691. *Alkaloid Biosynthesis. Part I. The Biosynthesis of Papaverine.*

By A. R. Battersby and B. J. T. Harper.

The background to the series of papers is outlined.

It is demonstrated that when DL-[2-^{14}C]tyrosine is fed to mature *Papaver somniferum* plants, radioactive papaverine is biosynthesised. Selective degradation of this alkaloid proves that it is labelled without appreciable randomisation at two positions only. Two Ar–C–C units, derivable in the plant from tyrosine, are thereby shown to be incorporated into papaverine.

Hofmann degradation of laudanosine (VI) yields a mixture of *trans*- and *cis*-stilbene derivatives. Other degradations of papaverine are described.

The alkaloid series displays a rich variety of structural types, and many valuable schemes have been suggested [1,2] to account for the biosynthesis of these molecules. These hypothetical biosynthetical routes have largely been based upon structural relations and upon mild reactions which are known and understood in the laboratory; they serve as working hypotheses for biosynthetical work carried out with living plants. It is remarkable that, though considerable progress had been made by the middle nineteen fifties in elucidating the biosynthetical routes used for several groups of natural products, far less was known about the alkaloids. Thus, the biosyntheses of many phenolic and enolic compounds,[3,4] lipids,[5] steroids and terpenes,[6] carbohydrates,[7] and porphyrins [8] had by that time been established in broad outline and for some cases in fair detail. In contrast, the published knowledge at the outset of the present work (1955) related to the origin of the pyrrolidine rings of nicotine [9] and hyoscyamine [10] and the biosynthesis of hordenine, *N*-methyltyramine, and gramine in barley.[11] The source of *N*- and *O*-methyl groups in several alkaloids was also known.[11]

Our purpose in the researches to be described is the elucidation, by experiments *in vivo*, of the biosynthetical routes to representative examples from the main groups of alkaloids. Some alkaloids outside the major groups have also been included in our study and their selection is due to the particularly interesting biosynthetical problems which they present; colchicine is an example. Many of the selected cases are those for which two or more biosynthetical schemes have been suggested.

The 1-benzylisoquinolines, *e.g.*, norlaudanosoline (IV), have for many years been used in biogenetical speculations as key intermediates for the large group of isoquinoline alkaloids. For example, mechanistically satisfactory schemes have been suggested [1,2] to

[1] See, for example, Robinson, "The Structural Relations of Natural Products," Clarendon Press, Oxford, 1955; Schöpf, *Angew. Chem.*, 1937, **50**, 787, 797; Woodward, *ibid.*, 1956, **68**, 13; Wenkert, *Experientia*, 1959, **15**, 165 and refs. therein.

[2] Manske, *J.*, 1954, 2987; Turner and Woodward, "The Alkaloids," ed. Manske and Holmes, Academic Press, New York, 1953, Vol. III, p. 54; Barton and Cohen, "Festschrift Arthur Stoll," Birkhäuser Verlag, Basel, 1959, p. 117.

[3] Birch, *Fortschr. Chem. org. Naturstoffe*, 1957, **14**, 186; Birch and Smith, *Chem. Soc. Special Pub* No. 12, 1958, p. 1.

[4] Davis and Sprinson, "Symposium on Amino Acid Metabolism," ed. McElroy and Glass, Johns Hopkins, Baltimore, U.S.A., 1955; Davis, *Adv. Enzymol.*, 1955, **16**, 247.

[5] Deuel, "The Lipids, Vol. III: Biochemistry," Interscience, New York, 1957.

[6] Würsch, Huang, and Bloch, *J. Biol. Chem.*, 1952, **195**, 439; Cornforth, Hunter, and Popjak, *Biochem. J.*, 1953, **54**, 597; Woodward and Bloch, *J. Amer. Chem. Soc.*, 1953, **75**, 2023; Cornforth, Cornforth, Pelter, Horning, and Popjak, *Tetrahedron*, 1959, **5**, 311.

[7] Calvin, *J.*, 1956, 1895.

