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THE BIOSYNTHESIS OF THE ALKALOIDS OF *CROTON SPARSIFLORUS* MORONG†

DEWAN S. BHAKUNI* and SUDHA JAIN
Central Drug Research Institute, Lucknow-226001, India

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Abstract—Incorporation of tyrosine, dopa, dopamine, 4-hydroxyphenylpyruvic acid, (±)-, norcoclaurine-1-carboxylic acid, -norcoclaurine, -coclaurine, and -N-methylcoclaurine into N-methylcrotsparine, N-methylcrotsparinine and N-methylsparsiflorine in *Croton sparsiflorus* Morong has been studied. The evidence supports the direct oxidative coupling of (+)-, and (−)-N-methylcoclaurines to give N-methylcrotsparine and N-methylcrotsparinine respectively. Tracer experiment show that N-methylcrotsparine undergoes dienone-phenol rearrangement to give N-methylsparsiflorine. A double labelling experiment with (±)-N[^{14}C]methyl[1-^{3}H]coclaurine demonstrated that the H atom at the asymmetric centre in the 1-benzylisoquinoline precursor is retained in the bioconversion. The intermediacy of norcoclaurine-1-carboxylic acid and specific incorporation of dehydro-N-methylcoclaurinium salt into the bases have been demonstrated.

Croton sparsiflorus Morong(Euphorbiaceae), a South American plant which has now completely naturalized throughout the plains of India has been extensively investigated for its alkaloidal constituents. The alkaloids isolated from the stems and leaves of the plant are: crotsparine[1,2] (**7**), N-methylcrotsparine[1] (**8**), NO-dimethylcrotsparine[1] (**9**), crotsparinine[3] (**15**), N-methylcrotsparinine[3] (**16**), sparsiflorine[4] (**11**) and N-methylsparsiflorine[1] (**12**). Of the isolated bases N-methylcrotsparine (**8**) is a potent hypotensive agent.[5] N-Methylcrotsparinine (**16**) produced an initial sharp transient fall of blood pressure followed by a gradual sustained hypotension.[5] NO-Dimethylcrotsparine (**26**) and NOO-trimethylsparsiflorine (**25**; X = I) methiodides also exhibited marked hypotensive activity. The structures of the proaporphine and dihydroproaporphine bases of *C. sparsiflorus* have been determined[1] and the absolute configuration at the asymmetric centre of the bases defined by chemical correlation.[6]

According to the most accepted biogenetic theory[7] oxidative cyclisation of coclaurine derivatives which in turn can derive from dopa and tyrosine,[8] can give rise to proaporphine bases. The dihydroproaporphines can form by selective reduction of one double bond of the dienone system. The aporphine bases of the sparsiflorine (**11**) type can arise from proaporphine (**7**) intermediate by dienone–phenol rearrangement.

Barton *et al.*[9] have shown that crotonosine, an isomer of crotsparine (**7**) is stereospecifically biosynthesised from (+)-coclaurine in *Croton linearis*. Preliminary tracer experiments by us demonstrated that crotsparine (**7**), crotsparinine (**15**) and sparsiflorine (**11**) are biosynthesised in *C. sparsiflorus* from (±)-coclaurine.[10] We now present a detailed report on the biosynthesis of N-methylcrotsparine (**8**), N-methylcrotsparinine (**16**) and N-methylsparsiflorine (**12**).

Labelled tyrosine (experiment 1) was initially fed to young cut branches of *Croton sparsiflorus* Morong (Euphorbiaceae) and it was found that N-methylcrotsparine (**8**), N-methylcrotsparinine (**16**) and N-methylsparsiflorine (**12**) were being actively biosynthesised by the plants. In subsequent experiments labelled hypothetical precursors were fed to young cut branches of *C. sparsiflorus*. The results of several feedings are recorded in Table 1. Dopa (experiment 2), dopamine (experiment 3) and 4-hydroxyphenylpyruvic acid (experiment 4) were efficiently incorporated into **8**, **12** and **16**. The regiospecificity of the labelling in biosynthetic N-methylsparsiflorine (**12**) derived from [2-^{14}C]dopa was shown as follows. The labelled N-methylsparsiflorine (**12**, experiment 2) was treated with diazomethane to give radioactive NOO-trimethylsparsiflorine (**13**) which was converted into its methiodide (**25**; X = I) and then into its hydroxide form (**25**, X = OH) with essentially no loss of radioactivity. Hofmann degradation of **25** (X = OH) yielded the methine-I (**22**) which had essentially the same radioactivity as that of the parent base. Second Hofmann degradation of methine-I (**22**) gave radioactive 3,4,6 - trimethoxy - 1 - vinylphenanthrene (**21**) with essentially no loss of radioactivity. Ozonolysis of radioactive **23** yielded radioactive formaldehyde trapped as its dimedone derivative (97.4% of the original activity).

The position of label in the biosynthetic N-methylcrotsparine (**8**) derived from [2-^{14}C]dopa (experiment 2) was established as follows. The labelled **8** was treated with 3N hydrochloric acid to give radioactive N-methylsparsiflorine (**12**) with essentially no loss of radioactivity. Radioactive **12** was degraded to radioactive 3,4,6 - trimethoxy - 1 - vinylphenanthrene (**23**) as above. Ozonolysis of radioactive **23** gave radioactive formaldehyde (98% of original activity).

