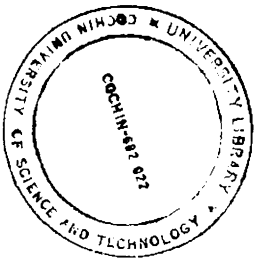
STUDIES ON THE SYNTHESIS AND CNS ACTIVITY OF STRYCHNINE DERIVATIVES

A 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

ANNAM CHACKO P



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MAY - 1987

CERTIFICATE

Certified that this thesis is based on the work done by Annam Chacko P. under my guidance in the Department of Applied Chemistry, Cochin University of Science and Technology, Cochin-22 and no part of this has been presented by her for any other degree.

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May 5, 1987.

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.

Annam Chacko P.

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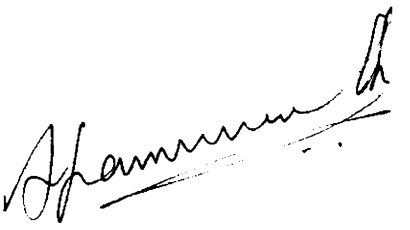


CONTENTS

CHAPTER I

INTRODUCTION

Strychnine, an alkaloid isolated from the seeds of Strychnos nuxvomica, L is known to stimulate all portions of the central nervous system with preference to the spinal cord. Its effects are believed to result from antagonism of an inhibitory transmitter, possibly glycine. It is a powerful convulsant and death results from asphyxia. At present strychnine has no therapeutic application in the western system of medicine. However, because of its convulsive effects, it is an important pharmacological tool as it plays a unique role as an inhibitor of post synaptic inhibitory impulses. It is useful to study inhibitory transmitter and receptor types. Strychnos nuxvomica trees grow naturally in this area and strychnine is isolated from the easily available seeds. The objective of this work was, therefore, to convert strychnine into a compound having CNS stimulant properties but with sufficiently low toxicity so that this locally available natural product may find some use in the preparation of a therapeutic agent.

Strychnine was isolated from its natural sources and was converted into a number of its derivatives by well established procedures in synthetic organic chemistry. As the lead compound is of extremely complex structure, the purification and structural determination of these derivatives required careful manipulations in the laboratory.

Derivatisation of strychnine involved modifications of the aromatic ring, and at positions 10,11,19 and 21-22. The biologically important sulphonamide substitution product was also prepared and characterised for the first time. Some derivatives were prepared with modification of more than one position in the parent molecule.

Prompted by reports that the N-oxide of strychnine, known as genostrychnine is less toxic and less convulsive than strychnine itself, all the derivatives of strychnine were converted into their N-oxides. Strychnine N-oxide however is not being used as a therapeutic agent because of the threat of convulsions at higher doses. The biological evaluation of other N-oxides was therefore considered worthwhile. It may be noted that although the pharmacology of a few strychnine derivatives have been reported, the pharmacology of these new N-oxides have remained unknown until now.

A systematic pharmacological investigation of strychnine and their N-oxides was carried out on frogs. Each compound was injected and the onset of action, convulsion pattern, duration of action and mortality rates were observed.

Some of the derivatives of strychnine were of considerably lower toxicity compared to strychnine.

They also exhibited reduced convulsive property. As expected, the N-oxides were found to be less toxic and less convulsive than their parent amines. Thus, this work has provided several new derivatives of strychnine with more desirable pharmacological properties.

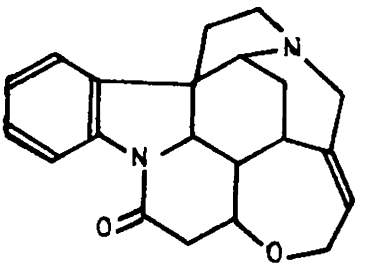
CHAPTER II

HISTORICAL REVIEW

2.1 SYNTHETIC STUDIES

2.1.1 Introduction

Strychnine (1), structurally one of the most complex alkaloids isolated so far from plants, was first obtained together with brucine (2) from the seeds and bark of Strychnos nuxvomica by Pelletier and Caventou[1] in 1819 and were fully characterised by Regnault[2] in 1838. The structure of strychnine was finally established more than hundred years later by Woodward and Brehm[3] in 1948. The total synthesis of strychnine was achieved in 1954 by Woodward and coworkers[4,5,6] and the absolute configuration was confirmed in 1963[7] . The structural elucidation of strychnine, achieved through the laborious work of many chemists over a long period of time, is considered to be a classical victory in the history of organic chemistry.



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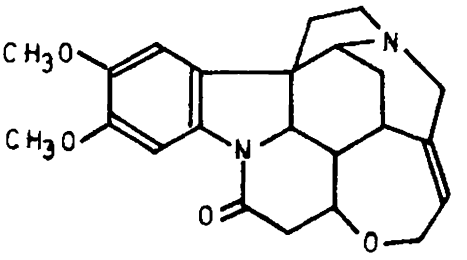
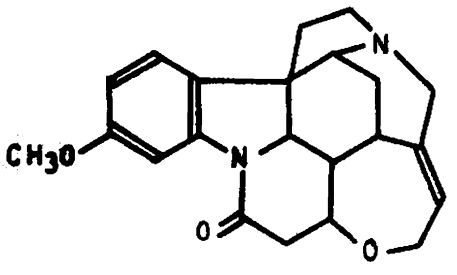


2

In addition to strychnine and brucine a few other alkaloids named as strychnos alkaloids have also been isolated from Strychnos nuxvomica[8] . They are ∝-colubrine (3), β-colubrine (4), vomicine (5) and pseudostrychnine (6). Recently a number of other alkaloids which are not structurally related to strychnine have also been found to occur in strychnos species[9].



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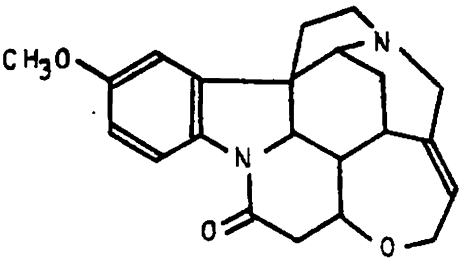

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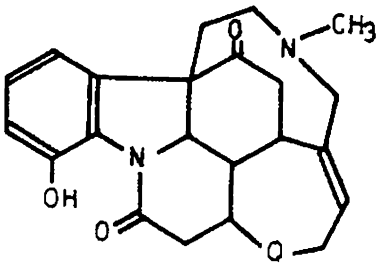

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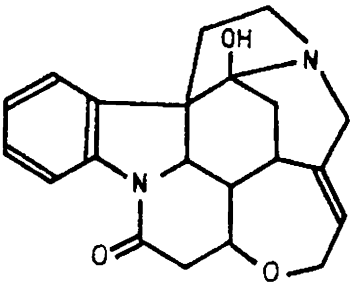
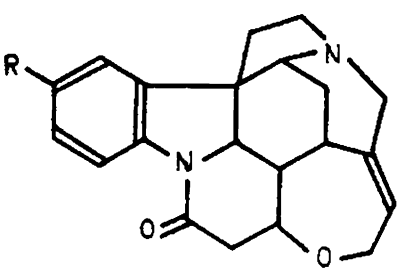


6

A large number of strychnine derivatives have been prepared both during the structural elucidation of the alkaloid and also for the interconversion of some of the strychnos alkaloids. An exhaustive survey of all the reactions of strychnine is too voluminous and have been reviewed elsewhere[9]. As our interest was to derivatise strychnine at positions 2,10,11,19 and 21-22, only related reactions will be presented here.

2.1.2 Reactions on the aromatic ring

Strychnine was found to undergo electrophilic substitution reactions at position 2, para to the aromatic nitrogen yielding various products. Thus the chlorination of strychnine hydrochloride solution with chlorine gave 2-chlorostrychnine (7)[10]. Bromine under similar conditions yielded 2-bromostrychnine (8)[11] and nitration of strychnine with nitric acid at 0° gave 2-nitrostrychnine (9)[12] which on reduction with zinc or sodium dithionite yielded 2-aminostrychnine (10)[13].



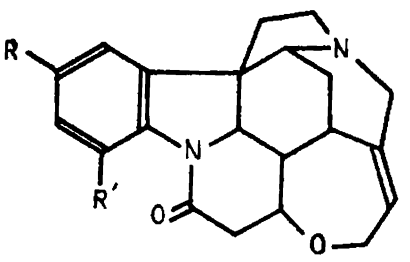
7, R = Cl

8, R = Br

9, R = NO_2

10, R = NH_2

In an attempt to establish the constitution of 2-bromostrychnine, Rosemund and Franke prepared several strychnine compounds substituted at 1 and 4 positions[14]. Thus 2-bromostrychnine (8) on treatment with 2N nitric acid and concentrated sulphuric acid at 0° gave 2-bromo-4-nitrostrychnine (11). This compound 11 on treatment with sodium amalgam in methanol yielded 4-aminostrychnine (12) which on diazotisation with sodium nitrite and subsequent treatment with cuprous chloride in concentrated hydrochloric acid produced 4-chlorostrychnine (13). 4-Aminostrychnine (12) on similar diazotisation and treatment with cuprous bromide in hydrobromic acid gave 4-bromostrychnine (14). Also 12 on diazotisation and treatment with potassium thiocyanate gave the 4-thiocyanate derivative 15.



11, R = Br, R' = NO_2

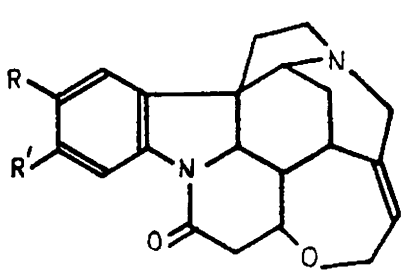
12, R = H, R' = NH_2

13, R = H, R' = Cl

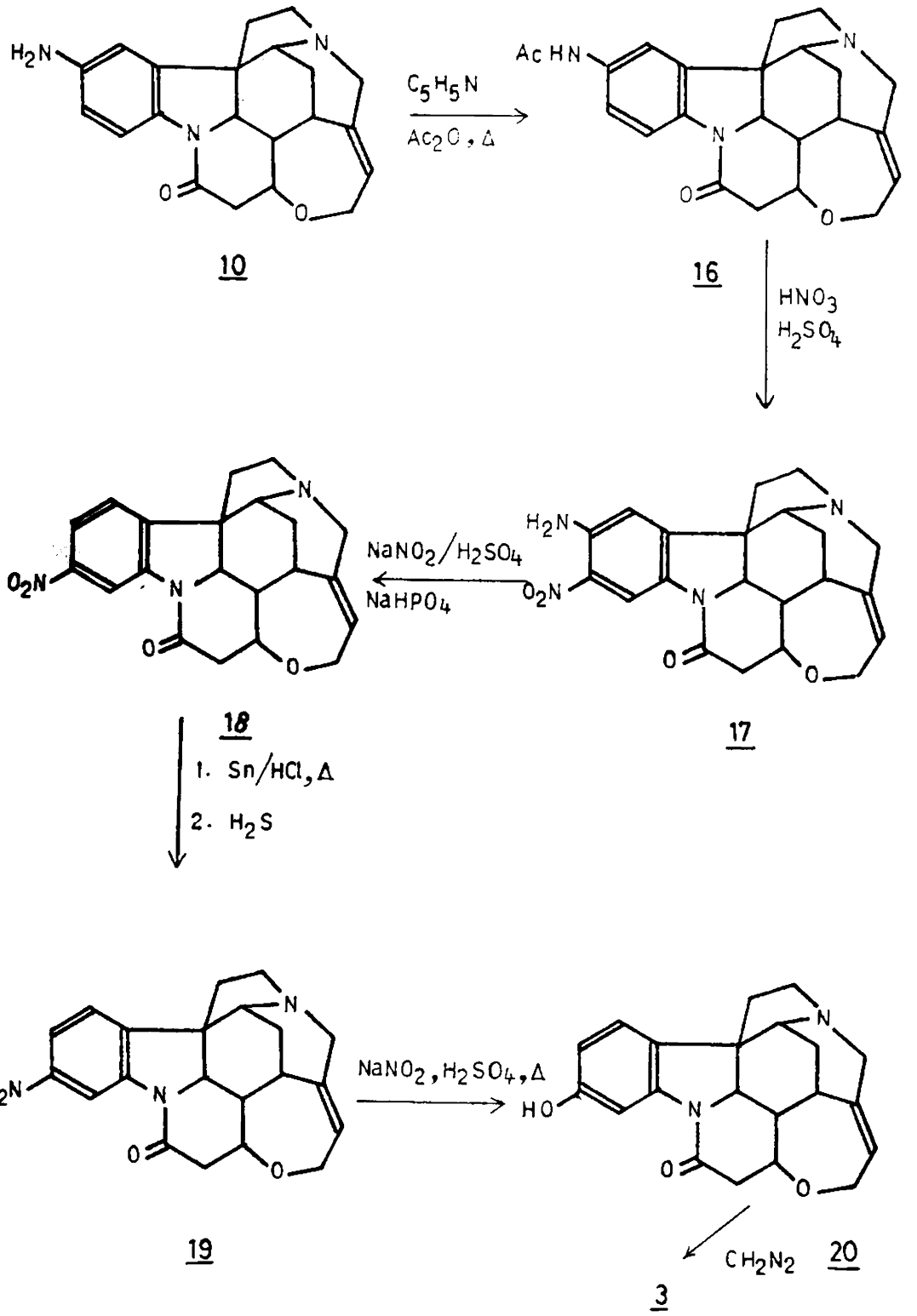
14, R = H, R' = Br

15, R = H, R' = SCN

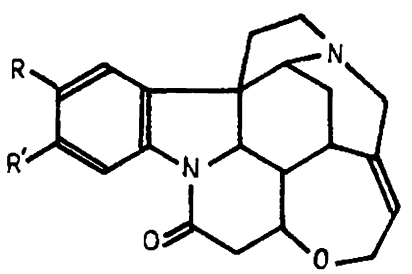
Strychnine was converted to ∝-colubrine (3) and in this process several substitution products of strychnine at the aromatic ring were prepared[15]. Thus 2-acetamido-strychnine (16) was prepared from 10 by boiling with acetic anhydride in pyridine. With nitric acid, 10 gave the nitrate at 0° and this product on treatment with sulphuric acid at 5° gave 2-amino-3-nitrostrychnine (17). The sulphate of 17 on diazotisation with 2N sulphuric acid and 5% aqueous sodium nitrite followed by treatment with sodium hydrogen phosphate gave 3-nitrostrychnine (18). Compound 18 on treatment with 3N hydrochloric acid and tin granules gave the tin double salt which when decomposed with hydrogen sulphide yielded 3-aminostrychnine (19). This compound 19 in 2N sulphuric acid was diazotised and the diazo derivative on refluxing yielded 3-hydroxystrychnine (20). Compound 20 on treatment with diazomethane in chloroform - methanol mixture at 5° gave the 3-methoxy derivative which was identical to the natural product, ∝-colubrine (3).



16, R = CH_3 CONH, R' = H
17, R = NH_2, R' = NO_2
18, R = H, R' = NO_2
19, R = H, R' = NH_2
20, R = H, R' = OH


H2N
N
O
O
10
C5H5N
Ac2O, Δ
AcHN
16
HNO3
H2SO4
O2N
18
NaNO2/H2SO4
NaHPO4
17
1. Sn/HCl, Δ
2. H2S
19
NaNO2, H2SO4, Δ
HO
3
CH2N2
20

2-Amino-3-nitrostrychnine (17) sulphate in 2N sulphuric acid on diazotisation with 5% aqueous sodium nitrite followed by the addition of cuprous bromide in 48% hydrobromic acid at 50° yielded 2-bromo-3-nitrostrychnine (21) as a lemon yellow product. Diazotisation of 21 in 2N sulphuric acid at 50° with 5% sodium nitrate and subsequent addition of cuprous chloride in 3N hydrochloric acid at room temperature followed by heating at 100° gave 3-chlorostrychnine (22). Similarly 21 in 2N sulphuric acid when added to saturated solution of potassium iodide and subsequent heating gave 3-iodostrychnine (23). Compound 23 in 2N sulphuric acid when diazotised and added to cuprous thiocyanate at 70° gave the thiocyanate derivative 24.



21, R = Br, R' = NO_2
22, R = H, R' = Cl
23, R = H, R' = I
24, R = H, R' = SCN

Several derivatives of strychnine substituted at positions 2 and 3 of the aromatic ring were prepared to convert strychnine to brucine (2)[13] . The goal was achieved by means of a double oxidation process with

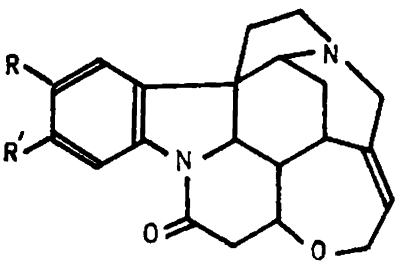
potassium nitrodisulphonate on strychnic acid and 2-hydroxystrychnine and final methylation of 2,3-dihydroxystrychnine to brucine.

2-Acetamido-3-nitrostrychnine (25) was prepared from 2-acetamido-strychnine nitrate by dissolving in acetic acid-sulphuric acid mixture at 10° followed by addition of ammonium hydroxide. 2-Dimethylamino-3-nitrostrychnine (26) was prepared from 25 by dissolving it in 30% formaldehyde and formic acid. This on refluxing and neutralisation with ammonium hydroxide yielded 26. 2-Chloro-3-nitrostrychnine (27) was prepared from 2-amino-3-nitrostrychnine (17) sulphate by diazotisation followed by treatment with cupric chloride in concentrated hydrochloric acid and subsequent addition of ammonium hydroxide to liberate the free base.

2,3-Diaminostrychnine (28) was synthesised by reduction of 2-amino-3-nitrostrychnine (17) with zinc and 3N hydrochloric acid, subsequent treatment with hydrogen sulphide and finally making it alkaline with ammonium hydroxide. 2,3-Diacetamido-strychnine (29) was prepared by reduction of 2-amino-3nitrostrychnine (17) sulphate using zinc dust in acetic acid and subsequent acetylation with acetic anhydride at 60°. Methyl-2-hydroxystrychnate (31) was synthesised by treating methyl strychnate with potassium nitrosodisulphonate(Fremy salt)

in potassium dihydrogen phosphate followed by the addition of sodium thiosulphate to convert the quinone to hydroxyl group. Compound 31 was converted to methyl 2-methoxystrychnate (32) by treating with diazomethane for 24 hours at room temperature. Fremy salt was also used to prepare 2-hydroxystrychnine (30).

2-Hydroxystrychnine (30) was also prepared from 2-aminostrychnine (10) by dissolving it in 30% sulphuric acid, diazotised with sodium nitrite and the excess sodium nitrite being destroyed with urea. 2-Acetoxystrychnine (33) another compound in this series was prepared by acetylation of 2-hydroxystrychnine (30) with acetic anhydride in dry pyridine. The N-oxide 34 of 30 was prepared using 30% hydrogen peroxide.



25, R = $NHCOCH_3$, R' = NO_2

26, R = $(CH_3)_2N$, R' = NO_2

27, R = Cl, R' = NO_2

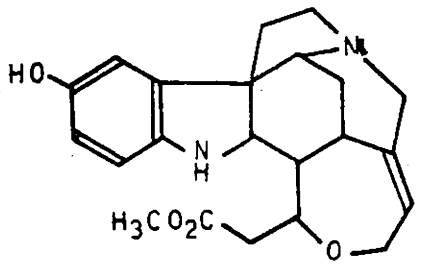
28, R = R' = NH_2

29, R = R' = CH_3CONH

30, R = OH, R' = H

33, R = $OCOCH_3$, R' = H

34, R = OH, R' = H, 19→O



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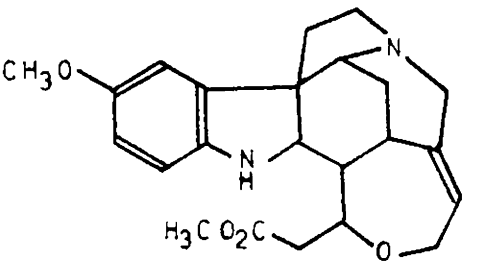
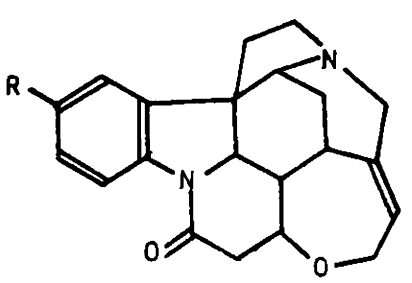


32

In 1981 Abdul *et al.* had achieved electrophilic substitution at position 2 of strychnine and groups like NO, CHO, CH_3CO, $PhCH_2$, Me_2CH etc. were introduced to prepare compounds 35a to 35e[16] . Later three of these compounds were reduced (NO to NH_2, CHO to CH_2OH and CH_3CO to CH_3CHOH) to get compounds 36a to 36c.



35a, R = NO

35b, R = CHO

35c, R = $COCH_3$

35d, R = $CH_2C_6H_5$

35e, R = $CH(CH_3)_2$

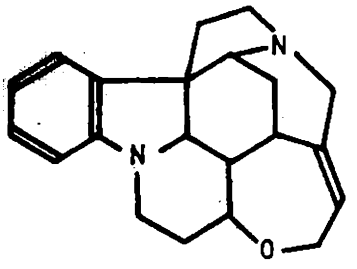
36a, R = NH_2

36b, R = CH_2OH

36c, R = $CH(OH)CH_3$

2.1.3 Modification of position 10

Electrolytic reduction was introduced by Tafel and was used to reduce the C-10 carbonyl group[17,19]. Before the discovery of lithium aluminium hydride this was the usually adopted method. It was first applied to the reduction of tetrahydrodeoxystrychnine to the corresponding oxygen free base and then for the reduction of strychnine to strychninidine (37) as a 2:1 mixture of 37 and tetrahydrostrychnine (38).



