethanol in the presence of potassium carbonate provided the 6-ethoxy derivative, 67. Treatment of 65 with different secondary amines such as morpholine, piperidine, pyrrolidine and diethyl amine also gave the dialkylamino derivatives 68-71 in good yield. These compounds will be submitted for screening their biological activity.



Finally, reduction of the chloro derivative 65 with sodium borohydride in boiling isopropanol displaced the chlorine with hydrogen and provided the parent heterocyclic system, 1H-1,5-benzodiazepino[2,3-b]quinoxaline (63) in 75% yield.



65 63

The mass spectrum of 63 gave a molecular ion peak at m/z 246 and the NMR spectrum showed only aromatic protons at δ 7.4-8.3 and the NH proton at δ 1.7.

CHAPTER IV

EXPERIMENTAL PROCEDURES

All melting points were taken using capillary tubes on a melting point bath containing liquid paraffin or silicon oil and are not corrected. Thin layer chromatography was performed on 5 x 20 cm glass plates coated with silica gel G. Chloroform was used as the developing solvent unless otherwise mentioned. Compounds were detected either by their colour or by developing with iodine. Mass spectra were recorded on a Varian MAT CH 7 Mass spectrometer. Nmr spectra were run in deuterio chloroform using Hitachi R-600 FT NMR spectrometer or a Varian FT 80A Spectrometer with tetramethylsilane as an internal standard. Infrared spectra were obtained on a Perkin Elmer Model 682 grating spectrophotometer. Ultraviolet spectra were obtained using a Hitachi Model 200 Spectrophotometer in methanol. Elemental analyses were performed at the Indian Institute of Science, Bangalore.

4.1 Ethyl mesoxalate[84] (3)

A mixture of 40 g (0.25 mol) of diethyl malonate and 14 g (0.125 mol) of selenium dioxide was heated at 120-130° for 2 hours. The precipitated selenium was removed by decantation. The decanted liquid was distilled under reduced pressure to give the fractions (A) upto 80°/45 mm, 2 ml; (B) 80-130°/36 mm, 25 ml and (C) 130-230°/36 mm, 4 ml; Fraction (C) was a complex, garlic smelling mixture of selenium containing compounds and was rejected. Fraction (B)

was extracted with water (7x10 ml) and the extracts were quickly evaporated under reduced pressure separately until they became viscous and yellow. On cooling in ice, they crystallised slowly giving 7.75 g (32.3%) of white ethyl mesoxalate hydrate, mp 56° (lit.[84] mp 56°). On drying the liquid remaining from the aqueous extract, 12 ml of diethyl malonate, bp 193-198° was recovered.

4.2 Ethyl 2-hydroxyquinoxaline-3-carboxylate[85] (4)

a) From ethyl mesoxalate (3)

A solution of 1.1 g (0.01 mol) of o-phenylene diamine in 30 ml of 1 N HCl and 1.75 g (0.01 mol) of ethyl mesoxalate (3) was stirred for 30 minutes at room temperature. Crystals of ethyl 2-hydroxyquinoxaline-3-carboxylate were formed. The mixture was cooled in a refrigerator overnight, filtered, washed with a little of ice cold water and recrystallised from hot water to give 1.96 g (93%) of ethyl 2-hydroxyquinoxaline-3-carboxylate (4), mp 176° (lit.[85] mp 175.5-176.5°).

b) From diethyl dibromomalonate (5)

A solution of 8.5 g (0.03 mol) of diethyl dibromomalonate (5) in methanol was added to 3.3 g of o-phenylene diamine and stirred for 24 hours. The reaction mixture

was added to the water and cooled in a freezing mixture. Solid product formed was filtered washed well with cold water and recrystallised from hot water to give 2.6 g (40%) of ethyl 2-hydroxyquinoxaline-3-carboxylate (4) mp 176°. A mmp with the product obtained from (a) above was undepressed.

4.3 2-Hydroxyquinoxaline-3-carbonylhydrazide (6)

A mixture of 2 g (0. 01 mol) of ethyl 2-hydroxyquinoxaline-3-carboxylate (4) in 40 ml of ethyl alcohol and 2 ml hydrazine hydrate was stirred for one hour at room temperature. The mixture was cooled, filtered, washed with cold ethanol, dried and recrystallised from methanol to give 1.75 g (85%) of 2-hydroxyquinoxaline-3-carbonylhydrazide (6), mp above 300°. (lit.[97] mp 343°).

MS: m/e 204 (M^+), 173 ($M^+ - NHNH_2$), 145 ($M^+ - CONHNH_2$).

IR(KBr): 3320 cm^{-1}(OH), 3250 cm^{-1}(NH), 1710 cm^{-1}(C=O).

Anal. Calcd. for $C_9H_8N_4O_2$: C, 52.94; H, 3.92; N, 27.45.
Found: C, 5289; H, 3.83; N, 28.01.

4.4. Attempted cyclisation of 2-hydroxyquinoxaline-3-carbonylhydrazide

a) Using acetic anhydride (6)

A mixture of 2.0 g (0. 01 mol) of 2-hydroxy-

quinoxaline-3-carbonylhydrazide (6) and 20 ml of acetic anhydride was heated on a boiling water bath for 2 hours. The reaction mixture was cooled and poured into 100 ml of water, cooled, filtered, washed with water and dried. Recrystallised from methanol to give 2.4 g (84%) of N-(2-acetyloxyquinoxaline-3-carbonyl)N'-acetylhydrazine (7), mp 236°.

MS: m/e 288 (M^+), 246 ($M^+ - COCH_3+H$), 204 ($M^+ - 2COCH_3$), 173 ($M^+ - 2COCH_3-NHNH+H$), 43 ($COCH_3$), 28 (CO).

IR(KBr): 3460 cm^{-1}, 3320 cm^{-1} (NHNH), 1750 ($OCOCH_3$), 1715 (CONH), 1670 ($NHCOCH_3$).

UV: λ_{max}^{MeOH} 206.4 nm (ϵ 1.83×10^4), 232 nm (ϵ 1.43×10^4), 295 nm (ϵ 4.49×10^3).

Anal. Calcd. for $C_{13}H_{12}N_4O_4$: C, 54.16; H, 4.16; N, 19.44.
Found: C, 53.78; H, 4.16; N, 19.28.

b) Using phosphorous oxychloride

A mixture of 1.0 gm (0. 005 mol) of 2-hydroxy-quinoxaline-3-carbonylhydrazide and 25 ml of freshly distilled phosphorous oxychloride was heated on a steam bath till the solid dissolved completely. The reaction mixture was cooled and poured into 200 gm of crushed ice with

stirring. It was filtered and the filtrate neutralised using sodium bicarbonate and was extracted with chloroform. The solvent was removed and the product recrystalised from chloroformhexane to give 550 mg (50%) of 2-chloroquinoxaline-3-carbonylhydrazide (8) mp 167°.

MS: m/e 222 (M^{+}), 224 (M^{+} + 2), 191 (M^{+} - $NHNH_2$), 163 (M^{+} - $CONHNH_2$), 129 (M^{+}-$CONHNH_2$-Cl+H). 28 (CO).

NMR($CDCl_3$): δ 1.3-2.2 (3, broad, $NHNH_2$), 8.1 (4, complex, aromatic Hs).

IR(KBr): 3320, 3220 cm^{-1} (NH), 1660 cm^{-1} (C=O).

UV: λ_{max}^{MeOH} 206.8 nm (ε $2.7x10^4$), 243 nm (ε $3.1x10^4$), 324.4 nm (ε $6.5x10^3$).

Anal. Calcd. for $C_9H_7N_4ClO$: C, 48.65; H, 3.15; N, 25.22. Found: C, 48.32; H, 3.02; N, 24.1.

4.5 1-Acetyl-3-hydroxy-1H-pyrazolo[3,4-b]quinoxaline (9)

A mixture of 200 mg (0. 001 mol) of 2-hydroxy-quinoxaline-3-carbonylhydrazide (6), 20 ml glacial acetic acid, 5 ml of acetic anhydride and 500 mg of p-toluene sulphonic acid was heated for 2½ hours on a boiling water

bath. The reaction mixture was cooled and poured into about 100 gms of crushed ice with stirring. The solution was neutralised with sodium bicarbonate and extracted with chloroform. The extract was dried and the solvent was distilled off. The product was recrystallised from methanol to give 110 mg (50%) of 1-acetyl-3-hydroxy-1H-pyrazolo[3,4-b]-quinoxaline (9), mp 313°.

MS: m/e 228($M^{+\cdot}$), 200 (M^{+}-C=O), 43 ($COCH_3$).

NMR($CDCl_3$): δ 2.8 (3, s, CH_3), 4.6 (1,s,OH), 7.7-8.2 (4, complex, aromatic).

IR(KBr): 3556 cm^{-1} (OH), 1678 cm^{-1} (C=O).

UV: λ_{max}^{MeOH} 206.6 nm (ε 3.2×10^4), 235.7 nm (ε 2.45×10^4), 311.2 (ε 1.33×10^4), 384.5 (ε 9.66×10^3).

Anal. Calcd. for $C_{11}H_8N_4O_2$: C, 57.89; H, 3.51; N, 24.56.
Found: C, 58.15; H, 3.44; N, 24.91.

4.6 1-Acetyl-3-chloro-1H-pyrazolo[3,4-b]quinoxaline (10)

A mixture of 2.2 g (0. 01 mol) of 1-acetyl-3-hydroxy-1H-pyrazolo[3,4-b]quinoxaline (9) and 40 ml of phosphorous oxychloride was heated under reflux on a steam bath for 45 minutes under a calcium chloride guard tube. The reaction

mixture was cooled and poured into 500 g of crushed ice with stirring. Cooled and filtered. Residue was dissolved in chloroform, purified by passing over a column of silica gel and recrystallised from chloroform- hexane to give 1.6 g (66%) of 1-acetyl-3-chloro-1H-pyrazolo[3,4-b]quinoxaline (10), mp 1.16°.

MS: m/e 246 (M^+), 248 ($M^+ + 2$), 204 ($M^+ - COCH_3 + H$), 43 ($COCH_3$), 28 (CO).

NMR ($CDCl_3$): δ 2.8 (3,s,CH_3), 8.1 (4,m,aromatic Hs).

UV: λ_{max}^{MeOH} 209.4 nm (ϵ 2.55×10^4), 253.9 nm (ϵ 2.76×10^4), 337.1 nm (ϵ 8.99×10^3).

Anal. Calcd. for $C_{11}H_7N_4ClO$: C, 53.65; H, 2.84; N, 22.76.
Found: C, 53.04; H, 2.81; N, 22.47.

4.7 1-Acetyl-3-methoxy-1H-pyrazolo[3,4-b]quinoxaline (11)

A solution of 500 mg (0. 002 mol) of 1-acetyl-3-chloro-1H-pyrazolo[3,4-b]quinoxaline in 200 ml of methanol was stirred with 75 ml of liquor ammonia in a closed vessel for 6 hours at room temperature. The reaction mixture was concentrated to 100 ml under reduced pressure. It was extracted with chloroform. The extract showed three

components on tlc. The mixture was separated by chromatographing on an alumina column. First component obtained on elution with chloroform after recrystalisation, from chloroform-hexane gave 360 mg (70%) of 1-acetyl -3-methoxy-1H-pyrazolo[3,4-b]quinoxaline (11), mp 180°.

MS: m/e 242 (M^{+}), 199 (M^{+} - $COCH_3$).

NMR ($CDCl_3$): δ 2.7 (3,s,$COCH_3$), 4.3 (3,s,OCH_3), 7.7-8.3 (4, m, aromatic Hs).

IR(Nujol): 1620 cm^{-1} (C=O).

UV: λ_{max}^{MeOH} 211.4 nm (ϵ 1.52×10^4), 251 nm (ϵ 1.16×10^4), 307.2 nm (ϵ 5.7×10^3), 352 nm (ϵ 4.87×10^3).

Anal. Cacld. for $C_{12}H_{10}N_4O_2$: C, 59.5; H, 4.13, N, 23.14.

Found: C, 59.05; H, 4.06; N, 23.58.

Further elution with chloroform gave the second component which on recrystallisation from methanol gave 20 mg (5%) of 1-acetyl-3-amino-1H-pyrazolo[3,4-b]quinoxaline (12), mp 260°. See under 4.8 for characterisation of compound 12. Continued elution with chloroform-methanol gave a third compound which after recrystallisation from chloroform-hexane gave 20 mg of 13 mp 200°.

MS: m/e 260 (M^+), 218 ($M^+ - COCH_3 + H$), 187 ($M^+ - N(COCH_3)NH_2$)

43 ($COCH_3$), 28 (CO).

NMR($CDCl_3$):δ1.7 (2,s,NH_2), 2.2 (s, $COCH_3$), 4.2 (3,s,$COOCH_3$)

7.7 (4,m,aromatic H).

IR(KBr): 3200 cm^{-1} (NH_2), 1680 cm^{-1} ($COOCH_3$), 1620 cm^{-1} ($NCOCH_3$).

UV: λ_{max}^{MeOH} 209.2 nm (ε 2.3x10^4), 227.6 nm (ε 1.38x10^4),

242.2 nm (ε 1.62x10^4), 301.4 nm (ε 4.74x10^3), 342.4 nm (ε 4.8x10^3).

Anal. Calcd. for $C_{12}H_{12}N_4O_3$: C, 55.38; H, 4.61; N, 21.53.
Found: C, 55.34; H, 4.33; N, 21.55.

