Tetrahedron Vol. 36, pp. 2153 to 2156
Pergamon Press Ltd., 1980. Printed in Great Britain

LATE STAGES IN THE BIOSYNTHESIS OF ABNORMAL *ERYTHRINA* ALKALOIDS

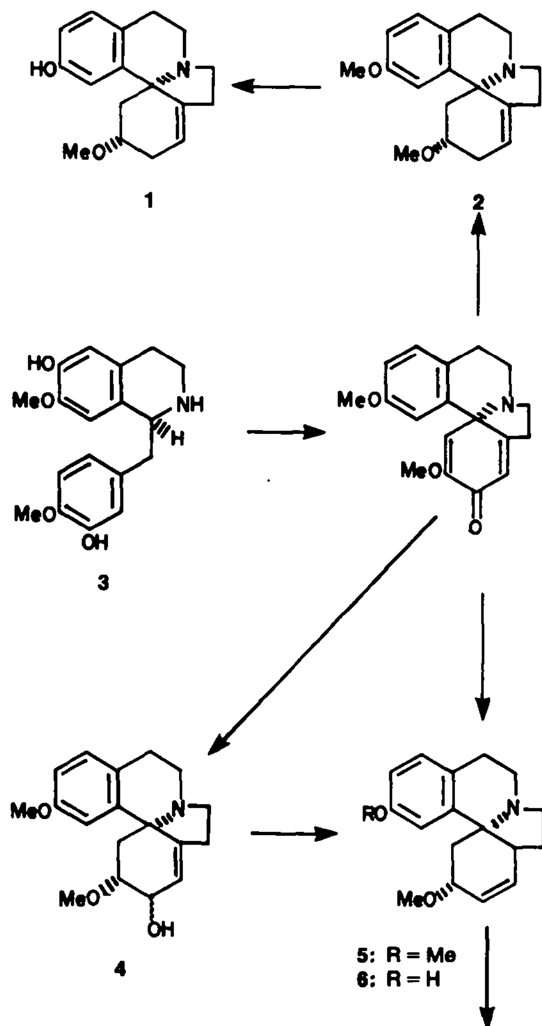
DEWAN S. BHAKUNI* and SUDHA JAIN
Central Drug Research Institute, Lucknow-226001, India

(*Received in UK* 10 *January* 1980)

Abstract—The incorporation of (±)-, N-norprotosinomenine, N-nor-orientaline, N-nor-reticuline, norlaudanosoline, protosinomenine, and N-[2-(3-hydroxy-4-methoxyphenyl)ethyl]-2-(4-hydroxyphenyl) ethylamine into coccuvine has been studied, and the specific utilisation of the (±)-norprotosinomenine demonstrated. A double labelling experiment with (±)-[1-^{3}H,4'-methoxy-^{14}C]-N-norprotosinomenine showed that the 4'-O-Me group of the precursor is retained in the bioconversion and the erythrinan ring system is not formed by addition of secondary amino function onto an *ortho*-quinone system. Feeding of (±)-[1-^{3}H, 7-methoxy-^{14}C]norprotosinomenine established that O-demethylation is the terminal step in the biosynthesis of coccuvine. Feeding of labelled abnormal *Erythrina* alkaloids revealed that isococculidine is converted into coccoline *via* coccuvinine and isococculine into coccolinine *via* coccuvine.

Abnormal *Erythrina* alkaloids cocculidine[1,2] (2), cocculine[1,2] (1), isococculidine[2] (5), isococculine[3] (6), coccuvinine[4] (9), coccuvine[5] (10), coccoline[2] (7), coccolinine[6] (8) and cocculitine[7] (4) have been isolated from the leaves of *Cocculus laurifolius* DC (Menispermaceae). The early stages of the biosynthesis of cocculidine[8] (2), cocculine[8] (1) and isococculidine[9] (5) have been studied. It has been demonstrated that cocculidine (2) and cocculine (1) and isococculidine (5) in *C. laurifolius* are stereospecifically biosynthesised from (+)-(S)-norprotosinomenine (3). We have examined the late stages of biosynthesis of abnormal *Erythrina* alkaloids. The results of these studies and also of tracer experiments which define the biosynthesis of coccuvine (10) are now presented.

