Tetrahedron Vol. 34, pp. 1409 to 1410. Pergamon Press 1978. Printed in Great Britain

ABSOLUTE CONFIGURATION AND BIOSYNTHESIS OF TILIAGEINE

D. S. BHAKUNI* and A. N. SINGH
Central Drug Research Institute, Lucknow, India

(*Received in the UK* 3 *November* 1977; *Accepted for publication* 10 *November* 1977)

Abstract—Tracer experiments on *Tiliacora racemosa* Colebr established that tiliageine has "S" and "R" configuration at the asymmetric centres C_1 and C_1' respectively. The experiments also prove that tiliageine is biosynthesised in the plants from N-methylcoclaurine.

Tiliageine, isolated from *Tiliacora dinklagei*[1,2] (Menispermaceae) has been assigned the structure **5**. The stereochemistry at the two asymmetric centres in tiliageine remained unsolved. The dimeric base represents a group of bisbenzylisoquinoline alkaloids in which the two benzylic "halves" are linked through a direct carbon to carbon bond rather than through the more common diaryl ether bridge.[3] This unusual structural feature precludes facile chemical interrelationship between tiliageine type bases and other bisbenzylisoquinolines of established structure and stereochemistry. Further the absolute configuration at the asymmetric centres of these dimeric bases cannot be determined by the usual sodium/liquid ammonia cleavage method.[4] The results which define the stereochemistry at the asymmetric centres in tiliageine by tracer experiments are now reported.

Tiliageine (**5**) can be formed in Nature from N-methylcoclaurine. Intermolecular oxidative coupling of **1** and **2** can form carbon to carbon bond in the benzylic "halves". The intermediate (**3**) so formed can in turn

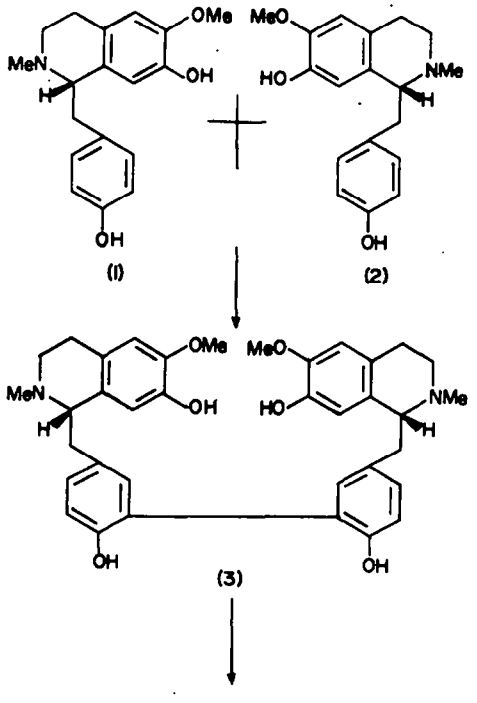
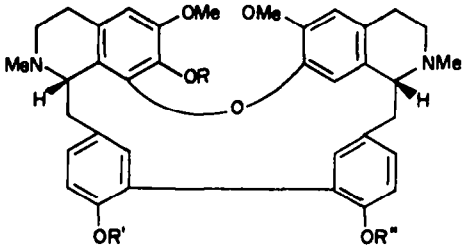


Fig. 1.



4: R = Me, R' = H, R" = Me
5: R = R' = H, R" = Me
6: R = R' = Me, R" = Me
7: R = R' = R" = H

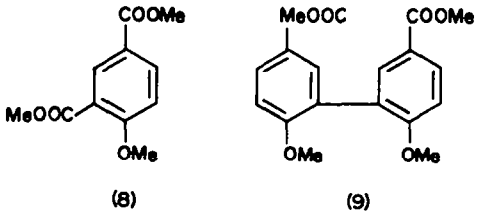


Fig. 2.

generate **7** by intramolecular oxidative coupling. Selective O-methylation of the phenolic groups in the benzylic portion of **7** can finally yield tiliageine (**5**).

Initially (±)-N-methylcoclaurine, an established precursor of bisbenzylisoquinoline alkaloids[5,6] was fed to young *Tiliacora racemosa* Colebr (Menispermaceae) plants and it was efficiently incorporated into tiliageine (**5**). Labelled **5** was converted into O,O-dimethyltiliageine (**6**) by treatment with ethereal diazomethane with no loss of radio activity. Alkaline permanganate oxidation of **6** followed by methylation with diazomethane of the acids so formed yielded 5,5 - dicarbomethoxy - 2,2' - dimethoxydiphenyl (**9**) having essentially 2/3 ratio activity of the parent base. The results thus established that (±)-N-methylcoclaurine is a specific precursor of tiliageine (**5**).

