

ISOLATION AND CHARACTERISATION
OF THE ALKALOIDAL CONSTITUENT
OF A PHARMACOACTIVE PLANT -
TRICLISIA SUBCORDATA

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T H E S I S R E C O M M E N D A T I O N

We hereby recommend that the thesis prepared by Mrs. F. W. Abdulrahman entitled "ISOLATION AND CHARACTERISATION OF THE ALKALOIDAL CONSTITUENT OF A PHARMACOACTIVE PLANT - TRICLISIA SUBCORDATA" be accepted in partial fulfilment of the requirements for the degree of M.Sc. Analytical Chemistry.

INTERNAL EXAMINER

EXTERNAL EXAMINER

Date

Date

DEDICATION

This thesis is sincerely dedicated to my Darling Husband
and Loving Children.

ACKNOWLEDGEMENT

My profound gratitude goes to my supervisor, Dr. S. K. Okwute for his constant guidance, cooperation, and for proving an untiring source of encouragement in many ways. He was very accessible and showed a lot of interest in the work.

I gratefully acknowledge my indebtedness to Professor C. Ikoku, my Co-Supervisor for his advice, useful suggestions and the help rendered in supplying some chemicals needed from Sokoto University and the efforts made in providing the plant materials investigated in this study.

My thanks also go to the Head of Department of Chemistry for providing the facilities necessary for the project, Mr. O. K. Abukkar, Mr. Yakubu of Physical Laboratory and other technical staff, too many to mention, who contributed immensely to the success of this work.

I'm most grateful to my mother and brothers for their understanding, endurance and the great help rendered during the course of this study.

A B S T R A C T

The roots of Trielisia subcordata have been exhaustively-extracted with methanol. Extraction of the methanol extract with dilute hydrochloric acid followed by Gasification gave an alkaloidal fraction which on Column Chromatography yielded a major compound (A). Based on chemical and spectroscopic evidence, (A) has been tentatively assigned structures (18-19).

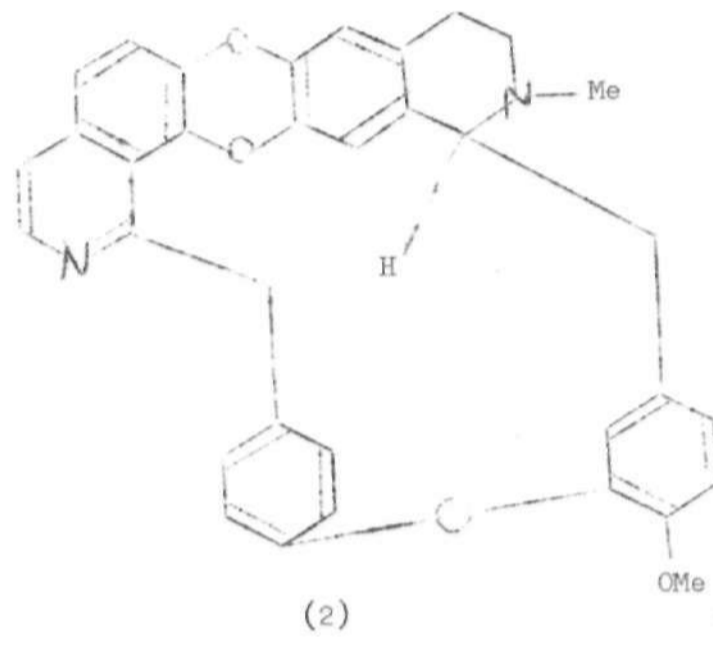
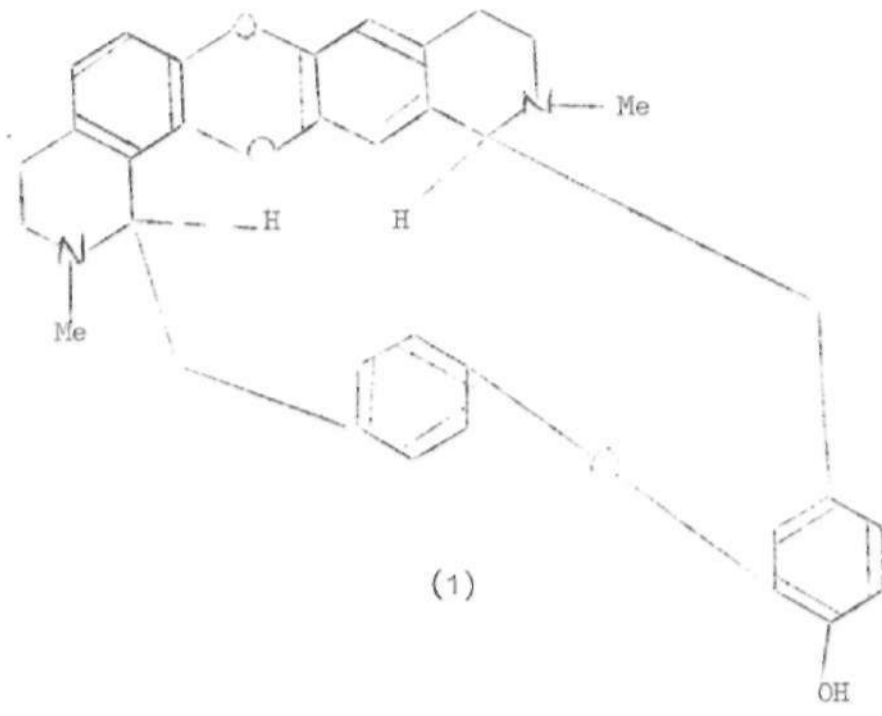
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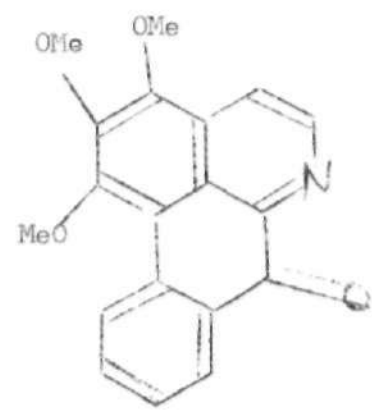
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INTRODUCTION

