Tetrahedron Vol 39, No 3, pp 455 to 459, 1983
Printed in Great Britain

0040-4020/83/030455-05$03 00/0

ABSOLUTE CONFIGURATION AND BIOSYNTHESIS OF (+)-SINACTINE†

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(*Received in UK 3 June* 1982)

Abstract—The incorporation of norreticuline, reticuline and nororientaline into sinactine in *Cocculus laurifolius* DC has been studied and specific utilization of reticuline demonstrated. Feeding with N-[$^{14}CH_3$]reticuline showed that the C atom 8 in sinactine is derived from N-Me group of reticuline. Feeding of (±)-[1-^{3}H, 3-^{14}C]norreticuline and degradation of biosynthetic sinactine established that the regiospecificity is maintained in the bioconversion of 1-benzyltetrahydroisoquinoline precursor into the tetrahydroprotoberberine alkaloid. Further feeding of doubly labelled precursor demonstrated that the H atom at the asymmetric centre in reticuline is retained in the bioconversion into sinactine. Feedings of labelled scoulerine and tetrahydropalmatrubine established the intermediacy of scoulerine in the biosynthesis and suggested that the methylenedioxy group formation probably precedes O-methylation in the bioconversion of scoulerine into sinactine. Parallel feedings of (*R*)-, and (*S*)-reticulines and chemical conversion of (+)-sinactine into (+)-(*R*)-tetrahydropalmatine established that (+)-sinactine has *R*-configuration at the asymmetric centre C-13a.

(−)-Sinactine[1-5] and (±)-sinactine[6,7] with established structure[8-11] have been isolated from several plant species. (+)-Sinactine (**4**) has been obtained by resolution[12] of (±)-sinactine. However, (+)-sinactine (**4**) as a natural product was isolated for the first time by us from *Corydalis meifolia* Wall.[13] According to Corrodi and Hardegger[14] tetrahydroprotoberberines which are laevorotatory have *S*-configuration at the asymmetric centre whereas tetrahydroprotoberberines which exhibit dextrorotation have *R*-configuration. Based on optical rotation (−)-sinactine was suggested to have *S*-configuration at carbon 13a. (+)-Sinactine (**4**) isolated by us from *Corydalis meifolia* Wall. on similar reasoning could have *R*-configuration at the asymmetric centre which has been confirmed by us by biosynthetic technique using labelled (*R*)-, and (*S*)-reticulines and by chemical transformation of (+)-sinactine (**4**) into (+)-(*R*)-tetrahydropalmatine (**5**). Experiments with labelled 1-benzyltetrahydroisoquinolines and tetrahydroprotoberberine precursors have also defined biosynthetic pathways of (+)-sinactine (**4**) from reticuline (**1**) in *Cocculus laurifolius* DC (Menispermaceae).

Sinactine (**4**) according to classical biogenetic theory[15] can be formed in nature from 1-benzyltetrahydroisoquinoline precursor, such as norreticuline (**12**) by condensation of one C unit. Tracer experiments, however, established that the so called "berberine bridge" (C atom 8) of berberine group of alkaloids is derived in nature from N-Me group of 1-benzyltetrahydroisoquinoline precursors.[16a]

(+)-Sinactine (**4**) can be formed in nature by alternate biosynthetic pathways from 1-benzyltetrahydroisoquinoline precursors as follows: Oxidation of (*R*)-reticuline (**1**) can give the corresponding iminium intermediate (**2**) which can cyclise to form tetrahydroprotoberberine nucleus of scoulerine (**6**) type. Cyclisation of O-methoxyphenol into methylenedioxy group[16b] followed by O-methylation of the phenolic group in **3** can finally yield (+)-sinactine (**4**). In the second possibility orientaline (**13**) can be oxidised to the corresponding iminium salt which can then cyclise to form the dienone (**15**). Dienone phenol rearrangement as shown in **15** can then afford tetrahydroprotoberberine nucleus of stepholidine type. Sinactine (**4**) can then be formed from this intermediate by unexceptional reactions.

