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189. *The Alkaloids of* Tylophora crebriflora: *Structure and Synthesis of Tylocrebrine, a New Phenanthroindolizidine Alkaloid.*

By E. Gellert, T. R. Govindachari, M. V. Lakshmikantham, I. S. Ragade,
R. Rudzats, and N. Viswanathan.

Tylophorine and a new alkaloid, tylocrebrine, have been isolated from *Tylophora crebriflora*. Structure (II), one of the two alternative structures assigned to tylocrebrine on the basis of analytical and spectroscopic evidence, has been confirmed by synthesis of the racemic base.

Tylophora crebriflora S. T. Blake[1] (family Asclepiadaceae) occurs in Northern Queensland. The vesicant phenanthroindolizidine alkaloids, tylophorine and tylophorinine, have been

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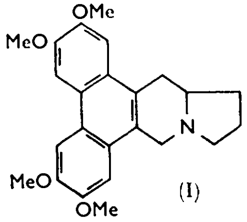
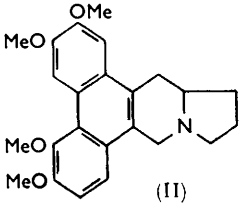
[1] Blake, *Proc. Roy. Soc. Queensland*, 1948, **59**, 168.

isolated from the related Indian species, *T. asthmatica.*[2] The vesicant action of *T. crebriflora*, although less pronounced than that of *T. asthmatica*, made handling of the material difficult.

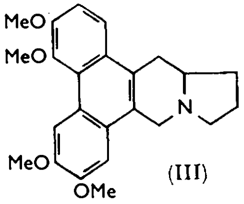
Although initial extraction in the laboratory indicated high alkaloidal content (0·33%), large-scale extractions from later collections gave yields not exceeding 0·15%. The crude alkaloids obtained by extraction of the whole plant with hot methanol were separated by partition chromatography in propan-1-ol–2N-acetic acid (15 : 85) on a cellulose column. The minor alkaloid was shown, by direct comparison, to be tylophorine (I).[3]



(I)

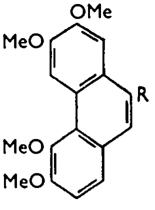


(II)

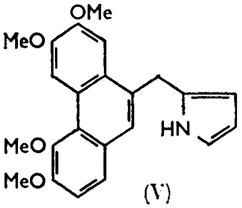


(III)

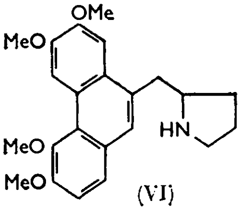
The major alkaloid, for which we propose the name tylocrebrine,[4] is isomeric with tylophorine. It contains four methoxyl groups (accounting for all the oxygen) but no *C*-methyl or *N*-methyl group or active hydrogen atom. Ready formation of a methiodide and the resistance to diazotisation and acetylation indicate that the nitrogen is tertiary and probably common to two rings, as in tylophorine. The ultraviolet absorption spectrum of the alkaloid (Fig. 1B) was practically superposable on that of 3,4,6,7-tetramethoxy-9-methylphenanthrene (Fig. 1D), indicating a common basic skeleton. The infrared spectrum (Fig. 2) confirmed the absence of hydroxyl, imino, and carbonyl groups. Resistance to catalytic hydrogenation showed the absence of double bonds. The alkaloid must therefore be pentacyclic, the phenanthrene part accounting for three of the rings.



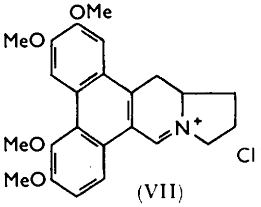
(IV)
(a: R = CO_2Et)
(b: R = $CH_2{\cdot}OH$)
(c: R = CH_2Cl)



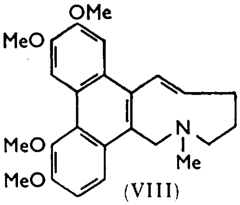
(V)



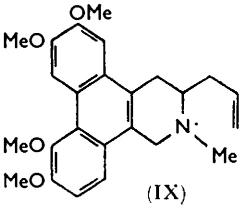
(VI)



(VII)



(VIII)



(IX)

Tylocrebrine methiodide was racemised by hot 20% aqueous potassium hydroxide.[5] Hofmann degradation by silver oxide gave the optically inactive methine, m. p. 145°.

