Natural Product Sciences
7(3) : 83~86 (2001)

Tiliacosine and Tiliasine, two New Bisbenzylisoquinoline Alkaloids from *Tiliacora racemosa*

Tapan Seal[1], Amarendra Patra[2], Gobinda Mukhopadhayay[3] and Biswapati Mukherjee[3]*

[1]Botanical Survey of India, Indian Botanic Garden , Howrah 711 103, India,
[2]Centre of Advanced Studies on Natural Products, Department of Pure Chemistry, University College of Science, Calcutta 700 009, India,
[3]S. N. Pradhan Centre for Neurosciences, B. C. Roy Post Graduate Institute of Basic Medical Sciences 244B, Acharya J. C. Bose Road, Calcutta 700 020, India

Abstract – Two new bisbenzylisoquinoline alkaloids tiliacosine (**1**) and tiliasine (**2**) were isolated from the leaves of *Tiliacora racemosa* Colebr.. The structures of these alkaloids were established on the basis of spectral evidence and by the correlation of their ^{1}H-NMR spectral data with those of the congeners *N*-methyltiliamosine (**3**), tiliamosine (**4**) and tiliacorine (**5**).
Key words – *Tiliacora racemosa*, Menispermaceae, alkaloids, tiliacosine, tiliasine.

Introduction

Tiliacora racemosa Colebr. (Menispermaceae) grows wildly in eastern India and is regarded as an antidote to snake bite and scorpion sting (Kirtikar *et al.*, 1933). It is a rich source of bisbenzylisoquinoline (BBI) alkaloids which are well known for their pharmacological activities such as antitumour (Kupchan et al., 1973), antimicrobial (Wu *et al.*, 1976, 1977) and hypotensive (Wu et al., 1976, 1977; Joshi et al., 1974) effects etc. The BBI alkaloids tiliacorine, tiliacorinine, tiliamosine, tiliaresine, tiliarine and N-methyltiliamosine were isolated earlier in our laboratory from different parts of the plant and were identified from detail spectral evidence (Guha *et al.*, 1976; Guinaudeau *et al.*, 1985; Ray *et al.*, 1989, 1990). In continuation of our studies, we wish to report here the isolation and identification of two new BBI alkaloids, tiliacosine (1) and tiliasine (2) from the leaves of this plant.

Experimental

General Experimental Procedures – The UV spectra of tiliacosine and tiliasine were recorded in Hitachi U 2000 spectrophotometer in aldehyde free alcohol. IR spectra were taken in Perkin Elmer 782 spectrophotometer in KBr pellets. ^{1}H-NMR spectra were recorded in d_4-MeOH solution on a Bruker AM 300 L spectrometer with TMS as internal standard. Mass spectra of the compounds were kindly supplied by Dr. B. C. Das, Institute de Chimie des Substances Naturelles, Gif-sur-Yvette, France at 70 eV using direct inlet system. TLC and preparative TLC were performed over silica gel G, using benzene-methanol (8:1) as solvent system.

Plant materials – Fresh leaves of Tiliacora racemosa were collected locally in the month of January, 2000 and identified by Mr. Aloke Bhattacharya, Botanist, Botanical Survey of India, Indian Botanic Garden, Howrah 711 103. A voucher specimen No. BM/UCM/005 has been preserved in our laboratory.

Extraction and isolation – Air-dried leaves (2 kg) were powdered and soxhleted with petroleum ether (60-80°C) for 48 h. Defatted leaves were then extracted with ethanol: acetic acid (95:5) for fifteen days by percolation. After removal of the solvent, the residual matter (50 g) was extracted with 5% citric acid (3×200 ml) and filtered. Acid extract was washed with petroleum ether (40-60°C) and basified with ammonium hydroxide to pH 8. A buff colored precipitate separated which was extracted with benzene (3×200 ml). Benzene was removed from the extract under reduced pressure to obtain the total base fraction (10 g). In a pilot experiment a part of the base (1 g) was churned with aqueous citric acid (10%, 3×100 ml) and filtered. The filtrate was washed with ethyl

*Author for correspondence.

acetate (6×50 ml) to remove non-alkaloidal coloring matters and made alkaline with ammonium hydroxide to pH 8. A buff colored precipitate separated which was extracted with benzene (5×100 ml). Benzene extract was washed with water, dried (Na_2SO_4) and concentrated to obtain a cream colored solid (0.3 g). On TLC it showed two spots of distinctly different R_f which were separated by PTLC using the solvent (C_6H_6-MeOH 8:1) as developer and iodine as indicator to afford tiliacosine (0.01 g) from the upper zone (R_f 0.47) and tiliasine (0.007 g) from the lower band (R_f 0.41).

