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BIOSYNTHESIS OF MAGNOFLORINE AND LAURIFOLINE

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Abstract- The incorporation of (±)-, nor-laudanosoline, nor-protosinomenine, nor-orientaline, nor-reticuline and reticuline and reticuline methiodide into magnoflorine and laurifoline has been studied and specific incorporation of nor-reticuline and reticuline demonstrated. Parallel feeding experiments with (+)-*S* and (−)-(*R*)-reticulines demonstrated specific incorporation of (+)-(*S*)-isomer into these bases.

According to the established biogentic theory,[1] the quaternary aporphine alkaloids magnoflorine[2] (**13**) and laurifoline,[2] (**8**) well known for their curarizing and hypotensive activities[3] may be formed in nature from 1-benzyltetrahydroisoquinoline precursors by alternate biosynthetic routes[4] as follows: Direct *ortho–para* and *ortho–ortho* oxidative coupling of reticuline (**1**) can give isoboldine (**4**) and corytuberine (**10**) skeletons respectively. Laurifoline (**8**) and magnoflorine (**13**) may then form from **4** and **10** respectively by quaternisation of tertiary N atoms. In the second possibility *ortho–para* oxidative coupling of orientaline (**2**) may afford orientalinone[5] (**7**). Dienone-phenol rearrangement as shown in **7** may then yield bracteoline (**5**) and isocorytuberine (**11**) skeletons. Compounds **8** and **13** may then form from **5** and **11** respectively by change in orientation of OH and OMe groups in ring D and quaternisation of the N atoms. The third possibility is that protosinomenine (**3**) by *para–para* and *ortho–para* oxidative couplings may give neoproaporphines **15** and **16**. Dienone-phenol rearrangement may then yield boldine (**6**) and N-methyllinocarpine (**12**) skeletons.[6] Laurifoline (**8**) and magnoflorine (**13**) may then form from **6** and **12** by change in position of OH and OMe groups in ring A and quaternisation of tertiary N atoms.

Quaternary aporphine alkaloids of magnoflorine and laurifoline types may also be formed in nature by oxidative coupling of suitably substituted quaternary 1-benzyltetrahydroisoquinoline precursors. Synthesis of (+)-laurifoline (**8**) from (+)-tembetarine[7] supports this idea.

Tracer experiments have shown that (±)-reticuline (**18**) in *Aquilegia* species[8] is specifically incorporated with high efficiency into magnoflorine (**13**) whereas the incorporation of norprotosinomenine (**20**) into **13** was negligible. We now report the results of tracer experiments which define the biosynthesis of magnoflorine (**13**) and laurifoline (**8**) in *C. laurifolius*.

Tyrosine (experiment 1) was initially fed to young cut branches of *C. laurifolius* (Menispermaceae) and it was found that the plants were biosynthesizing magnoflorine (**13**) and laurifoline (**8**). In subsequent experiments labelled hypothetical precursors were fed to young cut branches of *C. laurifolius* plants. The results of several feedings are recorded in the Table 1.

Feeding of tyrosine in parallel with (±)-, nor-orientaline (**19**) (experiment 7), non-protosinomenine (**20**) (experiment 6), and laudanosine (**21**) (experiment 10) revealed that these 1-benzyltetra-hydroisoquinoline derivatives are very poorly metabolised by the plants to form magnoflorine (**13**) and laurifoline (**8**). Feeding with norlaudanosoline (**17**) (experiment 8) and reticuline (**18**) (experiment 3) showed that **17** and **18** are efficient precursors of both **8** and **13**.

The regiospecificity of label in biosynthetic laurifoline (**8**) derived from the feeding of (±)-[3-^{14}C] nor-reticuline (**27**) (experiment 13) was determined as follows: **8** was treated with MeI–MeONa to give glaucine methoiodide (**9**, x = 1) which had essentially