Biosynthetic N-methylcrotsparine (**8**) derived from [1-^{14}C] dopamine (experiment 3) was treated with 3N HCl to give radioactive N-methylsparsiflorine (**12**) which was degraded as above to give radioactive formaldehyde (97.5% of original activity).

Biosynthetic N-methylsparsiflorine (**12**) derived from [1-^{14}C] dopamine (experiment 3) was treated with diazomethane to give radioactive NOO-trimethylsparsiflorine (**13**) and then degraded to radioactive 3,4,6 - trimethoxy - 1 - vinylphenanthrene (**23**) with essentially no loss of radioactivity. Ozonolysis of radioactive **23** yielded radioactive formaldehyde (98.4% of original activity).

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Table 1. Tracer experiments on *C. sparsiflorus* morong

Expt.	Precursor fed	Incorporation % N-Methylcrotsparine(8)	N-Methylcrotsparinine(16)	N-Methylsparsiflorine(12)
1	(±)-[2-^{14}C] Tyrosine	0.30	0.28	0.24
2	(±)-[2-^{14}C] Dopa	0.36	0.30	0.28
3	1-[^{14}C] Dopamine	0.38	0.22	0.26
4	[3,5-$^{3}H_2$] 4-Hydroxyphenylpyruvic acid	0.20	0.19	0.18
5	[3,5-$^{3}H_2$]Tyramine	0.05	0.03	0.04
6	(±)-[3-^{14}C] Norcoclaurine-1-carboxylic acid(6)	0.38	0.34	0.34
7	(±)-[1-^{3}H] Norcoclaurine(18)	0.48	0.38	0.33
8	(±)-[3',5',8-$^{3}H_3$] Coclaurine(19)	0.64	0.54	0.61
9	(±)-N-Methyl[3' 5',8-$^{3}H_3$] coclaurine(20)	0.88	0.83	0.81
10	(±)-NOO-Trimethoxy[3',5',8-$^{3}H_3$]coclaurine	0.0005	0.0007	0.001
11	(±)-N[^{14}C] Methylnorcoclaurine	0.006	0.004	0.008
12	(±)-N[^{14}C] Methylcoclaurine(20)	0.88	0.67	0.70
13	(±)-N[^{14}C] Methyl[1-^{3}H] coclaurine(20)	1.08	0.98	0.79
14	Didehydro-N-[^{14}C] methylcoclaurinium iodide(17)	0.42	0.36	0.42
15	(+)-(S)-N-Methyl[3',5',8-$^{3}H_3$]coclaurine(5)	1.97	0.019	1.48
16	(-)-(R)-N-Methyl[3',5',8-$^{3}H_3$]coclaurine (10)	0.022	1.69	0.0015
17	N-Methyl[5-^{14}C]crotsparine(8)	-	0.16	7.70
18	[5-^{14}C] Crotsparine(7)	6.20	0.12	7.06
19	N-Methyl[5-^{14}C] crotsparinine(16)	0.11	-	0.05

Specific incorporation of dopamine (**3**) and 4-hydroxyphenylpyruvic acid (**4**) into N-methylcrotsparine (**8**), N-methylsparsiflorine (**12**) and N-methylcrotsparinine (**16**) implies norcoclaurine - 1 - carboxylic acid (**6**) as an intermediate. The intermediacy of **6** in the biosynthesis of **8**, **12** and **16** was demonstrated as follows: (±)-[3-^{14}C] Norcoclaurine - 1 - carboxylic acid (**6**, experiment 6) was fed to young cut branches of *C. sparsiflorus* and the biosynthetic N-methylcrotsparine (**8**), N-methylsparsiflorine (**12**) and N-methylcrotsparinine (**16**) were isolated. Radioactive **8** was converted into radioactive **12** and then degraded to radioactive **23** with essentially no loss of radioactivity. Ozonolysis of radioactive **23** afforded radioactive formaldehyde (98.4% of the original activity). Biosynthetic **12** was similarly degraded to yield radioactive formaldehyde (97.6% of the original activity).

Feeding of (±)-norcoclaurine (**18**, experiment 7), (±)-coclaurine (**19**, experiment 8) and (±)-N-methylcoclaurine (**20**, experiment 9) demonstrated that **18**, **19** and **20** were efficient precursors of **8**, **12** and **16**. (±)-N-Methylnorcoclaurine (experiment 11) was not incorporated. The results showed that N-methylation of norcoclaurine does not precede O-methylation in the biosynthesis of N-methylcoclaurine. (±)-NOO-Trimethylcoclaurine (experiment 10), as expected, was not incorporated into **8**, **12** and **16**.

The regiospecificity of ^{14}C label in the biosynthetic N-methylcrotsparine (**8**) derived from the feeding of (±)-N [^{14}C] methylcoclaurine (**20**, experiment 12) was established as follows. Labelled N-methylcrotsparine (**8**) was treated with 3N HCl to give radioactive N-methylsparsiflorine (**12**) which was degraded as above to radioactive methine-I (**22**). Treatment of radioactive **22** with dimethyl sulphate-potassium hydroxide gave trimethylamine, trapped as its hydrochloride, which had essentially the same molar radioactivity as the parent base.

Biosynthetic N-methylsparsiflorine (**12**) derived from (±)-N [^{14}C] methylcoclaurine (experiment 12) was degraded as above to radioactive trimethylamine which had essentially the same molar radioactivity as the parent base.