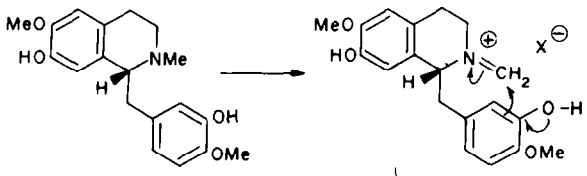
Initial feeding of (L)-[U-^{14}C]tyrosine (expt 1) (Table 1) to young cut branches of *C. laurifolius* DC showed that the plants were actively biosynthesizing (+)-sinactine (**4**) at the time of feeding. Feeding of tyrosine in parallel with (±)-reticuline (**11**) (expt 2), nororientaline (**14**) (expt 3) and norreticuline (**12**) (expt 4) demonstrated that **11** and **12** were efficiently incorporated into sinactine (**4**) in *C. laurifolius* DC while **14** was very poorly metabolised by the plant to form **4**.

(±)-[1-^{3}H, 3-^{14}C]Norreticuline (**12**) (expt 5) was fed to young cut branches of *C. laurifolius* DC and biosynthetic sinactine (**4**) was isolated. The ratios of the ^{14}C:^{3}H in the precursor was 1:9 while in the biosynthetic sinactine (**4**) 1:8 demonstrating thus that the H atom at the asymmetric centre, C-13a, essentially remained untouched during the biotransformation of **12** into **4**.

Biosynthetic sinactine (**4**) derived from (±)-[1-^{3}H, 3-^{14}C]norreticuline (**12**) (expt 5) was treated with methyl iodide to furnish sinactine methiodide (**16**) having essentially the same molar radioactivity as the parent base. **16** Was converted into its methohydroxide (**17**) by IR-410 anion exchange resin. Hofmann degradation of **17** gave radioactive methine (**18**) with essentially no loss of radioactivity. Ozonolysis of **18** afforded formaldehyde (dimedone derivative: 95% of original activity).

The C atom 8 in sinactine is formed by oxidative cyclization of N-Me group of reticuline was shown as follows: (±)-N-[$^{14}CH_3$]Reticuline (**11**) (expt 6) was fed to young twigs of *C. laurifolius* DC. Biosynthetic sinactine (**4**) was isolated and treated with BCl_3 in dry CH_2Cl_2. The resulting dihydroxy derivative was treated with an excess of ethereal CH_2N_2 to furnish (+)-tetrahydropalmatine (**5**) having essentially the same molar radioactivity as **4**. Radioactive **5** was oxidised with I_2 to radioactive palmatine (**19**) with practically no loss of radioactivity. Treatment of **19** with phenylmagnesium bromide afforded radioactive 8-phenyldihydropalmatine (**20**). Chromic acid oxidation of radioactive **20** in the usual manner (Kuhn-Roth) gave radioactive benzoic acid (97% original activity).

†CDRI Communication No. 3142.



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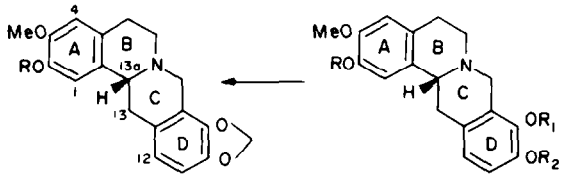
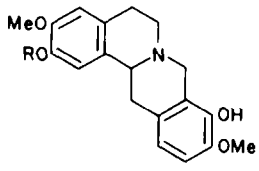
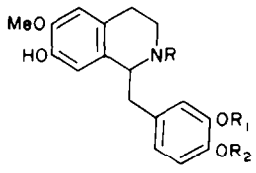


(3) R = H
(4) R = Me

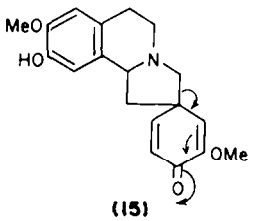
(5) R = R_1 = R_2 = Me
(6) R = R_1 = H; R_2 = Me
(7) R = Me; R_1 = R_2 = H
(8) R = R_1 = R_2 = H



