Biosynthesis. Part 23.[1] Degradative Studies on the Alkaloids Hasubanonine and Protostephanine from *Stephania japonica*

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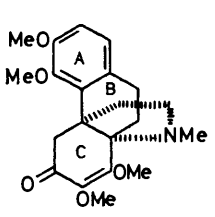
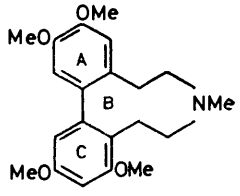
Degradative schemes are reported for the alkaloids hasubanonine (1) and protostephanine (2) of *Stephania japonica* to allow labelling patterns to be determined. Acetolysis of (1) leads eventually to a phenanthrene (5c) by removal of the ethanamine bridge. *N*-Methylation and Hofmann elimination of (2) gives one major olefinic product, assigned structure (6a) from ^{1}H n.m.r. data and confirmed by synthesis of specifically ^{13}C-labelled material.

Stephania japonica Miers (Menispermaceae) produces a wide variety of alkaloids,[2] among them hasubanonine (1) and the rare base protostephanine (2); (1) and (2) were respectively the first natural examples of the hasubanan and dibenz[*d*,*f*]azonine skeletons to be characterised. Hasubanonine,[3] the major alkaloid of the plant, bears a close structural relationship to the morphine group of bases [*e.g.* morphine itself (3)]. The vicinally trioxygenated ring C of hasubanonine is unusual but it is also found in other hasubanan alkaloids of *S. japonica* and a few different alkaloid types present in the plant show this same feature. Protostephanine[4] (2) remained a unique natural example of the dibenz[*d*,*f*]-azonine structure until 1971, when erybidine (4a) was isolated[5] from an *Erythrina* sp. and, more recently,[6] bases (4b—d) were added. These bases (4a—d) seem to be 'trapped' versions of intermediates on the pathway to the erythrina alkaloids.[6,7] The dibenzazonine system was well known in some rearrangement products of the morphine group,[8] a structural relationship which has been suggested[9b] to be of biosynthetic significance. Ring C of protostephanine (2) is interesting by having *meta*-oxygenation which could be derived from a 1,2,3-trisubstituted ring of shikimate origin, or possibly from the acetate–malonate pathway. The biosynthesis of these two unusual systems (1) and (2) has attracted lively speculation and many schemes have been proposed.[9] This and the following papers report our experimental studies of the biosynthetic problems.



(1)



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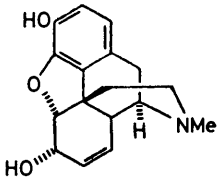

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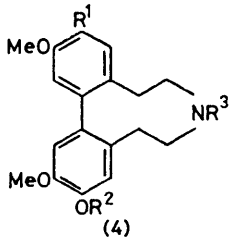


(4)

a; R^1 = OH, R^2 = Me, R^3 = Me
b; R^1 = H, R^2 = Me, R^3 = Me
c; R^1 = H, R^2 = Me, R^3 = H
d; R^1 = H, R^2 = H, R^3 = Me

(5)

a; R^1 = Ac, R^2 = H
b; R^1 = Ac, R^2 = OMe
c; R^1 = Me, R^2 = OMe

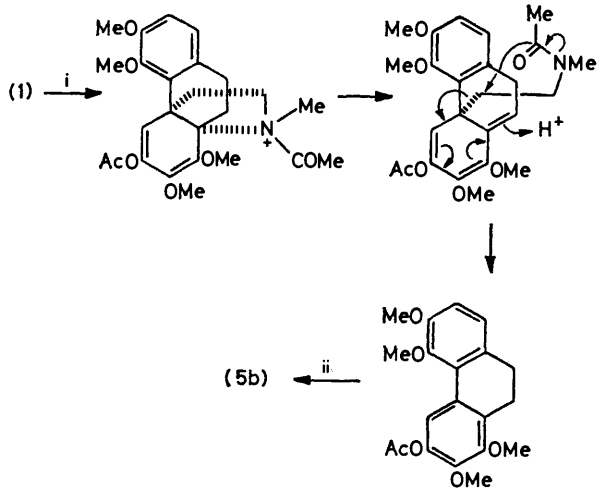
The structures of hasubanonine (1) and protostephanine (2) can be assembled in principle from two C_6–C_2 units and our planning took into account that incorporation experiments with C_6–C_2 and C_6–C_3 precursors would be needed in addition to work on the later intermediates. Labelling of these precursors was to be in the C_2 portion of the C_6–C_2 unit (and similarly for C_6–C_3) and successful incorporations would thus label the corresponding C_2 residues[10] of the alkaloids (1) and (2). Accordingly, it was essentially to develop unambiguous degradations of both alkaloids capable of distinguishing between these aliphatic parts of their molecules. It was important for the intermediates and degradation products to be crystalline and, particularly for the rare protostephanine (2), that the sequences could be handled on a small scale. This paper describes the successful degradations; their use in biosynthetic studies is described in the following papers.