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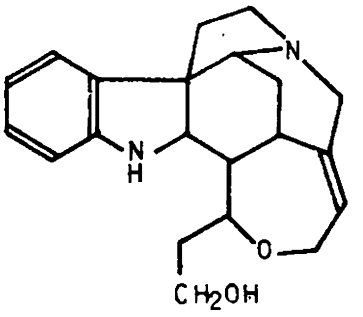

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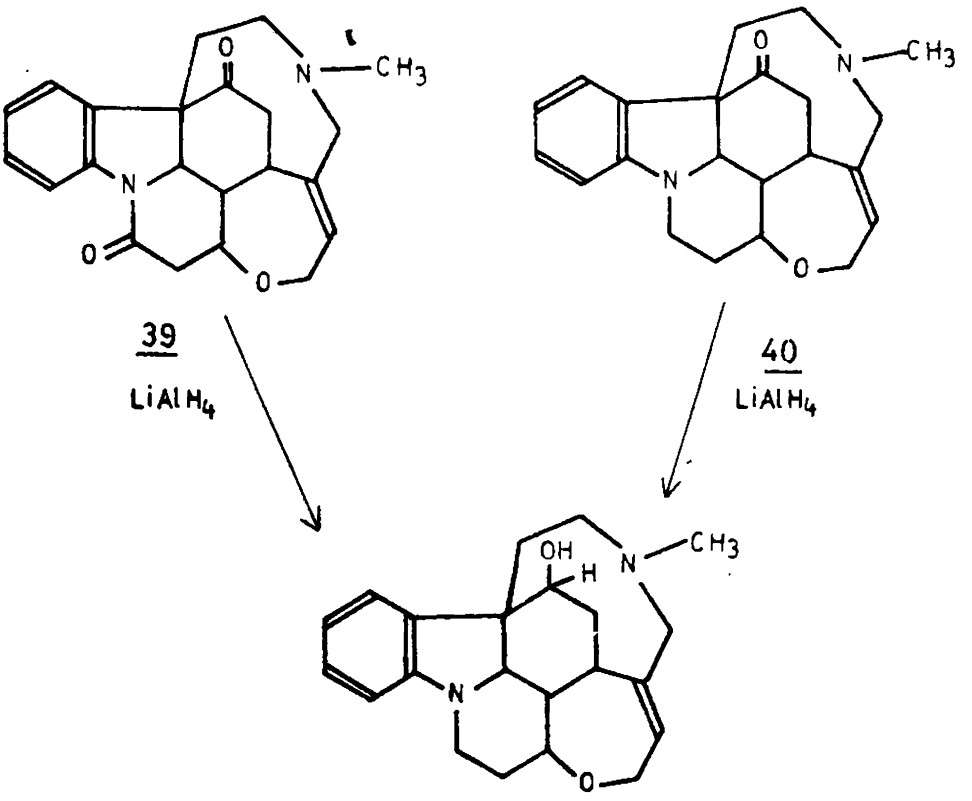
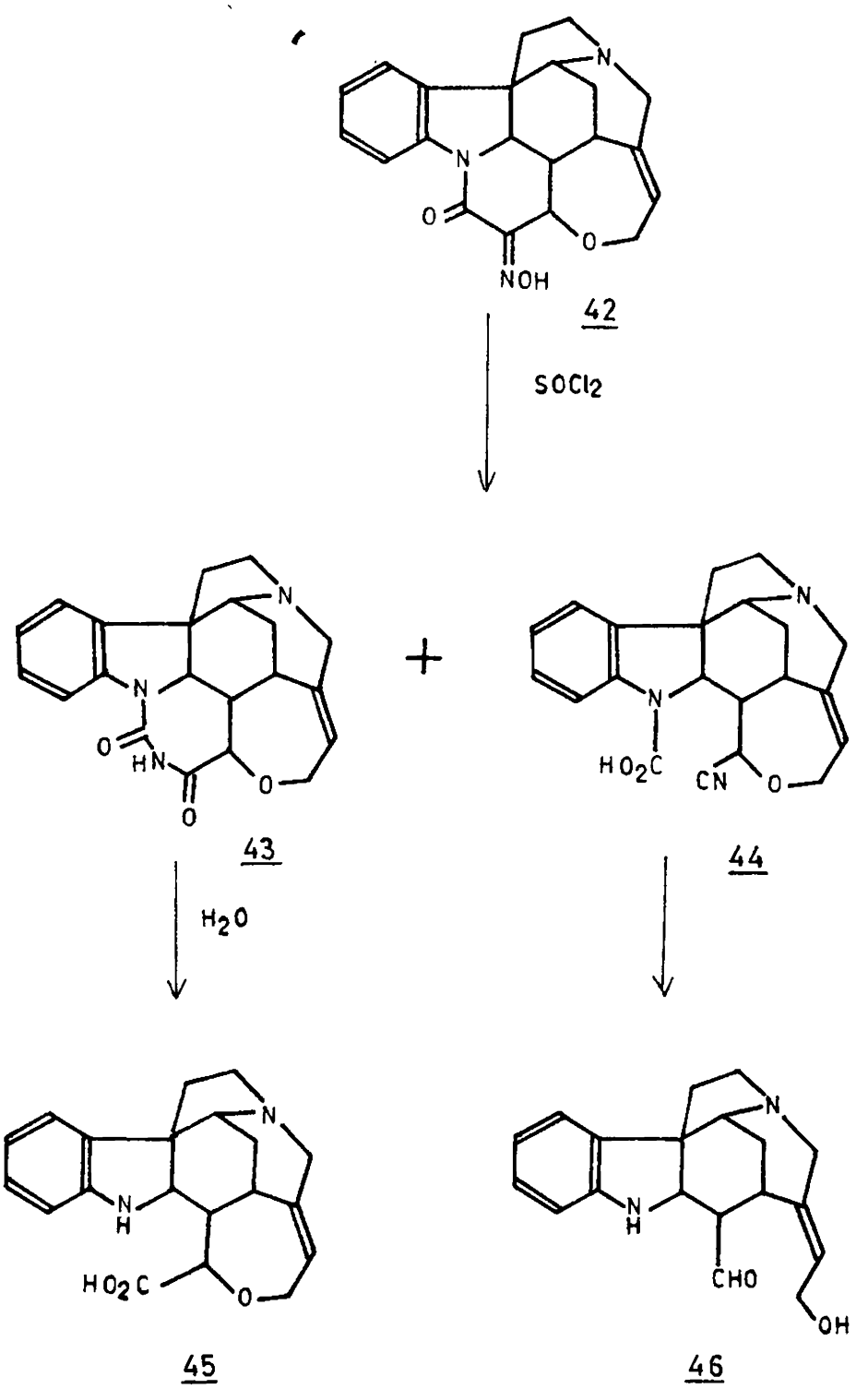
Lithium aluminium hydride was successfully used to reduce strychnine to strychnidine[18]. The reduction was effected by conducting the reaction with lithium aluminium hydride in tetrahydrofuran. It was shown that the action of lithium aluminium hydride on N-methyl secondary pseudostrychnine (39)[20] and N-methyl secondary pseudostrychnidine (40) gave the same product 41 which showed no carbonyl absorption in the IR spectra.

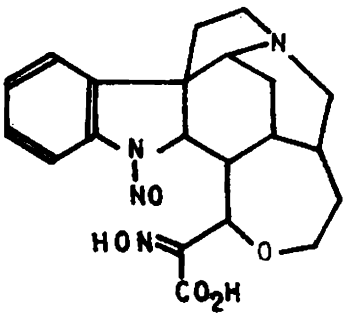


2.1.4 Reactions at position 11

Condensation of strychnine with amyl nitrite in presence of sodium ethoxide gave 11-oximinostrychnine (42)[21,22] . This oxime underwent Beckmann rearrangement with thionyl chloride to give two products 43 and 44 . On hydrolysis while 43 gave the amino acid 45, 44 with loss of carbon dioxide and hydrogen cyanide yielded Wieland - Gumlich aldehyde (46).


N
N
O
O
NOH
42
SOCl2
N
N
O
HN
O
O
43
+
N
N
HO2C
CN
O
44
H2O
N
N
H
HO2C
O
45
N
N
H
CHO
OH
46

The action of amyl nitrite and sodium ethoxide on dihydrostrychnine was entirely different. The product obtained was, quite unstable and not in the pure state. It was an amino acid containing a 9-nitroso group with probable structure 47[21] .



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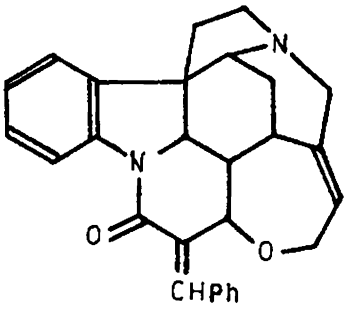

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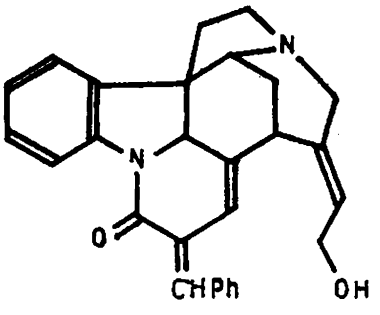

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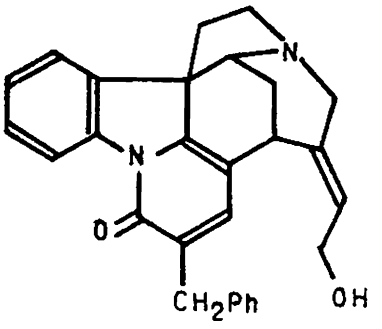


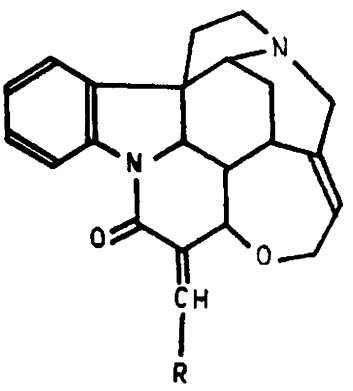
50

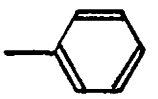
The C-11 methylene also condensed with benzaldehyde. Strychnine in hot aqueous alcoholic potassium hydroxide gave the yellow 11-benzylidene-strychnine (48)[23] . In mild conditions, isostrychnine condensed to give 49[24] . Under vigorous conditions, however the colourless pyridone derivative 50[25] was produced

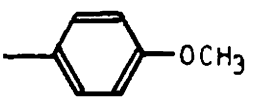
by double bond migration[26] . This easy isomerisation was of structural value in demonstrating clearly the presence of hydrogen atom on C-8.

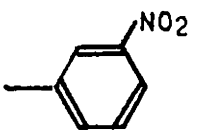
Knoevenagel reaction was effectively carried out on strychnine to give a number of condensation products at the C-11 position[27]. Perkin and Robinson had studied that strychnine and brucine could be condensed with benzaldehyde in presence of alcoholic sodium hydroxide to form the corresponding benzylidine derivatives[28]. Similarly by Knoevenagel reaction strychnine and brucine alkaloids were condensed with aromatic aldehydes in alkaline alcoholic solution in presence of piperidine to get optically active benzylidine type derivatives. Benzaldehyde, anisaldehyde, m-nitro benzaldehyde, p-hydroxy benzaldehyde, veratraldehyde, cinnamaldehyde and furfural were condensed with strychnine to give condensation products 48,51,52,53, 54,55 and 56 respectively. Similar reactions were carried out on brucine also.

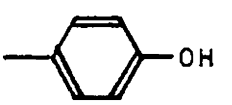
The alkylation of strychnine and brucine were studied earlier by Tafel[29,30], Perkin and Robinson[31] and Mitsuwa and coworkers[32]. The alkylation at active methylene carbon (C-11) was undertaken to prepare the optically active alkylated derivatives[33] . The reaction

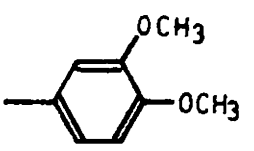


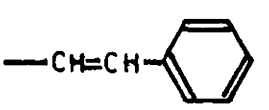
48, R = 

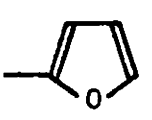
51, R = 

52, R = 

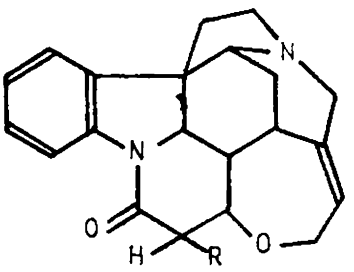
53, R = 

54, R = 

55, R = 

56, R = 

was effected by the action of alkyl halides in presence of sodium ethoxide in absolute ethanol. The reaction with alkyl halides-methyl iodide, ethyl iodide, propyl bromide, n-butyl bromide, and isoamyl bromide gave the alkylated derivatives 57,58,59,60 and 61 respectively.



57, R = CH_3

58, R = CH_2CH_3

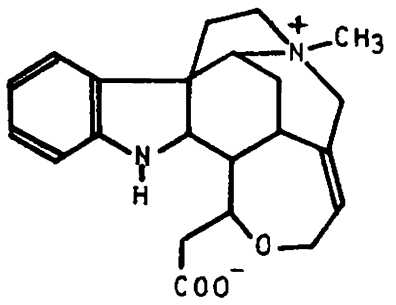
59, R = $CH_2CH_2CH_3$

60, R = $CH_2CH_2CH_2CH_3$

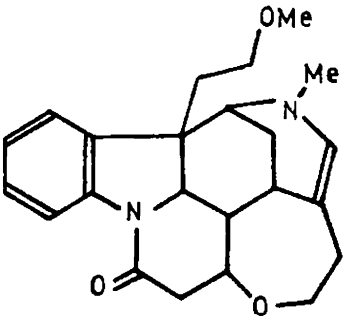
61, R = $CH_2CH_2CH(CH_3)_2$

2.1.5 Modification at Position 19

Strychnine N-oxide (62) (Genostrychnine) was prepared by Pectet et al.[34] . An improved method was suggested later[35] . 30% Hydrogen peroxide at 100° converted strychnine to its N-oxide. Strychnine methosalts, when heated with aqueous alkali, the lactam ring was opened to give strychnic acid methyl betaine (63)[36,37] but when either the betaine or the methosalts were treated with hot methanolic sodium methoxide, methoxylating fission occurred to give methoxymethyldihydroneostrychnine

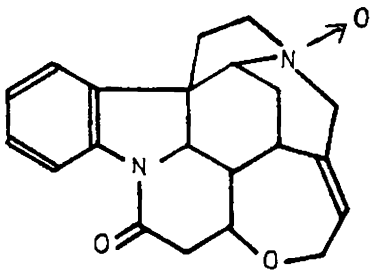


63

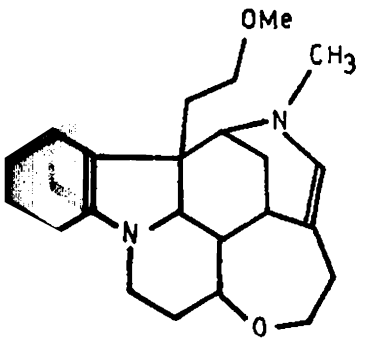


64

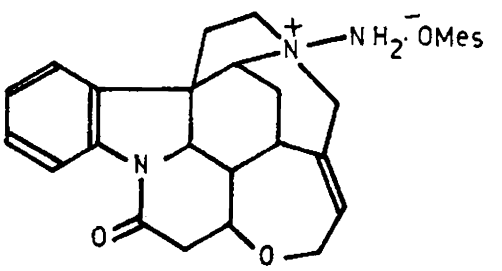
(64)[3,36,37] . The fission was probably preceded by the migration of the double bond to the neo position (C-20 to C-21).



62



65



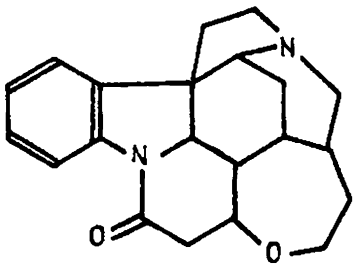
66

A parallel reaction occured when strychnidine 19-mono and 9,19 - dimethosalts were treated with sodium methoxide. Both gave methoxymethyldihydroneostrychnidine (65)[36] .

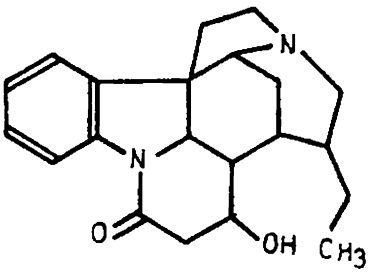
N-amination of strychnine was effected by o-mesitylene sulphonyl hydroxylamine in methylene chloride. Strychnine in methylene chloride was cooled to 0° and the above reagent in methylene chloride was added to get the N-amine 66[38] .

2.1.6 21,22 - Dihydrostrychnine and its derivatives

The straight forward hydrogenation of the 21,22 double bond in strychnine was effected by the action of hydrogen in presence of palladium-charcoal in aqueous acetic acid[39] . Strychnine was converted to dihydrostrychnine (67) and tetrahydrostrychnine (38) to hexahydrostrychnine.



67

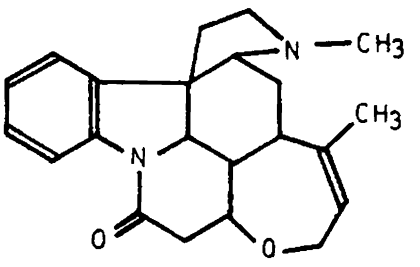


68

Strychnidine was not easily reduced with this catalyst and good results were obtained only when Adams PtO_2 catalyst was used in glacial acetic acid[40] . The hydrogenolysis of the allyl ether system was reported in which 3% of the tetrahydrostrychnine 68 was isolated from the mother liquor of the hydrogenation of strychnine to dihydrostrychnine[41] .

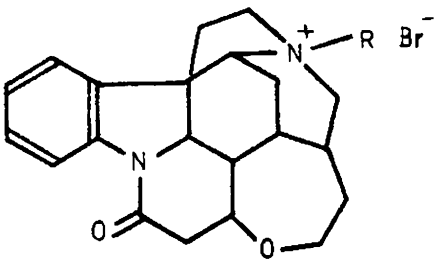
The 19-methyl strychninium ion with palladium as catalyst undergoes allylic hydrogenolysis to give 69

as the major product, together with small quantities of the 14,21 double bond isomer and two epimeric 21,22-dihydro derivatives[42] . Adams catalyst showed a different pattern in the reaction giving 3% of allylic hydrogenolysis in water at 70°, 40% of the dihydroderivative of 69 in methanolic ammonia, the remainder being 21,22-dihydrostrychnine methosalt[43] .



69

Some quarternary ammonium salts of 21,22-dihydrostrychnine were synthesised in 1962[44] . Dihydrostrychnine (67) in ethylmethyl ketone when treated with excess alkylbromide gave the quarternary alkyl derivatives. The following compounds (70 to 78) were synthesised by this procedure.



70, R = CH_3

71, R = CH_2CH_3

72, R = $CH_2CH_2CH_3$

73, R = $CH(CH_3)_2$

74, R = $CH{=}CHCH_3$

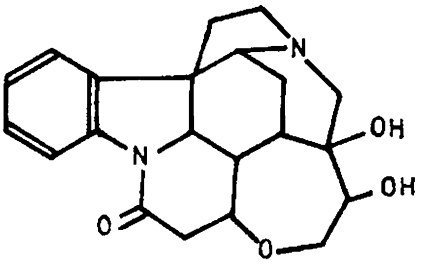
75, R = $CH_2CH_2CH_2CH_3$

76, R = $(CH_2)_5CH_3$

77, R = CH_2CH_2Br

78, R = $(CH_2)_6Br$

21,22-Dihydroxystrychnine (79)was synthesised by the hydroxylation of 21,22 double bond leaving the 19-methylene unaffected. Thus strychnine with potassium permaganate in neutral acetone gave the diol 79[45] .



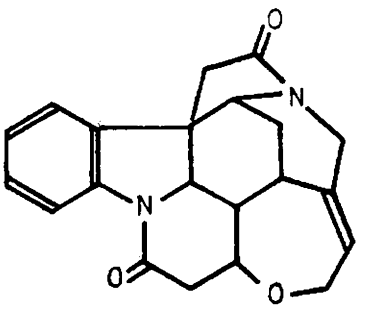
79

2.1.7 Spectral and chromatographic studies of strychnine derivatives

Chemical ionisation mass spectrometry of several alkaloid molecules like strychnine was studied using CH_4 (CH_5^+) as reactor gas[46] . In the chemical ionisation mass spectrum of strychnine the Q M^+ ion, m/e were located. Strychnine was known for its reluctance to fragment even in electron ionisation mass spectrometry. High resolution field desorption mass spectrometric studies were carried out on pharmacologically active compounds including strychnine[47] . In general the information obtained allowed the molecular weight of the intact salt to be determined. Some aspects of chemical ionisation mass spectroscopy using ammonia as reagent gas was found to be a valuable technique for biomedical and natural products studies[48] . Mass spectrometric characterisation data for some drugs and their biotransformation products as well as intermediate products were studied in 1983[49] .

The proton magnetic resonace spectra of strychnine and some of its derivatives were studied by Luther et al.[50]. The ^1H NMR spectra of 18-oxostrychnine (80) and neostrychnine (81) assignment helped the study of

^{1}H NMR spectrum of strychnine. The choice of the derivatives was made to provide further information about the hydrogens near the basic nitrogen.



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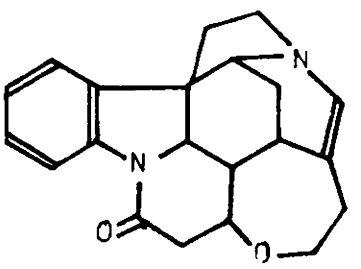


81

Both 80 and 81 has the same basic frame work of atoms as strychnine, yet provide significant change in the vinyl and aliphatic region. The spectra were recorded in 20% C_6D_6-80% $CDCl_3$ at 250 MHz. A reevaluation of the ^{1}H NMR assignments of strychnine was made at 250 MHz using a combination of aromatic solvent induced chemical shift changes, selective deuteration, double resonance technique and computer simulation of the spectra[51] .

^{13}C NMR spectra had been determined for strychnine and a series of fourteen derivatives[52]

The assignments were based on previously established data and on off-resonance decoupled spectra. The shifts resulting from the alterations in molecular structure were discussed. ^{13}C NMR data for a series of natural and semisynthetic aromatic, hydroxy and methoxy substituted strychnos alkaloids were presented and this was useful to determine substituent induced chemical shift values for the various substitution patterns[53] .

The effect of substitution on the retention in HPLC of strychnine derivatives were studies by Iskander *et al*.[54]. The capacity factor k, relative retentions α_{sp} values were measured on μ - porasil columns for 33 strychnine derivatives using $CHCl_3$ - MeOH (containing *ca* 2% NH_4OH)(93:7) as eluent in normal phase chromatography. A series of 2-carbamoyl strychnine derivatives, strychnine 2 and 3 substituted products, compounds substituted at 16 position of strychnine, 21,22 dihydrostrychnine and its derivatives and a few 19-substituted compounds were subjected to the HPLC studies[54] .

2.2 PHARMACOLOGICAL STUDIES

2.2.1 Introduction

Strychnine was probably introduced by Arabian physicians as a treatment for sores, boils, ulcers and abscesses (Nuxvomica = Ulcer nut)[55]. It was used in Europe from 1500 to 1800 to poison cows, dogs and rodents. It has an intensely bitter taste and has got a traditional but quite unjustified reputation as a bitter tonic[56]. Though useless as a therapeutic agent in modern medicine it is used for poisoning rats, moles and other pests. Strychnine is an important pharmacological tool as a central nervous system (CNS) stimulant and its significance is unique because its mechanism of action is well established[57].

A clear and vivid picture of the pharmacological actions of strychnine was achieved through the enormous work done on strychine during the span of nearly hundred and fifty years. The so called 'cruel poison'[55] labelled also as an 'out moded'[58] toxic drug has however been a topic of interest for pharmacologists as an indicator in studies on drug metabolising enzymes.

2.2.2 Action of strychnine

The therapeutic application of strychnine is highly limited because in most cases one cannot limit its

action to a single part of the Central Nervous System. It is impossible to stimulate the vasomotor centre by the use of strychnine without danger of inducing fatal convulsions as a result of the wide spread stimulationof the other areas. Stimulation with strychnine is usually followed by depression. Actually strychnine is not an emetic and the word vomica means depression or cavity, a feature of the strychnos seed attributed by legend to its digital imprint[57] .

On the CNS it produces excitation in all portions. This effect however, does not result from direct synaptic excitation. The neuronal level of excitability is increased by selectively blocking inhibition. Nerve impulses are normally confined to appropriate pathway by inhibitory influences. When inhibition is blocked by strychnine ongoing neuronal activity is enhanced and sensory stimuli produce exaggerated reflex effects.

The strychnine convulsions has a characteristic motor pattern and it differs in three respects from those induced by other analeptics. They are not accompanied by loss of consciousness, essentially reflex in nature and involve simultaneous contraction of the agonist and antagonist muscles. These features are a consequence of the fact that strychnine antagonises the action of glycine.

Glycine is a mediator of post synaptic inhibition in the spinal cord but not in appreciable amounts in the higher reaches of the brain. Convulsive movements though violent are co-ordinated. Intermittant thrusts are initiated by a sensory stimulus, usually when the strychnine concentration is lower than that required for the sustained tonic convulsion. Further toxic effects involve contraction of the diaphragmatic, thoracic and abdominal muscles leading to respiratory arrest and finally to medullary paralysis[59] .

The symptoms of poisoning may be sudden or may develop gradually. In the latter case there is stiffness of the jaw, face and neck, increased reflex and muscular twitching. The convulsive spasms are of reflex origin but they can be induced by the slightest sensory stimulus. This is a clear consequence of the fact that strychnine acts primarily to reduce inhibition in the spinal cord. During the strychnine convulsion, the body is arched rigidly backwards (opisthotonus) and the facial muscles are set in a fixed grin or grimace - the risus sardonicus. The muscles of respiration are tonically contracted so that breathing ceases during the convulsive spasm. Since consciousness is not lost, the person poisoned with strychnine suffer agonising pain from the violently contracting muscles. The calm which comes during the rela-

xation of the muscles is over shadowed by the knowledge that another period of convulsive spasm with its attendant agony is inevitable. The fact that consciousness is maintained in strychnine poisoning redoubles the torture. It eventually causes death from asphyxiation and exhaustion.

