Natural Product Sciences
5(3) : 142~147 (1999)

Some Pharmacological Studies with Tiliacorine, a Diphenylbisbenzylisoquinoline Alkaloid from *Tiliacora racemosa*

Arnab Khasnobis[1], Tapan Seal[2], J. Rajan Vedasiromoni[3], Malaya Gupta[4] and Biswapati Mukherjee[5]*

[1]Central Drugs Laboratory, 3 Kyd Street, Calcutta 700-016
[2]Botanical Survey of India, Indian Botanic Garden, Howrah 711-103
[3]Indian Institute of Chemical Biology, Calcutta 700-032
[4]Department of Pharmacy, Jadavpur University, Calcutta 700-032
[5]S. N. Pradhan Centre for Neurosciences, University College of Medicine, 244 B, Acharya J.C. Bose Road, Calcutta 700-020, India.

Abstract – *Tiliacora racemosa* Colebr. belonging to the family Menispermaceae is the biggest storehouse of diphenyl bisbenzylisoquinoline (DBBI) alkaloids. Exhaustive chemical processing of the root of *T. racemosa* by the application of modern separation techniques yielded a DBBI alkaloid which was identified as tiliacorine using sophisticated spectroscopic methods (UV, IR, ^{1}H-NMR, Mass). Tiliacorine potentiated the sleeping time induced by standard hypnotics *viz.* chlorpromazine (**CPZ**), pentobarbitone (**PB**) and diazepam (**DZ**) in a dose dependent manner. Tiliacorine potentiated the analgesic action of standard analgesic agents *viz.*, morphine and meperidine. It was also found to possess anticonvulsive activity in the strychnine induced convulsion model.
Key words – *Tiliacora racemosa*; tiliacorine; sedative-hypnotic; analgesic; anticonvulsant.

Introduction

Tiliacora racemosa Colebr. a plant belonging to the family Menispermaceae, is known to be the biggest storehouse of diphenylbisbenzylisoquinoline (DBBI) alkaloids which are well-known for their pharmacological activities such as antitumor (Kupchan *et al.*, 1973), antimicrobial (Wu, W.-N. *et al.* 1976) and hypotensive (Joshi *et al.*, 1974, Wu, W.-N. *et al.* 1976 & 1977) effects. The root of this plant having folkloric medicinal application, e.g., antidote to snake bite and scorpion sting (Kirtikar *et al.*1984), was chemically processed for the isolation and identification of alkaloids viz. tiliacorine, tiliacorinine, nor-tiliacorinine A, tiliamosine, *N*-methyltiliamosine, tiliaresine, tiliarine, *N*-methyltiliarine. In the present study we report some pharmacological activities of tiliacorine (**1**).

Experimental

Plant materials – Fresh roots of *Tiliacora racemosa* (Menispermaceae) were collected from Indian Botanic Garden, Howrah, and were identified by Mr. Aloke Bhattacharya, Botanist, Botanical Survey of India, Calcutta. A voucher specimen (No. BM/UCM/005) has been preserved in our laboratory.

Instrumentation – The UV spectrum of tiliacorine was recorded in Hitachi U 2000 spectrophotometer in aldehyde free alcohol. IR spectrum was taken in Perkin Elmer 782 spectrophotometer in KBr pellets. ^{1}H-NMR spectrum was recorded in $CDCl_3$ solution on a Bruker AM 300 L spectrometer with TMS as internal standard. Mass spectrum of the compound was kindly supplied by Dr. B. C. Das, Institute de Chimie des Substances Naturelles, Gif-Sur-Yvette, France carried out at 70 eV using direct inlet system. Column chromatography was performed over Silica gel (60-120 mesh), using mixture of chloroform and methanol of increasing polarity.

Animals – Swiss albino male mice (weighing 25-30 g) were used. The animals were fed standard pellet diet and water was provided *ad libitum*. The animals were housed in groups of 10 animals at 25±1°C. The animals were not provided with food for last 17 hours before the commencement of the experiment.

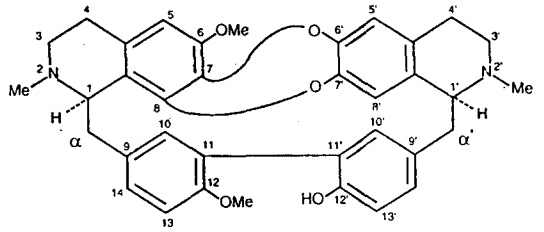
Drugs – The drugs used were acetylsalicylic acid

*Author for correspondence.

