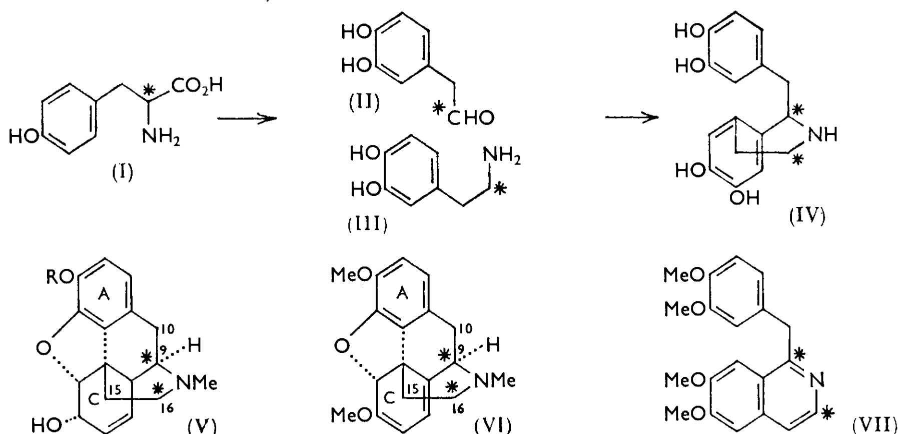
692. *Alkaloid Biosynthesis. Part II.* The Biosynthesis of Morphine.*

By A. R. BATTERSBY, R. BINKS, and B. J. T. HARPER.

DL-[2-^{14}C]Tyrosine fed to *Papaver somniferum* plants affords radioactive morphine which is shown by unambiguous degradation to be specifically labelled at positions 9 and 16. DL-[2-^{14}C]Phenylalanine is incorporated much less efficiently. The tracer results are interpreted as showing that the morphine skeleton is built from two C_6–C_2 units (Ar–C–C) derivable in the plant from tyrosine.

Recent comments on biosynthetical studies which make use of labelled precursors more complex than carbon dioxide are discussed.

FULL reviews are available [1,2] of the researches which led to the suggestion of structure (V; R = H) for morphine by Gulland and Robinson [3] in 1925. The validity of this structure has been established by two independent syntheses of the molecule [4,5] and by *X*-ray analysis [6] of morphine hydriodide. Stork [2] has surveyed the evidence for the relative and absolute stereochemistry shown in structure (V).



Many schemes have been suggested [7,8] for the biosynthesis of morphine and all save one [8] are based upon Gulland and Robinson's recognition of a close structural relation between the benzylisoquinolines and the morphine skeleton.[3] Thus, oxidative coupling [7] of the two aromatic rings in the tetrahydroxybenzylisoquinoline (IV) (known as norlaudanosoline), or some derivative thereof, could afford the morphine (V; R = H), codeine (V; R = Me), and thebaine (VI) systems. It must be stressed that the conversion

* Part I, preceding paper.

[1] Holmes, " The Alkaloids," ed. Manske and Holmes, Academic Press, New York, 1952, Vol. II, p. 2; Bentley, " The Chemistry of the Morphine Alkaloids," Clarendon Press, Oxford, 1954.

[2] Stork, " The Alkaloids," ed. Manske and Holmes, Academic Press, New York, 1952, Vol. II, p. 171; Stork, *ibid.*, 1960, Vol. VI, p. 233.

[3] Gulland and Robinson, *Mem. Proc. Manchester Lit. Phil. Soc.*, 1925, **69**, 79.

[4] Gates and Tschudi, *J. Amer. Chem. Soc.*, 1952, **74**, 1109; 1956, **78**, 1380.

[5] Elad and Ginsburg, *J. Amer. Chem. Soc.*, 1954, **76**, 312; *J.*, 1954, 3052.

[6] Mackay and Hodgkin, *J.*, 1955, 3261.

[7] (*a*) Robinson, " The Structural Relations of Natural Products," Clarendon Press, Oxford, 1955, and refs. therein; (*b*) Schöpf, *Naturwiss.*, 1952, **39**, 241; (*c*) Bentley, *Experientia*, 1956, **12**, 251; (*d*) Barton and Cohen, " Festschrift Arthur Stoll," Birkhäuser, Basle, 1957, p. 117; (*e*) Stork, " The Alkaloids," ed. Manske, Academic Press, New York, 1960, Vol. VI, p. 242.

[8] Cohen, *Chem. and Ind.*, 1956, 1391.

of norlaudanosoline into morphine is not a single step; indeed, it is probable [7,9,10] that the benzylisoquinoline which undergoes oxidative coupling in the plant is a methylated derivative of norlaudanosoline (IV). These aspects of the coupling step will be considered in future papers and the present one describes a study of simple substances which can act as precursors for the carbon skeleton of morphine. For reasons outlined in the preceding paper, DL-[2-^{14}C]tyrosine was chosen as the main precursor to be tested, and the soundness of this choice is discussed below in the light of recent biochemical knowledge. The illustrated steps from tyrosine (I) to norlaudanosoline (IV) by way of the aldehyde (II) and the amine (III) represent in their simplest form the early suggestions of Winterstein and Trier.[11] If tyrosine can be used by the plant to build morphine essentially as in the above scheme, but not necessarily following it in detail, then the morphine formed should be specifically labelled at the starred positions 9 and 16. An experimental test could be made on any type of poppy containing an appreciable amount of morphine, but, in order that the relationships of the opium alkaloids might be examined, it was necessary to select a poppy variety which yields appreciable amounts of morphine (V; R = H), codeine (V; R = Me), thebaine (VI), and papaverine (VII). Accordingly the total alkaloids of the varieties Noordster, Opiac, Flora, Mahndofor, and Peragis were examined by paper chromatography, and the first had the four bases present in convenient amounts.