RESULTS AND DISCUSSION

Degradation of Hasubanonine.—In the morphinan group of alkaloids, loss of the ethanamine side-chain on acetolysis of the free base (or a quaternary salt) is well known.[11] The remainder of the molecule is generally isolated as a fully-aromatic substituted phenanthrene. Similar aromatisation has also been observed in the hasubanan series; *e.g.* the product of Hofmann elimin-

ation from the methiodide of (1) could be acetolysed (acetic anhydride) to produce the phenanthrene (5a).[12]

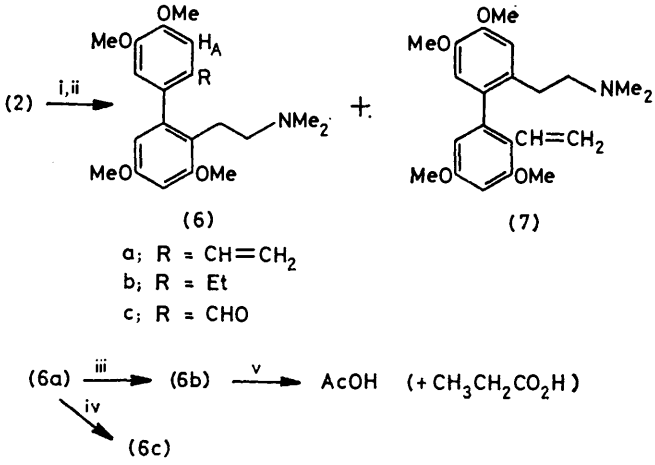
It was hoped that a similar degradation might be carried out directly on hasubanonine (1). Direct acetolysis (acetic anhydride with a trace of hydrochloric acid) under various conditions gave mixtures of products from which the penta-substituted phenanthrene (5b) was isolated in low and variable yield. Formation of a penta-oxygenated product requires a dehydrogenation (a plausible mechanism for the acetolysis is shown in Scheme 1). Of various methods tried to encourage aromatisation, 2,3,5,6-tetrachloro-1,4-benzoquinone (chloranil) was best and led to almost exclusive formation of (5b). The phenanthrene was characterised, after basic hydrolysis and *O*-methylation, as the penta-methoxy-derivative (5c) which gave a crystalline picrate in *ca.* 15% overall yield from hasubanonine (1). A practical method for distinguishing the two C_2-units of hasubanonine was thus available.



SCHEME 1 *Reagents:* i, Ac_2O; ii, oxidation

Degradation of Protostephanine.—A suitable sequence (Scheme 2) for separating the two similar C_2-units of ring B in protostephanine (2) was based on the studies by Takeda *et al.*[4] of the Hofmann elimination. Protostephanine methohydroxide underwent Hofmann elimination, in boiling aqueous sodium hydroxide solution, in somewhat better yield than that reported [4] for vacuum pyrolysis of the quaternary base. However, direct treatment of protostephanine methiodide with boiling aqueous sodium hydroxide gave a quantitative yield of a mixture of the two methines (6a) and (7). Conversion into the picrate gave the crystalline salt of the major isomer, designated protostephanine-α-methine, in 80% yield from the methiodide. The minor β-methine in the mother-liquors was not studied further.*

It is clearly of crucial importance for the biosynthetic studies to determine the direction of Hofmann elimination leading to the major α-methine since this step controls which carbon atoms are removed by the subsequent stages of degradation. The first structural pointers that the methine has structure (6a) came from