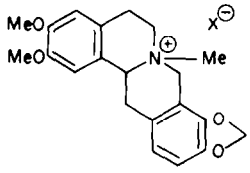
(9) R = H
(10) R = Me



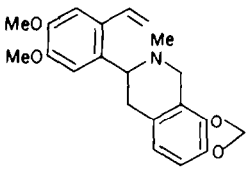
(11) R = R_2 = Me, R = H
(12) R = R_1 = H; R_2 = Me
(13) R = R_1 = Me; R_2 = H
(14) R = R_2 = H, R_1 = Me



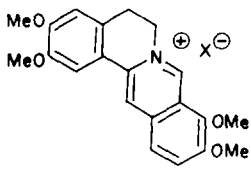
(15)



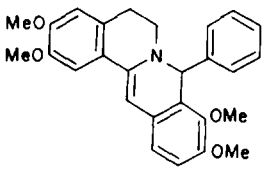
(16) X = I
(17) X = OH



(18)



(19)



(20)

Table 1.

Expt. No.	Precursor fed	% Incorporation into sinactine
1.	(L)-[U-^{14}C]Tyrosine	0.03
2.	(±)-[2',6',8-$^{3}H_3$]Reticuline (11)	0.17
3.	(±)-[5',8-$^{3}H_2$]Nororientaline (14)	0.004
4.	(±)-[2',6',8-$^{3}H_3$]Norreticuline (12)	0.40
5.	(±)-[1-^{3}H, 3-^{14}C]Norreticuline (12) (^{14}C : ^{3}H; 1:9)	0.47 (^{3}H), 0.72(^{14}C), (^{14}C : ^{3}H; 1:8)
6.	(±)-N-[$^{14}CH_3$]Reticuline (11)	0.045
7.	(±)-[Aryl-^{3}H]Scoulerine (9)	0.012
8.	(±)-[Aryl-^{3}H]Tetrahydropalmatrubine (10)	0.0013
9.	(R)-(-)-[2',6',8-$^{3}H_3$]Reticuline (1)	0.027
10.	(S)-(+)-[2',6',8-$^{3}H_3$]Reticuline	0.0018

The foregoing experiment with N-[$^{14}CH_3$]reticuline (**11**) (expt 6) established that sinactine (**4**) in *C. laurifolius* DC is specifically biosynthesized from reticuline. Further C atom 8 in **4** is formed by oxidative cyclisation of N-Me group of **11** probably by a mechanism as shown in **2**. Reticuline (**1**) is thus converted into scoulerine (**6**) in the biological system. Feeding of labelled scoulerine (**9**) (expt 7) established the intermediacy of **9** in the biosynthesis of **4**. Two possibilities exist by which **6** can be converted into **4**. In one selective O-methylation of phenolic function in ring A in **6** can occur to give tetrahydropalmatrubine (**10**) which can then be converted into **4** by cyclisation of O-methoxyphenol into a methylenedioxy group.[16] Alternately methylenedioxy group can first form to give cheilanthifoline (**3**) type of intermediate. O-Methylation of phenolic group in ring A can finally occur to give sinactine (**4**). Parallel feedings of [aryl-^{3}H]scoulerine (**9**) (expt 7) and [aryl-^{3}H]tetrahydropalmatrubine (**10**) (expt 8) support the second possibility.

Although the results discussed above demonstrated that reticuline (**11**) is a biological precursor of sinactine (**4**) in *C. laurifolius* DC, however, the precursors used were racemic. It would be expected that the enzyme system involved in the biosynthesis would be stereospecific and that only one of the two enantiomers should normally act as a direct substrate. Parallel feedings with (*R*)-(-), and (*S*)-(+)-, reticulines (expts **9** and **10** respectively) demonstrated that the stereospecificity is maintained in the bioconversion of 1-benzyltetrahydroisoquinoline precursor into sinactine (**4**). The former was incorporated about 15 times more efficiently than the latter. Incorporation of (*S*)-(+)-isomer was perhaps due to optical impurity of (*R*)-(-)-reticuline (**1**). The foregoing feeding experiments with reticulines of known absolute configuration thus suggested that (+)-sinactine (**4**) should have *R*-configuration at the asymmetric centre 13a. This was confirmed by chemical conversion of (+)-sinactine (**4**) into (*R*)-(+)-tetrahydropalmatine (**5**) as follows: (+)-Sinactine (**4**) on treatment with BCl_3 afforded a mixture of phenolic compounds (**7** and **8**). The position of OMe and OH in ring A in **8** is not confirmed. **7** Was, however, a major product. Treatment of **7** with an excess of ethereal CH_2N_2 furnished (*R*)-(+)-tetrahydropalmatine[17] (**5**).