Feeding of (±)-N[^{14}C]methyl[1-^{3}H]coclaurine (**20**, experiment 13) afforded N-methylcrotsparine (**8**), N-methylsparsiflorine (**12**) and N-methylcrotsparinine (**16**) each labelled both with ^{14}C and ^{3}H. Moreover, the ^{14}C:^{3}H ratios in the precursor and each of the biosynthetic bases were essentially unchanged. The experiment thus demonstrated that the H atom at the asymmetric centre in N-methylcoclaurine (**20**) is retained in the bioconversion of **20** into **8**, **12** and **16**.

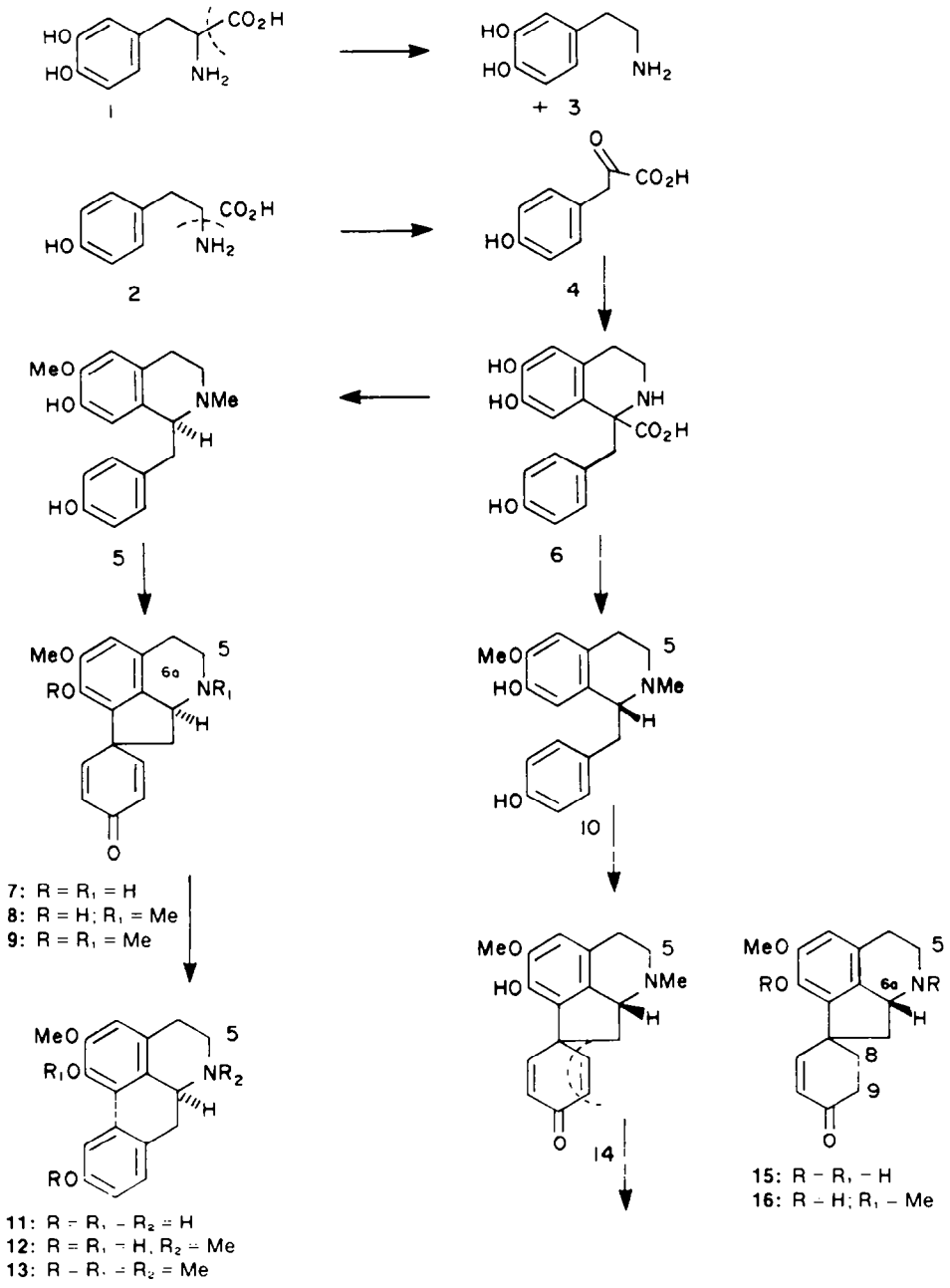
Feeding of didehydro - N - [^{14}C]methylcoclaurinium iodide (**17**, experiment 14) gave radioactive N-methylcrotsparine (**8**), N-methylsparsiflorine (**12**) and N-methylcrotsparinine (**16**). The position of the label in the biosynthetic N-methylcrotsparine (**8**) was determined as follows. Labelled **8** was treated with methyl iodide-sodium methoxide to give radioactive NO-dimethylcrotsparine methiodide (**26**) which was converted into its hydroxide form **27** by IR-410 anion exchange resin. Hofmann degradation of the radioactive **27** furnished the radioactive methine (**28**). Treatment of the radioactive **28** with dimethyl sulphate-potassium hydroxide gave trimethylamine, trapped as its hydrochloride which had essentially the same radioactivity as the parent base.

N-Methylsparsiflorine (**12**) derived from the feeding of didehydro - N[^{14}C]methylcoclaurinium iodide (**17**, experiment 14] was treated with diazomethane to give radioactive NOO-trimethylsparsiflorine (**13**). Radioactive **13** was degraded to radioactive methine-I (**22**) with essentially no loss of radioactivity. Treatment of radioactive **22** with dimethyl sulphate-potassium hydroxide gave the radioactive trimethylamine which had essentially the same molar radioactivity as the parent base.

