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ABSOLUTE CONFIGURATION OF SEBIFERINE

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Abstract—Absolute configuration of sebiferine has been determined.

During a search for hypotensive and neuromuscular blocking agents from the alkaloidal fraction of the leaves of *Cocculus laurifolius* (Menispermaceae) in which these activities[1] were concentrated led to the isolation of a morphinandienone alkaloid. The structure 1 (without stereochemistry) for the base was arrived on the basis of NMR, mass, UV and IR spectral data. The physical constant and spectral data of the base were almost identical with reported data of the morphinandienone alkaloid, sebiferine isolated by Sivakumaran and Gopinath[2] from the stem bark of *Litsea sebifera* (Lauraceae). Since the name sebiferine had the priority, the morphinandienone alkaloid isolated by us from *C. laurifolius* was called sebiferine. O-Methylflavinantine[3,4] a morphinandienone alkaloid isolated from the bark of *Nemuaron vieillardii* (Monimiaceae) is shown to be identical with sebiferine. O-Methylflavinantine was isolated in (−)-form from *N. vieillardii* and in (±)-form and (−)-form from the roots of *Rhigiocarya racemifera*[5,6] Miers (Menispermaceae).

Recent tracer experiments have shown that sebiferine (1) is specifically biosynthesised from reticuline (8) in *C. laurifolius*.[7] Parallel feedings with (+)- and (−)-reticulines showed that stereospecificity is not maintained in the biosynthesis of sebiferine from 1-benzylisoquinoline precursors. (+)-Reticuline (6) was incorporated into sebiferine (1) almost as efficiently as the (−)-enantiomer. The results were interpreted as showing that (−)-and (+)-reticulines were undergoing interconversion in the plant by oxidation and reduction presumably via the 1,2-dehydroderivative (4). (+)-Reticuline (6) was incorporated into sebiferine (1) by way of the intermediate (4). The occurrence of the racemic form in plants is most probably due to the presence of an active oxidation-reduction system as is present in Poppies.[8]

The structure 1 (without stereochemistry) for sebiferine has been confirmed by syntheses.[3,9] The absolute configuration of the base, however, remained unsolved. We now present the results which define the configuration as shown in 1 for sebiferine.

(±)-00-Dibenzylreticuline (7) was prepared by the usual route.[10] Resolution of the racemic mixture with (+)- and (−)-0,0-dibenzoyltartaric acids[8] separately afforded (−)- and (+)-0,0-dibenzylreticulines respectively. Acid catalysed hydrogenolysis of the benzyl ethers finally yielded (−)- and (+)-reticulines (3 and 6) respectively of known absolute configuration. (±)-Reticuline (8), (+)-reticuline (6) and (−)-reticuline (3) were then separately labelled with tritium *ortho*- and *para*- to phenolic OH group.[11]

Tritium labelled (±)-, (+) and (−)-reticulines were then separately oxidised with potassium ferricyanide in a two phase system.[12] The product so obtained in each case was treated with diazomethane to give O-Me derivatives. Sebiferine from the mixture was isolated by dilution technique. The radiochemical yields of sebiferine corrected for obligatory loss of tritium are recorded in Table 1.

Table 1.

Reticuline derivative	% Yield of sebiferine (1)
(±)-[2′,6′,8-$^3H_3$]Reticuline	2.22
(+)-[2′,6′,8-$^3H_3$]Reticuline	0.005
(−)-[2′,6′,8-$^3H_3$]Reticuline	3.86

Sebiferine (1) is obtained only from (±)- and (−)-(*R*)-reticulines. The absolute configuration of the base is, therefore, that of (−)-(*R*)-reticuline (3).

EXPERIMENTAL

Unless otherwise stated UV spectra refer to solns in EtOH, IR spectra to KBr discs and NMR spectra to solns in $CDCl_3$ and optical rotations were determined in MeOH. TLC was carried out, unless specified to the contrary, on silica G.F-254.

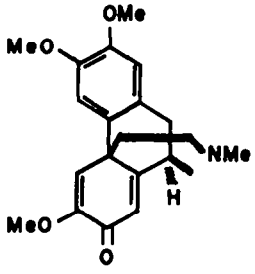
Extraction. Air dried leaves of *Cocculus laurifolius* DC (20 kg) which were collected from Dehra Dun, India, in September 1976, were extracted with EtOH. The solvent from the extract was removed *in vacuo* and the dark green viscous mass was extracted with 5% HCl. The aqueous acidic soln was defatted with petroleum ether and then basified with $NaHCO_3$ aq. The liberated bases were extracted with $CHCl_3$. The solvent from the extract was evaporated to give the alkaloidal mixture which was divided into Et_2O soluble and Et_2O insoluble fractions. The Et_2O soluble material was further separated into phenolic (15 g) and non-phenolic bases (40 g).