4.8 1-Acetyl-3-amino-1H-pyrazolo[3,4-b]quinoxaline (12)

a) By reaction of 10 with urea

A mixture of 250 mg (0.001 mol) of 1-acetyl-3-chloro-1H-pyrazolo[3,4-b]quinoxaline was mixed with 2 g of urea and heated at its melting point for 48 hours. Water (250 ml) was added to the reaction mixture and the mixture heated on a boiling water bath for 20 minutes, cooled and extracted with chloroform. The extract was dried (Na_2SO_4)

and the solvent removed under reduced pressure. Residue was purified by chromatographing on a silica gel column. Product obtained was recrystallised from methanol to give 190 mg (80%) of 1-acetyl-3-amino-1H-pyrazolo[3,4-b]quinoxaline (12), mp 260°.

MS: m/e227 (M^+), 185 ($M^+ - COHC_3+H$), 170 ($M^+-COCH_3-NH_2+2H$).

to dryness under reduced pressure. The residue was recrystallised from chloroform-hexane to give 25 mg (6%) of 2-p-chloroanilinoquinoxaline-3-carboxylic acid (31), mp 169°, identical with the sample prepared below.

b) From 1-p-chlorophenyl-1H-pyrazolo[3,4-b]quinoxaline (25)

A mixture of 500 mg (0.002 mol) of 1-p-chlorophenyl-1H-pyrazolo[3,4-b]quinoxaline (25), 50 ml of 10% sodium hydroxide solution and 4 ml of n-propanol was heated under reflux for 25 hours. The reaction mixture was concentrated to 50 ml, cooled in an ice bath, neutralised with hydrochloric acid and extracted with chloroform. The extract was dried with anhydrous sodium sulphate, evaporated to dryness under reduced pressure and the residue was recrystallised from chloroform-hexane to give 450 mg (85%) of 2-p-chloroanilinoquinoxaline-3-carboxylic acid (31), mp 169°.

MS: m/e 299 (M^+), 301 ($M^+ + 2$), 255 ($M^+ - CO_2$), 220 ($M^+ - CO_2 - Cl$).

IR(KBr): 3400 cm^{-1} (Borad,OH,NH), 1730 cm^{-1} (C=O).

UV: λ_{max}^{MeOH} 208 nm (ε 1.13×10^4), 222 nm (ε 1.23×10^4), 293 nm (ε 1.35×10^4), 412 nm (ε 1.44×10^3).

Anal. Calcd. for $C_{15}H_{10}ClN_3O_2$: C, 60.2; H, 3.34; N, 14.04.

Found: C, 60.4; H, 3.36; N, 14.2.

c) From 2-p-chloroanilinoquinoxaline-3-carboxamide (30)

A mixture of 200 mg (0.00065 mol) of 2-p-chloroanilinoquinoxaline-3-carboxamide (30), 50 ml of 10% of sodium hydroxide solution and 40 ml of n-propanol was heated on a boiling water bath for 4 hours. The reaction mixture was evaporated to 50 ml, cooled in an ice bath, neutralised with hydrochloric acid and extracted with chloroform. The extract was dried with anhydrous sodium sulphate, evaporated to dryness under reduced pressure and the residue recrystallised from chloroform-hexane to give 170 mg (85%) of 2-p-chloroanilinoquinoxaline-3-carboxylic acid (31), mp 169°. A mixed melting point determination with the sample from (a) above was undepressed.

d) From Ethyl 2-chloroanilinoquinoxaline-3-carboxylate (35)

A mixture of 325 mg (0.001 mol) of ethyl 2-p-chloroanilinoquinoxaline-3-carboxylate, 50 ml of 10% sodium hydroxide solution and 40 ml of n-propanol was heated on a boiling water bath for 4 hours. The reaction mixture was evaporated to 50 ml, cooled in an ice bath, neutralised with 2 N HCl

and extracted with chloroform. The extract was dried (Na_2SO_4) evaporated to dryness under reduced pressure and the residue recrystallised from chloroform-hexane to give 270 mg (90%) of 31, mp 169°, identical with the sample prepared above.

4.27 2-p-Chloroanilinoquinoxaline (32)

2-p-Chloroanilinoquinoxaline-3-carboxylic acid (31) (100 mg, 0.000375 mol) was heated in a test tube at 200° for 30 minutes in an oil bath. The solid mass obtained was dissolved in chloroform and purified by chromatographing over a column of silica gel using chloroform as eluent. The product was recrystallised from chloroform-hexane to give 60 mg (72%) of 2-p-chloroanilinoquinoxaline (32), mp 189°.

MS: m/e 255 (M^+), 257 ($M^+ + 2$), 254 ($M^+ - H$), 219 (M^+-H-Cl), 91 (C_6H_5N).

NMR($CDCl_3$): δ 7.4-8.1 (9,m,aromatic Hs), 8.4 (1,s,NH).

IR(KBr): 3280 cm^{-1} (NH).

UV: λ_{max}^{MeOH} 208 nm (ε 3.6×10^4), 287 nm (ε 2.7×10^4), 380 nm (ε 9.9×10^3).

Anal. Calcd. for $C_{14}H_{10}ClN_3$: C, 65.8; H, 3.9; N, 16.5.

Found: C, 65.3; H, 3.9; N, 16.8.

The samples of 32 obtained as described previously in the preparation of 30 were identical (lR,mmp) with the sample obtained in this reaction.

4.28 Ethyl 2-chloroquinoxaline-3-carboxylate[98] (34)

A mixture of 4.36 g (0.002 mol) of ethyl 2-hydroxy-quinoxaline-3-carboxylate and 50 ml of freshly distilled phosphorous oxychloride was heated on a steam bath for 3 hours, under a calcium chloride guard tube. The mixture was cooled and poured into 500 g of crushed ice with stirring. The precipitate was filtered, washed with ice cold water dried and recrystallised from hexane to give 3.7 g (85%) of ethyl 2-chloroquinoxaline-3-carboxylate (34), mp 42-43° (lit.[98] mp 42.5°).

4.29 Ethyl 2-p-chloroanilinoquinoxaline-3-carboxylate (35)

A mixture of 2.4 g (0.001 mol) of ethyl 2-chloro-quinoxaline-3-carboxylate and 2.5 g of p-chloroaniline was heated for 8 hours on a boiling water bath. The mixture

was cooled, 25 ml of 2 N hydrochloric acid was added, the product was extracted in chloroform and the extract was dried, concentrated and purified by chromatographing over a column of silica gel using chloroform as the eluent. The product obtained was recrystallised from chloroform-hexane to give 2.68 g (82%) of ethyl 2-p-chloroanilinoquinoxaline-3-carboxylate (35), mp 174°.

NMR($CDCl_3$): δ 1.5 (3,t,CH_3), 4.6 (2,q,OCH_2),
7.4-8 (9,complex, other Hs).

IR(KBr): 3240 cm^{-1} (NH), 1730 cm^{-1} (C=O).

Anal. Calcd. for $C_{17}H_{14}ClN_3O_2$: C, 62.4, H, 4.3; N, 12.8.
Found: C, 61.9; H, 4.1; N, 12.7.

4.30 Reaction of 3-chloro-1-phenyl-1H-pyrazolo[3,4-b]-quinoxaline (29) with sodium borohydride in isopropanol

A solution of 500 mg (0.002 mol) of 3-chloro-1-phenyl-1H-pyrazolo[3,4-b]quinoxaline (29) in 200 ml of isopropanol was mixed in 200 mg portions with 1.0 g of powdered sodium borohydride and the mixture was heated under reflux for 16 hours on a boiling water bath. The unreacted sodium borohydride was decomposed by the addition of a few ml of water, the isopropanol was removed under reduced

pressure and 50 ml of water was added to the residue. The precipitate was filtered, washed with water and dried. A tlc examination showed that the product contained two compounds which were separated on a column of silica gel. Elution of the column with carbon tetrachloride gave 300 mg (60%) of the first component, 3-isopropyloxy-1-phenyl-1H-pyrazolo[3,4-b]quinoxaline (36), mp 172°.

MS: m/e 304 ($M^{\ddagger}$), 245 (M^{+} - $OCH(CH_3)_2$), 91 (C_6H_5N).

NMR($CDCl_3$): δ 1.6 (6,d,c$(CH_3)_2$), 5.5 (1,seplat, OCH), 7.2-8.6 (9,m,aromatic Hs).

UV: λ_{max}^{MeOH} 207 nm (ε 4.08×10^4), 231 nm (ε 2.95×10^4), 279.6 nm (ε 7.47×10^4), 331.6 nm (ε 1.09×10^4), 440.2 nm (ε 4.75×10^3).

Anal. Calcd. for $C_{18}H_{16}N_4O$: C, 71.05; H, 5.26, N, 18.4.
Found: C, 71.2; H, 5.48; N, 18.8.

Further elution with chloroform gave 100 mg (20%) of 2-anilinoquinoxaline-3-carboxamide (37), mp 221° (lit.[63] mp 221°).

The mother liquor after separating the solids was cooled, acidified with hydrochloric acid and extracted

with chloroform. The extract was dried (Na_2SO_4) and evaporated to dryness under reduced pressure. The yellow residue was recrystallised from chloroform-hexane to give 50 mg (12%) of 2-anilinoquinoxaline-3-carboxylic acid (38), mp 165° (lit.[64] mp 165°).

4.31 2-Anilinoquinoxaline-3-carboxylic acid (38)

A mixture of 1 g (0.004 mol) of 1-phenyl-1H-pyrazolo-[3,4-b]quinoxaline (24), 100 ml of 10% sodium hydroxide solution and 80 ml of n-propanol was heated under reflux for 25 hours. The reaction mixture was concentrated to 100 ml, cooled in an ice bath, neutralised with hydrochloric acid and extracted with chloroform. The extract was dried with anhydrous sodium sulphate, evaporated to dryness under reduced pressure and recrystallised from 1:5 chloroform pentane to give 180 mg (83%) of 2-anilinoquinoxaline-3-carboxylic acid (38), mp 165° (lit. mp 165°).

4.32 Attempted hydrolysis of 3-chloro-1-phenyl-1H-pyrazolo-[3,4-b]quinoxaline (29)

A mixture of 100 mg (0.0004 mol) of 3-chloro-1-phenyl-1H-pyrazolo[3,4-b]quinoxaline (29), 10 ml of 10% sodium hydroxide solution and 10 ml of n-propanol was heated under

reflux for 30 hours. The reaction mixture did not indicate the formation of any new product on tlc. The reaction mixture was concentrated to 100 ml, cooled, neutralised with hydrochloric acid and extracted with chloroform to give back 95 mg of the starting material, mp 210°.

4.33 Attempted hydrolysis of 1-p-chlorophenyl-3-chloro-1H-pyrazolo[3,4-b]quinoxaline (26)

A mixture of 300 mg (0.001 mol) of 26, 30 ml of 10% sodium hydroxide solution and 25 ml of n-propanol was heated under reflux for 30 hours. The reaction mixture did not indicate the formation of any new product on tlc. The reaction mixture on work up done as above gave back 275 mg of the starting material, mp 200°.

4.34 Methyl-2-anilinoquinoxaline-3-carboxylate (40)

A mixture of 6 ml of 50% aqueous potassium hydroxide and 60 ml of ether was cooled to 5° and 2 g of nitrosomethyl urea was added with stirring. The ether layer containing diazomethane was added with stirring to a cold solution of 265 mg (0.001 mol) of 2-anilinoquinoxaline-3-carboxylic acid in ether. After the completion of addition, the mixture was stirred for 1 hour and the solvent was evaporated to dryness under reduced pressure. A 5% sodium

bicarbonate solution was added to the residue and stirred for 1 hour at room temperature. Filtered, washed with water, and purified by passing through a silica column which on recrystallisation from hexane gave 250 mg (90%) of methyl-2-anilinoquinoxaline-3-carboxylate (40), mp 116°.

MS: m/e 279 (M^+), 278 (M^+ - H), 219 (M^+-H-CO_2CH_3), 91 (C_6H_5N), 77 (C_6H_5), 28 (CO).

NMR($CDCl_3$): δ 4.2 (3,s,OCH_3), 7.2-8 (10,complex,other Hs).

IR(KBr): 3240 cm^{-1} (NH), 1680 cm^{-1} (C=O).

UV: λ_{max}^{MeOH} 209 nm (ϵ 3.37x10^4), 223 nm (ϵ 3.4x10^4), 290 nm (ϵ 3.2x10^4), 420 nm (ϵ 3.9x10^3).

Anal. Calcd. for $C_{16}H_{13}N_3O_2$: C, 68.8; H, 4.64; N, 15.05.
Found: C, 68.73; H, 4.52; N, 15.16.

4.35 Methyl 2-p-chloroanilinoquinoxaline-3-carboxylate (41)

A mixture of 2 ml of 50% aqueous potassium hydroxide and 60 ml of ether was cooled to 5° and 0.65 gm of nitroso-methyl urea was added with stirring. The ether layer containing diazomethane was added to a cold solution of 100 mg (0.00033 mol) of 2-p-chloroanilinoquinoxaline-3-carboxylic acid (31) in 25 ml of methanol. The work up as above gave

the product which was recrystallised from chloroform-hexane to give 88 mg (85%) of methyl 2-p-chloroanilinoquinoxaline-3-carboxylate (41), mp 207°.

MS: m/e 313 (M^+), 315 ($M^+ + 2$), 254 ($M^+ - CO_2CH_3$), 229 ($M^+ - CO_2CH_3$-Cl), 91 (C_6H_5N).

NMR($CDCl_3$): δ 4.1 (3,s,OCH_3), 7.3-8 (9,m,other Hs).

IR(KBr): 3400 cm^{-1} (NH), 1690 cm^{-1} (C=O).

UV: λ_{max}^{MeOH} 208 nm(ε $1.6x10^4$), 224 nm (ε $1.34x10^4$), 293 nm (ε $1.3x10^4$), 420 nm (ε $1.39x10^3$).

Anal. Calcd. for $C_{16}H_{12}ClN_3O_2$: C, 61.34; H, 3.83; N, 13.41.

Found: C, 61.28; H, 3.61; N, 13.3.