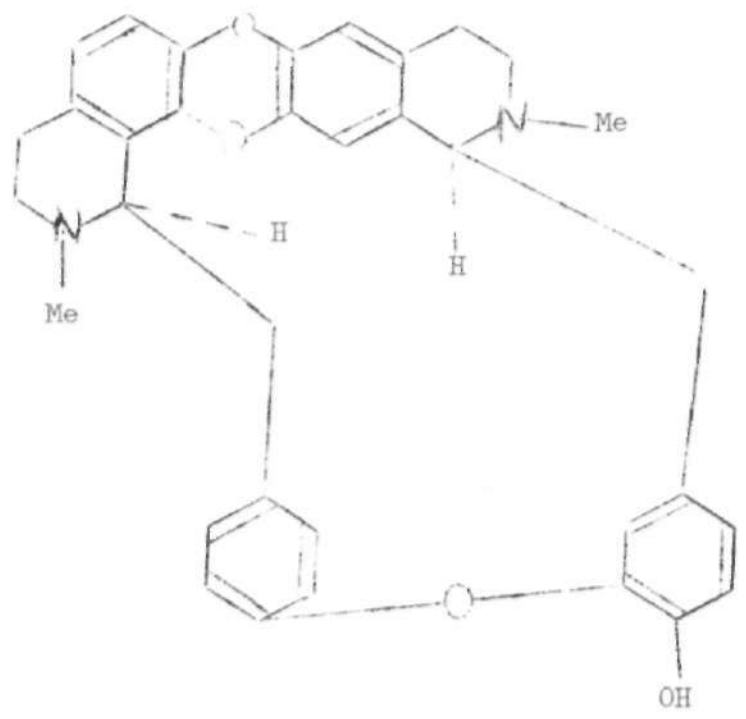
The genus Triclisia belongs to the family Menispermaceae (Order-Berberidales). Plants belonging to this family are twining or rarely erect shrubs. They are generally distributed throughout the tropics with a few species in warm temperate regions, but occurring mainly in evergreen forest areas of tropical Africa.¹ A considerable number of Menispermaceous alkaloid yielding plants have been used as crude drugs in the Far East. However, the alkaloids so far isolated from them, though pharmacologically active, do not seem to be of practical therapeutic value.²

In the past decade, a number of the species belonging to this family have been studied. Phytochemical investigations of these plants have revealed that Triclisia gillettii found in Ivory Coast and Southern Nigeria¹ contained Efirine³ (1) and Trigillettine⁴ (2), while Phenanthine and O-methylmoschatoline⁵ (3) have been isolated from the stems. Ethanolic extract of the roots of this specie has yielded Trigillettine⁶ (4). Investigation also revealed that a diquaternary alkaloid, N,N¹-dimethylpheanthine with muscle





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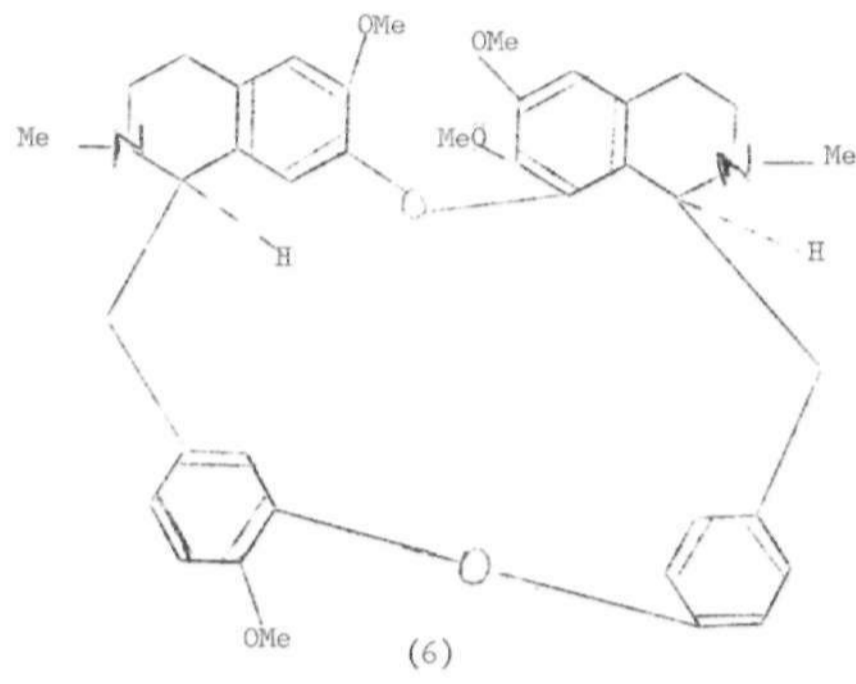
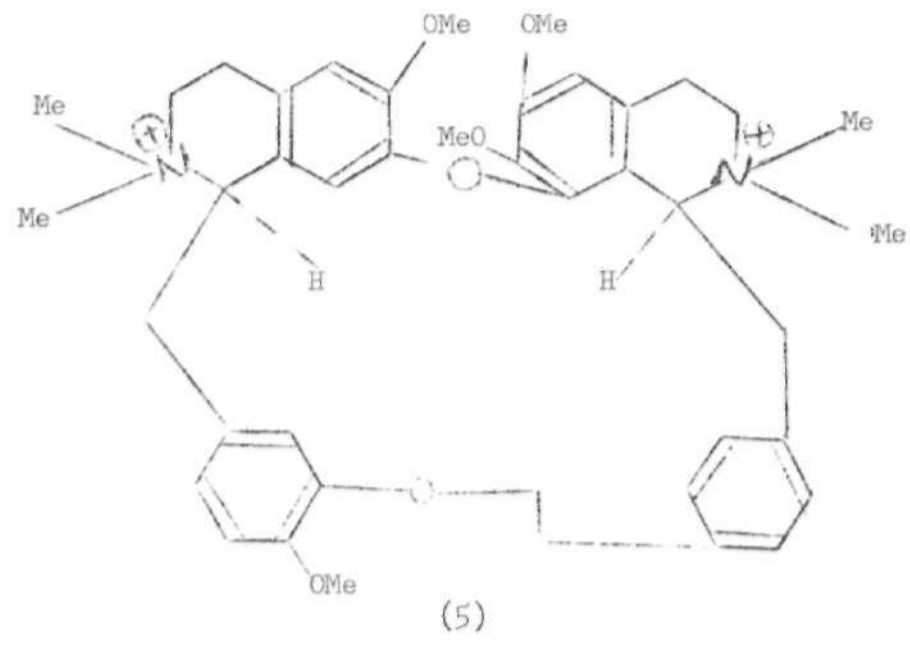