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[2] Ratnagiriswaran and Venkatachalam, *Indian J. Med. Res.*, 1935, **22**, 433; Govindachari, Nagarajan, and Pai, *J.*, 1954, 2801.

[3] (*a*) Govindachari, Lakshmikantham, Nagarajan, and Pai, *Tetrahedron*, 1958, **4**, 311; (*b*) Govindachari, Lakshmikantham, Pai, and Rajappa, *Tetrahedron*, 1960, **9**, 53; (*c*) Govindachari, Lakshmikantham, and Rajadurai, *Chem. and Ind.*, 1960, 664.

[4] Reported to the I.U.P.A.C. Internat. Symposium on the Chemistry of Natural Products, Melbourne, 1960.

[5] Gellert and Riggs, *Austral. J. Chem.*, 1954, **7**, 113; Gellert, *ibid.*, 1956, **9**, 489.

Attempts to degrade tylocrebrine to a phenanthrene derivative by oxidation with potassium permanganate or with chromic acid in acetic acid failed to give tractable products. However, the evidence outlined above, in conjunction with the biogenetic relationship of tylocrebrine to tylophorine, permitted assignment of structure (II) or (III) to tylocrebrine. The racemic bases corresponding to these structures were synthesised by the method used for the synthesis of tylophorine[3c] and deoxytylophorinine.[6] Structure (II) was found to represent tylocrebrine.

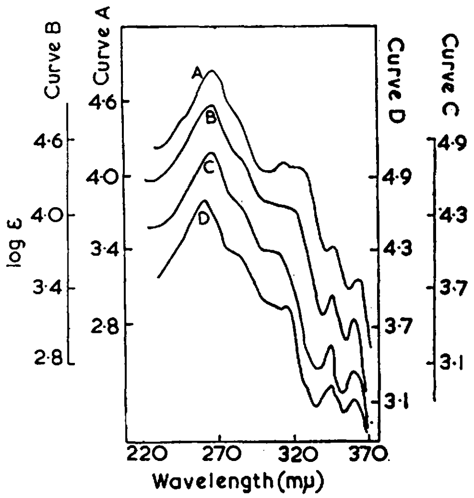

FIG. 1. Ultraviolet absorption of (A) tylocrebrine methine (natural or synthetic), (B) (−)-tylocrebrine, (C) (±)-tylocrebrine, and (D) 3,4,6,7-tetramethoxy-9-methylphenanthrene, in 95% EtOH.

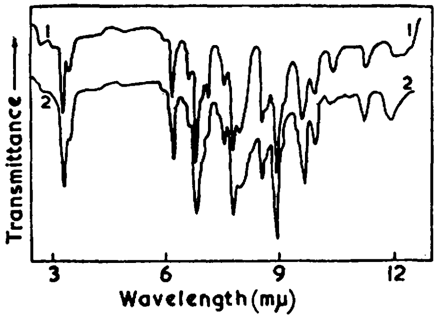

FIG. 2. Infrared spectra of (1) (−)- and (2) (±)-tylocrebrine, in $CHCl_3$.

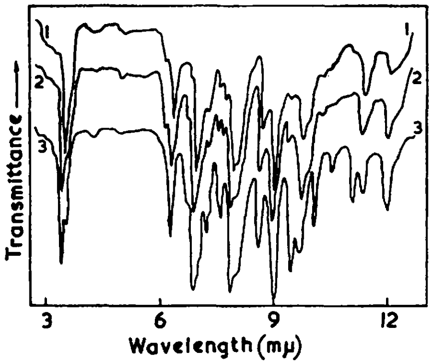

FIG. 3. Infrared spectra of (1) natural and (2) synthetic tylocrebrine methine, and (3) methine (XI), in $CHCl_3$.

Reduction of the phenanthrene ester[3a] (IVa) with lithium aluminium hydride gave the 9-hydroxymethyl derivative (IVb) which was converted into the chloride (IVc). Treatment of this with pyrrylmagnesium bromide gave 2-(3,4,6,7-tetramethoxy-9-phenanthrylmethyl)pyrrole (V), catalytic reduction of which gave the pyrrolidine (VI). The *N*-formyl derivative of this, on cyclisation with phosphorus oxychloride, afforded the quaternary chloride (VII) which on reduction with sodium borohydride gave compound (II), whose ultraviolet (Fig. 1C) and infrared (Fig. 2) absorption spectra were almost superposable on those of natural tylocrebrine. Hofmann degradation of the methiodide obtained from the synthetic base gave the methine, m. p. 144·5—145°, which was identical

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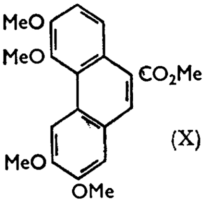
[6] Govindachari, Pai, Ragade, Rajappa, and Viswanathan, *Chem. and Ind.*, 1960, 966.

