Biosynthesis of the Abnormal *Erythrina* Alkaloids, Cocculidine and Cocculine

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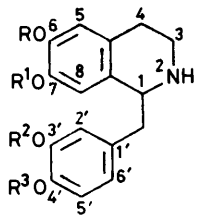
The incorporation of (±)-*N*-norprotosinomenine, (±)-*N*-nororientaline, (±)-*N*-nor-reticuline, (±)-norlaudanosoline, (±)-protosinomenine, and *N*-[2-(3-hydroxy-4-methoxyphenyl)ethyl]-2-(4-hydroxyphenyl)ethylamine into cocculidine has been studied, and the specific utilization of the (±)-*N*-norprotosinomenine demonstrated. A double labelling experiment with (±)-[1-^{3}H, 4'-*methoxy*-^{14}C]-*N*-norprotosinomenine showed that the 4'-*O*-methyl group of the precursor is retained in the bioconversion and the erythrinan ring system is not formed by addition of the secondary amino function onto an *ortho*-quinone system. Parallel experiments with (+)- and (−)-*N*-norprotosinomenine demonstrated specific incorporation of the (+)-isomer into cocculidine. High incorporation of cocculidine into cocculine revealed that *O*-demethylation is the terminal step in the biosynthesis of the latter. Feeding experiments also revealed that the plants can convert isococculidine into cocculidine with very high efficiency.

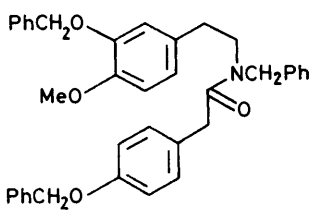
Cocculidine [1] (15) and cocculine [1] (16), the hypotensive principles of *Cocculus laurifolius* DC, can be formed in nature from norprotosinomenine (1), an established precursor of *Erythrina* alkaloids,[2] *via* the intermediates (7), (9), and (8) (Scheme 1). In the bioconversion of

[1] R. Razakov S. Yu Yunusov, S-M. Nasyrov, A. N. Chekhlov, V. G. Adrianov, and Y. T. Struchkov, *J.C.S. Chem. Comm.*, 1974, 150.

[2] D. H. R. Barton, R. James, G. W. Kirby, D. W. Turner, and D. A. Widdowson, *J. Chem. Soc.* (*C*), 1968, 1529.

norprotosinomenine (6) into the abnormal *Erythrina* alkaloids [3] one of the oxygen functions of the precursor can be eliminated by dienone–benzene rearrangement.

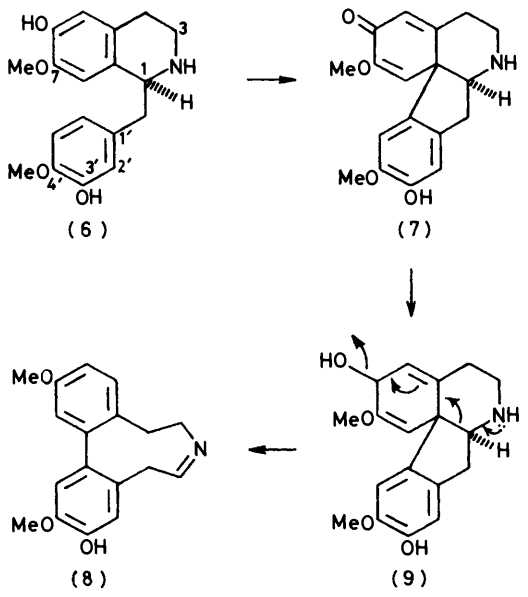




(5)

(1) $R = R^2 = H, R^1 = R^3 = Me$
(2) $R^1 = R^2 = H, R = R^3 = Me$
(3) $R^1 = R^3 = H, R = R^2 = Me$
(4) $R^1 = R^2 = R^3 = R = H$

However, the possibilities of the formation of (16) and (15) in nature from other 1-benzylisoquinolines [4] such as nor-reticuline (2) and nororientaline (3) and from *N*-[2-(3-hydroxy-4-methoxyphenyl)ethyl]-2-(4-hydroxyphenyl)ethylamine (18) cannot be ruled out.