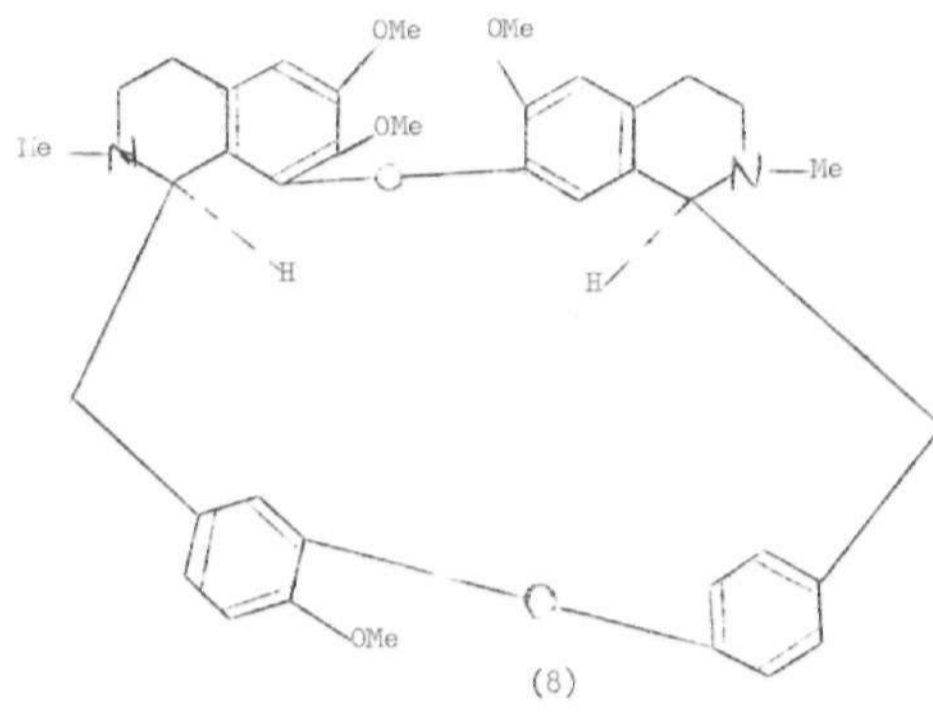
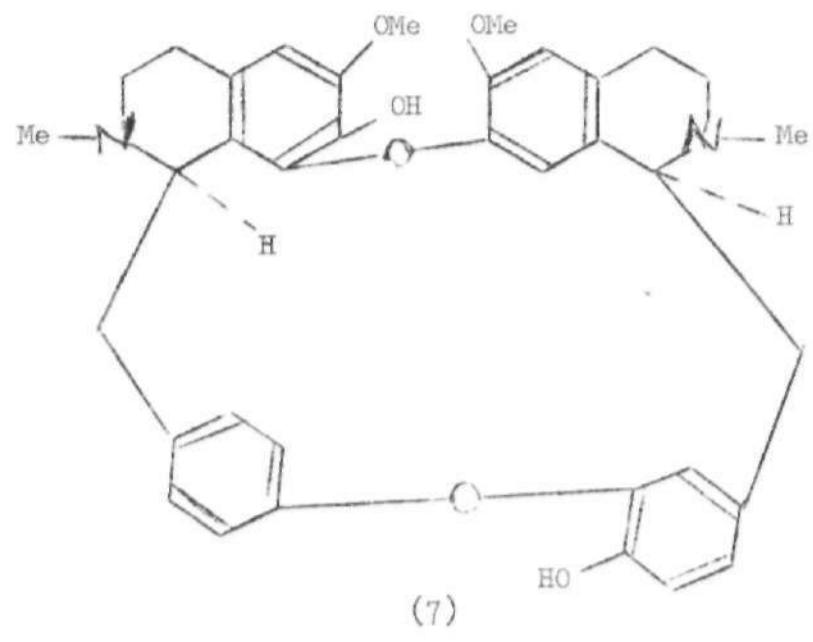
(4)

relaxant effect on rats and mice, Pheanthine⁷ (6), and Trigillettamine⁴ (2) have been isolated from T. patens (found in Liberia and Senegal). The ethanol extract of powdered leaves of the specie has yielded O-methylmoschatoline (Homoschatoline) (3) and Aromoline⁸ (7). Further studies of the Triclisia species showed that N,N¹-dimethylpheanthine (5) and pheanthine⁷ (6) have been isolated from T. dictyophylla. Extracts of the leaves and twigs also yielded Triclisine, Tricliseine and Nositol.⁹

Physiological studies on these Triclisia species have revealed that their extracts have been locally used in the treatment of Malaria, diarrhea, Pyorrhea, swelling in the extremities, anaemia joint pains, and as arrow poisons.¹⁰ The leaves and twigs of T. dictyophylla have been locally used for treating anaemia and oedema of legs.

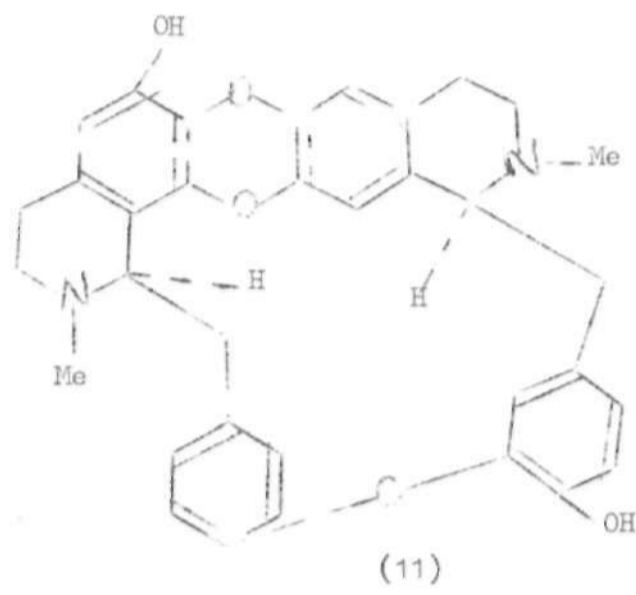
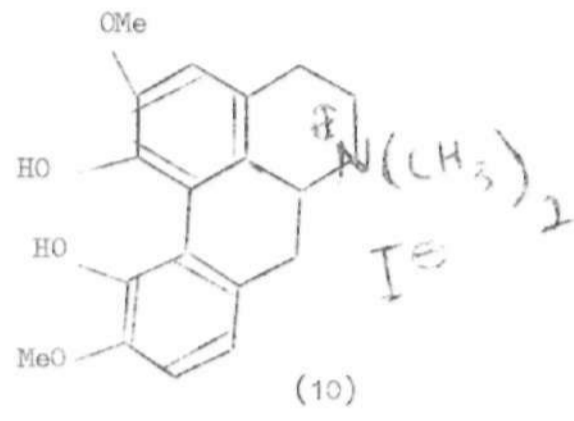
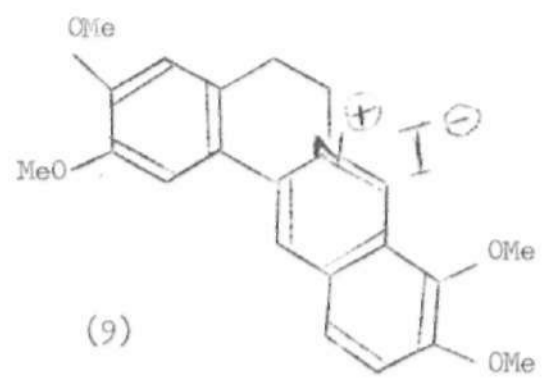
T. subcordata commonly called Alugborron in Yoruba is a slender woody twiner with brownish stems usually 6-15ft long with green flowers and orange fruits. It is found in Cape Coast, Achimota, Lome, Northern and Southern Nigeria.¹ The root has been successfully used locally, especially in the Eastern states of Nigeria as a smooth muscle relaxant to expel retained placenta and as smoothening