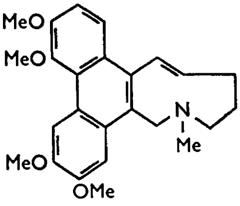
(mixed m. p., ultraviolet and infrared absorption spectra) with natural tylocrebrine methine (Figs. 1 and 3).

Structure (VIII), in preference to (IX), is ascribed to this methine as no formaldehyde could be detected when the natural methine was oxidised with periodic acid, and because its infrared spectrum shows no additional bonds in the regions where absorption characteristic of terminal methylene grouping occurs.[7] In addition, the mechanism proposed for the racemisation of methiodides of alkaloids of this type favours the formation of methine (VIII) under the conditions of the Hofmann degradation.[5,8]

The isomeric compound (III) was synthesised by a similar sequence of reactions from methyl 2,3,5,6-tetramethoxyphenanthrene-9-carboxylate (X). Its ultraviolet and infrared absorption spectra did not differ appreciably from those of tylocrebrine. However, Hofmann degradation of the methiodide gave a methine for which structure (XI) is proposed on basis of its infrared spectrum,[7] and this had m. p. 137—138°, which was depressed to 122—128° on admixture with natural tylocrebrine methine. The non-identity of the two methines was further shown by their infrared spectra (Fig. 3).



(X)



(XI)

EXPERIMENTAL

Microanalyses were carried out partly in the C.S.I.R.O. Microanalytical Laboratory under the direction of Dr. K. W. Zimmermann and partly at the Presidency College by Mr. S. Selvavinayakam. Ultraviolet absorption spectra were measured for 95% ethanol solutions; light petroleum had b. p. 40—60°.

Extraction of Tylophora crebriflora.—Milled plant material (21 lb.) was extracted with hot methanol and the extract concentrated to a small volume. The crude extract (4 l.) was diluted with water (~2 l.) and concentrated in a climbing-film evaporator to 800 ml. and, whilst warm, filtered (filtrate A) from the deposited solid (B). The filtrate (A) was acidified with dilute acetic acid and extracted exhaustively with carbon tetrachloride to remove non-basic material. The combined carbon tetrachloride extract was shaken with 2N-hydrochloric acid to recover any base extracted by carbon tetrachloride. The aqueous acidic layer was added to the previous acidic phase and the combined solutions were filtered. The filtrate gave a strong Mayer's test and showed two fluorescent spots when chromatographed on paper in butanol–acetic acid (R_F ~0·2 and 0·5). A solution of the sediment (B) in warm 2N-acetic acid was diluted with hot water, cooled, and filtered. This filtrate was extracted with carbon tetrachloride to remove non-basic material and combined with the acidic solution from fraction (A). The combined aqueous acidic solutions were basified with concentrated aqueous ammonia and the precipitate was filtered off. The crude alkaloid mixture (45 g.) was repeatedly precipitated from hot acetic acid solution with concentrated aqueous ammonia and finally extracted (Soxhlet) with methanol. Crystallisation from methanol gave impure dark-yellow crystals.

Isolation of the Alkaloids.—The alkaloids (in 2-g. batches) were separated on a 750 g. Whatman chromatography-grade cellulose-powder column (160 × 5 cm.). The column was developed with propan-1-ol–2N-acetic acid (15 : 85) (flow rate 500 ml./hr.), the progress of the fluorescent part of the first zone being followed under ultraviolet light. This caused partial separation into materials with R_F 0·2 and 0·5, severally. The fractions from several such

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[7] Bellamy, " The Infrared Spectra of Complex Molecules," Methuen, London, 1958; Cross, " Introduction to Practical Infra-red Spectroscopy," Butterworths Scientific Publns., London, 1960.

[8] Gellert, *Chem. and Ind.*, 1955, 983.

chromatograms were combined. After rechromatography of the intermediate fractions, two final fractions were obtained. The first was evaporated to dryness under reduced pressure and the residue dissolved in warm dilute acetic acid. Precipitation with concentrated aqueous ammonia yielded the alkaloid with R_F 0·5 in butan-1-ol–acetic acid (97 : 3). (Butan-1-ol is saturated with water.) The second yielded the alkaloid with R_F 0·2 in the same solvent.