Table 1. Tracer experiments on *Cocculus laurifolius*

Expt. No.	Precursor	(%) Incorporation	
		Magnoflorine	Laurifoline
1	(L)-[U-^{14}C] Tyrosine	0.079	0.06
2	[2-^{14}C] Dopamine	0.21	0.18
3	(±)-[2′,6′,8-$^{3}H_3$] Reticuline (**18**)	1.24	0.69
4	(±)-[2′,6′,8-$^{3}H_3$] Reticuline methoiodide (**23**)	0.075	0.082
5	(±)-[N-methyl-^{14}C] Reticuline (**18**)	1.17	0.52
6	(±)-[Aryl-^{3}H] Norprotosinomenine (**20**)	0.005	0.098
7	(±)-[Aryl-^{3}H] Nororientaline (**19**)	0.0014	0.001
8	(±)-[1-^{3}H] Norlaudanosoline (**17**)	0.32	0.21
9	(±)-[4′-methoxy-^{14}C, 1-^{3}H] Reticuline (**18**)	1.7	0.82
10	(±)-[2′,6′,8-$^{3}H_3$] Laudanosine (**21**)	0.004	0.002
11	(+)-[2′,6′,8-$^{3}H_3$] Reticuline (**1**)	1.46	0.87
12	(−)-[2′,6′,8-$^{3}H_3$] Reticuline	0.016	0.011
13	(±)-[3-^{14}C] Nor-reticuline (**27**)	0.84	0.36

the same radio-activity as the parent base. The labelled methoiodide was converted into methohydroxide (**9**, X=OH) by IR-4 10 anion exchange resin. Hofmann degradation of **9**, (X=OH) gave glaucine methine (**24**) with essentially no loss of radio-activity. Second Hofmann degradation of **24** yielded 3,4,6,7-tetramethoxy-1-vinylphenanthrene (**25**). Ozonolysis of **25** gave radioactive formaldehyde (dimedone derivative: 98% of original activity).

Biosynthetic laurifoline (**8**) derived from (±)-[N-methyl-^{14}C] reticuline (**18**) (experiment 5) was converted into glaucine methoiodide and then subjected to Hofmann degradation as above to give glaucine methine-1 (**24**) with essentially no loss of radioactivity. Treatment of labelled **24** with dimethyl sulphate–potassium hydroxide afforded 3,4,6,7-tetramethoxy-1-vinylphenthrene (**25**), essentially radio-inactive and radio-active trimethylamine hydrochloride (97% of original activity).

The regiospecificity of label in biosynthetic magnoflorine (**13**) derived from the feeding of (±)-[3-^{14}C] nor-reticuline (**27**) (experiment 13) was determined as follows: Labelled **13** was treated with MeI-MeONa to give 0,0-dimethylmagnoflorine iodide (**14**, X=I) which was converted into the corresponding methohydroxide and then subjected to double Hofmann degradation to give 3,4,5,6-tetramethoxy-1-vinylphenanthrene (**27**) with essentially no loss of radioactivity. Ozonolysis of **27** yielded radio-active formaldehyde (dimedone derivative: 97% of original activity).

Biosynthetic magnoflorine (**13**) derived from the feeding of (±)-N-methyl-^{14}C reticuline (**18**) (experiment 5) was converted into 0,0-dimethylmagnoflorine methohydroxide and then degraded under Hofmann condition to give a mixture of methine-I (**26**) and methine-II (**28**). Treatment of the mixture of methine-I and II with dimethyl sulphate–potassium hydroxide afforded 3,4,5,6-tetramethoxy-1-vinyl-phenanthrene (**27**) essentially radio-inactive and radio-active trimethylamine hydrochloride (97.6% original activity).

Incorporation of reticuline (**18**) into magnoflorine (**13**) and laurifoline (**8**) without degradation and demethylation was demonstrated by double labelling experiment as follows: (±)-[1-^{3}H, 4′-methoxy-^{14}C] reticuline (^{14}C:^{3}H ratio, 1:29) was fed to young cut branches of *C. laurifolius* plants and biosynthetic magnoflorine (**13**) and laurifoline (**8**) were isolated. The ratios of ^{14}C:^{3}H in biosynthetic **8** and **13** were 1:28 and 1:27 respectively.