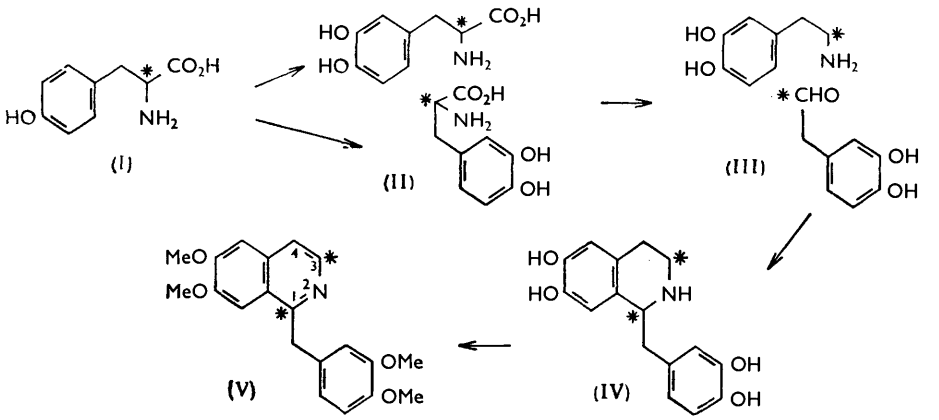
[8] Shemin and Russell, *J. Amer. Chem. Soc.*, 1953, **75**, 4873; Neuberger and Scott, *Nature*, 1953, **172**, 1093; Dresel and Falk, *Nature*, 1953, **172**, 1185.

[9] Leete, *Chem. and Ind.*, 1955, 537; Dewey, Byerrum, and Ball, *Biochim. Biophys. Acta*, 1955, **18**, 141.

[10] Leete, Marion, and Spenser, *Canad. J. Chem.*, 1954, **32**, 1116; Marion and Thomas, *ibid.*, 1955, **33**, 1853.

[11] Reviewed by Marion, *Bull. Soc. chim. France*, 1958, 109.

show how norlaudanosoline (IV) and its simple derivatives might be transformed into alkaloids of the protoberberine, aporphine, morphine–codeine–thebaine, and bisbenzylisoquinoline types, and also into several others. Thus, knowledge of the biosynthesis of a 1-benzylisoquinoline base has, in addition to its intrinsic value, a bearing upon future work in the isoquinoline series. Papaverine (V) was selected as the alkaloid for study and



our experiments were guided by Winterstein and Trier's suggestion [12] that two molecules of 3,4-dihydroxyphenylalanine (II) might be modified in the plant as shown at (III), or in some similar way. The products could then be used to form norlaudanosoline (IV). The further steps of dehydrogenation and methylation are required to give papaverine (V). The present work is concerned with the overall process, and DL-[2-^{14}C]tyrosine (I) was used as the test precursor since it is known [13] that tyrosine can be converted into 3,4-dihydroxyphenylalanine in living systems. The position of the label is marked with an asterisk and, on the basis of the above biosynthetical scheme, the precursor should be converted by the plant into papaverine (V) which is specifically labelled at positions 1 and 3.

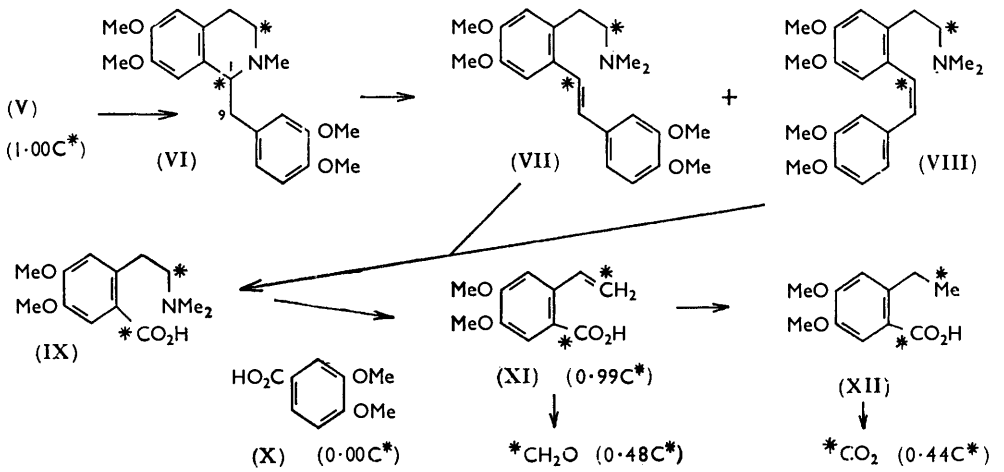
An aqueous solution of DL-[2-^{14}C]tyrosine (I) was injected into mature *Papaver somniferum* plants (variety Noordster) and two weeks later the plants were harvested. Details of the cultivation and feeding of the plants are given in Part II. The radioactive alkaloids were extracted and were separated by countercurrent distribution between ethyl acetate and aqueous acetate buffer; the yields of the alkaloids and the incorporations achieved are collected in the Table in Part II (p. 3535). After dilution of the papaverine fraction with inactive papaverine, it was crystallised to constant activity as the picrate. The total activity incorporated into this alkaloid corresponded to 0·16% of the activity fed. Activity was not lost during the chemical transformations of the active papaverine described below so that the radiochemical purity of the alkaloid is firmly established.*

The most satisfactory method for degrading the active papaverine was determined by experiments on inactive material. Some of the steps involved have been examined previously,[14] but the original methods have been considerably modified or replaced in order to achieve the highest yields. Methylation of papaverine gave the corresponding methiodide which was reduced by borohydride in methanol [15] to (±)-laudanosine (VI) in almost quantitative yield. This was quaternised with methyl iodide and, without

* A preliminary account has been published of part of the main results: Battersby and Harper, *Proc. Chem. Soc.*, 1959, 152.

