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CHEMICAL EXAMINATION OF *TYLOPHORA ASTHMATICA*—II

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Abstract—Tylophorine has been degraded to a compound, identified as 2:3:6:7-tetramethoxy-9-methylphenanthrene. In conjunction with other degradation results and biogenetic considerations, tylophorine is now formulated as 2:3:6:7-tetramethoxyphenanthro(9:10:6′:7′)indolizidine.

A synthesis of the basic ring system, phenanthro-(9:10:6′:7′)indolizidine, present in tylophorine has been achieved.

IN a previous paper[1] it was shown that the alkaloid tylophorine contains a tertiary nitrogen atom common to two rings. Careful repetition of this work has shown that during the second stage Hofmann degradation, the expected product de-N-methyl-tylophorine-methine is accompanied by a nitrogen-free product $C_{24}H_{24}O_5$ yielding a crystalline benzoyl derivative, which in all probability is formed by the replacement of a dimethylamino group by hydroxyl.[2] Application of the Hofmann degradation to de-N-methyl-tylophorinemethine yielded a colourless crystalline product analysing for the formula $C_{24}H_{24}O_4$, m.p. 181–188° and not 152–153° as reported earlier.

The ultra-violet absorption spectra of tylophorine, tylophorinemethine, de-N-methyl-tylophorinemethine and the compound $C_{24}H_{24}O_4$ are closely similar to that of phenanthrene. Tylophorine has no easily reducible unsaturation, but tylophorine-methine can be converted to a quaternary compound. The quaternary iodide, $C_{24}H_{27}O_4N,CH_3I$ is identical with tylophorine *iso*methiodide (vide infra). Reversion of a methine to an inactive form of the quaternary salt from which it is derived has been observed in the case of quinolizidine alkaloids of the canadine type.[3] Such a trans-annular interaction appears to be characteristic of eight, nine and ten-membered rings.[4] It may be concluded that tylophorinemethine has an eight-, nine- or ten-membered ring system, incorporating the nitrogen atom.

Treatment of tylophorine with cyanogen bromide gave a high yield of a bromo-cyanamide $(C_{24}H_{27}O_4N)\begin{matrix}CN\\Br.\end{matrix}$ with cleavage of one N—C— bond in the alkaloid. Reduction of the bromocyanamide under a variety of conditions failed to yield any pure product. Treatment of the bromocyanamide with sodium borohydride led only to replacement of the bromine by hydroxyl. The hydroxycyanamide on hydrolysis with sulphuric acid re-generated tylophorine, indicating the presence of a 1:4- or 1:5-amino-alcohol system in the compound. The bromocyanamide reacted readily

* A brief summary of this work was published in *Chem. & Ind.* 1484 (1957).

[1] T. R. Govindachari, B. R. Pai and K. Nagarajan, *J. Chem. Soc.* 2801–2803 (1954).

[2] R. H. F. Manske and H. L. Holmes, *The Alkaloids, Chemistry and Physiology* Vol. IV, p. 152. Academic Press, New York (1954).

[3] F. L. Pyman, *J. Chem. Soc.* **103**, 817 (1913) A. Voss and J. Gadamer, *Arch. Pharm.* **248**, 43 (1910).

[4] N. J. Leonard, R. C. Fox and M. Oki, *J. Amer. Chem. Soc.* **76**, 5708 (1954).

with diethylamine but the diethylaminocyanamide could be hydrolysed only to the urea and no further. The ready cleavage by cyanogen bromide indicates the presence of a benzylamine system in tylophorine and the high reactivity of the bromocyanamide, the presence in it of a $—CH_2Br$ group.[5]

Tylophorine methiodide was converted to an optically inactive *iso*methiodide by boiling with potassium hydroxide solution. That no deep-seated molecular rearrangement had taken place in the reaction was inferred from the superposability of the ultra-violet absorption spectra of the two compounds. The derived *iso*methochloride yields on Emde degradation *iso*dihydrohomotylophorine* and on dehydrogenation with palladised charcoal detetrahydro*iso*dihydrohomotylophorine. The latter has a typical phenanthrene spectrum, is non-basic, and contains a pyrrole ring (positive Ehrlich test). On reduction the original *iso*dihydrohomotylophorine is obtained showing that no alteration in ring size had taken place during dehydrogenation. These experiments establish beyond doubt the presence of a five-membered nitrogen-containing ring tylophorine.

After a Hofmann degradation of *iso*dihydrohomotylophorine the product was oxidised and the acidic fraction esterified with diazomethane giving a mixture of methyl esters. A *mono* ester $C_{21}H_{22}O_6$, m.p. 185–186° and a *diester* $C_{22}H_{22}O_8$, m.p. 246–247° were obtained. The latter on hydrolysis gave a dicarboxylic acid, which was converted to an anhydride under crystallisation conditions. This tendency has been noticed[6] in the case of phenanthrene-9:10-dicarboxylic acids. Attempts at decarboxylation of the dicarboxylic acid under a variety of conditions failed. The mono ester, however, hydrolyses and decarboxylates smoothly to yield a tetramethoxymethylphenanthrene, m.p. 188–189°. The identification of this compound was rendered easier by considering certain biogenetic aspects. Robinson[7] has envisaged the possibility of the phenanthrene ring in cryptopleurine[8] being derived from two phenylalanine units. Tylophorine, a phenanthrene derivative and containing four methoxy groups could conceivably be derived from two DOPA units and ornithine (or equivalent). The decarboxylation product should thus prove to be 2:3:6:7-, or 3:4:6:7-, or 3:4:5:6- or 2:3:5:6-tetramethoxy-9-methylphenanthrene. The first three 9-methylphenanthrenes were synthesised by the method of Buchanan *et al.*[9] from the corresponding 9-carboxylic acids and the 2:3:6:7-tetramethoxy-9-methylphenanthrene was found to be identical with the decarboxylation product m.p. 188–189° (mixed m.p., ultraviolet (Fig. 1) and infra-red spectra). This provides positive evidence about the presence of a phenanthrene ring, the orientation of the methoxy groups and one point of attachment of the heterobicyclic system in tylophorine.

Oxidation of tylophorine methohydroxide in aqueous pyridine with potassium permanganate yields two compounds one of which is considered to be 2:3:6:7-tetramethyoxyphenanthrene-9:10-dicarboxylic acid, the other gives an analysis corresponding to its imide. Vigorous oxidation of tylophorine methiodide with aqueous

* During the course of further degradation experiments (forthcoming publication) it has been shown that this compound is also obtained directly from tylophorine methochloride by Emde degradation.