Scheme 1

(±)-Tyrosine was initially fed to young cut branches of *C. laurifolius* DC (Menispermaceae) and it was found that the plants were actively biosynthesizing cocculidine (15). In subsequent experiments labelled hypothetical precursors were fed to young cut branches of *C. laurifolius* (Table 1). Feeding of (±)-tyrosine (experiment 1) in parallel with (±)-*N*-nor-reticuline (2) (experiment 4), (±)-*N*-nororientaline (3) (experiment 6), and *N*-[2-(3-hydroxy-4-methoxyphenyl)ethyl]-2-(4-hydroxyphenyl) ethylamine (18) (experiment 3) demonstrated that (2), (3), and (18) were very poorly metabolized by the plants. The very low incorporation (Table 1) of (2), (3), and (18) ruled out any significant involvement of these precursors in the biosynthesis of ' abnormal ' *Erythrina* alkaloids in *C. laurifolius*. Feeding of (±)-norprotosinomenine (1) (experiment 2) showed that the compound is an efficient precursor of (16) and (15). Feeding of (±)-protosinomenine (experiment 7) revealed that the plants cannot convert protosinomenine into *N*-norprotosinomenine (1).

Table 1

Tracer experiments on *C. laurifolius*

Expt.	Precursor fed	% Incorporation into cocculidine (16)
1	(±)-[U-^{14}C]Tyrosine	0.10
2	(±)-[1-^{3}H]-*N*-Norprotosinomenine (1)	0.19
3	[*aryl*-^{3}H]-*N*-[2-(3-Hydroxy-4-methoxyphenyl)ethyl]-2-(4-hydroxyphenyl)-ethylamine (11)	0.0004
4	(±)-[2′,6′,8-$^{3}H_3$]-*N*-Nor-reticuline (2)	0.005
5	(±)-[1-^{3}H]Noraudanosoline (4)	0.13
6	(±)-[5′ 8-$^{3}H_2$]-*N*-Nororientaline (3)	0.002
7	(±)-[*aryl*-^{3}H]Protosinomenine	0.006
8	(±)-[1-^{3}H,4′-*methoxy*-^{14}C]-*N*-Norprotosinomenine (1)	0.20
9	(±)-[1-^{3}H,7-*methoxy*-^{14}C]-*N*-Norprotosinomenine (1)	0.16
10	(+)-[*aryl*-^{3}H]-*N*-Norprotosinomenine (6)	0.27
11	(−)-[*aryl*-^{3}H]-*N*-Norprotosinomenine	0.045

Feeding of (±)-[1-^{3}H,7-*methoxy*-^{14}C]norprotosinomenine (1) (experiment 9) gave cocculidine (15) labelled with both ^{14}C and ^{3}H. The ^{14}C : ^{3}H ratio in the biosynthetic alkaloid was practically unchanged from that in the precursor. Zeisel cleavage of the methoxy-groups of (15) afforded radioactive methyl iodide, trapped as triethylmethylammonium iodide which had, as expected, essentially half the molar activity of (15). These results thus established that the 7-methoxy-group and the C-1 hydrogen atom (at the asymmetric centre) in norprotosinomenine (1) are retained in the bioconversion into (15).

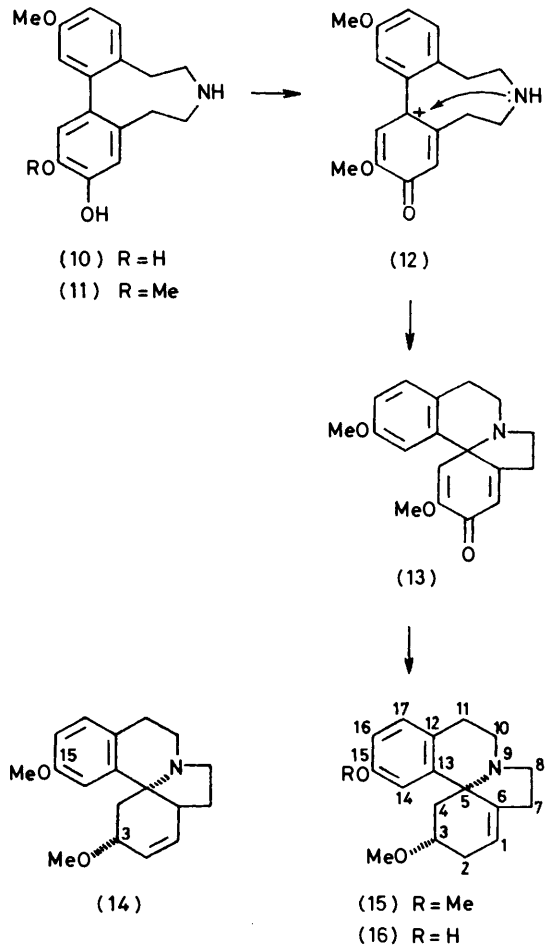
The foregoing experiments established that *N*-norprotosinomenine is a specific precursor of cocculidine in *C. laurifolius* plants. The precusors used, however, were racemic. Parallel feedings with (+)-*N*-norprotosinomenine (6) (experiment 10) and (−)-*N*-norprotosinomenine (experiment 11) demonstrated that the stereospecificity is maintained in the bioconversion of 1-benzylisoquinoline precursor into cocculidine. The former was incorporated about 60 times more efficiently than the latter.