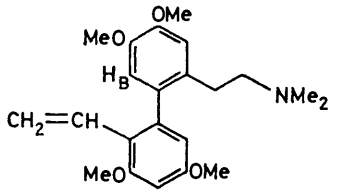
SCHEME 2 *Reagents:* i, MeI; ii, NaOH–H_2O; iii, H_2–Pt; iv, OsO_4–$NaIO_4$; v, CrO_3–H_2SO_4

the ^1H n.m.r. spectrum. The ring-A protons of protostephanine (2) appear as two singlets (δ 6.75 and 6.78) and those on ring C as two doublets at slightly higher field (centred at δ 6.34 and 6.45; *J* 2 Hz). In the spectrum of the α-methine the same pattern is observed for the aromatic protons. The ring-C protons are largely unaffected (δ 6.28 and 6.47; *J* 2 Hz) but one of the ring-A proton signals is shifted downfield by 0.4 p.p.m. to δ 7.14, the other remaining at δ 6.67. This shift is removed on hydrogenation of the α-methine to the dihydro-derivative (6b) (ring-A protons at δ 6.68 and 6.83). This entire behaviour of the signals is similarly reproduced in the n.m.r. spectra of the methiodides of (2), (6a), and (6b). The most probable explanation was that the vinyl group is attached to ring A [as in (6a)] and that the strongly shifted proton is *ortho* to the vinyl group (H_A) and deshielded by it. This requires the vinyl group to be in (or near) the plane of ring A; in support, the u.v. spectrum of the α-methine shows conjugation of the vinyl group to the dimethoxybenzene chromophore. N.m.r. data for the aldehyde (6c), obtained by oxidative cleavage of the α-methine (but not fully characterised), were consistent with this assignment; the ring-A protons were observed at δ 6.76 and 7.52, *i.e.* an even larger downfield shift (0.75 p.p.m.) of the same signal.

However, these spectroscopic arguments do not completely exclude the possibility that the vinyl group of the α-methine is attached to ring C; if conformation (8) is preferred, it is conceivable that proton H_B is being deshielded. Accordingly, protostephanine (2) was synthesised with an isotopic label unambiguously situated in one of the C_2 ethanamine portions so that its location could be followed through the Hofmann elimination

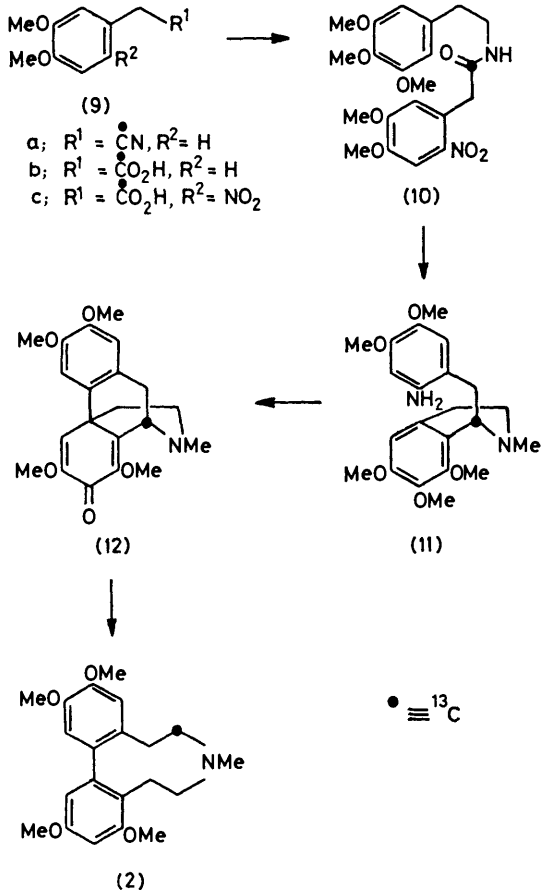
* Takeda *et al.*,[4] working on a much larger scale, isolated both isomers as their methiodides by fractional crystallisation but a structural assignment was not made. Our α-methine picrate was identical (m.p., mixed m.p.) with a sample kindly supplied from Dr. Takeda's collection by Dr. Natsume.