4.36 Methyl 2-(2,4-dichloroanilino)quinoxaline-3-carboxylate (42)

a) From methyl 2-anilinoquinoxaline-3-carboxylate (40)

A mixture of 500 mg (0.0018 mol) of methyl 2-anilinoquinoxaline-3-carboxylate (40) and 10 ml of thionyl chloride was heated under reflux for 120 hours. After the completion of the reaction excess thionyl chloride was removed under reduced pressure. The solid mass was dissolved in chloroform, washed well with water, dried, concentrated and purified by chromatographing over a silica gel column to

give 435 mg (70%) of methyl 2-(2,4-dichloroanilino)quinoxaline-3-carboxylate (42), mp 205° after recrystallisation from chloroform-hexane.

MS: m/e 347 (M^+), 349 (M^++2), 312 (M^+-Cl), 253 (M^+-Cl-CO_2CH_3).

NMR($CDCl_3$): δ 4.2 (3,s,OCH_3), 7.3-9.1 (8,m,other Hs).

IR (Nujol) : 3360 cm^{-1} (NH), 1720 cm^{-1} (C=O).

UV: λ_{max}^{MeOH} 209 nm (ϵ $1.7x10^4$), 224 nm (ϵ $1.7x10^4$), 295 nm (ϵ $1.8x10^4$), 415 nm (ϵ $2.3x10^3$).

Anal. Calcd. for $C_{16}H_{11}Cl_2N_3O_2$: C, 55.3; H, 3.17; N, 12.1.

Found: C, 55.6; H, 3.59; N, 11.61.

b) From methyl 2-p-chloroanilinoquinoxaline-3-carboxylate (41)

A mixture of 200 mg (0.0006 mol) of 2-p-chloroanilinoquinoxaline-3-carboxylate (41) and 5 ml of thionyl chloride was heated under reflux for 70 hours. After the completion of the reaction the mixture was worked up as in (a) above to give 160 mg (72%) of 2-(2,4-dichloroanilino)-quinoxaline-3-carboxylate (42) which was recrystallised from chloroform-hexane, mp 205° identical (mixed mp, IR) with that obtained by the previous method.

4.37 2-(2,4-dichloroanilino)quinoxaline-3-carboxylic acid (43)

a) By alkaline hydrolysis of methyl 2-(2,4-dichloroanilino)-quinoxalsine-3-carboxylate (42)

A mixture of 42 (200 mg, 0.0006 mol), 50 ml of 10% sodium hydroxide solution and 40 ml of isopropanol was heated under reflux for 6 hours, concentrated to 50 ml, cooled in ice, neutralised with 2 N hydrochloric acid and extracted with chloroform. The extract was dried (Na_2SO_4), evaporated to dryness under reduced pressure and the residue recrystallised from chloroform-hexane to give 175 mg (91%) of 2-(2,4-dichloroanilino)quinoxaline-3-carboxylic acid (43), mp 176°.

MS: m/e 333 ($M^{+\cdot}$), 335 ($M^+ + 2$), 288 (M^+-Cl), 253 ($M^+ - 2Cl$), 219 ($M^+ - 2Cl - CO_2$), 91 (C_6H_5N).

IR (Nujol): 1700 cm^{-1} (C=O).

UV: λ_{max}^{MeOH} 217 nm (ε 1.9×10^4), 296 nm (ε 1.83×10^4), 397 nm (ε 3.3×10^3).

Anal. Calcd. for $C_{15}H_9Cl_2N_3O_2$: C, 54.05; H, 2.7; N, 12.61.

Found: C, 53.85; H, 2.67; N, 12.77.

b) By the alkaline hydrolysis of 2-(2,4-dichloroanilino)-quinoxaline-3-carboxamide (46)

A mixture of 2-(2,4-dichloroanilino)quinoxaline-3-carboxamide (46) (200 mg, 0.0006 mol), 50 ml of 10% sodium hydroxide solution and 40 ml of isopropanol was heated under reflux for 10 hours, concentrated to 50 ml and worked up as in (a) above, to give 180 mg (90%) of 2-(2,4-dichloroanilino)quinoxaline-3-carboxylic acid (43) which was recrystallised from chloroform-hexane, mp 176° identical (mixed mp, IR) with the sample obtained from (a) above.

c) By the alkaline hydrolysis of 2-cyano-3-(2,4-dichloroanilino)quinoxaline (45)

Hydrolysis of 200 mg (0.0006 mol) of 45 as described above gave 175 mg (85%) of 43, mp 176° identical (mixed mp, IR) with the samples obtained in the above methods.

4.38 2-(2,4-dichloroanilino)quinoxaline (44)

a) From 2-p-chloroanilinoquinoxaline (32)

A mixture of 250 mg (0.0001 mol) of 2-p-chloroanilinoquinoxaline (32) and 5 ml of thionyl chloride was heated on a boiling water bath. Thionyl chloride was removed

under reduced pressure. The residue was dissolved in chloroform, washed with water, dried (Na_2SO_4), evaporated to dryness and purified by chromatographing over a column of silica gel to give 130 mg (45%) of 2-(2,4-dichloroanilino)-quinoxaline (44) which was recrystallised from chloroform-hexane, mp 169°.

MS: m/e 289 ($M^{+\cdot}$), 291 (M^++2), 254 (M^+-Cl), 219 (M^+-2Cl), 91 (C_6H_5N).

NMR($CDCl_3$): δ 7.3 - 8.9 (all Hs).

IR(KBr): 3350 cm^{-1} (NH).

UV: λ_{max}^{MeoH} 209 nm (ϵ $4.4x10^4$), 280 nm (ϵ $2.8x10^4$), 368 nm (ϵ $9.1x10^3$).

Anal. Calcd. for $C_{14}H_9Cl_2N_3$: C, 58.3; H, 3.1; N, 14.5.
Found: C, 58.0; H, 3.1; N, 14.7.

b) From 2-(2,4-dichloroanilino)quinoxaline-3-carboxylic acid (43)

2-(2,4-dichloroanilino)quinoxaline-3-carboxylic acid (43) (200 mg, 0.0006 mol) was heated in a test tube at 200° for 30 minutes in an oil bath. The solid mass obtained was dissolved in chloroform and purified by chromatographing

over a column of silica gel using chloroform as solvent. The product obtained was recrystallised from chloroform-hexane to give 150 mg (86%) of 2-(2,4-dichloroanilino)quinoxaline (44), mp 169°. A mixed melting point determination of the two samsples obtained by (a) and (b) was undepressed.

4.39 2-Anilinoquinoxaline-3-carboxamide (37)

A mixture of 280 mg(0.001 mol)of methyl 2-anilino-quinoxaline-3-carboxylate (40) dissolved in methanol and 25 ml of liquor ammonia was stirred for 48 hours in a closed vessel at room temperature. Volume of the reaction mixture was reduced to 25 ml, neutralised using 2 N hydrochloric acid and cooled. The crystalline product formed was filtered, washed with water, dried and purified by chromatographing through a column of silica gel. The product on recrystal-lisation from chloroform-hexane gave 80 mg (30%) of 2-anilino-quinoxaline-3-carboxamide (37), mp 152° (lit.[63] mp 152°).

The mother liquor was acidified with dilute HCl and extracted with chloroform. The extract was dried, concentrated and recrystallised from chloroform-hexane to give 160 mg (60%) of 2-anilinoquinoxaline-3-carboxylic acid (38), mp 165° identical (mixed mp) with the sample previously prepared.

4.40 Reaction of thionyl chloride with 2-anilinoquinoxaline-3-carboxamide (37)

A mixture of 1.0 g (0.0038 mol) of 2-anilinoquinoxaline-3-carboxamide and 10 ml of thionyl chloride was refluxed on a boiling water bath for 80 hours. After the completion of the reaction, the excess thionyl chloride was distilled off under reduced pressure. The residue was dissolved in chloroform, washed with water, dried (Na_2SO_4) and concentrated. This product was a mixture of two components as shown by tlc. The compounds were separated by chromatographing over a column of silica gel. Elution with carbon tetrachloride provided the first component 2-cyano-3-(2,4-dichloroanilino)quinoxaline (45) which was recrystallised from chloroform-hexane, mp 182°.

MS: m/e 314 (M^+), 316 (M^++2), 279 (M^+-Cl), 244 (M -2Cl)
91 (C_6H_5N).

IR(KBr): 3380 cm^{-1} (NH), 2230 cm^{-1} (C≡N).

UV: λ_{max}^{MeOH} 205 nm (ϵ 9.47x10^4), 219 nm (ϵ 8.75x10^4),
258 nm (ϵ 3.8x10^4), 389 nm (ϵ 6.8x10^4), 404 nm (ϵ 4.5x10^4).

Anal. Calcd. for $C_{15}H_8Cl_2N_4$: C, 57.32; H, 2.54; N, 17.83.
Found: C, 57.6; H, 2.45; N, 17.93.

Further elution of the column with chloroform gave the second component, 2-(2,4-dichloroanilino)quinoxaline-3-carboxamide (46) (300 mg, 24%), mp 268° which was recrystallised from chloroform-hexane.

MS: m/e 332 (M^+), 334 ($M^+ + 2$), 297 ($M^+ - Cl$), 262 ($M^+ - 2Cl$), 91 (C_6H_5N).

IR(KBr): 3440 cm^{-1} (NH_2), 3200 cm^{-1} (NH), 1690 (C=O).

UV: λ_{max}^{MeOH} 208 nm (ε 1.96×10^4), 224 nm (ε 1.98×10^4), 296 nm (ε 2×10^4), 401 nm (ε 6.96×10^4).

Anal. Calcd. for $C_{15}H_{10}Cl_2N_4O$: C, 54.21; H, 3.01; N, 16.86.
Found: C, 55.15; H, 3.6; N, 16.6.

4.41 Reaction of thionyl chloride with 2-p-chloroanilino-quinoxaline-3-carboxamide (31)

A mixture of 2-p-chloroanilinoquinoxaline-3-carboxamide (500 mg, 0.0016 mol) and 5 ml of thionyl chloride was heated under reflux for 16 hours on a boiling water bath. After the completion of reaction excess thionyl chloride was removed under reduced pressure. The residue was dissolved

in chloroform, washed with water, dried and concentrated. The product was a mixture of 2 components as shown by tlc which was separated using column chromatography. The first component collected by eluting with carbon tetrachloride (350 mg, 66%) and recrystallised from chloroform-hexane, was 2-cyano-3-(2,4-dichloroanilino)quinoxaline (45), mp 182° identical (mmp, IR) with the sample obtained in the method above.

Further elution with chloroform gave the second component 2-(2,4-dichloroanilino)quinoxaline-3-carboxamide (46) (100 mg, 18%), mp, mmp and IR was identical with the sample obtained in the method above.

4.42 Ethyl 2-hydrazinoquinoxaline-3-carboxylate (50)

A solution of 20 ml of 2% hydrazine hydrate in methanol was added dropwise to a solution of 1.2 g (0.005 mol) of ethyl 2-chloroquinoxaline-3-carboxylate (34) in methanol with stirring. Stirring was continued for 30 minutes and cooled in a freezing mixture. Yellow precipitate formed was filtered, washed with cold water and dried. It was recrystallised from hexane to give 250 mg (21%) of ethyl 2-hydrazinoquinoxaline-3-carboxylate (50), mp 141° (lit.[44] mp 141-142°).

The mother liquor was diluted with water, neutralised with dilute hydrochloric acid and extracted with chloroform. Solvent was removed and recrystallised from chloroform-hexane to give 150 mg (13%) of 2-chloroquinoxaline-3-carbonylhydrazide (8), mp 167° identical mixed mp with the sample obtained previously.

4.43 2-Hydrazinoquinoxaline-3-carbonylhydrazide (51)

A solution of 1.2 g (0.005 mol) of chloro ester 34 in ethanol was added to a solution of hydrazine hydrate taken in excess in ethanol, in small portions at 80° while stirring. Heating continued for 1 hour. Reaction mixture was cooled and added to cold water. The product formed was filtered, washed with water, dried and recrystallised from ethanol to give 1.0 g (90%) of 2-hydrazinoquinoxaline-3-carbonylhydrazide (51), mp 281° (lit.[44] 281-282°).

4.44 Ethyl 2-phenylhydrazinoquinoxaline-3-carboxylate (53)

A mixture of 2.4 g (0.01 mol) of ethyl 2-chloroquinoxaline-3-carboxylate and 2.0 g of freshly distislled phenylhydrazine was heated over a steam bath for 2 hours. The reaction mixture, after cooling was dissolved in chloroform and washed with 2 N hydrochloric acid, dried and

concentrated. On tlc examination it showed the presence of two components which were separated on an alumina column. Elution with chloroform gave the first component which was recrystallised from hexane to give 2.0 g (63%) of ethyl 2-phenylhydrazinoquinoxaline-3-carboxylate (53), mp 176°.

MS: m/e 308 ($M^{+\cdot}$), 263 ($M^{+}-OC_2H_5$), 77 (C_6H_5).

NMR($CDCl_3$): δ 1.5 (3,t,CH_3), 4.6 (2,q,CH_2), 6.9-8.1 (11, complex, other Hs).

IR(KBr): 3360 cm^{-1}, 3270 cm^{-1} (NH,NH), 1690 (C=O).

UV: λ_{max}^{MeOH} 205.6 nm (ε 1.48×10^4), 258.8 nm (ε 9.93×10^3).

Anal. Calcd. for $C_{17}H_{16}N_4O_2$: C, 66.23; H, 5.19; N, 18.18.

Found: C, 66.1; H, 5.31; N, 18.08.

4.45 2-Phenyl-3-oxo-3-pyrazolino[3,4-b]quinoxaline (54)

Ethyl 2-phenylhydrazinoquinoxaline-3-carboxylate (53) (300 mg, 0.001 mol) was heated in a test tube at 180° for 30 minutes in an oil bath. After cooling the reaction mixture, chloroform was added and boiled for two minutes.