(L)-Tyrosine (Experiment 1; Table 1) was initially fed to young cut branches of *C. laurifolius* DC and it was found that the plants were actively biosynthesising the alkaloids of interest. Parallel feedings of (±)-,N-norlaudanosoline (11; Exp 2), norprotosinomenine (12; Exp 3), N-nor-recticuline (13; Exp 4), N-nor-orientaline (14; Exp 5), protosinomenine (15; Exp 6) and N-[2-(3-hydroxy-4-methoxyphenyl) ethyl]-2-(4'-hydroxyphenyl)-ethylamine (16; Exp 7) demonstrated that only 11 and 12 are efficiently incorporated (Table 1) into coccuvine (10) in *C. laurifolius* plants. Feeding of (±)-[1-^{3}H, 7-methoxy-^{14}C] norprotosinomenine (12; Exp 8) gave coccuvine (10) labelled essentially with tritium. The results demonstrated that the OMe group at position 7 in norprotosinomenine (12) is demethylated in the bioconversion of 12 into 10. Feeding of (±)-[1-^{3}H,4'-methoxy-^{14}C]N-norprotosinomenine (12; Exp 9) gave coccuvine (10) labelled with ^{14}C and ^{3}H. The ^{14}C:^{3}H ratio in the precursor was 32:1 and in the biosynthetic coccuvine 30:1. Cleavage of the OMe group of 10 by the Ziezel method afforded radio active methyl iodide trapped as trimethylammonium iodide which had essentially the same molar activity as that of 10. The results thus established intact incorporation of 12 into 10 and demonstrated that the OMe group at position 7 and the H atom at the asymmetric centre C_1 in norprotosinomenine are retained in the bioconversion of 12 into 10.



Parallel feedings with (±)-N-norprotosinomenine (3; Exp 11) and (-)-N-norprotosinomenine (Exp 10) demonstrated that the stereospecificity is maintained in the bioconversion of norprotosinomenine (3) into coccuvine (10). The former was incorporated into 10 about 83 times more efficiently than the latter.

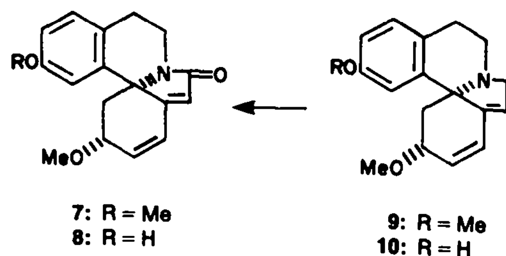
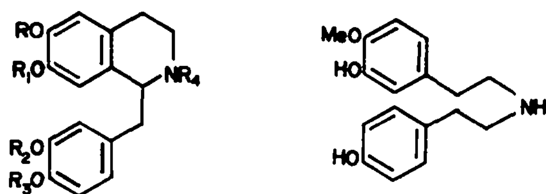


Fig. 1.



11: R = R1 = R2 = R4 = H
12: R1 = R3 = Me; R = R2 = R4 = H
13: R = R3 = Me; R1 = R2 = R4 = H
14: R = R2 = Me; R1 = R3 = R4 = H
15: R1 = R3 = R4 = Me; R1R2 = H

16

The bio-interconversions of isococculidine (5), cocculidine (2), and cocculine (1) have been studied earlier in *C. laurifolius*.[9] 2 is O-demethylated to give cocculine (1). The plants also convert isococculidine (5) into cocculidine (2) with very high efficiency.