The key dienone (13) from which hitherto known ' abnormal ' *Erythrina* alkaloids [1,5] can be formed in nature, can itself be formed from a dibenz[*d*,*f*]azonine intermediate [6] (11) as shown in (12). Feeding of (±)-[1-^{3}H,4′-*methoxy*-^{14}C]-*N*-norprotosinomenine (1) (experiment 8) gave cocculidine (15) labelled with ^{14}C and ^{3}H.

[3] D. S. Bhakuni, A. N. Singh, and R. S. Kapil, *J.C.S. Chem. Comm.*, 1977, 211.
[4] D. H. R. Barton, C. J. Potter, and D. A. Widdowson, *J.C.S. Perkin I*, 1974, 346.
[5] D. S. Bhakuni, H. Uprety, and D. A. Widdowson, *Phytochemistry*, 1976, **15**, 739; A. N. Singh, Hema Pande, and D. S. Bhakuni, *Experientia*, 1976, **32**, 1368.
[6] D. H. R. Barton, R. B. Boar, and D. A. Widdowson, *J. Chem. Soc.* (*C*), 1970, 1213.

The ^{14}C : ^{3}H ratio in the biosynthetic base was practically unchanged from that in the precursor. Zeisel cleavage of the methoxy groups of the biosynthetic cocculidine (15) as above afforded radioactive methyl iodide which had, as expected, essentially half the molar activity of the parent base.

In a study of the interconversion of bases in the biosystem, isococculidine (14), cocculidine (15), and cocculine (16) were fed to *C. laurifolius* (Table 2). It was found that the plants convert (14) into (15) and (15) into (16) (and *vice versa*). However, the high incorporation of (15) (experiment 2) into (16) suggested that de-*O*-methylation of (15) is the terminal step in the biosynthesis of (16). Feeding experiments also revealed that the plants convert isococculidine (14) (experiment 1) into cocculidine (15) with very high efficiency.



SCHEME 2

Trapping experiments by feeding (±)-tyrosine to *C. laurifolius* plants showed fairly high incorporation (0.40%) into norprotosinomenine (1). Thus, (1) is a true precursor of (15). The occurrence of dibenz[*d*,*f*]-azonine bases in *C. laurifolius* [7] supports indirectly the intermediacy of these bases in the biosynthesis of the 'abnormal' *Erythrina* alkaloids isococculidine [3] (14), cocculidine (15), and cocculine (16).

TABLE 2

Tracer experiments on *C. laurifolius*

		% Incorporation into		
Expt.	Alkaloid fed	cocculidine (15)	iso-cocculidine (14)	cocculine (16)
1	(+)-[*aryl*-^{3}H]-Isococculidine (14)	12.9		
2	(+)-[*aryl*-^{3}H]-Cocculidine (15)		1.13	5.13
3	(+)-[14,16-$^{3}H_2$]-Cocculine (16)	0.08		

The foregoing experiments thus strongly support the following sequence for the biosynthesis of cocculidine (15) and cocculine (16): tyrosine ⟶ (±)-norlaudanosoline (4) ⟶ (*S*)-*N*-norprotosinomenine (6) ⟶ cocculidine (15) ⟶ cocculine (16).

EXPERIMENTAL

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

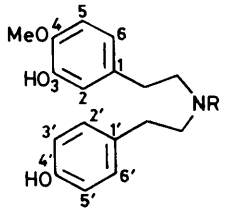
Synthesis of Precursors—Racemic *N*-norprotosinomenine,[9] *N*-nororientaline,[10] *N*-nor-reticuline,[11] norlaudanosoline,[12] and protosinomenine were prepared by standard methods.