The foregoing experiments established that reticuline (**18**) is specifically incorporated into magnoflorine (**13**) and laurifoline (**8**) in *C. laurifolius*. The precursors used, however, were racemic. Parallel feedings with (+)-(*S*)-reticuline (**1**) (experiment 11) and (−)-(*R*)-reticuline (experiment 12) demonstrated that stereospecificity is maintained in the bioconversion of 1-benzyltetrahydroisoquinoline precursors into magnoflorine (**13**) and laurifoline (**8**). (+)-(*S*)-Reticuline (**1**) was incorporated about 91 and 87 times more efficiently than (−)-(*R*)-reticuline into **8** and **13** respectively. Reticuline has been isolated from *C. laurifolius* plants.[9] Its presence in the plants was again confirmed by feeding (−)-U-^{14}C tyrosine (incorporation 0.34%). (+)-(*S*)-Reticuline (**1**) is, thus, a true precursor of magnoflorine (**13**) and laurifoline (**8**) in *C. laurifolius* DC. The foregoing results thus strongly support the following sequence for the biosynthesis of magnoflorine (**13**) and laurifoline (**8**) in *C. laurifolius*: Tyrosine → norlaudanosoline (**17**) → (+)-(*S*)-reticuline (**1**) magnoflorine (**13**) and laurifoline (**8**).

EXPERIMENTAL

For general directions (spectroscopy details, counting method, synthesis and labelling of precursors) see earlier papers in the series.[10]

Feeding experiments.—Labelled reticuline and norprotosinomenine were fed as their hydrochlorides. Nororientaline nor-reticuline and laudanosine were fed as their tartrate. Norlaudanosoline and reticuline methoiodide in H_2O (1 ml H_2O containing 0.2 ml of DMSO) were fed by stem cut method to young cut branches of *C. laurifolius* plants.

Isolation of magnoflorine (**13**). Young cut branches with leaves (typically 178 g wet wt) were macerated in EtOH (300 ml) with radio-inactive magnoflorine (110 mg) and left overnight. The alcohol was decanted and the plant material extracted with alcohol (5 × 250 ml). The combined alcoholic extract was concentrated under reduced pressure to give a greenish viscous mass which was extracted with 5% HCl (5 × 20 ml). The aqueous acidic soln was defatted with petroleum ether (4 × 20 ml), basified (pH 10) with Na_2CO_3 and extracted with $CHCl_3$ (3 × 25 ml). The aqueous alkaline soln was then extracted with n-BuOH (6 × 20 ml), washed with H_2O and solvent removed to give the crude base which was subjected to preparative tlc (*plates*: SiO_2; *solvent*: MeOH:NH_3:H_2O; 8:1:1) to give magnoflorine (82 mg). Base iodide m.p. 247 218° (dec) (lit[12] 248–49°) was crystallised from MeOH to constant activity.

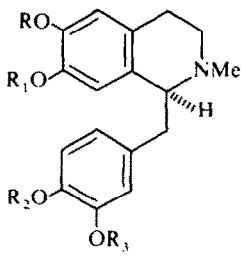
Isolation of laurifoline (**8**). Young cut branches with leaves (typically 175 g wet wt) were macerated with radio-inactive laurifoline (115 mg) in EtOH (300 ml) and worked up as above to give a mixture of quaternary alkaloids (n-BuOH extract) from which laurifoline (89 mg) was isolated by preparative tlc (*plates*: SiO_2; *solvent*: MeOH:NH_3:H_2O 8:1:1). Base chloride m.p. 252° (dec.) (lit[13] 253°) was crystallised from MeOH to constant activity.

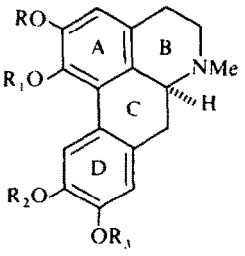
Feeding of (+)-[1-^{3}H, 4′-methoxy-^{14}C]reticuline. Young cut branches (7 nos) of *C. laurifolius* plants were fed with (±)-[1-^{3}H, 4′-methoxy-^{14}C] reticuline. After 8 days biosynthetic laurifoline (**8**) and magnoflorine (13) were isolated and counted for ^{14}C and ^{3}H activities. The ratios of the radio labels in the precursor and biosynthetic bases are given below (Table 2):

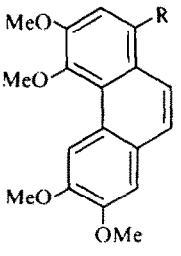
Table 2.