In the present study (Table 2) labelled isococculidine (5; Exp 2) coccuvinine (9; Exp 6) and cocculine (7; Exp 4) were fed to young cut branches of *C. laurifolius* and it was found that 5, 9 and 7 were efficiently incorporated into isococculine (6), coccuvine (10) and coccolinine (8) respectively. When labelled isococculine (6; Exp 3), coccuvine (10; Exp 7) and coccolinine (8; Exp 5) were fed to the plants, these were very poorly metabolised to form isococculidine (5), coccuvinine (9) and cocculine (7) respectively. These results, thus established that 5, 9 and 7 are O-demethylated in the plants to give isococculine (6), coccuvine (10) and coccolinine (8) respectively.

Coccuvinine (9) and coccuvine (10) have a conjugated diene system (C_{1-7} and C_{2-3}) whereas isococculidine (5) and cocculidine (2) have an isolated double bond in an erythrinan ring system at C_{2-3} and C_{1-2} respectively. Parallel feedings of labelled isococculidine (5; Exp 2) and cocculidine (2; Exp 1) demonstrated that 5 was incorporated with high efficiency into 9 and 10. The incorporation of 2 into 9 and 10 was relatively very poor (Table 2). Coccoline (7) and coccolinine (8) both have a CO function at C_8 conjugated with the diene system (C_{2-3}, C_{1-7}). Parallel feedings of labelled coccuvine (10; Exp 6) revealed that 10 and 9 were efficiently incorporated into coccolinine (8) and coccoline (7) respectively. When labelled coccoline (7; Exp 4) and coccolinine (8; Exp 5) were fed to the plants, these were not metabolised to form coccuvinine (9) and coccuvine (10; Table 2). The results thus established that in *C. laurifolius* coccoline (7) and coccolinine (8) are formed by oxidation of coccuvinine (9) and coccuvine (10) respectively.

Poor incorporation of labelled cocculidine (2; Exp 1) into cocculitine (4) in the plants showed that 4 is not formed by hydroxylation of 2.

The late stages of biosynthetic pathways of abnormal *Erythrina* alkaloids in *C. laurifolius*, confirmed by tracer experiments are shown in Fig. 1.

EXPERIMENTAL

For general directions (spectroscopy details and counting method) see Ref. 8.

Synthesis of precursors. The racemic 12,[10] 11,[11] 14,[12] 13,[13] 15 and 16[9] were prepared by known procedures.

Resolution. (±)-OO-Dibenzyl-N-nor-protosinomenine was resolved by (+)- and (−)-dibenzoyltartaric acids[14] to give (+)- and (−)-OO-dibenzyl-N-norprotosinomenines respectively.

(−)-N-*Norprotosinomeine*. (+)-OO-Dibenzyl-N-norprotosinomenine (150 mg) in MeOH (5 ml) was heated at 100° for 1 hr with 12 N HCl (3 ml) to give (−)-N-norprotosinomenine hydrochloride (90 mg); $[\alpha]_D$ −18.2° (*c*, 1.6 in EtOH) (lit.[15] −18°, *c*, 1.7 in EtOH).

(+)-N-*Norprotosinomenine* (3). (−)-OO-Dibenzyl-N-norprotosinomenine (130 mg) was hydrogenolysed with 12 N HCl (3 ml) to give (+)- 3 hydrochloride, $[\alpha]_D$ + 17.2° (*c*, 1.6 in EtOH) (lit.[15] 16°, *c*, 1.7 in EtOH).

Labelling of precursors

Tritiation. (±)- 14 (125 mg) in tritiated water (0.5 ml), activity 80 mCi) containing t-BuOK (200 mg) was heated under N_2 (sealed tube) for 110 hr at 100°. The resulting mixture was diluted with water, ammonium chloride was added (pH_7) and the liberated base was extracted with $CHCl_3$ (4 × 10 ml). The extract was washed with water and solvent removed. The crude product, so obtained, was passed through a column of alumina. Elution with $CHCl_3$–MeOH (96:4) gave the tritiated base which was treated with ethereal HCl to give (±)-[3′,8-$^3H_2$] 14. (±)-[2,6′,8-$^3H_3$] 13 was similarly prepared.

