## 697. *Alkaloid Biosynthesis. Part IV.* 1-Benzylisoquinolines as Precursors of Thebaine, Codeine, and Morphine.*

By A. R. Battersby, R. Binks, R. J. Francis, D. J. McCaldin, and H. Ramuz.

Details are given of experiments on *Papaver somniferum* with labelled norlaudanosoline (X) which led to the first demonstration *in vivo* of the conversion of a 1-benzylisoquinoline system into the hydrophenanthrene alkaloids, morphine, codeine, and thebaine.[1] The radioactive alkaloids are degraded unambiguously. It is shown that the incorporation into the alkaloids increases as the precursor fed to the plants is changed from norlaudanosoline to its di-*O*-methyl ether (XII; R = R′ = H) and to its *NOO*-trimethyl derivative (XII; R = H, R′ = Me), which is reticuline. In contrast, tetrahydropapaverine (XII; R = Me, R′ = H), which possesses no phenolic hydroxyl groups, is not significantly incorporated. The interlocking evidence in favour of reticuline being the base which undergoes phenol coupling is discussed.

Synthetic routes to the various labelled 1-benzylisoquinolines are described.

Experiments described in Part II [2] proved that the $C_{16}$ carbon skeleton of morphine (I; R = H) is synthesised in Nature from two $C_6$–$C_2$ units (Ar–C–C) as shown † which are derivable from tyrosine in the plant; independent studies by Leete [3] with this precursor gave results in full agreement with ours. The present Paper is concerned with the intermediates which lie on the biosynthetic pathway between these two $C_6$–$C_2$ units and the molecules of thebaine (VI), codeine (I; R = Me), and morphine (I; R = H). There has been much speculation concerning the formation of these alkaloids [4] and at the outset of our work, the main proposals in biogenetic theory had reached the following stage. Sir Robert Robinson's brilliant recognition [5] that morphine and the other hydrophenanthrene alkaloids are related to the 1-benzylisoquinolines (II) led to the proposal of the correct structure for morphine (I; R = H) and, further, to the postulate that these alkaloids are formed in the plant by oxidative ring-closure of the 1-benzylisoquinoline system.[6,7] A further important proposal came from Barton and Cohen [8] who, on the basis of earlier work with Pummerer's ketone,[9] suggested that the C-12–C-13 bond in these alkaloids is formed by phenol oxidation of a suitably protected 1-benzylisoquinoline (II). The groups R and R′ represent a residue, possibly part of an enzyme surface, which can be added to, or taken away from, a phenolic hydroxyl in order to provide protection adequate to ensure specific coupling of radicals. Their proposal then involves oxidation of the base (II) by some one-electron transfer system to generate radicals which, by coupling, would yield the dienone (III). The fifth ring may then be formed as indicated and, finally, adjustment of the oxidation level of the base (IV) is required to afford the hydrophenanthrene alkaloids of opium. There is an alternative sequence by which the biogenetic scheme may be continued from the dienone (III) stage [10,11]

* Part III, *J.*, 1964, 1595.

† It must be borne in mind at this stage that one of these residues which appears in the morphine skeleton as a $C_6$–$C_2$ unit may, at the time of combination with the other, be in the form of a $C_6$–$C_3$ residue (Ar–C–C–$CO_2$H), *e.g.*, 3,4-dihydroxyphenylpyruvic acid, and that one carbon could be lost in a subsequent decarboxylation step. This is indicated in the formulæ.

[1] For a preliminary account see Battersby and Binks, *Proc. Chem. Soc.*, 1960, 360.
[2] Battersby, Binks, and Harper, *J.*, 1962, 3534.
[3] Leete, *J. Amer. Chem. Soc.*, 1959, **81**, 3948.
[4] Bentley, "The Chemistry of the Morphine Alkaloids," Clarendon Press, Oxford, 1954, p. 394.
[5] Gulland and Robinson, *Mem. Proc. Manchester Lit. Phil. Soc.*, 1925, **69**, 79.
[6] Robinson and Sugasawa, *J.*, 1931, 3163; Robinson, *J.*, 1936, 1079.
[7] Robinson, "The Structural Relations of Natural Products," Clarendon Press, Oxford, 1955, and refs. therein.
[8] Barton and Cohen, "Festschrift Arthur Stoll," Birkhäuser, Basle, 1957, p. 117.
[9] Barton, Deflorin, and Edwards, *J.*, 1956, 530.
[10] Battersby, Tilden Lecture, *Proc. Chem. Soc.*, 1963, 189, and refs. therein.
[11] Ginsburg, "The Opium Alkaloids," Interscience, New York, 1962, p. 91.

but this is a very recent proposal which will be considered together with other new developments [10,12] in a future Paper; it is only necessary to summarise above the state of affairs when the work below was being planned and carried out.

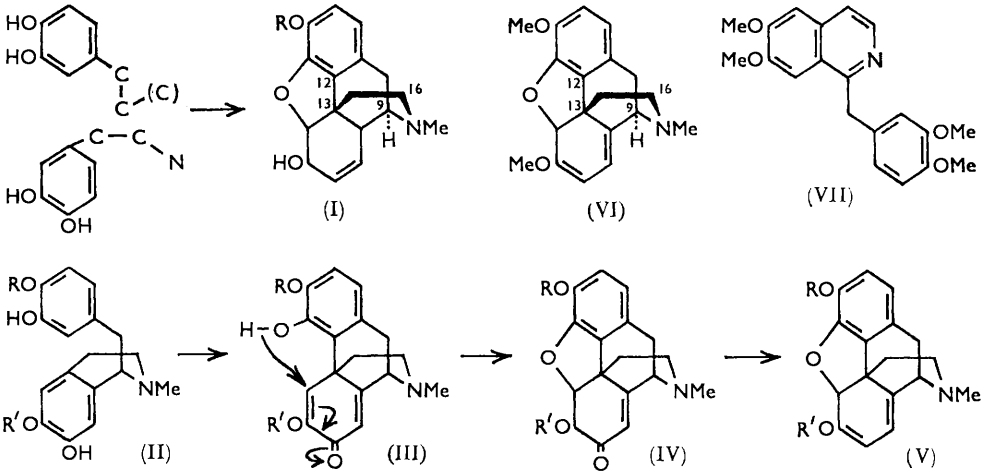
The first step in our study of the biosynthetic intermediates was to feed the so-called [1-$^{14}$C]norlaudanosoline (X) to *Papaver somniferum* plants since it was reasoned that this is

Tracer experiments on *Papaver somniferum* Noordster.