Tiliageine (**5**) has two asymmetric centres. The configuration at these two centres can be "*R,R*", "*S,S*", "*S,R*", "*R,S*". Since biosynthethic studies on the bisbenzylisoquinoline alkaloids[6] has established that the H atom at C_1 in the 1-benzyltetrahydroisoquinoline precursors is retained in the bioconversion of the precursors into these dimeric bases and the stereospecificity is maintained in the biosynthesis of bisbenzylisoquinoline

alkaloids from coclaurine derivatives, radioactive N-methylcoclaurines of known absolute configurations could be employed to determine the absolute configuration of tiliageine. (+)-(*S*)- and (−)-(*R*)-N-methylcoclaurines were, therefore, prepared and specifically labelled with tritium at *ortho*- and *para*-positions to the phenolic OH groups and were fed in parallel to *T. racemosa* plants. After 7 days the plants were harvested and worked up for tiliageine (5). It was found that both the (+)-(*S*)- and (−)-(*R*)-N-methylcoclaurines were almost equally and efficiently incorporated into 5 (Table 1).

Alkaline permanganate oxidation of the biosynthetic tiliageines separately which results in the cleavage of the ring containing the phenolic OH group yielded an acid which on treatment with diazomethane afforded dimethyl 4-methoxyisophthalate (8) in both the cases. Compound 8 obtained by the alkaline permanganate oxidation of tiliageine derived from (+)-(*S*)-N-methylcoclaurine (1) was essentially radio inactive whereas 8 obtained by the alkaline permanganate oxidation of tiliageine derived from (−)-(*R*)-N-methylcoclaurine (2) had one half the radio activity of the parent base. These results thus established "*S*" and "*R*" configuration at the asymmetric centres C_1 and C_1' in tiliageine (5). Since O-methylfuniferine (6) and O,O-dimethyltiliageine are identical, the absolute configuration at C_1 and C_1' in funiferine[7] (4) should also be "*S*" and "*R*" respectively.

Table 1. Tracer experiment on *T. racemosa*

Expt.	Precursor fed	Incorporation % into tiliageine (5)
1	(±)-(3′,5′,8-$^3H_3$) N-methylcoclaurine	0.14
2	(+)-(*S*)-3′,5′,8-$^3H_3$) N-methylcoclaurine (1)	0.17
3	(−)-(*R*)-(3′,5′,8-$^3H_3$) N-methylcoclaurine (2)	0.16

EXPERIMENTAL

For general directions, i.e. spectroscopy details and counting method see earlier papers in the series.[8,9]

Racemate of N-methylcoclaurine[10] was prepared by standard method.

Resolution. (±) - O,O - Bisbenzyl - N - methylcoclaurine was resolved by treatment with (−) - (*S*) - di - *p* - toluoyl - *l* - tartaric acid and (+) - (*R*) - di - *p* - toluoyl - *d* - tartaric acid. Hydrogenolysis of the O,O-bisbenzyl ethers with HCl furnished (−)-(*R*)-, and (+)-(*S*)-N-methylcoclaurines.[11]

Labelled precursors. (±)-(3′,5′,8-$^3H_3$) N-methylcoclaurine, (+)-(3′,5′,8-$^3H_3$) and (−)-(3′,5′,8-$^3H_3$) N-methylcoclaurines were prepared by base catalysed exchange method.[6]

Feeding experiment. For feeding purpose N-methylcoclaurine was dissolved in water (1 ml) containing tartaric acid (10 mg). The soln of the precursor was introduced into young *T. racemosa* plants by Wick Feeding. When uptake was complete the plants were left for 6–8 days and then worked up for tiliageine (5).

Isolation of tiliageine (5). Young plants (typically 130 g of wet wt.) of *T. racemosa* fed with precursors were macerated in EtOH (300 ml) with inactive tiliageine (100 mg) in 1 ml of 5% AcOH and left overnight. The EtOH was then decanted and the plant material was percolated with fresh EtOH (5 × 200 ml, containing 1% AcOH). The combined ethanolic extract was concentrated *in vacuo* to afford a greenish viscous mass which was extracted with water (3 × 10 ml) and remaining material was further extracted with 2% AcOH (3 × 10 ml). The combined aqueous acidic soln was defatted with ether (4 × 10 ml), basified with Na_2Co_3 (pH 8–9), the precipitated bases were extracted with $CHCl_3$:MeOH (90:10) (5 × 20 ml), washed with water, dried (anhy. Na_2SO_4) and solvent was removed to give the crude alkaloidal mixture (110 mg) which was chromatographed over a column of neutral Al_2O_3 (10.5 g). The residue from chloroform eluate was further subjected to preparative thin layer chromatography (silica gel GF254; solvent—$CHCl_3$:MeOH 90:10) to give *tiliageine* (5) (80 mg) m.p. 268–270° (lit.[1] 270°).