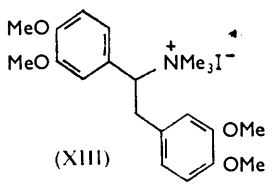
[12] Winterstein and Trier, "Die Alkaloid," Borntraeger, Berlin, 1910, p. 307.
[13] Rosenfeld, Leeper, and Udenfriend, *Arch. Biochem. Biophys.*, 1958, **74**, 252.
[14] Decker and Galatty, *Ber.*, 1909, **42**, 1179; Kondo and Mori, *J. Pharm. Soc. Japan*, 1931, **51**, 615.
[15] Mirza, *J.*, 1957, 4400; Garratt, B.Sc. Thesis, Bristol, 1956.

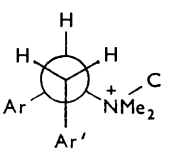
purification, the salt was subjected to Hofmann degradation. One basic product was separated, as its crystalline hydrochloride in 68% yield, and its properties show it to be identical with that obtained earlier.[14] It is now designated *trans*-laudanosinemethine (VII) because its ultraviolet spectrum is very similar to that of *trans*-3,3′,4,4′-tetramethoxystilbene,[16] and its infrared spectrum shows the expected strong band in the 960 cm.$^{-1}$



region. The methiodide of the *trans*-methine also showed this band which was absent in the spectrum of the dihydro-derivative (XV).

The mother liquor from the *trans*-methine (VII) afforded an isomeric base (9% yield) which was converted by catalytic hydrogenation into the same dihydro-derivative (XV) obtained above from the *trans*-isomer. That the new base was *cis*-laudanosinemethine (VIII) was confirmed by its ultraviolet spectrum, which corresponded closely to that of *cis*-3,3′,4,4′-tetramethoxystilbene,[16] and by its infrared spectrum and that of its methiodide, which both lacked strong bands in the 970—960 cm.$^{-1}$ region. Further evidence is adduced below. The formation of a *cis*-stilbene in this Hofmann degradation is somewhat surprising; similar degradation of the salt (XIII) yielded [16] no detectable amount of *cis*-3,3′,4,4′-tetramethoxystilbene. By viewing along the $C_{(9)}$–$C_{(1)}$ bond (see VI) it is clear that the conformation (XIV) for the corresponding quaternary salt, which would be required for *trans*-coplanar elimination to the *cis*-stilbene (VIII), is not a favoured one. The mechanism by which the *cis*-stilbene is formed warrants further investigation so that its relationship to other anomalous elimination reactions [17] may be known.





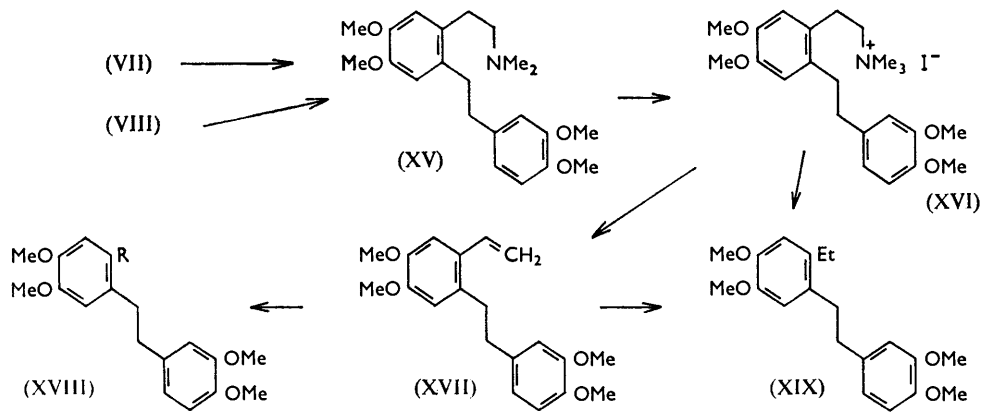
Both *trans*- and *cis*-laudanosinemethines, (VII) and (VIII) respectively, were oxidised by permanganate to veratric acid (X) and an amino-acid (IX) which, without isolation, was degraded by Hofmann's method [14] to the vinylbenzoic acid (XI). The evolved trimethylamine was collected as its picrate.