| Precursor | mc. | No. of plants | Year | Alkaloid | Wt. (mg.) | Incorporation (%) |
|---|---|---|---|---|---|---|
| (±)-[1-$^{14}$C]Norlaudanosoline (X) | 0·1 | 10 | 1960 | Morphine * | 220 | 3·9 |
| | | | | Codeine | 24 | 0·29 |
| | | | | Thebaine | 19 | 0·06 |
| | | | | Papaverine | 22 | 1·5 |
| (±)-[3-$^{14}$C]Norlaudanosoline (X) | 0·11 | 6 | 1961 | Morphine * | 198 | 2·2 |
| | | | | Codeine † | — | — |
| | | | | Thebaine † | — | — |
| (±)-[3-$^{14}$C]Nor-reticuline (XII; R = R′ = H) | 0·035 | 6 | 1961 | Morphine * | 231 | 3·6 |
| (±)-[3-$^{14}$C]Nor-reticuline (XII; R = R′ = H) | 0·015 | 3 | 1962 | Morphine * | 237 | 3·2 |
| (±)-[3-$^{14}$C]Reticuline (XII; R = H, R′ = Me) | 0·025 | 5 | 1962 | Morphine * | 313 | 7·3 |
| | | | | Codeine | 18 | 0·25 |
| | | | | Thebaine | 43 | 0·51 |
| (±)-[3-$^{14}$C]Tetrahydropapaverine (XII; R = Me, R′ = H) | 0·12 | 7 | 1961 | Morphine * | 172 | $<3·5 \times 10^{-3}$ |

* Hydrochloride. † Isolated by dilution.

the 1-benzylisoquinoline most probably formed first in the plant.[2,7,13] Experiments with derivatives of norlaudanosoline were planned to follow the successful incorporation of the parent compound into the hydrophenanthrene alkaloids. It should be realised that, at the




time of this work, labelled precursors of this size had not been introduced into the biosynthetic system of a higher plant. In fact, smooth incorporation was achieved and Grisebach and Patschke [14] similarly demonstrated the conversion of a chalcone glucoside into an isoflavone in red clover. More recently, this approach has been very rewarding for the study of other alkaloids, particularly those of the Amaryllidaceae.[15]

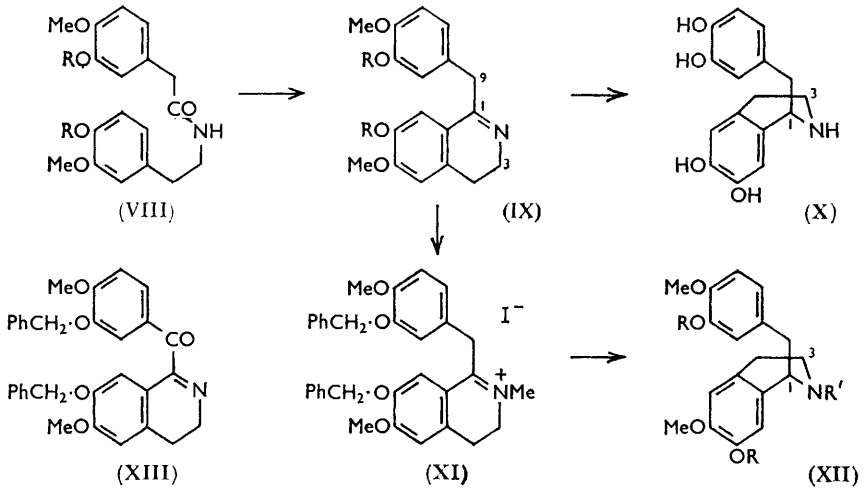
The chemical synthesis started from 3,4-dimethoxybenzyl chloride [16] which was treated

[12] Barton, Hugo Muller Lecture, *Proc. Chem. Soc.*, 1963, 293.
[13] Winterstein and Trier, "Die Alkaloide," Borntraeger, Berlin, 1910.
[14] Grisebach and Patschke, *Chem. Ber.*, 1960, **93**, 2326.
[15] Battersby, Binks, Breuer, Fales, and Wildman, *Proc. Chem. Soc.*, 1961, 243; Barton, Kirby, Taylor, and Thomas, *ibid.*, 1961, 254, and more recent Papers from both groups.
[16] Kröhnke, Schmeiss, and Gottstein, *Chem. Ber.*, 1951, **84**, 138.

with potassium [$^{14}$C]cyanide in dimethyl sulphoxide [17] and the resultant nitrile was hydrolysed to yield 3,4-dimethoxyphenyl[1-$^{14}$C]acetic acid. Reaction of the derived acid chloride with 3,4-dimethoxyphenethylamine gave the amide (VIII; R = Me) which was ring-closed with phosphorus oxychloride in boiling toluene to afford 3,4-[1-$^{14}$C]dihydropapaverine [18] (IX; R = Me). Substances of this type are prone to oxidation [19] and therefore the product was reduced without purification to give (±)-[1-$^{14}$C]tetrahydropapaverine (XII; R = Me, R′ = H), long known as an amorphous base in the radioinactive series,[20] but now obtained crystalline. Demethylation of this product with hot concentrated hydrochloric acid then yielded (±)-[1-$^{14}$C]norlaudanosoline (X) hydrochloride.

An aqueous solution of this material was injected [2] into the capsules of *P. somniferum* plants (variety Noordster) and after a period of growth, the plants were worked up for alkaloids. The incorporation into morphine (see Table) was higher than occurred earlier [2] from (±)-[2-$^{14}$C]tyrosine and this is in keeping with norlaudanosoline's standing closer to the final alkaloids than does tyrosine. The incorporations of activity from tyrosine into morphine under the same conditions [2] fell in the range 0·66—1·7%.

Chromatography of the non-phenolic alkaloids led to the isolation of radioactive codeine (I; R = Me) and thebaine (VI), whilst countercurrent distribution of the weakly basic fractions [2] yielded active papaverine (VII). Norlaudanosoline can thus serve as a precursor




of all the main hydrophenanthrene and 1-benzylisoquinoline alkaloids in *P. somniferum*. The studies on papaverine (by G. V. Parry) will be reported separately.

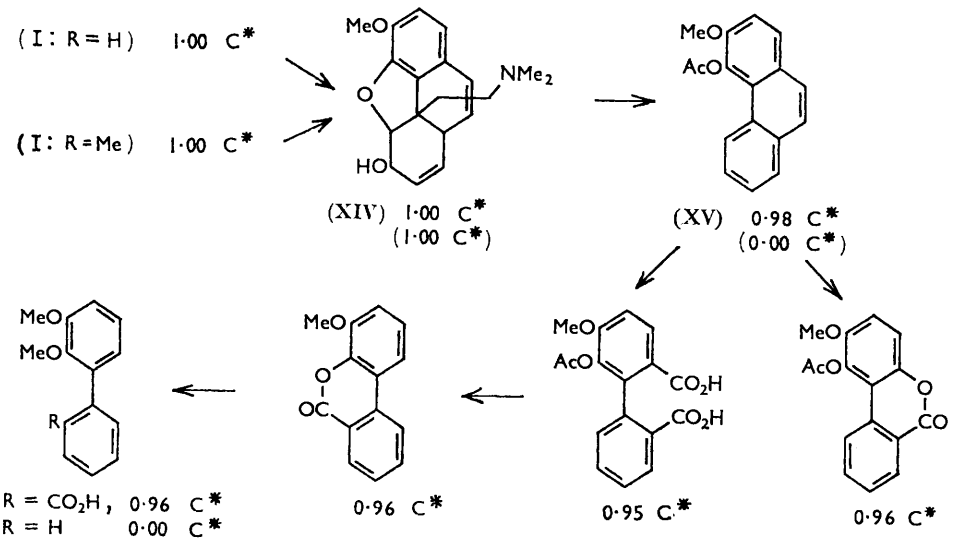
The radioactive morphine (I; R = H) was degraded to isolate the carbon atom from position 9 by the route described in Part II. It is therefore necessary here to show only the important degradation products, and their relative molar activities are recorded under the formulæ; the relative molar activities in parentheses are explained later. The results establish that the original morphine is labelled only at position 9 and that the first *in vivo* conversion of a 1-benzylisoquinoline system into the hydrophenanthrene alkaloids of the morphine group had been achieved; the main parts of the foregoing work have already been briefly reported.[1]