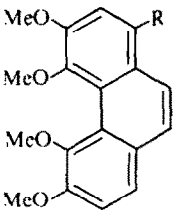
Label	Precursor	Biosynthetic	Alkaloid
	(±)-Reticuline	Magnoflorine (13)	Laurifoline (8)
^{14}C	1	1	1
^{3}H	29	27	28

Degradation of (+)-[5-^{14}C] laurifoline (**8**). A mixture of labelled **8** (227 mg) MeONa (15 ml) and MeI (5 ml) were refluxed on a water bath for 2 hr to give radio-active *glaucine methoiodide* (212 mg) m.p. 223–225° (lit[14] 221°). A soln of radio-active methoiodide (190 mg) in MeOH (30 ml) was passed through a column of freshly regenerated IR-410 anion-exchange resin (3 g) to afford radio-active glaucine methohydroxide. The methohydroxide in MeOH (10 ml) was refluxed for 2 hr with KOH (1.5 g). It was then cooled diluted with H_2O, extracted with ether: chloroform (3:1 V/V 5 × 50 ml). The extract was washed with H_2O, dried, and solvent removed to give radio-active **24** as an oil[14] (140 mg) 7.62 (6H,S,NMe_2), 6.52–7.54 (4H, m, 2-H_2), 6.08 (3H, S









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$R = R_3 = Me$: $R_1 = R_2 = H$ (5) $R = R_3 = Me$: $R_1 = R_2 = H$ | (25) $R = -CH=CH_2$ | (27) $R = -CH=CH_2$
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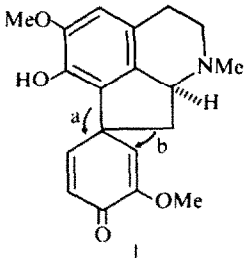

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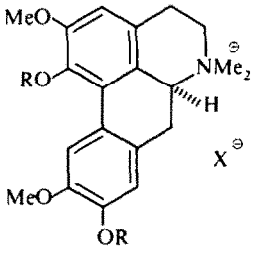

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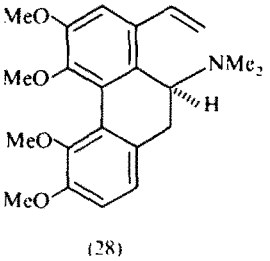
(9) R = R = Me



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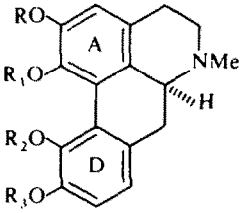

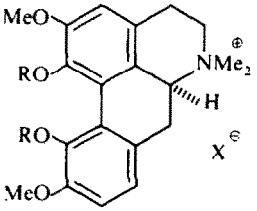

(10) $R = R_3 = Me$: $R_1 = R_2 = H$ (13) R = R = H
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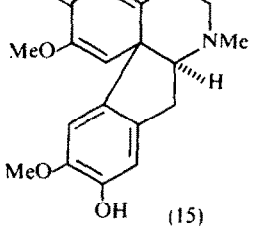

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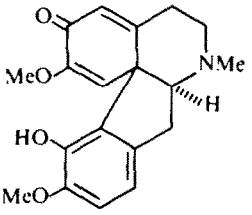

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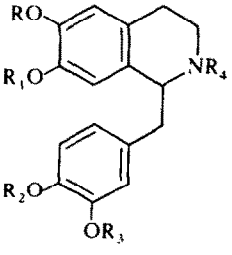

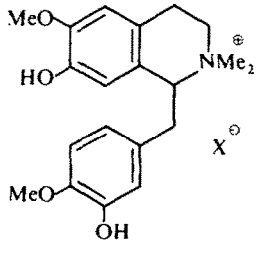

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(17) $R = R_1 = R_2 = R_3 = R_4 = H$
(18) $R = R_2 = R_4$: Me: $R_1 = R_3 = H$
(19) $R = R_3 = Me$: $R_1 = R_2 = R_4 = H$
(20) $R_1 = R_2 = Me$: $R = R_3 = R_4 = H$
(21) $R = R_1 = R_2 = R_3 = R_4 = Me$
(22) $R = R_2 = Me$, $R_1 = R_3 = R_4 = H$

OCH_3), 5.92 (6H, S, $2OCH_3$), 5.92 (3H, S, OCH_3), 2.78 (1H, S, 1- or 7-H), 2.72 (1H, S, 1- or 7-H), 2.34 (2H, 9-H and 10-H, J 16 Hz) and 0.78 (1H, S, 4-H).