The young seed capsules of Noordster poppy plants were injected [12] (summer 1957) with an aqueous solution containing 0·214 mc of DL-[2-^{14}C]tyrosine (I) and after two weeks the plants were worked up for alkaloids. These were fractionated by countercurrent distribution, and a complete separation of morphine, codeine, thebaine, and papaverine was obtained; all four were radioactive. In the Table are shown the amounts of the various fractions isolated, together with the percentage incorporation of activity into each alkaloid; this percentage is based upon crystalline materials purified to constant specific activity. Codeine and thebaine, unlike morphine and papaverine, were purified without dilution, and, because of handling losses on the small scale, the incorporations quoted are minimal values. The Table also includes results from three experiments in 1958 and from one where DL-[2-^{14}C]phenylalanine was the precursor tested. Attention is drawn to the incorporations achieved from tyrosine into total alkaloid (2·2—3·4%) and into pure morphine (0·66—1·7%).

Feeding experiments on *Papaver somniferum* Noordster.

Number of plants	20		5		5		5		20	
Year	1957		1958		1958		1958		1958	
Amount fed	0·214 mc Tyr.		0·05 mc Tyr.		0·05 mc Tyr.		0·50 mc Tyr.		0·2 mc PhA	
	Wt.[a] (mg.)	% incorpn.	Wt.[a] (mg.)	% incorpn.	Wt.[a] (mg.)	% incorpn.	Wt.[a] (mg.)	% incorpn.	Wt.[a] (mg.)	% incorpn.
Total crude alkaloid	646	2·2	471	2·6	455	3·0	298	3·4	1610	0·31
Morphine	144	0·66	201	1·6	205	1·4	174	1·7	800	0·13
Codeine	93	0·08 [d]	30	[c]	[b]	—	[b]	—	[b]	—
Thebaine	130	0·04 [d]	54	[c]	[b]	—	[b]	—	[b]	—
Papaverine	62	0·16	[b]	—	[b]	—	[b]	—	[b]	—

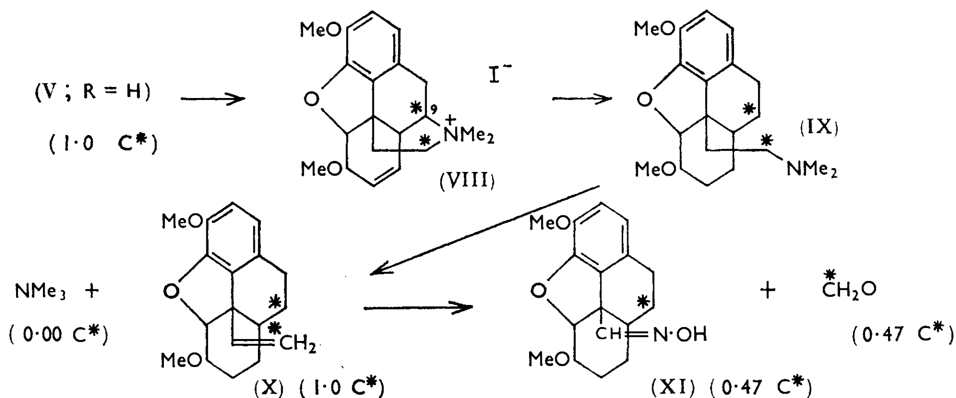
[a] Total weight of base recovered from countercurrent machine. [b] Not isolated. [c] Not further purified. [d] Minimal value.

In early experiments, an aqueous solution of labelled tyrosine was " painted " on to the leaves of poppy plants; there was no incorporation of activity into the alkaloids. Further trials involved mature poppy plants grown in hydroponic solution to which an aqueous solution of DL-[2-^{14}C]tyrosine was added. The activity was taken up over two days and the plants were harvested after ten days; this period covered the blooming phase. Extraction and fractionation of the alkaloids showed that the incorporation of activity

[9] Battersby, *Quart. Rev.*, 1961, **15**, 259.
[10] Battersby, Binks, and McCaldin, unpublished work.
[11] Winterstein and Trier, " Die Alkaloide," Borntraeger, Berlin, 1910.
[12] Cf. Kuzin and Merenova, *Biokhimiya*, 1954, **19**, 616.

into morphine in this case was only 0·01%. This result can be compared with Leete's value [13] of 0·017% incorporation of activity from DL-[2-^{14}C]tyrosine into morphine by the root-feeding method. Thus the technique used to introduce the labelled precursor into the living plant can greatly affect the incorporation achieved.

The isolation of the carbon atom from position-16 of morphine (V; R = H) followed a degradative route, the various steps of which were available in the literature; [14-16] modifications of certain stages are described in the Experimental section. Methylation of the active morphine (V; R = H) gave codeine methyl ether methiodide (VIII). Hofmann elimination cleaved the bond to carbon-9 and the methine was hydrogenated to yield tetrahydrocodeimethine methyl ether (IX). Further Hofmann degradation gave inactive trimethylamine and the morphenol derivative (X) which was degraded by way of the corresponding glycol. This allowed the isolation of formaldehyde from the original position-16 of morphine (V; R = H), together with the rest of the molecule as the oxime (XI). The relative activities of morphine and its degradation products are shown under the formulæ in this scheme and in the following one.