[16] Battersby and Binks, *J.*, 1958, 4333; Battersby and Greenock, *J.*, 1961, 2592.
[17] *E.g.*, Weinstock and Bordwell, *J. Amer. Chem. Soc.*, 1955, **77**, 6706; Hughes and Maynard, *J.*, 1960, 4087; Hughes and Wilby, *J.*, 1960, 4094; Weygand, Daniel, and Simon, *Chem. Ber.*, 1958, **91**, 1691.

Exploratory work on the degradation of papaverine is shown in the scheme below. Dihydrolaudanosinemethine (XV) was converted into its methiodide (XVI) which was degraded by Hofmann's method. Ozonolysis of the resultant styrene (XVII) gave formaldehyde, and the other product is presumably the aldehyde (XVIII; R = CHO) though no attempt was made to isolate it. Instead, the non-volatile material from the ozonolysis was oxidised by permanganate to the acid (XVIII; R = CO_2H) which was identical with synthetic material of unequivocal structure.[18] Hydrogenation of the styrene (XVII) gave 1-(2-ethyl-4,5-dimethoxyphenyl)-2-(3,4-dimethoxyphenyl)ethane (XIX), also obtained by Emde degradation of dihydrolaudanosinemethine methiodide using sodium in liquid ammonia.[19]

When the radioactive papaverine (V) was degraded according to the above scheme part of the vinylbenzoic acid (XI) so obtained was catalytically hydrogenated to 6-ethylveratric acid (XII), identical with a synthetic specimen.[20] Decarboxylation of the acid (XII) by a Schmidt reaction gave the carbon atom from position-1 of the original papaverine (V) as carbon dioxide which was trapped as barium carbonate. Finally, the carbon atom from position-3 of the papaverine (V) was isolated as formaldehyde (dimedone derivative) by ozonolysis of the remaining vinylbenzoic acid (XI).

The results have been calculated by multiplying the observed counts per 100 sec. for the standard barium carbonate disc (see Experimental) by the number of carbon atoms in the molecule combusted. The figures so obtained are directly proportional to the molar activities, and the degradative scheme (V) ⟶ (XII) shows the relative activity values. The activities at positions 1 and 3 of papaverine differ only slightly from equality, but because of the experimental error (±5%) in determination of activities a ratio of 5 : 6 of the activity at position-1 to that at position-3 is also in keeping with our results. The true ratio thus lies on or between the limits 1 : 1 and 5 : 6. It is obvious by inspection that the tyrosine fed to the plants must be modified on its way to papaverine and probably the two " halves " take different paths (see Discussion in Part II). This could result in different dilutions by endogenous materials so that a ratio of activities differing from 1 : 1



is possible as Gear and Spenser [21] have pointed out for the case of hydrastine. At most, only slightly different dilutions can have occurred in our experiments and further study of papaverine at a much higher level of activity would be required to decide between the very similar ratios given above.

Independently of the finer quantitative aspects, our present results lead to two

[18] Battersby and Binks, *J.*, 1955, 2896.
[19] Clayson, *J.*, 1949. 2016.
[20] Battersby and Openshaw, *J.*, 1949, S 59.
[21] Gear and Spenser, *Nature*, 1961, **191**, 1393.

conclusions. First, the work of Davis, Sprinson, and their co-workers [4,22] on micro-organisms, and of Neish, his co-workers, and others [23] on higher plants, has firmly established that the $C_{(6)}$–$C_{(3)}$ systems of phenylalanine and tyrosine are built up from shikimic acid by way of prephenic acid. The specific incorporation of activity from tyrosine into papaverine thus makes it highly probable that this alkaloid is a product of the shikimic–prephenic acid pathway in which the precursors reach the aromatic state. Secondly, the fact that the labels are not scattered during the biosynthesis of papaverine is sound evidence against breakdown of the tyrosine into fragments which are then incorporated into the alkaloid. Our results are therefore interpreted as proof that the C_{16}-skeleton of papaverine (V) is built from two C_6–C_2 units (Ar–C–C) derivable in the plant from tyrosine. We have gone no further with this statement than our results strictly allow and its form must not be taken as implying that the biosynthetical step in which the benzylisoquinoline system is formed cannot involve, for example, a condensation of 3,4-dihydroxyphenylpyruvic acid (C_6–C_3) with 3,4-dihydroxyphenethylamine (dopamine) with a subsequent decarboxylation step. This is one of several reasonable possibilities which can be envisaged for the formation of what is probably a 1,2,3,4-tetrahydro-1-benzylisoquinoline initially (*e.g.*, IV); experimental evidence on this point will be presented in Part IV. After the completion of our work, Kleinschmidt and Mothes [24] reported the incorporation of generally-labelled tyrosine into the phthalide isoquinoline alkaloid, narcotoline; the general labelling allows their results to be interpreted in a number of ways. More recently, a brief account has been given [21] of Gear and Spenser's work which shows that singly-labelled tyrosine will serve as a specific precursor of the related phthalide isoquinoline, hydrastine, and that the two units which are built into the alkaloid have different labelling levels.