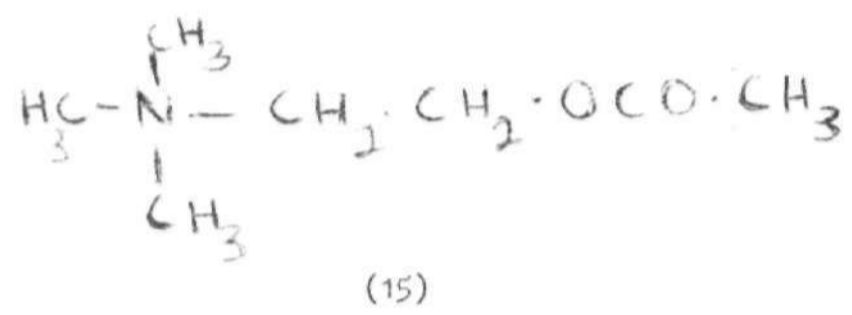
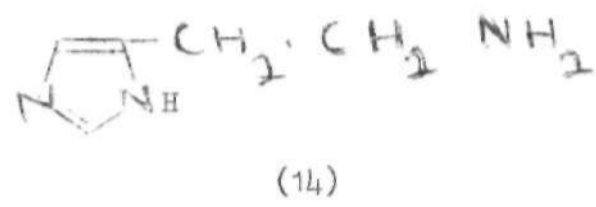
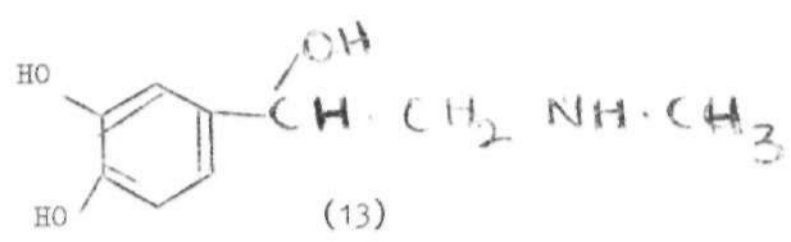
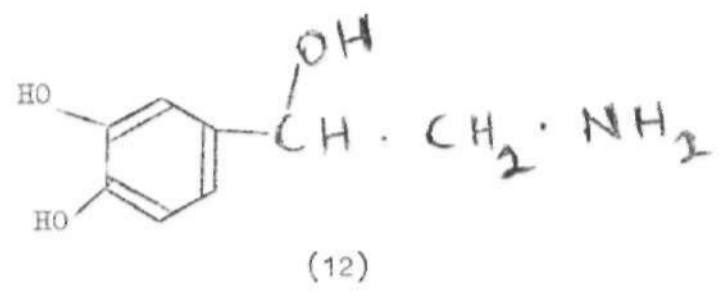




agent when rubbed on the body either alone or with palm kernel oil. In the Northern States, it is used as a laxative. Recent phytochemical investigations on the plant led to the isolation of a few alkaloids. The chloroform extract of the powdered roots has yielded Tetrandrine⁸ (8). Adsorption column chromatography of the quaternary iodide fraction over silicic acid gave Planatine iodide (9), Magnoflorine iodide (10) and two incompletely characterised alkaloids.¹¹ Ethanol extract of the roots also yielded Tricordatine⁶ (11).

The responses to the water extract of the plant of isolated tissues (rabbit-aortic strip, guinea-pig ileum, guinea pig uterus etc.) have been found to be higher than the responses to known drugs¹² such as Noradrenaline (12), Adrenaline (13), Serotonin, Histamine (14) Isoprenaline and Acetylcholine (15). Pharmacological studies on Tetrandrine have revealed that it has a pronounced irritant action on mucous membrane, is slightly antipyretic, produces hyperglycemia in rabbits, induces emesis in pigeons, depresses cardiac activity, reduces blood pressure and depresses the smooth muscle of isolated rabbit intestine and guinea pig or rabbit Uterus.² It has also been found to cause reversal of the





polymorphonuclear cells and lymphocytes in rabbits four or six hours after injection. It has also been shown pharmacologically¹³ that acetylcholine, adrenaline and Noradrenaline are concerned with the transmission of impulses in the human nervous system. Noradrenaline is secreted by the adrenal medulla and released at the sympathetic nerve endings, producing a variety of physiological effects, including a rise in blood pressure and blood sugar, dilation of the pupil of the eyes, acceleration of the heart rate, relaxation of the bronchial muscles and constriction of some blood vessels. Adrenaline is mixed with local anaesthetic in dentistry and surgery, resulting in reduced bleeding.¹³

The centre of interest of this work is in the muscle relaxing and anti-acid effects of the plant extract. Consequently we have decided to look at the alkaloidal constituents of the root with the primary aim of isolating the characterising any pure alkaloid(s) for subsequent pharmacological studies.

In most alkaloid-bearing plants, the alkaloids constitute only a small percentage in terms of dry weight. In their extraction and isolation, there is first the problem of separating them from the bulk of non-alkaloidal materials. Also, very few plants (if any) produce just one single alkaloid. In the great majority of alkaloid-bearing plants, several alkaloids which are usually

rather closely related to one another in chemical structures occur in the same plant.¹⁴ Therefore, there is the further problem of separating the individual alkaloids. In any extraction process, the plant material needs to be reduced to a moderate coarse powder to facilitate effective contact of solvent with alkaloid-containing tissues and cells. The extraction and isolation of the total alkaloid (i.e., mixture of all or nearly all the alkaloids present in a given sample of plant material) reasonably free from inorganic and non-alkaloidal organic matter may be effected by certain general procedures largely based on the alkaline nature of most alkaloids, the consequent ability to form salts with acids and on the relative solubilities of the alkaloid bases and their salts in water and in various organic solvents. These procedures can be grouped into two types.¹⁴

- (a) Using water - immiscible organic solvent for the initial extraction.
- (b) Using aqueous or alcoholic medium for the initial extraction.

In this project, procedure (b) is followed because it is easier to work with small volume than with large volume, since in this case, the alcoholic extract is

first concentrated to small volume before the acid solution is added. While in the other case, the acid is added to chloroform extract. Shaking of the mixture easily causes formation of emulsion with large volumes, while insufficient mixing of the two phases would result in ineffective extraction of the alkaloid from one phase to the other.

RESULTS AND DISCUSSIONS

Repeated column chromatographic separation of the alkaleidal fraction from the methanol extract of the roots of Triclisia subcordata afforded compound (A) recrystallisable from benzene - petroleum ether mixture as white needle - like crystals (m.p. 274-277°C).

Compound (A), being basic was soluble in dilute hydrochloric acid, and when heated on a nickel spatula in bunsen flame gave a very sooty flame indicating its aromaticity. It gave a positive Lassaigne test for nitrogen. It exhibited positive Mayer's and Dragendorff's tests for alkaloids. No evolution of carbon dioxide was observed when it was shaken with sodium hydrogen carbonate solution, thus carboxylic acid was absent. On addition of ferric chloride solution to an alcoholic solution of compound (A), no characteristic colour (violet, green, blue) was observed giving a negative test for phenol. It also gave negative tests for both primary and secondary amines and also exhibited a negative reaction for an amide test. From the above chemical properties, it could be deduced that compound (A) is neither an acid, nor an amide but an

an alkaloid possessing a tertiary nitrogen atom.

Some spectrophotometric studies on compound (A) gave the following results (Figures 1-3).

$$\nu_{\max} \text{ (nujol)} \quad \text{cm}^{-1} - 1600-1420, 1380, 1220, 1200-1070.$$

In this spectrum, there are no characteristic bands between 3600-3000 cm^{-1} thus indicating the absence of O-H and N-H thus the compound (A) is neither phenolic nor acidic nor a primary or secondary amine. The absence of bands at 1720-1700 cm^{-1} is also suggestive that the compound (A) is not an amide. The bands between 1600-1420 cm^{-1} indicate the C=C stretch of the arenes while that at 1380 cm^{-1} shows the C-H stretch of the methyl groups. The bands between 1200-1070 have been attributed to C-O stretching of the ether linkages. The IR spectrum gives a hint that although the compound is neither an acid nor an amide, it contains ether linkages in the system and is probably aromatic.