The undissolved solid product was filtered and washed well with chloroform and methanol to give 65 mg (25%) of 2-phenyl-3-oxo-3-pyrazolino[3,4-b]quinoxaline (54) which did not melt below 280°.

MS: m/e 262 ($M^{+\cdot}$), 234 (M^{+}-CO), 77 (C_6H_5).

NMR was not determined because of insufficient solubility in $CDCl_3$.

IR(KBr): 3300 cm^{-1} (NH), 1675 cm^{-1} (C=O).

UV: λ_{max}^{MeOH} 203.8 nm (ε 7.89x10^3), 284.8 nm (ε 1.21x10^4), 363.2 nm (ε 4.19x10^3).

Anal. Calcd. for $C_{15}H_{10}N_4O$: C, 68.7; H, 3.81; N, 21.37.
Found: C, 68.52; H, 3.73; N, 21.62.

4.46 Preparation of substituted phenylhydrazines[99,100,101]

A suspension of 0.2 mol of finely powdered substituted aniline (p-bromo-, p-chloro or p-methyl) in 70 ml of concentrated hydrochloric acid was warmed to about 60° for 1 hour and was then cooled in a freezing mixture. An ice-cold solution of 20 g of sodium nitrite in 50 ml of water was added dropwise with vigorous stirring. The diazonium salt formed was filtered and added slowly to

a solution of sodium sulphate (prepared by passing SO_2 into a solution of 45 g of sodium hydroxide in 300 ml of water until the solution turned acidic as indicated by phenolphthalein). The resulting solution was warmed to 60°, made acidic to litmus by the addition of about 20 ml of concentrated hydrochloric acid and heated for about an hour. About 100 ml of concentrated hydrochloric acid was added and the mixture was allowed to cool. The p-substituted phenylhydrazine hydrochloride crystallised as a lump of small needles which were filtered and redissolved in minimum quantity of hot water and cooled. After neutralisation with 50% sodium hydroxide solution, the mixture was cooled in freezing mixture and the crystals formed were filtered and recrystallised from hot water. By this method, p-chlorophenylhydrazine (81%) mp 86°, p-bromophenylhydrazine (75%) mp 108° and p-tolylhydrazine (86%) mp 61°, were prepared.

4.47 Ethyl 2-p-tolylhydrazinoquinoxaline-3-carboxylate (55)

A mixture of 2.4 g (0.01 mol) of ethyl 2-chloro-quinoxaline-3-carboxylate (34) and 2.5 g of p-tolylhydrazine was heated on a boiling water bath for 8 hours. Reaction mixture after work up as above was separated on an alumina

column. Elution with chloroform gave the first component which was recrystallised from chloroform-hexane to give 850 mg (25%) of ethyl 2-p-tolylhydrazinoquinoxaline-3-carboxylate (55), mp 179°.

NMR($CDCl_3$): δ 1.4 (3,t,CH_3), 2.5 (3,s,$ArCH_3$), 4.5 (2,q,CH_2), 7.1-8.3 (10,complex,other Hs).

IR(KBr): 3350 cm^{-1}, 3260 cm^{-1} (NH,NH), 1680 (C=O).

Anal. Calcd. for $C_{18}H_{18}N_4O_2$: C, 67.08; H, 5.59; N, 17.39.

Found: C, 66.1; H, 5.51; N, 17.15.

Further elution of the column with chloroform-methanol gave 100 mg (4%) of 2-p-tolyl-3-oxo-3-pyrazolino[3,4-b]-quinoxaline (56), which did not melt upto 280°. See 4.47 for structural characteristics.

4.48 2-p-tolyl-3-oxo-3-pyrazolino[3,4-b]quinoxaline (56)

Ethyl 2-p-tolylhydrazinoquinoxaline-3-carboxylate (55) (320 mg, 0.001 mol) was heated in a test tube at 180°

for 30 minutes in an oil bath. After cooling the reaction mixture, chloroform was added and boiled for 2 minutes. The undissolved solid product was filtered, washed with chloroform and dried to give 100 mg (40%) of 2-p-tolyl-3-oxo-3-pyrazolino[3,4-b]quinoxaline (56) which did not melt below 280°.

MS: m/e 274 ($M^{+\cdot}$), 246 (M^{+}-CO), 89 ($C_6H_4CH_3$).

NMR was not determined because of insufficient solubility in $CDCl_3$.

IR(KBr): 3310 cm^{-1} (NH), 1680 cm^{-1} (C=O).

UV: λ_{max}^{MeOH} 207 nm, 284 nm, 352 nm, 547 nm.

Anal. Calcd. for $C_{16}H_{12}N_4O$: C, 69.56; H, 4.35; N, 20.29.
Found: C, 69.7; H, 4.31; N, 20.1.

4.49 Ethyl 2-p-bromophenylhydrazinoquinoxaline-3-carboxylate (57)

A mixture of 2.4 g (0.01 mol) of ethyl 2-chloroquinoxaline-3-carboxylate and 3 g of p-bromophenylhydrazine was heated on a boiling water bath for 8 hours. Reaction mixture was worked up as above and separated on an alumina

column. Elution with chloroform gave the first component which was recrystallised from chloroform hexane to give 1.9 g (49%) of ethyl 2-p-bromophenylhydrazinoquinoxaline-3-carboxylate (57), mp 173°.

NMR($CDCl_3$): δ 1.5 (3,t,CH_3), 4.6 (2,q,CH_2), 6.8-8.1 (10,complex, other Hs).

IR(Nujol): 3400 cm^{-1}, 3360 cm^{-1} (NH,NH), 1710 cm^{-1} (C=O).

UV: λ_{max}^{MeOH} 206.2 nm (ε 1.37×10^4), 241.2 nm (ε 8.28×10^3), 310 nm (ε 1.04×10^4), 345 nm (ε 1.21×10^4).

Anal. Calcd. for $C_{17}H_{15}BrN_4O_2$: C, 52.7; H, 3.87; N, 14.47.

Found: C, 52.8; H, 3.84; N, 14.1.

4.50 2-p-Bromophenyl-3-oxo-3-pyrazolino[3,4-b]-quinoxaline (58)

Ethyl 2-p-bromophenylhydrazinoquinoxaline-3-carboxylate (57) (400 mg, 0.001 mol) was heated in a test tube at 180° for 30 minutes in an oil bath. After cooling the reaction mixture, chloroform was added and boiled for 2 minutes. The undissolved solid product was filtered,

washed with chloroform and dried to give 70 mg (20%) of 2-p-bromophenyl-3-oxo-3-pyrazolino[3,4-b]quinoxaline (58) which did not melt below 280°.

MS: m/e 341 ($M^{+\cdot}$), 343 (M^{+}+2), 261 (M^{+}-Br), 76 (C_6H_4).

NMR was not determined because of insufficient solubility in $CDCl_3$

IR(KBr): 3310 cm^{-1} (NH), 1680 cm^{-1} (C=O).

UV: λ_{max}^{MeOH} 207 nm, 287 nm, 363 nm, 551 nm.

Anal. Calcd. for $C_{15}H_9BrN_4O$: C, 52.78; H, 2.6; N, 16.42.

Found: C. 53.1; H, 2.51; N, 16.2.

4.51 Ethyl 2-p-chlorophenylhydrazinoquinoxaline-3-carboxylate (60)

A mixture of 2.4 g (0.01 mol) of ethyl 2-chloroquinoxaline-3-carboxylate (34) and 2.5 g of p-chlorophenylhydrazine was heated on a boiling water bath for 8 hours. Reaction mixture after work up as above was separated on an alumina column. Elution with chloroform gave the first

component which was recrystallised from chloroform-hexane to give 850 mg (25%) of ethyl 2-p-chlorophenylhydrazino-quinoxaline-3-carboxylate (60) mp 183°.

NMR ($CDCl_3$): δ 1.5 (3,t,CH_3), 4.6 (2,q,CH_2), 6.8-8.1 (10, complex, other Hs).

IR (Nujol): 3380 cm^{-1}, 3330 cm^{-1} (NH), 1700 cm^{-1} (C=O).

UV: λ_{max}^{MeOH} 205.6 nm (ε 1.61×10^4), 249 nm (ε 9.76×10^3), 262 nm (ε 9.44×10^3), 309 nm (ε 1.09×10^4), 349.8 nm (ε 1.21×10^4).

Anal. Calcd. for $C_{17}H_{15}ClN_4O_2$: C, 59.64; H, 4.38; N, 16.37.
Found: C, 59.1; H, 4.24; N, 16.31.

On further elution with chloroform gave 680 mg (20%) of 2-chloroquinoxaline-3-phenylhydrazide (59) which was recrystallised from chloroform-hexane, mp 213°.

MS: m/e 332 (M^+), 334 (M^++2), 191 (M^+-$NHNHC_6H_4Cl$).

NMR($CDCl_3$):δ6.9-8.3 (complex).

IR(Nujol): 3300 cm^{-1}, 3220 cm^{-1} (NH,NH), 1670 cm^{-1} (C=O).

UV: λ_{max}^{MeOH} 205.4 nm (ε 1.19x10^4), 235.2 nm(ε5.87x10^3), 287.4 nm (ε 8.94x10^3), 359.2 nm(ε 4.89x10^3).

4.52 2-p-Chlorophenyl-3-oxo-3-pyrazolino[3,4-b]quinoxaline (61)

Ethyl 2-p-chlorophenylhydrazinoquinoxaline-3-carboxylate (60) (350 mg, 0.001 mol) was heated in a test tube at 180° for 30 minutes in an oil bath. After cooling the reaction mixture, chloroform was added and boiled for 2 minutes. The undissolved solid product was filtered, washed with chloroform and dried to give 120 mg (40%) of 2-p-chlorophenyl-3-oxo-3-pyrazolino[3,4-b]quinoxaline (61) which did not melt below 280°.

MS: m/e 296 (M^+), 298 (M^++2), 270 (M^+-CO).

NMR was not determined due to insufficient solubility in $CDCl_3$

IR(KBr): 3250 cm^{-1} (NH), 1670 cm^{-1} (C=O).

UV: λ_{max}^{MeOH} 204.4 nm (ε 1.72x10^4), 288 nm (ε 2.27x10^4), 363.8 nm (ε 8.99x10^3).

4.54 6-Hydroxy-1H-1,5-benzodiazepino[2,3-b]quinoxaline (64)

a) From ethyl 2-hydroxyquinoxaline-3-carboxylate (4)

A mixture of 2.2 g (0.01 mol) of ethyl 2-hydroxyquinoxaline-3-carboxylate (4) and 4 g of o-phenylene diamine was heated in a boiling tube at 170° for 1 hour in an oil bath. After cooling the reaction mixture 100 ml of water was added and boiled for 2-3 minutes. Solid product obtained was filtered washed well with hot water to remove excess o-phenylene diamine, dried and purified by recrystallisation from methanol to give 2 g (80%) of 6-hydroxy-1H-1,5-benzodiazepino[2,3-b]quinoxaline (64) which did not melt below 280°.

MS: m/e 262 ($M^{+\cdot}$)

NMR:not determined due to insufficient solubility in $CDCl_3$

IR(KBr); 3260 cm^{-1} (NH), 1700 cm^{-1} (C=O).

UV: λ_{max}^{MeOH} 218.8 nm (ϵ 3.5×10^4), 397 nm (ϵ 1.7×10^4), 415 nm (ϵ 1.1×10^4).

Anal. Calcd. for $C_{15}H_{14}N_4O$: C, 68.7; H.3.81; N, 21.37.

Found: C, 68.75; H, 3.8; N.21.54.

b) From ethyl 2-chloroquinoxaline-3-carboxylate (34)

A mixture of 1.2 g (0.005 mol) of ethyl 2-chloroquinoxaline-3-carboxylate (34) and 2 g of o-phenylene diamine was heated in a boiling tube at 120° for 3 hours in an oil bath. Reaction mixture was poured in 50 ml of water and boiled for 2-3 minutes. Solid product obtained was filtered, washed well with hot water, dried and purified by recrystallisation from methanol to give 900 mg (70%) of 6-hydroxy-1H-1,5-benzodiazepino[2,3-b]quinoxaline (64), identical (IR, UV) with the sample from (a) above.

4.55 6-Chloro-1H-1,5-benzodiazepino[2,3-b]quinoxaline (65)

A mixture of 2.6 g (0.01 mol) of 64 and 50 ml of freshly distilled phosphorous oxychloride was heated 20 hours under a $CaCl_2$ guard tube on a steam bath. The reaction mixture was cooled and poured into 500 g of crushed ice with stirring. The solid product formed was filtered, washed with water and dried. It was dissolved in chloroform, purified by passing over a column of silica gel and recrystallised from methanol to give 1.96 g (70%) of 6-chloro-1H-1,5-benzodiazepino[2,3-b]-quinoxaline (65), mp 213°.

MS: m/e 280 (M^+), 282 (M^++2), 245 (M^+-Cl).

NMR($CDCl_3$): δ 7.4-9.1 (complex).

IR(KBr): 3400 cm^{-1} (NH).

UV: λ_{max}^{MeOH} 208.6 nm (ϵ 3.1x10^4), 247.2 nm (ϵ 2.9x10^4), 273.6 nm (ϵ 9.88x10^3), 296.2 nm (ϵ 1.03x10^4), 363.6 nm (ϵ 1.57x10^4).

Anal. Calcd. for $C_{15}H_9ClN_4$: C, 64.2; H, 3.21; N, 20.
Found: C, 63.92; H, 3.13; N, 20.02.