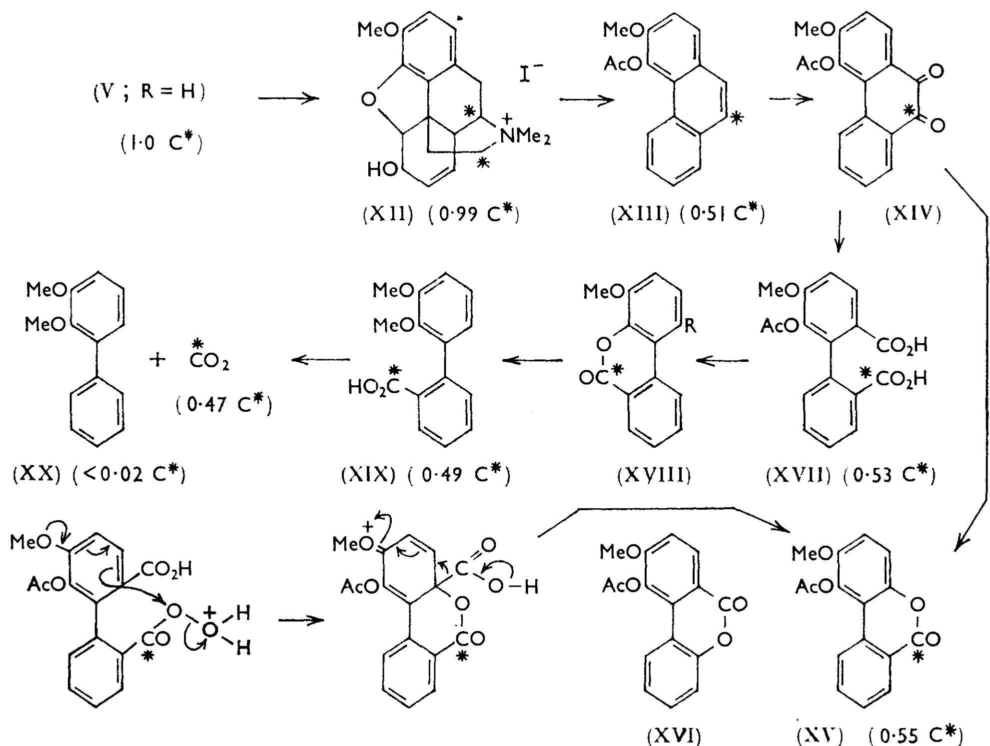


The second degradation required for the isolation of the carbon atom from position-9 involved the conversion of radioactive morphine by known methods [17] into acetylmethylmorphol (XIII), which was isolated by an improved method. Oxidation of this product to the quinone (XIV) had been carried out previously [18] in unstated yield, but in our hands the method was unsatisfactory (15% yield); oxidation by chromium trioxide at 20—40° raised the yield to 30—35%. The further steps to the acid (XIX) were modelled upon the work of Bentley and Robinson [19] on phenyldihydrothebaine. Thus the quinone (XIV) was oxidised by peracetic acid to give mainly the diphenic acid (XVII) and a neutral substance, $C_{16}H_{12}O_5$, as a minor by-product. The latter showed no hydroxyl bands in its infrared spectrum and the shape of the carbonyl band (1755 cm.$^{-1}$, shoulder on low wave-number side) suggested the presence of two carbonyl groups. The by-product can thus be the structure (XV) or the isomer (XVI), and, since the material was radioactive, the proof below of the position of the label shows that the former is correct. Coumarin formation by the action of peracids on diphenic acids has been previously observed [19-21] and the

[13] Leete, *J. Amer. Chem. Soc.*, 1959, **81**, 3948.
[14] Pschorr and Dickhäuser, *Ber.*, 1911, **44**, 2633.
[15] Faltis and Suppan, *Pharm. Monatsh.*, 1923, **4**, 189; Rapoport, *J. Org. Chem.*, 1948, **13**, 714.
[16] Rapoport and Payne, *J. Amer. Chem. Soc.*, 1952, **74**, 2630.
[17] Knorr, *Ber.*, 1894, **27**, 1144; *ibid.*, 1904, **37**, 3494.
[18] Vongerichten, *Ber.*, 1898, **31**, 51.
[19] Bentley and Robinson, *J.*, 1952, 947.
[20] Fieser, *J. Amer. Chem. Soc.*, 1929, **51**, 2471; Adelson, Hasselstrom, and Bogert, *ibid.*, 1936, **58**, 871.
[21] Kenner, Murray, and Tylor, *Tetrahedron*, 1957, **1**, 259.

formation here of coumarin (XV) by attack at the more electron-rich benzene ring supports the view [21] that the reaction is an electrophilic substitution as indicated.

The acidic product (XVII) from the oxidative cleavage was initially free from the coumarin (XVIII; R = CO_2H) as shown by infrared spectral measurements, but ring-closure to this product gradually occurred during manipulation. This change was more conveniently carried out by alkaline hydrolysis of the acid (XVII) followed by heating of



the acidified solution. The resultant coumarin was decarboxylated when warmed with concentrated sulphuric acid to afford the neutral coumarin (XVIII; R = H). This same product was formed when the acid (XVII) was treated with concentrated sulphuric acid and the shorter route was used for the degradation of radioactive material. Hydrolysis of the coumarin (XVIII; R = H) and methylation released the required carboxyl group to give the acid (XIX) which was then decarboxylated over copper chromite in boiling quinoline. Carbon dioxide corresponding to the original position-9 of morphine was thus isolated. The main part of the molecule (XX) was identical with a synthetic sample of 2,3-dimethoxybiphenyl kindly supplied by Dr. Bruce.[22]

Leete also studied the incorporation of activity from DL-[2-^{14}C]tyrosine into morphine concurrently with the above work,[23] and his degradative results [13,24] and ours are in full agreement.

Our two degradations above show that the radioactive morphine is labelled specifically and about equally at positions 9 and 16; the ratio of activities can differ only slightly from 1 : 1. Not more than a trace of activity is present in the rest of the molecule and we

[22] Bruce and Sutcliffe, *J.*, 1955, 4435.
[23] For preliminary communications see Battersby and Harper, *Chem. and Ind.*, 1958, 364; Battersby, Binks, and Le Count, *Proc. Chem. Soc.*, 1960, 287.
[24] Leete, *Chem. and Ind.*, 1958, 977.

wish to emphasise this finding of specificity in the labelling pattern. These results are interpreted as showing that the C_{16}-skeleton of morphine is built from two aromatic C_6–C_2 units (Ar–C–C) produced by the shikimic acid–prephenic acid pathway [25] and which can be derived from tyrosine in the living system. The reasons for this interpretation are discussed in the preceding paper and what is said there, including the proviso about C_6–C_3 units, holds equally well here. The low incorporation of activity from phenylalanine into morphine found here (see Table) agrees with Leete's result [13] and is understandable since this amino-acid is known to be converted poorly into tyrosine in higher plants.[26]

Discussion

Some of the foregoing results and those of other workers in this field [13,29] have recently been discussed by Rapoport and his co-workers [27,28] and they were led to a viewpoint which differs from ours. The following relevant points were made to support their case: (*a*) The incorporation of activity from tyrosine into morphine was said to be low and the values 0·017% [13] and 0·23% [29] were quoted. These were considered [27] to be " more in line with what might be expected from a minor precursor or even through an aberrant synthesis." (*b*) Degradation of morphine isolated from poppy plants which had taken up ^{14}C-carbon dioxide showed that the total activity in the eight carbon atoms (six of ring A, and positions 9 and 10) (V; R = H) was about twice that in the other eight skeletal carbons.[28] The plants were supplied for six hours with unnaturally high concentrations of labelled carbon dioxide in air and were then transferred to normal air for a further 5—10 days. Since the incorporation of activity from tyrosine affords morphine which is effectively equally labelled in the two " halves," it was suggested [28] that either this " may be the result of long-term feeding or tyrosine incorporation may represent only a minor or aberrant biosynthetic pathway."