sequence. A ^{13}C-label was chosen to allow a clear distinction to be made (by chemical shift) between a label situated at an sp^2 (vinyl group) or sp^3 (ethanamine) site, in the labelled α-methine. The route used was based on that described in the preceding paper.[13] 3,4-Dimethoxybenzyl chloride was converted using



(8)

$Na^{13}CN$ into the [^{13}C]phenylacetonitrile (9a) with an enrichment of *ca.* 20 atom %. Hydrolysis to the corresponding acid (9b) followed by nitration to (9c) and coupling to 2-(3,4,5-trimethoxyphenyl)ethylamine produced the amide (10) (Scheme 3). The subsequent



SCHEME 3

sequence of steps, namely cyclisation of (10) in Bischler–Napieralski fashion to a dihydroisoquinoline, *N*-methylation, borohydride reduction, and catalytic hydrogenation of the nitro-group, led to the aminotetrahydroisoquinoline (11) which could be diazotised and coupled in a copper-catalysed Pschorr reaction. The [^{13}C]-morphinandienone (12) was isolated by dilution with unlabelled material to give 5 atom % ^{13}C at the position illustrated. In this way, sufficient material was available for conversion into protostephanine (2) and α-methine (6a).* The protostephanine (2) so obtained was unambiguously labelled as shown in the ring-A ethanamine side-chain.

The α-methine (6a) was prepared from this sample as previously described, and a comparison of the ^{13}C n.m.r. spectrum of the specifically enriched α-methine with that of natural abundance material showed clearly the enhancement of one resonance (*ca.* 4-fold) at 112.3 p.p.m., *i.e.* of a signal from an sp^2 rather than an sp^3 centre. The vinyl group of the α-methine has thus been labelled, and must be attached to ring A; the structure (6a) is thereby confirmed.

Removal of a benzylic proton by base is an essential step in the present Hofmann elimination, and the preference for elimination involving the ring-A side-chain of (2) is presumably the result of lesser hindrance to approach of a hydroxide ion than is the case for the ring-C side-chain.†

Having in the α-methine (6a) an unambiguous differentiation of the two C_2-units in protostephanine, the degradation was completed as follows. Attempted cleavage of the vinyl group with ozone was unsatisfactory, and although aldehyde (6c) could be produced using osmium tetraoxide–periodate, it could not be purified sufficiently to be suitable for radiochemical studies. However, Kuhn–Roth oxidation[15] of the dihydro-α-methine (6b) allowed isolation of the ring-A side-chain as acetic acid,‡ characterised as the *p*-bromophenacyl ester; a smaller quantity of propionic acid was also formed.

The methods developed here for specific degradation of hasubanonine (1) and protostephanine (2) were then used to determine the labelling patterns of the radioactive alkaloids obtained in the biosynthetic experiments.[10]

EXPERIMENTAL

Organic solutions were dried over anhydrous sodium or magnesium sulphate and evaporated under reduced pressure below 40 °C. M.p.s were determined on a Kofler hot-stage. T.l.c. was carried out on plates coated with Kieselgel F_{254} (Merck), and preparative t.l.c. on 20 × 20 × 0.1 cm plates coated with Kieselgel GF_{254} (Merck). Alumina for column chromatography was normally grade III neutral. Unless otherwise stated, u.v. spectra were recorded on Unicam SP800 and SP8000 instruments for solutions in 95% ethanol, i.r. spectra for solutions in chloroform on a

* Attempts to improve on the Pschorr reaction, for example by thallium(III) coupling[14] of suitable phenolic substances, were not successful.

† Similar Hofmann degradation of laurifonine (4b) is reported[6] to give a mixture of methines in unspecified proportions.

‡ Special care had to be taken (see Experimental section) to avoid contamination of the dihydro-α-methine with carbon compounds (notably ethanol) that could give rise to acetic acid on oxidation.

Perkin-Elmer 257 spectrometer, and n.m.r. spectra for solutions in deuteriochloroform on Varian HA100 or XL100 spectrometers for ^1H spectra and a CFT20 instrument for ^{13}C spectra (tetramethyl-silane as reference). Mass spectra were determined by direct insertion in A.E.I. MS9, MS30, and MS902 machines.