Additional evidence was gained by the preparation of (±)-[3-$^{14}$C]norlaudanosoline (X). 3,4-[1-$^{14}$C]Dimethoxyphenylacetonitrile was reduced catalytically to the corresponding

[17] Smiley and Arnold, *J. Org. Chem.*, 1960, **25**, 257; Friedman and Shechter, *ibid.*, 1960, **25**, 877.
[18] Pictet and Finkelstein, *Ber.*, 1909, **42**, 1979; Späth and Burger, *ibid.*, 1927, **60**, 704.
[19] Buck, Haworth, and Perkin, *J.*, 1924, **125**, 2176; Lindemann, *Helv. Chim. Acta*, 1949, **32**, 69.
[20] Pyman, *J.*, 1909, **95**, 1619, and refs. therein.

primary amine which was converted into (±)-[3-$^{14}$C]tetrahydropapaverine (XII; R = Me, R′ = H) by the method used above. Part was resolved (see Experimental section) to give optically active labelled materials for other experiments and the rest was demethylated as before to afford the required (±)-[3-$^{14}$C]precursor (X). This was fed to *P. somniferum* plants and again was incorporated well into the opium alkaloids (see Table). The complete degradation was carried out on the radioactive thebaine (VI) by conversion first into methebenine (XVI; R = Me); control of the conditions raised the yield obtainable in this valuable rearrangement [21] to 72%. The remaining steps were based upon the work of Gulland and Virden [22] though modifications of certain stages are described in the Experimental section. Ozonolysis of the vinylphenanthrene (XVII) yielded formaldehyde, which was not produced when the hydrogenated product (XIX) was treated with ozone. Thus, the formaldehyde is derived solely from the asterisked carbon atom of the olefin (XVII) and this corresponds to position 16 of thebaine (VI). The aldehyde (XVIII) could not be isolated from the ozonolysis products and the infrared spectrum of the crude mixture suggested that cleavage of the dimethoxylated ring had occurred, a well-known reaction in this series.[23] Accordingly, the olefin (XVII) was oxidised by permangangate [22] to yield the aldehyde (XVIII) which no doubt is protected sterically against further oxidation. In the annexed scheme, the reaction sequence and the relative activities of the product are shown. These results prove that the radioactive thebaine is labelled specifically at position 16, as expected.

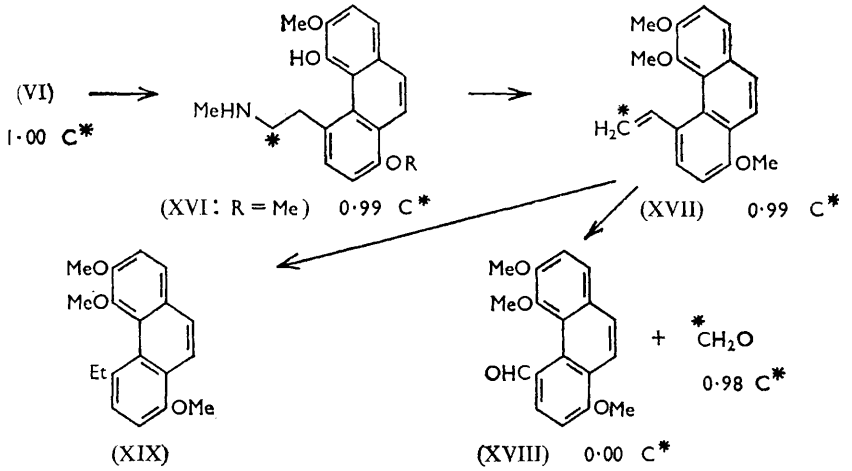
An alternative route to the olefin (XVII) involves rearrangement of thebaine by aqueous acid to yield thebenine [21] (XVI; R = H), followed by complete *O*- and *N*-methylation (dimethyl sulphate) and Hofmann degradation. Both routes are very satisfactory.




Codeine (I; R = Me), isolated from the experiment using (±)-[3-$^{14}$C]norlaudanosoline (X), was also degraded by the route used above for morphine *via* α-codeimethine (XIV) to the point where elimination of the ethanamine side-chain occurs. The resultant *O*-acetylmethylmorphol (XV) was totally inactive, in keeping with the expected specific labelling at position 16 of codeine (I; R = Me); the relative molar activities of the substances in this series are given in parentheses below the formulæ. Thus, the incorporation of norlaudanosoline (X), without randomisation of the label, is demonstrated into thebaine, codeine, and

[21] Hesse, *Annalen*, 1870, **153**, 47; Knorr, *Ber.*, 1903, **36**, 3074; Freund and Holtoff, *ibid.*, 1899, **32**, 168.
[22] Gulland and Virden, *J.*, 1928, 921.
[23] Speyer and Popp, *Ber.*, 1926, **59**, 390.

morphine. Moreover, the good incorporations into morphine (see Table) make it probable that norlaudanosoline lies on the direct biosynthetic pathway, but parallel work gave strong indications that it is modified before the oxidative coupling step occurs. These came from studies of the order of formation of the hydrophenanthrene alkaloids in the opium poppy. It was proved by the work of Rapoport and his colleagues [24] and by Battersby and Harper [25] that thebaine (VI) is the first hydrophenanthrene alkaloid to be




formed. This is de-*O*-methylated as one step in the formation of codeine (I; R = Me) and a second demethylation affords morphine (I; R = H). Thus, the conclusion can be drawn that the 1-benzylisoquinoline which undergoes phenol oxidation is suitably methylated to generate thebaine (VI); this requires participation of the base (XII; R = H, R′ = Me), which had been isolated earlier from *Anona reticulata* and named reticuline.[26]

The foregoing results and conclusions fall satisfyingly into line with theoretical considerations, for it was part of Barton and Cohen's proposal [8] that the 1-benzylisoquinoline precursor (II) should carry protective groups R and R′, and in reticuline these are both methyl groups. The idea that Nature uses *O*-methylation as the protecting device was tested by synthesising (±)-[3-$^{14}$C]norreticuline (XII; R = R′ = H) and (±)-[3-$^{14}$C]-reticuline (XII; R = H, R′ = Me). Both had been synthesised previously without labelled atoms [27] but it was necessary to modify parts of the route so that labels could be introduced at the 3-position for the present work and at other positions for later experiments. Because of the earlier work, the method used will be described very briefly.