These two points will be considered in turn. (*a*) Tyrosine occurs free in the latex of *P. somniferum* plants and is present both free and bound in other parts of the poppy.[30] In our experiments, [^{14}C]tyrosine was added to this natural tyrosine supply by injecting it as an aqueous solution directly into the capsule and into the released latex. The amount fed to each large plant (3—4 ft. tall) was in trace quantity (0·2 mg. per injection corresponding to 7·7 μg. of L-tyrosine nitrogen; two injections were given, two days apart). In order to carry out an " aberrant synthesis " of labelled morphine, the plant would have to select labelled C_6–C_2 and/or C_6–C_3 units and use them for a synthesis not normally carried out from the plant's natural supplies of these intermediates. Further, if aberrant synthesis is suggested for morphine in *P. somniferum* then it must be considered for the many other tracer studies on biosynthesis in plants [9,26] and in different living systems (*e.g.*, see refs. 3—8, Part I). Yet the general experience has been that the normal natural product is produced in these tracer experiments and, moreover, is *specifically* labelled. The combined evidence against aberrant synthesis seems to be very strong.

Discussion of whether the use of two Ar–C–C units derivable from tyrosine is a minor route to morphine depends partly upon the level of incorporation achieved and partly upon the way in which it occurs. The opinion [27] that incorporation of activity from tyrosine is low was based upon two percentages reported by other workers [13,29] whereas the values reported here are much higher. Our conclusions concerning the biosynthesis of morphine (above) can be further supported by collecting three sets of incorporation values: from

[25] Davis, *Arch. Biochem. Biophys.*, 1958, **78**, 497; Sprinson, *Adv. Carbohydrate Chem.*, 1960, **15**, 235.
[26] Gamborg and Neish, *Canad. J. Biochem. Physiol.*, 1959, **37**, 1277; Neish, *Ann. Rev. Plant Physiol.*, 1960, **11**, 55.
[27] Stermitz and Rapoport, *J. Amer. Chem. Soc.*, 1961, **83**, 4045; cf. *Nature*, 1961, **189**, 310.
[28] Rapoport, Levy, and Stermitz, *J. Amer. Chem. Soc.*, 1961, **83**, 4298.
[29] Kleinschmidt and Mothes, *Z. Naturforsch.*, 1959, **14*b***, 52.
[30] Kleinschmidt, *Planta medica*, 1960, **8**, 114.

[^{14}C]carbon dioxide into the skeleton of morphine [27,31] 0·031—0·14%,* from DL-[2-^{14}C]-tyrosine into morphine 0·66—1·7%, from thebaine (VI), in which the main skeleton is already built, into codeine (V; R = Me) and morphine (V; R = H), 7·5% and 5·0%, respectively.[27]

Finally, if the two units derivable from tyrosine are built into morphine by the normal biosynthetical route, the activity would be expected to pass through the pools of compounds which immediately precede morphine in the same way as occurs from [^{14}C]carbon dioxide. In fact, incorporation of activity from labelled tyrosine [32] occurs first into thebaine (VI), next into codeine (V; R = Me), and last into morphine (V; R = H); the same rate-pattern has been demonstrated from carbon dioxide.[31]

(*b*) For the second point, it is necessary to show that a scheme, based upon the knowledge that two Ar–C–C units are used by the plant to construct morphine, will also serve to explain the results from the experiments with carbon dioxide. Such a scheme below shows the main aromatic compounds which probably lie on the pathway between carbon dioxide and morphine; all the arrows can represent a few or many stages.† There is direct evidence to prove that almost all the conversions shown can occur in higher plants and for the rest there is ample biochemical analogy,[25,33,34] particularly from the work of Davis, Sprinson, and Neish. For example, the formation of dopamine (XXIII) by decarboxylation of the corresponding amino-acid is based upon similar conversions firmly established in higher plants [35] and in other living systems.[36] Also, the co-occurrence of phenethylamines with isoquinolines in several plants [37] gives some support to the view that the former are precursors of the latter.[7a,38] 3,4-Dihydroxyphenylpyruvic acid (XXII) and the amine (XXIII) could be used to form the benzylisoquinoline (XXIV) with a decarboxylation step at some stage, and this base could then be converted, by way of thebaine and codeine,[27,31,32] into morphine. The biosynthesis of the benzylisoquinoline papaverine (VII) from two Ar–C–C units is described in Part I. The above scheme represents a working hypothesis to be modified if necessary as more tracer results become available; moreover, slight variations of it will also account for the experimental results.