Tylophorine. The crude alkaloid from the second fraction was recrystallised thrice from chloroform–methanol, yielding colourless crystals, m. p. 282—284° (decomp.) undepressed on admixture with tylophorine, $\lambda_{max.}$ 257, 290, 340, 355 mμ (log ε 4·82, 4·51, 3·43, 2·96). The infrared absorption spectrum and the *X*-ray powder diagram were identical with those of tylophorine.

The methiodide, prepared in chloroform and crystallised from water, had m. p. 278—280° undepressed on admixture with tylophorine methiodide.

Tylocrebrine. The crude alkaloid from the first fraction, on three crystallisations from methanol, yielded colourless *tylocrebrine*, m. p. 218—220° (decomp.) (Found: C, 73·2; H, 7·0; N, 3·5; O, 16·6; OMe, 30·9; *N*-Me, 0; *C*-Me, 0; active H, 0. $C_{24}H_{27}NO_4$ requires C, 73·3; H, 6·9; N, 3·6; O, 16·3; 4OMe, 31·5%), $\lambda_{max.}$ 263, 342, 360 mμ (log ε 4·81, 3·25, 3·09), $[\alpha]_D^{24}$ $-45° \pm 2°$ (*c* 0·74 in $CHCl_3$), pK_a in 50% aqueous ethanol 6·7.

The yellow *hydriodide*, recrystallised from aqueous methanol, had m. p. 214—217° (decomp.) (Found: C, 54·2; H, 5·7; N, 2·4; O, 14·5. $C_{24}H_{28}NO_4I,CH_3\cdot OH$ requires C, 54·2; H, 5·8; N, 2·5; O, 14·4%). The *perchlorate*, prepared in hot aqueous acetone–acetic acid, had m. p. 262—264° (decomp.) (from aqueous acetone) (Found: C, 55·6; H, 6·0; N, 2·6; Cl, 6·7. $C_{24}H_{28}ClNO_8,1\frac{1}{2}H_2O$ requires C, 55·3; H, 6·0; N, 2·7; Cl, 6·8%). The picrate, crystallised from acetone containing a little methanol, had m. p. 134—136°.

Tylocrebrine was recovered quantitatively after treatment with nitrous acid or with acetic anhydride–pyridine.

The alkaloid was refluxed on a water-bath with an excess of methyl iodide in methanol until dissolved and then for 30 min. more, concentrated, and allowed to cool. The precipitated yellow *methiodide*, recrystallised thrice from methanol, had m. p. 255—258° (decomp.), $[\alpha]_D^{21}$ $-30° \pm 2°$ (*c* 0·30 in MeOH) (Found: C, 55·2; H, 5·7; N, 2·5; O, 13·6; I, 23·0; OMe, 22·6; *N*-Me, 2·6. $C_{25}H_{30}INO_4,1\frac{1}{2}H_2O$ requires C, 55·2; H, 5·7; N, 2·6; O, 13·2; I, 23·3; 4OMe, 22·8; *N*-Me, 2·8%).

This methiodide was refluxed in 20% aqueous sodium hydroxide for 30 min. The deposited crystals of (±)-*methiodide* were collected, washed with water, then with ether, and crystallised from methanol; they had m. p. 264—266° (decomp.), $[\alpha]_D^{21}$ 0° (*c* 0·10 in MeOH) (Found: C, 56·0; H, 5·6; N, 2·4; O, 12·1; I, 23·6; OMe, 23·0; *N*-Me, 2·7. $C_{25}H_{30}INO_4$ requires C, 56·1; H, 5·6; N, 2·6; O, 12·0; I, 23·7; 4OMe, 23·2; *N*-Me, 2·8%).

Hofmann Degradation of Tylocrebrine Methiodide.—(*a*) The methiodide (1·4 g.) was converted into the methochloride by silver chloride in refluxing aqueous methanolic solution, then into the methohydroxide (by shaking the aqueous solution with silver oxide). The solution was evaporated to dryness, and the residue heated at 240°/0·2 mm. for 3 min. The product was chromatographed in benzene on basic alumina, yielding the *methine* (VIII) (400 mg.), m. p. 139—141°. Two recrystallisations from benzene–light petroleum (b. p. 60—80°) followed by two from light petroleum gave material of m. p. 144·5—145° (Found: C, 74·0; H, 7·3; N, 3·6; O, 15·6; *N*-Me, 3·5. $C_{25}H_{29}NO_4$ requires C, 73·7; H, 7·2; N, 3·4; O, 15·6; *N*-Me, 3·7%).