4.56 6-Amino-1H-1,5-benzodiazepino[2,3-b]quinoxaline (66)

A mixture of 1.4 g (0.005 mol) of 65 and an excess quantity of urea was heated in a test tube at 130° for 60 hours in an oil bath. After the completion of the reaction (T.L.C. monitoring) water was added and boiled. A solid product formed was filtered, washed with water and dried. It was dissolved in chloroform and purified by passing through a column of silica gel using chloroform as eluent. The product obtained was recrystallised from methanol to give 1.0 g (80%) of 6-amino-1H-1,5-benzodiazepino[2,3-b]quinoxaline (66) which did not melt below 280°.

MS: m/e 261 ($M^{+\cdot}$), 245 ($M^{+}-NH_2$).

NMR($CDCl_3$): δ 1.6 (2,s,NH_2), 7.8 (9,complex, other Hs).

IR(Nujol): 3380 cm^{-1} (NH_2).

UV: λ_{max}^{MeOH} 225.2 nm (ε 3.3×10^4), 251.2 nm (ε 1.4×10^4), 382 nm (ε 1×10^4), 346.6 nm (ε 9.7×10^3), 399 nm (ε 1.3×10^4).

Anal. Calcd. for $C_{15}H_{11}N_5$: C, 68.96; H, 4.24; N, 26.8.
Found: C, 68.1; H, 4.27; N, 26.93.

4.57 6-Ethoxy-1H-1,5-benzodiazepino[2,3-b]quinoxaline (67)

A mixture of 280 mg (0.001 mol) of 65, 1.0 g of anhydrous potassium carbonate and 50 ml of absolute ethanol was refluxed on a boiling water bath for 24 hours. Ethanol was distilled off and 100 ml of water added to the residue and the solution was extracted with chloroform. The extract was dried and the chloroform distilled off. Solid residue was purified by chromatographing through a column of silica gel using chloroform as the eluent. Product obtained was recrystallised from chloroform-hexane to give 230 mg (80%) of 6-ethoxy-1H-1,5-benzodiazepino[2,3-b]quinoxaline (67), mp 210°.

MS: m/e 290 (M^+), 245 ($M^+-OC_2H_5$).

NMR($CDCl_3$): δ 1.6 (6,t,$2CH_3$), 4.8 (4,q,$O(CH_2)_2$),
7.9-8.2 (9,complex,other Hs).

UV: λ_{max}^{MeOH} 219 nm (ε 2.49×10^4), 250 nm (ε 1.2×10^4),
367 nm (ε 1.07×10^4).

Anal. Calcd. for $C_{17}H_{14}N_4O$: C, 70.34; H, 4.82; N, 19.31.

Found: C, 70.1; H, 4.71; N, 19.4.

4.58 6-(N-Morpholinyl)-1H-1,5-benzodiazepino[2,3-b]quinoxaline (68)

A mixture of 280 mg (0.001 mol) of 65 and 10 ml of morpholine was heated for 2 hours on a boiling water bath. The mixture was cooled and poured into about 100 ml of ice cold water. The precipitate formed was filtered, washed with water and dried. Residue was dissolved in chloroform and purified by chromatographing over a column of silica gel using chloroform as eluent. The product on recrystallisation from chloroform hexane gave 300 mg (90%) of 6-(N-morpholinyl)-1H-1,5-benzodiazepino[2,3-b]quinoxaline (68), mp 230°.

MS: m/e 331 (M^+).

NMR($CDCl_3$): δ 3.6 (4,t,$N(CH_2)_2$), 4 (4,t,$O(CH_2)_2$),
7.3-8 (9,complex,other Hs).

UV: λ_{max}^{MeOH} 206 nm (ε 1.9×10^4), 227.8 nm (ε 2.69×10^4),
280.4 nm (ε 1.62×10^4), 327.2 nm (ε 8.14×10^3),
391.8 nm (8.01×10^3).

Anal. Calcd. for $C_{19}H_{17}N_5O$: C, 68.88; H, 5.13; N, 21.15.

Found: C, 69.2; H, 5.1; N, 21.

4.59 6-(N-Piperidyl)-1H-1,5-benzodiazepino[2,3-b]quinoxaline (69)

A mixture of 280 mg (0.001 mol) of 65 and 10 ml of piperedine was heated for 2 hours on a boiling water bath. The reaction mixture after work up as above was purified by passing through a column of silica gel using chloroform as eluent. The product was recrystallised from chloroform-hexane to give 900 mg (90%) of 6-(N-piperidyl)-1H-1,5-benzodiazepino-[2,3-b]quinoxaline (69), mp 230°.

NMR($CDCl_3$): δ 1.7 (6,m,CH_2-CH_2-CH_2), 3.4 (4,t,CH_2-N-CH_2),

7.2-8 (9,complex,other Hs).

UV: λ_{max}^{MeOH} 205 nm (ε 1.83x10^4), 228.2 nm (ε 2.64x10^4),

282.4 nm (ε 1.61x10^4), 321 nm (ε 7.15x10^3),

395.2 nm (ε 6.56x10^3).

Anal. Calcd. for $C_{20}H_{19}N_5$: C, 72.95; H, 5.78; N, 21.28.

Found: C, 72.8; H, 5.8; N, 21.1.

4.60 6-(N-Pyrrolidyl)-1H-1,5-benzodiazepino[2,3-b]quinoxaline (70)

A mixture of 280 mg (0.001 mol) of 65 and 5 ml of pyrrolidine was heated for 2 hours on a boiling water bath. The reaction mixture after work up as above was purified by passing through a column of silica gel using chloroform as the eluent. The product was recrystallised from chloroform-hexane to give 270 mg (85%) of 6-(N-pyrrolidyl)-1H-1,5-benzodiazepino[2,3-b]quinoxaline (70), mp 206°.

MS: m/e 315 ($M^{+\cdot}$), 246 ($M^+ - C_4H_8N + H$).

NMR ($CDCl_3$): δ 1.9 (4,m,CH_2), 3.6 (4,t,$N(CH_2)_2$),

7.2-7.9 (9,complex,other Hs).

UV: λ_{max}^{MeOH} 207.5 nm (ϵ $2.4x10^4$), 226.2 nm (ϵ $3.2x10^4$),

276.4 nm (ϵ $2.1x10^4$), 319.5 nm (ϵ $7x10^3$),

412.6 nm (ϵ $8.87x10^3$).

Anal. Calcd. for $C_{19}H_{17}N_5$: C, 72.38; H, 5.4; N, 22.22.

Found: C, 72.34; H, 5.41; N, 22.1.

4.61 6-(N-Diethylamino)-1H-1,5-benzodiazepino[2,3-b]-quinoxaline (71)

A mixture of 280 mg (0.001 mol) of 65 and 5 ml of diethylamine was heated for 2 hours on a boiling water bath. The reaction mixture after work up as above was purified by chromatographing over a column of silica gel using chloroform as eluent. The product on recrystallisation from chloroform hexane gave 260 mg (82%) of 6-(N-diethylamino)-1H-1,5-benzodiazepino[2,3-b]-quinoxaline (71), mp 212°.

NMR ($CDCl_3$): δ 1.2 (6,t,$2CH_3$), 3.5 (4,q,$N(CH_2)_2$),
7.2-8 (9,complex,other Hs).

UV: λ_{max}^{MeOH} 206 nm (ε 2.23×10^4), 227 nm (ε 3.2×10^4),
281 nm (ε 1.99×10^4), 312 nm (ε 7.68×10^3),
403 nm (ε 7.7×10^3).

Anal. Calcd. for $C_{19}H_{19}N_5$: C, 71.9; H, 5.99; N, 22.08.
Found: C, 71.7; H, 5.91; N, 21.9.

4.62 1H-1,5-Benzodiazepino[2,3-b]quinoxaline (63)

A solution of 560 mg (0.002 mol) of 65 in isopropanol (250 ml) was mixed with 1.0 g of powdered sodium borohydride and heated under reflux on a boiling water bath for 24 hours. The unreacted $NaBH_4$ was decomposed by the addition of a few ml of water. The isopropanol was removed under reduced pressure and 50 ml of water was added to the residue. The solution was extracted in chloroform. Extract was dried (Na_2SO_4) and the solvent distilled off. Residue was purified by chromatographing over a column of silica gel using chloroform as eluent. Product obtained was recrystallised from chloroform-hexane to give 370 mg (75%) of 1H-1,5-benzodiazepino-[2,3-b]quinoxaline (63), mp 242°.

MS: m/e 246 ($M^{+\cdot}$).

NMR ($CDCl_3$): δ 7.5-8.3 (complex).

UV: λ_{max}^{MeOH} 208.8 nm (ε 3.26x10^4), 248 nm (ε 3.25x10^4), 270.2 nm (ε 8.56x10^3), 294.2 nm (ε 1.29x10^4), 359.8 nm (ε 1.92x10^4).

Anal. Calcd. for $C_{15}H_{10}N_4$: C, 73.17; H, 4.06; N, 22.76.

Found: C, 73.1; H, 3.96; N, 22.5.

CHAPTER V

SUMMARY AND CONCLUSIONS

Many 1H-pyrazolo[3,4-b]quinoxalines have been reported to have useful biological activity. Only very few methods are available for the synthesis of unsubstituted 1H-pyrazolo-[3,4-b]quinoxaline derivatives. Therefore, it is necessary to develop new methods for their synthesis, especially for 3-substituted pyrazoloquinoxalines which are expected to have improved biological properties. Thus ethyl 2-hydroxyquinoxaline-3-carboxylate, the key starting material for the synthesis of pyrazoloquinoxalines was prepared easily from o-phenylene diamine and diethyl dibromomalonate, which in turn was obtained by the bromination of diethylmalonate. 2-Hydroxyquinoxaline-3-carbonylhydrazide obtained from ethyl 2-hydroxyquinoxaline-3-carboxylate was cyclised to 1-acetyl-3-hydroxy-1H-pyrazolo-[3,4-b]quinoxaline. Treatment of this compound with phosphorous oxychloride and subsequent displacement of the chlorine atom provided for the first time, 1-acetylpyrazoloquinoxalines with different substituents at position 3 such as the chloro, amino, hydroxy, methoxy, N-piperidyl, N-morpholiyl, N-pyrrolidyl and N-diethylamino groups. Though various methods were attempted to remove the acetyl group from position 1 and obtain the 3-substituted 1H-pyrazolo[3,4-b]quinoxalines, only one method was found to be successful, in which sodium carbonate in methanol was used for hydrolysis. In all other cases

the pyrazole ring of the pyrazoloquinoxaline got ruptured. Although opening of pyrazole ring in pyrazoloquinoxalines with breakage of the N,N bond, have been reported earlier, ring cleavage with breakage of the C=N bond has been observed for the first time. Hence hydrolysis of the pyrazole ring of the pyrazoloquinoxaline was further studied. While 1-aryl-1H-pyrazolo[3,4-b]quinoxaline was hydrolysed to 2-anilino-quinoxaline-3-carboxylic acid with aqueous sodium hydroxide, 3-chloro-1-aryl-1H-pyrazolo[3,4-b]quinoxalines were unaffected under the same conditions. However, sodium borohydride in boiling isopropanol hydrolysed all these compounds to give the respective 2-arylamino derivatives of quinoxaline-3-carbox-amide and its hydrolysis product the carboxylic acid. Thus in all the cases studied previously, pyrazole ring was found to rupture at the N,N bond and not at the C=N bond. Mechanisms have been suggested for the different ring opening reactions.

Chlorination of pyrazoloquinoxalines and anilino-quinoxalines using thionyl chloride as the chlorinating agent has been studied as a new synthetic method for chlorination. A number of chloro derivatives of pyrazoloquinoxalines and anilinoquinoxalines were prepared. 1-p-Chlorophenyl pyrazolo-quinoxaline and 3-chloro-1-p-chlorophenyl pyrazoloquinoxaline were obtained from 1-phenyl pyrazoloquinoxaline in good yields.

3-Chloro-1-phenyl pyrazoloquinoxaline also gave 3-chloro-1-p-chlorophenyl pyrazoloquinoxaline. 2-Anilinoquinoxaline-3-carboxamide gave 2-cyano-3-(2,4-dichloroanilino)quinoxaline and 2-(2,4-dichloroanilino)quinoxaline. Similarly methyl esters of the anilinoquinoxaline-3-carboxylic acid and 2-p-chloroanilinoquinoxaline-3-carboxylic acid gave methyl 2-(2,4-dichloroanilino)quinoxaline-3-carboxylate. 2-p-Chloroanilino-quinoxaline gave 2-(2,4-dichloroanilino)quinoxaline. All the compounds being new were also prepared by unambiguous methods starting from ethyl 2-chloroquinoxaline-3-carboxylate. The spectral data of these compounds have been discussed and a mechanism for chlorination reactions using thionyl chloride has been suggested.

As a related structure, synthesis of 2-aryl substituted 3-oxo-pyrazolinoquinoxalines were achieved starting from ethyl 2-chloroquinoxaline-3-carboxylate. Ethyl 2-hydrazino-quinoxaline-3-carboxylate, 2-chloroquinoxaline-3-carbonyl-hydrazide, ethyl 2-phenylhydrazinoquinoxaline-3-carboxylates with substituents such as p-Cl, p-Br, p-CH_3 and 2,4-dinitro groups on the phenyl group were prepared by condensation with the respective hydrazines. Ethyl 2-phenylhydrazinoquinoxaline 3-carboxylate as well as esters with substituents such as p-Cl, p-Br, p-CH_3 groups were cyclised to give 2-aryl-3-oxo-3-pyrazolino[3,4-b]quinoxalines. As these compounds give

rise to sharp colour changes under acid and basic media they may be used as acid-base indicators. An explanation has been provided for the difference in colour under acid and basic media.

A new heterocyclic system 1H-1,5-benzodiazepino-[2,3-b]quinoxaline has been prepared for the first time. The synthesis of this system was achieved by the condensation of ethyl 2-chloroquinoxaline-3-carboxylate with o-phenylene diamine to give the 6-hydroxy derivative. Benzodiazepino-quinoxalines with different substituents at position 6 such as amino, chloro, hydroxy, ethoxy, morpholino, piperidyl, pyrrolidyl and diethylamino have also been prepared. The parent compound was prepared by the dechlorination of the 6-chloro-derivative using sodium borohydride and the product has been fully characterised.