A dose of 0.25 mg of strychnine injected subcutaneously is sufficient to produce convulsions in the frog. The effects of strychnine are often ascribed to a spinal locus of action and the convulsion is frequently termed a spinal convulsion. The medulla is affected by strychnine at dosages that produce hyper excitability throughout the CNS. However as strychnine does not selectively stimulate the medulla, the drug is not therapeutically useful as a respiratory analeptic.

Strychnine has no direct effect on the heart or the blood vessels (cardiovascular system). Complex changes in blood pressure that occur in strychnine convulsions are related to the effects of the drug on the vasomotor centre including those of the spinal cord. A stimulatory effect was presumed on the gastro-intestinal system and was employed for atonic constipation. Experiments in both animals and man have failed to demonstrate such stimulation with concentrations that can be applied clinically. The bitter taste of strychnine detectable in

very dilute solutions has led to the use of the drug as a stomachic and bitter. Bitters are supposed to stimulate the taste buds, increase the appetite and reflexly stimulate the gastric secretion. Convulsive doses of strychnine have no detectable effect on skeletal muscle. Increased muscle tone is purely the result of central action of the drug. In supra convulsive doses a curariform action on the neuro muscular junction is observed.

2.2.3 Mechanism of action

The convulsant action of strychnine has often been attributed to interference with central inhibitary process. Blockade of spinal inhibition by subconvulsive doses of strychnine was first demonstrated by Eccles and coworkers[60]. Strychnine interferes only with post synaptic inhibition which is mediated by many known pathways in the brain and spinal cord. Well known examples of post synaptic inhibition are the inhibitory influences existing between the motoneurons of antagonistic muscle group and recurrent spinal inhibition mediated by the Renshaw cells. These cells liberate acetylcholine, strychnine blocks recurrent inhibition at the Renshaw cell-motoneuron synapse.

Glycine is the predominant post synaptic inhibitory transmitter to motoneurons and interneuros in the spinal cord. An important part of this evidence is the ability of strychnine to block selectively both synaptically

evoked post synaptic inhibition and the identical inhibitory effects of glycine on spinal neurons. Strychnine acts as a competitive antagonist of the inhibitory transmitter at post synaptic inhibitory sites in the same manner as curare blocks acetylcholine at the neuromuscular junction[61] . Tetanus toxin also blocks post synaptic inhibition but it acts by preventing release of glycine from inhibitory interneurons. The pharmacology of post synaptic inhibition has been reviewed by Curtis[62] . The relation between the effect of drugs on post synaptic inhibition and on peripheral cholinergic synapses are discussed by Esplin et al.[63] .

The glycine receptor antagonist ^{3}H labelled strychnine was bound irreversibly to rat spinal cord membranes upon UV illumination[64] . The incorporation of ^{3}H labelled strychnine into these membranes could be inhibited by glycine. The study of Kehne et al. showed that glycine receptors are primarily located in the caudal region of the CNS[65] . It was also proved that glycine may exert a tonic inhibitory influence. In 1981 the glycine receptors were located by auto radiograph in the rat central nervous system using ^{3}H labelled strychnine[66] . The distribution of glycine receptor is greatest in the grey matter of the spinal cord. The anatomical localisation

of binding sites may help to explain many of the signs and symptoms of strychnine ingestion. An interaction of the anthelmintic avermectin with glycine receptor was reported by Graham et al.[67] . The mechanism of glycine binding sites were clearly understood by fluorescence studies on strychnine[68].

Strychnine is readily absorbed from the gastro-intestinal tract and parentral site of injection. Both plasma and erythrocytes carry it and readily leaves the circulation for the tissues. There is no high concen - tration of the drug in the CNS. Strychnine is readily metabolised, mainly by the enzymes of the hepatic micro- somes[69] . Approximately 20% of the alkaloid escapes into the urine. The rate of destruction of strychnine is such that approximately two lethal doses can be given over a period of 24 hours without noticable toxic symptoms or cumulative effects.

2.2.4 Poisoning and treatment

Poisoning from strychnine occurs from rodenticides and sugar coated proprietary cathartic and tonic tablets. The majority of accidental cases of poisoning are in children. Even 15mg of strychnine may be fatal. The fatal adult dose is about 50 - 100mg. But 30mg has been proved to be lethal[70] . The prevention of

convulsion and support of respiration are most urgent in the treatment of strychnine poisoning. CNS depressants antagonise the effect of strychnine so that effective respiration is possible. Short acting barbiturates were preferred for combating strychnine convulsions.

The study of mutual antagonism between barbiturates and strychnine is of two fold interest. Barbiturates have been used as an antidote for strychnine poisoning and strychnine has been used as antidote for barbiturate poisoning. Barbiturates antagonise strychnine convulsion and may even raise the lethal dose of strychnine. Phenobarbitone has been successfully used for the suppression of convulsions in strychnine poisoning[71] . It has been proved that pentylenetetrazol enhances the life saving action of barbiturates in strychnine poisoning[72] . The antagonistic action of strychnine against barbiturate have been studied by various workers[73-80] . But it has not been demonstrated as a life saving drug in barbiturate intoxication[77] . There are reports of recovery from severe strychnine poisoning with barbiturate treatment[71] . Phenobarbitone in moderate doses are given first and anaesthetic doses followed when required.

Although short acting barbiturates have long been used for combating strychnine convulsions, the limited

clinical experience indicates that diazepam is superior to barbiturates and it is considered as the drug of choice in poisoning. Prompt and continuous control of convulsions by diazepam has proved successful in the management of strychnine poisoning[81-83] . A more rational therapeutic approach is based on the inhibition of tonic activity by diazepam. The drug appears to have a localised effect at the spinal cord level, where the effects of strychnine are more prominant[84] . The antagonistic action of diazepam in strychnine poisoning is probably effected by the centrally acting muscle relaxant properties.

All forms of sensory stimulations has to be minimised in treatment. Intubation and mechanical respiration may be required. Gastric lavage may be effective after the convulsions are controlled. Potassium permanganate is an effective chemical antidote and may be used for gastric lavage in a 1:5000 concentration. Iodine Tincture, tannic acid in the form of strong tea or activated charcoal are other supportive measures.

2.2.5 Chemical changes during convulsions

The level of gama amino butyric acid (GABA), prostaglandins, dopamine, acetylcholine etc. in the brain during the strychnine convulsions were of great significance in understanding the brain functions. The GABA

content in the brain is considerably reduced during strychnine convulsions[85]. During convulsions induced by strychnine (2mg/Kg subcutaneously) a significant decrease in the brain GABA level was found in comparison to controls.

An increase in the brain prostglandins during strychnine convulsions was reported by Forstermann et al [86]. This is thought to be due to increased neuronal activity and not to hypoxia. The levels of prostglandins D_2 (PGD_2) and prostaglandin $F_{2\alpha}$ ($PGF_{2\alpha}$) being the major prostglandins formed in mouse brain invivo were determined using a radio-immunological assay technique. Under basal conditions, they were less than 8.49mg/g for PGD_2 and less than 3.76mg/g for $PGF_{2\alpha}$. If convulsions were induced with spinal cord convulsants like strychnine, no increase in brain prostaglandins was seen although the occuring hypoxia were very similar. Therefore hypoxia does not seem to play a significant role in prostaglandin increase.

Effect of dopamine on strychnine induced seizures was studied on domestic fowl [87]. The experiment was done on young chicks. The susceptibility of chicks to strychnine seizure decreased profoundly with age. Dopamine protected the chicks against strychnine seizures dose-dependantly. The anti convulsant effect of dopamine against

strychnine seizure decreased with age of chicks. Pimozide effectevely blocked the anti convulsant effect of dopamine against strychnine seizure.

The acetylcholine content of the spinal cord during the seizure caused by strychnine was evaluated in rats with intact spinal cord and rats with spinal cord cut at thorcic level[88] . A reduction in acetylcholine was observed during seizure in rats with intact spinal cord.

As a novel method for the recognition of organic molecules on bio membranes the fluorescence studies were introduced[68,89] . The binding of small confirmatory rigid molecules to biological membranes was examined by using strychnine which was labelled directly with a fluoresent probe to mark the strychnine binding sites in rat spinal cord and brain stem. In another experiment 3-aminostrychnine was coupled with fluorescein isothiocynate to mark strychnine binding sites on the rats spinal cord[90] . Specific binding of strychnine could be demonstrated. In all these cases the addition of glycine to strychnine labelled synaptosomal fraction caused a decrease in fluorescence indicating a displacement of labelled strychnine by glycine. Addition of GABA had no effect on fluorescence.

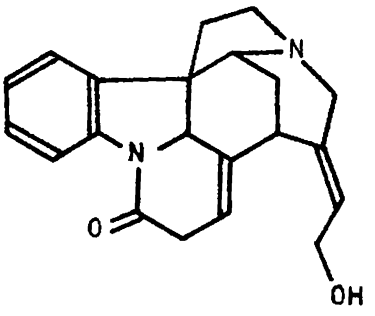
The influence of sex difference on the pharmacological action and metabolism of some drugs were studied by Kato et al[91]. Sex hormone treatment modified this effect.

Adult male rats were found to have much higher tolerance for strychnine than females, after subcutaneous and intraperitonial administration but not following intravenous dosage[92] . The result indicates that this difference is due to the higher strychnine metabolic activity in the liver microsomes of male rats compared with those from the female rather than to a difference in sensitivity of their respective nervous systems to the drug or penetration of the drug. The difference is due to the greater activity of the microsomes. An earlier study on frog demonstrated that the female animals possess the greater sensitivity to poison[93] . The toxicity studies were done in male and female Rana temporaria.

2.2.6 Pharmacological action of derivatives

The pharmacological action of some of the strychnine derivatives were studied in comparison to that of strychnine (1). Strychnine N-oxide (62) was found to be a promising derivative with attenuated toxicity and was reported in 1925 by Polonowski[94] . Genostrychnine (strychnine N-oxide) is similar to strychnine in its pharmacological action but it is less toxic. The biological test of genostrychnine showed that its lethal dose was much less than that of strychnine[95] . The minimum lethal dose

of strychnine (in rat weighing 160g) was 0.000385g while that of genostrychnine was 0.02g. The lower toxicity of strychnine N-oxide (62) in comparison to strychnine was confirmed by experiments on mice and white rats[96,97] . A comparitive study of the action of strychnine derivatives like strychnine N-oxide (62), isostrychnine (82), methyl strychnine, strychnine methyl sulphate (83), strychnic acid (84), isostrychnic acid (85) were done in rats, mice, rabbits, cats and dogs[98] . All these produce tetany and other effects like strychnine. Strychnic acid is having stronger action on frogs than strychnine.



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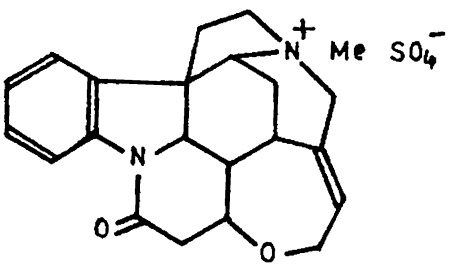

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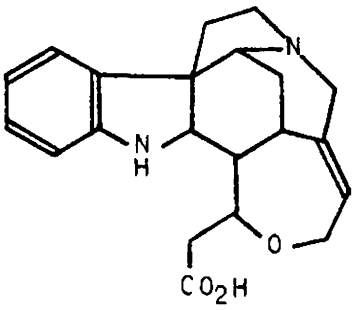

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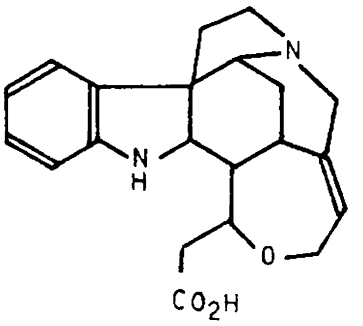

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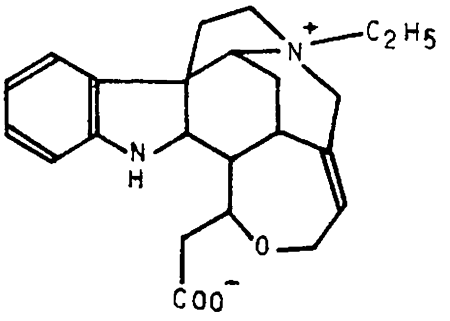

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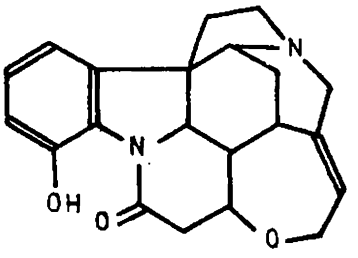
Ethylbetaine of strychnic acid (86) is reported to have relaxation effect on the striated muscles in 20 to 30 minutes which appears to be a paradoxical reaction of the strychnine derivative where strychnine causes muscular rigidity[99] . In toads treated with ethylstrychnine no convulsion was observed. Action of salts of quarternary

ammonium derivatives of strychnine and strychnine N-oxide on muscular contractibility were studied[97] . When carbon number is 4,5,10 etc the derivatives had curarizing action. Lower members showed convulsive property. In general derivatives of genostrychnine were less toxic than those of strychnine.

A comparitive study of twenty strychnos alkaloids including 4-hydroxy strychnine were subcutaneously administered to mice and the effects were studied[100]. 4-hydroxy strychnine was found to be the most effective.



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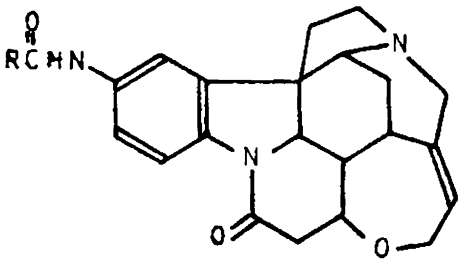
2-Carboxamide strychnine derivatives 88,89,90 were tested for their ability to inhibit choline uptake in the mouse brain synaptosomes[101] . A non competitive inhibition of the high affinity choline uptake by strychnine and some of its derivatives were observed.



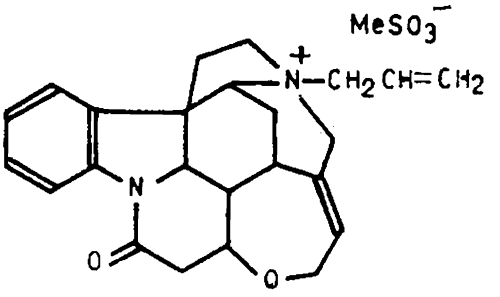
<u>88</u>, R = H

<u>89</u>, R = $(CH_2)_m CH_3$ m=0-1

<u>90</u>, R = $(CH_2)_n CH(CH_3)_2$

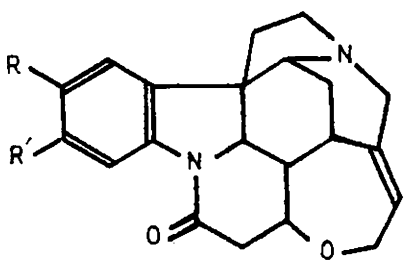
n = 1,2,3

The pharmacology of allyl strychnine mythyl sulfonate (<u>91</u>), a derivative of strychnine was reported to be almost similar to those of strychnine sulfate in lab animals[102] . This compound was found to be less potent



<u>91</u>

and less toxic than strychnine. The difference being maximum with intragastric administration. The convulsive effect of 2- and3- substituted strychnine derivatives 8,9, 10,16,18,19,20,30,92 and 93 were studied. Strychnine derivatives substituted in 2 or 3 positions were synthesised and tested for convulsant activity on mice[103] .



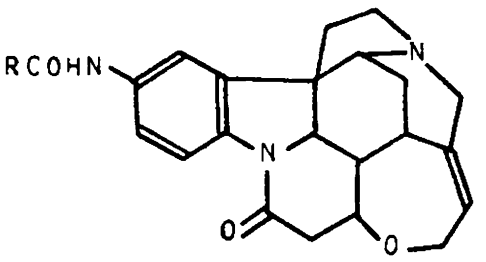
92, R = H, R' = Br

93, R = H, R' = $NHCOCH_3$

3-substituted compounds were found to be more active than the 2-substituted derivatives. A bulky group in the 2 position such as benzamido or para toluene sulphonamido decreased the activity that they showed no convulsion upto a dose of 200 mg/Kg. The 2-acetamido and 3-acetamido substituted compounds had almost no convulsant activity but had muscle relaxant effect.

Muscle relaxant activity was observed in some

of the carboxamide derivative of strychnine[104] . Ethyl, propyl and butyl derivatives 94, 95, 96 were prepared by acylation of the 2-aminostrychnine (10) with the corresponding acyl chorides. An increase in the chain length of 2-carboxamido function caused a decrease of both the LD_{50} values and ED_{50} values.



94, R = CH_2CH_3
95, R = $CH_2CH_2CH_3$
96, R = $CH_2CH_2CH_2CH_3$

The memory storage process after strychnine administration was studied. The strychnine sulfate doses are known either to enhance or disrupt memory storage process in experimental animals[105] . Differential facilitation of memories by strychnine at different times of the day were studied in adult male albino rats[106] . The retention performance at different times of the day was observed. There may be differential facilitation of retention test performance at different times of the day. In recent years strychnine has been used for the treatment

of nonketonic hyperglycemia in children, a rare metabolic disorder in infants[107] .

2.2.7 Structure activity relationship

The relation of chemical structure of strychnine to its biological action was investigated by Szabo et al.[108] . The action of strychnine is mainly due to the lactam group. It was found that the action was abolished by quarternisation. Other transformations had little influence. The role of hydroindole nitrogen in the biological activity was studied in several strychnine analogues[109] . The acid amide link of the dihydroindole nitrogen or a carbonyl group attached near to this link was found necessary to produce cerebrospinal convulsion in the frog, while the unsaturated lactam was thought to be necessary for hypertensive effect in the cat.

Structure activity relationship of strychnine derivatives modified in the non aromatic part was investigated by Iskender et al.[110]. The alteration in the non aromatic part of strychnine molecule caused less convulsion and lethal effect as compared to strychnine itself. The quarternary N-alkyl salts of these strychnine derivatives were found to have muscle relaxant property.

2.2.8 Studies on N-oxides

The discovery of geneserine (eserine N-oxide) and the study of many other alkaloid N-oxides by Polonovski and Polonovski[111] in the 1920's initiated an interesting line of research and the potential pharmacotherapeutic applications. Geneserine and the N-oxide of tropane and strychnos alkaloids were reported to exert an action similar to that of their respective tertiary amines without the toxicity[111] . The interesting principle of retained pharmacological activity and remarkable reduction in the toxicity was reported by the above authors. The N-oxides of strychnine, atropine, hyoscyamine, scopolamine and morphine, most of which have been prepared synthetically were found to have decreased toxic effects[112]. Other synthetic N-oxides investigated were those of cinchonine, acetyl morphine, aconitine, arecoline[113] and emetine[114] .

The decreased toxicity of the N-oxides was explained by their water solubility and increased excretion. The highlight of these findings was clearly the higher therapeutic index of these compounds and therefore the possibility of a broader clinical use of many such alkaloids. Morphine N-oxide was a particularly obvious case which was reported to have the same action as the parent compound,

one fourth of its activity, low toxicity and no habit forming properties[115]. It was even proposed for the treatment of addiction. The promising era of alkaloid N-oxides has thus become a historical interlude. The discovery that naturally occuring N-oxides, iodinine[116] an antibacterial and the antibiotic aspergillic acid[117] inhibit certain gram positive and gram negative bacteria as well as tubercle bacilli further revealed the significance of N-oxides. The search followed in this line led to the discovery of a number of useful chemotherapeutic complex N-oxides of benzotriazines[118], quinoxalines[119] and pyridylalanine[120] .

A synthetic N-oxide, 4-nitroquinoline N-oxide investigated by Japanese workers was found to have antibacterial[121] and antifungal[122,123] properties. Later its carcinostatic and carcinogenic properties were discovered[124]. The observation that the N-oxide group is essential for the activity has been confirmed by several workers[125,126,127]. The N-oxides of nitrogen mustards have twice the curative effect and one tenth the toxicity of its carcinostatic parent compound[128] .

In all major psychotropic drugs, tranquilisers, neuroleptics and thymoleptics there are pharmacologically

active N-oxides. The tranquliser chlordiazepoxide is an example. The corresponding compound benzodiazepine derivative without oxygen attached to the nitrogen atom is also active[129]. One of its metabolites is a lactam which retains both N-oxide and psychotropic activity[130]. The neuroleptic drug chlorpromazine is transformed into numerous metabolites including the N-oxide which was found in the urine of patients[131] . It is less potent than the parent compound or desmethyl chlorpromazine but more potent than the chlorpromazine sulfoxide[132] . The same workers found that the N-oxide is the only major chloropromazine metabolite showing a lag in onset of action. The lag suggests that it has no activity *per se* but is transformed to active metabolite.

Another drug forming an active N-oxide metabolite is the vasodialator diallyl melamine. Its N-oxide formed in rats and dogs is twenty times more potent than the parent compound[133]. A vast number of N-oxides which are more active and less toxic are reported.

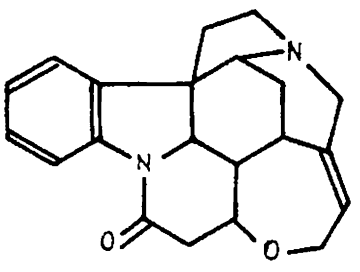
CHAPTER III

SYNTHETIC STUDIES

3.1 RESULTS AND DISCUSSION

3.1.1 Introduction

Strychnine (1) the major alkaloid present in the seeds, bark and leaves of strychnos nuxvomica was extracted from the seeds and purified to get 1.5% of strychnine according to the procedure reported by Ikan[134]. As this was a tedious method and the yield was poor, strychnine was purchased as its hydrochloride for our synthetic purposes[135]. Strychnine has been reported to possess interesting biological activity as a central nervous system stimulant. However, because of its extreme toxicity it has no application in current therapeutics. As strychnine N-oxide(2) shows improved biological properties with reduced toxicity[96,97], several derivatives of strychnine were prepared by (a) electrophilic substitution at the aromatic ring, (b) reduction



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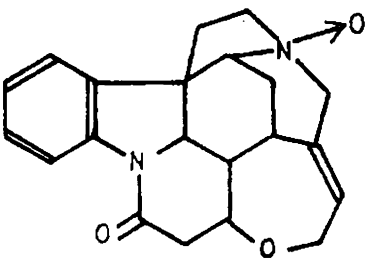

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of the carbonyl function at position 10 to give strychnidine, (c) modification of 11 position by introducing oximino group and benzylidene groups, (d) hydrogenation of the 21,22 double bond to the saturated dihydrostrychnine and further conversion into its derivatives and the final conversion of all these derivatives to their N-oxides by treatment with either hydrogen peroxide or meta-chloroperbenzoic acid.

The structures of the known compounds were established by comparison with the reported data and new compounds were fully characterised by elemental and spectral analysis. All the compounds were then tested for their CNS stimulant activity.