[5] R. C. Elderfield and M. Green, *J. Org. Chem.* **17**, 431 (1952).

[6] J. Szmuszkovicz and E. J. Modest, *J. Amer. Chem. Soc.* **70**, 2542 (1948); A. Jeanes and R. Adams, *Ibid.* **59**, 2608 (1937).

[7] Sir Robert Robinson, *The Structural Relations of Natural Products* p. 78. Oxford University Press (1955).

[8] E. Gellert and N. V. Riggs, *Aust. J. Chem.* **7**, 113 (1954); J. Friedrichsons and A. Mathieson, *Nature, Lond.* **173**, 732 (1954); E. Gellert, *Aust. J. Chem.* **9**, 489 (1956).

[9] G. L. Buchanan, J. W. Cook and J. D. Loudon, *J. Chem. Soc.* 321 (1944).

potassium permanganate yields *m*-hemipinic acid. The most careful search failed to reveal the presence of any other aromatic acid. Oxidation of tylophorinemethine with potassium permanganate in pyridine yields a neutral, nitrogenous substance, $C_{25}H_{27}O_5N$, apparently formed by the oxidation of a methylene group (adjacent to the nitrogen atom) to a carbonyl group, since reduction with lithium aluminium hydride yielded the original methine.

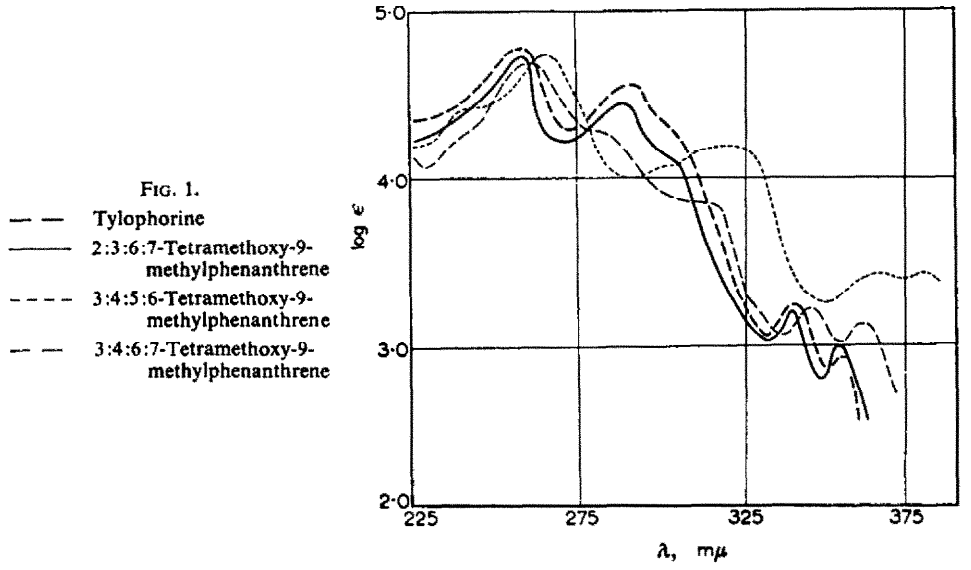
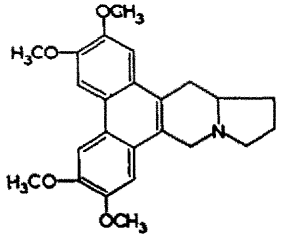
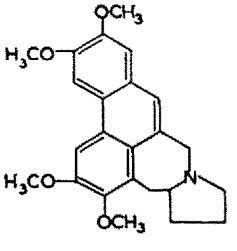


FIG. 1.

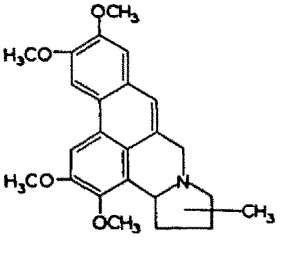
On the basis of the foregoing degradation studies tylophorine can be formulated as 2:3:6:7-tetramethoxyphenanthro(9:10:6′:7′)-indolizidine (I). Although positive evidence that the heterobicyclic system is attached not only to the 9- but also to the 10-position of the phenanthrene ring has not been provided, this is the only structure consistent with biogenetic derivation from DOPA and ornithine and *all* the degradation results. Structures of the type (II) or (III) could also lead to 2:3:6:7-tetramethoxy-9-methylphenanthrene by the sequence employed, but both these structures should yield in addition to *m*-hemipinic acid, a tricarboxylic acid. The methine derived from structure (II) cannot revert to the original quaternary salt. Whilst the methine derived from structure (III) can revert to the original quaternary salt, the structure is inadmissible since tylophorine does not contain a C-methyl group (Kuhn–Roth, infra-red). Both structures (II) and (III) are inconsistent with the biogenetic scheme envisaged.



I

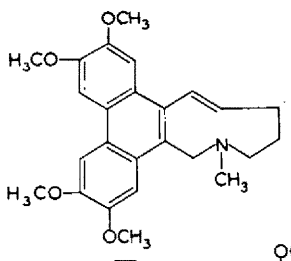


II

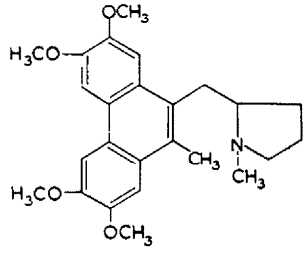


III

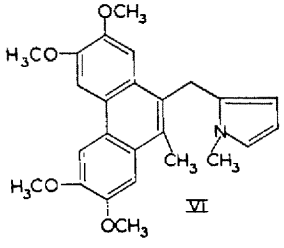
On the basis of structure (I), tylophorinemethine should be (IV), *iso*dihydrohomotylophorine (V) and detetrahydro*iso*dihydrohomotylophorine (VI).



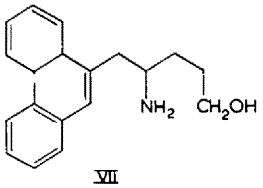
IV



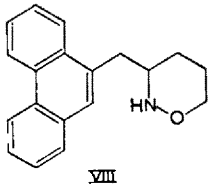
V



VI



VII



VIII

The synthesis of phenanthro(9:10:6′:7′)indolizidine as a model for the synthesis of tylophorine itself has been completed. Phenanthrene-9-aldehyde was condensed with δ-nitrobutyl benzoate. and the resulting nitropentene reduced with lithium aluminium hydride to a mixture of 4-amino-5-(9-phenanthryl)pentanol (VII) and 3-(9-phenanthrylmethyl)-1:2-oxazine (VIII). (VII) was converted to a diformyl derivative which on partial hydrolysis yielded 4-formamido-5-(9-phenanthryl)pentanol. Cyclisation of the latter yielded a quaternary salt which was reduced catalytically to phenanthro(9:10:6′:7′)indolizidine.