Experimental

Solutions in organic solvents were dried (Na_2SO_4) and evaporated below 40° under nitrogen at reduced pressure. Analytical samples were dried at 100° *in vacuo* unless otherwise stated. The m. p.s are uncorrected.

Radioactive Assay.—Radioactive samples were burnt to carbon dioxide which was trapped as barium [^{14}C]carbonate. This was collected so as to provide an " infinitely thick " disc by the method described by Andrews and Hough.[25] Counting equipment consisted of the Dynatron power unit P2000A, scaler of type 1009B, probe-unit 1014A with a thin end-window (3 mg./cm.^{2}) Geiger–Müller tube (Mullard). The counting system was calibrated by use of poly([^{14}C]methyl methacrylate) (Radiochemical Centre, Amersham) of known specific activity.

Isolation of Papaverine (V).—The contents of tubes 81—99 from the countercurrent distribution at pH 6·7 described in Part II were combined and the aqueous layer was adjusted to pH 9 with sodium carbonate. After equilibration, the upper layer was separated and the lower layer was extracted with ethyl acetate (2 × 100 ml.). Evaporation of the dried solutions in ethyl acetate left the weak bases (222 mg.) from the *Papaver somniferum* plants. This product (scattered in 4 tubes) was fractionated for 96 transfers between ethyl acetate and 0·2M-buffer made from 0·2N-acetic acid (9·5 vol.) and 0·2N-sodium acetate (0·5 vol.). A well separated peak was found between tubes 36 and 60 and the radioactive papaverine (61 mg.) was recovered from these tubes as above. This was dissolved in ethanol (10 ml.) together with inactive papaverine (440 mg.), and the solution was treated with picric acid (378 mg.) dissolved in ethanol (10 ml.). After recrystallisation of the picrate twice from ethanol, the crystals (730 mg.), m. p. 186—187°, reached constant activity.

(±)-*Laudanosine* (VI).—The foregoing picrate (730 mg.) was shaken with ether (200 ml.) and 50% aqueous ethanolamine (25 ml.), and the aqueous layer was further extracted with

[22] Davis, *Arch. Biochem. Biophys.*, 1958, **78**, 497; Schwinck and Adams, *Biochim. Biophys. Acta*, 1959, **36**, 102; Sprinson, *Adv. Carbohydrate Chem.*, 1960, **15**, 235.
[23] Reviewed by Neish, *Ann. Rev. Plant Physiol.*, 1960, **11**, 55.
[24] Kleinschmidt and Mothes, *Z. Naturforsch*, 1959, **14**b, 52.
[25] Andrews and Hough, *J.*, 1958, 4483.

ether. After the combined ethereal layers had been extracted with 50% aqueous ethanolamine (2 × 10 ml.) they were washed with water until neutral, dried, and evaporated. The residue (410 mg.) was diluted with inactive papaverine (404 mg.) by dissolving the mixture in ethanol and evaporating the solution to dryness; a sample was assayed by combustion (Found: $17{\cdot}9 \times 10^2$ counts).

A solution of the papaverine (802 mg.) in methanol (10 ml.) and methyl iodide (2 ml.) was heated under reflux for 2 hr. The solution was concentrated until crystallisation occurred and, after being kept at 0° overnight, the papaverine methiodide (1·05 g.), m. p. 119—120° (air-dried), was collected. This was dissolved in methanol (30 ml.), and potassium borohydride (394 mg.) was added portionwise during 0·5 hr. After the solution had been kept for 0·5 hr. at room temperature, it was evaporated to 5 ml. and poured into N-potassium hydroxide (100 ml.). Extraction with 3 : 1 ether–chloroform (3 × 100 ml.) gave crystalline (±)-laudanosine (722 mg.), m. p. 114—115°.