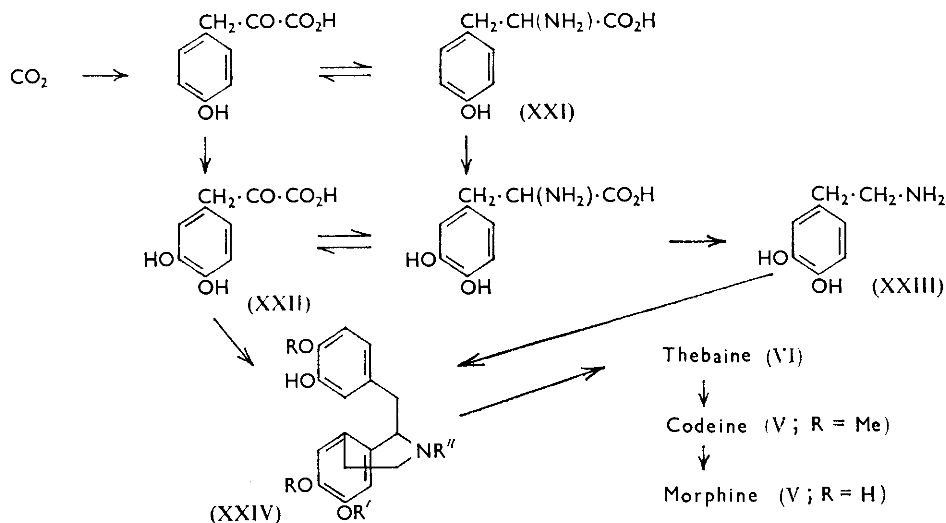
The coupling step and the nature of the groups R, R′, and R″ in (XXIV) will be discussed in Part IV. Our present concern is with the formation of the benzylisoquinoline (XXIV) and with the rate at which the specific activities of the various pools of intermediates will change after labelled material has been absorbed by the plant. All the evidence suggests that in mature *P. somniferum* plants the turnover rate of at least some of the pools preceding morphine is fairly slow. This is supported by the time taken to achieve maximum incorporation of activity from tyrosine into morphine [32] (5—11 days), by the estimated turnover rate of thebaine [27] (1—2 days), by the minute incorporation of activity into the morphine skeleton ($0{\cdot}93 \times 10^{-3}$%) after a 6 hr. metabolic period [31] (cf. longer periods above), and by analogy with rate studies on secondary plant products in other higher plants.[34,39] Thus, in the experiments using [^{14}C]carbon dioxide activity will pass gradually along the scheme and the early intermediates will become highly

* The results from feeding experiments lasting a few hours have not been included; these give much lower incorporations, *e.g.*, $0{\cdot}41 \times 10^{-4}$% into the morphine skeleton for a 2 hr. experiment.[27]

† For simplicity, the many other relationships involved are not shown. Thus, the pools of phenylpropanoid compounds and possibly the 3,4-dihydroxyphenethylamine (XXIII) are being drawn upon to provide materials for many plant products such as lignin, pigments, and protein.[26]

[31] Rapoport, Stermitz, and Baker, *J. Amer. Chem. Soc.*, 1960, **82**, 2765.
[32] Battersby and Harper, *Tetrahedron Letters*, 1960, No. 27, 21.
[33] Weinstein, Porter, and Laurencot, *Nature*, 1959, **183**, 326.
[34] McCalla and Neish, *Canad. J. Biochem. Physiol.*, 1959, **37**, 531.
[35] Leete, Kirkwood, and Marion, *Canad. J. Chem.*, 1952, **30**, 749; Leete and Marion, *ibid.*, 1953, **31**, 126; Massicot and Marion, *ibid.*, 1957, **35**, 1; Leete, *Chem. and Ind.*, 1959, 604.
[36] Senoh, Creveling, Udenfriend, and Witkop, *J. Amer. Chem. Soc.*, 1959, **81**, 6236, and refs. therein.
[37] *E.g.*, Reti, " The Alkaloids," ed. Manske and Holmes, Academic Press, New York, 1954, Vol. IV, pp. 7—28.
[38] Manske, *J.*, 1954, 2987.
[39] Brown, Towers, and Wright, *Canad. J. Biochem. Physiol.*, 1960, **38**, 143.

labelled before the later ones. During the first part of the metabolic period, material at a high level of specific activity could be drawn from the pool of the ketone (XXII) to be combined with material of low specific activity from the pool of dopamine (XXIII). The ratio of activities in the two " halves " of morphine produced at this stage could therefore



be much greater than 2 : 1. The ratio of activities should fall with time as the pool of the amine (XXIII) increases in specific activity, and the result found of 2 : 1 (see above) is in keeping with these considerations. Several other explanations of the 2 : 1 ratio could be devised weighing the effects of pool sizes, free and bound forms of intermediates, and the use of intermediates for other biosyntheses, all of which are important.[40] With present knowledge it is not possible to select one of these explanations as the best and our aim has been to indicate that the results from the experiments with carbon dioxide are not incompatible with ours.

In considering the work with labelled tyrosine, it must be made clear that incorporation of activity into morphine does not mean that two identical units are involved in the step which produces the benzylisoquinoline system. Indeed, we have repeatedly stressed (*e.g.*, ref. 9) that it is not possible on present evidence to distinguish between tyrosine and its close biological relatives, and the interpretation of our results takes account of this fact. All biosynthetical schemes [7,11a] have included suggestions as to the probable nature of the *different* molecules involved in building the system (XXIV); for it is obvious on simple structural grounds that modification of tyrosine must occur. Radioactive tyrosine introduced into the plant lies on the above scheme between, and two stages away from, each of the units (XXII) and (XXIII) which are considered to form the benzylisoquinoline (XXIV). Given suitable rates and pool sizes the ratio of 1 : 1 found above for the activities in the two " halves " of morphine (V; R = H) can be explained; this ratio is fixed by the ratio established in the benzylisoquinoline precursor. Alkaloids which show different levels of activity in two units derived from labelled tyrosine are now being found, *e.g.*, in hydrastine [40] and in cephæline,[41] and these results are doubtless reflections of the effects of pool sizes, rates of turnover, etc. (cf. ref. 40).

Thus, our conclusion is that morphine is biosynthesised from two Ar–C–C units and it is argued that the results reported by Rapoport and his co-workers [27,28] are not in conflict with this view.

[40] Gear and Spenser, *Nature*, 1961, **191**, 1394.
[41] Battersby and Binks, unpublished work.

EXPERIMENTAL

For general directions see Battersby and Harper.[42] The counting equipment described there was used for part of the work and the rest was carried out using scaler N530F, probe unit N558, and shielded Geiger–Müller tube holder N620 (Ekco Electronics Limited). The efficiencies of the two counters were correlated by using standard samples.