Sebiferine (1). The non-phenolic alkaloidal mixture (40 g) was chromatographed on neutral Al_2O_3 (800 g) and the column was eluted with solvent of increasing polarity. Elution with C_6H_6:$CHCl_3$ (1:1) (fr 151–165) gave 1 (200 mg) m.p. 112–13°; $[\alpha]_D^{25}$ +17° (*c*, 1.02 in MeOH). (Found: C, 70.24; H, 6.68; N, 3.92. $C_{20}H_{23}NO_4$ requires: C, 70.38; H, 6.74; N, 4.10%). λ_{max} 239 and 283 nm. ν_{max} 2910, 2820, 1450, 1666, 1642, 1620 (dienone) and 1250 (OCH_3) cm^{-1}. τ 7.55 (s, 3H, N-CH_3), 6.20 (s, 3H, aromatic OCH_3), 6.15 (s, 3H, aromatic OCH_3), 6.12 (s, 3H, aromatic OCH_3), 3.70 (s, 1H, C_5–H), 3.65 (s, 1H, aromatic H), 3.37 (s, 1H, aromatic H) and 3.19 (s, 1H, C_8–H). *m/e* 341 (M^+, base peak), 326 (M^+–15), 313 (M^+–28), 298 (M^+–43) and 281.

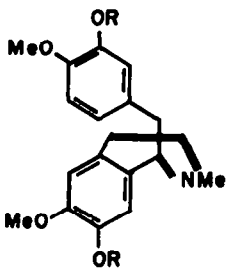
(±)-0,0-*Dibenzylreticuline* (7). The corresponding dihydroisoquinoline[10] in MeOH at 0° was reduced with $NaBH_4$ to give (±) 7 crystallized from EtOH as plates m.p. 90–91° (lit.[10] 89°).

Resolution of (±)-0,0-*dibenzylreticuline*

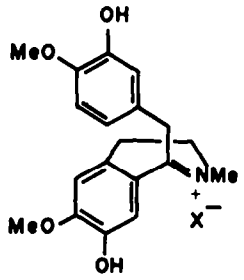
(±)-0,0-Dibenzylreticuline 7 (1.0 g) in MeOH (5 ml) was treated with (−)-0,0-dibenzoyltartaric acid (0.71 g) in MeOH (5 ml). The



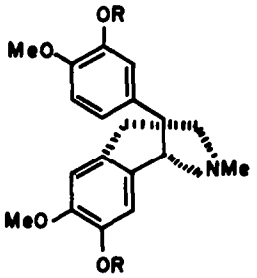
1



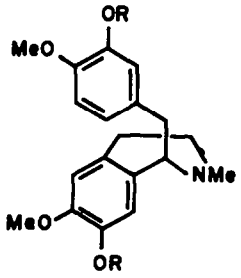
2: R = —$CH_2C_6H_5$
3: R = H



4



5: R = —$CH_2C_6H_5$
6: R = H



7: R = —$CH_2C_6H_5$
8: R = H

resulting salt was crystallized (15 times) from MeOH-Et_2O to give the tartarate salt of (+)-0,0-dibenzylreticuline m.p. 130–34°; $[\alpha]_D$ +25° (*c*, 1.2, $CHCl_3$) (lit.[8] 130°; $[\alpha]_D$ +20° in $CHCl_3$). The salt was decomposed with 4N NaOH, the liberated base extracted with $CHCl_3$ and chromatographed on neutral Al_2O_3. The purified product crystallized from C_6H_6-petroleum ether to give (+) **5** (180 mg) m.p. 90–92°; $[\alpha]_D$ +45° (*c*, 1.4 in $CHCl_3$) (lit.[8] 89°; $[\alpha]_D$ +44° in $CHCl_3$).

(+)-*Reticuline* (**6**). Compound **5** (150 mg) in MeOH (5 ml) was refluxed with 12N HCl (4 ml) for 2 hr to afford (+) **6** (100 mg) as an amorphous powder; $[\alpha]_D$ +40° (*c*, 1.04 in $CHCl_3$). Base hydrochloride $[\alpha]_D$ +75.5° (*c*, 1.4 in H_2O) (lit.[8] $[\alpha]_D$ +73° in H_2O).