EXPERIMENTAL

Hofmann degradation of tylophorine

Tylophorine methiodide (2·5 g) was converted into the methohydroxide and decomposed as described previously.[1] The product was extracted with benzene and filtered. Tylophorinemethine (1·45 g), m.p. 185–188°, $[\alpha]_D^{30} = 0$, was obtained by evaporation of the filtrate. The benzene-insoluble residue (0·4 g) was completely soluble in water and identified as tylophorine *iso*methohydroxide by conversion to the *iso*methiodide, m.p. and mixed m.p., 268–270° (decomp) (Found: C, 56·2; H, 5·9. Calc. for $C_{25}H_{30}O_4NI$; C, 56·1; H, 5·6%). Hofmann degradation of the methine (2 g) was found to give in addition to varying yields of de-N-methyltylophorinemethine (0·15 to 0·75 g), m.p. 177° (with softening above 167°), a nitrogen-free non-basic alcohol (0·65 g), which on crystallisation from methanol, melted indefinitely

at 158–165° (Found: C, 73·5; H, 6·6. $C_{24}H_{25}O_5$ requires C, 73·3; H, 6·4%), but yielded a crystalline *benzoate* (with benzoylchloride in pyridine at 100°), m.p. 188–190° (from methanol) (Found: C, 75·0; H, 6·0 $C_{31}H_{30}O_6$ requires C, 74·7; H, 6·0%). De-N-methyltylophorinemethine methiodide (0·84 g) was converted into the metho-hydroxide and decomposed *in vacuo*. The product, m.p. 145–150°, was extracted with benzene and shaken with dilute acid. The residue (0·26 g) from the benzene extract was chromatographed over alumina in the same solvent. Elution with benzene gave a colourless *product*, which was crystallised from methanol to give needles, m.p. 181–188° (Found: C, 76·5; H, 6·4; N, 0·0; $C_{24}H_{24}O_4$ requires C, 76·6; H, 6·4%). Benzene containing 0·5% alcohol moved a yellow band. Evaporation gave a pale yellow substance (0·11 g), part (12 mg) of which sublimed at 170–240° at 10^{-5} mm and melted indefinitely at 88–115° (Found: C, 76·0; H, 6·5%). The non-volatile fraction (50 mg) could not be crystallised and had m.p. 230° (decomp; shrinking above 190°) (Found: C, 74·0; H, 7·0; N, 4·4%).

Attempted catalytic reduction of tylophorinemethine

A solution of the methine (0·65 g) in alcohol (100 ml) containing acetic acid (1 ml) was shaken with hyrogen at 60 lb/in^2 in presence of Adams catalyst (0·1 g) for 6 hr. The solution was filtered, methanol removed *in vacuo*, the residue dissolved in water, and shaken once with benzene. The aqueous acid solution was made alkaline by addition of sodium carbonate, saturated with potassium chloride and extracted with chloroform (1 l.). The solid (0·7 g) recovered from the chloroform extract was recrystallised from alcohol to give *tylophorine* iso*methochloride*, m.p. 208–210° (Found: C, 59·7; H, 7·3. $C_{25}H_{30}O_4NCl$, $3H_2O$ requires C, 60·3; H, 7·2%), identified by conversion to tylophorine isomethiodide, m.p. and mixed m.p. 270–272° (decomp). The use of alcohol alone as solvent for the reduction resulted in recovery of the methine.

Tylophorine bromocyanamide

Tylophorine (1 g) in chloroform (50 ml) was added gradually with stirring to cyanogen bromide (1·5 g) in the same solvent (25 ml) during 2 hr. Next day the solution was evaporated. The residue was ground with dilute acid, filtered and then washed with ammonium hydroxide and water. *The bromocyanamide* (1·05 g) on recrystallisation from benzene melted at 163° (Found: C, 60.4; H, 5·4. $C_{25}H_{27}O_4N_2Br$ requires C, 60·1; H, 5·4%).

Action of sodium borohydride on tylophorine bromocyanamide

The above cyanamide (1·05 g) and sodium borohydride (2·5 g) in methanol (150 ml) were left for 24 hr with occasional shaking. The solution was evaporated *in vacuo* at 30°. The residue was washed with acid and then with water and dried. A benzene solution of the solid was filtered through a column of alumina and the eluate evaporated. Two crystallisations of the solid from methanol gave the *hydroxy-cyanamide* as a white compound (0·25 g), m.p. 195–198°, with sintering above 187° (Found: C, 68·6; H, 6·7. $C_{25}H_{28}O_5N_2$ requires C, 68·8; H, 6·4%). The hydroxy-cyanamide (0·25 g) in sulphuric acid (4 N; 5 ml) was refluxed for 3 hr. The clear yellow solution was cooled, shaken once with benzene and then made alkaline. An

initially colourless precipitate which turned yellow was obtained. This had m.p. and mixed m.p. with tylophorine, 283–285° (decomp).

Action of zinc dust on the bromocyanamide

To a warm solution of the bromocyanamide (0·5 g) in acetic acid (50 ml) was gradually added zinc dust (2 g). The mixture was heated to boiling and filtered. The filtrate was evaporated *in vacuo* and the residue dissolved in water and made alkaline. Crystallisation of the precipitate from chloroform–alcohol mixture gave tylophorine, m.p. and mixed m.p. 283–285° (decomp).

Action of diethylamine on tylophorine bromocyanamide

The bromocyanamide (1 g) was refluxed with diethylamine (15 ml) for 3 hr. Excess amine was distilled and the residue taken up in ether and extracted with dilute acid. The acid layer was made alkaline and extracted with ether. Evaporation of the dried (sodium sulphate) ether layer gave a froth (0·88 g), which on crystallisation from methanol gave the *diethylamino-compound* (0·45 g) as colourless thick plates, m.p. 157° (Found: C, 71·0; H, 7·8; N, 8·9. $C_{29}H_{37}O_4N_3$ requires C, 70·9; H, 7·5; N, 8·6%).