Cleavage of the N-methyl group of the biosynthetic N-methylcrotsparinine (**16**) derived from didehydro - N[^{14}C]methylcoclaurinium iodide (**17**, experiment 14) by Ziesel method afforded radioactive methyl iodide which had, as expected, essentially half the molar activity of **16**.

N-Methylcrotsparine (**8**) and N-methylcrotsparinine (**16**) have opposite configuration at the asymmetric centre while N-methylcrotsparine (**8**) and N-methylsparsiflorine (**12**) have the same configuration at the corresponding asymmetric centres.[6] If N-methylcrotsparine (**8**) is a biosynthetic precursor of N-methylcrotsparinine (**16**), a change of configuration should occur at position 6a in the proaporphine (**8**) during the course of biochemical transformations. Alternately N-methylcrotsparine (**8**) and N-methylcrotsparinine (**16**) could be biosynthesized by independent routes from (*S*)-N-methylcoclaurine (**5**) and (*R*)-N-methylcoclaurine (**10**) respectively. Stuart and Graham[11,12] have reported that in *Croton linearis* the dihydroproaporphine, linearisine having opposite configuration at asymmetric centre is converted into proaporphine alkaloid, crotonosine. If the same enzyme system is present in *C. sparsiflorus* N-methylcrotsparinine (**16**) should be incorporated into N-methylcrotsparine (**8**). Feeding experiments with N-methyl[5-^{14}C]crotsparine (**8**, experiment 17) and N-methyl[5-^{14}C]crotsparinine (**16**, experiment 19) showed that **8** and **16** are not interconvertible and probably an intermediate of the type **21** is not involved in the biosynthesis of these bases.

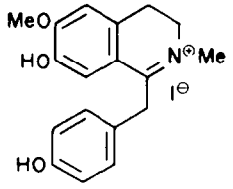
Parallel feedings with (+)-(*S*)-, and (−)-(*R*)-, N-methylcoclaurines **5** and **10** respectively demonstrated



that the stereospecificity is maintained in the biosynthesis of proaporphine, dihydroproaporphine and aporphine alkaloids of *C. sparsiflorus*. (*S*)(+)-N-Methylcoclaurine (**5**) was incorporated into N-methylcrotsparine (**8**, experiment 15) and N-methylsparsiflorine (**12**, experiment 15) about 89 and 95 times respectively, more efficiently than *R*-isomer (**10**). (*R*)-N-Methylcoclaurine (**10**) was incorporated into N-methylcrotsparinine (**16**, experiment 16) about 86 times more efficiently than the *S*-isomer.

Stereospecific incorporation of (*S*)-N-methylcoclaurine (**5**) into N-methylsparsiflorine (**12**) implies (*S*)-N-methylcrotsparine (**8**) as an intermediate. When (*S*)-N-methyl[5-^{14}C]crotsparine (**8**, experiment 17) fed to young cut branches of *C. sparsiflorus* it was efficiently incorporated into **12**. [5-^{14}C]Crotsparine (**7**, experiment 18) was also efficiently incorporated into **12**. N-Methyl[5-^{14}C]crotsparinine (**16**, experiment 19) which had the opposite configuration at the asymmetric centre to that of **8** and **12** and a double bond at C_8–C_9 was not utilized by the plants to form **8** and **12**. The regiospecificity of the label in the biosynthetic N-methylsparsiflorine (**12**) derived from N-methyl[5-^{14}C]crotsparine (**8**) was demonstrated as follows. Labelled **12** was treated with diazomethane to give radioactive NOO-trimethylsparsiflorine (**13**) with essentially no loss of radioactivity. Radioactive **13** was degraded to radioactive 3,4,6 - trimethoxy - 1 - vinylphenanthrene (**13**). Ozonolysis of **23** gave radioactive formaldehyde (98.3% of original activity).

N-Methylcoclaurine (**20**) was specifically incorporated into N-methylcrotsparine (**8**), N-methylsparsiflorine (**12**) and N-methylcrotsparinine (**16**). The presence of N-methylcoclaurine (**20**) in *C. sparsiflorus* was established by trapping experiments. Thus **20** was a true precursor of **8**, **12** and **16**. The foregoing tracer experiments strongly support the following sequence for the biosynthesis of N-methylcrotsparine (**8**), N-methylsparsiflorine (**12**) and N-methylcrotsparinine (**16**) in *C. sparsiflorus* Morong. Tyrosine + dopa → 4-hydroxyphenylpyruvic acid +



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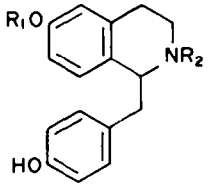
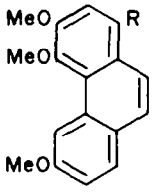
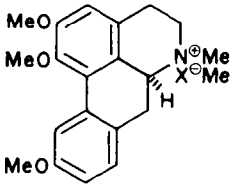


18: R = R_1 = R_2 = H
19: R = R_2 = H; R_1 = Me
20: R = H; R_1 = R_2 = Me



22: R = $-(CH_2)_2NMe_2$
23: R = $-CH=CH_2$
24: R = CHO

21



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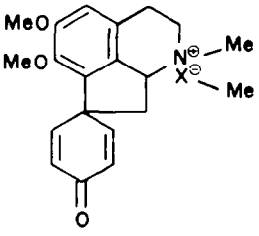
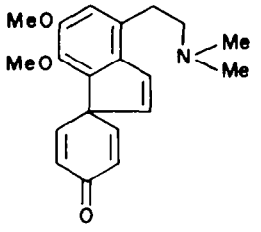


26: X = I
27: X = OH



28

dopamine → norcoclaurine - 1 - carboxylic acid (**6**) → norcoclaurine (**18**) → coclaurine (**19**) → (*S*) - (+) - N - methylcoclaurine (**5**) → N - methylcrotsparine (**8**) → N-methylsparsiflorine (**12**). (*R*)-(−)-N-Methylcoclaurine (**10**) → N-methylcrotsparinine (**16**).