The NMR spectrum of compound (A) in CDCl_3 showed the following signals: - δ 2.59; 3.55; 3.88; 4.2-4.5; 5.8-7.56 and 2.7-3.39. It has been observed¹⁵ that a methoxyl group attached to a benzene ring has a normal chemical shift of 3.8 on the δ scale. This chemical shift is an average value for the various orientations

of the methoxyl groups with respect to the benzene ring reached by rotation about the aromatic C-O bond. Benzene rings are magnetically very anisotropic because applied magnetic fields readily generate currents in the π -electron system. The sense of this anisotropy is such that the hydrogen nuclei of a methoxyl group lying in the plane of the ring will have a somewhat lower chemical shift than those of a methoxyl group above or below the ring i.e. lying so that the plane of the C-O-C atoms is perpendicular to that of the ring. The presence of bulky substituents near such a group will tend to force it out of the aromatic plane i.e. to favour lower δ values by a few hundredths of a unit. More pronounced shifts to lower δ values by several tenths of a unit are found when a methoxyl group can take up configurations so that its hydrogen atoms fall more directly over the top of a second adjacent benzene ring. This is the predominant cause of the marked spread of methoxyl resonances ($\delta = 3.02 - 3.95$) observed with both Bisbenzylisoquinoline and Aporphine alkaloids. The methylamino resonances also exhibit a range of values ($\delta = 2.25-2.65$) for the same reasons. It has also been established¹⁵ that for both classes of alkaloids the chemical shifts of methoxyl groups adjacent to two benzene rings at positions 5 and have consistently lower chemical shifts

($\delta = 3.42-3.63$) than those at 2,3 or 6(3.72-3.89) on scale. Those at position 4 have intermediate chemical shifts (3.65-3.72) for the Aporphine Alkaloids. All methylamino alkaloids lie in the range $\delta = 2.35-2.55$. From the foregoing discussions, it could be rightly said that there are two methoxyl group signals in the NMR spectrum of compound (A) i.e. the singlets at $\delta = 3.55$ and 3.88 and that one of them is attached to position 5, as the general δ value of methoxyl group at this position is 3.63 - 3.42 and the other one is attached to either position 2,3 or 6 as the general δ values for methoxyl groups at these positions range from 3.72 to 3.89. In this compound, there can be no methoxyl group at position 6 because of the absence of the two singlets at $\delta = 6.6$ and 7.4 which are characteristic of two para protons on tetrasubstituted benzene. The third singlet at $\delta = 2.59$ is also due to the methylamino group. On the basis of the above information and using methoxyl (OMe) and Methylamino (NMe) groups as standards, the doublet at $\delta = 4.2-4.5$ represents one proton (1H) and may be due to the methine proton coupling with the benzyl methylene protons. The Multiplet at $\delta = 5.8-7.56$ (6H) is attributable to the aromatic protons, and that at 2.7-3.39 (6H) has been assigned to the aliphatic Methylenes.

The qualitative U.V. spectrum of Compound (A) in methanol gave absorptions at the following wavelengths.

$\lambda_{\text{max}}^{\text{nm}}$:- 229, 275, and 284. The maximum peak is at 229, the other two peaks at 275 and 284 are small shoulders. Generally speaking,¹⁶ the spectral of Aporphine Alkaloids (type 17) with no substitution at position 3 and 4 ($R^1=R^2=R^3=R^4=R^5=H$) show one peak at $270\text{-}280\text{cm}^{-1}$. Most of the aporphine alkaloids are substituted at positions 3 and 4 and all the known alkaloids are invariably substituted at positions 5 and 6. It was recognised¹⁶ that the spectra of the other aporphines fall into two characteristic types having maxima at 220 and two in the region $270\text{-}310\text{cm}^{-1}$. The shapes of the curve and the intensity of the latter two maxima depend on the substitution in ring D.

Considering the above values, i.e. the literature values and the values in question, compound (A) might be an Aporphine - type alkaloid with substituents at positions 2 or 3 and 5.

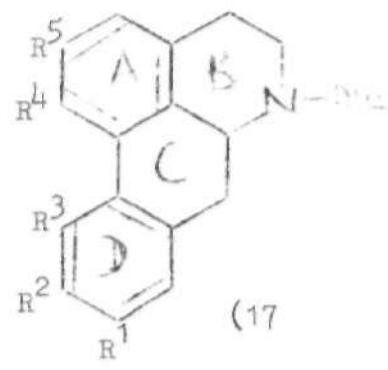
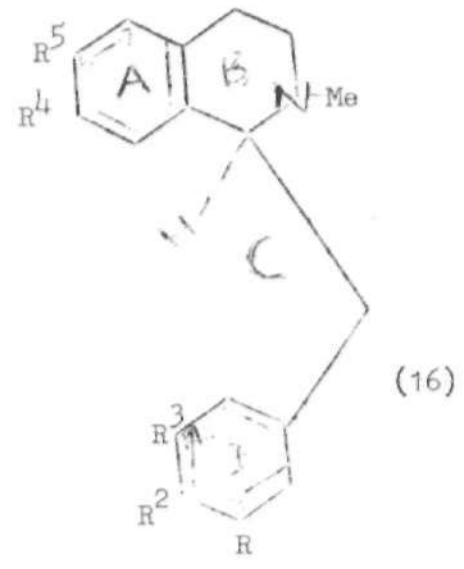
Comparing the spectroscopic data obtained for compound (A) with those recorded for other alkaloids, it is possible to give tentative structures to compound A.

TABLE 1: THE SPECTROSCOPIC DATA AND MELTING POINTS OF SOME BISBENZYLISOQUINOLINE ALKALOIDS INCLUDING THOSE OF COMPOUND (A).

Compound	NMR(CDCl ₃) 60 mHZ	U.V. (mech max nm (log t))	IR KBr cm ⁻¹ V _{max}	Melting Point °C
Tetrandrine ⁸ (8)	2.29(S, 3H, NMe); 2.58(S, 3H, NMe); 3.15(S, 3H, OMe) 3.31 (S, 3H, OMe) 3.69(S, 3H, OMe); 3.87(S, 3H, OMe); 5.91-7.35 (M, 10H, ArH)	214(4.78) 283(3.91)	1608, 1585 1505, 1273 1235, 1213, 1135, 1128 1113, 1070 1028, 845	218
Tricordatine ⁶ (11)		227(-4.60) 275(369)sh) 284(3.71) 304(3.44)sh)		280
Efirine ³ (1)	2.4(S, 3H, NMe); 2.58(S, 3H, NMe); 3.86(S, 3H, OMe); 6.0-7.7(M, 10H, ArH); 4.2-4.65(1H, OH)	260(3.7) 285(3.9) 300(3.8)	3450, 2950 1600, 1500 1410, 1340 1250, 1140 1100, 800	268, 5 -269
Isotrilobine	2.4(S, 3H, NMe), 2.58(S, 3H, NMe), 3.80(S, 3H, OMe) 3.94(S, 3H, OMe), 6.0-7.15(M, 10H, ArH)			
Compound (A)	2.59(S, 3H, NMe) 3.55(S, 3H, OMe), 3.88(S, 3H, OMe); 4.2-4.5(a, 1H, CH); 5.8-7.56(M, 6H, ArH) and 2.7-3.39(M, 6H, (CH ₂) ₃)	229 275 and 284	1600-1420 1380, 1220 1200-1010	274-277