Hydrolysis of the diethylamino compound

The above compound (0·5 g) was refluxed with sulphuric acid (20%; 10 ml) for 4 hr. The solution was made alkaline and extracted with ether. The residue (0·27 g) from the ether layer on two crystallisations from aqueous methanol gave the *product*, m.p. 199–200° (decomp) (Found: C, 68·5; H, 7·8; N, 8·4. $C_{29}H_{39}O_5N_3$ requires C, 68·5; H, 7·7; N, 8·3%).

Emde degradation of tylophorine

(a) *Tylophorine* iso*methiodide*. Tylophorine methiodide[1] (3·5 g) was refluxed in methanol (150 ml) and water (150 ml) containing potassium hydroxide (50 g) for 4 hr. The solution was then concentrated to a volume of 100 ml and cooled. The precipitate was filtered and washed with ice-water. Further concentration of the filtrate gave more solid. The combined iso*methiodide* (3·2 g) was recrystallised from water to give white crystals, m.p. 268–270° (decomp), λ_{max} 260, 288, 340, 355 mμ (log ε 4·81, 4·52, 3·39, 3·19) (Found: C, 56·2; H, 5·9. $C_{25}H_{30}O_4NI$ requires C, 56·1; H, 5·6%).

(b) iso*Dihydrohomotylophorine*. The crude *iso*methiodide (3·2 g) was refluxed with silver chloride (from 10 g of silver nitrate) in water (60 ml) and alcohol (60 ml) for 4 hr. The mixture was filtered and the filtrate boiled to remove the alcohol. The aqueous solution of the *iso*methochloride was diluted to 100 ml and heated at 100° with sodium amalgam (5%; 100 g) for several hours, with occasional addition of another 100 g of the amalgam. The crystalline froth was filtered, dried and recrystallised from benzene-petroleum ether (b.p. 40–60°) to yield the *Emde base* (1·4 g), m.p. 200–202°, $[\alpha]_D^{30°}$ ±0°; λ_{max} 260, 290, 340, 360 mμ (log ε 4·80, 4·54, 3·24, 3·12) (Found: C, 72·9; 72·9; H, 7·5, 7·5; N, 3·5; C—CH_3 1·9. $C_{25}H_{31}O_4N$ requires C, 73·4; H, 7·6; N, 3·4; 1 C—CH_3 3·7%).

(c) *de-Tetrahydro*iso*dihydrohomotylophorine*. The Emde base (0·32 g) in *p*-cymene (45 ml) was dehydrogenated with palladium-on-charcoal (0·5 g; 5%) at 220–240°,

till evolution of hydrogen ceased (4 hr). The mixture was filtered and the catalyst washed with benzene. The combined filtrates were evaporated to dryness, the residue ground with dilute hydrochloric acid and filtered. The non-basic residue (0·25 g) was recrystallised from benzene to give *de-tetrahydroisodihydrohomotylophorine* as colourless cubes, m.p. 235°; λ_{max} 255, 290, 340, 355 mμ (log ε 4·79, 4·54, 3·10, 2·90), giving positive pine-splinter and Ehrlich tests (Found: C, 74·2, 74·2 74·0; H, 6·9, 6·6, 6·4; N, 3·2. $C_{25}H_{27}O_4N$ requires C, 74·1; H, 6·7; N, 3·5%).

The above compound (0·1 g) in acetic acid (30 ml) containing Adams catalyst (0·1 g) was shaken with hydrogen at a pressure of 60 lb/in^2 for 7 hr, with addition of more catalyst (0·05 g) at the end of 4 hr. The solution was filtered and evaporated to dryness *in vacuo*. The residue was dissolved in sulphuric acid (2 N; 10 ml) and shaken with ether. The acid layer was cooled and basified. The precipitate was filtered and washed to give *iso*dihydrohomotylophorine (0·08 g), m.p. and mixed m.p. 198–200°.

Hofmann degradation of iso*dihydrohomotylophorine*

The Emde base (0·95 g) was refluxed with methyl iodide (4 ml) in chloroform (50 ml) for 4 hr and cooled. The precipitate was filtered and washed with chloroform to give the *methiodide* (1·25 g), white cubes (from methanol-ether), m.p. 278–279° (decomp); λ_{max} 260, 290, 340, 355 mμ (log ε 4·85, 4·57, 3·25, 3·02) (Found: C, 56·5; H, 6·1. $C_{26}H_{34}O_4NI$ requires C, 56·6; H, 6·2%). The methiodide (1·2 g) in water (120 ml) was shaken with silver oxide (from 5 g of silver nitrate) for 4 hr. The mixture was filtered and the filtrate evaporated at 50° *in vacuo*. The residue was heated at 100° at 0·5 mm for 1 hr. The product was boiled with benzene, filtered and the filtrate evaporated to give the crude methine (0·5 g), m.p. 127–132° which was chromatographed in benzene through alumina (80 g). After washing with benzene (400 ml) and benzene containing 0·25% ethanol (200 ml), elution with benzene containing 0·75% ethanol (250 ml) gave white crystals, m.p. 136–140°. These were recrystallised from ether to give iso*dihydrohomotylophorinemethine* (0·25 g), m.p. 142° (Found: C, 74·3; H, 7·6. $C_{26}H_{33}O_4N$ requires C, 73·8; H, 7·8%). Further elution of the column with benzene containing 10% ethanol gave a solid (50 mg) which was not further investigated.

Oxidation of iso*dihydrohomotylophorinemethine*

The methine (0·2 g) in acetone (15 ml) was treated gradually with potassium permanganate (1 g) in acetone (200 ml). After 72 hr, the mixture was filtered. The manganese dioxide residue was digested with sodium bicarbonate solution and filtered. The filtrate was extracted with chloroform to remove non-acidic material, concentrated to a small volume and acidified. The precipitated acid (0·1 g) was filtered, suspended in methanol and treated with ethereal diazomethane (from 10 g nitrosomethylurea). After 24 hr the solvents were evaporated and the residue washed with dilute ammonium hydroxide and then with water to give a solid (65 mg), m.p. 180–215°. This was extracted with boiling methanol (10 ml) and filtered. On cooling the methanolic filtrate deposited *methyl 2:3:6:7-tetramethoxy-9-methylphenanthrene-10-carboxylate* (8 mg) as pale yellow cubes, m.p. 185–186°, λ_{max} 260, 290, 340, 358 mμ (log ε 4·75, 4·54, 3·40, 3·15) (Found: C, 68·4; H, 5·8. $C_{21}H_{22}O_6$ requires C, 68·1; H, 5·9%). The methanol-insoluble fraction was recrystallised

twice from benzene to give *dimethyl* 2:3:6:7-*tetramethoxyphenanthrene*-9:10-*dicarboxylate* (8 mg) as pale yellow rectangular plates, m.p. 246–247°, λ_{max} 260, 290, 330 mμ (log ε 4·61, 4·45, 3·80) (Found: C, 63·6; H, 4·9. $C_{22}H_{22}O_8$ requires C, 63·8; H, 5·3%). Separation of the two esters (total 150 mg) could also be achieved by chromatography over alumina, the methyl ester (90 mg), m.p. 185–186° being eluted by benzene containing 0·1% methanol and the dimethyl ester (5 mg), m.p. 245–247°, by benzene containing 0·5% methanol. The oxidation of the methine gave the two acids in irreproducible ratio, in most experiments only the dimethyl ester being obtained.