EXPERIMENTAL

For general directions (spectroscopy details and counting method) see Ref. 16.

Synthesis of precursors. The racemates of **19**,[13] **18**[14] and **20**[15] were prepared by standard procedures.

(±)-N-Methylnorcoclaurine, **17**[16] and **6**[8] were prepared by the procedure described earlier.

Resolution—(±) - Di - O - bisbenzyl - N - methylcoclaurine was resolved by treatment with (−)-, and (+) - di - p - toluoyltartaric acid. Hydrogenolysis of the benzyl ethers with HCl furnished (−)-(*R*) - N - *methylcoclaurine* (**10**), m.p. 177–178° and $[\alpha]_D$ − 120° (*c*, 1.0 in MeOH) (lit.[15] 178° and 121.8° in MeOH) and (+) - (*S*) - N - *methylcoclaurine* (**5**), m.p. 178°; $[\alpha]_D$ + 123° (*c*, 0.5 in MeOH) (lit.[15] 178–179° and + 124° in MeOH).

Labelling of precursors—Tritiation. (±)-**19** hydrochloride (120 mg) in tritiated H_2O (0.5 ml, 80 mCi) containing t-BuOK (210 mg) was heated under N_2 (sealed tube) for 120 hr at 100° and worked up as earlier[10] to give (±)-[3′,5′,8-$^3H_3$]coclaurine hydrochloride (80 mg). The other 1-benzylisoquinoline precursors were tritiated in the same way.

(±)-N-Methyl[1-^{3}H]coclaurine and (±)-[1-^{3}H]norcoclaurine were prepared by reduction of the corresponding dihydroisoquinolines with potassium[^{3}H]borohydride in dry dimethyl formamide.

(±) - N - [^{14}C]Methylcoclaurine, (±) - N - [^{14}C]methylnorcoclaurine and didehydro - N - [^{14}C]methylcoclaurinium iodide were prepared by treating the corresponding dihydroisoquinolines with [^{14}C]MeI and subsequent reduction of the methiodides with $NaBH_4$.

(±) - N - [^{14}C]Methyl[1-^{3}H]coclaurine was prepared by mixing (±) - N[^{14}C]methylcoclaurine and (±) - N - methyl[1-^{3}H]coclaurine. (±) - NOO - Trimethyl[3′,5′,8-$^3H_3$]coclaurine was prepared by treating (±) - N - methyl[3′,5′,8-$^3H_3$]coclaurine with CH_2N_2.

(*S*) - [5-^{14}C] - **8**, (*R*) - [5-^{14}C] - **16** and (*S*) - [5-^{14}C] - **7** were prepared by feeding (±) - [3-^{14}C] - **20** and (±)[3-^{14}C] - **19** to young cut branches of *C. sparsiflorus*.

Feeding experiment. For feeding purposes tyrosine, dopa, dopamine, N-methylcoclaurine, NOO-trimethylcoclaurine were dissolved in H_2O (1 m) containing tartaric acid (12 mg). Coclaurine hydrochloride, didehydro - N - methylcoclaurinium iodide, norcoclaurine, N - methylnorcoclaurine and norcoclaurine - 1 - carboxylic acid hydrochlorides, N - methylcrotsparine, N - methylcrotsparinine, crotsparine, and 4 - hydroxyphenylpyruvic acid were dissolved in H_2O (1 ml) and dimethylsulphoxide (0.2 ml). Freshly cut young branches of *C. sparsiflorus* were dipped into the soln of the precursors to take up the precursor. When uptake was complete H_2O was added for washing. The twigs were then dipped in H_2O, left for 6 to 8 days and worked up for N - methylcrotsparine, N - methylcrotsparinine and N - methylsparsiflorine.

Isolation of alkaloids

N-Methylcrotsparine (**8**). The twigs (typically 114 g, wet wt.) of *C. sparsiflorus* were macerated in EtOH (250 ml) with radioinactive **8**, (125 mg) and left for 10 hr. The EtOH was decanted and the plant material was percolated with fresh EtOH (6 × 200 ml). The extract was concentrated under reduced pressure to give a greenish viscous mass which was extracted with 10% tartaric acid (5 × 15 ml). The aqueous acidic extract was defatted with hexane (4 × 10 ml), basified with Na_2CO_3 (pH 8) and extracted with $CHCl_3$ (6 × 10 ml). The $CHCl_3$ extract was washed with H_2O (3 × 15 ml), dried (Na_2SO_4) and the solvent removed. The crude base (140 mg), so obtained, was taken in $CHCl_3$, passed through a column of neutral Al_2O_3 and the purified material subjected to preparative tlc on SiO_2 plates (solvent: $CHCl_3$:MeOH, 94:6). The region containing the desired alkaloid was cut and eluted with $CHCl_3$:MeOH (80:20). The solvent from the eluate was removed and the residue crystallized from EtOAc to give N - *methylcrotsparine* (**8**) (78 mg), m.p. 224–226° (lit.[2] 223–225°).