(−)-0,0-*Dibenzylreticuline* (**2**). Compound **2** (750 mg) enriched with (−)-enantiomer was treated with (+)-0,0-dibenzoyltartaric acid (500 mg) and crystallized from MeOH-Et_2O (10 times) to give the salt (400 mg) m.p. 134°; $[\alpha]_D$ −25° (*c*, 1.20 in $CHCl_3$). The salt was decomposed with 4N NaOH to give (−) **2** (190 mg), m.p. 90–92°; $[\alpha]_D$ −45° (*c*, 1.04 in $CHCl_3$) (lit.[8] 89°, $[\alpha]_D$ −44° in $CHCl_3$).

(−)-*Reticuline* (**3**). Compound (−)- **2** (145 mg) in MeOH (4 ml) was refluxed with 12N HCl (3.5 ml) for 2 hr to afford (−)-*reticuline hydrochloride* (120 mg), $[\alpha]_D$ −75.5° (*c*, 0.88 in H_2O) (lit.[8] $[\alpha]_D$ −73° in H_2O).

Tritiation of reticuline

Tritium in **8** was introduced by the technique published earlier.[11] (±)-Reticuline (120 mg) in T_2O (0.5 ml, 200 mCi) containing t-BuOK (180 mg) was heated under N_2 (sealed tube) for 110 hr at 100° to give (±)-[2′,6′,8-$^3H_3$]-*reticuline*; Specific activity = 0.018 mCi/mg.

(+)-[2′,6′,8-$^3H_3$]*Reticuline*. (+)- **6** (86 mg), T_2O (0.4 ml, 175 mCi) and t-BuOK (140 mg) were heated for 130 hr. The resulting mixture was worked up as above to give (+)-[2′,6′,8-$^3H_3$]*reticuline* (55 mg); Specific activity = 0.02 mCi/mg.

(−)-[2′,6′,8-$^3H_3$]*Reticuline*. (−) **3** (100 mg), T_2O (0.4 ml, 175 mCi) and t-BuOK (150 mg) were heated for 125 hr. The resulting mixture was worked up as above to give (−)-[2′,6′,8-$^3H_3$]-reticuline (70 mg); Specific activity = 0.024 mCi/mg.

Oxidation of (±)-[2′,6′,8-$^3H_3$]*reticuline*

(±)-[2′,6′,8-$^3H_3$]Reticuline (13 mg) diluted with (±)- **8** (40 mg) in 8% NH_4AcO (3 ml), was added dropwise to a mixture of $K_3Fe(CN)_6$ (350 mg), 28% NH_4OH (0.8 ml) and $CHCl_3$ (25 ml) at 0° with vigorous stirring under N_2. Stirring was continued for 3 hr. **1** (58 mg) was then added, the $CHCl_3$ layer collected and the aqueous layer extracted with $CHCl_3$ (4 × 30 ml). The combined $CHCl_3$ extract was washed with sat NaCl aq, dried (anhyd

K_2CO_3) and solvent removed. The residue in MeOH (2 ml) was treated with an excess of ethereal diazomethane and left for 20 hr. The resulting product was passed through a short column of neutral Al_2O_3 and then subjected to preparative TLC of SiO_2 (solvent: MeOH:$CHCl_3$, 7:93) to give *sebiferine* (18 mg), m.p. 112–13°. The radiochemical yield of sebiferine was 2.22%.

Oxidation of (+)-[2′,6′,8-$^3H_3$]*reticuline*

(+)-(*S*)-[2′,6′,8-$^3H_3$] Reticuline **8** (6 mg, activity 0.0353 mCi/mg) diluted with radioinactive (±)- **8** was oxidized with $K_3Fe(CN)_6$ (300 mg) under identical condition as above. Inactive **1** (50 mg) was added at the end of the reaction. The product was extracted with $CHCl_3$ and treated with ethereal diazomethane in MeOH to give radioactive *sebiferine* (15 mg). The radiochemical yield of sebiferine was 0.005%.

Oxidation of (−)-(R)-[2′,6′,8-$^3H_3$] reticuline

(−)-(*R*)-[2′,6′,8-$^3H_3$] Reticuline (8.2 mg, activity 0.041 mCi/mg) diluted with radioinactive (±)- **8** was oxidized with $K_3Fe(CN)_6$ (350 mg) under identical condition as above. Inactive **1** (55 mg) was added at the end of the reaction. The product was treated with diazomethane and sebiferine (17 mg) was isolated as above. The radiochemical yield of sebiferine was 3.86%.

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