trans- *and* cis-*Laudanosinemethine* (VII) *and* (VIII).—A solution of the foregoing laudanosine (722 mg.) in methanol (10 ml.) and methyl iodide (1 ml.) was heated under reflux for 1 hr.; a further portion of methyl iodide was then added and the heating was continued for 1 hr. Concentration of the solution afforded laudanosine methiodide (932 mg.) which, without purification, was shaken as a suspension in water (10 ml.) with silver oxide (from 1·5 g. of silver nitrate) for 1·5 hr. After the solution had been filtered, it was treated with potassium hydroxide (15 g.) and heated under reflux for 2 hr. The cooled mixture was extracted with 3 : 1 ether–chloroform (3 × 100 ml.), and the crude laudanosinemethines (0·8 g.), recovered by evaporation, were dissolved in ethyl acetate (20 ml.). An excess of dry hydrogen chloride was passed into the solution which was then concentrated until crystallisation started. The precipitated hydrochlorides (567 mg.) were collected and crystallised from methanol (8 ml.) to give *trans*-laudanosinemethine hydrochloride (520 mg.), m. p. 220—221° (Decker and Galatty [14] report m. p. 220°) (Found: C, 62·4; H, 7·0; N, 3·8. Calc. for $C_{22}H_{30}ClNO_4,H_2O$: C, 62·0; H, 7·5; N, 3·4%); $\lambda_{max.}$ 223, 333, $\lambda_{min.}$ 262 mμ (log ε 4·30, 4·43, 3·76), in ethanol.

The mother liquors from the *trans*-methine were not examined in the radioactive case. However, degradation of inactive laudanosine methiodide (2·03 g.) yielded the *trans*-methine hydrochloride (974 mg., 60%) and a methanolic mother liquor which was evaporated to dryness. A solution of the residue in dilute hydrochloric acid was extracted thrice with 3 : 1 (v/v) ether–chloroform, then made strongly alkaline and extracted thrice again with ether–chloroform. Evaporation of the latter extracts left a gum which was warmed with light petroleum (b. p. 60—80°), the insoluble matter being rejected. Concentration of the solution yielded crystals which were recrystallised from aqueous ethanol to give cis-*laudanosinemethine* (140 mg.; 9%), m. p. 95—96° (Found, in material dried at 78°: C, 71·5; H, 7·9; N, 3·5. $C_{22}H_{29}NO_4$ requires C, 71·1; H, 7·9; N, 3·8%), $\lambda_{max.}$ 215, 294, $\lambda_{min.}$ 251 mμ (log ε 4·37, 4·04, 3·76), in ethanol.

trans- *and* cis-*Laudanosinemethine Methiodide*.—An aqueous solution of the *trans*-methine hydrochloride (460 mg.) was made strongly alkaline and extracted with ether to yield the solid free-base (407 mg.) which was heated under reflux in methanol (10 ml.) with methyl iodide (4 ml.) for 2 hr. Concentration of the solution gave trans-*laudanosinemethine methiodide*, (406 mg.), m. p. 236—237° (from methanol) (Found: C, 53·6; H, 6·3; N, 2·5. $C_{23}H_{32}INO_4$ requires C, 53·8; H, 6·3; N, 2·7%).

cis-Laudanosinemethine (25 mg.) was dissolved in ether (2 ml.) and treated with methyl iodide (0·1 ml.) at room temperature for 48 hr. to give cis-*laudanosinemethine methiodide* (28 mg.), m. p. 199—200° (from methanol–ether) (Found: C, 53·6; H, 6·2; N, 2·3%).

Oxidation of trans- *and* cis-*Laudanosinemethine* (VII) *and* (VIII).—The radioactive *trans*-laudanosinemethine was recovered from its hydrochloride (521 mg.) as above to give crystals (466 mg.). A stirred solution of this base in 50% (by vol.) aqueous pyridine (10 ml.) was treated at room temperature during 1 hr. with a solution of potassium permanganate (595 mg.) in 50% aqueous pyridine (50 ml.). The manganese dioxide was filtered off and washed with 50% aqueous pyridine (2 × 10 ml.) and N-potassium hydroxide (2 × 10 ml.). The filtrate was freed from pyridine by evaporation, and the aqueous solution was extracted with 3 : 1 ether–chloroform (3 × 100 ml.). After extraction of the combined organic solution with N-hydrochloric acid (3 × 20 ml.), it was evaporated to leave the neutral products (139 mg.). The basic products (32 mg.) were recovered in the usual way from the acidic extracts.

The alkaline aqueous solution (above) was acidified, and extracted with 3 : 1 ether–chloroform (3 × 100 ml.) to yield veratric acid (X) (133 mg.) which was sublimed at 120°

(bath)/1·0 mm. and gave the pure acid, m. p. 181—182° (from aqueous ethanol) which was burnt for assay (Found: 0·08 × 10^2 counts).

In an inactive run, *cis*-laudanosinemethine (VIII) was oxidised in the same way with effectively the same results.