*O*-Benzylvanillin [28] was reduced by borohydride to the corresponding alcohol which, with thionyl chloride, yielded 3-methoxy-4-benzyloxybenzyl chloride. This underwent exchange with potassium [$^{14}$C]cyanide in dimethyl sulphoxide, and the derived nitrile was reduced by lithium aluminium hydride to 3-methoxy-4-benzyloxyphen[1-$^{14}$C]ethylamine. The amide (VIII; R = $CH_2Ph$) was then formed by treatment of the radioactive amine with 3-benzyloxy-4-methoxyphenylacetyl chloride. The latter was prepared by the published method [29] or, more conveniently, from *O*-benzylisovanillin by way of the corresponding alcohol, chloride, and nitrile. Ring-closure of the amide gave 3,4-[3-$^{14}$C]dihydroisoquinoline (IX; R = $CH_2Ph$) as its hydrochloride or hydriodide. The corresponding

[24] Rapoport, Stermitz, and Baker, *J. Amer. Chem. Soc.*, 1960, **82**, 2765; Stermitz and Rapoport, *Nature*, 1961, **189**, 310; *J. Amer. Chem. Soc.*, 1961, **83**, 4045.
[25] Battersby and Harper, *Tetrahedron Letters*, 1960, No. 27, 21.
[26] Gopinath, Govindachari, Pai, and Viswanathan, *Chem. Ber.*, 1959, **92**, 776.
[27] Tomita and Kikkawa, *Pharm. Bull. (Japan)*, 1956, **4**, 230; *J. Pharm. Soc. Japan*, 1957, **77**, 195; Gopinath, Govindachari, and Viswanathan, *Chem. Ber.*, 1959, **92**, 1657; Tomita and Kunimoto, *J. Pharm. Soc. Japan*, 1960, **80**, 1238; Kunitomo, *ibid.*, 1961, **81**, 1253; Jain, *J.*, 1962, 2203.
[28] Finkelstein, *J. Amer. Chem. Soc.*, 1951, **73**, 550.
[29] Robinson and Sugasawa, *J.*, 1931, 3163.

free base was readily oxidised by air, and in trial experiments with radio-inactive materials, a yellow salt having the properties and colour reactions [19] corresponding to the ketone (XIII) was isolated. Hydrogenation of the radioactive base (IX; R = $CH_2Ph$) hydrochloride over palladised charcoal resulted in the sharp uptake of three moles of hydrogen and the formation of (±)-[3-$^{14}$C]norreticuline (XII; R = R′ = H) hydrochloride; the crystalline picrate was also prepared. The dihydroisoquinoline (IX; R = $CH_2Ph$) was also converted into (±)-[3-$^{14}$C]reticuline (XII; R = H, R′ = Me); details of this synthetic work and also of the degradation of the isolated radioactive morphine will be reported in a joint Paper with Professor D. H. R. Barton, Dr. G. W. Kirby, and their co-workers.

Aqueous solutions of the labelled norreticuline hydrochloride and reticuline hydrochloride were injected into *P. somniferum* plants and the alkaloids were later isolated.[2] The Table shows that these precursors are incorporated well into the hydrophenanthrene alkaloids and that (±)-reticuline gives a higher incorporation into morphine than does (±)-norreticuline or (±)-norlaudanosoline. The main parts of this work have been briefly outlined.* [30] By far the greatest part of the activity appears in morphine in these experiments and in the earlier ones with norlaudanosoline. This is because morphine is the last hydrophenanthrene alkaloid to be formed [24,25] and a relatively long period (3—4 weeks) elapsed between feeding and harvesting the plants.

The incorporation of (±)-reticuline into morphine was also observed by Barton and his co-workers; [31,32] the labels, at the *N*-methyl group ($^{14}$C) and at position 1 ($^{3}$H) were not randomised in the biosynthesis. They have also shown, in recent elegant experiments,[32] that reticuline (XII; R = H, R′ = Me) can undergo phenol coupling in the laboratory to yield the dienone (III; R = R′ = Me).

In reticuline (XII; R = H, R′ = Me), two free hydroxyl groups are available to allow the 12,13-bond in thebaine to be formed by the coupling of phenolate radicals [8] and it is of interest to determine the effect of changing this arrangement. (±)-[3-$^{14}$C]Tetrahydropapaverine (XII; R = Me, R′ = H), obtained in the synthesis above, was fed as the hydrochloride to poppy plants under the normal conditions. The incorporation into morphine was less than $3{\cdot}5 \times 10^{-3}$%, compared with 7·3% from reticuline. Thus, as was hoped, the biosynthesis is blocked by the methylation of all the phenolic hydroxyl groups. De-*O*-methylation does occur in poppy plants for the change thebaine ⟶ codeine ⟶ morphine.[24,25] However, it is clearly a specific process, for the foregoing experiment shows that the conversion of tetrahydropapaverine (XII; R = Me, R′ = H) into norreticuline (XII; R = R′ = H) or reticuline (XII; R = H, R′ = Me) does not occur to any significant extent.

These studies firmly establish the conversion of 1-benzylisoquinolines into thebaine (VI), codeine (I; R = Me), and morphine (I; R = H) and the incorporations are such as to leave little doubt that this is the normal route used in the living system. The results also give strong support to the view that reticuline (XII; R = H, R′ = Me) is the substance upon which the phenol oxidation step is carried out. Thus, the combined evidence and that presented earlier [2] is in keeping with a biosynthetic route leading from tyrosine, 3,4-dihydroxyphenylalanine, and the corresponding keto-acids to norlaudanosoline, (X) which is *N*- and *O*-methylated to yield reticuline. Phenol coupling and modification † of the initially formed dienone then affords thebaine, the precursor [24,25] of codeine and morphine.

* The experiments with (±)-[3-$^{14}$C]norlaudanosoline and (±)-[3-$^{14}$C]norreticuline were reported at the Anniversary Meeting of The Chemical Society, Sheffield, April 1962 (cf. ref. 10).

† Tracer experiments which establish the mechanism of these late stages in the biosynthesis of thebaine, codeine, and morphine will also be covered in the joint paper with the Imperial College group.

[30] Battersby, The Donegani Lectures on Biosynthesis, Milan, Sept. 1962; Acad. Naz. dei Lincei, vii° Corso Estivo di Chimica, 1964, p. 37; Battersby, Binks, Foulkes, Francis, McCaldin, and Ramuz, *Proc. Chem. Soc.*, 1963, 203.