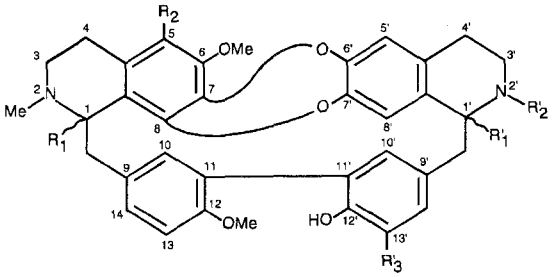
Results and Discussion

Tiliacosine $[\alpha]_D$ +5.12° (MeOH) and tiliasine $[\alpha]_D$ +4.10° (MeOH) were amorphous. These two alkaloids produced transient blue coloration when their solutions in cold concentrated sulfuric acid were treated with traces of cold conc. nitric acid. This is indicative of the presence of dibenzo-*p*-dioxin system (Anjaneyulu *et al.*, 1969). The UV spectra of tiliacosine [λ_{max} (log ε) at 291.0 (3.62) and 225.4(4.68) nm, λ_{min} (log ε) at 273.0 (2.84) nm] and tiliasine [λ_{max} (log ε) at 290(3.48) and 235 sh (4.70) nm] were similar to those of BBI alkaloids having dibenzo-*p*-dioxin (C-8, C-7' & C-7, C-6') and biphenyl (C-11, C-11') system (Guha *et al.*, 1979). The IR spectra displayed [tiliacosine 3400 cm^{-1} and tiliasine 3350 cm^{-1}] absorption due to hydrogen bonded phenolic -OH group. The 300 MHz ^{1}H-NMR spectrum of the alkaloid indicated the presence of two -NMe groups (δ 2.26, 2.67), three -OMe groups (δ 3.74, 3.81, 3.91) and seven aromatic protons (δ 6.60- 8.03). The IR and ^{1}H-NMR spectra suggested that tiliacosine was very much similar to 1*S*, 1*S* *N*-methyltiliamosine (**3**) (Ray *et al.*, 1989) excepting that the former had seven aromatic protons whereas there were eight in the latter. The mass spectrum (70 eV) of tiliacosine exhibited highest ion peak at *m/z* 620 with base peak at *m/z* 379. The latter ion peak originated from the upper half of the molecule as in the case of *N*-methyltiliamosine, arising out of the double benzylic cleavage. Thus, two -OMe groups, two aromatic protons and two -NMe groups of tiliacosine are in the upper half of the molecule and the third -OMe is located in its lower half which is having one aromatic proton less than that of *N*-methyltiliamosine.

Assignment of resonances for seven aromatic protons (δ 6.60→H-5', δ 8.03→H-8', δ 7.49→H-10', δ 7.43→H-10, δ 6.99→H-13, δ 7.29→H-14, δ 7.38 →H-14'), and positions of -OMe (δ 3.74, 3.81, 3.91') of this alkaloid were settled by comparison with the spectral scenario of *N*-methyltiliamosine (Table 1) complemented by homo decoupling experiments in appropriate cases. Tiliacosine exhibited highest ion peak at 14 a.m.u higher than that observed for *N*-methyltiliamosine (*m/z* 606). Since tiliacosine (R_f 0.47) was polar relative to *N*-methyltiliamosine (R_f 0.60), an additional -OH group might be present in tiliacosine, substituing the aromatic proton at C 13' in the lower half of *N*-methyltiliamosine. Further the presence of a catechol unit would readily account for the loss of two a.m.u. from molecular ion peak at *m/z* 622 to generate highest ion peak at *m/z* 620. Therefore, tiliacosine may be represented by structure **1**. The UV spectrum of tiliasine [λ_{max} 290 (3.48), 235 sh (4.70) nm] was again indicative of the presence of dibenzo-*p*-dioxin and biphenyl systems. The IR spectrum displayed absorption at 3350 cm^{-1} due to hydrogen bonded phenolic -OH group. The 300 MHz ^{1}H-NMR spectrum of tiliasine showed the presence of one -NMe (δ 2.25), three -OMe (δ 3.76, 3.84, 3.89) and seven aromatic protons (δ 6.69-8.09). The IR and ^{1}H-NMR spectrum of tiliasine was very much similar to tiliacosine except the presence of an additional -NMe in the latter. The mass spectrum of tiliasine exhibited a highest ion peak *m/z* 606 with base peak at *m/z* 365. The latter representing the top half of the molecule as in the case of tiliamosine (**4**) that resulted from the double benzylic cleavages. So, two -OMe, two aromatic protons and one -NMe group