Cultivation of Papaver somniferum *Plants and Administration of Labelled Precursors.*—(a) *Hydroponic work.* The seeds were sown early in March in shallow trays containing a 1 : 1 mixture of sand and sieved soil, and after 6—8 weeks the seedlings were transplanted into sand in pots where they remained until a flower bud was visible. The plants were fed throughout this period by being watered with nutrient solution [43] which was modified by replacing the ferrous sulphate of the original mixture with ferric potassium ethylenediaminetetra-acetate (10 parts per million). Before the plants were transferred to glass jars containing the same nutrient solution (500 ml. per plant), the root system was washed gently with water to remove the sand. The plants remained healthy in hydroponic culture and produced many new roots. When the plants flowered (July), an aqueous solution of 0·11 mc of DL-[2-^{14}C]tyrosine (21·8 mg.) was divided equally between the nutrient solutions of eleven plants and the activity was shown to be largely removed from the solution after 2 days. The plants were harvested after 10 days.

(b) *Injection of precursors.* The seeds of *P. somniferum*, variety Noordster, were sown early in March in good soil in a sheltered garden. Protection against rain was provided during the feeding experiments by a cover of thin Polythene sheet stretched over metal frames. A solution of DL-[2-^{14}C]tyrosine (0·214 mc; 9·14 mg.) in water (20 ml.) was administered to 20 plants by injecting portions (0·5 ml.) into the capsule at petal fall with a fine hypodermic needle. Two days later a second injection (0·5 ml.) was made and the plants were harvested 2 weeks later. The 1958 experiments reported in the Table were carried out in the same way.

DL-[2-^{14}C]Phenylalanine (0·2 mc; 13·8 mg.) in water (5 ml.) was similarly fed to 20 Noordster plants which were harvested 15 days after injection.

Chromatography of Opium Alkaloids.—The upper layer of the solvent system n-butanol–ethanol–water (40 : 11 : 30 by vol.) used in descending chromatography on Whatman No. 1 paper gave good separations of morphine, codeine, and thebaine (R_F 0·54, 0·60, 0·72, respectively). Narcotine and papaverine overlapped (R_F 0·89) but were well separated from the rest. A mixture of ethyl acetate (12 vol.), acetic acid (2 vol.), and water (2 vol.) gave a greater separation of morphine (R_F 0·26) from the other alkaloids (R_F: codeine, 0·45; thebaine, 0·68; papaverine, 0·75; narcotine, 0·81). The bases were detected with Dragendorff's reagent.[44]

Extraction and Separation of Alkaloids.—Batches of 5 plants were worked up. The whole plants were cut up finely in a rotatory bean slicer or, in the later experiments, by maceration with water in a Waring blender, and then packed into a glass column. Aqueous acetic acid (5% by vol.; 5—6 l.) was percolated through the column at room temperature during 2—3 days. The percolate from 5 plants (or the combined percolate from up to 4 batches) was evaporated to *ca.* 150 ml., diluted with water (500 ml.), and acidified with concentrated hydrochloric acid (25 ml.). Extraction with 3 : 1 (by vol.) ether–chloroform (3 × 1 l.; back-washed with 3 × 50 ml. of water) removed the neutral material, and the combined aqueous solution was adjusted to pH 9 with sodium carbonate and was then extracted with ethyl acetate (1 × 2 l., 2 × 1 l.; back-washed with water 3 × 50 ml.). Evaporation of the dried ethyl acetate extracts gave the total alkaloids as a gum (see Table for weights).

The total bases (1957 experiment, 646 mg.) were fractionated by countercurrent distribution (scattered in first 5 tubes; 10 ml. phases) for 95 transfers between ethyl acetate and phosphate buffer [0·5M-KH_2PO_4 (1 vol.) and 0·5M-K_2HPO_4 (1 vol.)]. Analysis showed morphine and codeine in tubes 0—25, thebaine in tubes 50—80, and the weak bases (narcotine, papaverine, etc.) in tubes 81—99; the contents of tubes 50—80 and 81—99 were removed and reserved (see below). After the empty tubes had been refilled with the solvent system the fractionation was continued for a further 200 transfers to separate morphine (tubes 1—25) from codeine (tubes 26—55).

[42] Battersby and Harper, preceding paper.
[43] Leete, Marion, and Spenser, *Canad. J. Chem.*, 1954, **32**, 1116.
[44] Munier and Macheboeuf, *Bull. Soc. Chim. biol.*, 1949, **31**, 1144.

The bases were recovered from each of the four fractions obtained above by adjustment of the aqueous layer to pH 9 with sodium carbonate followed by extraction first with the upper layer from the same tubes and then twice with fresh ethyl acetate. For morphine, dipotassium hydrogen phosphate was added to the aqueous layer (3 g. per 20 ml.) before the pH was adjusted. Evaporation of the dried extracts gave gums (see Table for weights).

Purification of Alkaloids to Constant Specific Activity.—(a) *Morphine.* A solution of the active morphine fraction (144 mg.) and pure inactive morphine (600 mg.) in ethanol (30 ml.) was treated with picric acid (651 mg.), and the picrate was recrystallised twice to yield morphine picrate (1·18 g.), m. p. 160—161° (decomp.); the specific activity was unchanged by the second recrystallisation.

(b) *Codeine.* Picric acid (0·1 g.) was added to a solution of the active codeine fraction (93 mg.) in ethanol (5 ml.), and after the solution had been concentrated the crystals (57 mg.) were collected and recrystallised from 1 : 1 aqueous ethanol without change of specific activity; they had m. p. 194° (decomp.).

(c) *Thebaine.* The thebaine fraction (130 mg.) in ethanol (10 ml.) was treated with picric acid (110 mg.), and the solution was concentrated to afford thebaine picrate (100 mg.) which did not change in specific activity on recrystallisation twice from methanol; this had m. p. 215—216° (decomp.).