A large number of previously prepared pyrazolo-quinoxaline derivatives have been reported to possess biological activity as diuretic, anti-inflammatory, analgesic, antileukaemic, tuberculostatic and immunochemical agents. Also the pyrazolinoquinoxalines, anilinoquinoxalines and benzodiazepinoquinoxalines are expected to have significant

biological properties. Therefore all the new compounds reported in this work will be submitted for studying their pharmacological activities.

REFERENCES

1. H.Ohle and M.Heilscher, Ber., 74, 13 (1941).

2. H.Ohle and G.Melkonian, Ber., 74, 279 (1941).

3. P.Nordin, Methods Carbohyd.Chem., 2, 136 (1965).

4. J.H.Pazur and D.French, J.Biol.Chem., 196, 265 (1952).

5. H.Ohle and G.Melkonian, Ber., 74, 398 (1941).

6. G.Henseke, W.Dose and K.Dittrich, Angew.Chem., 69, 479 (1957).

7. T.Kappe, E.Lender and E.Ziegler, Monatsch.Chem., 99, 157 (1968); Chem.Abstr., 70, 68089 (1969).

8. J.Klicnar, Coll.Czech.Chem.Commun., 30, 3087 (1965).

9. V.Vanicek, Coll.Czech.Chem.Commun., 27, 2699 (1962).

10. H.Ohle and R.Liebig, Ber., 75, 1536 (1942).

11. H.Ohle and J.J.Kruyff, Ber., 77, 507 (1944).

12. G.Neumuller, Arkiv.Kemi.Mineral.Geol., 21A, 13 (1946); Chem.Abstr., 41, 1210 (1947).

13. U.Rosenqvist, G.Neumuller and K.Myrback, Arkiv.Kemi.Mineral. Geol., 24A, 9 (1946); Chem.Abstr., 42, 5425 (1948).

14. G.Henseke and E.Brose, Chem.Ber., 91, 2273 (1958).

15. R.L.Whistler and J.L.Hickson, J.Amer.Chem.Soc., 76, 1611 (1954).

16. J.E.Courtois and U.Ariyoshi, Ann.Pharm.france, 16, 385 (1958); Chem.Abstr., 53, 8003 (1959).

17. D.French, G.M.Wild and W.J.James, J.Amer.Chem.Soc., 75, 3664 (1953).

18. P.Nordin and D.French, J.Amer.Chem.Soc., 80, 1445 (1958).

19. J.Farkas, K.Sebesta, K.Horska, Z.Samek, L.Dolejs and F.Sorm, Coll.Czech.Chem.Commun., 34, 118 (1969).

20. G.Henseke and K.J.Bahner, Chem.Ber., 91, 1605 (1958).

21. H.H.Credner, Chem.Ber., 104, 2640 (1971).

22. H.H.Credner and W.Pueschel, German Patent, 2, 109, 455 (1972); Chem.Abstr., 78, 5406 (1973).

23. B.Teichmann, K.Himmelspach and O.Westphal, J.Prakt.Chem., 313, 940 (1971).

24. B.Teichmann, K.Himmelspach and O.Westphal, Z.Chem., 11, 380 (1971).

25. B.Teichmann, K.Himmelspach and O.Westphal, J.Prakt.Chem., 315, 517 (1973).

26. M.A.E.Sallam, Carbohyd.Res., 67, 79 (1978).

27. M.A.E.Sallam, R.L.Whistler and J.L.Markley, Carbohyd.Res., 87, 87 (1980).

28. M.A.E.Sallam, Nucleosides Nucleotides, 1, 297 (1982); Chem.Abstr., 98, 198646 (1983).

29. J.H.Pazur, J.Amer.Chem.Soc., 77, 1055 (1955).

30. M.von Saltza, J.D.Dutcher, J.Reid and O.Wintersteiner, J.Org.Chem., 28, 999 (1968).

31. H.S.Blair and P.J.Watt, J.Macromol.Sci.Chem., 11A, 679 (1977).

32. G.Henseke and W.Lemke, Chem.Ber., 91, 102 (1958).

33. G.Henseke and W.Lemke, Chem.Ber., 91, 113 (1958).

34. G.Henseke and C.Bauer, Chem.Ber., 92, 501 (1959).

35. P.M.Pillai and P.Ramabhadran, Indian J.Chem., 25B, 960 (1986).

36. P.Ramabhadran, Synthesis and Reactions of Flavazoles, Ph.D. Thesis, University of Cochin, 1984.

37. P.M.Pillai and P.Ramabhadran, Indian.J.Chem., 25B, 901 (1986).

38. H.Ohle and A.Iltgen, Ber., 76, 1 (1943).

39. W.Sauer and G.Henseke, Z.Chem., 10, 381 (1970).

40. V.D.Romanenko and S.I.Burmistrov, Khim.Geterotsiki.Soedin., 6, 852 (1973); Chem.Abstr., 79, 92158 (1973).

41. N.P.Buu-Hoi, J.N.Vallat, G.S.Ruf and G.Lambelin, Chim.Ther., 6, 245 (1971); Chem.Abstr., 76, 3794 (1972).

42. G.R.Wendt and K.W.Ludig, U.S.Patent, 3, 431, 262 (1969); Chem.Abstra., 70, 106512 (1969).

43. W.Sauer and G.Henseke, Z.Chem., 10, 381 (1970).

44. K.Yoshida and H.Otomasu, Chem.Pharm.Bull., 32(9), 3361 (1970).

45. G.Henseke and K.Dittrich, Chem.Ber., 92, 1550 (1959).

46. G.Henseke, D.Lehmann and K.Dittrich, Chem.Ber., 94, 1743 (1961).

47. E.S.H.El Ashry, I.E.El Kholy and Y.El Kilany, Carbohyd. Res., 67, 495 (1978).

48. E.S.H.El Ashry, I.E.El Kholy and Y.El Kilany, Carbohyd. Res., 60, 303 (1978).

49. E.S.H.El Ashry, M.M.A.Rahman, N.Rashed and A.Amer, Carbohyd. Res., 67, 423 (1978).

50. E.S.H.El Ashry, M.M.A.Rahman, M.A.Nasser and A.Amer, Carbohyd. Res., 67, 403 (1978).

51. R.Boliman, E.S.H.El Ashry, I.E.El Kholy and Y.El Kilany, Carbohyd.Res., 67, 179 (1978).

52. E.S.H.El Ashry, M.M.A.Rahman, Y.El Kilany and A.Amer, Carbohyd.Res., 87, C_5-C_7 (1981).

53. F.Weyand, K.Fehr and J.F.Klebe, Z.Naturforsch, 14B, 217 (1959); Chem.Abstr., 54, 1328 (1960).

54. H.J.Binte, G.Henseke, W.Bauer and K.Koenke, Z.Chem., 8, 104 (1968).

55. N.P.Buu-Hoi, J.N.Vallat, G.S.Ruf and G.Lambelin, Chim.Ther., 7, 210 (1972); Chem.Abstr., 77, 152114 (1972).

56. J.M.Bemiller, D.R.Smith, V.K.Ghauta, T.O.Lyles and E.R.Doyle, Carbohyd.Res., 35, 255 (1974).

57. S.Hanessian and A.G.Pernet, Advances in Carbohyd.Chem.and Biochem., 33, 111 (1976).

58. E.S.H.El Ashry, M.M.A.Rahman and N.Rashed, Carbohyd.Res., 82, 15 (1980).

59. E.S.H.El Ashry, M.M.A.Rahman and N.Rashed, Sci.Pharm., 48, 126 (1980).

60. B.Kohlstock and G.Henseke, Z.Chem., 13, 100 (1973).

61. B.Kohlstock and G.Henseke, Z.Chem., 12, 385 (1972).

62. B.Kohlstock, G.Henseke and R.Starke, Z.Chem., 13, 11 (1973).

63. P.M.Pillai and P.Ramabhadran, Indian J.Chem., 25B, 215 (1986).

64. H.El Khadem, Advances in Carbohyd.Chem., 20, 139 (1965).

65. H.Dahn and J.P.Fumeaux, Bull.Soc.Vaud.Sci.Natur., 70, 313 (1970); Chem.Abstr., 75, 140791 (1971).

66. H.Simon, G.Heubach and H.Wacker, Chem.Ber., 100, 3106 (1967).

67. H.Dahn and H.Moll, Helv.Chim.Acta., 49, 2426 (1966).

68. H.Dahn and J.Nussbaum, Helv.Chim.Acta., 52, 1661 (1969).

69. J.March, 'Advanced Organic Chemistry: Reactions, Mechanism and Structure', 2nd Edn., McGraw Hill Kogakusha Ltd., Tokyo, 1977, p.1064.

70. L.Dolejs, Z.Veisova and J.Farkas, Org.Mass Spectrom., 3, 1535 (1970).

71. P.Nordin and M.Doty, Science, 134, 112 (1961).

72. M.A.E.Sallam, Carbohyd.Res., 66, C_4-C_6 (1979).

73. W.S.Chilton and R.C.Krahn, J.Amer.Chem.Soc., 89, 4129 (1967).

74. T.Kobayashi, T.Haneishi and M.Saito, Nippon Nogeikaguku Kaishi, 36, 189 (1962); Chem.Abstr., 61, 10759 (1964).

75. V.G.Nair and S.Bernstein, U.S.Patent, 4, 304, 903 (1981); Chem.Abstr., 96, 104686 (1982).

76. V.G.Nair and S.Bernstein, U.S.Patent, 4, 304, 904 (1981); Chem.Abstr., 96, 104685 (1982).

77. J.E.Holchin, J.Gen.Microbiol., 5, 609 (1951).

78. J.McIntosh and F.R.Selbie, Brit.J.Exptl.Path., 27, 46 (1946).

79. C.Moreno, C.Hale, R.Hewett and D.Cussell, Europ.J. Immunol., 9, 916 (1979).

80. B.Teichmann, K.Himmelspach and O.Westphal, J.Prakt. Chem., 314, 877 (1972).

81. K.Himmelspach, O.Westphal and B.Teichmann, Europ.J.Immunol., 1, 106 (1971).

82. V.Harisdangkul and E.A.Kabat, J.Immunol., 108, 1232 (1972).

83. B.Teichmann, J.Prakt.Chem., 316, 821 (1974).

84. S.Astin, A.C.C.Newmann and H.L.Riley, J.Chem.Soc., 393 (1933).

85. H.Ohle and W.Gross, Ber., 68, 2262 (1935).

86. C.Bruckner, Ber., 24, 3001 (1891).

87. H.Kano and M.Fujimoto, Pharm.Bull.(Tokyo)., 5, 389 (1957).

88. M.Fujimoto, Bull.Chem.Soc.Japan, 32, 294 (1959).

89. A.Burger, 'Medicinal Chemistry', Part I, 3rd Edn, Wiley Interscience, New York, 1970, p.71.

90. P.M.Pillai and P.Ramabhadran, Indian J.Chem., 25B, 901 (1986).

91. M.Fieser and L.Fieser, 'Reagents for Organic Synthesis', Vol.I, Wiley Interscience, New York, 1969, p.1128 and references cited therein.

92. C.L.Leese and H.N.Rydon, J.Chem.Soc., 303 (1955).

PUBLICATIONS ARISING OUT OF THIS WORK

1. Synthesis of Flavazoles(1H-pyrazolo[3,4-b]quinoxalines) starting from 2-hydroxyquinoxaline-3-carboxylic acid derivatives, P.M.Pillai and V.S.Bhat, Abstracts, Symposium on Recent Trends in Heterocyclic Chemistry, Varanasi, 1986, p.39.

2. Chlorination of heterocyclic compounds using thionyl chloride, V.S.Bhat and P.M.Pillai, Abstracts, National Seminar on Heterocyclic Chemistry in Nature and Industry, Nagpur, 1988, p.28.

3. Synthesis and reactions of 3-substituted 1-acetyl-flavazoles.(1H-pyrazolo[3,4-b]quinoxalines), V.S.Bhat and P.M.Pillai, Abstracts, Section of Physical Sciences, National Academy of Sciences, India, 58th Annual Session, Jammu, 1988, p.68.

4. Ring opening reactions of flavazoles, P.M.Pillai and V.S.Bhat, Abstracts, National Seminar on Current Research in Heterocyclic Compounds, Tiruchirapalli, 1989, p.30.

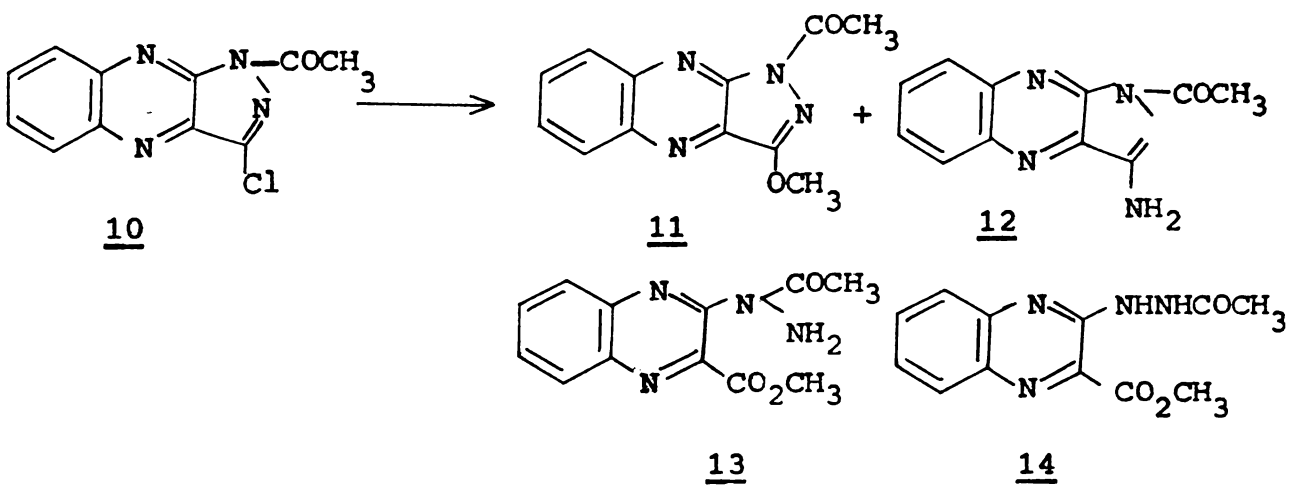
5. Synthesis and reactions of 1,H-benzodiazepino[6,7-b]-quinoxaline, a new heterocyclic system, V.S.Bhat and P.M.Pillai, Abstracts, Symposium on Trends in Heterocyclic Chemistry, Hyderabad, 1989, p.70.