3.1.2 Modification of the aromatic ring

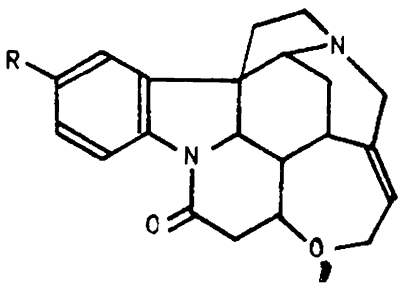
Strychnine hydrochloride[135] was treated with sodium hydroxide and the liberated free base was purified by recrystallisation from chloroform ether. This was converted to the N-oxide 2 also known as genostrychnine[96]. This N-oxide nas been isolated from the natural source, Strychnos wallichiana[136], and has also been reported to be a microbial transformation product of strychnine[137]. Nitration of strychnine using a nitrating mixture containing concentrated nitric acid and concentrated sulphuric acid in the ratio 1:2 gave 2-nitrostrychnine(3)[12] in

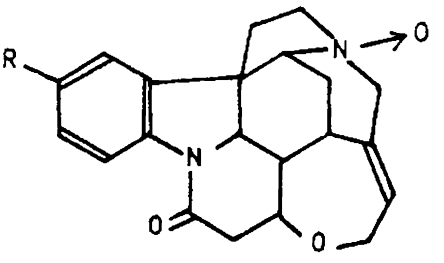
80% yield. Preparation of 2-nitrostrychnine N-oxide (4) was attempted both by N-oxidation of 3 using hydrogen peroxide and also by nitration of 2. However the second method in which strychnine N-oxide (2) was treated with a mixture of concentrated nitric acid and concentrated sulphuric acid in the ratio 1:1 was found to be a better one and the N-oxide 4 was obtained in 85.7% yield. In the infrared spectrum of 4 showed characteristic band at 1530 cm^{-1}(NO_2) and 930 cm^{-1} (N-oxide)[148].

Reduction of 3 to the 2-aminostrychnine (5) was carried out using two procedures. Reduction of 2-nitro-strychnine (3) using either sodium dithionite[13] or freshly prepared Raney Nickel [138] gave 2-Aminostrychnine (5). The sodium dithionite method gave 80% of the product where as reduction with Raney Nickel provided only 40% pure compound after recrystallisation. The identity of the products were established by a mixed melting point determination and comparison of the infrared spectra. Acylation of 5 using acetic anhydride in the presence of 20% sodium acetate in water gave 60% of the 2-acetamido derivative 6[13]. The conversion of 2-acetamido strychnine to its 19-N-oxide 7 was accomplished by oxidation with meta-chloroperbenzoic acid in chloroform according to a

procedure described by Craig et al.[139].

The infrared spectrum of 7 showed absorption at 3410 cm^{-1} (NH), 1640 (NHC=O), 1650 cm^{-1} (C=O), 925 cm^{-1} (N→O).





3, R = NO_2

4, R = NO_2

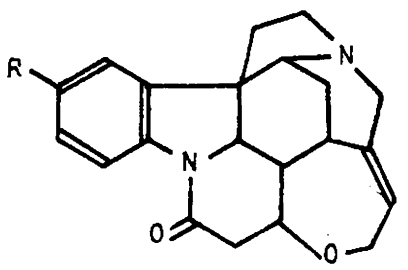
5, R = NH_2

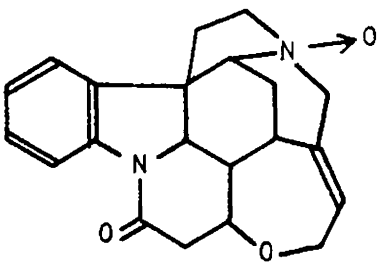
7, R = $NHCOCH_3$

6, R = $NHCOCH_3$

The treatment of strychnine with chlorosulphonic acid [140] at 0° followed by decomposition of the excess chlorosulphonic acid with ice gave 2-chlorosulphonyl strychnine (8) as a white precipitate which was not fully characterised at this stage. Treatment of 8 with concentrated ammonia solution provided strychnine 2-sulphonamide (9). The preparation of 9 is of special interest because attempted sulphonation of strychnine had

been unsuccessful so far[141]. Also as sulphonamide derivatives are of well known biological activity[142], incorporating a sulphonamide group into the aromatic ring of strychnine molecule was highly desirable. The structure of the sulphonamide 9 was established by its spectral data and elemental analysis. Conversion of 9 to its 19-N-oxide was also accomplished by oxidation with 30% hydrogen peroxide at 100°. The N-oxide 10 was fully characterised using spectral and elemental analysis.





8 , R = SO_2 Cl

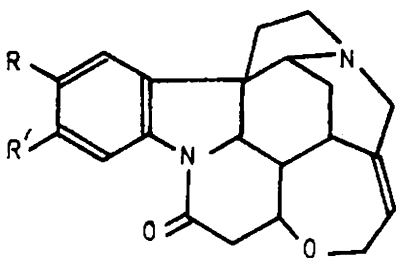
9 , R = SO_2NH_2

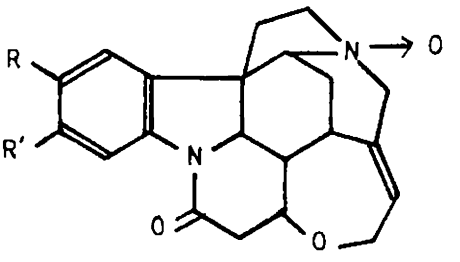
10 , R = SO_2 NH_2

Bromination of strychnine with bromine in hydrobromic acid gave the known 2-bromostrychnine (11) in 75%

yield[11]. 2-Bromostrychnine (11) was converted into its N-oxide in 50% yield by oxidation with hydrogen peroxide at 100°.

Another strychnine derivative with modified aromatic ring that was evaluated pharmacologically was brucine, (13)[57]. This is present in Strychnos nuxvomica along with strychnine. Brucine for the experimental work was obtained commercially[135] and purified. Brucine was oxidised with hydrogen peroxide to its N-oxide 14 in 60% yield[34]. Brucine N-oxide was fully characterised through its spectral and chemical analysis.





11 R = Br, R' = H

12 R = Br, R' = H

13 R = R' = OCH_3

14 R = R' = OCH_3

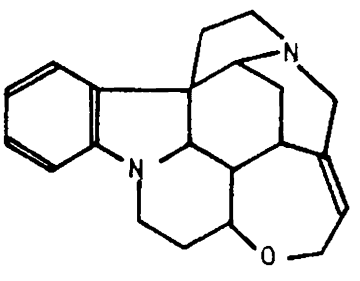
As there are reports[110] that substitution on the aromatic ring in the strychnine molecule do not substant-

ially alter the pharmacological activity or toxicity, substitution products at other positions in the aromatic ring were not prepared.

3.1.3 Modification of position 10

The only modification attempted at position 10 was reduction of the carbonyl group to a methylene group. The two methods reported for this reduction are the electrochemical method[17] and lithium aluminium hydride reduction[18]. As lithium aluminium hydride reduction is much easier, conversion of strychnine (1) to strychnidine (15) was achieved by this method. Strychnidine was obtained in 58% yield. Although some pharmacological studies on strychnidine have been carried out, the preparation or pharmacology of strychnidine 19-N-oxide has not been reported.

Strychnidine N-oxide (16) was prepared in 50% yield by treatment of strychnidine with 30% hydrogen peroxide. The infrared spectrum of strychnidine N-oxide did not show any absorption for the amide carbonyl group



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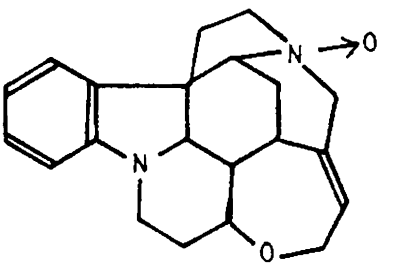
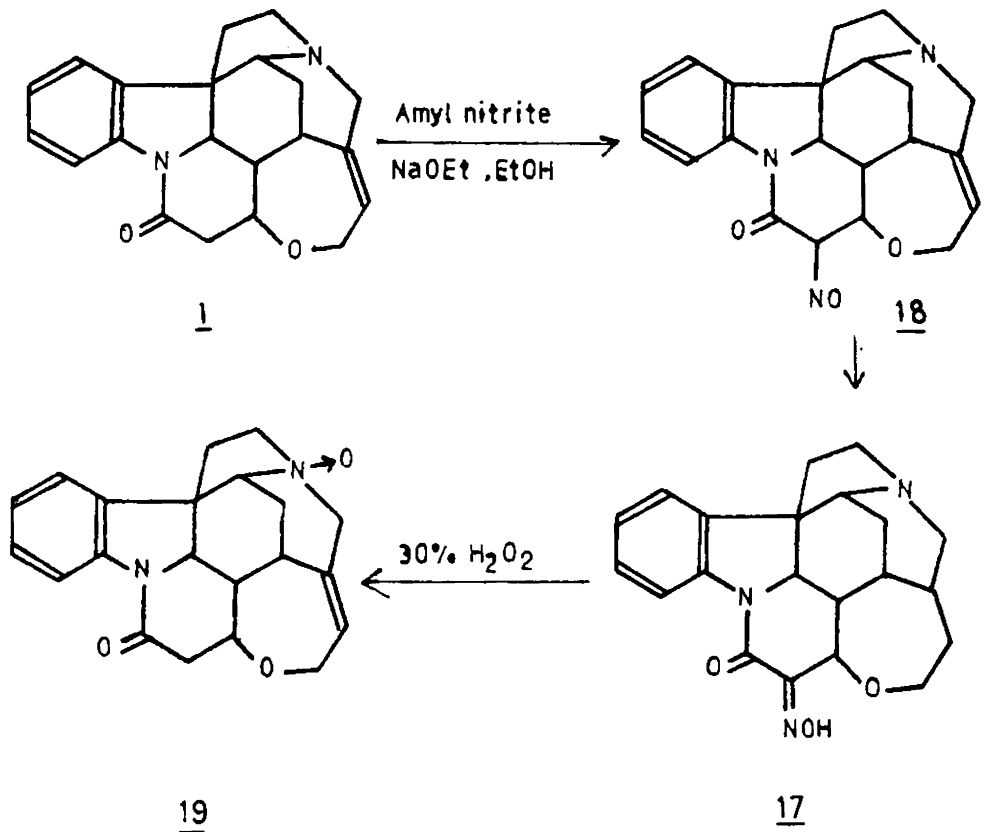


16

at 1640 cm^{-1}. The elemental analysis was also in agreement with molecular formula .

3.1.4 Modification of position 11

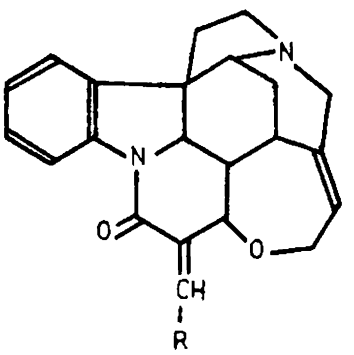
In order to study the pharmacological effect of change at the 11 position of strychnine molecule, a few derivatives were prepared by making use of the reactivity of the active methylene group. Thus treatment of strychnine with amyl nitrite in the presence of sodium ethoxide in ethanol provided the 11-oximino strychnine (17) which must have been formed through the 11-nitrosostrychnine(18) by tautomerism. Oxidation of 11-oximinostrychnine (17)

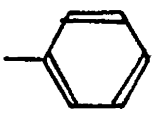


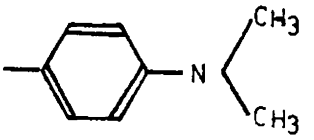
1 18 19 17

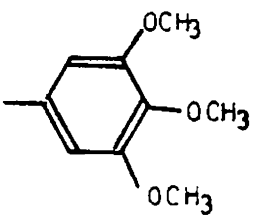
using 30% hydrogen peroxide gave the N-oxide 19 in 60% yield.

The treatment of strychnine with benzaldehyde in presence of piperidine in absolute alcohol gave the condensation product, 11-benzylidene strychnine (20) in 60% yield. Similar treatment of strychnine with para-dimethylamino benzaldehyde and 3,4,5 trimethoxy benzaldehyde also produced the corresponding condensation products 21 and 22 respectively in 65.8 and 48% yields.

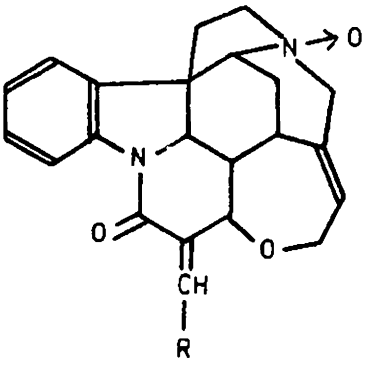


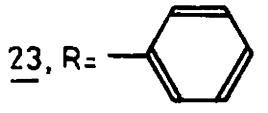
20, R = 

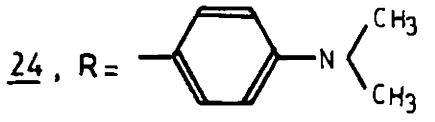
21, R = 

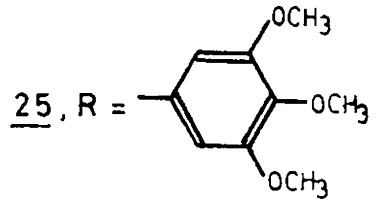
22, R = 

These benzylidene derivatives were then treated with m-chloroperbenzoic acid when their N-oxides 23,24 and 25 were obtained in 85%, 47.8% and 66.6% yields respectively. The structures of the benzylidene derivatives and their N-oxides were established by spectral and chemical analysis.





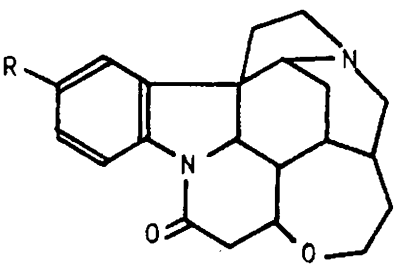




Attempted Mannich reactions in strychnine with a view to introduce amino methyl groups at position 11 were not successful.

3.1.5 Derivatives of 21,22-dihydrostrychnine

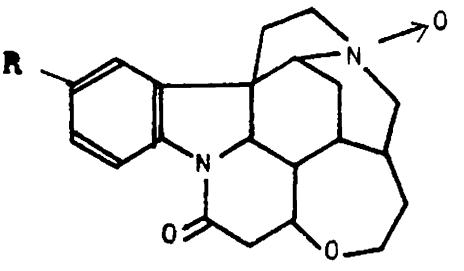
A systematic pharmacological study of 21,22-dihydrostrychnine and its derivatives have not been reported. 21,22-Dihydrostrychnine 26 was therefore prepared by the known procedure[39] of hydrogenation of strychnine in the presence of 5% palladium on carbon as catalyst using 50% acetic acid as solvent. Dihydrostrychnine was obtained in 70% yield and had the reported physical data[39]. The N-oxide 27 of dihydrostrychnine was prepared in 75% yield by the oxidation of 26 with 30% hydrogen peroxide. Also nitration of the dihydrostrychnine with 1:1 mixture of concentrated nitric acid and concentrated sulphuric acid gave 2-nitro - 21, 22-dihydrostrychnine (28) in 40% yield. Treatment of the nitro compound with



26 , R = H

28 , R = NO_2

either 30% hydrogen peroxide or m-chloroperbenzoic acid was not successful in getting a solid N-oxide. Therefore the corresponding N-oxide 29 was prepared by nitration of 21,22-dihydrostrychnine N-oxide using a nitrating mixture of concentrated nitric acid and concentrated sulphuric acid. The N-oxide 29 was characterised by its infrared spectrum which showed absorption at 1510 cm^{-1}(NO_2) and 960 cm^{-1} (N-oxide).



27 , R = H

29, R = NO_2

3.1.6 Miscellaneous Reactions

Treatment of strychnine with benzoyl peroxide in chloroform gave a product 30 that appeared to be a trichloro methyl derivative from its elemental analysis.

Attempts to get an insight into its structure by hydrolysis under acidic and basic condition gave back strychnine. Thus treatment of the above product with orthophosporic acid, triphenyl phosphine, para-toluene sulphonic acid in methanol and morpholine yielded a product which corresponded to strychnine which may be formed by hydrolysis followed by decarboxylation.

The reaction of chloroform in the presence of benzoyl peroxide is known to add a CCl_3 group to a double bond[143] or at a position that will produce a stable free radical[144]. The mechanism of the reaction has been proposed to take place by the formation of CCl_3 radical[144]. The structure of 30 has not been established because being a very complex molecule its ^1H spectra cannot be completely interpreted. Also a useful mass spectrum was not received as the molecule was highly non volatile. The infrared spectrum was consistant with structural features of strychnine and the elemental analysis of the product indicated a molecular formula $C_{22}\ H_{21}\ Cl_3\ N_2\ O_2$. However, because of its significantly reduced toxicity, it was converted into its N-oxide 31 by treatment with 30% hydrogen peroxide. The N-oxide also has an elemental analysis corresponding to the molecular formula $C_{22}\ H_{21}\ Cl_3\ N_2\ O_3$. Because of their interesting pharmacological

activity, these compounds are included in this thesis although their structures have not been established so far.

3.2 EXPERIMENTAL

3.2.1 General

All melting points were taken using capillary tubes on a melting point bath containing liquid paraffin and are not corrected. Thin layer chromatography was performed on 5.0 x 20 cm glass plates coated with silicagel G using chloroform-methanol (different proportions as mentioned) as the developing solvent. Compounds were detected either by their colour or by developing with iodine. NMR spectra were run in deuterio chloroform or dimethyl sulfoxide-d_6 using a Hitachi R-600 FT NMR spectrometer and Varian FT 80 A spectrometer at 60 MHz or a Jeol FX 90 Q NMR spectrometer at 90 MHz with tetramethyl silane as internal standard. Infrared spectra were recorded on a Perkin Elmer Model 682 or 781 grating spectrophotometer. Ultra violet spectra were obtained using Hitachi 200-20 Model UV-Vis spectrophotometer in methanol. Optical rotation was measured on a Digital Type 71 Jobin Yvon Polarimeter in a 3 ml capacity cell at the Raman Institute, Bangalore. Elemental analyses were performed at the Central Food Technological Research Institute, Mysore, National Chemical Laboratory, Pune and Indian Institute of Science, Bangalore.

3.2.2 Isolation of strychnine (1)

(a) From Strychnos nuxvomica seeds[134]

Ground nuts of Strychnos nuxvomica (200 g) were throughly mixed with 200 ml suspension of 10% calcium hydroxide in water and left overnight at room temperature. After air drying, the slurry was extracted with chloroform in a Soxhlet extractor for 3 hours. The chloroform solution was extracted several times with 5% sulphuric acid solution and subsequently basified with 10% aqueous sodium hydroxide. After cooling, the crystals were separated, 1.5 volume of 50% ethyl alcohol was added and the mixture was refluxed until most of the solid had dissolved. After addition of a little activated charcoal, the solution was filtered hot and left overnight. The crystals of strychnine were filtered and washed with a little 50% ethyl alcohol. The mother liquor and washings were kept for the isolation of brucine. The yield of this crude material was 3 g.

The crude strychnine was dissolved in 9 volumes of boiling water and 15% hydrochloric acid was added slowly until the mixture was slightly acidic to congo red. Activated charcoal was added and the solution was refluxed 1 hour and filtered hot. The hydrochloride which crystallised on cooling was filtered and washed with cold water.

This hydrochloride was dissolved in 15 volumes of water at 80° and neutralised with 10% aqueous sodium carbonate, after addition of charcoal, the solution was filtered hot. Strychnine precipitated on addition of aqueous sodium carbonate and cooling. The precipitate was filtered and washed with cold water, recrystallisation from ethanol yielded 2 g of strychnine of m.p. 286 - 288° (lit.[145] m.p. 286 - 288°).

(b) From commercially available strychnine hydrochloride

Strychnine free base was also prepared by dissolving strychnine hydrochloride[135] in water followed by filtration of the solution and treatment with ammonium hydroxide to pH 8. The precipitated free base was filtered, washed with cold water and dried. It was recrystallised from chloroform-ether to get pure strychnine. The compound charred at 240° and melted with decomposition above 270°.

$[\alpha]_D^{20}$ = -138.8°, c 0.13 ($CHCl_3$)

UV : λ_{max} : 254 nm ($\epsilon = 2.8 \times 10^4$), 211 nm ($\epsilon = 5.7 \times 10^4$)

IR (KBr) : 1670 cm^{-1} (C = O)

(Lit.[145] m.p. 286 - 288°)

$[\alpha]_D^{18}$ = -139.9° ($CHCl_3$), $[\alpha]_D^{20}$ = -104 (EtOH)

λ_{max}^{EtOH} 254 nm (ϵ = 1.5 x 10^4), 278 nm (ϵ = 4.3 x 10^3)

288 nm (ϵ = 3.4 x 10^3)

3.2.3 Strychnine N-oxide (2)[34]

A mixture of 3.34 g (0.01 mol) of strychnine (1) and 8 ml of water was made into a paste and heated on a boiling water bath. 30% Hydrogen peroxide solution was added drop by drop with thorough shaking while heating at 100°. When 3 to 3.5 ml of H_2O_2 was added a clear solution was obtained which was further heated for 30 minutes. The solution was cooled and the crystals formed was filtered and dried to give 2.5 g (75%) of strychnine N-oxide, m.p. 205 - 208° (Lit.[34] m.p. 209 - 212°) TLC ($CHCl_3$ - MeOH, 8:2) showed a single spot running slightly below strychnine.

$[\alpha]_D^{20}$ = +17.4°, c 0.17 (MeOH)

UV : λ_{max} 254 nm (ϵ = 2.3 x 10^4), 210 nm (ϵ = 4.7 x 10^4)

IR (KBr) : 1670 cm^{-1} (C = O), 980 cm^{-1} (N→O)

3.2.4 2-Nitrostrychnine (3)[12]

Strychnine 2.5 g (0.0075 mol) was added in portions to 10 ml of a nitrating mixture (H_2SO_4 - HNO_3, 1:2) at 0^o with stirring. The addition required 3 to 4 hours at 0^o with good stirring. Towards the end of the reaction the temperature was allowed to rise to 20^o. The viscous product was poured into 75 ml of warm water (50^o) and then cooled. The crystals of nitrostrychnine formed were collected by filteration, washed with chilled water and recrystallised from boiling water containing small amount of nitric acid. On cooling 2.0 g. (80%) of 2-nitrostrychnine was obtained as fine yellow crystals melting at 241 - 243^o (lit.[12] m.p. 240 - 43^o).

$[\alpha]_D^{20} = -65.8^o$, c 0.15 (DMF)

UV : λ_{max} 339 nm ($\epsilon = 2.7 \times 10^4$), 231 nm ($\epsilon = 2.8 \times 10^4$)
207 nm ($\epsilon = 4.1 \times 10^4$)

IR (KBr) : 1680 cm^{-1} (C = O), 1530 cm^{-1} (NO_2)

3.2.5 2-Nitrostrychnine N-oxide (4)

1.75 g (0.005 mol) of strychnine N-oxide was added in portions to 7 ml of ice cold mixture of nitric acid and sulphuric acid(1:1). The reaction mixture was stirred at 0^o for 3 hours. This viscous liquid formed was poured into 60 ml of water at 50^o. Fine crystals

formed were filtered and dried. The yield was 1.5 g (85.7%).TLC ($CHCl_3$ - MeOH, 8:2) showed a single spot having an Rf value between that of strychnine N-oxide and nitro strychnine. The compound darkened from 200°, no melting upto 270°.

$[\alpha]_D^{20}$ = -42.5°, c 0.59 (DMF)

UV : λ_{max} 328 nm (ϵ = 2.5 x 10^4), 210 nm (ϵ = 5.2x10^4)

IR (KBr) : 1680 cm^{-1}(C = O), 1530 cm^{-1}(NO_2), 930 cm^{-1}(N→O)

Anal. Calcd. for C_{21} H_{21} N_3 O_5 : C, 63.80; H, 5.31; N, 10.63

Found : C, 63.40; H, 5.29; N, 10.24

3.2.6 2-Aminostrychnine (5)[13]

(a) A suspension of 1.5 g (0.004 mol) of 2-nitro-strychnine in 10 ml of hot water was dissolved by adding few drops of 10% hydrochloric acid adjusting the pH to 3 - 4. Sodium dithionite (3 g.) was added in small portions while stirring. The addition was continued till the yellow colour disappeared. 10 ml of 50% sulphuric acid was added and the reaction mixture was heated on a boiling water bath for 3 hours. The solution was filtered while hot and ammonium hydroxide solution was added to pH 8. The crystalline precipitate obtained was recrystallised from chloroform-ether containing traces of methanol to

give 1.2 g (80%) of the product, melting at 274 - 277°, TLC examination ($CHCl_3$ - MeOH, 6:4) indicated complete conversion of nitrostrychnine to aminostrychnine.