*Degradation of Morphine to Isolate the Position-*16 *Carbon.*—A solution of the above morphine picrate (596 mg.) in water (60 ml.) and 2N-hydrochloric acid (30 ml.) was extracted continuously with ether for 6 hr. The ether extract was evaporated and the residue was dissolved in aqueous acid as above before being re-extracted continuously with ether until free from picric acid. Dipotassium hydrogen phosphate (24 g.) was added to the combined aqueous solutions (250 ml.) which were then adjusted to pH 9 with sodium carbonate and extracted with ethyl acetate (4 × 500 ml.). Evaporation of the extracts gave morphine base (328·1 mg.), most of which (321·3 mg.) was diluted further with inactive morphine (461·9 mg.) (Found, in diluted material: $14{\cdot}0 \times 10^{2}$ counts).

The foregoing morphine (783 mg.) was methylated by Pschorr and Dickhäuser's method [14] to yield codeine methyl ether methiodide which was subjected to Hofmann degradation.[14] A solution of the resultant α-codeimethine methyl ether (512 mg.), m. p. 93° (lit.[14] m. p. 94°), in ethanol (20 ml.) was shaken at room temperature and pressure (cf. ref. 15) with hydrogen and platinum oxide (73 mg.); uptake, 1·03 mol. The catalyst was filtered off, the filtrate was evaporated to dryness, and the gum was redissolved in ethyl acetate (20 ml.). This solution was saturated with dry hydrogen chloride and then concentrated to yield *tetrahydrocodeimethine methyl ether hydrochloride* (as IX; 565 mg.), m. p. 234—235° (Found, in material dried at 110°: C, 65·2; H, 8·1; N, 3·7. $C_{20}H_{30}ClNO_3$ requires C, 65·3; H, 8·2; N, 3·8%).

The free base recovered as usual from all the foregoing hydrochloride was converted [15] into its methiodide (650 mg.), m. p. 249—251° (lit.[15] m. p. 246—248°), which was degraded [15] to 6-methoxy-13-vinyloctahydromethylmorphenol (X; 294 mg.) and trimethylamine, which was trapped as the picrate, m. p. 217—218° (Found: 0·0 counts). The neutral fragment (X) was distilled at 160° (bath)/0·1 mm. for combustion (Found: $14{\cdot}1 \times 10^{2}$ counts). Part (110 mg.) of the remainder was converted by Rapoport and Payne's method [16] into the corresponding glycol (58 mg.), m. p. 158—159° (lit.[16] m. p. 157—158°). This was dissolved in ethanol (4 ml.) and a solution of sodium metaperiodate (56 mg.) in water was added, followed by saturated aqueous sodium hydrogen carbonate (2 ml.). After being kept at room temperature for 3 hr. the solution was treated dropwise with a saturated aqueous solution of lead nitrate until precipitation ceased. The solids were collected by centrifugation and were washed with water (2 × 5 ml.). The combined aqueous solution was distilled to half volume and the distillate was run directly under the surface of a solution of dimedone (0·3 g.) in ethanol (8 ml.) and water (20 ml.) contained in a Dreschel bottle; the solution in the distillation flask was then made up to the original volume with water and the process was repeated. Collection of the precipitate (46 mg.) from the Dreschel bottle, and recrystallisation from aqueous ethanol, gave formaldehyde dimethone, m. p. 191—192° (Found: $6{\cdot}52 \times 10^{2}$ counts). The suspension in the distillation flask was extracted with ether (3 × 100 ml.) and the ether dried and evaporated to leave a gum (49 mg.). This was converted into its oxime (30 mg.) as originally done; [16] it had m. p. 118—119° (lit.[16] m. p. 118—120°) (Found: $6{\cdot}57 \times 10^{2}$ counts).

*Degradation of Morphine to Isolate the Position-*9 *Carbon.*—(a) *Acetylmethylmorphol* (XIII). Radioactive morphine (2·05 g.; found $7{\cdot}9 \times 10^{3}$ counts) was prepared as above by recovery

from the picrate and dilution with inactive alkaloid. This was converted [17] into codeine methiodide (2·54 g.; Found, 7·8 × 10^3 counts), m. p. 268—270° (decomp.) (lit.[17] m. p. 270°, decomp.) which was degraded [17] to α-codeimethine (1·34 g.), m. p. 118·5° (lit.[17] m. p. 118·5°). Fission to yield crude methylmorphol (370 mg.) was carried out by Knorr's method [17] and this product was heated on the steam-bath for 2 hr. with acetic anhydride (3 ml.) and anhydrous sodium acetate (0·15 g.). The excess of anhydride was evaporated and the residue was partitioned between water and ether–chloroform (3 : 1); evaporation of the organic extracts gave a semi-crystalline mass. This, in benzene (5 ml.), was run on to a column (15 cm. × 2 cm.) of silica gel which was eluted with benzene–light petroleum (b. p. 60—80°) (3 : 1 by vol.). A trace of oil was eluted before crystalline acetylmethylmorphol (375 mg.; Found, 4·0 × 10^3 counts), m. p. 128—129° (lit.[17] m. p. 131°), $\nu_{max.}$ 1760 $cm.^{-1}$ (OAc).

(b) 8-*Methoxy*-3,4-*benzocoumarin* (XVIII; R = H). A solution of acetylmethylmorphol (363 mg.) in acetic acid (3·75 ml.) at 40° was treated dropwise during 5 min. with a solution of chromium trioxide (0·538 g.) in water (0·47 ml.) and acetic acid (0·47 ml.). After being kept at 20° for 16 hr. the suspension was warmed to 40° and again kept at 20° for a further 28 hr. Water (25 ml.) was then added, the solution was extracted with chloroform (5 × 50 ml.), and after the combined extracts had been extracted with 0·5M-sodium hydrogen carbonate (2 × 100 ml.) they were dried and evaporated. A solution of the residue (0·222 g.) in benzene (100 ml.) was run on to a column of silica gel (100 g.), the elution being continued with chloroform–benzene (1 : 4 by vol.). The main fraction (175 mg.) crystallised from benzene to give the quinone (XIV) as orange needles (117 mg.), m. p. 214—216° (lit.[18] m. p. 205—207°), $\nu_{max.}$ 1765 $cm.^{-1}$ (OAc), 1692, 1671 $cm.^{-1}$ (2 >CO) (Found: C, 68·5; H, 4·0. Calc. for $C_{17}H_{12}O_5$: C, 68·9; H, 4·1%).