TABLE 2: THE CHEMICAL SHIFT (δ -VALUES FOR ALKALOIDS OF THE BENZYLISOQUINOLINE, APORPHINE SERIES AND COMPOUND (A)

	Type	R ¹	R ²	R ³	R ⁴	R ⁵	2 Δ	3 Δ	4	5	6	NMe	OCH ₂ O
+ Laudanosine ¹⁵	16	H	OMe	OMe	OMe	OMe	-	3.82	3.78	3.55	3.82	2.52	-
Dicentrine ¹⁵	17	OMe	OMe	H	OCH ₂ O		3.80	3.80	-	-	-	2.28	5.95 & 5.80 (Doublet)
Glaucine ¹⁶	17	OMe	OMe	H	OMe	OMe	3.80	3.75	-	3.55	3.80	2.43	-
Compound(A)							= 2.59, 3.55, 3.88, 4.2-4.5, 5.8-7.6 and 2.7-3.39.						



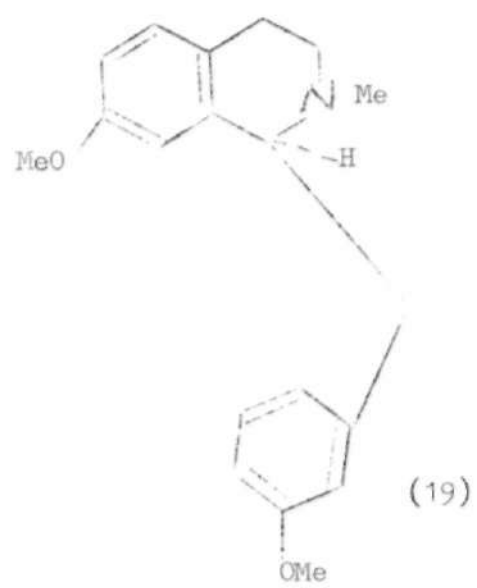
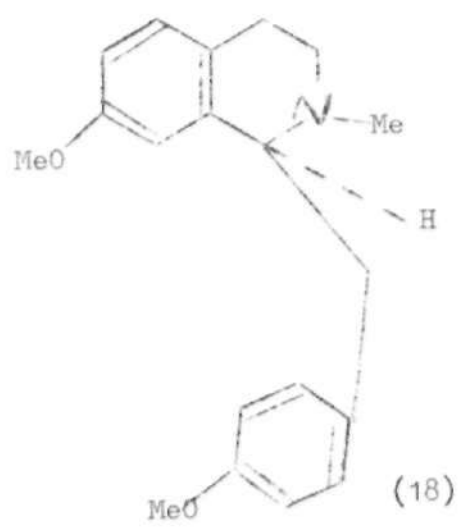
From the literature (Table 1), It has been found that the spectral values obtained for each of the alkaloids are indicative of of the Bisbenzylisoquinoline alkaloid of the Isotrilobine type. It can be seen that Efirine (1) and Isotrilobine have the same basic skeleton except for the OH signal at $\delta = 4.2 - 4.62$ in Efirine and the methoxyl (OMe) signal at $\delta = 3.94$ in Isotrilobine. Based on spectra data, compound (A) cannot be either of the alkaloids in Table 1 because Tetrandrine (8) has four methoxyl groups with ten aromatic protons. Efirine (1) is monomethoxy, mono-phenolic, with two methylamino groups and ten aromatic protons. While Tricordatine (11) is diphenolic, has two methylamino groups with ten aromatic protons, and Isotrilobine is dimethoxy, has ten aromatic protons and two methylamino groups. On the contrary, compound (A) has one methylamino group, two methoxyl groups, six aromatic protons and is non-phenolic.

Previous chemical studies on Triclisia subcordata led to the isolation of Tetrandrine (8), Tricordatine (11), Palmatine (9), and Magnoflorine (10). Tetrandrine and Tricordatine belong to the class of Bisbenzylisoquinoline alkaloids and they generally have ten aromatic protons in the molecule as well as two methylamino groups. Palmatine and Magnoflorine are Aporphine - type

alkaloids possessing six and three aromatic protons respectively. Unsubstituted Aporphine (16) ($R^1=R^2=R^3=R^4=R^5=H$) has nine aromatic protons when ring (c) is open but seven aromatic protons with ring (c) closed (17).

From the available evidence, Compound (A) probably belongs to the class of Aporphine alkaloids because it has six aromatic protons as observed in its NMR spectrum. It is however neither Palmatine nor Magnoflorine since it has two methoxyl groups and one methylamino group. Palmatine is tetramethoxy with a quaternary nitrogen atom in its structure while Magnoflorine is dimethoxy, diphenolic and has two methylamino groups.

Based on chemical and spectral data, Compound (A) can be grouped among the aporphine alkaloids (16) and the following tentative structures (18-19) have been assigned to it. A more conclusive structure could not be drawn in this work because of the inaccessibility of the mass spectrum and Microanalysis. Also, future work involving chemical degradation will help to locate the position of the methoxyl groups.



CONCLUSIONS

Compound (A) does not belong to the Bisbenzylisoquinoline - type alkaloids because it does not contain ten aromatic protons, neither does it have two methylamino groups typical of this class of alkaloids. It could be conclusively said to belong to the aporphine class of alkaloid of type (16) (benzylisoquinolines) because it has six aromatic protons with three substituents and one methylamino group as observed in most Aporphine - type alkaloids.

Alkaloids have long been studied and found to be of pharmacological importance and of therapeutic value. Although, the pharmacological activities of compound (A) were not investigated partly because of the time limit for the project, the traditional medicinal properties of this plant may not be unrelated to its alkaloidal constituents. Also, the presence of an Aporphine alkaloid of type (16) in a plant previously known to contain Aporphines of type (17) i.e. palmatine¹¹ (19) and Magnoflorine¹¹ (10), as well as Bisbenzylisoquinolines i.e. Tetrandrine⁸ (8) and Tricordatine⁶ (11) tends to provide a common biogenetic precursor

for Aporphines of type (17) and the Bisbenzylisoquinoline through oxidative couplings of two aromatic rings of the same molecule or of two benzylisoquinolines.

E X P E R I M E N T A L

The roots of Triclisia subcordata were collected from Imo State of Nigeria, sun dried and powdered. Ether refers to di-isopropyl ether. Dragendorff's and Mayer's reagents were prepared according to reference (14). Neutral Alumina refers to Brochman Activity I.

Rf - value was determined on silica gel with fluorescent indicator 254nm (DC-cards SIF 10x20cm). Melting point was determined on a hot stage apparatus (Olympus) and was uncorrected.