2:3:6:7-*Tetramethoxy*-9-*methylphenanthrene*. The methyl ester, m.p. 186° (50 mg) was refluxed with alcohol (10 ml) and potassium hydroxide (2 g) for 8 hr. As hydrolysis proceeded, the sparingly soluble potassium salt separated. The solvent was evaporated, the residue dissolved in hot water, filtered and the filtrate acidified to give the acid (40 mg), m.p. 225–227°. This was dissolved in quinoline (2 ml) and heated with copper sulphate (20 mg) for 3 hr. Benzene (100 ml) was added to the mixture and the solution washed successively with dilute acid, water, dilute sodium hydroxide and water. The benzene layer was dried (sodium sulphate) and evaporated and the residue filtered in benzene solution through alumina. Evaporation of the eluate and recrystallisation from methanol gave the *methylphenanthrene* (10 mg) as almost colourless needles, m.p. 188–189°, λ_{max} 255, 285, 340, 355 mμ (log ε 4·88, 4·50, 3·20, 2·97), undepressed by admixture with a synthetic specimen (see below).

2:3:6:7-*Tetramethoxyphenanthrene*-9:10-*dicarboxylic acid*

The diester, m.p. 245–247° (0·12 g) was refluxed with potassium hydroxide (2 g) in alcohol (10 ml) for 4 hr. Water (50 ml) was added to the solution which was boiled to remove the alcohol. The cooled aqueous solution was extracted with ether and then acidified. The yellow precipitate (100 mg) was recrystallised from acetic acid or anhydride to give the *anhydride* as yellow needles, which shrank above 290°, and melted indefinitely at 315–325° (decomp) (Found: C, 64·8; H, 4·3. $C_{20}H_{16}O_7$ requires C, 65·2; H, 4·4%).

Oxidation of tylophorine

A suspension of tylophorine (1·3 g) in water (20 ml) was treated with potassium permanganate solution (5%; 210 ml) with stirring and slight warming when the rate of oxidation slackened. The mixture was filtered from the manganese dioxide residue. No acidic material could be recovered from the filtrate. The manganese dioxide residue from the oxidation was extracted with chloroform. The extract was concentrated and passed through an alumina column. Evaporation of the first 100 ml of the eluate gave a colourless solid which on recrystallisation from chloroform-alcohol mixture gave a *substance* (10 mg), colourless needles, m.p. 249° (Found: C, 64·3; H, 5·3. $C_{20}H_{20}O_7$ requires C, 64·5; H, 5·4%). Further elution with chloroform gave a solid which crystallised from chloroform-alcohol mixture as deep brown needles (10 mg) of another *substance*, m.p. 253–255° (Found: C, 62·2; H, 5·0; N, 1·2%).

Oxidation of tylophorine iso*methohydroxide*

The *iso*methohydroxide (1 g) in water (25 ml) and pyridine (15 ml) was treated with aqueous potassium permanganate (N/10) first at 30° and then with warming.

Addition of the oxidant was stopped when a faint pink colour persisted. The mixture was filtered and the filtrate evaporated *in vacuo* and acidified. The gum that separated was treated with sodium bicarbonate solution and extracted with ether. The aqueous layer was acidified and the precipitate crystallised from acetic acid to yield the dicarboxylic anhydride (6 mg), m.p. 320–325° (decomp). The manganese dioxide residue from the oxidation was extracted with hot chloroform and the extract washed with dilute acid, dried and evaporated. The residue on crystallisation from acetic acid or pyridine gave 2:3:6:7-*tetramethoxyphenanthrene*-9:10-*dicarboxylimide* (12 mg), m.p. 355° (decomp), with shrinking above 330° (Found: C, 65·0, 64·9; N, 5·2, 5·1; N, 4·0. $C_{20}H_{17}O_6N$ requires C, 65·4; H, 4·6; N, 3·8%).

Oxidation of tylophorine methiodide

Tylophorine methiodide (0·5 g) in water (150 ml) was treated with aqueous potassium permanganate (3%; 130 ml) with stirring at 30° and then at 100° till a faint pink colour persisted. The mixture was filtered and the manganese dioxide residue washed thoroughly with hot water. The filtrate was evaporated to dryness, acidified with dilute sulphuric acid and extracted continuously with ether for 12 hr. The extract was dried (sodium sulphate) and evaporated. The residue from two such batches was sublimed in a high vacuum at 180–200° to give a white sublimate (0·1 g) which was taken up in alcohol (5 ml) containing ethylamine (2 ml). The solution was evaporated and the residue treated again with alcoholic ethylamine. The solvents were evaporated and the residue heated at 180° for 5 min, and sublimed *in vacuo* at 150–200°. The sublimate on one crystallisation from alcohol gave pale yellow needles (30 mg), m.p. 225–227°, with shrinking above 220°. Filtration through an alumina column and a second crystallisation from alcohol gave N-*ethyl*-m-*hemipinimide* (15 mg), as colourless needles, m.p. and mixed m.p. 230–231° (Found: C, 61·4; H, 5·1. Calc. for $C_{12}H_{13}O_4N$ C, 61·3; H, 5·5%). A sample for comparison was prepared by oxidising as before 3:4-dihydro-6:7-dimethoxy*iso*-quinoline (1 g). Treatment of the acidic product (0·55 g) with ethylamine and vacuum sublimation gave N-ethyl-*m*-hemininimide (0·3 g).