N-*Methylcrotsparinine* (**16**). The twigs (105 g, wet wt.) of *C. sparsiflorus* were macerated in EtOH (250 ml) with radioinactive **16** (95 mg). The plant material was extracted with EtOH and the extract worked up as above to give N - *methylcrotsparinine* (**16**) (60 mg) m.p. 160–161° (lit.[3] 160–161°).

N-*Methylsparsiflorine* (**12**). The twigs (127 g, wet wt.) were macerated in EtOH (250 ml) with radioinactive **12** (122 mg). The plant material was extracted with EtOH and the extract worked up as above to give N - *methylsparsiflorine* (**12**) (81 mg) m.p. 148–150° (lit.[1] 148–151°).

Degradations of the biosynthetic alkaloids

1. *N - Methyl*[5-^{14}C]*sparsiflorine* (**12**) *derived from* (±)[2-^{14}C]*dopa.* Labelled **12** (180 mg; molar activity 8.68×10^{-2} μCi m mol^{-1}) (experiment 2) in MeOH (3 ml) was treated with an excess of ethereal CH_2N_2 to give the radioactive NOO-*trimethyl sparsiflorine* (**13**; 168 mg; molar activity 8.62×10^{-2} μCi m mol^{-1}).

Radioactive **13** (165 mg) in MeOH (3 ml) was refluxed with MeI (1 ml) for 2 hr to give radioactive NOO - *trimethylsparsiflorine methiodide* (**25**; X = I) (168 mg) m.p. 216–217° (dec) (molar activity 8.50×10^{-2} μCi m mol^{-1}) (lit.[4] 218°).

A soln of the radioactive **25** (160 mg) in MeOH was passed through a column of freshly generated IR-410 anion exchange resin (OH^- form) (10 g) to give the corresponding radioactive methohydroxide (**25**; X = OH). Radioactive **25** (X = OH) was heated with KOH (1.5 g in 10 ml H_2O) at 100° for 4 hr to give the radioactive *methine-I* (**22**; 130 mg) as an oil (molar activity 8.60×10^{-2} μCi m mol^{-1}).

Radioactive methine-I (**22**; 125 mg) in MeOH (2 ml) was heated with MeI (1 ml) to give the corresponding radioactive *methine-*I *methiodide* (121 mg) m.p. 276° (molar activity 8.45×10^{-2} μCi m mol^{-1}) which was converted into its hydroxide form with IR-410 anion exchange resin. The radioactive methohydroxide was heated with KOH (1.4 g in 10 ml H_2O) at 100° for 2 hr. The resulting mixture was cooled, diluted with H_2O and extracted with Et_2O (5 × 30 ml). The ether extract was washed with H_2O, dried (Na_2SO_4) and the solvent removed to give the radioactive 3,4,6 - *trimethoxy* - 1 - *vinylphenanthrene* (**23**; 81 mg) m.p. 90° (molar activity 8.42×10^{-2} μCi m mol^{-1}).

Ozonized O_2 was passed through a soln of radioactive **23** (74 mg) in EtOAc (7 ml) at − 78° for 10 min. The solvent from the resulting mixture was removed under reduced pressure. To the residue H_2O (30 ml), Zn dust (320 mg) and $AgNO_3$ (13 mg) were added. The mixture was refluxed for 20 min, the liberated radioactive HCHO distilled and the distillate was collected in a soln of dimedone (300 mg) in aqueous EtOH (50 ml). After 1 hr the soln was concentrated to 10 ml and then left for 12 hr. The ppt was taken in $CHCl_3$ and chromatographed over SiO_2 column. Elution with $CHCl_3$ (tlc control) gave radioactive *formaldehyde dimethone* m.p. 192–193° (molar activity 8.45×10^{-2} μCi m mol^{-1}; 97.4% of original).

2. N-*Methylcrotsparine* (**8**) *derived from* (±)-[2-^{14}C]*dopa.* Labelled **8** (270 mg; experiment 2) was heated with 3N HCl to give radioactive N-*methylsparsiflorine* (**12**; 262 mg) hydrochloride. Radioactive **12** was degraded as above to 3,4,6 - *trimethoxy* - 1 - *vinylphenanthrene* (**23**). Ozonolysis of the radioactive **23** yielded radioactive HCHO. The radioactivity of the degradation products is given in Table 2.

3. N-*Methyl*[5-^{14}C]*sparsiflorine* (**12**) *derived from* [1-^{14}C]*dopamine.* Labelled **12** (295 mg, experiment 3) was degraded as above to give the radioactive HCHO. The radioactivity of the degradation products is given in Table 3.

4. N-*Methyl*[5-^{14}C]*crotsparine* (**8**) *derived from* [1-^{14}C]*dopamine.* Labelled **8** (295 mg; experiment 3) was heated with 3N HCl to give the radioactive N-*methylsparsiflorine* (**12**) *hydrochloride* (272 mg). Radioactive **12** was degraded as above to give the radioactive HCHO. The radioactivity of the degradation products is given in Table 4.

5. N-[^{14}C]*Methylsparsiflorine* (**12**) *derived from didehydro*-N[^{14}C]*methylcoclaurinium iodide* (**17**). Labelled **12** (310 mg;

Table 2.