[31] Barton and Kirby, The Donegani Lectures on Biosynthesis, Milan, Sept. 1962; Acad. Naz. dei Lincei, vii° Corso Estivo di Chimica, 1964, p. 17; see also ref. 12.

[32] Barton, Kirby, Steglich, and Thomas, *Proc. Chem. Soc.*, 1963, 203.

## Experimental

For general directions and the method used for calculation of incorporations, see Part III.[33] The methods described there for proof of purity of the labelled precursors and of the isolated alkaloids were also used in the present work. Early radioactive assays were carried out as recorded in Part II [2] but most of the counting was done as in Part III.[33] Cultivation of the plants and administration of the labelled precursors have already been described in Part II.[2]

*Extraction and Separation of Alkaloids.*—The method used previously [2] was followed to the point where a solution of the total alkaloids in aqueous acid (this time aqueous phosphoric acid) had been extracted with ether–chloroform (3 : 1, v/v); the further stages have been simplified and a typical run on five poppies is now described. The aqueous solution was adjusted to *ca.* pH 12 by the addition of concentrated potassium hydroxide solution and extracted four times with equal volumes of chloroform. Emulsions which tended to form were overcome by filtering the mixture through a thin pad of Filtercel. When a large amount of solid was precipitated and collected at this stage, it was redissolved in dilute phosphoric acid, the solution was adjusted to pH 12 as before, and the chloroform extraction repeated as before. The combined chloroform solutions were extracted with N-sodium hydroxide (2 × 50 ml.) and washed with water. Evaporation of the dried organic solution left the non-phenolic alkaloids as a gum (202 mg.) which was examined as described below.

The combined aqueous alkaline solutions were adjusted to pH 8·3—8·6 by the addition of phosphoric acid and extracted with chloroform–propan-2-ol (4 × 1 l.; 4 : 1, v/v). The dried extracts were evaporated to leave the phenolic alkaloids (549 mg.) which were dissolved in the minimum volume of ethanol and treated with a slight excess of concentrated hydrochloric acid. Morphine hydrochloride crystallised (292 mg.) and the alkaloid was further purified by dissolving the hydrochloride in a small volume of water and adding ammonium hydroxide. Morphine monohydrate separated (234 mg.) and was recrystallised from aqueous methanol to give pure morphine (216 mg.). The combined mother-liquors were worked up for morphine base and the crude material was diluted with pure inactive morphine (usually 0·05—0·2 g., depending on scale of extraction). The formation of the hydrochloride and recovery of the free base was then repeated as above. Alternatively, the ethanolic mother-liquor from the first crop of morphine hydrochloride above was further worked up for radioactive alkaloid by dilution with inactive morphine followed by the addition of a slight excess of concentrated hydrochloric acid. From the activity of the undiluted morphine isolated from the plants and that of the material isolated by dilution, the quantity of morphine present in the mother-liquors can be calculated.

Ethyl acetate was shaken with an equal volume of aqueous 0·2N-acetate buffer (pH 3·4) until equilibrium was achieved. A solution of the non-phenolic bases above in the wet ethyl acetate (50 ml.) was shaken with the buffer (50 ml.) and the aqueous layer was back-extracted with the wet ethyl acetate (2 × 50 ml.). After the combined solutions in ethyl acetate had been shaken with more equilibrated buffer (50 ml.), they were washed with water (20 ml.), dried, and evaporated to give the weakly basic non-phenolic alkaloids. Thin-layer chromatograms (silica gel, 4 : 1 (v/v) benzene–methanol) showed narcotine and/or papaverine to be the main component(s).

Potassium carbonate was added to the combined buffer solutions and washings until the pH was 10. Extraction with ethyl acetate (4 × 50 ml.) gave the strongly basic non-phenolic alkaloids, shown by thin-layer chromatography to contain mainly codeine and thebaine. These were separated by chromatography on alumina.[24]

*Purification of Alkaloids to Constant Specific Activity.*—(*a*) *Morphine.* This was purified by repetition of the hydrochloride formation and reconversion into the free base as above. In some cases, picrate formation [2] was used and the free base was recovered by passing a solution of the picrate (*ca.* 600 mg.) in chloroform–methanol (3 : 1, v/v) through a column of neutral alumina and eluting with the same solvent (700 ml.). Evaporation of the colourless eluate gave pure morphine.

(*b*) *Codeine.* This is best handled as the picrolonate. A solution of codeine (50 mg.) in ethanol (5 ml.) was mixed with a solution of picrolonic acid (60 mg.) in ethanol (4 ml.). The *precipitate* (102 mg.) had m. p. 232—233° (from aqueous ethanol) (Found: C, 59·85; H, 5·45; N, 12·3. $C_{28}H_{29}N_5O_8$ requires C, 59·7; H, 5·2; N, 12·4%). Codeine was recovered by passing a solution of the picrolonate in chloroform–methanol (6 : 1, v/v) over neutral alumina and

[33] Battersby, Binks, Breuer, Fales, Wildman, and Highet, *J.*, 1964, 1595.

eluting with chloroform. The residue obtained by evaporation was recrystallised from aqueous ethanol.

(*c*) *Thebaine.* This was purified as the picrate (see Part II [2]), and the free base, obtained from the salt as in (*b*) using chloroform throughout, was recrystallised from ethanol, m. p. 195—196° (Found: C, 73·5; H, 6·7. Calc. for $C_{19}H_{21}NO_3$: C, 73·3; H, 6·8%).

**3,4-*Dimethoxyphenyl*[1-$^{14}$C]*acetic Acid*.**—Potassium cyanide (30·5 mg.) was stirred for 5 min. with dimethyl sulphoxide (2 ml.) and potassium [$^{14}$C]cyanide (3 mg., 1·0 mc.) was added and washed in with dimethyl sulphoxide (4 ml.). After 10 min., freshly distilled 3,4-dimethoxybenzyl chloride (95 mg.) was added, and the solution was stirred at room temperature for 6 hr. It was shaken with water (50 ml.) and ether–light petroleum (b. p. 60—80°) (50 ml.; 1 : 1, v/v) and the aqueous layer was further extracted thrice with the same solvent, backwashing each time with water (10 ml.). Evaporation of the combined, dried extracts left 3,4-dimethoxyphenyl[1-$^{14}$C]-acetonitrile (85·3 mg.), shown in radio-inactive runs to be identical with authentic material. All the active sample was dissolved in ethane-1,2-diol (2 ml.) and water (0·5 ml.) and heated with potassium hydroxide (0·45 g.) under reflux (bath temp. 135°) for 12 hr. The cooled solution was partitioned between water and ether and the aqueous phase, after acidification, was extracted four times with ether to afford 3,4-dimethoxyphenyl[1-$^{14}$C]acetic acid (90·1 mg.; 0·86 mc.), m. p 89—91°, which was sufficiently pure for the next stage.