$KMNO_4$ *Oxidation of* O,O-*dimethyltiliageine.* Tiliageine (5) (molar activity 8.86×10^{-2} μCi m mol^{-1}) derived from (±)-(3′,5′,8-$^3H_3$) N-methylcoclaurine feeding was treated with ethereal diazomethane to give the O,O-*dimethyltiliageine* (6) m.p. 169–170° (lit.[1] 170–172°) (molar activity 8.82×10^{-2} μCi m mol^{-1}). Compound 6 in 1% H_2SO_4 (10 ml) (pH 5–6 adjusted with NaOH) was oxidised with 2% $KMnO_4$ aq (6 ml) as described earlier[7] to give *5,5′-dicarbomethoxy-2,2′-dimethoxydiphenyl* (9) m.p. 167–168° (lit.[1] 168–169°). (Molar activity 5.86×10^{-2} μCi m mol^{-1}). The radio chemical purity of 9 was checked by dilution technique.

$KMnO_4$ *Oxidation of tiliageine derived from* (+)-(*S*)-(3′,5′,8-$^3H_3$) N-*methylcoclaurine feeding.* A well stirred soln of 5 (molar activity 9.82×10^{-2} μCi m mol^{-1}) in 1% H_2SO_4 (adjusted to pH 5–6 by the addition of NaOH) was oxidised with a 2% $KMnO_4$ (5 ml) according to the method[7] described to give the *dimethyl-4-methoxyisophthalate* (8) m.p. 94° (lit.[7] 94–95°) (essentially radio inactive).

$KMNO_4$ *Oxidation of tiliageine derived from* (−)-(R)-(3′,5′,8-$^3H_3$) N-*methylcoclaurine feeding.* Tiliageine 5 (molar activity 6.30×10^{-2} μCi m mol^{-1}) in 1% H_2SO_4 (adjusted to pH 5–6 with NaOH) was oxidised with 2% $KMNO_4$ to give the *dimethyl-4-methoxyisophthalate* (8) m.p. 94–95° (lit.[7] 94–95°) (molar activity 3.10×10^{-2} μCi m mol^{-1}). The radio chemical purity of 8 was checked by dilution method.

Acknowledgement—Grateful thanks are due to Drs. P. L. Schiff, Jr. and D. Dwuma-Badu for the generous gift of a sample of tiliageine and to Miss Sudha Jain for the isolation of tiliageine.

REFERENCES

[1]A. N. Tackie, D. Dwuma-Badu, T. T. Dabra, J. E. Knapp, D. J. Slatkin and P. L. Schiff Jr., *Experientia* **30**, 847 (1974).
[2]A. N. Tackie, D. Dwuma-Badu, J. S. K. Ayim, T. T. Dabra, J. E. Knapp, D. J. Slatkin and P. L. Schiff Jr., *Lloydia*, p. 138. **38**, 210 (1975).
[3]M. Shamma, *The Isoquinoline Alkaloids*, p. 138. Academic Press, New York (1972).
[4]Y. Inubushi, K. Nomura and M. Miyawaki, *J. Pharm. Soc. Japan* **83**, 1282 (1963).
[5]D. H. R. Barton, G. W. Kirby and A. Wiechers, *J. Chem. Soc.* (C), 2313 (1966).
[6]D. S. Bhakuni, V. M. Labroo, A. N. Singh and R. S. Kapil, *Ibid.* Perkin I, 3469 (1977).
[7]A. N. Tackie, D. Dwuma-Badu, J. E. Knapp and P. L. Schiff, Jr., *Lloydia* **36**, 66 (1973).
[8]D. S. Bhakuni, S. Tewari and R. S. Kapil, *J. Chem. Soc.* Perkin I. 706 (1977).
[9]D. S. Bhakuni, S. Tewari, A. N. Singh and R. S. Kapil, *Ibid.* Perkin I, 1662 (1977).
[10]H. Yamaguchi, *J. Pharm. Soc. Japan* **78**, 678 (1958).
[11]H. Yamaguchi, *Ibid.* **78**, 692 (1958).