(*b*) The methiodide (100 mg.) was converted directly into the methohydroxide [by silver oxide in water (10 ml.); 5 hr.]. The mixture was filtered, evaporated to dryness *in vacuo* at 50°, and the residue heated at 100°/0·05 mm. for 30 min. The product was repeatedly extracted with hot benzene and chromatographed in benzene over alumina. Two recrystallisations of the product from benzene–light petroleum gave the methine (10 mg.), m. p. and mixed m. p. 144·5—145°.

The methine (50 mg.) in glacial acetic acid (3 ml.) was heated with an excess of periodic acid at 125° for 5 min., and the vapours formed were absorbed in an aqueous dimedone. No precipitate had been formed even on prolonged storage. (Quinine gave, under the same conditions, an immediate positive test.)

Synthesis of (±)-*Tylocrebrine.*—9-*Hydroxymethyl*-3,4,6,7-*tetramethoxyphenanthrene.* A solution of ethyl 3,4,6,7-tetramethoxyphenanthrene-9-carboxylate [3a] (3 g.) in dry tetrahydrofuran (25 ml.) was added to a stirred suspension of lithium aluminium hydride (1·5 g.) in tetrahydrofuran (15 ml.). After 4 hours' stirring the complex was decomposed by ether and water.

The ether–tetrahydrofuran mixture was decanted and evaporated, to yield the *hydroxymethylphenanthrene* (2·2 g.), needles (from benzene), m. p. 164—165° (Found: C, 69·6; H, 6·2. $C_{19}H_{20}O_5$ requires C, 69·5; H, 6·1%).

9-*Chloromethyl*-3,4,6,7-*tetramethoxyphenanthrene*. A solution of the above alcohol (5 g.), thionyl chloride (4 ml.), and pyridine (0·5 ml.) in chloroform (120 ml.) was heated at 40—60° for 15 min., then cooled, poured into water, and extracted with chloroform. The dried extract was concentrated to a small volume and the chloride precipitated with an excess of light petroleum. Crystallisation from benzene–light petroleum yielded the *chloromethylphenanthrene* (4·2 g.) as needles, m. p. 148° (decomp.) (Found: C, 66·3; H, 5·6. $C_{19}H_{19}ClO_4$ requires C, 65·9; H, 5·5%).

2-(3,4,6,7-*Tetramethoxy*-9-*phenanthrylmethyl*)*pyrrole*. The chloromethylphenanthrene (4 g.) in dry tetrahydrofuran (40 ml.) was added dropwise to an ice-cooled, stirred solution of pyrrylmagnesium bromide (from magnesium 1·8 g., ethyl bromide 5·8 ml., and freshly distilled pyrrole 5·26 ml.) in ether, under nitrogen. After 3 hours' stirring, during which the mixture was allowed to reach room temperature, ether was added and the oily complex decomposed by saturated ammonium chloride solution. The organic layer was separated, washed with water, dried, and evaporated. Chromatography of the residue in chloroform over alumina yielded the *pyrrole* (2 g.), white cubes (from benzene–light petroleum), m. p. 155—156° (Found: C, 73·5; H, 5·8. $C_{23}H_{23}NO_4$ requires C, 73·2; H, 6·1%).

2-(3,4,6,7-*Tetramethoxy*-9-*phenanthrylmethyl*)*pyrrolidine*. The foregoing pyrrole (0·4 g.) in acetic acid (30 ml.) was shaken with Adams catalyst (0·25 g.) in presence of hydrogen (60 lb./in.^{2}) at room temperature for 8 hr. The mixture was filtered and the solvent removed *in vacuo*. The residue was extracted repeatedly with hot dilute hydrochloric acid. The acid extract was filtered, basified with aqueous ammonia, and extracted with chloroform, to yield the pyrrolidine (0·25 g.) which failed to crystallise even after chromatography. The *picrate* crystallised from acetic acid as yellow needles, m. p. 247—249° (Found: C, 57·5; H, 5·3. $C_{29}H_{30}N_4O_{11}$ requires C, 57·1; H, 4·9%).