(b) Aminostrychnine was also prepared by reduction[138] of 2-nitrostrychnine with Raney nickel. 0.3 g (0.0008 mol) of nitrostrychnine base was added to 5 g of freshly prepared Raney nickel[146] in 2-propanol under stirring. The temperature was maintained at 60° and the reaction was allowed to proceed for 3 hours. The Raney nickel residue was repeatedly washed with 2-propanol and then with methanol. The washings were collected and concentrated. TLC ($CHCl_3$ - MeOH, 6:4) showed a major spot corresponding to aminostrychnine with traces of nitrostrychnine. This material was purified by crystallisation from methanol-chloroform mixture to give 0.12 g (40%) of the product, m.p. 275 - 277°. Mixed melting point with the previously prepared sample was undepressed.

$[\alpha]_D^{20}$ = -159.5°, c 0.09 (DMF)

UV : λ_{max} 272 nm ($\epsilon = 2.8 \times 10^4$), 263 nm ($\epsilon = 1.4 \times 10^4$)
209 nm ($\epsilon = 1.0 \times 10^4$)

IR (Nujol) : 3430 & 3310 cm^{-1} (NH_2), 1650 cm^{-1} (C = O)

NMR : See fig. 3.1 ($CDCl_3$, 90 MHz)

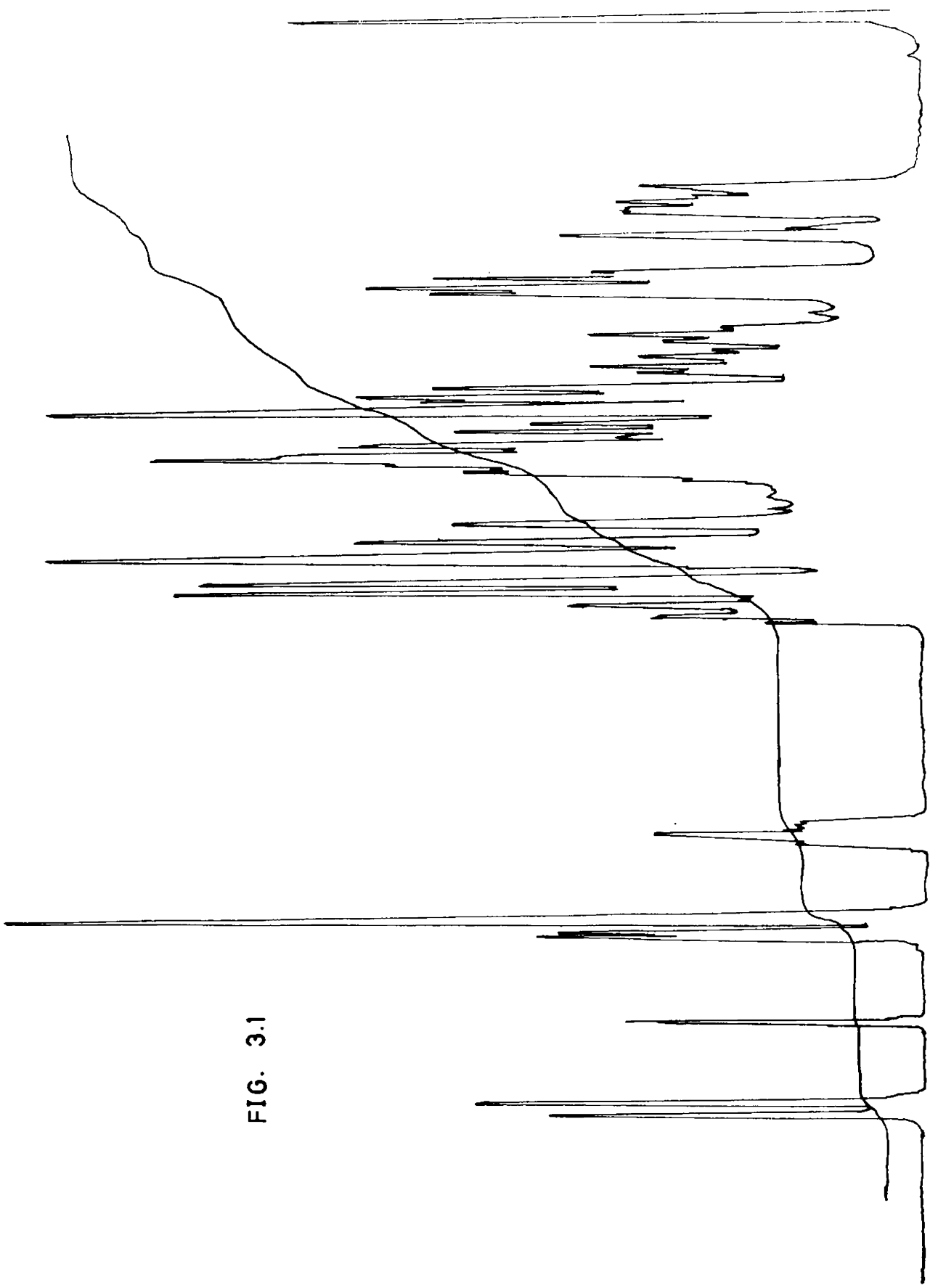


FIG. 3.1

3.2.7 2-Acetamido-strychnine (6)[13]

A mixture of 2-aminostrychnine, 2 g. (0.0057 mol) in 50 ml of water, 1 ml of acetic acid, 1 ml of acetic anhydride and 20 ml of 25% sodium acetate was heated on a water bath for 4 hours. The reaction mixture was cooled and neutralised with 10% sodium hydroxide solution to get a precipitate. The product was filtered and recrystallised from methanol to give 1.2 g (60%) of 2-acetamido-strychnine melting at 180 - 183°, (lit.[13] m.p. 183 -184°).

$[\alpha]_D^{20} = -134.9°$, c 0.13 ($CHCl_3$)

UV : λ_{max} 273 nm ($\epsilon = 1.9 \times 10^4$)

208 nm ($\epsilon = 4.1 \times 10^4$)

IR (Nujol) : 3420 cm^{-1}(NH), 1670 cm^{-1}(C = O),1650 cm^{-1}(C=O)

3.2.8 2-Acetamidostrychnine N-oxide (7)

A solution of 0.433 g (0.0025 mol) of metachloro-perbenzoic acid indry chloroform was added in portions to an ice cold solution of 0.977 g (0.0025 mol) of 2-acetamido-strychnine in chloroform. The reaction mixture was stirred for 2 hours and an additional amount 0.108 g of metachloro-perbenzoic acid was added and reaction was continued at 20° overnight. The chloroform solution was washed with sodium bicarbonate, dried and concentrated. The residue obtained was crystallised from ether-chloroform mixture containing

traces of methanol to give 0.4 g (41%) of 2-acetamido-strychnine N-oxide, m.p. 189 - 192°.

$[\alpha]_D^{20} = -120°$, c 0.11 (DMF)

UV : λ_{max} 263 nm ($\epsilon = 1.8 \times 10^4$), 207 nm ($\epsilon = 4.2 \times 10^4$)

IR (Nujol) : 3410 cm^{-1}(NH), 1650 cm^{-1}(C = O),

1640 cm^{-1}(C = O), 925 cm^{-1}(N→O)

Anal. Calcd. for $C_{23}H_{25}N_3O_4$: C, 67.81; H, 6.14; N, 10.32.

Found : C, 67.32 ; H, 6.54 ; N, 10.0

3.2.9 Strychnine-2-sulphonamide (9)

Chlorosulphonic acid[140] (5ml) was added dropwise at intervals with frequent shaking to strychnine hydrochloride, 2 g (0.0054 mol) at 0°. A clean viscous solution was formed in 2 hours. This reaction mixture was kept overnight. The viscous liquid was poured into crushed ice with stirring. The white precipitate of strychnine-2-sulphonylchloride formed was filtered and washed with chilled water. This material was treated with concentrated ammonia (10 ml) and heated on a water bath for 1 hour. The product was filtered, washed with cold water and dried. In TLC ($CHCl_3$ - MeOH, 6:4 with drops of diethylamine) it showed a single spot at the origin. The compound did not melt upto 270°.

$[\alpha]_D^{20}$ = -79.9°, c 0.19 (DMSO)

UV : λ_{max} 213 nm (ϵ = 6.1 x 10^3), 270 nm (ϵ = 3.5 x 10^3)

IR (Nujol) : 3600 - 3200 cm^{-1}(NH_2); 1670 cm^{-1}(C = O)

Anal. Calcd. for C_{21} H_{33} N_3 O_4 S : C, 61.02; H, 5.57; N, 10.17

Found : C, 61.12 ; H, 5.57 ; N, 9.82

3.2.10 Strychnine-2-sulphonamide N-oxide (10)

To a mixture of 1 g (0.0024 mol) of the amide(24) with 5 ml water was added 30% hydrogen peroxide solution dropwise while heating the mixture on a boiling water bath. After the addition of 3 ml of hydrogen peroxide solution, a clear solution was formed. This was cooled and the solid separated was filtered, washed with cold water and dried to give 0.4 g (40%) of the N-oxide charring at 235°.

$[\alpha]_D^{20}$ = -21.1°, c 0.19 (DMSO)

UV : λ_{max} 266 nm (ϵ = 6.9 x 10^3), 210 nm (ϵ = 1.4 x 10^4)

IR (Nujol) : 3300 - 3100 cm^{-1}(NH_2); 1680 cm^{-1} (C = O);
960 cm^{-1} (N→O)

Anal. Calcd. for C_{21} H_{23} N_3 O_5 S : C, 58.74 ; H,5.36; N, 9.79

Found : C, 58.37; H,5.40; N, 9.84

3.2.11 2-Bromostrychnine (11)[11]

To a solution of 3.34 g (0.01 mol) strychnine in 60 ml of water containing 4 ml hydrobromic acid was added slowly with stirring a solution of 2 ml of bromine in 60 ml

of water. The addition was done during the course of 2 hours keeping the temperature steady at 80^{o}. At the end of the reaction a clean rose red solution was obtained. On cooling this solution,a crystalline product was formed. These crystals were dissolved in water and the solution was made alkaline with ammonia to pH 8. The white precipitate formed was filtered and recrystallised from chloroform ether containing traces of methanol to give 2.5 g. (75%) of 2-bromostrychnine m.p. 212 - 215^{o} (Lit. m.p. 221 - 222^{o}).

$[\alpha]_{D}^{20} = -150^{o}$, c 0.10 ($CHCl_3$)

UV : λ_{max} 263 nm ($\epsilon = 3.9 \times 10^4$), 210 nm ($\epsilon = 4.8 \times 10^4$)

IR(Nujol) : 1680 cm^{-1} (C = O)

NMR : See fig. 3.2 ($CDCl_3$, 90 MHz)

3.2.12 2-Bromostrychnine N-oxide (12)

2-Bromostrychnine (11) 1 g (0.0023 mol) was mixed throughly with 5 ml of water and heated on a boiling waterbath. 30% Hydrogen peroxide solution was added(3 ml) until a complete solution was effected and heating was continued for 30 minutes. The mixture was cooled and ether added to get a white precipitate. It was filtered and crystallised from ethanol-ether mixture to give 0.5 g(50%)

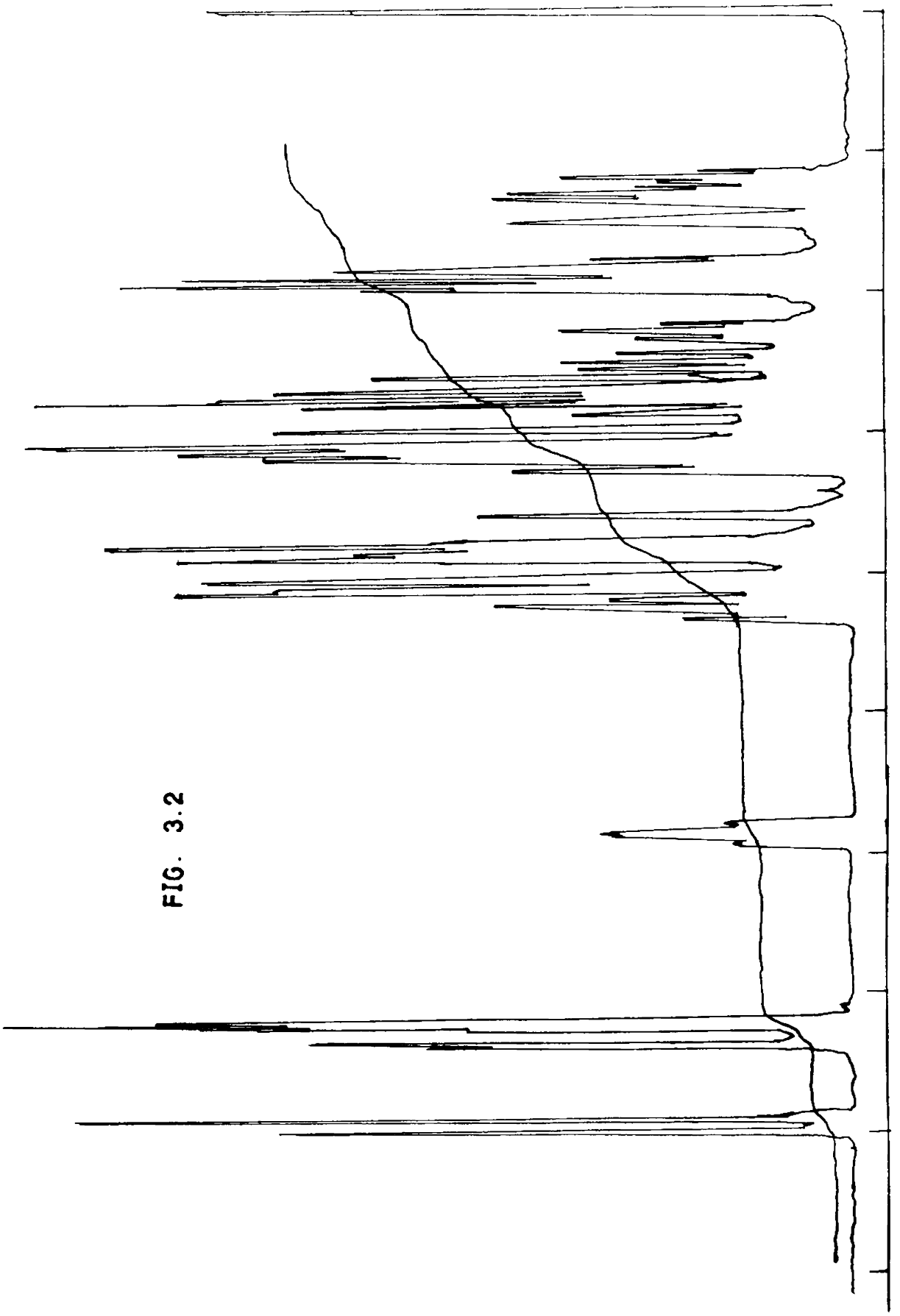


FIG. 3.2

of 12 melting at 189 - 191°. TLC ($CHCl_3$ - MeOH; 9:1) showed a single spot moving below bromostrychnine.

$[\alpha]_D^{20}$ = +24.3°, c 0.08 (DMF)

UV : λ_{max} 210 nm (ϵ = 1.0 x 10^4)

IR(Nujol) : 1670 cm^{-1} (C = O); 930 cm^{-1} (N→O)

Anal. Calcd. for C_{21} H_{21} Br N_2 O_3 : C,58.74; H,4.90; N,6.53;

Found : C, 58.45, H, 5.38; N, 6.78

3.2.13 Brucine (13)

Brucine free base was prepared by dissolving brucine hydrochloride[135] in water followed by filtration or the solution and treatment with ammonium hydroxide to pH 8. The precipitated free base was filtered, washed with cold water and dried. It was recrystallised from acetone-water to get pure brucine. The compound melted at 176 - 178° (lit. m.p. 178°).[145].

$[\alpha]_D^{20}$ = -126.3°, c 0.13($CHCl_3$), lit.[145] $[\alpha]_D^{20}$ = -127°($CHCl_3$)

UV : λ_{max} 303 nm (ϵ = 9.4 x 10^3)

Lit.[145] λ_{max} 301nm(ϵ = 8.5 x 10^3), 263 nm(ϵ = 1.2 x 10^4)

3.2.14 Brucine N-oxide (14)

1.97 g(0.005 mol) of brucine was mixed with 5 ml water and heated at 100° adding 30% hydrogen peroxide solution drop by drop with thorough stirring. A clear solution was obtained which was further heated for 30 minutes. The solution was cooled and the crystals formed were filtered and dried to get 1.3 g (66%) of brucine N-oxide melting at 174 - 176°.

3.2.15 Strychnidine (15)[18]

A suspension of 2.4 g (0.072 mol) of strychnine in 150 ml of dry tetrahydrofuran was added slowly to a refluxing solution of 2.0 g of lithium aluminium hydride in 50 ml of THF in about 4 hours. The reaction was allowed to continue for 10 hours. The mixture was cooled and the excess $LiAlH_4$ was decomposed with wet ether, ethyl acetate and few drops of water. The mixture was stirred for 1 hour and filtered. The filtrate was evaporated under vacuum and the residue was recrystallised from $CHCl_3$ containing few drops of methanol and excess ether. After repeated crystallisations 1.4 g (58%) of strychnidine was obtained, m.p. 253° - 254° (Lit.[18] m.p. 256°)

$[\alpha]_D^{20}$ = +110.5°, c, 0.10 ($CHCl_3$)

UV : λ_{max} 259 nm (ϵ = 1.4 x 10^4), 209 nm (ϵ = 4.6 x 10^4)

NMR : See fig. 3.3 ($CDCl_3$, 90 MHz)

3.2.16 Strychnidine N-oxide (16)

1 g (0.003 mol) of strychnidine was made into a paste with 5 ml water and heated on a boiling water bath. To this mixture, 30% hydrogen peroxide solution (2 ml) was added drop wise till a complete solution was effected. Heating was continued for 30 minutes and the mixture was allowed to cool. The crystalline compound formed was filtered, washed with chilled water and dried. Recrystallisation from MeOH - $CHCl_3$ - ether gave 0.5 g (50%) of the N-oxide, m.p. 172°- 174°. TLC ($CHCl_3$ - MeOH, 7:3) showed a single spot below that of strychnidine.

$[\alpha]_D^{20}$ = +23.8°, c 0.13 (DMF)

UV : λ_{max} 259 nm (ϵ=1.1 x 10^4), 206 nm (ϵ = 2.5 x 10^4)

Anal. Calcd. for C_{21} H_{24} N_2 O_2 : C,75.00; H,7.14; N, 8.33

Found : C,74.20; H, 7.05; N, 8.05

3.2.17 11-Oximino strychnine (17)[21,22]

A mixture of 600 mg (0.0018 mol) of strychnine in 15 ml of absolute alcohol and 2 ml of amyl nitrite was heated on a water bath at 40° using a reflux condenser

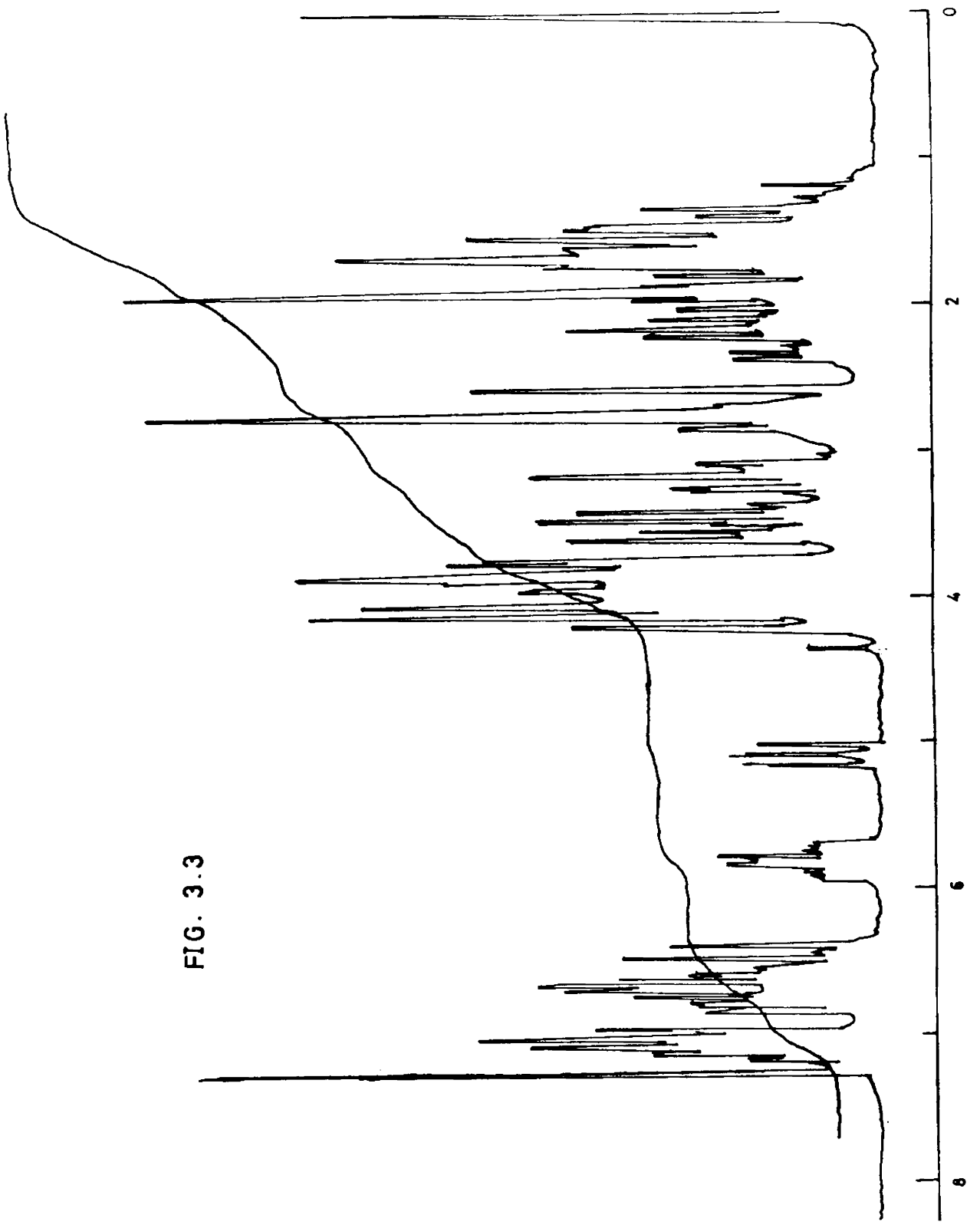


FIG. 3.3

through which ice cold water was circulated. Sodium ethoxide prepared from 2 g of sodium and 80 ml alcohol was added to the reaction mixture slowly during the course of 3 hours. The reaction mixture was then stirred on a magnetic stirrer and the temperature was slowly raised to 80°. Since the reaction was not complete amyl nitrite (1 ml) was added and the reaction was allowed to continue for one more hour. The mixture was cooled and filtered to remove the unreacted strychnine. The filtrate was concentrated under vacuum and the residue was dissolved in water and filtered. On addition of solid sodium bicarbonate to the filtrate the product was precipitated which was filtered, washed with water, dried and recrystallised from chloroform-ethanol to give 400 mg(66%) of 11-oximinostrychnine, not melting even at 300° (lit.[21] charring at 200° but no melting even at 300°)

$[\alpha]_D^{20}$ = -183.2°, c 0.14 (DMF)

UV : λ_{max} 314 nm (ϵ = 1.2 x 10^4), 333 nm (ϵ = 3.1 x 10^4)
207 nm (ϵ = 3.9 x 10^4)

IR (Nujol) : 3580 and 3480 cm^{-1}(OH); 1650 cm^{-1}(C = O)

3.2.18 11-Oximino strychnine N-oxide (19)

A mixture of 1 g (0.00275 mol) of 11-oximino-strychnine and 5 ml water was made into a paste. While heating on a boiling water bath 30% hydrogen peroxide was added drop by drop with vigorous shaking. After adding nearly 2 ml of hydrogen peroxide a complete solution was obtained. Heating was continued for some more time and then the mixture was allowed to cool. The crystalline precipitate obtained was filtered and washed with chilled water repeatedly. Recrystallised from DMF - methanol-ether mixture to yield 600 mg (60%) of the N-oxide (19). The compound turned black at 290° but not melting.