Degradation of Hasubanonine (1).—Hasubanonine (127 mg, 0.34 mmol) was dissolved in acetic anhydride (15 ml) to which had been added concentrated hydrochloric acid (5 drops) and chloranil (100 mg, 0.41 mmol). The mixture was heated under reflux for 12 h, then the excess of acetic anhydride was decomposed with water (50 ml), the mixture was extracted with dichloromethane (3 × 50 ml), and the organic extracts were washed with water (2 × 50 ml). The residue from the dichloromethane was dissolved in ethanol (20 ml) and potassium hydroxide (500 mg) added. After the mixture had been heated under reflux for 2 h, methyl iodide (3 ml) was added, heating was continued for a further 3 h and water (100 ml) was added before extraction with dichloromethane (3 × 100 ml). The residue from the organic solvent was fractionated by preparative t.l.c. (developed in chloroform) to give the phenanthrene as the major band (R_F 0.6). This in hot ethanol (0.5 ml) was treated with picric acid (20 mg) to give the *picrate* of 1,2,3,-5,6-*pentamethoxyphenanthrene* (5c) (27 mg), m.p. 135—136 °C (from ethanol) (Found: C, 54.1; H, 4.2; N, 7.65. $C_{25}H_{23}N_3O_{12}$ requires C, 53.9; H, 4.2; N, 7.55%). The free phenanthrene was regenerated by passing the picrate (38 mg) in dichloromethane (5 ml) through a plug of basic alumina (grade I) (1 g) which was further eluted with dichloromethane (20 ml). Evaporation afforded 1,2,3,5,6-pentamethoxyphenanthrene as a colourless gum (20 mg), $\lambda_{max.}$ 238, 258sh, 264, 290sh, 302, 315, 333sh, 347, and 368 nm; δ 3.92, 3.98, and 4.02 (15 H, 3s, 5 × OCH_3), 7.28, 7.52, 7.60, and 7.91 (each 1 H, d, *J* 8 Hz, 7-, 8-, 9-, and 10-H), and 9.10 (1 H, s, 4-H); *m/e* 328 (M^+).

Degradation of Protostephanine (2).—The protostephanine used to develop the degradation was synthetic material [16] with the following physical properties: $\nu_{max.}$ 2 835, 2 780, 1 600, 1 585, and 1 510 cm^{-1}; $\lambda_{max.}$ 224 and 284 nm; δ 2.5—2.8 (8 H, m, 4 × CH_2), 2.32 (3 H, s, NCH_3), 3.79 and 3.90 (each 3 H, s, OCH_3), 3.83 (6 H, s, 2 × OCH_3), 6.34 and 6.45 (each 1 H, d, *J* 2 Hz, ArH of ring c), and 6.68 and 6.75 (each 1 H, s, ArH of ring A); *m/e* 357 (M^+).

Protostephanine Methiodide.—Protostephanine (91 mg) in ethyl acetate (2 ml) and methyl iodide (0.2 ml) was kept in the dark at 20 °C for 18 h and the methiodide (123 mg, 97%) was collected, m.p. 219—220 °C (lit.,[17] 220—221 °C) (from methanol) (Found: C, 52.6; H, 6.0; I, 25.35; N, 2.9. Calc. for $C_{22}H_{30}INO_4$: C, 52.9; H, 6.05; I, 25.4; N, 2.8%); $\nu_{max.}$ 1 600, 1 580, and 1 510 cm^{-1}; $\lambda_{max.}$ 226 and 284 nm; δ 3.38 and 3.45 (each 3 H, s, NCH_3), 3.82, 3.86, 3.90, and 3.99 (each 3 H, s, OCH_3), 6.36 and 6.52 (each 1 H, d, *J* 2 Hz, ArH of ring c), and 6.68 and 6.70 (each 1 H, s, ArH of ring A); *m/e* 357 ($M^+ - CH_3I$).