N-Benzyl-N-[2-(3-benzyloxy-4-methoxyphenyl)ethyl]-2-(4-benzyloxyphenyl)acetamide (5). 4-Benzyloxyphenylacetyl chloride (1.25 g) in dry benzene (50 ml) was added dropwise to a stirred solution of *N*-benzyl-2-(3-benzyloxy-4-methoxyphenyl)ethylamine hydrochloride [13] (1.4 g) in aqueous 4N-sodium hydroxide (50 ml). Stirring was continued for 3 h, then the benzene layer was separated, washed with N-hydrochloric acid and finally water, dried (Na_2SO_4), and evaporated to give the *amide* (5) (1.80 g) as an oil; $\nu_{max.}$ (neat) 2 994, 1 656, 1 600, 1 517, 1 462, 1 244, 1 094, and 1 027 cm^{-1}; τ($CDCl_3$) 2.5—3.4 (22 H, m, ArH), 4.95 (2 H, s, OCH_2Ph), 5.04 (2 H, s, OCH_2Ph), 5.44 (1 H, s) and 5.74 (1 H, s, NCH_2Ph), 6.19 (3 H, s, OCH_3), and 6.34—6.70 (4 H, m) and 7.12—7.54 (2 H, m) (methylene protons) (Found: M^+, 571.2127. $C_{38}H_{37}NO_4$ requires *M*, 571.2113).

N-Benzyl-N-[2-(3-benzyloxy-4-methoxyphenyl)ethyl]-2-(4-benzyloxyphenyl)ethylamine (17).—To a stirred solution of lithium aluminium hydride (400 mg) in dry ether (10 ml) was added dropwise a solution of the preceding amide (700 mg) in dry ether (10 ml). The mixture was heated under reflux for 1 h, cooled, and treated with aqueous 4N-sodium hydroxide. The product was extracted into ether, washed with water, and dried (Na_2SO_4). The solvent was removed

[7] Hema Pande and D. S. Bhakuni, *J.C.S. Perkin I*, 1976, 2197.
[8] D. S. Bhakuni, A. N. Singh, S. Tewari, and R. S. Kapil, *J.C.S. Perkin I*, 1977, 1662.
[9] D. H. R. Barton, A. J. Kirby, and G. W. Kirby, *J. Chem. Soc.* (*C*), 1968, 929.
[10] M. Tomita and J. Kunitomo, *J. Pharm. Soc. Japan*, 1952, **72**, 1081.
[11] A. R. Battersby, R. Binks, R. J. Francis, D. J. McCaldin, and H. Ramuz, *J. Chem. Soc.*, 1964, 3600.
[12] S. Teitel, J. O'Brien, and A. Brossi, *J. Medicin Chem.*, 1972, **15**, 845.
[13] G. W. Kirby and L. Ogunkoya, *J. Chem. Soc.*, 1965, 6914.

and the residue was crystallized from ethanol–ether to give the *amine* (17) (450 mg), m.p. 76—77°; $\nu_{max.}$ (neat) 2 865, 1 603, 1 503, 1 451, 1 372, 1 229, 1 138, and 1 021 cm^{-1}; $\tau(CDCl_3)$ 2.57—3.32 (22 H, m, ArH), 4.94 (2 H, s, OCH_2Ph), 4.99 (2 H, s, OCH_2Ph), 6.15 (3 H, s, OCH_3), 6.32 (2 H, s, NCH_2Ph), and 7.3 (8 H, m, methylene); *m/e* 557 (M^+) (Found: C, 81.4; H, 7.4; N, 2.7. $C_{38}H_{39}NO_3$ requires C, 81.8; H, 7.0; N, 2.5%).



(17) R = CH_2Ph
(18) R = H

N-[2-(3-*Hydroxy*-4-*methoxyphenyl*)*ethyl*]-2-(4-*hydroxyphenyl*)*ethylamine* (18) *Hydrochloride*.—The amine (17) (550 mg) in ethanol (100 ml) containing 12N-hydrochloric acid (0.5 ml) was hydrogenated over 10% palladium–charcoal (300 mg). After 2 h, when the reaction was complete, the catalyst was filtered off, the solvent from the filtrate removed under reduced pressure, and the *amine* (18) hydrochloride was crystallized from ethanol–ether as plates (250 mg), m.p. 184—185°; $\nu_{max.}$ 3 460, 2 950, 2 747, 1 603, 1 506, 1 441, 1 244, 1 106, and 1 016 cm^{-1}; $\tau(D_2O)$ 2.5—3.2 (7 H, m, ArH), 6.1 (3 H, s, OCH_3), and 6.5—7.25 (8 H, m, methylene).