9,11,12,13,13a,14-*Hexahydro*-2,3,5,6-*tetramethoxydibenzo*(f,h)*pyrrolo*(1,2-b)*isoquinoline*. (±)-*Tylocrebrine*.—The above pyrrolidine (0·5 g.) was heated with 98% formic acid (3 ml.) at 180° for 1½ hr., cooled, and taken up in chloroform. The *N*-formyl derivative obtained by evaporation of the washed and dried chloroform extract did not crystallise and was cyclised by refluxing it with phosphorus oxychloride (4 ml.) and toluene (15 ml.) for 1½ hr. The quaternary chloride, precipitated by addition of an excess of light petroleum to the cooled mixture, was dried *in vacuo* and reduced with sodium borohydride (0·8 g.) in methanol (30 ml.). The methanol was removed *in vacuo* and the residue taken up in chloroform. The chloroform extract was washed with water, dried, and evaporated. Chromatography of the residue in chloroform over alumina followed by crystallisation from chloroform–methanol yielded (±)-*tylocrebrine* (0·2 g.) as white needles, m. p. 219—221° (Found: C, 72·9; H, 6·9. $C_{24}H_{27}NO_4$ requires C, 73·2; H, 6·9%).

Hofmann Degradation of (±)-*Tylocrebrine*.—(±)-Tylocrebrine (200 mg.) in chloroform (10 ml.) was refluxed with methyl iodide (2 ml.) for 3 hr. on a water-bath and left overnight at 30°. The methiodide obtained by evaporation of the solution was shaken with silver oxide (from 1 g. of silver nitrate) and water (10 ml.) for 5 hr. and subjected to the Hofmann degradation as in the case of natural tylocrebrine methiodide, to yield the *methine* (40 mg.), needles (from benzene–light petroleum), m. p. 144·5—145° undepressed on admixture with natural tylocrebrine methine (Found: C, 74·1; H, 7·4. $C_{25}H_{29}NO_4$ requires C, 73·7; H, 7·2%).

Synthesis of Compound (III).—2-Amino-α-(3,4-dimethoxyphenyl)-4,5-dimethoxycinnamic acid [3a] was diazotised in acetone solution with n-butyl nitrite and subjected to the Pschorr ring closure according to the procedure of Bradsher and Berger.[9] The yield of 2,3,5,6-tetramethoxyphenanthrene-9-carboxylic acid obtained by this method was higher than that reported earlier.

This acid (5 g.) was refluxed with concentrated sulphuric acid (4 ml.) in methanol (150 ml.) for 4 hr. The solution was concentrated, diluted with water, and extracted with chloroform, to yield the *methyl ester* (4·2 g.), m. p. 150° (from ethanol) (Found: C, 67·5; H, 5·9. $C_{20}H_{20}O_6$ requires C, 67·4; H, 5·6%).

9,11,12,13,13a,14-*Hexahydro*-3,4,6,7-*tetramethoxydibenzo*(f,h)*pyrrolo*(1,2-b)*isoquinoline*, m. p. 219° (from chloroform–methanol) (Found: C, 73·1; H, 6·9. $C_{24}H_{27}NO_4$ requires C, 73·2; H, 6·9%), was then prepared, under essentially the conditions described for (±)-tylocrebrine,

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[9] Bradsher and Berger, *J. Amer. Chem. Soc.*, 1958, **78**, 930.

the intermediates being 9-*hydroxymethyl*-2,3,5,6-*tetramethoxyphenanthrene*, m. p. 133° (from benzene) (Found: C, 69·2; H, 6·0%), 9-*chloromethyl*-2,3,5,6-*tetramethoxyphenanthrene*, m. p. 163—164° (from benzene–light petroleum) (Found: C, 65·7; H, 5·6%), 2-(2,3,5,6-*tetramethoxy-9-phenanthrylmethyl*)*pyrrole*, m. p. 173—174° (from chloroform–benzene) (Found: C, 72·9; H, 6·3%), and 2-(2,3,5,6-tetramethoxy-9-phenanthrylmethyl)pyrrolidine which was isolated as a *picrate*, m. p. 218° (decomp.) (from acetic acid–ethanol) (Found: C, 57·0; H, 4·8%).

Compound (III) (200 mg.) was converted into the methiodide and subjected to the Hofmann degradation as usual, to give the *methine* (XI) (30 mg.), 137—138° (from benzene–light petroleum) (Found: C, 74·0; H, 7·1%).

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