Protostephanine-α-methine (6a).—Protostephanine methiodide (50 mg) was dissolved in boiling water (5 ml); sodium hydroxide pellets (2 g) were added *carefully* to the hot solution and the mixture was heated under reflux for a further 2 h. The resultant cooled emulsion was poured into water (20 ml) and extracted with ether (3 × 25 ml) to yield a clear gum (33 mg) which, in methanol (0.2 ml), was treated with picric acid (22 mg) in methanol (0.3 ml). The α-methine picrate crystallised (48 mg, 80%), m.p. 165—167 °C (lit.,[4] 166—167 °C) (from methanol) (Found: C, 56.2; H, 5.4; N, 9.3. Calc. for $C_{28}H_{32}N_4O_{11}$: C, 56.0; H, 5.4; N, 9.3%). The amorphous free base was quantitatively recovered from the picrate by chromatography on alumina (1 g for 10 mg picrate), eluting with chloroform; $\nu_{max.}$ 2 820, 2 770, 1 600, 1 585, and 1 510 cm^{-1}; $\lambda_{max.}$ 218, 263, and 303 nm; δ 2.2—2.8 (4 H, m, 2 × CH_2), 2.07 (6 H, s, 2 × NCH_3), 3.76 and 3.93 (each 3 H, s, OCH_3), 3.85 (6 H, s, 2 × OCH_3), 5.00 (1 H, d, *J* 11 Hz, CH=CH_2), 5.50 (1 H, d, *J* 17.5 Hz, CH=CH_2), 6.24—6.57 (1 H, dd, *J* 11 and 17.5 Hz, C*H*=CH_2), 6.28 and 6.47 (each 1 H, d, *J* 2 Hz, ArH of ring c), and 6.67 and 7.14 (each 1 H, s, ArH of ring A); *m/e* 371 (M^+).

Protostephanine-α-methine Methiodide.—Protostephanine-α-methine was recovered from the picrate (40 mg) as above and dissolved in methanol (0.2 ml) and methyl iodide (1.5 ml). Evaporation of the solvents and trituration of the resultant gum with ether gave the *methiodide* (33 mg, 97%), m.p. 119—121 °C (Found: C, 52.8; H, 6.6; N, 2.7; $C_{23}H_{32}INO_4 \cdot \frac{1}{2}H_2O$ requires C, 52.9; H, 6.4; N, 2.7%); $\nu_{max.}$ 2 730, 1 595, 1 585, and 1 505 cm^{-1}; $\lambda_{max.}$ 224, 265/270, and 303 nm; δ 3.24 (9 H, s, 3 × NCH_3), 3.79 3.90, 3.92, and 3.96 (each 3 H, s, OCH_3), 5.00 (1 H, d, *J* 11 Hz, CH=CH_2), 5.50 (1 H, d, *J* 17 Hz, CH=CH_2), 6.26—6.54 (1 H, dd, *J* 11 and 17 Hz, C*H*=CH_2), 6.40 and 6.48 (each 1 H, d, *J* 2 Hz, ArH of ring c), 6.58 and 7.15 (each 1 H, s, ArH of ring A); *m/e* 388 and 371 ($M^+ - CH_3I$).

Protostephanine Dihydro-α-methine (6b).—Protostephanine-α-methine was recovered from its picrate (40 mg) as above and, in ethanol (4 ml), was injected into a suspension of platinum [from Adams catalyst (14 mg)] in ethanol (4 ml). After the suspension had been stirred under hydrogen (1 atm) for 2 h (complete reduction by u.v. spectroscopy), it was filtered and the solids were washed with ethanol. Evaporation of the filtrates gave a gum which in ethanol (0.3 ml) was mixed with picric acid (27.5 mg) in ethanol (0.5 ml) to give the *picrate* (33 mg, 82%), m.p. 166—168 °C (from ethanol) (Found: C, 55.8; H, 5.8; N, 9.15. $C_{28}H_{34}N_4O_{11}$ requires C, 55.8; H, 5.7; N, 9.3%). The amorphous free base was regenerated from the picrate as described for protostephanine-α-methine; $\nu_{max.}$ 2 820, 2 770, 1 600, 1 585, and 1 510 cm^{-1}; $\lambda_{max.}$ 233 and 284 nm; δ 1.09 (3 H, t, *J* 8 Hz, CH_2CH_3), 2.09 (6 H, s, 2 × NCH_3), 2.37 (2 H, q, *J* 8 Hz, CH_2CH_3), 3.78 and 3.92 (each 3 H, s, OCH_3), 3.84 (6 H, s, 2 × OCH_3), 6.34 and 6.50 (each 1 H, d, *J* 2 Hz, ArH of ring c), and 6.68 and 6.83 (each 1 H, s, ArH of ring A); *m/e* 373 (M^+).