Resolution. – (±)-*OO*-Dibenzyl-*N*-norprotosinomenine was resolved by (+)- and (−)-dibenzoyltartaric acids according to the method of Barton and his co-workers.[6]

(−)-N-*Norprotosinomenine*.— (+)-*OO*-Dibenzyl-*N*-norprotosinomenine (150 mg) in methanol (5 ml) was heated at 100 °C for 1 h with 12N-hydrochloric acid (3 ml) to give (−)-*N*-norprotosinomenine hydrochloride (80 mg), $[\alpha]_D$ −18.2° (*c* 1.9 in EtOH) [lit.,[6] −18° (*c* 1.7 in EtOH)].

(+)-N-*Norprotosinomenine*.— (−)-*OO*-Dibenzyl-*N*-norprotosinomenine (100 mg) was similarly hydrogenolysed with 12N-hydrochloric acid (3.2 ml) to give (+)-*N*-norprotosinomenine hydrochloride, $[\alpha]_D$ −16.8° (*c* 1.0 in EtOH) [lit.,[6] 16° (*c* 1.7 in EtOH)].

Labelling of Precursors.—Tritiation. (±)-Nor-reticuline (120 mg) in tritiated water (0.6 ml; activity 65 mCi) containing potassium t-butoxide (200 mg) was heated under nitrogen (sealed tube) for 110 h at 100 °C. The mixture was diluted with water, ammonium chloride was added (pH 7), and the liberated base was extracted with chloroform (4 × 15 ml). The extracts were washed with water and evaporated. The crude product was chromatographed on a column of neutral alumina. Elution with chloroform–methanol (90 : 10) afforded material which was further purified through its hydrochloride to give (±)-[2′,6′,8-$^3H_3$]norreticuline (2). (±)-[5′,8-$^3H_2$]Nororientaline (3) was similarly prepared.

(+)-[*aryl*-^{3}H]-N-*Norprotosinomenine*.—Thionyl chloride (0.1 ml) was added to tritiated water (0.6 ml). To the solution was added (+)-*N*-norprotosinomenine (90 mg) and the mixture was heated under nitrogen (sealed tube) at 100 °C for 95 h. Water was added to the resulting mixture, which was then basified with aqueous sodium hydrogen carbonate. The liberated base was extracted with chloroform, washed with water, dried (Na_2SO_4), and evaporated. The residue was taken up in ethanol and treated with ethereal hydrogen chloride to give (+)-[*aryl*-^{3}H]norprotosinomenine hydrochloride (50 ml), which was crystallized from ethanol–ether. (−)-[*aryl*-^{3}H]Norprotosinomenine and (±)-[*aryl*-^{3}H]protosinomenine hydrochloride were similarly prepared.

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

(±)-[4′-*methoxy*-^{14}C]Norprotosinomenine was prepared by a standard procedure. (±)-[1-^{3}H, 4′-*methoxy*-^{14}C]-*N*-norprotosinomenine was prepared by mixing (±)-[4′-*methoxy*-^{14}C]-*N*-norprotosinomenine and (±)-[1-^{3}H]-*N*-norprotosinomenine.

[*aryl*-^{3}H]-*N*-[2-(3-Hydroxy-4-methoxyphenyl)ethyl]-2-(4-hydroxyphenyl)ethylamine (18) was prepared by acid-catalysed tritiation as described above.

(+)-[*aryl*-^{3}H]*Cocculidine*.—Thionyl chloride (0.1 ml) was added to tritiated water (0.5 ml). To the solution was added (+)-cocculidine (15) (90 mg), and the mixture was heated (sealed tube) under nitrogen at 100 °C for 95 h then diluted with water and basified with aqueous sodium hydrogen carbonate. The liberated base was extracted with chloroform. The extract was washed with water, dried, and evaporated. The crude product was chromatographed over a column of neutral alumina. Elution with benzene gave (+)-[*aryl*-^{3}H]cocculidine. (+)-[*aryl*-^{3}H]Isococculidine (14) was similarly prepared.

(+)-[14,16-$^3H_2$]*Cocculine*.—Tritium was introduced into cocculine (16) by the method of Kirby and Ogunkoya.[13] Cocculine (16) (80 mg) in tritiated water (0.4 ml) and dimethylformamide (0.15 ml) was heated (sealed tube) under nitrogen for 90 h at 100 °C. The solvent and water were removed from the resulting mixture. The residue was taken up in chloroform–methanol and passed through neutral alumina to give (+)-[14,16-$^3H_2$]-cocculine.