Compound	Molar activity (μCi m mol^{-1})
N-Methylcrotsparine (8)	9.68×10^{-2}
N-Methylsparsiflorine (12)	9.60×10^{-2}
NOO-Trimethylsparsiflorine (13)	9.52×10^{-2}
Methine-I (22)	9.50×10^{-2}
3,4,6-Trimethoxy-1-vinylphenanthrene (23)	9.58×10^{-2}
Formaldehyde–dimethone	9.48×10^{-2}

Table 3.

Compound	Molar activity (μCi m mol^{-1})
N-Methylsparsiflorine (12)	8.36×10^{-2}
NOO-Trimethylsparsiflorine (13)	8.30×10^{-2}
Methine-I (22)	8.26×10^{-2}
3,4,6-Trimethoxy-1-vinylphenanthrene (23)	8.20×10^{-2}
Formaldehyde–dimethone	8.13×10^{-2}

Table 4.

Compound	Molar activity (μCi m mol^{-1})
N-Methylcrotsparine (8)	9.87×10^{-2}
N-Methylsparsiflorine (12)	9.82×10^{-2}
NOO-Trimethylsparsiflorine (13)	9.77×10^{-2}
Methine-I (22)	9.70×10^{-2}
3,4,6-Trimethoxy-1-vinylphenanthrene (23)	9.74×10^{-2}
Formaldehyde–dimethone	8.62×10^{-2}

experiment 14) was degraded to the radioactive methine-I (22; 248 mg). The radioactive 22 (240 mg) in aqueous EtOH (10 ml) at 0° was stirred with $(Me)_2SO_4$ (0.5 ml) and 10N KOH (0.25 ml) for 1 hr. At hourly intervals $(Me)_2SO_4$ (0.25 ml) and 10N KOH (0.13 ml) were added, 3 times. After 5 hr KOH (6 g) was added and the mixture refluxed for 2 hr. The radioactive trimethylamine, so formed, was distilled and collected in 20% HCl. The remaining alkaline soln was extracted with Et_2O (4 × 50 ml), washed with H_2O, dried (Na_2SO_4) and the solvent removed to give the radioactive 3,4,6 - *trimethoxy* - 1 - *vinylphenanthrene* (23). The radioactivity of the degradation products is given in Table 5.

6. N-[^{14}C]*Methylcrotsparine* (8) *derived from didehydro*-N[^{14}C]*methylcoclaurinium iodide* (17). A mixture of labelled 8 (283 mg; experiment 14), MeOH (3 ml) MeI (1 ml) and MeONa (2 ml) was refluxed for 10 hr to give radioactive NO-*dimethylcrotsparine methiodide* (26; 276 mg) m.p. 170°.

Radioactive 26 (263 mg) in MeOH was passed through a column of freshly generated IR-410 anion exchange resin (OH form; 10 g) and the process was repeated five times. The column was finally washed with MeOH (100 ml). The solvent from the methanolic soln was removed to afford 27. Radioactive 27 in MeOH (10 ml) was refluxed with KOH (3.5 g) for 2 hr to give the radioactive *methine* (28; 197 mg) m.p. 90° [Found: C, 73.60; H, 7.32; N, 4.14. $C_{20}H_{23}NO_3$ requires; C, 73.84; H, 7.07; N, 4.30%]; ν max 2900, 1660, 1620, 1600, 1495, 1220 and 1122 cm^{-1}; λ_{max} 230 and 300 nm; $[\alpha]_D^{25}$ 0° (*c*, 1.1 in $CHCl_3$); NMR τ7.85 (s, 6H, NMe_2), 6.42 (s, 3H, C_1-OCH_3), 6.20 (s, 3H, C_2-OCH_3), 2.97 (s, 1H, aryl-H) and six olefinic protons multiplets centred at τ3.32, 3.79 and 4.74.

A mixture of radioactive 28 (190 mg), H_2O (2 ml), Me_2SO_4 (1 ml) at pH 10 adjusted with KOH aq was stirred for 1 hr. At hourly intervals Me_2SO_4 (0.5 ml) and 10N KOH (0.25 ml) were added. The resulting mixture was refluxed for 2 hr after adding KOH (6 g). The radioactive trimethylamine, so evolved, was distilled and collected in 15% HCl. The radioactivity of the degradation products is given in Table 6.

7. N-*Methyl*[5-^{14}C]*crotsparine* (8) *derived from* (±)[3-^{14}C]*norcoclaurine* - 1 - *carboxylic acid* (6). Labelled 8 (299 mg; experiment 6) was heated with 3N HCl to give the radioactive N-*methylsparsiflorine* (12) HCl (268 mg) which was degraded to give the radioactive HCHO as described above. The radioactivity of the degradation products is given in Table 7.

8. N-*Methyl*[5-^{14}C]*sparsiflorine* (12) *derived from* (±)-[3-^{14}C]*norcoclaurine* - 1 - *carboxylic acid* (6). Labelled 12 (293 mg; experiment 6) was degraded as above to give the radioactive HCHO. The radioactivity of the degradation products is given in Table 8.

9. N-*Methylcrotsparine* (12) *derived from* (±)-N-[^{14}C]*methylcoclaurine* (20). Labelled 12 (268 mg; experiment 12) was treated with 3N HCl to give radioactive N-*methylsparsiflorine* (12; 252 mg) which was degraded as above to give radioactive *trimethylamine*. The radioactivity of the degradation products is given in Table 9.