Oxidation of tylophorinemethine

(a) *With potassium permanganate.* The methine (0·5 g) in dry pyridine (25 ml) was treated gradually with potassium permanganate (1 g). When no pink colour persisted, the solution was filtered and the filtrate evaporated. The residue was washed successively with acid, alkali and water. The neutral *product* (0·35 g) on crystallisation from benzene–petroleum ether (b.p. 40–60°), had m.p. 241–244°, with partial decomposition above 230° (Found: C, 71·5; H, 6·5; N, 3·5. $C_{25}H_{27}O_5N$ requires C, 71·3; H, 6·4; N, 3·3%), unaffected by refluxing with 20% alcoholic potassium hydroxide. The use of acetone instead of pyridine gave the same product together with partial recovery of the methine, while in aqueous acid solution, no product could be obtained. A solution of the lactam (0·1 g) in tetrahydrofuran (30 ml) reduced with lithium aluminium hydride (0·8 g) gave tylophorinemethine (30 mg) as colourless needles (from benzene–petroleum ether, b.p. 40–60°), m.p. and mixed m.p. 187–188°.

(b) *With alkaline hydrogen peroxide.* The methine (0·7 g) in tetrahydrofuran (50 ml) was made alkaline with a concentrated aqueous solution of sodium hydroxide

and treated with hydrogen peroxide (30%; 365 ml) at 100° with vigorous stirring. The solution was concentrated *in vacuo* to 30 ml and extracted with ether. The aqueous layer was acidified and extracted with ether (10 × 150 ml). The ether layer was evaporated to give an acid (10 mg), which on treatment with ethereal diazomethane and crystallisation of the product from benzene-methanol gave pale yellow needles (2 mg), m.p. 247–248°, undepressed by admixture with dimethyl 2:3:6:7-tetramethoxyphenanthrene-9:10-dicarboxylate reported above.

Syntheses of Tetramethoxymethylphenanthrenes

2:3:6:7-*Tetramethoxy-9-methylphenanthrene*

(a) 3:4-*Dimethoxy-α-(3:4-dimethoxyphenyl)-6-nitrocinnamic acid.* A mixture of 6-nitroveratraldehyde (21 g), homoveratric acid (21 g), acetic anhydride (40 ml) and triethylamine (20 ml) was heated for 16 hr at 100°, protected from moisture. Water (20 ml) was added and the mixture, after remaining for some time, poured into potassium carbonate (160 g) in water (1 l.) and heated till all the gummy material dissolved. The red solution was cooled, shaken with ether (2 × 100 ml) and acidfied with hydrochloric acid. Two crystallisations of the separated gum from methanol gave the *cinnamic acid* (28 g), m.p. 185° (Found: C, 58·3; H, 5·0. $C_{19}H_{19}O_8N$ requires C, 58·6; H, 4·9%).

(b) 2:3:6:7-*Tetramethoxyphenanthrene-9-carboxylic acid.* The above nitro acid (10 g) in ammonium hydroxide (4 N; 180 ml) was added with stirring to a hot mixture of concentrated aqueous ammonia (d 0·88; 180 ml) and ferrous sulphate (75 g) in water (180 ml). After stirring for ¾ hr at 90°, the mixture was filtered. The filtrate was cooled, shaken once with benzene, and then carefully made neutral with concentrated hydrochloric acid. The precipitated amino acid was filtered, washed first with water, then with cold methanol and allowed to dry. To a mixture of the amino acid (3 g) in dioxane (90 ml) and concentrated sulphuric acid (1 ml) at 30° was added dropwise with stirring, freshly prepared *iso*amyl nitrite (2 ml) in dioxane (10 ml). The diazonium solution was added at 40° with stirring to a mixture of freshly precipitated copper (1 g) and sodium hypophosphite (20 g) in water (20 ml) (heated initially to 90° and cooled to 40–50°). The stirring was continued for several hr at 40° and then for 1 hr at 90°. The mixture was then poured into water (1 l) containing sufficient ammonium hydroxide solution to dissolve the acidic product. The mixture was filtered, and the filtrate acidified with concentrated hydrochloric acid. The dark precipitate was collected and boiled with three portions (20 ml) of methanol and filtered. The residue was dissolved in dilute sodium carbonate solution, extracted once with benzene and acidified. Crystallisation of the precipitate from acetic acid gave 2:3:6:7-*tetramethoxyphenanthrene-9-carboxylic acid* (0·4 g), m.p. 285° (Found: C, 66·7; H, 5·3. $C_{19}H_{18}O_6$ requires C, 66·7; H, 5·3%). The methanolic filtrate was evaporated and the residue recrystallised from acetone, to give 3:4:6:7-*tetramethoxyphenanthrene-10-carboxylic acid* (0·3 g) m.p. 210° (Found: C, 66·7; H, 5·3%), which on decarboxylation by a procedure similar to that described below, gave 3:4:6:7-tetramethoxyphenanthrene, m.p. and mixed m.p. with an authentic sample, 121°.

(c) 2:3:6:7-*Tetramethoxyphenanthrene.* The acid, m.p. 285° (0·1 g), from the above experiment was refluxed in quinoline (3 ml) in the presence of copper sulphate

(0·1 g) for 1 hr. The mixture was cooled, poured into excess hydrochloric acid and extracted with benzene (3 × 25 ml). The benzene extract was washed with dilute alkali and water. The dried (sodium sulphate) extract gave on evaporation and crystallisation of the residue from methanol, the *phenanthrene* (30 mg), m.p. 178°, λ_{max} 255, 285, 300, 335, 350 mμ (log ε 4·86, 4·38, 4·16, 3·08, 2·78) (Found: C, 72·4; H, 6·1. $C_{18}H_{18}O_4$ requires C, 72·5; H, 6·0%).

(d) *Methyl* 2:3:6:7-*tetramethoxyphenanthrene*-9-*carboxylate*. The acid (2 g) in methanol (20 ml) was treated during 24 hr with ethereal diazomethane (from 20 g of nitrosomethylurea), to give as the neutral product, the *methyl ester* (1·8 g) which crystallised from alcohol had m.p. 195° (Found: C, 67·7; H, 5·4. $C_{20}H_{20}O_6$ requires C, 67·4; H, 5·6%).

(e) 2:3:6:7-*Tetramethoxyphenanthrene*-9-*carboxylic acid hydrazide*. The foregoing ester (0·5 g), hydrazine hydrate (85%; 1·5 ml) and *iso*amyl alcohol (5 ml) were refluxed for 4 hr, cooled and treated with mehtanol (20 ml). The solid was crystallised from ethanol to give the *hydrazide* (0·3 g), m.p. 246° (decomp) (Found: C, 63·9; H, 5·8. $C_{19}H_{20}N_2O_5$ requires C, 64·0; H, 5·6%). The hydrazide (0·3 g), dried at 140° *in vacuo*, was left in pyridine solution (10 ml) with benzenesulphonyl chloride (0·4 g) at 0° for 24 hr, and poured into ice and hydrochloric acid. The precipitate was recrystallised from acetone–methanol to give the *benzenesulphonyl derivative* (0·2 g), m.p. 265° (decomp) (Found: C, 60·4; H, 4·6. $C_{25}H_{24}N_2SO_7$ requires C, 60·5; H, 4·8%).