[*Aryl*-^{3}H]N-[2(3-*hydroxy-4-methoxyphenyl*)*ethyl*]-2-(4′-*hydroxyphenyl*)*ethylamine* (16). $SOCl_2$ (0.1 ml) was added to tritiated water (0.5 ml, activity 80 mCi). To this was added 16

Table 1. Tracer experiments on *C. laurifolius*

Expt.	Precursor fed	Incorporation % into (10)
1.	(L)-[U-^{14}C]Tyrosine	0.11
2.	(±)-[1-^{3}H]Norlaudanosoline (11)	0.20
3.	(±)-[1-^{3}H]N-Norprotosinomenine (12)	0.38
4.	(±)-[2′,6′,8-$^3H_3$]N-Nor-reticuline (13)	0.004
5.	(±)-[3′,8-$^3H_2$]N-Nor-orientaline (14)	0.002
6.	(±)-[*Aryl*-^{3}H]Protosinomenine (15)	0.005
7.	[Aryl-^{3}H]N-[2-(3-hydroxy-4-methoxyphenyl)ethyl]-2-(4′-hydroxyphenyl)ethylamine (16)	0.003
8.	(±)-[1-^{3}H, 7-OCH_3-^{14}C]N-Norprotosinomenine (12)	0.35
9.	(±)-[1-^{3}H, 4′-OCH_3-^{14}C]-N-Nor-protosinomenine (12)	0.42
10.	(−)-(R)-[*Aryl*-^{3}H]N-Norprotosinomenine	0.007
11.	(+)-(S)-[*Aryl*-^{3}H]N-Norprotosinomenine (3)	0.66

Table 2. Tracer experiment on *C. laurifolius*

Expt.	Alkaloid fed	Incorporation (%) into alkaloids (5)	(6)	(7)	(8)	(9)	(10)
	* †					* †	* †
	1:28					1:30	1:27
1.	Cocculidine (2)	—	—	—	—	0.14	0.16
	* †			*	*	* †	* †
	1:32					1:30	1:33
2.	Isococculidine (5)	—	—	4.10	3.37	4.25	3.87
	* †				*		* †
	1:26						1:28
3.	Isococculine (6)	0.02	—	0.005	3.56	0.07	4.20
4.	Cocculine (7)	0.005	0.006	—	6.5	—	—
5.	Coccolinine (8)	0.006	0.005	0.06	—	—	—
	* †		* †	*	*		
	1:29		1:28				
6.	Coccuvinine(9)	—	4.20	4.80	3.20	—	—
	* †			*	*		
	1:28						
7.	Coccuvine (10)	0.005	0.004	0.074	5.5	—	—

*^{14}C activity.
†^{3}H activity.
* †^{14}C:^{3}H ratio.

(120 mg) and the resulting mixture was heated under N_2 (sealed tube) at 100° for 100 hr. Water was added to the resulting mixture and basified with $NaHCO_3$aq. The liberated base was extracted with $CHCl_3$, washed with water, dried (Na_2SO_4) and the solvent removed. The residue was dissolved in EtOH and treated with ethereal HCl to give (±)-[aryl-^{3}H] **16** hydrochloride (70 mg) m.p. 184–185° (lit.[8] 184–185°).

(±)-[Aryl-^{3}H]Protosinomenine, (+)- and (−)-[aryl-^{3}H]N-norprotosinomenines were prepared by acid catalysed tritiation as above.

(±)-[1-^{3}H] **12**, (±)-[1-^{3}H] **11** were prepared by reduction of the corresponding dihydroisoquinoline derivatives in dry dimethyl formamide with sodium-[^{3}H]borohydride.

(±)-[1-^{3}H] **12**, and (±)-[1-^{3}H] **12** were prepared by reduction of the corresponding dihydroisoquinoline derivatives in dry dimethyl formamide with sodium[^{3}H]borohydride.