4,5-*Dimethoxy*-2-*vinylbenzoic Acid* (XI).—The final acidic aqueous solution from the foregoing experiment, which must contain the amino-acid (IX), was concentrated to 20 ml., adjusted to pH 10 with potassium hydroxide, and then stirred at 0° with dimethyl sulphate (1 ml.) and 10N-potassium hydroxide (0·5 ml.) for 1 hr. At hourly intervals, three more portions of dimethyl sulphate (each 0·5 ml.) and 10N-potassium hydroxide (0·25 ml.) were added, and after a total of 4·5 hr. potassium hydroxide (10 g.) was added and the solution was heated under reflux for 1·75 hr. The evolved trimethylamine was collected in dilute hydrochloric acid, recovered as the hydrochloride by evaporation, and precipitated from aqueous solution as the picrate by the addition of saturated sodium picrate solution. Recrystallisation from ethanol gave trimethylamine picrate (30 mg.), m. p. and mixed m. p. 217—218°.

The aqueous alkaline solution was acidified and extracted with 3 : 1 ether–chloroform (3 × 100 ml.), and the combined extracts were evaporated to yield 4,5-dimethoxy-2-vinylbenzoic acid (XI) (156 mg.). Recrystallisation from benzene afforded the pure acid, m. p. 183—184° (lit.[14] 184°) which was burnt for assay (Found: 17·7 × 10^2 counts).

Ozonolysis of the Acid (XI).—Ozonised oxygen was passed through a solution of 4,5-dimethoxy-2-vinylbenzoic acid (48 mg.) in ethyl acetate (5 ml.) at −78° and then through acidified potassium iodide solution. The gas was passed for three times the interval required to produce the first colour of iodine in the potassium iodide solution. After the ethyl acetate had been evaporated, the ozonide was decomposed by being heated under reflux for 0·5 hr. with water (20 ml.), zinc dust (0·2 g.), and silver nitrate (10 mg.). Half of the water was then distilled at atmospheric pressure into a solution of dimedone (0·3 g.) in water (20 ml.) and ethanol (8 ml.), water (10 ml.) was added to the distilling flask and the distillation to half volume was repeated into the same dimedone solution. The formaldehyde dimethone (31 mg.) separated during 15 hr. and was collected and recrystallised from 50% aqueous ethanol to afford the pure derivative (20 mg.) for combustion, m. p. and mixed m. p. 188° (Found: 8·6 × 10^2 counts).

A parallel run of the same duration in which 2-ethyl-4,5-dimethoxybenzoic acid (XII) replaced the unsaturated acid (XI) yielded no formaldehyde dimethone.

Preparation and Decarboxylation of 2-*Ethyl*-4,5-*dimethoxybenzoic Acid* (XII).—A solution of radioactive 4,5-dimethoxy-2-vinylbenzoic acid (XI) (57·8 mg.) in ethanol (10 ml.) was hydrogenated at 22°/760 mm. over Adams platinum (uptake 1·0 mol.). The filtered solution was evaporated to leave the 2-ethyl-acid, m. p. and mixed m. p. with synthetic [20] material, 140—141°. Sodium azide (42 mg.) and benzene (2 ml.) were added to this acid, in equipment being swept with carbon dioxide-free nitrogen, and at 0° concentrated sulphuric acid (0·6 ml.) was added. The reaction flask was then warmed to 45° for 45 min. and the carbon dioxide evolved was collected in saturated (at 20°) aqueous barium hydroxide solution which had previously been heated to 80°. The further steps then followed those used in the combustion assay above (Found: 7·9 × 10^2 counts).

Dihydrolaudanosinemethine (XV) *Picrate and Methiodide* (XVI).—A solution of the *trans*-methine base (VII) (4·1 g.) in ethanol (60 ml.) was shaken at 20°/750 mm. with hydrogen and platinic oxide (0·5 g.). Uptake (0·95 mol.) ceased after 7 hr. The catalyst was filtered off, the concentrated filtrate was treated with a slight excess of picric acid, and the precipitate, crystallised (from ethanol), was *dihydrolaudanosinemethine picrate* (5·24 g.), m. p. 153—156° (Found: C, 55·9; H, 5·6; N, 8·8. $C_{28}H_{34}N_4O_{11}$ requires C, 55·8; H, 5·7; N, 9·3%).