(f) 2:3:6:7-*Tetramethoxyphenanthrene*-9-*aldehyde*. The above sulphonyl derivative (0·2 g) dried at 140° *in vacuo* was treated in ethylene glycol solution (10 ml) at 160° with anhydrous sodium carbonate (0·2 g). After 80 sec, warm water was added. The mixture was cooled and extracted with chloroform. The chloroform layer was washed with water, dried (sodium sulphate) and evaporated and the residue crystallised from acetic acid giving the *aldehyde* (0·15 g), m.p. 210° (Found: C, 69·6; H, 5·3. $C_{19}H_{18}O_5$ requires C, 69·9; H, 5·5%).

(g) 2:3:6:7-*Tetramethoxy*-9-*methylphenanthrene*. The foregoing aldehyde (0·1 g) was refluxed with hydrazine hydrate (85%; 1 ml) in absolute alcohol (5 ml) for 2 hr. The solvent was removed *in vacuo*. Powdered potassium hydroxide (0·2 g) was added to the residue which was then heated at 190° for ½ hr. Water (10 ml) was added and the solid product extracted with benzene. The benzene extract was washed with water, dried (sodium sulphate), evaporated and the residue sublimed *in vacuo*. Recrystallisation of the sublimate from methanol gave 9-*methylphenanthrene* (20 mg), m.p. 188–189° λ_{max} 255, 285, 340, 355 mμ (log ε 4·74, 4·41, 3·19, 2·99) (Found: C, 72·8; H, 6·4. $C_{19}H_{20}O_4$ requires C, 73·1; H, 6·4%).

3:4:5:6-*Tetramethoxy*-9-*methylphenanthrene*

(a) *Methyl* 3:4:5:6-*tetramethyoxyphenanthrene*-9-*carboxylate*. 2-Amino-α-(6-bromo-3:4-dimethoxyphenyl)-3:4-dimethoxycinnamic acid[10] (11 g) was diazotised with *iso*amyl nitrite (6 ml) and treated with sodium hypophosphite-copper mixture to give the crude acidic product (5 g). Repeated fractional crystallisation from methanol gave as the more soluble fraction, 8-*bromo*-3:4:5:6-*tetramethoxyphenanthrene*-9-*carboxylic acid* (1 g), m.p. 187° and 3:4:5:6-*tetramethoxyphenanthrene*-9-*carboxylic acid* (2 g), m.p. 236° as the less soluble fraction. This acid (2·5 g) was refluxed for

[10] H. Kondo and E. Ochiai, *Liebigs Ann.* **470**, 224 (1929).

2 hr with dry methanol (25 ml) containing concentrated sulphuric acid (1 ml). The methanol was distilled and water was added to the residue, which was then extracted with chloroform. The chloroform layer was washed with sodium carbonate solution and water, and dried (sodium sulphate) and evaporated. The residual gum crystallised from dilute alcohol to give the *methyl ester* (1·5 g), m.p. 70° (Found: C, 67·6; H, 5·3%).

(b) 3:4:5:6-*Tetramethoxyphenanthrene-9-carboxylic acid hydrazide.* The ester (1·5 g) was refluxed with hydrazine hydrate (64%; 15 ml) in absolute alcohol (15 ml) for 4 hr, to yield the *hydrazide* (1·3 g), m.p. 175° (from benzene) (Found: C, 61·0; H, 6·0. $C_{19}H_{20}N_2O_5 \cdot H_2O$ requires C, 61·0; H, 5·9%).

(c) N-*Benzenesulphonyl*-3:4:5:6-*tetramethoxyphenanthrene*-9-*carboxylic acid hydrazide.* The benzenesulphonyl derivative (0·7 g) from 1·1 g of hydrazide, with 0·6 ml benzenesulphonyl chloride in pyridine (15 ml) crystallised from alcohol and had m.p. 217° (Found: C, 60·3; H, 5·2%).

(d) 3:4:5:6-*Tetramethoxyphenanthrene-9-aldehyde.* The foregoing benzensulphonyl derivative (0·7 g) was rearranged as previously, in ethylene glycol solution (25 ml) with sodium carbonate (0·6 g), to yield the *aldehyde* (0·3 g), m.p. 127° (from alcohol) (Found: C, 69·9; H, 5·5%).

(e) 3:4:5:6-*Tetramethoxy-9-methylphenanthrene.* The foregoing aldehyde (0·3 g), anhydrous hydrazine (0·6 ml) and absolute alcohol (6 ml) were refluxed for 2 hr and the solvent removed *in vacuo*. Powdered potassium hydroxide (0·3 g) was added to the residue maintained at 120–125°. After 25 min, water was added and the suspension extracted with ether. The residue from the ether extract was filtered through an alumina column, in benzene solution and the product obtained by evaporation of the benzene was crystallised from methanol to give 3:4:5:6-*tetramethoxy-9-methylphenanthrene* (80 mg), m.p. 87–88°, λ_{max} 265, 310, 325, 368, 380 mμ (log ε 4·73, 4·16, 4·16, 3·41, 3·43) (Found: C, 72·9; H, 6·5%).

3:4:6:7-*Tetramethoxy-9-methylphenanthrene*

3:4:6:7-Tetramethoxyphenanthrene-9-carboxylic acid (2·5 g), m.p. 210° was obtained by subjecting 2-amino-α-(3:4-dimethoxyphenyl)-3:4-dimethoxycinnamic acid[10] (15 g) to the Pschorr reaction. The *ethyl ester*, made as usual had m.p. 105° (Found: C, 68·0; H, 5·9. $C_{21}H_{22}O_6$ requires C, 68·1; H, 6·0%). This was converted into the *hydrazide* (0·5 g from 0·6 g ester), m.p. 219° (from alcohol) (Found: C, 64·0; H, 5·8%), thence into the *benzenesulphonyl derivative* (0·2 g from 0·4 g hydrazide), m.p. 245° (from alcohol-dioxane) (Found: C, 60·6; H, 5·0%). This was rearranged as before into 3:4:6:7-*tetramethoxyphenanthrene-9-aldehyde* (0·1 g) which, on crystallisation from alcohol, melted at 148° (Found: C, 69·6; H, 5·3%), which was then reduced by the usual Wolff–Kishner method to give 3:4:6:7-*tetramethoxy-9-methylphenanthrene*, m.p. 123° (from methanol), λ_{max} 260, 315, 345, 360 mμ (log ε 4·70, 3·86, 3·21, 3·10) (Found: C, 72·7; H, 6·3%).