(±)-[7-OCH_3-^{14}C]N-Nor-protosinomenine and (±)-[4′-OCH_3-^{14}C]N-norprotosinomenine were prepared by complete syntheses. (±)-[1-^{3}H, 7-OCH_3-^{14}C]N-norprotosinomenine was prepared by mixing (±)-[7-OCH_3-^{14}C]N-norprotosinomenine and (±)-N-nor[1-^{3}H]protosinomenine. (±)-[1-H, 4′-OCH_3-^{14}C]N-Norprotosinomenine was prepared by mixing (±)-[4′-OCH_3-^{14}C]N-norprotosinomenine and (±)-[1-^{3}H]-N-norprotosinomenine.

[4-OCH_3-^{14}C]coccoline (7) and [4-OCH_3-^{14}C]coccolinine (8) (±)-[7-OCH_3-^{14}C] **12** hydrochloride dissolved in water (1 ml) containing tartaric acid (12 mg) was fed to young cut branches of *C. laurifolius*. After 8 days the plants were worked up and radioactive [4-OCH_3-^{14}C] **7** and [4-OCH_3-^{14}C] **8** were isolated by dilution technique. [4-OCH_3-^{14}C, 8-^{3}H] **5**, [4-OCH_3-^{14}C, 8-^{3}H] **6**, [4-OCH_3-^{14}C, 8-^{3}H] **9** and [4-OCH_3-^{14}C, 8-^{3}H] **10** were prepared by feeding (±)-[1-^{3}H, 4′-OCH_3-^{14}C] **12** and [15-OCH_3-^{14}C, 8-^{3}H] **2** was prepared by feeding (±)-[1-^{3}H, 7-OCH_3-^{14}C] **12** to young cut branches of *C. laurifolius*.

Feeding experiments. For feeding purposes, norprotosinomenine, norreticuline and protosinomenine were dissolved in water (1 ml) containing tartaric acid (15 mg). Nor-orientaline, nor-laudanosoline, N-[2-(3-hydroxy-4-methoxyphenyl)ethyl]-2-(4′-hydroxyphenyl)ethylamine, compounds **2**, **5**, **6**, **9**, **10**, **7** and **8** were dissolved in dimethyl sulphoxide (0.2 ml) and water (1 ml). Freshly cut young branches of *C. laurifolius* plants were dipped into the solns of the precursors. When up-take was complete water was added for washing the precursor. The plants were then dipped into water, left for 7 to 8 days and worked up for the alkaloids of interest.

Isolation and purification

Isococculidine (**5**). Young branches and leaves (120 g wet wt) of *C. laurifolius* plants were macerated in EtOH (250 ml) with inactive isococculidine (110 mg) and left for 18 hr. The EtOH was then decanted and the plant material was percolated with EtOH (4 × 200). The combined ethanolic extract was concentrated *in vacuo* to afford a greenish viscous residue which was extracted with 5% HCl (4 × 15 ml). The acidic extract was defatted with petroleum ether (4 × 15 ml) and basified (pH 10) with Na_2CO_3. The liberated bases were extracted with $CHCl_3$ (5 × 20 ml), washed with water, dried and solvent removed. The crude bases (115 mg), so obtained, were purified on neutral alumina column and then subjected to preparative tlc (plates: silica; solvent: $CHCl_3$: EtOH, 97:3). The desired band was cut and eluated with $CHCl_3$:MeOH (90:10) to give **5** (80 mg) m.p. 94–95° (lit[2] 95–96°). The radio chemical purity of the biosynthetic isococculidine was checked by dilution method.

Coccuvinine (**9**). Young cut branches of *C. laurifolius* plants (130 g wet wt) were macerated in EtOH (250 ml) with **9** (90 mg). The plant material was worked-up as above to give crude bases (85 mg) which were subjected to tlc (plates: silica gel; solvent: $CHCl_3$:MeOH, 96:4) to give **9** (60 mg) m.p. 102–103° (lit[4] 103–104°).