6. Chlorination of 1-phenyl-1H-pyrazolo[3,4-b]quinoxaline and related compounds using thionyl chloride, P.M.Pillai and V.S.Bhat, Indian Journal of Chemistry, to be published in December 1989.

7. Synthesis of 2-aryl-3-oxo-3-pyrazolino[3,4-b]quinoxaline, V.S.Bhat and P.M.Pillai, accepted for 77th Session, Indian Science Congress Association, Cochin, 1990.

on a boiling water bath for 2 hours provided 1-acetyl-3-chloro-1H-pyrazolo[3,4-b]quinoxaline (10) in about 66% yield. The mass spectrum of 10 showed characteristic m/z peaks for a monochloro derivative in that the $[M]^{+\cdot}$ appeared at m/z 246 and an M+2 peak of about 1/3 intensity at m/z 248. The other spectral data were also in complete agreement with the structure as given in the experimental section.

Treatment of the chloro derivative 10 with 30% of liquor ammonia in methanol at room temperature in an attempt to remove the N-acetyl group at position 1, not only did not yield the desired result but provided a mixture consisting of 1-acetyl-3-methoxy-1H-pyrazolo[3,4-b]quinoxaline (11), 1-acetyl-3-amino-1H-pyrazolo[3,4-b]quinoxaline (12) and a ring opened product which has a structure of either 13 or 14. These compounds were separated by column



and the solvent removed under reduced pressure. Residue was purified by chromatographing on a silica gel column. Product obtained was recrystallised from methanol to give 190 mg (80%) of 1-acetyl-3-amino-1H-pyrazolo[3,4-b]quinoxaline (12), mp 260°.

MS: m/e227 (M^+), 185 ($M^+ - COHC_3+H$), 170 ($M^+-COCH_3-NH_2+2H$).

NMR($CDCl_3$): δ 1.7 (2,s,NH_2), 2.7 (3,s,$COCH_3$), 7.7 (4,m, aromatic).

IR(KBr): 3420 cm^{-1}, 3320 cm^{-1}, (NH_2), 1630 cm^{-1} (C=O).

UV: λ_{max}^{MeOH} 223 nm (ε $1.3x10^4$), 253.4 nm (ε $1.4x10^4$), 305 nm (ε $5.6x10^3$).

Anal. Calcd. for $C_{11}H_9N_5O$: C, 58.14; H, 3.96; N, 30,83.

Found: C, 58; H, 3.85; N, 30.56.

b) By reaction of 10 with ammonia

A mixture of 500 mg (0.002 mol) of 1-acetyl-3-chloro-1H-pyrazolo[3,4-b]quinoxaline and 250 ml of liquor ammonia was kept for 10 days in a closed vessel at room temperature. Ammonia was removed under vacuum and neutralised using dilute hydrochloric acid. It was extracted using chloroform. The extract showed three components on tlc.

Solvent was distilled off. The mixture was separated by column chromatography on alumina. Elution with chloroform gave 220 mg of 1-acetyl-3-chloro-1H-pyrazolo[3,4-b]quinoxaline (10) and 120 mg (26%) of 1-acetyl-3-amino-1H-pyrazolo[3,4-b]-quinoxaline (12) which was recrystallised from chloroform-hexane, mp 260°, mmp identical with the sample obtained from (a) above.

Further elution with chloroform methanol gave 100 mg (22%) of 1-acetyl-3-hydroxy-1H-pyrazolo[3,4-b]-quinoxaline (9), mp 312°. A mixed mp with the sample from 4.5 was undepressed.

4.9 2-Hydrazinoquinoxaline-3-carboxamide (15)

A mixture of 225 mg (0.001 mol) of 1-acetyl-3-amino-1H-pyrazolo[3,4-b]quinoxaline and 10 ml of 2 N hydrochloric acid was heated over a water bath for 1 hour. The reaction mixture was cooled, neutralised using sodium bicarbonate and extracted using chloroform. The solvent was distilled off and recrystallised from chloroform-hexane to give 140 mg (70%) of 2-hydrazinoquinoxaline-3-carboxamide (15), mp 180°.

MS: m/e 203 (M^+), 175 (M^+ - CO).

NMR($CDCl_3$): δ 1.2-1.6 (3,m,$NHNH_2$), 7.6-8 (4,complex, aromatic Hs), 9.1 (2,broad, $CONH_2$).

IR (KBr): 3380 cm^{-1} (NH), 3300 cm^{-1} (NH_2), 3260 ($CONH_2$), 1670 cm^{-1} (C=O).

UV: λ_{max}^{MeOH} 216 nm (ε 2.5×10^4), 251 nm (ε 1.8×10^4), 306.6 nm (ε 3.1×10^4), 398 nm (ε 4.9×10^4).

Anal. Calcd. for $C_9H_9N_5O$: C, 53.20; H, 4.43; N, 34.48.
Found: C, 52.93; H, 4.31; N, 34.24.

4.10 1-Acetyl-3-(N-piperidyl)-1H-pyrazolo[3,4-b]quinoxaline (16)

A mixture of 250 mg (0.001 mol) of 1-acetyl-3-chloro-1H-pyrazolo[3,4-b]quinoxaline (10) and 10 ml of piperidine was heated on a boiling water bath for 1 hour. The reaction mixture was cooled and poured into about 100 ml of ice cold water. The crystals formed were filtered washed with water and dried. Solid product was purified by chromatographing on a column of silica gel using chloroform as the eluent. The product was recrystallised from chloroform-hexane to give 260 mg (88%) of 1-acetyl-3-piperidyl-1H-pyrazolo[3,4-b]quinoxaline (16), mp 100°.

MS: m/e 295 (M^+), 211 ($M^+ - C_5H_{10}N$), 43 ($COCH_3$).

NMR($CDCl_3$): δ 1.7 (6,m,CH_2-CH_2-CH_2), 2.7 (3,s,CH_3), 3.4 (4,t,N-$(CH_2)_2$), 7.6-8.1 (4,m, aromatic Hs).

IR(KBr): 1630 cm^{-1} (C=O).

UV: λ_{max}^{MeOH} 226.8 nm (ϵ 2.08×10^4), 278 nm (ϵ 1.9×10^4), 405 nm (ϵ 5.13×10^3).

Anal. Calcd. for $C_{16}H_{17}N_5O$: C, 65.08; H, 5.76; N, 23.72.
Found: C, 64.42; H, 5.71; N, 23.34.

4.11 1-Acetyl-3-(N-morpholinyl)-1H-pyrazolo[3,4-b]-quinoxaline (17)

A mixture of 250 mg (0.001 mol) of 1-acetyl-3-chloro-1H-pyrazolo[3,4-b]quinoxaline (10) and 10 ml of morpholine was heated on a boiling water bath for one hour. The reaction mixture on work up as done above gave 250 mg (85%) of 1-acetyl-3-(N-morpholinyl)-1H-pyrazolo[3,4-b]-quinoxaline (17), mp 130°.

NMR($CDCl_3$): δ 2.7 (3,s,CH_3), 3.5 (4,q,N$(CH_2)_2$), 3.9 (4,q,O$(CH_2)_2$), 7.6-8.2 (4,m,aromatic Hs).

IR(KBr): 1630 cm^{-1} (C=O).

UV: λ_{max}^{MeOH} 227.2 nm (ε 1.01x10^4), 258.2 nm (ε 9.98 x10^3), 272.2 nm (ε 9.41x10^3), 394.2 nm (ε 2.74x10^3).

Anal. Calcd. for $C_{15}H_{15}N_5O_2$: C, 60.6; H, 5.05; N, 23.56.
Found: C, 60.1; H, 4.98; N, 23.34.

4.12 1-Acetyl-3-(N-pyrrolidyl)-1H-pyrazolo[3,4-b]quinoxaline (18)

A mixture of 250 mg (0.001 mol) of 1-acetyl-3-chloro-1H-pyrazolo[3,4-b]quinoxaline (10) and 10 ml of pyrrolidine was heated on a boiling water bath for 1 hour. The reaction mixture on work up as done above gave 125 mg (50%) of 1-acetyl-3-(N-pyrrolidyl)-1H-pyrazolo[3,4-b]-quinoxaline (18), mp 143°.

NMR ($CDCl_3$): δ 1.9 (4,m,CH_2-CH_2), 2.7 (3,s,CH_3), 3.5 (4,t, $N(CH_2)_2$, 7.5-8.1 (4,m,aromatic Hs).

IR(KBr): 1630 cm^{-1} (C=O).

Anal. Calcd. for $C_{15}H_{15}N_5O$: C, 64.05; H, 5.34; N, 24.91.
Found: C, 63.85; H, 5.06; N, 24.68.

4.13 1-Acetyl-3-(N,N-diethylamino)-1H-pyrazolo[3,4-b]-quinoxaline (19)

A mixture of 250 mg (0. 001 mol) of 1-acetyl-3-chloro-1H-pyrazolo[3,4-b]quinoxaline (10) and 10 ml of diethylamine was heated for one hour on a boiling water bath. The reaction mixture was cooled and poured into about 100 ml of ice cold water. The cyrstals were filtered, washed with water, dried and recrystallised from hexane to give 125 mg (50%) of 1-acetyl-3-(N,N-diethylamino)-1H-pyrazolo-[3,4-b]quinoxaline (19), mp 86°.

NMR($CDCl_3$): δ 1.2 (6,t,$2CH_3$), 2.7 (3,s,$COCH_3$), 3.4 (4,q, $N(CH_2)_2$), 7.6-8.1 (4,m,aromatic Hs).

IR(KBr): 1630 cm^{-1} (C=O).

UV: λ_{max}^{MeOH} 220.3 nm (ε 2.77 x10^4), 275.4 nm (ε 2.51x10^4), 408.5 nm (ε 6.62x10^3).

Anal. Calcd. for $C_{15}H_{17}N_5O$: C, 63.6; H, 6.0; N, 24.73.

Found: C, 63.42; H, 5.81; N, 24.34.

4.14 3-Amino-1H-pyrazolo[3,4-b]quinoxaline (20)

A solution of 225 mg (0. 001 mol) of 1-acetyl-3-amino-1H-pyrazolo[3,4-b]quinoxaline (12) and 500 mg of

Na_2CO_3 in methanol was refluxed on a boiling water bath for 20 hours. After the completion of the reaction methanol was distilled off under reduced pressure and water was added to the reaction mixture to dissolve the Na_2CO_3. Aqueous solution was extracted with chloroform. The extract was dried (anhyd: Na_2SO_4), concentrated under reduced pressure and purified by passing over a column of silica gel using chloroform as the eluent to give 120 mg (65%) of 3-amino-1H-pyrazolo[3,4-b]quinoxaline (20), mp 253°.

MS: m/e 185 (M^+), 170 ($M^+ - NH_2 + H$).

NMR($CDCl_3$): δ 1.6 (2,s,NH_2), 7.3-8.2 (5,m,aromatic Hs).

IR(KBr): 3420 cm^{-1}, 3320 cm^{-1} (broad) (NH_2,NH).

Anal. Calcd. for $C_9H_7N_5$: C, 58.37; H, 3.78; N, 37.84.

Found: C, 57.96; H, 3.63; N, 37.45.

4.15 2-(D-Arabino-tetrahydroxybutyl)quinoxaline[1] (21)

A solution of 36.0 g (0.2 mol) of D-glucose in 54.0 ml of water was mixed with 6.0 ml of glacial acetic acid, 21.6 g (0.2 mol) of o-phenylene diamine, 5.0 ml (0.1 mol) of hydrazine hydrate and a pinch of sodium bicarbonate and the mixture was heated under reflux for 5.0 hours on a boiling water bath. The solution was cooled

in ice and the precipitated product was filtered and washed with water. It was recrystallised from hot water and dried to give 17.0 g (34%) of 2-(D-arabino-tetrahydroxybutyl)-quinoxaline (21), mp 192° (d) (lit.[1] mp 192°).

4.16 Quinoxaline-2-carboxaldehyde (22)

A mixture of 5.0 g (0.02 mol) of 2-(D-arabino-tetrahydroxybutyl)quinoxaline (21) and 13.0 g (0.06 mol) of sodium metaperiodate in 300 ml of water and 10 ml of glacial acetic acid was kept at room temperature with occasional shaking for 16 hours. The mixture was filtered and the filtrate neutralised with sodium bicarbonate. The neutral solution was extracted with ether, the ether extract was dried with anhydrous sodium sulphate, and evaporated to dryness. The residue was recrystallised from petroleum ether (60-80°) to give 2.0 g (63%) of quinoxaline-2-carboxaldehyde (22), mp 107° (lit.[92] mp 107-108°).