Phenanthro(9:10:6′:7′)*indolizidine*

(a) *δ-Nitrobutyl benzoate*

Powdered silver nitrite (48 g) was added, with stirring, at 70–80°, to *δ*-bromobutyl benzoate[11] (78 g) in the course of 5 hr. The reaction mixture was heated for 4 hr,

[11] J. B. Cloke and F. J. Pilgrim, *J. Amer. Chem. Soc.* **61**, 2667 (1939).

cooled and the product taken up in ether, was filtered from silver bromide. The ether was evaporated and the residue fractionated twice under reduced pressure (metal or oil bath for heating) and the fraction distilling at 156–157°/1·5 mm collected (Found: C, 59·0; H, 6·0. $C_{11}H_{13}O_4N$ requires C, 59·2; H, 5·8%).

(b) 5-*Benzoyloxy*-2-*nitro*-1-(9-*phenanthryl*)-Δ′-*pentene*

Phenanthrene-9-aldehyde (10 g), δ-nitrobutyl benzoate (10 ml) and ammonium acetate (5 g) were refluxed in acetic acid (60 ml) for 2 hr. The red solution was poured into water, and the heavy oil that separated was washed several times with water and mixed with ethanol. The solid that separated was filtered and recrystallised from alcohol and had m.p. 118° (Found: C, 76·3; H, 5·3. $C_{26}H_{21}O_4N$ requires C, 75·9; H, 5·1%).

(c) 4-*Amino*-5-(9-*phenanthryl*)*pentanol*

(i) *By electrolytic reduction of* (b). A mixture of the foregoing nitropentene (5 g), acetic acid (200 ml), ethanol (50 ml) and concentrated hydrochloric acid (15 ml) was placed in the cathode compartment and dilute sulphuric acid (20%) in the anode compartment, maintaining the level of the anolyte slightly higher than that of the catholyte. Using lead electrodes, a steady current of 5 amperes was passed continually for 18 hr, while the catholyte was stirred. The catholyte was filtered and evaporated *in vacuo*; the residue was ground well with aqueous ammonia and the basic material taken up in ether. The ether was removed and the residue refluxed with dilute sulphuric acid (4 N; 70 ml) for 2 hr. The cooled acid solution was shaken with ether and then basified with ammonia. The base was extracted with ether and purified by crystallisation from benzene; it melted at 120° (Found: C, 81·6; H, 7·4; N, 5·0. $C_{19}H_{21}ON$ requires C, 81·7; H, 7·5; N, 5·0%).

The *picrate* made as usual had m.p. 175° (from alcohol). (Found: C, 59·3; H, 4·9; N, 11·3. $C_{25}H_{24}O_8N_4$ requires C, 59·1; H, 4·7; N, 11·0%).

(ii) *By lithium aluminium hydride reduction of* (b). A solution of the nitropentene (8·5 g) in dry tetrahydrofuran (50 ml) was added with stirring to a suspension of lithium aluminium hydride (3 g) in tetrahydrofuran (30 ml) kept protected from moisture. After decomposing with water, the tetrahydrofuran solution was filtered and concentrated almost to dryness. Ether was added and the solution cooled to 10°. The solid that separated was filtered and recrystallised from benzene to give 3-(9-*phenanthrylmethyl*)-1:2-*oxazine* (1·2 g), m.p. 185° (λ_{max} 255, 300, 350, mμ log ε 4·73, 4·03, 2·56) (Found: C, 82·4; H, 6·9; N, 5·2. $C_{19}H_{19}ON$ requires C, 82·3; H, 6·9; N, 5·0%).

The mother liquor after separation of the oxazine, was left at 0° overnight. The solid that separated from it was recrystallised from benzene and had m.p. 120° and it was identical with the electrolytic reduction product. The amino-alcohol gave a *monobenzoyl derivative*, m.p. 155° (from benzene) (Found: C, 81·7; H, 6·5. $C_{26}H_{25}O_2N$ requires C, 81·5; H, 6·5%).

(d) 4-*Formamido*-5-(9-*phenanthryl*)*pentanol*

The amino-alcohol (1 g), was heated with formic acid (98%; 3 ml) at 180° for 2 hr. The neutral product isolated, was recrystallised from benzene to give the O:N-*diformyl derivative* (1 g), m.p. 145° (Found: C, 75·3; H, 6·4; N, 3·9. $C_{21}H_{21}O_3N$

requires C, 75·2; H, 6·0; N, 4·2%). This was refluxed with aqueous sodium hydroxide (10%; 10 ml) for 1 hr. The gum that separated was washed well with water and mixed with benzene to give a solid, which was recrystallised from a very large volume of benzene giving the N-*formyl compound* (0·6 g), m.p. 150° (Found: C, 77·9; H, 6·9. $C_{20}H_{21}O_2N$ requires C, 78·2; H, 7·0%).

(e) *Phenanthro*(9:10·6′:7′)*indolizidine*

The foregoing monoformyl derivative (0·5 g) was refluxed for 1 hr with phosphorus oxychloride (5 ml) in sulphur-free dry toluene (5 ml). The toluene layer was diluted with a large excess of dry petroleum ether. The gummy material was washed well with dry petroleum ether, and then extracted with hot water. The aqueous extract was cooled, neutralised and treated with excess potassium iodide to give the quaternary iodide (0·2 g), which was separated, washed and reduced catalytically in methanolic suspension using Adams catalyst (50 mg) at a hydrogen pressure of 50 lb/in^2. The catalyst was filtered, the solvent removed and the residue ground with alkali and extracted with chloroform. After removal of the chloroform the residue was recrystallised from methanol to give *phenanthro*(9:10:6′:7′)*indolizidine* (50 mg), m.p. 170°, λ_{max} 255, 270, 300, 335, 340, 350 mμ (log ε 4·73, 4·56, 3·98 2·6, 2·6, 2·68) (Found: C, 87·8; H, 6·8. $C_{20}H_{19}N$ requires C, 87·9; H, 6·9%).

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