Reticuline has been isolated from *C. laurifolius* DC.[18] Its presence in the plant was again confirmed by feeding (L)-[U-^{14}C]tyrosine (incorporation 0.34%). (*R*)-(-)-Reticuline (**1**) is, thus, a biological precursor of (+)-sinactine (**4**) in *C. laurifolius* DC. The foregoing results thus strongly support the following sequence for the biosynthesis of (+)-sinactine (**4**) in *C. laurifolius* DC.

Tyrosine → norreticuline (**12**) → (*R*)-reticuline (**1**) → scoulerine (**6**) → cheilanthifoline (**3**) → (*R*)-sinactine (**4**).

EXPERIMENTAL

For general directions (spectroscopy details, counting method, synthesis and labelling of precursors) see earlier papers in the series.[19,20]

Feeding experiments. Labelled reticuline was fed as its hydrochloride. Nororientaline and norreticuline were fed as their tartrates. Scoulerine and tetrahydropalmatrubine were fed by dissolving in H_2O (1 ml H_2O containing 0.2 ml of DMSO). The precursors were fed by stem cut technique to young cut branches of *C. laurifolius* DC. The plants were kept alive for 6–7 days to metabolise the precursors.

Isolation of (+)-sinactine (**4**). Young cut branches with leaves (typically 180 g wet wt) were macerated in EtOH (300 ml) with radioinactive **4** (110 mg) and left overnight. The alcohol was decanted and the plant material extracted with alcohol (5 × 250 ml). The 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 9.5) with Na_2CO_3 and extracted with $CHCl_3$ (5 × 25 ml). The $CHCl_3$ extract was washed with H_2O, dried (Na_2SO_4) and the solvent removed to give the crude base which was subjected to preparative TLC (*plates*: SiO_2; *solvent*: $CHCl_3$:MeOH, 99:1). The major band from the plates was cut, extracted with $CHCl_3$:MeOH, 75:25) and the solvent removed. The residue, thus obtained, was crystallized from MeOH to give *sinactine* (74 mg), m.p. 177–78°; $[\alpha]_D$ + 298° (*c*, 1.04 in $CHCl_3$) (lit.[12] $[\alpha]_D$ + 302° ($CHCl_3$)).

Feeding of (±)-[1-^{3}H, 3-^{14}C]norreticuline. Freshly cut young

branches of *C. laurifolius* DC were fed with (±)-[1-^{3}H, 3-^{14}C]norreticuline (activity: ^{3}H 0.037 mCi and ^{14}C 0.0035 mCi) by stem cut technique. The twigs were kept alive for 7 days and harvested. Sinactine (70 mg) was added and reisolated and counted for ^{14}C and ^{3}H activities. The ratios of the radio labels in the precursor and biosynthetic base is given below (Table 2).

Degradation of (+)-[13a-^{3}H, 6-^{14}C]*sinactine* (**4**). Labelled sinactine (34.8 mg) derived from feeding of (±)-[1-^{3}H, 3-^{14}C]norreticuline (expt 5) was diluted with radioinactive **4** (220 mg) to give radioactive **4** (250 mg).

A mixture of **4** (250 mg), MeONa (15 ml) and MeI (2 ml) were refluxed for 4 hr to give radioactive **16** (245 mg), m.p. 250° (dec). A soln of radioactive **16** (240 mg) in MeOH (30 ml) was passed through a column of freshly regenerated IR-410 anion exchange resin (3 g) to afford radioactive **17**. 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:$CHCl_3$ (3:1 v/v; 5 × 50 ml). The extract was washed with H_2O, dried and solvent removed to give radioactive **18** (130 mg), m.p. 140–142° (MeOH–C_6H_6). ^{1}H NMR ($CDCl_3$): τ 7.90 (s, 3H, N-CH_3), 6.24 (s, 3H, OCH_3), 6.20 (s, 3H, OCH_3), 4.42–4.96 (m, 3H, $CH=CH_2$), 4.15 (s, 2H, O-CH_2O) and 2.48 to 2.88 (m, 4H, ArH); MS: *m/z* 352 (M$^+$), 205 and 148 (base peak).