Feeding Experiments.—For feeding purposes, norreticuline, norprotosinomenine hydrochloride, protosinomenine, cocculidine, and cocculine were dissolved in water (1 ml) containing tartaric acid. Nororientaline, norlaudanosoline, isococculidine, and *N*-[2-(3-hydroxy-4-methoxyphenyl)ethyl]-2-(4-hydroxyphenyl)ethylamine were dissolved in water (1 ml) containing dimethyl sulphoxide (0.2 ml). Freshly cut young branches of *C. laurifolius* DC were dipped into the solutions of the precursors. When uptake was complete, water was added for washing. The young cut branches were then dipped into water, left for 5—6 days, and worked up for cocculidine and cocculine.

Isolation and Purification of Cocculidine (15).—Young branches and leaves (typically 120 g wet wt.) of the plant were macerated in ethanol (300 ml) with inactive cocculidine (15) (80 mg) and left for 20 h. The ethanol was then decanted and the plant material was percolated with fresh ethanol (4 × 250 ml). The combined ethanolic extract was concentrated *in vacuo* to afford a greenish viscous residue which was extracted with 5% hydrochloric acid (4 × 15 ml). The acidic extract was defatted with light petroleum (5 × 20 ml) and then basified with sodium carbonate. The liberated bases were extracted with chloroform (5 × 25 ml). The extracts were washed with water, dried, and evaporated to give crude cocculidine (75 mg), which was chromatographed over a column of neutral alumina (8 g). Elution with benzene gave cocculidine (15)

(50 mg), m.p. 86—87° (lit.,[14] 86—87°). The base was crystallized from light petroleum to constant activity. The radiochemical purity of the biosynthetic cocculidine was checked by the dilution method.

Isolation and Purification of Isococculidine (14).—Young branches and leaves (100 g wet wt.) of the plant were macerated in ethanol with inactive isococculidine (125 mg) and worked up as above to give the crude base (112 mg), which was chromatographed over a column of neutral alumina (10 g). Elution with hexane–benzene (50 : 50) gave isococculidine (14) (85 mg), m.p. 94—95° (lit.,[5] 95—96°), which was crystallized from light petroleum to constant activity. The radiochemical purity of the biosynthetic isococculidine was checked by the dilution method.

Isolation and Purification of Cocculine (16).—Young branches and leaves (130 g wet wt.) of the plant were macerated in ethanol (350 ml) with inactive cocculine (16) (110 mg). The plant material was then extracted with ethanol. The ethanolic extract was worked up as above to give the crude base (90 mg), which was chromatographed over neutral alumina (10 g). Elution with ethyl acetate–methanol (95 : 5) gave cocculine (16) (60 mg), m.p. 220—222° (lit.,[14] 217—218°), which was crystallized from ethyl acetate to constant activity.

Isolation of Norprotosinomenine (1).—Young cut branches of *C. laurifolius* plant were dipped into an aqueous solution (1 ml) of (±)-[U-^{14}C]tyrosine (0.1 mCi). When uptake was complete, water was added for washing. The twigs were then dipped into water, left for 4 days, and harvested. The plant material (110 g wet wt.) was macerated in methanol (300 ml) with radioinactive (±)-*N*-norprotosinomenine (105 mg) (dissolved in 2% hydrochloric acid in methanol) and left for 5 h. The methanol was decanted and the plant material was percolated with fresh methanol (4 × 200 ml) containing concentrated hydrochloric acid (1 ml). The combined percolate was concentrated *in vacuo*, diluted with water (30 ml), and extracted with ether (4 × 30 ml). The defatted acidic solution was basified with aqueous sodium hydrogen carbonate. The liberated bases were extracted with chloroform–methanol (90 : 10; 4 × 25 ml). The combined extract was washed with water, dried, and evaporated to give the crude base, which was treated with ethereal hydrogen chloride to give *N*-norprotosinomenine hydrochloride (50 mg), m.p. 240—241° (lit.,[2] 241°), which was crystallized from methanol–ether to constant activity; specific activity 8.88 × 10^3 disint. min^{-1} mg^{-1}; incorporation 0.40%.

[7/1077 *Received*, 22*nd June*, 1977]

[14] S. Yunusov, *Zhur. obshchei Khim.*, 1950, **20**, 368 (*Chem. Abs.*, 1950, **44** 6582).