(±)-[1-$^{14}$C]*Tetrahydropapaverine* (XII; R = Me, R′ = H).—The foregoing acid was warmed on a steam-bath for 20 min. with thionyl chloride (1 ml.) and the excess of reagent was evaporated at *ca.* 80 mm. A solution of the residue in anhydrous ether (4 ml.) was added dropwise to a stirred solution of 3,4-dimethoxyphenethylamine (270 mg.) in anhydrous ether (2 ml.) at 0°. The mixture was shaken with 2N-hydrochloric acid (10 ml.) and ethyl acetate (50 ml.), and the aqueous layer was extracted thrice with ethyl acetate. After the combined extracts had been shaken with an excess of aqueous potassium carbonate and water, they were dried and evaporated to yield *N*-(3,4-dimethoxyphenethyl)-3,4-dimethoxyphenyl[*carbonyl*-$^{14}$C]acetamide (VIII) as a solid (157 mg.). Recrystallisation from ethyl acetate gave the amide (125 mg.; 0·65 mc.), m. p. 125—126°, suitable for ring-closure. A solution of this product (124 mg.) in anhydrous toluene (5 ml.) was evaporated to 3 ml. and heated under reflux for 1·5 hr. with freshly distilled phosphorus oxychloride (0·7 ml.). The cooled mixture was partitioned between dilute hydrochloric acid and ether and, after the aqueous layer had been extracted twice with ether, it was basified (under nitrogen) and extracted thrice with ether to afford crude 3,4-[1-$^{14}$C]dihydropapaverine (IX; R = Me) as a gum (118 mg.). This, in methanol (10 ml.), was treated with sodium borohydride (0·1 g.) and after the solution had been kept overnight, it was worked for base as above to yield (±)-1,2,3,4-[1-$^{14}$C]tetrahydropapaverine (94 mg.). Trial runs on inactive material showed that the product from this sequence was of good quality suitable for use in the demethylation step. The base obtained in the trial runs had m. p. 90° (from ether); this readily afforded the known [20] hydriodide, m. p. 256° (decomp.) [lit.,[20] 259° (decomp.)].

(±)-[1-$^{14}$C]*Norlaudanosoline* (X).—The foregoing active base (94 mg.) was heated at 170—175° for 2·5 hr. in an evacuated sealed tube with AnalaR concentrated hydrochloric acid (1 ml.); the temperature used in later preparations was 165° for 1 hr. and this afforded a cleaner product. The crystals which separated from the cooled tube were collected, dried over potassium hydroxide, and recrystallised from water to yield colourless (±)-[1-$^{14}$C]norlaudanosoline hydrochloride (70 mg.; 0·4 mc.), m. p. 278—281° (decomp.) [lit.,[20] 291—293° (corr.) after sintering and decomposing at 280°] (Found: OMe, 0·0%).

*Degradation of Radioactive Morphine from* (±)-[1-$^{14}$C]*Norlaudanosoline*.—This was carried out as in Part II [2] to give similar yields of degradation products which were identified by direct comparison with the samples previously obtained.

(+)-, (−)-, *and* (±)-[3-$^{14}$C]*Tetrahydropapaverine* (XII, R = Me, R′ = H).—N-Hydrochloric acid (3·5 ml.) was added to a solution of 3,4-dimethoxyphenyl[1-$^{14}$C]acetonitrile, prepared as above, (412 mg.; 5·5 mc.) in ethanol (20 ml.). This was shaken with hydrogen and 10% palladised charcoal (350 mg.) until uptake (2 mol.) ceased, and the catalyst was then filtered off. The alcohol was evaporated from the filtrate and a solution of the residue in dilute hydrochloric acid was extracted thrice with ether to remove neutral material (42 mg.). Evaporation of the aqueous layer gave crystalline 3,4-dimethoxyphen[1-$^{14}$C]ethylamine hydrochloride which was identified in an identical radio-inactive run by conversion into the picrate, m. p. and mixed m. p. 167—168° (decomp.).

3,4-Dimethoxyphenylacetic acid (750 mg.) was converted into its acid chloride as above and

added in anhydrous dioxan (3 ml.) to a stirred solution of the radioactive amine hydrochloride in 10% aqueous sodium hydroxide (10 ml.) and dioxan (7 ml.). After being stirred for 15 min., the solution was acidified and freed from dioxan by evaporation. The suspension was extracted with ethyl acetate (3 × 100 ml.) and the combined extracts were washed with 0·5N-sodium hydroxide (2 × 20 ml.) and water (2 × 15 ml.). Evaporation of the dried solution in ethyl acetate left a solid (600 mg.) which was recrystallised from ethyl acetate to yield *N*-(3,4-dimethoxyphen[1-$^{14}$C]ethyl)-3,4-dimethoxyphenylacetamide (VIII) (550 mg.; 3·6 mc.).

This was cyclised as for the same amide above (different $^{14}$C-label), with proportionately greater quantities of solvents and reagents, to yield crude 3,4-[3-$^{14}$C]dihydropapaverine (IX; R = Me) as a gum (420 mg.) which was reduced as before to give 1,2,3,4-[3-$^{14}$C]tetrahydropapaverine (404 mg.; 2·8 mc.). Of this, part (17·8 mg.; 0·12 mc.) was dissolved in dilute hydrochloric acid (0·3 ml.) and the solution was evaporated to dryness to yield the hydrochloride for administration to the plants.

The remainder (386 mg.) was resolved by an adaptation of the method of Corrodi and Hardegger.[34] This base, in methanol (2·5 ml.), was treated with *N*-acetyl-L-leucine [35] (193 mg.) and ether (3·5 ml.) was added to the clear solution. After 2 days, the crystals (176 mg.) were collected and recrystallised twice from methanol–ether to yield the salt (95·4 mg.), m. p. 178—180° of (−)-[3-$^{14}$C]tetrahydropapaverine identical with radio-inactive material prepared in a trial run. The base was recovered into ether as usual and on evaporation of the solution, (−)-[3-$^{14}$C]-1,2,3,4-tetrahydropapaverine was obtained as crystals (65 mg.; 0·44 mc.), m. p. 97·5—98·5°.

The mother-liquor from the 176 mg. crop of crystals above was worked for base which was obtained as a gum (252 mg.). To a solution of this in methanol (1·8 ml.) was added *N*-acetyl-D-leucine [35] (130 mg.) and the clear solution was diluted with ether (2·5 ml.). The crystals which separated overnight were collected (142 mg.) and recrystallised twice from methanol ether to afford the salt (78 mg.), m. p. 178—180°, of (+)-[3-$^{14}$C]tetrahydropapaverine. The free base, (+)-1,2,3,4-[3-$^{14}$C]tetrahydropapaverine, was recovered as above as a crystalline solid (53 mg.; 0·36 mc.), m. p. 97·5—98·5°, $[\alpha]_D$ +26° ±8° ($CHCl_3$) {lit.,[34] $[\alpha]_D$ +21° ($CHCl_3$)}.