10. N-[^{14}C]*Methylsparsiflorine* (12) *derived from* (±)-N-[^{14}C]*methylcoclaurine* (20). Labelled 12 (282 mg; experiment 12) was degraded as above to give radioactive *trimethylamine*. The radioactivity of the degradation products is given in Table 10.

Table 5.

Compound	Molar activity (μCi m mol^{-1})
N-Methylsparsiflorine (12)	8.77×10^{-2}
NOO-Trimethylsparsiflorine (13)	8.60×10^{-2}
Methine-I (22)	8.54×10^{-2}
3,4,6-Trimethoxy-1-vinylphenanthrene (23)	8.58×10^{-2}
Trimethylamine HCl	8.50×10^{-2}

Table 6.

Compound	Molar activity (μCi m mol^{-1})
N-Methylcrotsparine (8)	9.62×10^{-2}
NO-Dimethylcrotsparine methiodide (26)	9.68×10^{-2}
Methine (28)	9.54×10^{-2}
Trimethylamine HCl	9.50×10^{-2}

Table 7.

Compound	Molar activity (μCi m mol^{-1})
N-Methylcrotsparine (8)	7.55×10^{-2}
N-Methylsparsiflorine (12)	7.50×10^{-2}
NOO-Trimethylsparsiflorine (13)	7.45×10^{-2}
Methine-I (22)	7.48×10^{-2}
3,4,6-Trimethoxy-1-vinylphenanthrene (23)	7.38×10^{-2}
Formaldehyde–dimethone	7.40×10^{-2}

Table 8.

Compound	Molar activity (μCi m mol^{-1})
N-Methylsparsiflorine (12)	9.32×10^{-2}
NOO-Trimethylsparsiflorine (13)	9.20×10^{-2}
Methine-I (22)	9.17×10^{-2}
3,4,6-Trimethoxy-1-vinylphenanthrene (23)	9.25×10^{-2}
Formaldehyde–dimethone	9.10×10^{-2}

Table 9.

Compound	Molar activity (μCi m mol^{-1})
N-Methylcrotsparine (**8**)	8.49×10^{-2}
N-Methylsparsiflorine (**12**)	8.30×10^{-2}
NOO-Trimethylsparsiflorine (**13**)	8.28×10^{-2}
Methine-I (**22**)	8.33×10^{-2}
Trimethylamine HCl	8.36×10^{-2}

Table 10.

Compound	Molar activity (μCi m mol^{-1})
N-Methylsparsiflorine (**12**)	10.38×10^{-2}
NOO-Trimethylsparsiflorine (**13**)	10.30×10^{-2}
Methine-I (**22**)	10.22×10^{-2}
Trimethylamine HCl	10.10×10^{-2}

Table 11.

Compound	Molar activity (μCi m mol^{-1})
N-Methylsparsiflorine (**12**)	10.32×10^{-2}
NOO-Trimethylsparsiflorine (**13**)	10.20×10^{-2}
Methine-I (**22**)	10.25×10^{-2}
3,4,6-Trimethoxy-1-vinylphenanthrene (**23**)	10.16×10^{-2}
Formaldehyde–dimethone	10.11×10^{-2}

11. N-*Methyl*[5-^{14}C]*sparsiflorine* (**12**) *derived from* N-*methyl*[5-^{14}C]*crotsparine* (**8**). Labelled **12** (278 mg; experiment 17) was degraded as above to give radioactive 3,4,6 - *trimethoxy* - 1 - *vinylphenanthrene* (**23**). Radioactive **23** was degraded to radioactive HCHO. The radioactivity of the degradation products is given in Table 11.

Feeding of N - [^{14}C]*methyl*[1-^{3}H]*coclaurine*. (±) - N[^{14}C]Methyl[1-^{3}H]coclaurine (**20**; experiment 13) (activity ^{14}C 0.016 mCi and ^{3}H 0.40 mCi) was dissolved in H_2O (1 ml) containing tartaric acid (10 mg). Freshly cut young branches of *C. sparsiflorus* were dipped in the soln of the precursor to take up the precursor; when uptake was complete water was added for washing. The plants were then left for 8 days and worked up for **8**, **12** and **16**. The ^{14}C:^{3}H ratios in the precursor and biosynthetic bases determined. The values are recorded in Table 12.

Trapping experiment. (L)-[U-^{14}C]Tyrosine (activity 0.1 mCi) was fed to young cut branches of *C. sparsiflorus*. After 7 days the plants were harvested. The plant material (120 g, wet wt.) was macerated in EtOH (200 ml) with inactive (±)-N **20** (120 mg). After 10 hr EtOH was decanted and the plant material percolated with fresh EtOH (6 × 150 ml) containing 2% AcOH. The solvent from the ethanolic extract was distilled under reduced pressure. The greenish viscous mass, so obtained, was extracted with AcOH (5 × 10 ml). The aqueous acidic soln was defatted with hexane and basified with Na_2CO_3. The liberated bases were extracted with $CHCl_3$, washed with H_2O, dried (Na_2SO_4) and the solvent removed. The crude base, so obtained, was subjected to preparative tlc of SiO_2 plates to give N-*methylcoclaurine* (99 mg) m.p. 177–178° (lit.[15] 178–179°) (molar activity 10.27×10^{-2} μCi m mol^{-1}; incorporation 0.2%).

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Table 12.

Label	N-Methylcoclaurine (**20**)	N-methylcrotsparine (**8**)	N-methylcrotsparinine (**16**)	N-methylsparsiflorine (**12**)
^{14}C	1	1	1	1
^{3}H	30	28	27	31