Oxidative Cleavage of Protostephanine-α-methine (6a).—Protostephanine-α-methine (64 mg), regenerated as described above from its picrate (103 mg), was dissolved in a mixture of tetrahydrofuran (7 ml), acetic acid (2.75 ml; 0.2M), and aqueous sodium acetate (5.6 ml; 0.2M) to give a solution at pH 5. This was stirred during the addition of osmium tetraoxide (12 mg) in t-butyl alcohol (2 ml), when a brown colour developed. After 5 min, sodium metaperiodate (150 mg) in water (5 ml) was added, whereupon the colour changed to yellow. The mixture was stirred overnight and then poured into saturated aqueous arsenous oxide solution (40 ml). The solution was basified first with solid sodium hydrogen carbonate, and then aqueous sodium hydroxide (10 ml; 30% w/v), to pH 12 and extracted with dichloromethane (4 × 50 ml). The organic layers were washed with water (1 × 50 ml), dried, and evaporated to leave a gum (90 mg). T.l.c. indicated one major component which reacted with 2,4-dinitrophenylhydrazine, but no crystalline derivative of this aldehyde could be formed;

δ 2.10 (6 H, s, 2 × NCH_3), 3.8—4.0 (12 H, 4s, 4 × OCH_3), 6.35 and 6.55 (each 1 H, d, *J* 2.5 Hz, ArH of ring C), 6.76 and 7.52 (each 1 H, s, ArH of ring A), and 9.61 (1 H, s, CHO).

Kuhn–Roth Degradation of Protostephanine Dihydro-α-methine (6b).—Protostephanine-α-methine picrate was converted into the free base by chromatography on alumina as usual, but using as eluant chloroform (A.R. grade) which had been freed from ethanol by passage over neutral alumina (activity I). Platinum oxide (32 mg) was added to a solution of the α-methine (65 mg, 0.175 mmol) in methanol (A.R. grade) (11 ml) and the suspension was stirred under hydrogen (1 atm.) for 2 h to give (as above) the dihydro-α-methine which was dried to constant weight (65 mg). This was transferred to a 3-neck flask and oxidised by addition of chromic acid [25 ml of a solution of chromium trioxide (33.6 g) in concentrated sulphuric acid (40 ml) and water (200 ml)]. The mixture was heated under reflux for 1.75 h and then cooled by addition of water (50 ml; CO_2-free) *via* the condenser (to rinse it out). Using a splash-head, the mixture was distilled and 100-ml fractions were collected. Water (CO_2-free) was added to the distillation flask from a dropping funnel as necessary. The fractions (usually four) were titrated against lithium hydroxide solution (0.05M), with phenolphthalein as indicator, to determine the recovery of acetic and propionic acids (usually quantitative) and then taken to pH 10 with excess of lithium hydroxide solution. The basic solutions were combined, evaporated to a small volume (*ca.* 20 ml), and the pH adjusted to the end-point of phenolphthalein using 1N-hydrochloric acid before evaporation to dryness.

The residue in water (20 drops) and ethanol (10 ml) was mixed with *p*-bromophenacyl bromide (70 mg, 0.35 mmol, freshly recrystallised from ethanol) dissolved in ethanol (6 ml) and the mixture was heated under reflux for 1 h, and then cooled. Removal of the solvents left a residue which was chromatographed on alumina (10 g) using chloroform (A.R. grade) as eluant to yield a gum (59 mg) which was further fractionated by preparative t.l.c. on silica plates which had been activated at 180 °C for 30 min prior to use; development three times with benzene allowed the separation of *p*-bromophenacyl acetate (19 mg, 42%), m.p. 84—85 °C (lit.,[18] 86 °C) [from light petroleum (b.p. 60—80 °C)] and *p*-bromophenacyl propionate (5 mg), m.p. 57—59 °C (lit.,[18] 63 °C) [from light petroleum (b.p. 40—60 °C)].