Radio-active **24** (115 mg) in MeOH (3 ml) was refluxed with MeI for 2 hr to yield radio active glaucine methyl methine methoiodide (113 mg) m.p. 277–279° (lit[14] 276–280°). The methoiodide was converted into methohydroxide by IR-410 anion-exchange resin and then refluxed in MeOH (6 ml) with KOH (1.2 g) for 2 hr. The resulting mixture was cooled, diluted with H_2O and extracted with ether: chloroform (3:1, V/V; 5 × 50 ml). The extract was washed with H_2O, dried and evaporated to give **25** (65 mg) m.p. 142–143° (lit[14] 143°).

Ozonised O_2 was passed through a soln of radio-active **25** (130 mg) in EtOAc (8 ml) at −78° for 10 min. The solvent from the resulting mixture was removed under reduced pressure and to the residue H_2O, Zn dust (325 mg) and $AgNO_3$ (16 mg) were added. The mixture was refluxed for 20 min and then distilled. The distillate was collected in a soln of dimedone (320 mg) in aqueous EtOH (80 ml). After 1 hr it was concentrated to 10 ml and left for 12 hr. The ppt in $CHCl_3$ was chromatographed over silica. Elution with $CHCl_3$ (tlc control) afforded formaldehyde dimedone derivative m.p. 193–194° as needles from EtOH (98% original activity). The radio-activity of the degradation products is given below (Table 3).

Table 3. Activity of degradation products of 5-^{14}C laurifoline

Compound	Molar activity (disint. min^{-1} m mol^{-1})
Laurifoline iodide	3.38×10^5
Methine-I (**24**)	3.30×10^5
Methine-I methoiodide	3.39×10^5
3,4,6,7-Tetramethoxy 1-vinylphenanthrene (**25**)	3.29×10^5

Degradation of [N-*methyl*-^{14}C] *laurifoline* (**8**). Labelled **8** (217 mg) was converted into glaucine methoiodide by treatment with MeI–MeONa and then degraded to **24**. The radio-active **24** (169 mg) suspended in water (10 ml) pH 10 (adjusted with KOH) at 0° was stirred with Me_2SO_4 (0.8 ml) and 10N KOH (0.25 ml) were added. The mixture was refluxed for 2 hr. The trimethylamine, so obtained was

collected in 15% HCl. The radio-activity of the degradation products is given below (Table 4).

Table 4. Activities of degradation products of [N-methyl-^{14}C]laurifoline:

Compound	Molar activity (disint. min^{-1} m mol^{-1})
Laurifoline (**8**) iodide	3.58×10^4
Glaucine methoiodide (**9**, X=I)	3.60×10^4
Glaucine methyl methine (**24**)	3.44×10^4
Trimethylamine hydrochloride	1.73×10^4

Degradation of [5-^{14}C] *magnoflorine* (**13**). Labelled **13** (238 mg) was refluxed with MeONa and MeI (5 ml) to give 0,0-dimethyl-magnoflorine iodide (209 mg), m.p. 250–252° (dec) (lit[15] 252–253°) which was then converted into its hydroxide form by IR-410 anion-exchange resin. The base methohydroxide was refluxed with 15% methanolic KOH (10 ml) to give a mixture of methine-I as an oil[15]; base methoiodide, m.p. 276–278° (dec.) (Lit[15] 279–280°) and methine-II m.p. 76–77° (Lit[15] 75–76°); base methoiodide m.p. 170° (dec) (Lit[15] 168°). The mixture of methine methoiodides was converted into the hydroxide form by IR-410 anion exchange resin and then refluxed in 25% methanolic KOH (10 ml) to give **27** m.p. 67–68° (lit[15] 69°).

Radio-active **27** was ozonised as **25** above to give radio-active formaldehyde, dimedone derivative (97% original activity).

Degradation of [N-*methyl*-^{14}C] *magnoflorine*. Labelled magnoflorine (273 mg) was treated with MeI–MeONa to give radio-active 0,0-dimethylmagnoflorine iodide which was subjected to Hofmann degradation as above to give a mixture of **26** and Me_2SO_4 **28**. The mixture of methines was then heated with Me_2SO_4 and 10N KOH as above to give trimethylamine hydrochloride (97.6% original activity) 3,4,5,6-Tetramethoxy-1-vinylphenanthrene (**27**) was essentially radio-active.

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