IR spectra were obtained with Perkin Elmer (SP 700). NMR spectra were recorded with variance (SP 700) model with TMS as the internal reference, values are quoted in (δ). U.V. spectra were obtained with Perkin Elmer (SP 800).

EXTRACTION

The sun dried root (530g) was crushed into fine powder. It was shaken in portions with redistilled methanol (6 litres) on a mechanical shaker for three days. The methanolic solution of the extract so obtained was filtered and the filtrate evaporated to dryness with a rota vapor. The extract was acidified to pH 2 with 2M hydrochloric acid to convert the alkaloids to their salts. The resulting solution was extracted with ether ($4 \times 100 \text{cm}^3$) and then with chloroform. ($3 \times 100 \text{cm}^3$) to ensure total extraction of non alkaloidal organic components. The ether and chloroform extracts were separately dried over anhydrous sodium sulphate and evaporated. The ether extract weighed (5.965g, 1.126% of the dry weight of plant). The aqueous acidic (Alkaloidal) solution was basified with 10% sodium carbonate to pH 12 and extracted with chloroform ($4 \times 10 \text{cm}^3$). The chloroform solution of the alkaloids was dried over anhydrous sodium sulphate, filtered and filtrate evaporated to dryness. The residue weighed (6.14g; 0.158% of dry weight of plant).

ISOLATION

In this procedure, the major task was the problem of finding a suitable solvent system with best column materials. Thin layer chromatography of the crude alkaloidal extract showed a major compound with several other compounds in traces. In the bulk separation process, the column (58x5.5cm) was packed with neutral alumina (300g) as a petroleum ether slurry. The alkaloidal extract (3.43g) was dissolved in benzene and spotted on the column. It was first eluted with chloroform and then with methanol - chloroform mixture collecting 100cm³ of eluent each time. Pure chloroform eluted the major compound (A) (0.5933g; 0.112% of dry weight of plant) which was recrystallisable from benzene petroleum ether as white needles (m.p. 274-277°C).

Rf value = 0.60 (methanol - chloroform 1:7).

ν_{\max} (nujol) cm⁻¹: - 1600-1420, 1380, 1220, 1200-1070.

NMR (CDCl₃): - 2.59(s, 3H, NMe); 3.55 (s, 3H, OMe), 3.88 (s, 3H, OMe); 4.2-4.5 (d, 1H, CH); 5.8-7.56 (m, 6H, ArH) and 2.7-3.39 (m, 6H, (CH₂)₃)

λ_{\max} (MeOH) nm: - 229, 275, and 284.

CHEMICAL TESTS ON COMPOUND (A)

Compound (A) was soluble in dilute hydrochloric acid, gave positive reaction for, Lassaigne nitrogen test, exhibited positive results for Mayer's and Dragendorff's tests for alkaloids. No evolution of carbon dioxide gas on shaking a solution of compound (A) with sodium hydrogen carbonate (NaHCO_3) solution. It gave a negative test with ferric chloride (FeCl_3) solution. On heating compound (A) in bunsen flame, a very sooty flame was observed. It showed negative reaction for primary and secondary amine and amide tests.

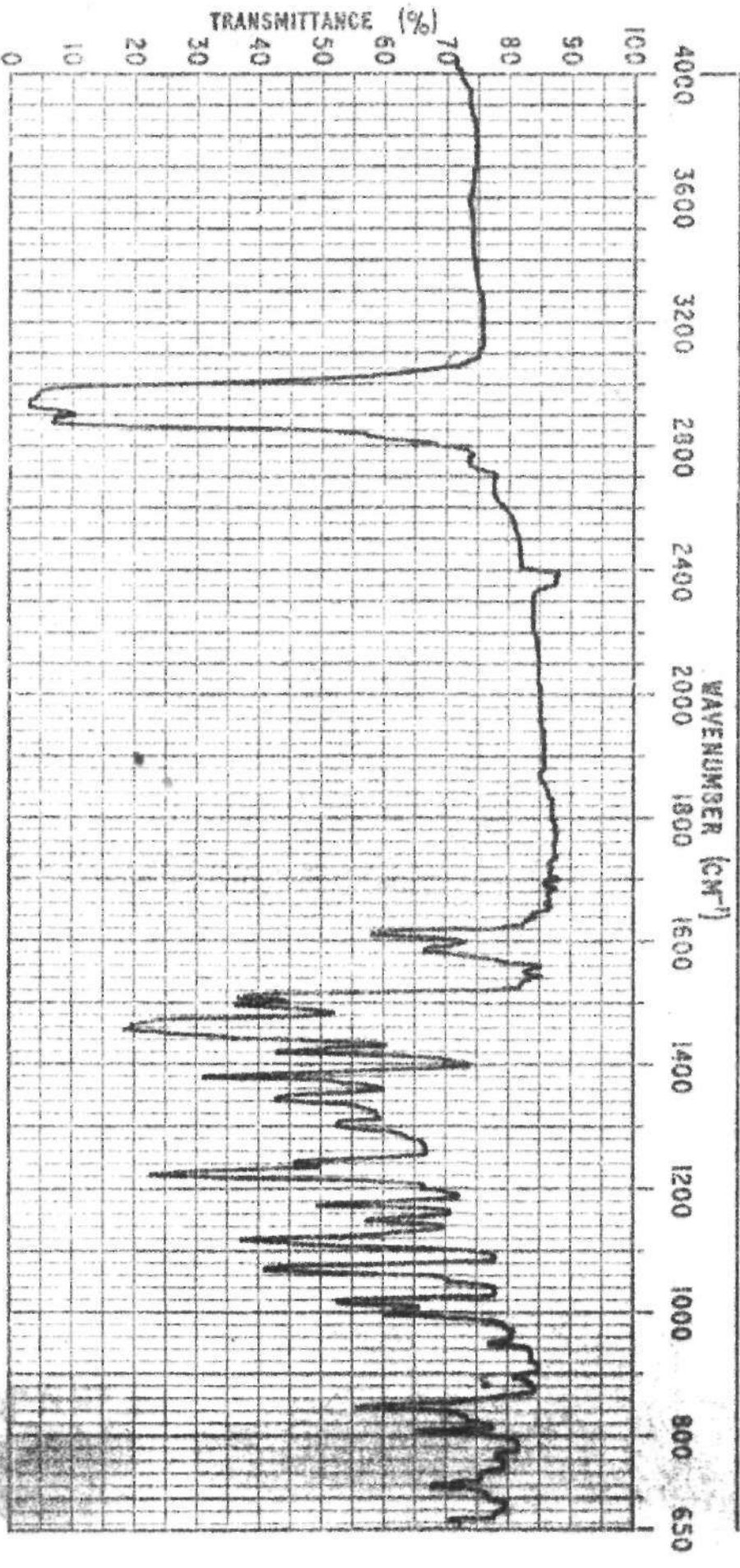
Further elution with either chloroform or chloroform - methanol mixture gave only traces of other alkaloids which were not further investigated.