3,4-*Dimethoxyphenyl*[1-^{13}C]*acetonitrile* (9a).—3,4-Dimethoxybenzyl chloride (4.0 g, 21.5 mmol) in dimethylformamide (100 ml) was treated with sodium cyanide (115 mg, 2.4 mmol) and the mixture was stirred at 20 °C with the exclusion of moisture for 24 h. Sodium [^{13}C]cyanide (230 mg, 4.6 mmol; 90 atom %) was then added and the stirring continued for a further 48 h; unenriched sodium cyanide (705 mg, 14.4 mmol) was then added. After another 48 h, a final portion of sodium cyanide (1.05 g, 21.4 mmol) was added and the mixture was stirred for 48 h. It was then poured into saturated brine (500 ml) and extracted with ethyl acetate (3 × 100 ml). The combined organic layers were washed with water (2 × 100 ml), dried, and evaporated to yield the nitrile (3.25 g, 86%), m.p. 48—50 °C (lit.,[19] 48—51 °C) (from methanol); $\nu_{max.}$ (Nujol) 2 260, 2 190 (^{13}CN stretch), 1 615, and 1 600 cm^{-1}; *m/e* 177 (M^+ for ^{12}C material) and 178 (M^+ for ^{13}C material) (1 : 0.36).

3,4-*Dimethoxyphenyl*[1-^{13}C]*acetic Acid* (9b).—The foregoing nitrile (3.16 g, 0.018 mol) in ethylene glycol (120 ml) was treated with potassium hydroxide (10 g, 0.18 mol) in water (30 ml) and the mixture was heated under reflux for 18 h. The cooled solution was then poured into water (600 ml) and washed with ether (2 × 400 ml). The aqueous layer was acidified with concentrated hydrochloric acid, extracted with ether–chloroform (3 : 1 v/v) (4 × 200 ml), and these latter combined organic layers were washed with water (1 × 500 ml, 2 × 300 ml), dried, and evaporated. Recrystallisation of the residue from benzene–light petroleum (b.p. 60—80 °C) gave the phenylacetic acid (2.6 g, 75%), m.p. 97—98 °C (lit.,[20] 98—99 °C), $\nu_{max.}$ (Nujol) 3 400—2 900br, 1 720, 1 650, 1 620, and 1 600 cm^{-1}; δ_H([$^2H_6$]dimethyl sulphoxide) 3.46 (2 H, s, CH_2CO_2H), 3.74 (6 H, s, 2 × OCH_3), and 6.82 (3 H, s, ArH); *m/e* 196 (M^+ for ^{12}C material) and 197 (M^+ for ^{13}C material) (1 : 0.36).

Unenriched material had δ_C 40.6, 55.1, 55.9, 111.5, 112.8, 121.6, 125.8, and 177.8. Labelled material showed enhancement of one signal (δ_C 177.8).

[^{13}C]*Protostephanine.*—The foregoing acid was converted as detailed in the preceding paper [13] into protostephanine (2) *via* the dienone protostephanone (12). [^{13}C]Protostephanone was diluted with unlabelled material to 5 atom % ^{13}C to provide sufficient compound for rearrangement to form (2) and for degradation of this to the α-methine (6a). Protostephanone (12) had $\delta_C(C_6D_6)$ 33.3, 41.0, 42.1, 46.2, 53.5, 55.0, 55.8, 111.3, 112.0, and 119.0. Labelled material showed enhancement of the signal at δ_C 53.5, and *m/e* 371 (M^+ for ^{12}C material) and 372 (M^+ for ^{13}C material). Protostephanine (2) had $\delta_C(C_6D_6)$ 29.1, 34.2, 47.4, 55.1, 55.8, 57.2, 58.6, 98.2, 105.9, and 113.7. Labelled material showed *ca.* 4-fold enhancement of the resonance at δ_C 58.1, and *m/e* 357 (M^+ for ^{12}C material) and 358 (M^+ for ^{13}C material).

[^{13}C]*Protostephanine-α-methine.*—[^{13}C]Protostephanine (5 atom % ^{13}C) was converted into the α-methine, characterised as its picrate, as described above. Unlabelled α-methine free base had δ_C 23.7, 29.8, 44.3, 55.4, 55.5, 56.1, 58.3, 97.7, 106.5, 107.2, 112.3, 112.7, and 134.7, and the labelled sample showed *ca.* 4-fold enhancement of the signal at δ_C 112.3.

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