The picrate (6·98 g.) was shaken with ether (250 ml.) and 50% (by vol.) aqueous ethanolamine (150 ml.), and the clear separated aqueous layer was further extracted with ether (3 × 100 ml.). After the combined ethereal solution had been shaken with more ethanolamine solution (4 × 100 ml.), it was washed with water until the washings were neutral. The dihydromethine was recovered by evaporation from the dried solution as a gum (3·94 g.). A solution of this in methanol (20 ml.) was heated under reflux for 3 hr. with methyl iodide (10 ml.) and then evaporated to dryness to leave crystals (5·13 g.). Part (620 mg.) was recrystallised thrice from methanol to afford pure *dihydrolaudanosinemethine methiodide*, m. p. 197° (Found: C, 54·0; H, 6·7; N, 2·5. $C_{23}H_{34}INO_4$ requires C, 53·6; H, 6·7; N, 2·7%).

Hydrogenation of the *cis*-laudanosinemethine (VIII) as above (uptake 1·03 mol.) gave a gum, part of which was converted into the picrate, m. p. 153—156° and part into the methiodide,

m. p. 197°. These were proved to be identical with the corresponding salts above by mixed m. p.s and infrared spectra.

1-(4,5-*Dimethoxy*-2-*vinylphenyl*)-2-(3,4-*dimethoxyphenyl*)*ethane* (XVII).—A solution of the remaining methiodide (XVI) (4·51 g.) in water (50 ml.) was shaken with fresh silver oxide (from 10 g. of silver nitrate) for 15 min. and then filtered. The solution and washings (now 150 ml.) were treated with potassium hydroxide (75 g.) and heated under reflux for 2 hr. Extraction of the cooled mixture thrice with benzene yielded a resin (2·64 g.), the ethanol-soluble part of which crystallised from ethanol to give the *diphenylethane derivative* (XVII) as plates (1·9 g.), m. p. 74° (Found, in material dried at 56°: C, 73·6; H, 7·1. $C_{20}H_{24}O_4$ requires C, 73·2; H, 7·4%).

Oxidation of the Vinyldiphenylethane (XVII).—Ozonised oxygen was passed through a solution of the *diphenylethane* derivative (XVII) (0·3 g.) in ethyl chloride (15 ml.) at −78° and then through acidified potassium iodide solution. The gas was passed for twice as long as was required to release the first trace of iodine in the iodide solution. After the ethyl chloride had been evaporated, the ozonide was decomposed as in the ozonolysis of the acid (XI) to give formaldehyde dimethone (69 mg.), m. p. and mixed m. p. 188°.

The aqueous suspension containing the products not volatile in steam was extracted thrice with 4 : 1 ether–chloroform to give a gum (181 mg.) which was dissolved in 50% aqueous pyridine. To this stirred solution at room temperature was gradually added potassium permanganate (187 mg.) in 50% aqueous pyridine (35 ml.). The excess of permanganate was destroyed by the addition of a few drops of hydrogen peroxide, and the manganese dioxide was filtered off. The pad was washed with hot acetone and dilute sodium hydroxide, and the filtrate was freed from organic solvents by evaporation. It was extracted thrice with ether, acidified to Congo Red, and re-extracted thrice with ether. Evaporation of the latter extracts left a resin which crystallised from ethanol to give 2-(3,4-dimethoxyphenethyl)-4,5-dimethoxybenzoic acid (XVIII; R = CO_2H) (89 mg.), m. p. and mixed m. p. with synthetic [18] material 158—159°.

1-(2-*Ethyl*-4,5-*dimethoxyphenyl*)-2-(3,4-*dimethoxyphenyl*)*ethane* (XIX).—(*a*) *By hydrogenation.* Hydrogen (0·93 mol.) was absorbed when the styrene (XVII) (158 mg.) was catalytically reduced in ethanol (as above) over platinum. The catalyst was filtered off, and concentration of the filtrate caused crystallisation of the diphenylethane (XIX) (114 mg.), m. p. 78° (Found, in material dried at 56°: C, 72·7; H, 7·6. Calc. for $C_{20}H_{26}O_4$: C, 72·7; H, 7·9%). Robinson and Sugasawa,[26] who prepared this compound by a different route, record m. p. 78°.

(*b*) *By Emde degradation.* A solution of the methiodide (XVI) (36 mg.) in liquid ammonia (5 ml.) was treated with small pieces of sodium until the blue colour persisted. The ammonia was then allowed to evaporate, the residue was partitioned between water and ether, and the aqueous layer was further extracted with ether. After the combined ethereal solution had been washed with an excess of acid and then with water, it was dried and evaporated. Crystallisation of the residue from aqueous ethanol gave the diphenylethane (XIX) (13 mg.), m. p. 76—77° raised to 77—78° on admixture with the sample from (*a*). The infrared spectra of the two samples were identical.

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[26] Robinson and Sugasawa, *J.*, 1932, 789.