$[\alpha]_D^{20} = -137.2^\circ$ c 0.10 (DMSO)

UV : λ_{max} 310 nm ($\epsilon = 1.8 \times 10^4$), 220 nm ($\epsilon = 3.0 \times 10^4$)

209 nm ($\epsilon = 4.4 \times 10^4$)

IR (Nujol) : 3540 and 3360 cm^{-1} (OH), 1670 cm^{-1} (C = O),

920 cm^{-1} (N→O)

Anal. Calcd. for $C_{21}H_{21}N_3O_4$: C, 66.49; H, 5.54, N, 11.08

Found : C, 66.20; H, 5.72; N, 11.06

3.2.19 11-Benzylidene strychnine (20)[23,147]

A solution of 3.34 g (0.01 mol) strychnine in 100 ml of absolute alcohol was added to 25 ml of alcohol containing 2.1 ml(0.02 mol) of benzaldehyde and 10 drops of piperidine. The mixture was refluxed on a water bath at a temperature not exceeding 85° for 4 hours. A crystalline product was separated. The reaction mixture was kept as such for few hours. The crystals formed were filtered and recrystallisation from chloroform ethanol mixture, gave 2 g of (60%) of 11-benzalidene strychnine as a lemon yellow product melting at 233 - 36°(lit.[147] m.p. 235°). TLC ($CHCl_3$ - MeOH; 9:1) showed an yellow spot slightly above that of strychnine.

$[\alpha]_D^{20}$ = -236.2°, c 0.12 ($CHCl_3$),

Lit.[147] $[\alpha]_D^{20}$ = -233.1°($CHCl_3$)

UV : λ_{max} 335 nm (ϵ = 5.1 x 10^4), 207 nm (ϵ = 4.9 x 10^4)

IR (Nujol) : 1660 cm^{-1} (C = O), 1620 cm^{-1}(C = C)

NMR : See fig. 3.4($CDCl_3$, 90 MHz)

3.2.20 11-(4-Dimethylaminobenzylidene) strychnine (21)

A solution of 3.34 g (0.1 mol) of strychnine in 100 ml absolute alcohol was added to 25 ml of alcohol

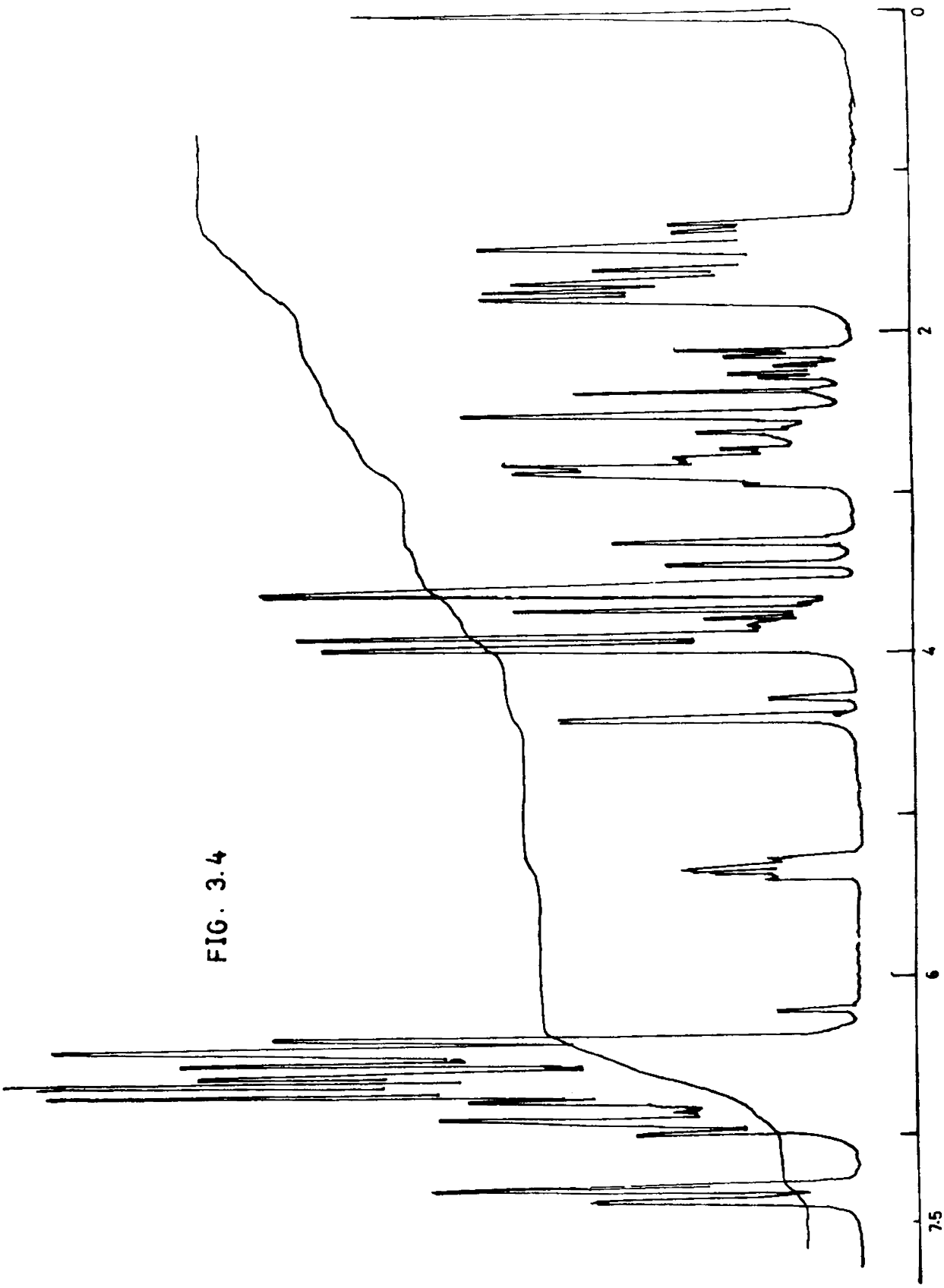


FIG. 3.4

containing 3g(0.02 mol) of 4-dimethylaminobenzaldehyde and 10 drops of piperdine. The mixture was heated on a water bath at a temperature not exceeding 80° for 6 hours. A crystalline yellow compound separated during the reaction. The reaction mixture was shaken at intervals. When the reaction was complete as shown by TLC, the reaction mixture was cooled and filtered. A second crop of the product was obtained by concentrating the filtrate, cooling and filtering. This crop of the product contained some unreacted strychnine. This was purified by dissolving in chloroform-ethanol mixture, concentrating the solution and cooling when yellow crystals separated. The total yield was 2.2 g (66%). The yellow product darkened at 245°, not melting upto 260°.

$[\alpha]_D^{20}$ = -435.4, c 0.11 ($CHCl_3$)

UV : λ_{max} 234 nm ($\epsilon = 1.68 \times 10^4$), 207 nm ($\epsilon = 4.01 \times 10^4$)

IR (Nujol):1640 cm^{-1} (C = O), 1620 cm^{-1} (C = C)

Anal. Calcd. for $C_{30}H_{31}N_3O_2$: C, 77.42; H, 6.67; N,9.03

Found : C, 77.0; H, 6.80; N, 9.36

3.2.21 11-(3,4,5-Trimethoxy benzylidene)strychnine (22)

A solution of 1.67 g (0.005 mol) of strychnine in 50 ml of dry ethanol was added to 1.96 g (0.01 mol) of trimethoxy benzaldehyde in 25 ml alcohol containing

6 drops of piperdine. An additional amount of alcohol (25 ml) was added and the mixture was heated for 5 hours below 80°. The reaction mixture was shaken at intervals. The solution was concentrated and the crystals formed were separated. After recrystallisation from chloroform-ether containing traces of methanol was found to be a single compound by TLC ($CHCl_3$ - MeOH, 9:1). Lemon yellow crystals of 22 obtained in 48% yield (800 mg) melted at 160 - 162°. The reaction mixture was concentrated and diluted with water to get a second crop of crystals containing some unreacted strychnine. This was purified by recrystallisation.

$[\alpha]_D^{20}$ = -304.1°, c 0.12 (DMF)

UV : λ_{max} 257 nm (ϵ = 1.6 x 10^4), 207 nm (ϵ = 3.3 x 10^4)

IR (Nujol) : 1640 cm^{-1} (C = O), 1620 cm^{-1} (C = C)

Anal. Calcd. for C_{31} H_{32} N_2 O_5 : C,72.66;H,6.25; N,5.47

Found : C, 72.76; H, 5.94; N, 5.07

3.2.22 11-Benzylidene strychnine N-oxide (23)

A solution of 0.867 g (0.005 mol) of metachloroperbenzoic acid[139] in dry chloroform was added in portions to an ice cold stirred solution of 2.11 g (0.005 mol) of 11-benzylidene strychnine (20). After stirring for 3 hours,

an additional amount of 0.120 g (0.0007 mol) of metachloroperbenzoic acid was added and the reaction was continued for another hour. The chloroform solution was washed with sodium bicarbonate solution, dried and concentrated. A solid product was obtained when ether was added dropwise. Further purification by recrystallisation from chloroform ether mixture (with drops methanol) gave 1.8 g (85%) of the N-oxide melting at 180 - 185°.

$[\alpha]_D^{20} = -321.7°$, c 0.12 (DMF)

UV : λ_{max} 327 nm ($\epsilon = 4.6 \times 10^4$), 207 nm ($\epsilon = 5.0 \times 10^4$)

IR (Nujol) : 1670 cm^{-1} (C = O), 930 cm^{-1} (N→O)

Anal. Calcd. for $C_{28} H_{26} N_2 O_3$: C,76.71; H,5.94; N, 6.39

Found : C, 76.32; H, 5.57; N, 6.80

3.2.23 11-(4-Dimethyl aminobenzylidene) strychnine N-oxide(24)

A solution of 0.216 g (0.00125 mol) of metachloroperbenzoic acid in 5 ml of chloroform was gradually added at 0.5° to an ice cold stirred solution of 0.585 g (0.00125 mol) of 11-(4-Dimethyl amino benzylidene) strychnine (21) in 15 ml of chloroform. The addition was done slowly with uniform stirring and the reaction was allowed to continue for 4 hours. The mixture was allowed to attain room

temperature. The solution was washed ($NaHCO_3$) and the chloroform layer dried (Na_2SO_4), and concentrated. The residue was recrystallised from chloroform-methanol to give 0.280 g (48%) of the 11-(4-dimethylaminobenzylidene) strychnine N-oxide, charring begins at 220° complete charring at 240°.

$[\alpha]_D^{20} = -339^\circ$, c 0.12 (DMF)

UV : λ_{max} 233 nm ($\epsilon = 7.2 \times 10^3$), 210 nm ($\epsilon = 1.1 \times 10^5$)

IR (Nujol) : 1650 cm^{-1} (C = O), 930 cm^{-1} (N→O)

Anal. Calcd. for $C_{30}H_{31}N_3O_3$: C, 74.84; H, 6.44; N,8.73

Found : C, 74.39; H, 6.80; N, 9.00

3.2.24 11-(3,4,5 Trimethoxy benzylidene) strychnine N-oxide(25)

To a solution of 0.120 g (0.00025 mol) of(22) in 5 ml of dry chloroform kept at 0° was added 0.045 g (0.00025 mol) metachloroperbenzoic acid in chloroform. The solution was stirred for 3 hours. An additional amount of 0.045 gm of metachloroperbenzoic acid was added and the reaction mixture was kept overnight. The chloroform solution was washed with sodium bicarbonate solution, dried and concentrated. The residue was dissolved in chloroform-methanol mixture and on adding ether, a solid product separated. It was filtered, washed and dried to get 80 mg (66.6%) of 25, and TLC showed a spot running just below

trimethoxy benzylidine strychnine ($CHCl_3$ - MeOH, 8:2). The compound melted at 193 - 195°.

$[\alpha]_D^{20}$ = -197.8°, c 0.10 (DMF)

UV : λ_{max} 207 nm ($\epsilon = 2.1 \times 10^4$)

IR (Nujol) : 1650 cm^{-1} (C = O), 930 cm^{-1} (N→O)

Anal. Calcd. for $C_{31}H_{32}N_2O_6$: C,70.45; H, 6.06; N,5.30

Found : C, 70.05; H, 5.97; N, 5.60.

3.2.25 Mannich Reaction

A solution of 3.67 g (0.01 mol) of strychnine hydrochloride in 50 ml of alcohol,0.2 ml of concentrated HCl, 2 ml of 40% formaldehyde solution and 0.55 g of ammonium chloride was refluxed for 3 hours[148]. A product running below strychnine was found in minute quantities (TLC;$CHCl_3$: MeOH, 8:2). The reaction was continued for 5 hours and a slight excess of formaldehyde and ammonium chloride were added. No significant increase in the quantity of the product was observed. The reaction was repeated using diethylamine instead of ammonium chloride. A very small amount of a product with lower Rf value when compared to strychnine was formed (TLC) but the isolation and purification of this product was not achieved. Repeating the reaction using pyrrolidine and

piperidine as bases and paraformaldehyde instead of formaldehyde solution were also unsuccessful.

3.2.26 21,22-Dihydrostrychnine (26)[39]

A solution of 5 g (0.015 mol) of strychnine in 20 ml of 50% acetic acid was hydrogenated in presence of 1.5 g of 5% palladium on carbon. Nearly 600 ml of hydrogen was absorbed during the course of 10 hours. The reaction mixture was filtered to remove the catalyst and washed with 50% acetic acid. The filtrate was made alkaline (pH 8) with ammonium hydroxide. The white precipitate formed was washed with cold water and recrystallised from methanol-water to give 3.5 g (70%) of dihydrostrychnine, m.p. 119 - 21°, (lit.[39] m.p. 220 - 22°). TLC ($CHCl_3$ - MeOH, 7:3) showed a spot running slightly below strychnine.

$[\alpha]_D^{20}$ = +17.5°, c 0.11 (MeOH)

UV : λ_{max} 254 nm ($\epsilon = 2.7 \times 10^4$), 212 nm ($\epsilon = 4.3 \times 10^4$)

IR(KBr) : 1660 cm^{-1} (C = O)

NMR : See fig. 3.5 ($CDCl_3$, 60 MHz)

3.2.27 21,22-Dihydrostrychnine N-oxide (27)

A mixture of 1 g (0.003 mol) of dihydrostrychnine and 5 ml of water was heated on a boiling water bath. 2 ml of 30% hydrogen peroxide was added dropwise. The

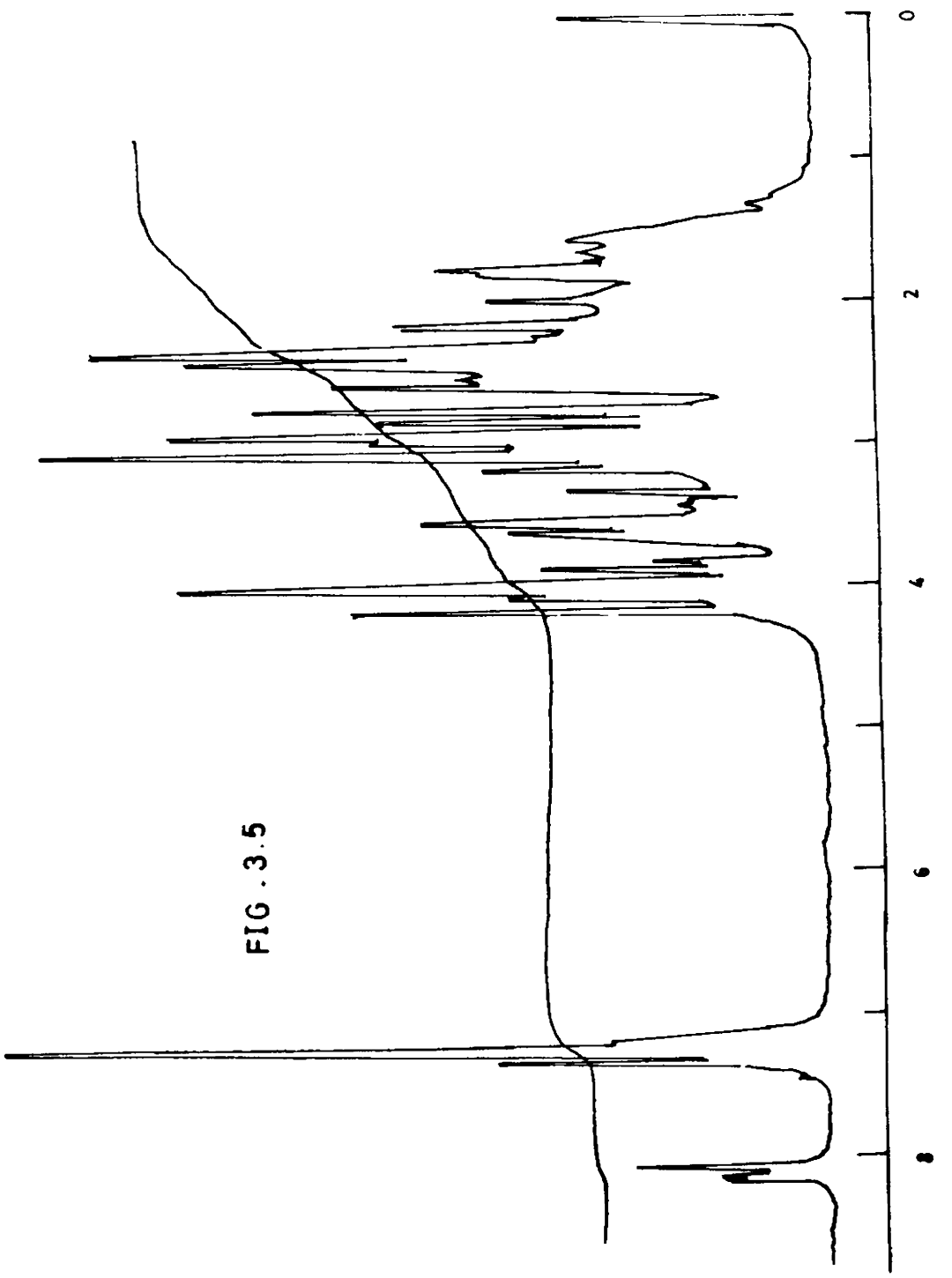


FIG. 3.5

mixture was heated again on a boiling water bath until a clear solution was effected. On cooling, crystals were separated, filtered and dried. This gave 0.75 g (75%) of dihydrostrychnine N-oxide. TLC examination of the N-oxide showed a spot just below that of dihydrostrychnine. The compound 27 darkened at 210°, melted with charring at 227°.

$[\alpha]_D^{20}$ = +115.9, c 0.07 (DMSO)

UV : λ_{max} 254 nm ($\epsilon = 3.2 \times 10^4$), 211 nm ($\epsilon = 4.8 \times 10^4$)

IR (KBr) : 1660 cm^{-1} (C = O), 940 cm^{-1} (N→O)

NMR : See fig.3.6 (DMSO-d_6, 90 MHz)

Anal. Calcd. for $C_{21}H_{24}N_2O_3$: C, 71.59; H, 6.80; N, 7.95

Found : C, 71.44; H, 6.49; N, 7.92

3.2.28 2-Nitro-21,22-dihydrostrychnine (28)

Dihydrostrychnine 1 g (0.003 mol) was added slowly with stirring to 4 ml of a nitrating mixture (H_2SO_4 - HNO_3, 1:1) kept at 0°. The reaction was allowed to continue for 2 hours. By the end of the reaction the temperature was allowed to rise to 20°. The viscous liquid was poured into water (30 ml at 50°) and the crystalline product separated was filtered, washed with cold water. On recrystallisation from boiling water

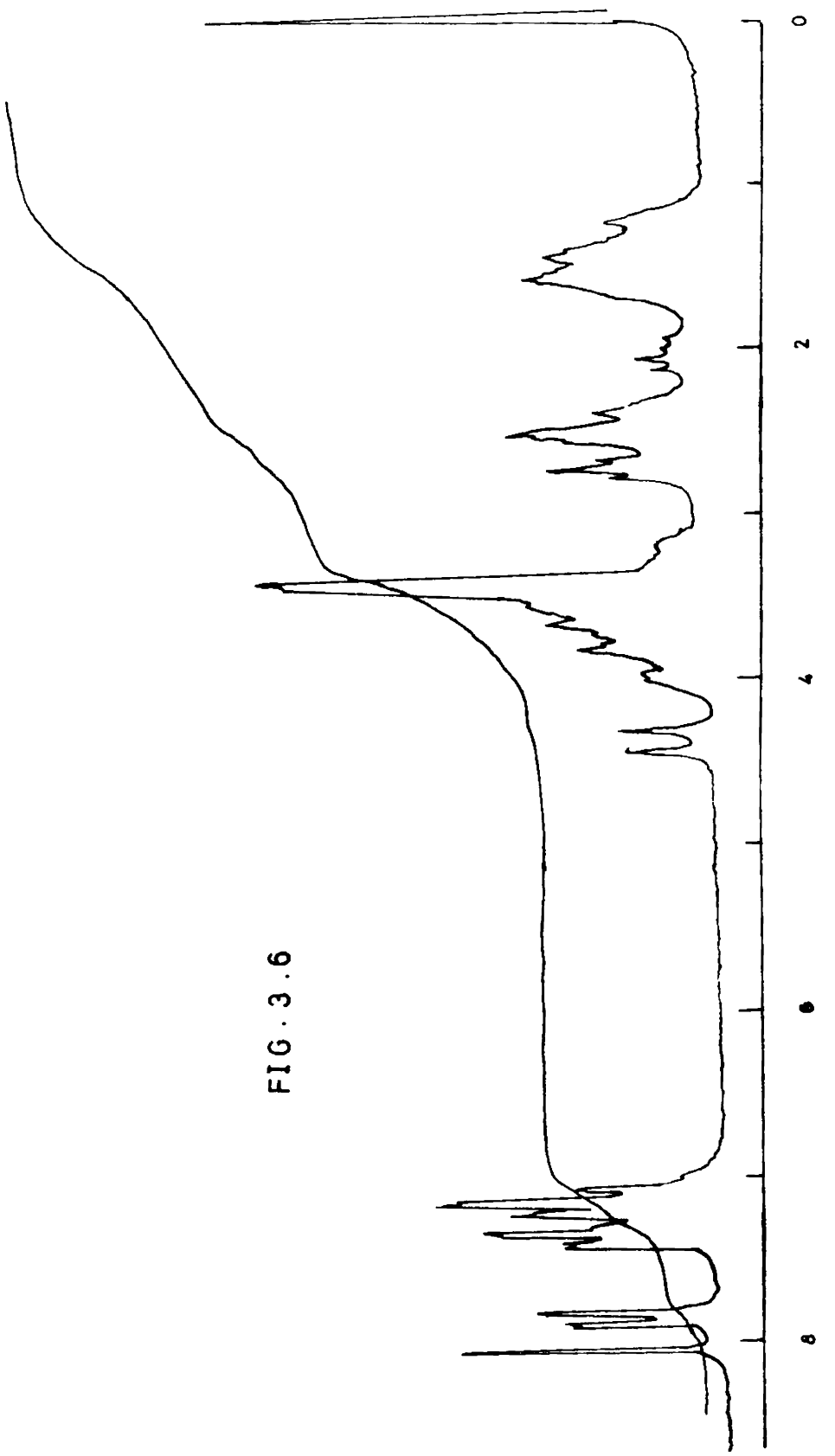


FIG. 3.6

containing traces of nitric acid, 400 mg (40%) of a powder was obtained. TLC ($CHCl_3$ - MeOH, 7:3) gave a single spot running slightly above dihydrostrychnine. The compound darkened at 210°, melted with charring at 235°.