Table 1. 300 MHz ^{1}H-NMR spectral signals (δ in ppm) of tiliacosine (**1**), tiliasine (**2**), *N*-methyltiliamosine (**3**), tiliamosine (**4**) and tiliacorine (**5**)

Alkaloid	1	2	3	4	5
2-NMe	2.26	2.25	2.20	2.23	2.35
2'-NMe	2.67	2'-NH	2.51	2'-NH	2.71
1-H	3.58	3.59	3.20	3.36	4.09
1'-H	–	4.10	3.35	3.99	3.41
5-OMe	3.74	3.76	3.72	3.72	5-H (6.30)
5'-H	6.60	6.69	6.54	6.58	6.72
8'-H	8.03	8.09	7.99	8.04	7.02
10-H	7.49	7.49	7.59	7.51	7.93
13-H	6.99	6.99	6.88	6.88	6.93
14-H	7.29	7.31	7.27	7.32	7.26
10'-H	7.43	7.44	7.50	7.48	7.28
13'-H	13'-OH	13'-OH	6.91	6.88	7.10
14'-H	7.38	7.44	7.17	7.27	7.26
6-OMe	3.81	3.84	3.82	3.87	3.87
12-OMe	3.91	3.89	3.89	3.82	3.97



Tiliacosine (**1**) : R_1 = H ▶ , R_1' = H ▷ , R_2 = OMe, R_2' = Me, R_3' = OH
Tiliasine (**2**) : R_1 = H ▶ , R_1' = H ▷ , R_2 = OMe, R_2' = H, R_3' = OH
N-methyltilamosine (**3**): R_1 = H ▶ , R_1' = H ▷ , R_2 = OMe, R_2' = Me, R_3' = H
Tiliamosine (**4**) : R_1 = H ▶ , R_1' = H ▷ , R_2 = OMe, R_2' = H, R_3' = H
Tiliacorine (**5**) : R_1 = R_1' = H ▷ , R_2 = R_3' = H, R_2' = Me

are in the upper half of tiliasine whereas the third -OMe and another five aromatic protons are placed in the lower half of the molecule. Three aromatic proton resonances at δ 7.49 (1H, d, *J*=2.0 Hz), 7.31 (1H, dd, *J*=8.4 Hz and 2.0 Hz), and 6.99 (1H, d, *J*=8.4 Hz) were for a 1, 3, 4 trisubstituted benzenoid system, very similar to those of H-10, H-14 and H-13 in 1 as well as in *N*-methyltiliamosine and tiliamosine but different from those of tiliacorine (**5**) (vide Table 1). Such proton arrangement were established by homodecoupling experiments. Two aromatic *m*-oriented protons resonated at δ 7.49 and 7.44 in 2 as broad singlets. The position of -OMe groups of tiliasine was settled by comparing ^{1}H-NMR spectrum of tiliacosine with that of tiliamosine. Considering the above discussions tiliasine may be represented by structure **2**. The resonance positions for H-10 and H-10 in both tiliacosine and tiliasine are very close as in the *SS* series (H-1 and H-1' oppositely placed, anti) alkaloids **3** and **4** in complete contrast to the markedly different resonance positions for H-10 and H-10' in the *RS* series (H-1 and H-1' on same side, syn) alkaloid tiliacorine indicating their chirality to *SS* at C-1 and C-1' i.e. H-1 and H-1' are oppositely placed (Bhakuni *et al.*, 1978).

Tiliacosine (**1**): UV λ_{max} (log ε) 291.0 (0.780), 225.4 (3.14) nm; IR (KBr) υ_{max} 3400, 2915, 1500, 1450, 1270 cm^{-1}; ^{1}H-NMR (d_4-MeOH, 300 MHz) δ 2.26 (2-NMe), 2.67 (2-NMe), 2.36-3.25 (6×CH_2), 3.74 (5-OMe), 3.81 (6-OMe), 3.91 (12-OMe), 6.60 (H-5'), 6.99 (d, *J*=8.4 Hz, H-13), 7.29 (dd, *J*=8.4 & 2.0 Hz, H-14), 7.38 (H-14'), 7.43 (d, *J*=8.4 Hz, H-10'), 7.49 (d, *J*=2.0 Hz, H-10), 8.03 (H-8'); MS *m/z* 620(M^+-2), 380, 379, 190.