4.17 Quinoxaline-2-carboxaldehyde phenylhydrazone (23)

A solution of 1.6 g (0.01 mol) of quinoxaline-2-carboxaldehyde (22) and 1.1 g (0.01 mol) of phenylhydrazine in 20 ml of methanol was stirred at room temperature for 1 hour using a magnetic stirrer. Yellow crystals of

quinoxaline-2-carboxaldehyde phenylhydrazone were formed. The mixture was cooled in ice, filtered and washed with a small amount of ice cold methanol and the product recrystallised from methanol to give 2.0 g (81%) of quinoxaline-2-carboxaldehyde phenylhydrazone (23), mp 230° (lit.[93] mp 229-230°).

4.18 1-Phenyl-1H-pyrazolo[3,4-b]quinoxaline[36] (24)

A mixture of 0.5 g (0.002 mol) of quinoxaline-2-carboxaldehyde phenylhydrazone (23), 0.37 g (0.002 mol) of azobenzene, 50 ml of 60% aqueous 1-propanol, 0.5 ml of glacial acetic acid and 5.0 ml of 1 N HCl was heated under reflux for 10 hours on a boiling water bath and then refrigerated overnight. The product was filtered, washed with water followed by a small quantity of cold 50% aqueous n-propanol, dried and recrystallised from 50% acetic acid to give 0.46 g (93%) of 1-phenyl-1H-pyrazolo[3,4-b]quinoxaline (24), mp 152° (lit.[36] mp 152°).

4.19 1-p-Chlorophenyl-1H-pyrazolo[3,4-b]quinoxaline (25)

a) By chlorination of 24 with thionyl chloride

A mixture of 1-phenyl-1H-pyrazolo[3,4-b]quinoxaline (24) (1.0 g, 0.0004 mol) and 10 ml of thionyl chloride was kept at room temperature for 120 hours. Excess thionyl

chloride was removed under reduced pressure. The solid product obtained was dissolved in $CHCl_3$, washed with water, dried over Na_2SO_4 and evaporated to dryness under reduced pressure and the residue was purified by chromatographing over a column of silica gel and the product recrystallised from chloroform hexane to give 960 mg (85%) of 1-p-chlorophenyl-1H-pyrazolo[3,4-b]quinoxaline (25), mp 198° (lit.[37] mp 198°).

b) By oxidative cyclisation of quinoxaline-2-carboxaldehyde p-chlorophenylhydrazone[37] (27)

A mixture of 560 mg (0.002 mol) of quinoxaline-2-carboxaldehyde p-chlorophenylhydrazone (27), 0.37 g (0.002 mol) of azobenzene, 50 ml of 60% aqueous 1-propanol, 0.5 ml of glacial acetic acid and 5.0 ml of 1 N HCl was heated under reflux for 10 hours on a boiling water bath and refrigerated overnight. The product was filtered, washed with 50% aqueous 1-propanol , dried and recrystallised from 50% acetic acid to give 500 mg (90%) of 1-p-chlorophenyl-1H-pyrazolo[3,4-b]quinoxaline (25), mp 198° (lit.[37] mp 198°).

4.20 1-p-Chlorophenyl-3-chloro-1H-pyrazolo[3,4-b]-quinoxaline (26)

a) From 1-phenyl-1H-pyrazolo[3,4-b]quinoxaline (24)

A mixture of 1.0 g (0.004 mol) of 1-phenyl-1H-pyrazolo[3,4-b]quinoxaline (24) and 10 ml of thionyl chloride

was refluxed for 120 hour on a boiling water bath. Thionyl chloride was removed under vacuum. The residue was dissolved in chloroform, washed with water, dried (Na_2SO_4) and evaporated to dryness. This product was a mixture of two components as shown by tlc. The two compounds were separated by chromatographing over a column of silica gel. Elution with carbon tetrachloride provided the first component, 800 mg (63%), mp 200° which was shown to be 1-p-chlorophenyl-3-chloro-1H-pyrazolo[3,4-b]quinoxaline (26).

MS: m/e 314 (M^+), 316 ($M^+ + 2$), 279 ($M^+ - Cl$), 234 ($M^+ - 2Cl$), 91 (C_6H_5N).

UV: λ_{max}^{MeOH} 208 nm (ε 5.52×10^4), 233 nm (ε 4.73×10^4), 273 nm (ε 6.84×10^4).

Anal. Calcd. for $C_{15}H_8N_4Cl_2$: C, 57.3; H, 2.5; N, 17.8.

Found: C, 56.8; H, 2.48; N, 18.2.

Further elution of the column with chloroform gave 150 mg (16%) of 1-p-chlorophenyl-1H-pyrazolo[3,4-b]quinoxaline (25), mp 198°.

b) From 1-p-chlorophenyl-1H-pyrazolo[3,4-b]quinoxaline (25)

A mixture of 500 mg (0.0016 mol) of 1-p-chlorophenyl-1H-pyrazolo[3,4-b]quinoxaline (25) and 5 ml of thionyl chloride was heated under reflux for 80 hours on a boiling water bath. The excess thionyl chloride was removed under reduced pressure. The solid mass was dissolved in chloroform, washed with water, dried over Na_2SO_4, concentrated and the residue purified by column chromatography to give 1-p-chlorophenyl-3-chloro-1H-pyrazolo[3,4-b]quinoxaline (26), 460 mg (82%), mp 200°. A mmp with the product obtained from (a) above was undepressed.

c) From 3-chloro-1-phenyl-1H-pyrazolo[3,4-b]quinoxaline (29)

A mixture of 500 mg(0.0016 mol) of 3-chloro-1-phenyl-1H-pyrazolo[3,4-b]quinoxaline (29) and 5 ml of thionyl chloride was heated under reflux on a boiling water bath for 24 hours. After the completion of the reaction as shown by tlc, the excess thionyl chloride was removed under vacuum. The solid product was dissolved in chloroform, washed with water, dried, concentrated and purified by column chromatography on silica gel to give 26 (450 mg, 80%), mp 200°. A mixed mp with the product obtained in (a) above was undepressed.

4.21 Quinoxaline-2-carboxaldehyde p-chlorophenylhydrazone[37] (27

A solution of 3.9 g (0.025 mol) of quinoxaline-2- carboxaldehyde (22) in 50 ml of methanol and 3.6 g (0.025 mol) of p-chlorophenylhydrazine was stirred at room temperature for 1 hour. The mixture was diluted to 250 ml with water, stirred 2 hours more and refrigerated overnight. The precipitated material was filtered, washed with water, dried and recrystallised from methanol to give 5.8 g (83%) of quinoxaline-2-carboxaldehyde p-chlorophenylhydrazone (27), mp 236° (lit.[37] 230°).

4.22 2-Hydroxyquinoxaline-3-carbonylphenylhydrazide (28)

A mixture of 1.0 g (0.005 mol) of ethyl 2-hydroxyquinoxaline-3-carboxylate (4) and 4.0 ml of freshly distilled phenylhydrazine was heated 2 hours on a boiling water bath. The mixture was cooled, 200 ml of 1 N HCl was added and shaken well to dissolve the unreacted phenylhydrazine. The suspended dark brown product was filtered, washed with water, dried and recrystallised from methanol to give 1.15 g (82%) of 2-hydroxyquinoxaline-3-carbonylphenylhydrazide (28), mp 250° (d) (lit.[35] mp 250°).

4.23 3-Chloro-1-phenyl-1H-pyrazolo[3,4-b]quinoxaline (29)

A mixture of 1.12 g (0.004 mol) of 2-hydroxyquinoxaline-3-carbonylphenylhydrazide (28) and 20 ml of freshly distilled phosphorous oxychloride was heated 12 hours under a $CaCl_2$ guard tube on a steam bath. The reaction mixture was cooled and poured into 200 g of crushed ice with stirring. The crystalline material was filtered, washed with water and dried. It was dissolved in 50 ml of carbon tetrachloride, purified by passing over a column of silica gel and recrystallised from hexane to give 800 mg (71%) of 3-chloro-1-phenyl-1H-pyrazolo[3,4-b]quinoxaline (29), mp 210° (lit.[35] mp 210°).

4.24 Reaction of 3-chloro-1-p-chlorophenyl-1H-pyrazolo-[3,4-b]quinoxaline (26) with $NaBH_4$ in isopropanol

A solution of 500 mg (0.0016 mol) of 3-chloro-1-p-chlorophenyl-1H-pyrazolo[3,4-b]quinoxaline (26) in 200 ml of isopropanol was heated with 1.2 g of powdered sodium borohydride in 200 mg portions heating the mixture under reflux for 30 hours on a boiling water bath. The reaction mixture was concentrated under reduced pressure. 100 ml of water was added and stirred for 1 hour. The precipitate was filtered, washed with water and dried. A tlc examination showed that the product contained two compounds which were

separated on a column of silica gel. Elution with chloroform gave 160 mg (30%) of 1-p-chloroanilinoquinoxaline 3-carboxamide (30), mp 254°.

MS: m/e 298 (M^+), 300 ($M^+ + 2$), 253 ($M^+ + H - CONH_2$), 218 ($M^+ + H - CONH_2$-Cl), 91 (C_6H_5N).

IR(KBr): 3420 cm^{-1} (NH_2), 3240 (NH), 1680 (C=O).

UV: λ_{max}^{MeOH} 208 nm (ε 5.0x10^3), 224 nm (ε 8.1x10^3), 294 nm (ε 9.3x10^3), 420 nm (ε 1.2x10^3).

Anal. Calcd. for $C_{15}H_{11}ClN_4O$: C, 60.4; H, 3.7; N, 18.8.
Found: C, 60.9; H, 3.72; N, 18.6.

Further elution of the column with chloroform-methanol gave 200 mg (50%) of 2-p-chloroanilinoquinoxaline (32), mp 198°. See section 4.27 for structural characteristics.

4.25 Reaction of 1-p-chlorophenyl-1H-pyrazolo[3,4-b]-quinoxaline (25) with $NaBH_4$ in isopropanol

A solution of 400 mg (0.0014 mol) of 1-p-chlorophenyl-1H-pyrazolo[3,4-b]quinoxaline (25) in 200 ml of isopropanol was heated with 1.2 g of powdered sodium borohydride in

200 mg portion while heating the mixture under reflux for 30 hours on a boiling water bath. The reaction mixture was concentrated under reduced pressure. 25 ml of water was added and stirred for 1 hour. The precipitate was filtered, washed with water and dried. A tlc examination showed that the product contained two compounds which were separated on a column of silica gel. Using chloroform as eluent 2-p-chloroanilinoquinoxaline-3-carboxamide (125 mg (25%)) was obtained which was recrystallised from chloroform-hexane, mp 254°, identical with the sample obtained above.

Further elution of the column with chloroform-methanol gave 250 mg (60%) of 2-p-chloroaniliroquinoxaline (32), mp 198°. A mixed mp with the sample obtained from above was undepressed.

4.26 2-p-Chloroanilinoquinoxaline-3-carboxylic acid (31)

a) From the mother liquors of the above two reactions for the preparation of 30

The mother liquor after separating 30 and 32 was cooled acidified with hydrochloric acid and extracted with chloroform. The extract was dried (Na_2SO_4) and evaporated

to dryness under reduced pressure. The residue was recrystallised from chloroform-hexane to give 25 mg (6%) of 2-p-chloroanilinoquinoxaline-3-carboxylic acid (31), mp 169°, identical with the sample prepared below.

b) From 1-p-chlorophenyl-1H-pyrazolo[3,4-b]quinoxaline (25)

A mixture of 500 mg (0.002 mol) of 1-p-chlorophenyl-1H-pyrazolo[3,4-b]quinoxaline (25), 50 ml of 10% sodium hydroxide solution and 4 ml of n-propanol was heated under reflux for 25 hours. The reaction mixture was concentrated to 50 ml, cooled in an ice bath, neutralised with hydrochloric acid and extracted with chloroform. The extract was dried with anhydrous sodium sulphate, evaporated to dryness under reduced pressure and the residue was recrystallised from chloroform-hexane to give 450 mg (85%) of 2-p-chloroanilinoquinoxaline-3-carboxylic acid (31), mp 169°.

MS: m/e 299 (M^+), 301 ($M^+ + 2$), 255 ($M^+ - CO_2$), 220 ($M^+ - CO_2 - Cl$).

IR(KBr): 3400 cm^{-1} (Borad,OH,NH), 1730 cm^{-1} (C=O).

UV: λ_{max}^{MeOH} 208 nm (ε 1.13×10^4), 222 nm (ε 1.23×10^4), 293 nm (ε 1.35×10^4), 412 nm (ε 1.44×10^3).

4.53 Ethyl 2-(2:4-dinitrophenylhydrazino)quinoxaline-3-carboxylate (62)

A mixture of 2.4 g (0.01 mol) of ethyl 2-chloro-quinoxaline-3-carboxylate (34) and 2.0 g of 2:4 dinitro phenylhydrazine was heated on a boiling water bath for 16 hours. A tlc examination showed that the product contained two compounds which were separated on a column of silica gel. Elution of the column with chloroform gave 600 mg (25%) of the starting material. Further elution of the column with chloroform gave 2.8 g (70%) of ethyl 2-(2;4-dinitrophenylhydrazino)quinoxaline-3-carboxylate (62) after recrystallisation from chloroform-hexane, mp 222°.

MS: m/e 398 (M^{+})

NMR($CDCl_3$): δ 1.6(3,t,CH_3), 4.7 (2,q,CH_2), 7.4-9.8 (9,complex,Hs).

IR(KBr): 3300 cm^{-1} (NH), 1700 cm^{-1} (C=O).

UV: λ_{max}^{MeOH} 216 nm (ε 2.24×10^4), 253.4 nm (ε 1.86×10^4), 343 nm (ε 1.05×10^4).

Anal. Calcd. for $C_{17}H_{14}N_6O_6$: C, 51.25; H, 3.52; N, 21.1.
Found: C, 51.2; H, 3.5; N, 21.4.