Ozonised O_2 was passed through a soln of radioactive **18** (120 mg) in EtOAc (7 ml) at −78° for 40 min. The solvent from the resulting mixture was removed under reduced pressure and to the residue H_2O, Zn dust (318 mg) and $AgNO_3$ (13 mg) were added. The mixture was refluxed for 1 hr and then distilled. The distillate was collected in a soln of dimedone (290 mg) in aqueous EtOH (70 ml). Work-up in the usual manner afforded formaldehyde dimethone derivative, m.p. 192–193° as needles from EtOH (95% original activity). The radioactivity of the degradation products is given below (Table 3).

Conversion of (+)-*sinactine* (**4**) *into* (+)-*tetrahydropalmatine* (**5**). To a soln of **4** (100 mg) in dry CH_2Cl_2 (5 ml) was added a soln of BCl_3 in dry CH_2Cl_2 (5 ml) at room temp. The mixture was stirred at ambient temp for 1.5 hr, MeOH (3 ml) added slowly to decompose excess of BCl_3 and then evaporated. The residue, thus obtained, was refluxed for 20 min with N HCl cooled and the acid neutralised with $NaHCO_3$. The product, so obtained, was extracted with $CHCl_3$ (5 × 20 ml) to give **7**; MS: 327 (M$^+$), 194, 136 and 135. **7** Was dissolved in MeOH (8 ml), an excess of ethereal soln of CH_2N_2 was added to it and left at ambient temp for 3 days. The resulting mixture was worked up in the usual manner to give (+)- **5** (35 mg), m.p. 142° (lit.[17], m.p. 142°), $[\alpha]_D$ + 289° (*c*, 0.95 in EtOH) (lit.[17] $[\alpha]_D$ + 292.5° in EtOH); CD maxima (EtOH): λ nm[θ], 275 (−0.15), 230 (+5.1) and 203 (+37.8) [lit.[21], CD maxima (EtOH): λ nm[θ], 280 (−0.22), 233 (+6.71) and 205 (+45.0)].

Degradation of [8-^{14}C]*sinactine* (**4**). Labelled **4** (80 mg) (molar activity 4.08×10^4 disint. min^{-1} mmol^{-1}) derived from feeding of (±)-N[^{14}CH$_3$]reticuline (expt 6) was converted into radioactive (+)- **5** (40 mg) (molar activity 3.98×10^4 disint. min^{-1} mmol^{-1}) as described above. Radioactive **5** (36.5 mg) was diluted with radioinactive **5** (129 mg). Radioactive **5** (162 mg) in EtOH (10 ml) was refluxed with I_2 (100 mg) to give radioactive **19** (155 mg), m.p. 238–240° (lit.,[22] m.p. 241° (dec.)). Radioactive **19** was treated with PhMgBr to give radioactive **20** (82 mg), m.p. 158–159° (lit.,[22] m.p. 158–160°. Kuhn–Roth oxidation of radioactive **20** in the usual manner afforded radioactive benzoic acid (97% of original activity). The radioactivity of the degradation products is given below (Table 4).

Table 2.

Label	(±)-Reticuline	Biosynthetic sinactine (4)
^{14}C	9	8
^{3}H	1	1

Table 3. Activity of degradation products of [5-^{14}C]sinactine

Compound	Molar activity (disint. min^{-1} mmol^{-1})
Sinactine (4)	3.25×10^5
Sinactine methiodide (16)	3.20×10^5
Methine (18)	3.18×10^5
Formaldehyde dimethone	3.09×10^5

Table 4. Activity of degradation products of [8-^{14}C]sinactine

Compound	Molar activity (disint. min^{-1} mmol^{-1})
Tetrahydropalmatine (5)	8.37×10^3
8-Phenyldihydropalmatine (20)	8.28×10^3
Benzoic acid	8.10×10^3

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