$[\alpha]_D^{20}$ = -23.8, c 0.17 (DMF)

UV : λ_{max} 334 nm (ϵ = 1.9 x 10^4), 208 nm (ϵ = 3.2 x 10^4)

IR (KBr) : 1670 cm^{-1} (C = O)

Anal. Calcd. for C_{21} H_{23} N_3 O_4 : C, 66.14; H,6.04; N,11.02

Found : C, 65.82, H, 6.36; N, 10.83.

3.2.29 2-Nitro-21,22-dihydrostrychnine N-oxide (29)

21, 22-Dihydrostrychnine N-oxide 500 mg (0.0015 mol) was added slowly with stirring to 3 ml of nitrating mixture (HNO_3 - H_2SO_4, 1:1) kept at 0°. After 3 hours the reaction mixture was allowed to warm upto 20° and poured into 30 ml of water at 50°, when an amorphous product was obtained. It was recrystallised from methanol water mixture. The product obtained after repeated purification was shown to be a single compound by TLC ($CHCl_3$ - MeOH, 7:3). The yield was 200 mg (40%) and the compound charring at 220° and no melting upto 270°.

$[\alpha]_D^{20}$ = +6.57, c 0.15 (DMF)

UV : λ_{max} 327 nm (ϵ = 3.1 x 10^4), 209 nm (ϵ = 5.2 x 10^4)

IR (KBr) : 1680 cm^{-1} (C = O), 940 cm^{-1} (N→O)

Anal. Calcd. for C_{21} H_{23} N_3 O_5 : C,63.48; H,5.79; N,10.58

Found : C, 63.08; H, 5.74; N, 10.24

3.2.30 Reaction product of strychnine with Benzoyl peroxide in chloroform (30)

To a solution of 3 g (0.009 mol) of strychnine in 200 ml of dry chloroform, 1 g of benzoyl peroxide was added and the mixture was refluxed for 1 hour. 200 mg Benzoyl peroxide was again added in portions to the reaction mixture at intervals of 1 hour. The addition was continued until a total of 2 g of benzoyl peroxide was added during the course of 6 hours. The course of the reaction was monitored by TLC. The reaction was continued for 6 hours, the reaction mixture concentrated and the residue chromatographed over a column of 50 g of silicagel using $CHCl_3$ as solvent. The elution was carried out with CCl_4 and different combinations of $CHCl_3$: MeOH and finally with pure methanol. The pure methanol fraction on concentration on a rotary vacuum evaporator gave a

solid product which on recrystallisation from MeOH gave 1.5 g (50%) of the product. m.p. 189 -191° with charring.

$[\alpha]_D^{20} = -22.8^\circ$, c 0.09 ($CHCl_3$)

UV : λ_{max} 255 nm ($\epsilon = 5.07 \times 10^4$), 208 nm ($\epsilon = 2.0 \times 10^4$)

IR (Nujol) : 1670 cm^{-1} (C = O)

NMR : See fig 3.7 (DMSO-d_6, 90 MHz)

Anal. Calcd. for $C_{22} H_{21} N_2 O_2 Cl_3$: C,58.47; H,4,65; N,6.20.

Found : C, 58.92; H, 4.93; N, 6.47.

A mixture of compound 30 (100 mg) and 8 ml of 90% orthophosphoric acid was heated at 150° for 6 hours on an oil bath and kept at room temperature overnight. The thick brown liquid formed was poured into ice and made alkaline with sodium bicarbonate and extracted with chloroform. The chloroform extract contained a good amount of strychnine identified by m.p., mixed m.p. and tlc examination.

Another solution of 100 mg of 30 in 5 ml of methanol and 10 mg of p- toluene sulphonic acid were refluxed for 4 hours and a solid product obtained on purification was identified as strychnine (TLC and mixed melting point).

A solution of 90 mg of 30, in 5 ml of methanol was refluxed with 25 mg of triphenyl phosphine. The reaction was continued for 5 hours and the solid separated on

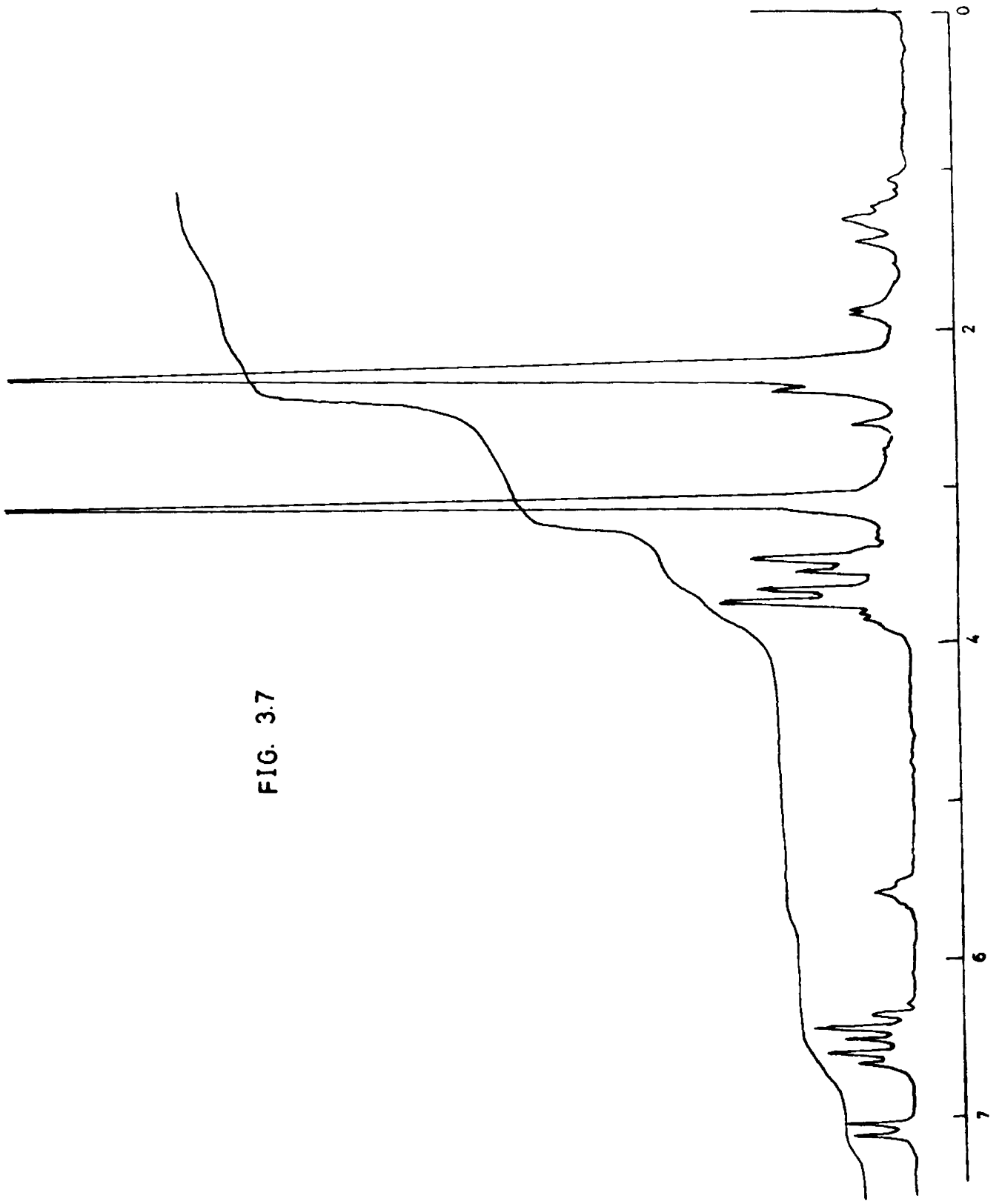


FIG. 3.7

purification was identified as strychnine.

Compound 30 was also treated with 10% sodium hydroxide in methanol,heated for 2 hours. The starting material almost disappeared (TLC). The reaction mixture was concentrated,acidified with acetic acid and extracted with chloroform after neutralisation, gave strychnine as the product (TLC and mixed melting point).

Treatment with methanolic ammonia also gave back strychnine.

3.2.31 N-oxide of 30 (31)

A mixture of 1 g (0.0022 mol) of 30 was mixed with 5 ml of water and heated on a boiling water bath. 30% hydrogen peroxide solution (3 ml) was added drop by drop till a complete solution was effected. It was further heated for 30 minutes and then cooled when a crystalline product was obtained which was filtered, washed with chilled water and dried. Recrystallisation from MeOH - $CHCl_3$ gave 0.5 g (50%) of the product melting at 185 - 187° with charring.

$[\alpha]_D^{20} = -119.4°$, c 0.07 (DMSO)

UV : λ_{max} 253 nm ($\epsilon = 2.6 \times 10^4$), 210 nm ($\epsilon = 5.7 \times 10^4$)

IR : 1670 cm^{-1} (C = O), 940 cm^{-1} (N→O)

Anal. Calcd. for $C_{22} H_{22} Cl_3 N_2 O_3$: C,56.47; H,4.49; N,5.99

Found : C, 57.00; H, 4.89; N, 6.20

CHAPTER IV

PHARMACOLOGICAL STUDIES OF STRYCHNINE DERIVATIVES

4.1 RESULTS AND DISCUSSION

4.1.1 Introduction

The pharmacological studies were carried out on strychnine derivatives and their N-oxides, the preparations of which are described in Chapter 3. These studies were mainly concentrated on the action on CNS because strychnine is a powerful spinal convulsant[150]. The experiments were carried out on frogs weighing 40 - 100 g and were done in a systematic manner. For comparison of the effects of the different derivatives of strychnine, identical conditions were maintained. Each dose was given to a number of frogs as uniform suspensions. Equimolar quantities were administered and strychnine was kept as control.

Each compound was injected into the Ventral lymph sac of the frog[150]. The onset of action, convulsion pattern, duration of action and mortality rates were observed for 24 to 48 hours. The details of procedures and observations are reported in Section II.

4.1.2 Results

Strychnine (1) produced hyperactivity at the lowest dose of 1.25 mg/100 g. A dose of 3 mg caused convulsions and there was 50% mortality. A 6 mg dose was fatal and the convulsions produced were very strong. The

convulsion pattern of strychnine had the following characteristics, viz. tonic and violent extension of the hind limbs and flexion of the fore limbs. In many cases of convulsions there was a marked proconvulsive strained respiration and the body was arched backwards (opisthotonus)[150].

In the case of strychnine N-oxide(2) there was no significant hyperaction or convulsion even upto a dose of 10 mg. But at this dose the mortality rate was as high as 75%. A substitution with nitrogroup at the 2-position of the aromatic ring in strychnine did not alter the convulsion pattern or the mortality rate compared to strychnine. Also the convulsions were stronger and similar to that of strychnine. There was 100% mortality at a dose of 10 mg. The corresponding N-oxide 4 showed no convulsions but a 6 mg dose was almost fatal. 2-Aminostrychnine (5) was more toxic when compared to strychnine, convulsions were very strong and the mortality rates were high reaching 100% at a dose of 10 mg. In this case, however, instead of muscular rigidity a relaxation effect was observed.

2-Acetamido strychnine (6) was found to be less convulsive and less toxic compared to 5. It showed muscle relaxant effect[103] at 10 mg doses, there was only

50% mortality and this occured only after 24 hours. The N-oxide of this compound, 7 was of low toxicity and there was no typical strychnine like convulsion. At a dose of 6 mg there was no mortality observed and a 10 mg dose produced only 25% mortality after 24 hours.

The sulphonamide substitution product, 9 which is expected to have interesting biological properties[142], was found to reduce the convulsions. Out of the 16 frogs only 2 showed convulsion at a dose of 10 mg and 75% mortality was observed. The N-oxide of this compound 10 was also of low toxicity as expected and there was no convulsion or mortality at a dose of 6 mg.

2-Bromostrychnine (11) showed considerably increased toxicity, the onset of convulsions were very quick and there was 100% mortality at a dose of 6 mg. The hyperactions and convulsions were also stronger when compared to strychnine. The N-oxide of bromostrychnine(12) was found to be of less toxicity as expected and the convulsive patterns were milder when compared to bromostrychnine. At a dose of 10 mg there was only 50% mortality.

Brucine (13), as reported[57], was found to have less convulsive property and toxicity was also nil at a

dose of 6 mg. There was no sign of convulsions at a dose of 3 mg or 6 mg. The N-oxide of this compound (14) did not manifest significant convulsions. Only 2 cases out of 12 recorded convulsion when a dose of 6 mg was administered.

Reduction of strychnine at the 10 position to strychnidine (15) showed a different pattern in the pharmacological action. Though there was convulsion at a dose of 6 mg and 10 mg and 25% mortality was observed, the surviving frogs completely recovered and were found to be alert after 12 hours. Strychnidine N-oxide (16) was of reduced toxicity and showed no convulsion at a dose of 3 mg or 6 mg. There was no mortality at these doses. The frogs were alert even after 24 hours.

The 11-oximinostrychnine (17) was found to be a promising derivative in the case of toxicity and convulsion. Practically there was no convulsion at doses of 3 mg or 6 mg. The mortality rate was nil at these doses. No hyperactivity was observed and the frogs were alert after 24 hours. The corresponding N-oxide 19 showed no convulsion at doses upto 6 mg. There was no considerable excitation or mortality. Benzylidene substitution at the 11th position of strychnine showed variations in the convulsive and toxic pattern of strychnine. 11-Benzylidene strychnine (20)

was found to be more convulsive and more toxic than strychnine. Even at a dose of 1.25 mg there was immediate hyperaction and convulsions occured in 30 minutes. Strong hyperaction and severe convulsions were manifested at a dose of 3 mg and there was 100% mortality even at this dose. In the series of compounds subjected to pharmacological studies this was found to be the most powerful convulsant and most toxic. The corresponding N-oxide 23 was of less toxicity when compared to 20. In a few cases there was convulsion and 50% mortality occured at a dose of 10 mg.

The 4-dimethyl aminobenzylidene derivative, 21 was not as toxic as the benzylidine derivative. It showed strong convulsions at a dose of 10 mg and the mortality rate was 75% at this dose while 6 mg dose showed 50% mortality. The N-oxide 24 of this compound did not show much change in the convulsions, but the convulsions were not as strong as that of 21. The mortality was 50% at a dose of 10 mg.

Contrary to the other benzylidene substitution products, the 3,4,5-trimethoxy benzylidene derivative 22 was of least toxicity in this series as it showed no convulsion at a dose of 6 mg or 10 mg and no marked hyperactivity was observed. The mortality rates were nil

at doses of 6 mg and 10 mg. No mortality was observed in 48 hours. The N-oxide of this compound 25 was also of least toxicity which showed no convulsion at dose of 3 mg or 6 mg. Mild convulsion was observed in one case and the mortality rate was only 25% at a dose of 6 mg.

The hydrogenation of the 21,22 double bond did not reduce the toxicity or convulsions compared to strychnine. The convulsions produced by dihydro-strychnine (26) at doses of 3 mg and 6 mg were equally strong as the strychnine convulsions at the corresponding doses. There was 75% mortality at 3 mg dose and 100% mortality in 6 mg dose. The N-oxide 27 of dihydro-strychnine was also subjected to pharmacological action. The N-oxide did not show convulsions at doses 3 mg or 6 mg. But there was 75% mortality at a dose of 6 mg. A slight muscle relaxant activity was also observed in this case. On comparison with strychnine N-oxide (2) dihydrostrychnine N-oxide (27) was found to be more toxic.

Nitration of the 21,22-dihydroderivative still increased the toxicity which showed strong convulsion at a dose of 3 mg and there was 75% mortality. At a dose of 6 mg there was strong convulsion and the rate of mortality increased to 100%. The 2-nitro-21,22 dihydro-strychnine N-oxide (29) was found to be less toxic when

compared to the parent compound 28. There was no convulsion at doses of 3 mg and 6 mg. but 75% mortality was observed during the course of 2 to 12 hours. Also, there was no hyperactvity.

The reaction product of strychnine with benzoyl peroxide and chloroform was found to be much less toxic than strychnine. There was no marked convulsions at doses of 3,6 and 10 mg. The mortality rates were also minimum. Only 25% mortality was observed at a dose of 10 mg. The N-oxide of this compound 31 showed mild convulsions in some cases but these convulsions were not strong and mortality was minimum.

Since the above trichloromethyl derivative 30 was found to be of lower toxicity and showed reduced convulsion it was given to frogs along with strychnine to observe whether it alters the pattern of action of strychnine. 3 mg strychnine was administered followed by 3 mg of 30. The onset of hyperaction and convulsion were delayed. Frogs were found to be more alert but 50% mortality occured in 24 hours. Prior treatment of the frogs with 3 mg of 30 followed by 3 mg of strychnine was also attempted. Onset of convulsion and hyperaction were again delayed; but 75% mortality occured in this combined dose. At a dose of 3 mg of strychnine and 6 mg of the trichloromethyl compound

the same effect was observed. Prior treatment of trichloromethyl compound (6 mg) followed by a dose of strychnine (3 mg) again delayed the onset of convulsions and hyperactivity. This effect was observed at a dose of 10 mg of the trichloromethyl compound followed by 3 mg of strychnine. Onset of convulsions and mortality were delayed when compared to the effect of administration of strychnine alone.

4.1.3 Structure activity relationship

In general, the N-oxides of all the above compounds were less toxic and most of them showed no convulsions. This was in accordance with the reports that N-oxides of alkaloids are less toxic when compared to the parent amines[94]. The nitro, bromo and amino substitutions on the 2-positions did not alter the pharmacological actions. They were as toxic or slightly more toxic when compared to strychnine. But their N-oxides showed less toxicity. Brucine and its N-oxide did not manifest the strychnine type convulsions or fatality. This was in accordance with earlier reports[57]. 2-Acetamido compound as reported[103] had muscle relaxant property and showed minimum toxicity. A subsequent reduced toxicity in the case of its N-oxide was observed.

2-Sulphonamido compound 9 and its N-oxide 10 were also of lesser toxicity as expected[142]. Strychnidine though showed some convulsions could completely be recovered to normal state. This may be in accordance with the earlier report that the action of strychnine is due to the lactam group[108]. The N-oxide of this compound 16 was of lesser toxicity. The 11-oximino strychnine was of very minute toxicity and its N-oxide was still less toxic. Alteration of the non aromatic part of strychnine molecule has been reported to cause less convulsion and lethal effect[110]. The benzaldehyde condensation product 20 was very strongly toxic while the 4-dimethyl aminoanalogue 21 was less toxic and 3,4,5-trimethoxy benzylidene derivative 22 was of least toxicity and the corresponding N-oxides of these compounds showed reduction in toxicity and convulsions compared to the parent amines. Trimethoxy derivative and its N-oxide showed minimum toxicity and convulsions. Hydrogenation of the 21,22 double bond in strychnine did not reduce the toxicity compared to strychnine.

The trichloromethyl derivative was found to be of minimum toxicity and the convulsions were of very mild type. The N-oxide of this compound was of least toxicity.

Administration of combined doses of trichloromethyl derivative and strychnine in different combinations was found to delay the onset of action or convulsion due to strychnine. As the position of the trichloromethyl group has not been conclusively established, the change in pharmacological action with respect to the structural modification of strychnine cannot be related at this time.

4.2 EXPERIMENTAL

4.2.1 Experimental procedures

A comparitive study of the pharmacological action of the different strychnine derivatives and their N-oxides were carried out. Frogs weighing 40 to 100 g were used for the screening and the dose dependant responses like the hyper activity, type of convulsions and mortality rates were observed.

Each dose was given to a number of frogs and the different doses of the same drug was given to different groups of the experimental animals. Depending on the body weight equimolar doses were injected to the ventral lymph sac of frog. The solutions were given as uniform suspension in 2% aqueous acacia. The frogs were watched 24 to 48 hours and changes were observed. The onset of action, extent of hyper action and type of convulsion were noted in each case.

The results obtained are reported in the following charts. Conclusions were arrived at depending on the convulsive pattern and the mortality rate, the duration in which the hyper action, convulsion or mortality occured was considered as a decisive factor and is indicated in the charts. The notations mentioned in the charts are explained in the first chart. The weights of the frogs and the equivalent doses given are also mentioned in each case. The hyper action or convulsion is usually initiated by the external stimuli.

4.2.2 Pharmacological studies of strychnine and its derivatives

Explanation of notations

	-	No convulsion
	-	No mortality
Strychnine type tonic convulsion	+	Mortality
	+	Mild convulsion
	++	Strong convulsion
	+++	Very strong convulsion, usually leading to asphyxia and death.