The mother-liquors from the (+)- and (−)-*N*-acetyl-leucine salts above were combined in the correct proportions to regenerate the racemic base which was recovered into ether as usual. This base was highly coloured, and purification over neutral alumina (activity III) in benzene–chloroform (3 : 1, v/v) gave material (160 mg.) suitable for demethylation as above (1·6 ml. concentrated acid). Recrystallisation of the coloured hydrochloride from water yielded (±)-[3-$^{14}$C]norlaudanosoline hydrochloride (90 mg.; 0·65 mc.).

3-*Benzyloxy*-4-*methoxyphenylacetic Acid*.—This, m. p. 127—128°, was prepared by Robinson and Sugasawa's method [29] or, more readily, as follows. A stirred solution of *O*-benzylisovanillin [29] (50 g.), in methanol (400 ml.) and benzene (100 ml.), was treated portionwise with sodium borohydride (7 g.) over 1·5 hr. The solution was warmed at 40° for 0·5 hr., acidified with hydrochloric acid, and treated with potassium hydroxide (60 g.) in water (100 ml.). After the mixture had been heated on a steam-bath for 3·5 hr., the organic solvents were evaporated, the aqueous suspension was almost saturated by the addition of potassium carbonate and the product was extracted with ether–chloroform (4 : 1, v/v). It was recrystallised from ether and light petroleum (b. p. 40—60°) to yield 3-benzyloxy-4-methoxybenzyl alcohol (35·4 g.), m. p. 72—73° (lit.,[36] 70—71°).

Thionyl chloride (40 ml.) was added dropwise during 30 min. to a rapidly stirred suspension of the foregoing alcohol (35 g.) in ether (70 ml.). After a further 30 min., the clear solution was evaporated to yield 3-benzyloxy-4-methoxybenzyl chloride, m. p. 72—73° (lit.,[36] 70—75°), which was sufficiently pure for the next stage.

This chloride (16 g.) reacted with potassium cyanide (5·94 g.) in dimethyl sulphoxide (240 ml.) as for the exchange reaction on p. 3607. Recrystallisation of the crude product, m. p. 70—76°, from chloroform–light petroleum (b. p. 60—80°) and chromatography in benzene on silica gel gave 3-*benzyloxy*-4-*methoxyphenylacetonitrile*, m. p. 79·5—80·5° (Found: C, 75·9; H, 6·2. $C_{16}H_{15}NO_2$ requires C, 75·8; H, 6·0%).

A solution of the nitrile (15·4 g.) in ethylene glycol (320 ml.) and water (80 ml.) was heated under reflux for 12 hr. with potassium hydroxide (8 g.). After dilution of the cooled solution

[34] Corrodi and Hardegger, *Helv. Chim. Acta*, 1956, **39**, 889.
[35] DeWitt and Ingersoll, *J. Amer. Chem. Soc.*, 1951, **73**, 3359.
[36] Bersch, *Arch. Pharm.*, 1939, **277**, 271.

with water, it was extracted thrice with ether, then acidified and extracted again with ether. The combined second set of extracts gave 3-benzyloxy-4-methoxyphenylacetic acid which was recrystallised from benzene–light petroleum to give crystals (14·1 g.), identical with the product from the other route.

3-*Methoxy*-4-*benzyloxybenzyl Alcohol* (with G. V. PARRY).—A solution of *O*-benzylvanillin [28] (5 g.) in methanol (35 ml.) was treated with sodium borohydride (0·7 g.). After being kept at room temperature overnight, the *alcohol* (5·0 g.) was isolated as for the isomer above and had m. p. 72—73° [from ether–light petroleum (b. p. 40—60°)] (Found: C, 73·8; H, 6·6. $C_{15}H_{16}O_3$ requires C, 73·9; H, 6·5%).

3-*Methoxy*-4-*benzyloxybenzyl Chloride*.—The crude chloride was prepared, as for its isomer above, from the foregoing alcohol (5 g.), ether (10 ml.), and thionyl chloride (5 ml.). The product, in the minimum volume of benzene (avoid heat), was passed through a column of silica gel and the percolate (250 ml.) was evaporated. Recrystallisation of the residue from chloroform–light petroleum (b. p. 40—60°) yielded the chloride (2·68 g.), m. p. 71·5—72·5° (lit.,[37] 72—74°) (Found: C, 69·0; H, 5·6. Calc. for $C_{15}H_{15}ClO_2$: C, 68·8; H, 5·7%).

3-*Methoxy*-4-*benzyloxyphenyl*[1-$^{14}$C]*acetonitrile*.—This (151 mg.) was prepared as for 3,4-dimethoxyphenyl[1-$^{14}$C]acetonitrile above, from the foregoing chloride (185·5 mg.) and potassium [$^{14}$C]cyanide (3·8 mg.; *ca.* 1·0 mc.). Radio-inactive trial runs showed that the cyanide crystallised from ether–light petroleum (b. p. 40—60°), m. p. 68—69° (lit.,[37] 67—68°) (Found: C, 75·8, 75·9; H, 6·0, 5·9; N, 5·5, 5·4. Calc. for $C_{16}H_{15}NO_2$: C, 75·9; H, 6·0; N, 5·5%).

3-*Methoxy*-4-*benzyloxyphen*[1-$^{14}$C]*ethylamine*.—A solution of the foregoing nitrile (150 mg.) in anhydrous ether (35 ml.) was added to a stirred solution of lithium aluminium hydride (0·5 g.) in ether (100 ml.). After the mixture had been heated under reflux for 3·5 hr., it was cooled, treated with an excess of saturated aqueous sodium potassium tartrate and extracted thrice with ether. The combined ether extracts were shaken with an excess of 0·5N-hydrochloric acid, and the acidic extracts were basified and extracted thoroughly with ether to yield the amine (99 mg.; 0·7 mc.). This base, from radio-inactive runs, was characterised as the oxalate, m. p. 159—161° (lit.,[27] 160—165°).

1,2,3,4-*Tetrahydro*-7-*hydroxy*-1-(3-*hydroxy*-4-*methoxybenzyl*)-6-*methoxy*[3-$^{14}$C] *isoquinoline* (*Norreticuline*; XII; R = R′ = H).—The foregoing amine was reacted with the acid chloride prepared from 3-benzyloxy-4-methoxyphenylacetic acid (318 mg.), using the conditions described above for the preparation of *N*-(3,4-dimethoxyphen[1-$^{14}$C]ethyl)-3,4-dimethoxyphenylacetamide. The crude amide was chromatographed in benzene on neutral alumina (activity III) to remove a trace of oil. Chloroform–benzene (1 : 1) eluted the pure amide (VIII; R = $CH_2Ph$) which was recrystallised from ethyl acetate, and the mother-liquor was further worked for radioactive amide by the addition of inactive amide (50 mg.) and re-isolation. The combined crops (203 mg.) had m. p. 140·5—141·5° (lit.,[27] 134·5—136·5°) (Found: C, 75·0; H, 6·4. Calc. for $C_{32}H_{35}NO_5$: C, 75·1; H, 6·5%).