Hydrogen peroxide (0·3 ml.; 100 vol.) was added to a solution of the quinone (117 mg.) in acetic acid (3 ml.), and the solution was heated at 80—90° for 1·5 hr. before more hydrogen peroxide (0·3 ml.) was added. After the solution had been heated for a further 2 hr., it was diluted with water (6 ml.), and the precipitated crystals were collected (14·5 mg.). Recrystallisation from aqueous acetic acid gave 5-*acetoxy*-6-*methoxy*-3,4-*benzocoumarin* (XV), m. p. 223—224° (Found: C, 67·8; H, 4·4. $C_{16}H_{12}O_5$ requires C, 67·6; H, 4·3%) (Found: 4·35 × 10^3 counts).

Palladium black (50 mg.) was added to the filtrate from the coumarin (XV) and when effervescence ceased the solution was filtered and evaporated to dryness. A solution of the residue in ~N-potassium carbonate was extracted thrice with ether, acidified, and then re-extracted thrice with ether. Evaporation of the latter extracts yielded the crystalline acid (XVII) (96 mg.), m. p. 181—183°, $\nu_{max.}$ 1770 (OAc) and 1690 $cm.^{-1}$ (broad, 2Ar·CO_2H), no band at 1745 $cm.^{-1}$ where the coumarin (XVIII; R = CO_2H) absorbs strongly (Found: 4·2 × 10^3 counts). Trial experiments with inactive material showed that recrystallisation of this product from aqueous ethanol gave mixtures. Fractional crystallisation of the high-melting crops gave a solid *A*, m. p. 261°, which is identified under (c) below.

A solution of the diphenic acid above (55·2 mg.) in concentrated sulphuric acid (1·85 ml.) was heated at 68° for 30 min., then cooled, diluted with water (25 ml.), and extracted with ether (4 × 25 ml.). The combined ethereal solution was shaken with 0·5M-sodium carbonate (3 × 10 ml.), dried, and evaporated. Recrystallisation of the residue (21 mg.) from ethanol gave 8-*methoxy*-3,4-*benzocoumarin* (XVIII; R = H) as needles (17·2 mg.), m. p. 168—169° $\nu_{max.}$ 1735 $cm.^{-1}$ (>CO) (Found: C, 74·5; H, 4·45. $C_{14}H_{10}O_3$ requires C, 74·3; H, 4·45%).

(c) 8-*Methoxy*-3,4-*benzocoumarin*-5-*carboxylic acid* (XVIII; R = CO_2H). The acid (XVII) (0·1 g.) was heated on the steam-bath for 2 hr. with 2N-sodium hydroxide (5 ml.), and the solution was cooled and acidified. A precipitate formed which by infrared analysis was shown to contain a hydroxy-acid, presumably (XVII; H replacing Ac). Slowly at room temperature, or rapidly on heating in aqueous acid or in ethanol, this product yielded 8-*methoxy*-3,4-*benzocoumarin*-5-*carboxylic acid* (XVIII; R = CO_2H) (57 mg.), m. p. 263—265°, $\nu_{max.}$ 1745 (>CO), 1687 $cm.^{-1}$ (Ar·CO_2H) (Found: C, 66·6; H, 3·6. $C_{15}H_{10}O_5$ requires C, 66·7; H, 3·7%).

The benzocoumarin (XVIII; R = CO_2H) was shown to be identical with solid *A* under (b) above.

(d) 2′,3′-*Dimethoxybiphenyl*-2-*carboxylic Acid* (XIX) *and its Decarboxylation*.—8-Methoxy-3,4-benzocoumarin [124·1 mg. containing 13·65 mg. of the carbon-14 sample from (b) above] was suspended in 2N-sodium hydroxide (5 ml.), dimethyl sulphate (0·25 ml.) was added, and the mixture was heated under reflux for 10 min. After 30 min., further portions of dimethyl

sulphate (0·25 ml.) and 2N-sodium hydroxide (2 ml.) were added, and these additions were repeated after 1 hr. and 1·5 hr. Solid sodium hydroxide (0·2 g.) was added after 2 hr., and after 2·5 hr. the solution was cooled, acidified, and extracted with ether (4 × 50 ml.). The combined ethereal solution was extracted with 0·5M-sodium carbonate (3 × 40 ml.) and the aqueous alkaline extracts were acidified and extracted thrice with ether. Evaporation of the dried extracts left a solid (128 mg.) which crystallised from aqueous ethanol to give *2',3'-dimethoxybiphenyl-2-carboxylic Acid* (116 mg.), m. p. 168—169°, $\nu_{max.}$ 1680 cm.$^{-1}$ (Ar·CO_2H) (Found: C, 69·7; H, 5·7. $C_{15}H_{14}O_4$ requires C, 69·75; H, 5·5%) (Found, after allowance for dilution: 3·9 × 10^3 counts).

Suitable all-glass equipment was built to allow the above biphenylcarboxylic acid (98·7 mg.) to be dropped into a suspension of copper chromite (50 mg.) in boiling quinoline (10 ml.) under an atmosphere of nitrogen. The nitrogen stream carried the evolved carbon dioxide into saturated barium hydroxide (freshly filtered); the reaction was complete after 20 min. The barium carbonate was collected and dried as for combustion assay [42] (69·8 mg.; found after allowance for dilution: 3·7 × 10^3 counts).

After the quinoline solution had been filtered, it was dissolved in an excess of hydrochloric acid and this solution was extracted thrice with ether. The ether extracts were washed with 0·5M-sodium carbonate, dried, and evaporated. A solution of the residue (78 mg.) in light petroleum (b. p. 60—80°) was run on to a column (15 cm. × 2 cm.) of silica gel which was eluted with benzene–light petroleum (b. p. 60—80°) (1 : 1 by vol.). This eluted one sharp fraction (59 mg.) which was distilled on a short-path still at 110° (bath)/0·5 mm. to yield 2,3-dimethoxybiphenyl (51 mg.), m. p. and mixed m. p. 47°, infrared spectrum identical with an authentic sample.[22] (Found, after allowance for dilution: $<0{\cdot}16 \times 10^3$ counts.)

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