1. Strychnine (1)

Weight of frog (g)	Dose given (mg/100g)	Onset of action	Convulsion Type (onset time in hrs.)	Mortality in hrs.	% Mortality
60	1.25	Slight response to external stimuli. Hyper activity in 20 mts.	-	-	
60	"	"	-	-	0
60	"	"	-	-	
60	"	"	+(6)	-	
80	3	Proconvulsive strained respiration after 10 mts.	-	-(24)	
60	"	Hyper activity in 10 mts.	++(2)	+(24)	
60	"	"	++(2)	+(24)	50
80	"	"	++(2)	-	
60	6	Immediate strong hyper activity	+++(10mts)	+(0.5)	
60	"	"	+++(10mts)	+(0.5)	100
80	"	"	+++(10mts)	+(0.5)	
80	"	"	+++(10mts)	+(0.5)	

2. Strychnine N-oxide (2)

Weight of frog (g)	Dose given (mg/100g)	Onset of action	Convulsion Type (onset time in hrs.)	Mortality in hrs.	% Mortality
80	1.25	No hyper activity	-	-	
80	"	"	-	-	0
80	"	"	-	-	
60	"	"	-	-	
100	3	No hyper activity	-	-	
80	"	"	-	-	0
100	"	"	-	-	
80	"	"	-	-	
80	6	No hyper action	-	-	
60	"	Slight hyper r action	+(12)	+(24)	25
80	"	"	-	-	
80	"	"	-	-	
70	10	No hyper action	-	+(4)	
80	"	"	-	-	75
75	"	"	-	+(4)	
60	"	"	-	+(4)	

3. 2-Nitrostrychnine (3)

Weight of frog (g)	Dose given (mg/100g)	Onset of action	Convulsion Type (onset time in hrs.)	Mortality in hrs.	% Mortality
70	1.25	Response to external stumuli. Hyper activity in 30 mts.	-	-	
70	"	"	-	-	0
60	"	"	-	-	
60	"	"	+(2)	-	
70	3	Hyper activity in 10 mts	++(2)	+(12)	
70	"	Hyper activity in 20 mts	++(2)	+(4)	50
80	"	Hyper activity in 10 mts	++(2)	-(24)	
60	"	"	++(2)	-(24)	
80	6	Immediate hyper action on external stimuli	+++(0.5)	+(1)	
80	"	"	+++(0.5)	+(1)	100
70	"	"	+++(0.5)	+(1)	
80	"	"	+++(0.5)	+(1)	

4. 2-Nitrostrychnine N-oxide (4)

Weight of frog (g)	Dose given (mg/100g)	Onset of action	Convulsion Type (onset time in hrs.)	Mortality in hrs.	% Mortality
70	1.25	No hyper action	-	-	
65	"	"	-	-	0
60	"	"	-	-	
60	"	"	-	-	
70	3	No typical hyper activity	-	+(1)	
80	"	"	-	+(1)	50
80	"	"	-	-	
60	"	"	-	-	
80	6	No typical hyper activity	-	+(0.5)	
75	"	"	-	+(0.5)	75
70	"	"	-	+(0.5)	
80	"	"	-	-(24)	

5. 2-Aminostrychnine (5)

Weight of frog (g)	Dose given (mg/100g)	Onset of action	Convulsion Type (onset time in hrs.)	Mortality in hrs.	% Mortality
50	1.25	Immediate hyper action and extension of the hind limbs	++(0.5)	-	
50	"	"	+(4)	-	25
45	"	"	+( )	-	
45	"	"	+(4)	+(10)	
40	3	Immediate hyper action	+(3)	+(10)	
45	"	"	+(10)	-	50
45	"	Slight hyper action	+(3)	+(5)	
55	"	"	+(3)	-	
60	6	Hyper action in 30 mts.	+(3)	+(6)	
65	"	"	++(6)	+(10)	75
70	"	Hyper action	-	-	
60	"	"	+(3)	+(6)	
60	10	Immediate hyper action	++(1)	+(2)	
65	"	"	++(1)	+(4)	100
70	"	"	+++(0.5)	+(2)	
75	"	"	+++(0.5)	+(3)	

6. 2-Acetamido-strychnine (6)

Weight of frog (g)	Dose given (mg/100g)	Onset of action	Convulsion Type (onset time in hrs.)	Mortality in hrs.	% Mortality
50	1.25	No hyper action on external stimuli	-	-	
60	"	"	-	-	0
60	"	"	-	-	
65	"	"	-	-	
45	3	No hyper action	-	-	
50	"	Slight hyper action	-	-	25
55	"	"	-	+(24)	
55	"	No hyper action	-	-	
50	6	Slight hyper action	-	-	
55	"	Hyper action	+(18)	+(24)	25
50	"	No hyper action	-	-	
50	"	"	-	-	
60	10	Hyper action	+(18)	+(24)	
70	"	"	+(18)	+(24)	50
65	"	"	-	-	
70	"	"	-	-	

7. 2-Acetamido-strychnine N-oxide (7)

Weight of frog (g)	Dose given (mg/100g)	Onset of action	Convulsion Type (onset time in hrs.)	Mortality in hrs.	% Mortality
80	1.25	No change on external stimuli, no hyper action	-	-	
75	"	"	-	-	0
90	"	"	-	-	
75	"	"	-	-	
85	5	No change on external stimuli, no hyper action	-	-	
70	"	"	-	-	0
75	"	"	-	-	
75	"	"	-	-	
70	"	Slight hyper action on external stimuli	-	-	
75	"	"	-	-	0
70	"	"	-	-	
80	"	No hyper action	-	-	
60	10	Hyper action on external stimuli	+(20)	-	
65	"	"	-	-	25
70	"	"	+(20)	+(24)	
80	"	"	-	-	

8. Strychnine 2-sulphonamide (9)

Weight of frog (g)	Dose given (mg/100g)	Onset of action	Convulsion Type (onset time in hrs.)	Mortality in hrs.	% Mortality
50	1.25	No hyper action and excitability on external stimuli	-	-	
45		"	-	-	0
50	"	"	-	-	
50	"	"	-	-	
55	3	No hyper action or excitability on external stimuli	-	-	
45	"	"	-	-	25
65	"	"	-	-	
50	"	Showed inactivity	-	+(10)	
40	6	Immediate hyper action	+(12)	+(14)	
45	"	"	+(12)	+(14)	50
65	"	No hyper action	-	-	
70	"	"	-	-	
50	10	No hyper action, showed inactivity	-	+(12)	
50	"	"	-	+(12)	75
45	"	No hyper action	-	-	
50	"	"	-	+(12)	

9. Strychnine 2-sulphonamide N-oxide (10)

Weight of frog (g)	Dose given (mg/100g)	Onset of action	Convulsion Type (onset time in hrs.)	Mortality in hrs.	% Mortality
55	1.25	No hyper action, no excitability on external stimuli	-	-	
50	"	"	-	-	0
55	"	"	-	-	
60	"	"	-	-	
60	3	No excitability, no hyper action	-	-	
50	"	"	-	-	0
50	"	"	-	-	
50	"	"	-	-	
60	6	Slight hyper action	-	-	
65	"	"	-	-	0
60	"	Mild excitability	-	-	
65	"	"	-	-	

10. 2-Bromostrychnine (11)

Weight of frog (g)	Dose given (mg/100g)	Onset of action	Convulsion Type (onset time in hrs.)	Mortality in hrs.	% Mortality
80	1.25	Immediate reaction to external stimuli	+(5mts)	+(4)	
75	"	"	+(10mts)	+(4)	50
75	"	"	+(5mts)	-	
80	"	"	+(5mts)	-	
80	"	Immediate hyper action	+(5mts)	+(6)	
75	"	"	+(5mts)	+(4)	75
70	"	"	+(5mts)	-	
70	"	"	++(5mts)	+(4)	
60	6	Immediate hyper action	++(5mts)	+(1)	
60	"	"	++(5mts)	+(1)	100
70	"	"	+++(5mts)	+(1)	
70	"	"	+++(5mts)	+(1)	

11. 2-Bromostrychnine N-oxide (12)

Weight of frog (g)	Dose given (mg/100g)	Onset of action	Convulsion Type (onset time in hrs.)	Mortality in hrs.	% Mortality
100	1.25	No hyper action	-	-	
80	"	"	-	-	0
120	"	"	-	-	
80	"	"	-	-	
70	3	Slight hyper action on external stimuli	+(1)	+(12)	
70	"	"	-	-	25
60	"	No hyper action	-	-	
60	"	"	-	-	
60	6	Hyper action on external stimuli after 1 hour.	+(2)	+(10)	
70	"	"	+(6)	-	25
80	"	No hyper action	-	-	
80	"	"	-	-	
60	10	Hyper action in one hour	+(0.5)	+(12)	
60	"	"	+(2)	+(24)	50
70	"	No hyper action	+(6)	-	
65	"	"	+(6)	-	

12. Brucine (13)

Weight of frog (g)	Dose given (mg/100g)	Onset of action	Convulsion Type (onset time in hrs.)	Mortality in hrs.	% Mortality
55	1.255	No hyper action or alertness on external stimuli	-	-	
50	"	"	-	-	0
55	"	"	-	-	
55	"	"	-	-	
60	3	No hyper action or excitability	-	-	
55	"	"	-	-	0
55	"	"	-	-	
50	"	"	-	-	
65	6	No typical excitation like strychnine.Frogs were alert	-	-	
60	"	"	-	-	0
65	"	"	-	-	
60	"	"	-	-	

13. Brucine N-oxide (14)

Weight of frog (g)	Dose given (mg/100g)	Onset of action	Convulsion Type (onset time in hrs.)	Mortality in hrs.	% Mortality
40	1.25	No hyper action or excitability No strychnine type actions.	-	-	
40	"	"	-	-	0
45	"	"	-	-	
40	"	"	-	-	
50	3	No excitability or hyper action No typical strychnine like action.	-	-	
55	"	"	-	-	0
50	"	"	-	-	
50	"	"	-	-	
60	6	Mild hyper action and excitability	+(12)	-	
65	"	"	+(12)	-	0
50	"	No hyper action	-	-	
55	"	"	-	-	

14. Strychnidine (15)

Weight of frog (g)	Dose given (mg/100g)	Onset of action	Convulsion Type (onset time in hrs.)	Mortality in hrs.	% Mortality
60	1.25	No hyper action, no excitability	-	-	
50	"	"	-	-	0
65	"	Intermittant hyper action	-	-	
50	"	"	-	-	
70	3	Hyper action & excitability on external stimuli.	+(4)	-	
75	"	"	+(4)	-	0
60	"	No hyper action	-	-	
65	"	"	-	-	
75	6	Hyper action and excitability	+(4)	-	
60	"	"	++(2)	+(24)	25
75	"	"	+(4)	-	
60	"	"	+(4)	-	
50	10	Hyper action and excitability	++(2)	+(24)	
55	"	"	+(4)	-	25
60	"	"	+(2)	-	
60	"	"	+(2)	-	

15. Strychnidine N-oxide (16)

Weight of frog (g)	Dose given (mg/100g)	Onset of action	Convulsion Type (onset time in hrs.)	Mortality in hrs.	% Mortality
45	1.25	No excitability or hyper action on external sti-stimuli.	-	-	
45	"	"	-	-	0
50	"	"	-	-	
50	"	"	-	-	
50	"	No hyper action No excitability Frogs were alert	-	-	
50	"	"	-	-	0
45	"	"	-	-	
55	"	"	-	-	
60	6	No hyper action or excitability	-	-	
65	"	"	-	-	0
55	"	"	-	-	
55	"	Slight hyper action	-	-	

16. 11-Oximinostrychnine (17)

Weight of frog (g)	Dose given (mg/100g)	Onset of action	Convulsion Type (onset time in hrs.)	Mortality in hrs.	% Mortality
60	1.25	No hyper action, no convulsion	-	-	
50	"	"	-	-	0
50	"	"	-	-	
60	"	"	-	-	
55	3	No hyper action	-	-	
50	"	"	-	-	0
60	"	"	-	-	
65	"	Slight hyper action	+(24)	-	
50	6	Slight hyper action	-	-	
55	"	"	+(24)	-	0
50	"	No hyper action	-	-	
55	"	"	-	-	

17. 11-Oximinostrychnine N-oxide (19)

Weight of frog (g)	Dose given (mg/100g)	Onset of action	Convulsion Type (onset time in hrs.)	Mortality in hrs.	% Mortality
45	1.25	No hyper action, no convulsion on external stimuli	-	-	
50	"	"	-	-	0
50	"	"	-	-	
55	"	"	-	-	
50	3	No hyper action	-	-	
60	"	"	-	-	0
60	"	"	-	-	
55	"	"	-	-	
55	6	Slight hyper action occasionally on external stimuli	-	-	
60	"	"	-	-	0
60	"	"	-	-	
65	"	"	-	-	

18. 11-Benzylidene strychnine (20)

Weight of frog (g)	Dose given (mg/100g)	Onset of action	Convulsion Type (onset time in hrs.)	Mortality in hrs.	% Mortality
40	1.25	Immediate hyper excitability on external stimuli	++(0.50)	+(2)	
40	"	Hyper action	++(0.5)	+(2)	50
50	"	"	++(6)	-	
40	"	No hyper action	-	-	
60	3	Strong hyper action and excitability	+++ (Immediate)	+(2)	
70	"	"	++(1)	+(6)	100
70	"	"	++(0.5)	+(6)	
80	"	"	++(0.5)	+(6)	

19. 11-Benzylidene strychnine N-oxide (23)

Weight of frog (g)	Dose given (mg/100g)	Onset of action	Convulsion Type (onset time in hrs.)	Mortality in hrs.	% Mortality
50	1.25	No hyper action	-	-	
50	"	"	-	-	0
40	"	"	-	-	
50	"	"	-	-	
70	3	No hyper action	-	-	
75	"	"	-	-	25
50	"	"	-	-	
50	"	Slight hyper action	+(12)	+(24)	
50	6	No hyper action	-	-	
50	"	"	-	-	25
50	"	"	+	+(24)	
60	"	"	-	-	
60	10	No hyper action	-	-	
70	"	"	-	-	50
60	"	"	-	+(12)	
70	"	"	-	+(18)	

20. 11-(4-Dimethylamino benzylidene) strychnine (21)

Weight of frog (g)	Dose given (mg/100g)	Onset of action	Convulsion Type (onset time in hrs.)	Mortality in hrs.	% Mortality
40	1.25	No hyper action	-	-	
50	"	"	-	-	0
50	"	"	-	-	
40	"	"	-	-	
70	3	No hyper action	-	-	
60	"	"	-	-	50
65	"	"	+	+(14)	
70	"	Hyper activity	+(24)	+(14)	
40	6	No hyper action	-	-	
50	"	"	-	-	50
40	"	Hyper action	+(12)	+(24)	
50	"	"	++(6)	+(12)	
40	10	Immediate hyper activity	++(6)	+(12)	
40	"	"	++(6)	+(12)	75
45	"	"	++(8)	+(12)	
40	"	"	++(6)	-	

21. 11-(4-Dimethylamino benzylidene) strychnine N-oxide (24)

Weight of frog (g)	Dose given (mg/100g)	Onset of action	Convulsion Type (onset time in hrs.)	Mortality in hrs.	% Mortality
40	1.25	No hyper activity	-	-	
50	"	"	-	-	0
50	"	"	-	-	
45	"	"	-	-	
50	3	No hyper activity	-	-	
60	"	"	-	-	25
50	"	Mild hyper activity	+(24)	+(48)	
60	"	"	-	-	
50	6	Hyper activity after 12 hours	+	-	
60	"	"	+	-	50
50	"	Hyper activity after 2 hours	+(12)	+(6)	
60	"	Hyper activity after 6 hours	+(12)	+(12)	
50	10	Hyper activity in 6 hours	+(12)	+(24)	
50	"	"	++(10)	+(6)	50
40	"	"	+	-	
50	"	"	+	-	

22. 11-(3,4,5-Trimethoxybenzylidene) strychnine (22)

Weight of frog (g)	Dose given (mg/100g)	Onset of action	Convulsion Type (onset time in hrs.)	Mortality in hrs.	% Mortality
50	1.25	No hyper action	-	-	
45	"	"	-	-	0
45	"	"	-	-	
50	"	"	-	-	
50	3	Hyper action after 8 hours	+(12)	-	
55	"	No hyper action	-	-	0
55	"	"	-	-	
60	"	"	-	-	
50	6	No hyper action	-	-	
55	"	"	-	-	0
55	"	"	-	-	
60	"	"	-	-	
60	10	No hyper action	-	-	
65	"	"	-	-	0
60	"	"	-	-	
60	"	"	-	-	

23. 11-(3,4,5-Trimethoxybenzylidene) strychnine N-oxide (25)

Weight of frog (g)	Dose given (mg/100g)	Onset of action	Convulsion Type (onset time in hrs.)	Mortality in hrs.	% Mortality
60	1.25	No hyper action	-	-	
65	"	"	-	-	0
55	"	"	-	-	
60	"	"	-	-	
60	3	No hyper action	-	-	
55	"	"	-	-	0
55	"	"	-	-	
55	"	"	+	-	
60	6	Slight hyper action	-	-	
65	"	"	+(24)	+(48)	25
60	"	No hyper action	-	-	
55	"	"	-	-	

24. 21,22-Dihydrostrychnine (26)

Weight of frog (g)	Dose given (mg/100g)	Onset of action	Convulsion Type (onset time in hrs.)	Mortality in hrs.	% Mortality
50	1.25	No hyper action	-	-	
50	"	"	-	-	0
50	"	No excitability	-	-	
60	"	"	-	-	
55	3	Mild hyper action after 1 hour	+(2)	+(5)	
70	"	"	+(3)	+(6)	75
50	"	Strong hyper action after 2 hours	+(4)	+(12)	
60	"	"	+(8)	-	
60	6	Strong hyper action after 30 minutes	++(1)	+(4)	
70	"	"	++(2)	+(6)	100
65	"	"	++(0.5)	+(0.5)	
70	"	"	++(0.5)	+(0.5)	

25. 21,22-Dihydrostrychnine N-oxide (27)

Weight of frog (g)	Dose given (mg/100g)	Onset of action	Convulsion Type (onset time in hrs.)	Mortality in hrs.	% Mortality
80	1.25	No hyper action, no excitability	-	-	
75	"	"	-	-	0
80	"	"	-	-	
80	"	"	-	-	
70	3	No hyper activity, no response to external stimuli	-	-	
75	"	"	-	-	0
80	"	"	-	-	
60	"	"	-	-	
70	6	No hyper activity	-	+(6)	
60	"	"	-	+(4)	75
50	"	"	-	+(6)	
75	"	"	-	-	

26. 2-Nitro-21,22-dihydrostrychnine (28)

Weight of frog (g)	Dose given (mg/100g)	Onset of action	Convulsion Type (onset time in hrs.)	Mortality in hrs.	% Mortality
40	1.25	No immediate hyper action	-	-	
50	"	"	-	-	25
45	"	"	-	-	
50	"	Hyper action after 30 minutes	+(6)	+(12)	
60	3	Immediate hyper action and excitability	++(0.5)	+(12)	
50	"	Hyper action and excitability after 30 minutes	++(2)	+(12)	75
65 5	"	"	++(2)	+(12)	
60	"	"	++(6)	-	
70	6	Hyper action and typical excitation on external stimuli	++(0.5)	+(2)	
75	"	"	++(1)	+(2)	100
80	"	"	+++(0.5)	+(2)	
80	"	"	+++(0.5)	+(2)	

27. 2-Nitro-21,22-dihydrostrychnine N-oxide (29)

Weight of frog (g)	Dose given (mg/100g)	Onset of action	Convulsion Type (onset time in hrs.)	Mortality in hrs.	% Mortality
60	1.25	No hyper action	-	-	
65	"	"	-	-	0
65	"	"	-	-	
50	"	"	-	-	
70	3	No hyper action	-	-	
70	"	"	-	-	25
60	"	"	-	-	
50	"	"	-	+(4)	
80	6	No hyper action	-	+(12)	
80	"	"	-	+(6)	75
100	"	"	-	+(2)	
60	"	"	-	-	

28. Reaction product of strychnine with benzoyl peroxide in chloroform (30)

Weight of frog (g)	Dose given (mg/100g)	Onset of action	Convulsion Type (onset time in hrs.)	Mortality in hrs.	% Mortality
60	1.25	No excitability, no hyper action frogs were alert	-	-	
65 5	"	"	-	-	0
50	"	"	-	-	
60	"	"	-	-	
60	3	Slight hyper action	-	-	
50	"	No hyper action, frogs were alert	-	-	0
55	"	"	-	-	
55	"	"	-	-	
50	6	No hyper action	-	-	
55	"	"	-	-	0
55	"	"	-	-	
45	"	Slight hyper action	+	-	
60	10	No hyper action	-	-	
70	"	Hyper action after 6 hours	+	-	25
70	"	Hyper action after 12 hours	+	+	
80	"	No hyper action	-	-	

29. N-oxide of the reaction product of strychnine with benzoyl peroxide in chloroform (31)

Weight of frog (g)	Dose given (mg/100g)	Onset of action	Convulsion Type (onset time in hrs.)	Mortality in hrs.	% Mor ality
40	1.25	No hyper action, no excitability	-	-	
45	"	"	-	-	0
45	"	"	-	-	
50	"	"	-	-	
45	3	No hyper action	-	-	
50	"	"	-	-	0
60	"	Hyper action after 6 hours	-	-	
45	"	"	+(12)	-	
40	6	No hyper action	-	-	
45	"	Hyper action and excitability	+(12)	+(24)	25
45	"	"	+(12)	-	
50	"	No hyper action	-	-	
60	10	No hyper action	-	-	
70	"	Hyper action in 6 hours	+(12)	-	25
65	"	"	+(12)	-	
60	"	"	+(12)	+(24)	

30. Combination of strychnine(1) and 30

Weight of frog	Dose given mg/100g)	Onset of action	Convulsion Type (onset time in hrs.)	Mortality in hrs.	% Mortality
100	3(1)+3(30)	Hyper action after two hours	+(5)	+(12)	
100	"	"	+(5)	+(12)	50
100	"	Hyper action after four hours	-	-	
80	"	"	+(6)	-	
60	3(30)+3(1)	Hyper action after five hours	+(6)	+(8)	
60	"	"	+(6)	+(24)	75
60	"	Hyper action after 3 hours	+(6)	+(25)	
80	"	Hyper action after six hours	+(6)	-	
60	3(1)+6(30)	No hyper action upto four hours	+(3)	+(24)	
60	"	"	+(4)	+(24)	75
60	"	Hyper action in two hours	+(6)	+(24)	
60	"	"	+(8)	-	
60	6(30)+3(1)	No hyper action upto 3 hours	+(6)	+(12)	
60	"	"	+(6)	+(14)	50
60	"	Hyper action after two hours	-	-	
80	"	Hyper action after 24 hours	-	-	

Combination of strychnine(1) and 30 Table continued

70	3(1)+10(30)	Hyper action after 3 hours	+(6)	-	
80	"	"	+(8)	+(24)	50
100	"	Hyper action in 1 hour	+(4)	+(24)	
80	"	"	+(4)	-	
80	10(30)+3(1)	Hyper action in 1 hour	+(4)	-	
80	"	"	+(4)	+(30)	50
75	"	Hyper action in 2 hours	+(5)	+(30)	
75	"	"	+(5)	-	

CHAPTER V

SUMMARY AND CONCLUSIONS

Strychnine, the major alkaloid present in Strychnos nuxvomica seeds has been reported to stimulate the entire central nervous system with preference for the spinal cord. It is a powerful convulsant and because of this property, it is an important pharmacological tool as it plays a unique role as an inhibitor of post synaptic inhibitory impulses. It is useful to study inhibitory transmitter and receptor types. However, because of its extreme toxicity, strychnine does not have any therapeutic application in the Western system of medicine.

The present work was undertaken with a view to obtaining strychnine derivatives having CNS stimulating properties but with sufficiently low toxicity so that they may eventually find some application in medicine. As strychnine is isolated from the locally available Strychnos nuxvomica seeds, its possible utilization in therapeutics will have considerable commercial significance.

A number of derivatives of strychnine were prepared. These included products with nitro, amino, acetamido, bromo and sulphonamido groups introduced at the 2 position by electrophilic aromatic substitutions and subsequent chemical conversions as required. Also reduction of the amide carbonyl group at position 10 using lithium aluminium hydride

provided strychnidine. Treatment of strychnine with benzaldehyde and substituted benzaldehydes yielded derivatives with benzylidene groups attached at 11-position. Similarly an oxime was obtained at this position by reaction with amylnitrite. Catalytic hydrogenation of strychnine in the presence of palladium on carbon provided the known 21,22-dihydro-strychnine which on nitration gave another derivative having nitrogroup at 2-position and with the 21,22 double bond saturated. The structures of all new compounds were established with the help of spectral data and elemental analysis. Finally a reaction of strychnine with chloro-form in the presence of benzoyl peroxide yielded a tri-chloromethyl derivatives, but the exact position of this group has not been determined. These reactions have thus yielded structural analogue of strychnine with modifications at the aromatic ring and at position 10,11 and 21-22.

There have been reports that the N-oxides of alkaloids generally modify their pharamacological properties and the N-oxide of strychnine (known as genostrychnine) is less toxic and less convulsive than strychnine itself. However, strychnine N-oxide is not being used as a therapeutic agent now probably because

of the threat of convulsions at higher doses. All the derivatives of strychnine which were prepared as mentioned earlier were, therefore,converted into their N-oxides by well established procedures. Brucine N-oxide was also prepared from brucine for the sake of comparitive studies.

Pharmacological studies were carried out on the derivatives of strychnine and their N-oxides. These experiments were conducted systematically on frogs weighing 40-100 g. Each dose was given to a number of frogs as uniform suspension and equimolar quantities were administered and strychnine was kept as control.

Generally, the N-oxides of all compounds were less toxic and most of them showed no convulsions in accordance with earlier reports. The nitro, bromo and amino substituents at 2-positions of strychnine did not substantially change the pharmacological activity. 2-Amino strychnine, 2-acetamidostrychnine and its N-oxide manifested muscle relaxant properties as well. The acetamido derivative and its N-oxide were less toxic compared to strychnine. Brucine and its N-oxide did not exhibit strychnine type convulsions or toxicity.

The introduction of the biologically important sulphonamido group also reduced the toxicity of the

strychnine molecule and the N-oxide further decreased the toxicity as expected. Although the benzaldehyde condensation product at position 11 showed increased toxicity and convulsive property, substituted benzaldehyde condensation products provided compounds with substantially lower toxicity compared to strychnine. Their N-oxides were found to have further decreased toxicity with a similar effect on the convulsant activity. The 11-oximino derivative and its N-oxide also had reduced toxicity. Saturation of the double bond at the 21,22 position made the molecule more toxic and produced stronger convulsions. These effects were demonstrated both by dihydrostrychnine and the 2-nitrodihydrostrychnine. The N-oxides of the saturated derivative also had correspondingly increased toxicity compared to their unsaturated analogues.

One of the products with lowest toxicity was the trichloromethyl derivative and it produced only very minor convulsions on frogs. The toxicity and convulsive property was further decreased when the trichloromethyl derivative was converted into its N-oxide. A complete pharmacological screening of these compounds are required for their further biological evaluation.

This work has provided several new compounds which are significantly less toxic than strychnine and its N-oxide as shown from the pharmacological studies. As they also possessed CNS stimulating properties, they are well suited for further screening to assess their potential as valuable therapeutic agents.

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