The amide was cyclised by Jain's method [27] and, after removing the volatile materials, the residue was dissolved in ethanol (0·8 ml.) and treated with 0·5N-hydrochloric acid (5 ml.). The precipitated hydrochloride was recrystallised twice from chloroform–ethyl acetate to give the [3-$^{14}$C]dihydroisoquinoline (IX; R = $CH_2Ph$) hydrochloride as prisms (168 mg.; 0·36 mc.), m. p. 198—200° (lit.,[27] 201—203°) (Found: C, 72·4; H, 6·1. Calc. for $C_{32}H_{32}ClNO_4$: C, 72·6; H, 6·1%).

A solution of the foregoing hydrochloride (47 mg.; radio-inactive) in water was treated with potassium iodide, and the precipitate was recrystallised from chloroform–ethanol to give the 3,4-*dihydroisoquinoline* (IX; R = $CH_2Ph$) *hydroiodide*, m. p. 200—202° (decomp.) (Found: C, 61·8; H, 5·3. $C_{32}H_{32}INO_4$ requires C, 61·8; H, 5·2%).

Part of the [3-$^{14}$C]dihydroisoquinoline hydrochloride was reserved for the synthesis of (±)-[3-$^{14}$C]reticuline (XII; R = H, R′ = Me) and the remainder (34 mg.), in ethanol (15 ml.), was shaken with hydrogen and 10% palladised charcoal at room temperature and pressure. Uptake (3·0 mol.) was complete in 1 hr. Removal of the catalyst and evaporation of the solution left a resin which crystallised on addition of water to give (±)-[3-$^{14}$C]norreticuline hydrochloride monohydrate (19 mg.), m. p. 165° (lit.,[27] 165—166°).

In trial runs, the product was further characterised as the *picrate*, m. p. 202—205° after a colour change at 121° (from ethanol) (Found: C, 52·45; H, 4·6. $C_{24}H_{24}N_4O_{11}$ requires C, 52·9; H, 4·45%).

*Degradation of Codeine*.—A solution of radioactive codeine (545 mg.) in methanol (4 ml.) and

[37] Strukov, *Zhur. obshchei Khim.*, 1961, **31**, 2709.

methyl iodide (1 ml.) was heated under reflux for 1 hr. The precipitated codeine methiodide was recrystallised from methanol to give the pure salt (759 mg.), m. p. 262—264°.

The further steps were as described in Part II.[2]

*Degradation of Thebaine.*—(*a*) *Methebenine* (XVI; R = Me) *hydrochloride.* Methanol was saturated at room temperature with gaseous hydrogen chloride and part (1·5 ml.) was diluted with methanol (1·5 ml.). Thebaine (0·6 g.) was dissolved in this solution and heated in an evacuated sealed tube at 100° for 3 hr. The crystals of methebenine hydrochloride which separated from the solution at 0° (413 mg.) had m. p. 228—231° (from ethanol) (Found: C, 65·4; H, 6·3. Calc. for $C_{19}H_{21}ClNO_3$: C, 65·6; H, 6·4%).

(*b*) (i) N,O-*Dimethylmethebenine methosulphate.* Aqueous sodium hydroxide (30% w/v; 0·6 ml.) was added to a solution of methebenine hydrochloride (395 mg.) in water (1·5 ml.) and followed immediately by the addition of dimethyl sulphate (0·6 ml.). The mixture was shaken vigorously until a homogeneous solution was formed and from the cooled solution the methosulphate crystallised (325 mg.), m. p. 273—275°, though this is variable and m. p.s at 225—228° have been observed (Found: C, 59·6; H, 6·7. Calc. for $C_{23}H_{31}NO_7S$: C, 59·3; H, 6·7%).

(ii) (With D. M. Foulkes.) An alternative route involves the conversion [21] of thebaine into thebenine (XVI; R = H) hydrochloride. A solution of this (20 mg.) in water (0·2 ml.) was treated with aqueous sodium hydroxide (30% w/v; 0·2 ml.) followed immediately by dimethyl sulphate (0·2 ml.). After a homogeneous solution formed, a further portion (0·2 ml.) of dimethyl sulphate was added and the mixture was heated at 40° to effect solution. The crystals which separated from the cooled solution were recrystallised from ethanol–ether to give the methosulphate (17 mg.), m. p. 225—228° (Found: C, 59·4; H, 6·8%). This sample underwent degradation to the 5-vinylphenanthrene as for the material prepared by the other route above.

(*c*) 3,4,8-*Trimethoxy-5-vinylphenanthrene.* The foregoing methosulphate (0·3 g.) was degraded by Gulland and Virden's method [22] to yield the phenanthrene (170 mg.), m. p. 120—121° (lit.,[22] 121°) (Found: C, 77·4; H, 6·1. Calc. for $C_{19}H_{18}O_3$: C, 77·5; H, 6·2%). Hydrogenation yielded the known [22] 5-ethylphenanthrene (XIX), m. p. 112—113° (lit.,[22] 112—113°).

(*d*) *Oxidation of the Vinylphenanthrene.* Ozonised oxygen was passed for 10 min. through a solution of the foregoing 5-vinylphenanthrene (80 mg.) in ethyl acetate (5 ml.) at −78°. The solvent was evaporated and water (20 ml.), zinc dust (0·2 g.), and a crystal (*ca.* 10 mg.) of silver nitrate were added to the residue. After the mixture had been heated under reflux for 20 min., the aqueous solution was distilled into a solution of dimedone (0·2 g.) in aqueous alcohol (3 : 1) (50 ml.). Formaldehyde dimethone (36 mg.) separated and was recrystallised from ethanol, m. p. 193—194° (Found: C, 70·1; H, 8·1. Calc. for $C_{17}H_{24}O_4$: C, 69·8; H, 8·3%). Under the same conditions, the foregoing 5-ethylphenanthrene yielded no formaldehyde dimethone.

Powdered potassium permanganate (0·1 g.) was added to a solution of the 5-vinylphenanthrene (78 mg.) in acetone (5 ml.) and the mixture was shaken at room temperature for 18 hr. The solids were extracted (Soxhlet) with acetone and the combined acetone solutions were evaporated to yield a gum (24 mg.). This was fractionated on neutral alumina in benzene–chloroform (4 : 1) and the first band yielded 3,4,8-trimethoxyphenanthrene-5-aldehyde, m. p. 157—159° (from ethanol) (lit.,[22] 151°) (Found: C, 73·0; H, 5·4. Calc. for $C_{18}H_{16}O_4$: C, 73·0; H, 5·45%) $\nu_{max.}$ 1690 cm.$^{-1}$ (Ar·CHO).

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