REFERENCES

1. Dalziel J. M. (1937); The Useful Plants of West Tropical Africa, London.
 2. Thomas Anderson, H. (1949); The Plant Alkaloids Fourth Edition, London.
 3. Huls, R. and Detry C. (1975); Alkaloids of Triclisia gillettii, Bulletin de la Societe Royale de Science de liege (Belg.) 42 (1-2), 73-9.
 4. Elsohly Mahmoud A. (1975); The Isolation and Characterisation of Some Constituents from Ruscus Aculeatus L. and Cocculus Carolinus D. C. and the Structural elucidation of Trigillettamine from Triclisia gillettii (De wild) Staner and Triclisia Patens Oliver, 138 pp. (England).
 5. Huls, R. (1972); Alkaloids Extracted from Triclisia gillettii Bulletin de la Societe Royale de Science Liege, 41(11-12), 686-93 (Fr.)
 6. Tackie A. N. et. al. (1973); Constituents of West African Medicinal Plants, Phytochemistry 12(10), 2509-1), (England).
 7. Kronlund, Anders et. al. (1979); New Simple Method for Estimation of Muscle Relaxant Effect. Acta Pharm. Sciecia, 7(3), 279-84, (England).
 8. D. Dwuma-Badu et. al. (1975); Additional Alkaloids of Triclisia Patens and Triclisia Subcordata, Phytochemistry 14(11), 2524-5; (England).
 9. ~~Singh S. G.~~ (1960); Medicinal Plants of Nigeria.
 10. Irvine, F. R. (1961); Woody Plants of Ghana. Oxford University Press, London, pp. 37.
9. MEDICINAL PLANTS IN NIGERIA (SERIES OF 4 LECTURES AT IBADAN), (1960)

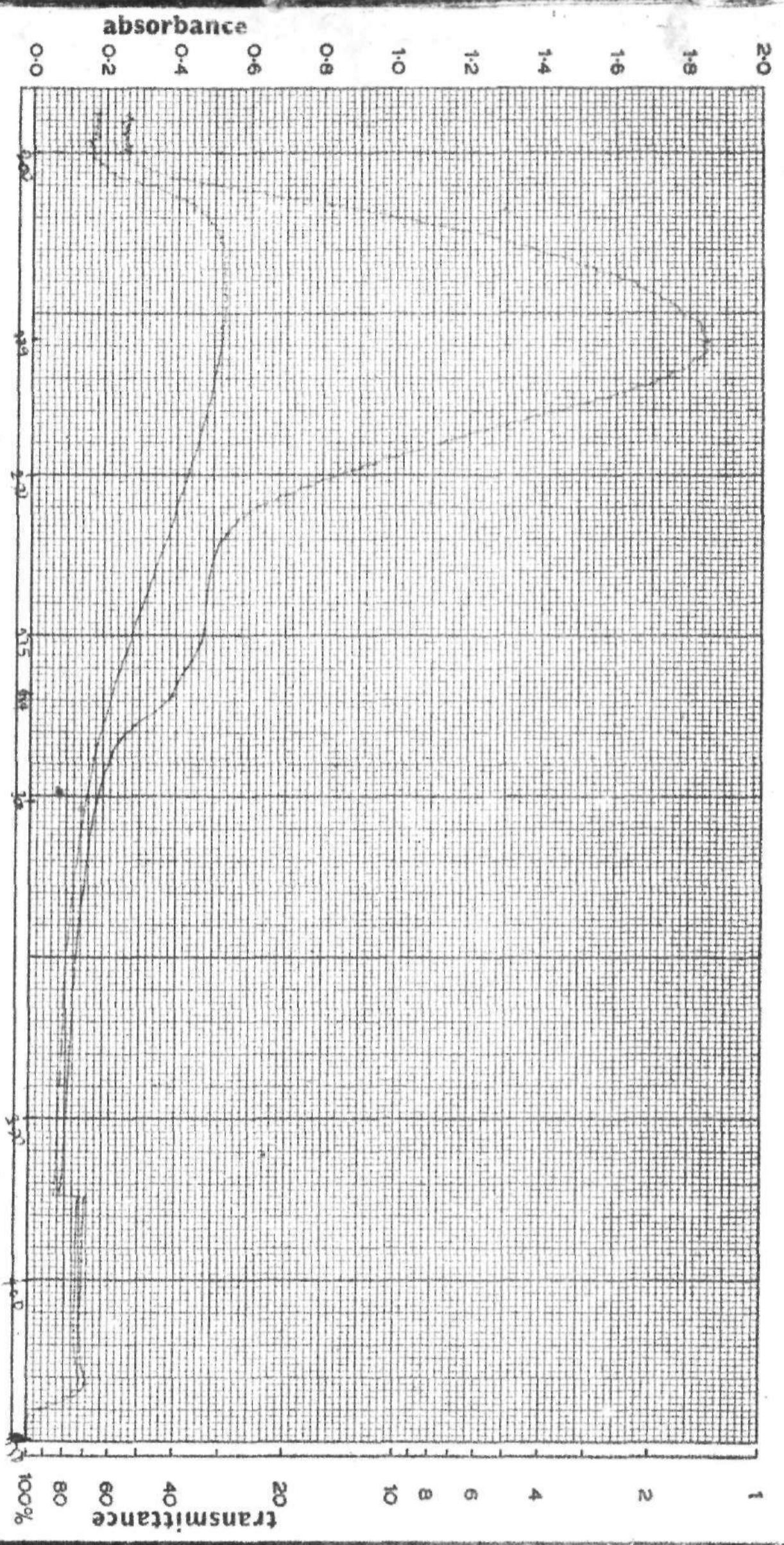
11. Okarter and Temple Uchechuku (1976). Chemical Constituents of West African Medicinal Plants. Griffonia simplicifolia Bail; (Caesalpinaceae) Sphenocentrum jollyanum Pierre (Menispermaceae) and Triclisia subcordata Oliver, (Menispermaceae), 114 pp.
12. Ikoku, C. and W. Davison, Personal Communication on Pharmacological Investigation of Aqueous Extract of Triclisia subcordata.
13. Swan G. A. (1967). An Introduction to the Alkaloids. Blackwell (Chapter 2 on Phenethylamines and Ephedra Alkaloids).
14. Sim S. K. (1968). Medicinal Plant Alkaloids University of Toronto Press. pp. 13-20, Toronto.
15. I. R. J. Bick et. al. (1961); Structural Correlations in the N.M.R. Spectra of Bisbenzyl-isoquinoline and Aporphine Alkaloids. Journal of Chemical Society, pg. 1896.
16. Alfred W. Sangster and Kenneth L. Stuart (1965). Ultraviolet Spectra of Alkaloids, Chemical Review 65, 69, Jamaica.

REMARKS <i>Dist, NH out</i> <i>aliphatic</i>	
ORIGIN <i>From root of</i> <i>Trichilia splendens</i>	PERKIN-ELMERA
PURITY _____	SPECTRUM NO. <i>Fig 1</i>
PHASE <i>NuSol</i>	SAMPLE 1 _____
CONCENTRATION _____	DATE _____
THICKNESS _____	OPERATOR _____
SCAN SPEED _____	
SLIT _____	



SAMPLE _____ SPECTRUM NO. _____

ETHIOPIA POWER
LNICAM SP 800



ALSO WITH REEF
ON THE RECORD

SAMPLE AND FORMULA

CONCENTRATION
REFERENCE

SCANNED FAST SLOW

DATE

Catalogue No. 400868

Fig. 3