Coccuvine (**10**). Young cut branches of *C. laurifolius* plants (110 g wt, wet) were macerated in EtOH (250 ml) with inactive **10** (85 mg) and worked up as above to give crude bases (80 mg) which were chromatographed on preparative thin layer silica gel plates (solvent: $CHCl_3$:MeOH, 94:6) to give **10** (60 mg) m.p. 217–218° (lit[5] 216–217°). The base was crystallized from benzene-petroleum ether to constant activity.

Coccoline (**7**). Young cut branches of *C. laurifolius* plants (143 g, wt. wet) were macerated in EtOH (300 ml) with inactive **7** (97 mg) and worked up as above to give crude bases (90 mg) which were purified by preparative tlc (plates: silica; solvent: $CHCl_3$:MeOH, 98:2) to give coccoline (63 mg) m.p. 246–247° (lit[2] 245–246°).

Coccolinine (**8**). Young cut branches of *C. laurifolius* plants (127 g, wt. wet) were macerated with inactive **8** (93 mg) in EtOH (250 ml) and worked up as above to give crude bases (90 mg) which were subjected to preparative tlc (plates: silica gel; solvent: $CHCl_3$:MeOH, 96:4) to give **8** (58 mg) m.p. 173–174° (lit[6] 174–175°) crystallised from benzene:hexane to constant activity.

Cocculitine (**4**). Young cut branches of *C. laurifolius* plants (132 g wt. wet) were macerated with inactive **4** (93 mg) in EtOH (280 ml). The plant material was worked up in the usual manner to give the crude bases (100 mg), which were subjected to tlc (plates: silica; solvent: $CHCl_3$:MeOH, 96:4) to give **4** (60 mg) m.p. 142–143° (lit[7] 142–143°), crystallised from EtOAc:petroleum ether to constant activity.

Isococculine (6). Young cut branches of *C. laurifolius* plants (145 g wt. wet) were macerated with inactive 6 (80 mg) in EtOH (250 ml). The plant material was worked up as above to give the crude bases (89 mg) which were subjected to preparative tlc (plates: silica; solvent: $CHCl_3$:MeOH, 94:6), to give 6 (48 mg) m.p. 183–185° (lit[3] 182–184°).

REFERENCES

[1]R. Razakov, S. Yu. Yunusov, S. M. Nasyrov, A. N. Cheklov, V. G. Andrianov and Y. T. Struchkov, *J. Chem. Soc.* Chem. Commun. 150 (1974).
[2]D. S. Bhakuni, H. Uprety and D. A. Widdowson, *Phytochemistry* **15**, 739 (1976).
[3]R. S. Singh, S. Jain and D. S. Bhakuni, *Nat. Acad. Sci. Letters* **1**, 93 (1978).
[4]A. N. Singh and D. S. Bhakuni, *Ind. J. Chem.* **15B**, 388 (1977).
[5]A. N. Singh, H. Pande and D. S. Bhakuni, *Experientia* 468 (1976).
[6]H. Pande, N. K. Saxena and D. S. Bhakuni, *Ind. J. Chem.* **14B**, 366 (1976).
[7]A. N. Singh, H. Pande and D. S. Bhakuni, *Lloydia* 322 (1977).
[8]D. S. Bhakuni and A. N. Singh, *J. Chem. Soc.* Perkin I, 1977 (1978).
[9]D. S. Bhakuni, A. N. Singh, S. Jain and R. S. Kapil, *Ibid.* Chem. Commun. 211 (1977).
[10]D. H. R. Barton, A. J. Kirby and G. W. Kirby, *Ibid.* (C), 929 (1968).
[11]S. Teitel, J. O'Brien and A. Bross, *J. Med. Chem.* **15**, 845 (1972).
[12]M. Tomita and J. Kunitoma, *J. Pharm. Soc. Japan*, **72**, 1081 (1952).
[13]A. R. Battersby, R. Bink, R. J. Francis, D. J. McCaldin and H. Ramuz, *J. Chem. Soc.* 3600 (1964).
[14]D. H. R. Barton, R. B. Boar and D. A. Widdowson, *Ibid.* (C), 1213 (1970).