Tiliasine (2): UV λ_{max} (log ε) 290.0(3.48), 2.35 sh (4.70) nm; IR (KBr) υ_{max} 3350, 2800, 1560, 1400 cm^{-1}; ^{1}H-NMR (d_4-MeOH, 300 MHz): δ 2.25 (2-NMe), 3.59 (H-1), 4.10 (H-1'), 3.76 (5-OMe), 3.84 (6-OMe), 3.89 (12-OMe), 6.69 (H-5'), 6.99 (d, J=8.4 Hz, H-13), 7.31 (dd, J=8.4 & 2.0 Hz, H-14), 7.44 (H-14'), 7.44 (H-10'), 7.49 (d, J=2.0 Hz, H-10), 8.09 (H-8'); MS m/z: 606 (M^+-2), 366, 365,183.

Acknowledgements

The authors wish to thank the International Foundation for Science, Sweden for financing the project and Centre of Advanced Studies on Natural Products, Department of Pure Chemistry, Calcutta University, for the spectral data.

References

Anjaneyulu, B., Govindachari, T. R., Sathe, S. S., Viswanathan, N., Gopinath, K. W. and Pai, B. R., Alkaloids of *Tiliacora racemosa* Colebr. *Tetrahedron* **25**, 3091-3105 (1969).

Bhakuni, D. S., Singh, A. N., Jain, S. and Kapil, R. S., Absolute configuration and biosynthesis of Tiliacorine and Tiliacorinine. *J. Chem. Soc. Chem. Commun.* 226-228 (1978).

Guha, K. P., Das, P. C., Mukherjee, B., Mukherjee, R., Juneau, G. P. and Bhacca, N. S., Structure of Tiliamosine: A new diphenylbisbenzylisoquinoline alkaloid from *Tiliacora racemosa*. *Tetrahedron Letters* **47**, 4241-4244 (1976).

Guha, K. P., Mukherjee, B. and Mukherjee, R., Bisbenzylisoquinoline alkaloids: A review. *J. Nat. Prod.* **42**, 1-84 (1979).

Guinaudeau, H., Freyer, A. J., Shamma, M., Mitra, S. K., Ray, A. K. and Mukherjee, B., The structure of (+)-Tiliarine. *J. Nat. Prod*, **48**, 651-53 (1985).

Joshi, P.P., Bhakuni, D. S. and Dhar, M. M., Structure and streochemistry of Cocsulinin. *Indian J. Chem*, **12**, 517-518 (1974).

Kirtikar, K. R. and Basu, B. D., Indian Medicinal Plants. Vol. 1, p. 83, Lolit Mohan Basu, Allahabad, India (1933).

Kupchan, S. M. and Atland, H. W., Structural requirements for tumor-inhibitory activity among benzylisoquinoline alkaloids and related synthetic compounds. *J. Med. Chem.* **16**(8), 913-917 (1973).

Ray, A. K., Mukhopadhayay, G., Mitra, S. K., Guha, K. P., Mukherjee, B., Rahman, A. and Nelofar, A., (+)-*N*-Methyltiliamosine, an alkaloid from *Tiliacora racemosa Phytochemistry* **28**, 675-76 (1989).

Ray, A. K., Mukhopadhayay, G., Mitra, S. K., Guha, K.

P., Mukherjee, B., Rahman, A. and Nelofar, A. A., Diphenylbisbenzylisoquinoline alkaloid from *Tiliacora racemosa*. *Phytochemistry* **29**, 1020-22 (1990).

Wu, W.-N., Beal, J. L. and Doskotch, R. W., Alkaloids of *Thalictrum*. XVI. Isolation of two additional alkaloids from the root of *Thalictrum lucidum*. *Lloydia* **39**(5), 378-379 (1976).

Wu, W.-N., Beal, J. L., Leu, R.-P. and Doskotch, R. W., Alkaloids of *Thalictrum*. XXI. Isolation and characterisation of alkaloids from the roots of *Thalictrum podocarpum*. *Lloydia* **40**, 384-394 (1977).

(Accepted July 21, 2001)