(**Disprin**), chlorpromazine hydrochloride (**CPZ**), diazepam (**DZ**), glacial acetic acid, meperidine hydrochloride, morphine sulphate, pentobarbitone sodium (**PB**), propylene glycol, sodium chloride, strychnine hydrochloride, tiliacorine and d-tubocurarine chloride (d-TC).

Isolation and Identification of Tiliacorine

Finely ground air-dried roots (1 kg) were powdered and soxhleted with petroleum ether (60-80°C) for 48h. Defatted roots were then extracted with ethanol : acetic acid (95:5) for fifteen days by percolation. After removal of the solvent, the residual matter (20 g) was churned with 5% citric acid (3×200 ml) and filtered. Acid extract was washed with petroleum-ether (40-60°C) and basified with ammonium hydroxide (pH 8). The buff coloured precipitate was extracted with chloroform (3×200 ml). Chloroform was removed under reduced pressure to obtain the total base fraction (5 g), which was subjected to column chromatography using silica gel (60-120 mesh) as adsorbent and mixture of chloroform and methanol of increasing polarity as solvent. The compound (R_f 0.56 , Adsorbent : Silica gel G, Developer : benzene : chloroform : methanol :: 6:3:2, Indicator : Dragendorff reagent) that migrated was purified by repeated crystallisation (ethylacetate-acetone) and identified as tiliacorine (**1**) (100 mg; yield 0.01%) ($C_{36}H_{36}N_2O_5$, mp. 262-264°C) from spectroscopic analysis and by comparing with authentic samples (Guha *et al.*, 1976, Guinaudeau *et al.* 1985 , Ray *et al.*, 1989 & 1990).



1

Tiliacorine – Mol. formula : $C_{36}H_{36}N_2O_5$; mp.: 262-264°C; [α]: +71.2° (Pyridine); UV (EtOH) : 295 nm (3.91); CD : +216 (230), +754 (250), +461 (291) nm; IR (cm^{-1}) : 3400; ^{1}H-NMR (300 MHz, $CDCl_3$) : 2.35, 2.71, 3.41, 3.87, 3.97, 4.09, 6.30, 6.72, 6.93, 7.02, 7.10, 7.26, 7.26, 7.28, 7.93; MS (m/z) : 576 (M^+), 350, 349, 335, 288, 175.

Preparation of drug – Tiliacorine (75 mg) was dissolved in minimum volume of acetone and excess methyliodide was added to it, filtered and the residue (200 mg) was dried. Methiodide salt of tiliacorine thus prepared was freely soluble in water and was taken as drug for pharmacological investigations.

Pharmacological Studies

Safety evaluation – LD_{50} was determined by the standard method of Litchfield and Wilcoxon (Litchifield *et al.*,1949) .d- Tubocurarine chloride (d-TC) was taken as the reference drug for comparison with tiliacorine. The drugs were administered i.v. to groups of 10 albino mice at doses upto 12.5 mg/kg for tiliacorine and 0.22 mg/kg for dTC and the animals were observed 24h for mortality.

Barbiturate potentiation – Albino mice were used and they were divided into twenty eight groups, each group containing ten mice. Chlorpromazine hydrochloride (**CPZ**), diazepam (**DZ**) and pentobarbitone sodium (**PB**) were used as the standard sleep inducing agents. Three doses of tiliacorine, viz. 1.33, 1.77 and 2.65 mg/kg were administered i.p. to three different groups of animals respectively. Control group received 10 ml/kg normal saline and the animals in the reference groups received i.p. 10 mg/kg **CPZ**, 5 mg/kg **DZ** and 50 mg/kg **PB** respectively. The criterion for sleep in mice was loss of righting reflex and the sleeping time was measured. Potentiation of sleeping time induced by different reference standards was also noted with respect to d-TC at doses of 0.0237, 0.0316 and 0.0475 mg/kg respectively.

Analgesic activity – The analgesic activity was tested by the following methods:

i) Acetic acid induced writhing (chemical stimulus)

Albino male mice were divided into seven groups, each group containing ten mice. Writhing response was induced by 2% acetic acid at a dose of 200 mg/kg i.p. (Turner *et al.*, 1965). The test and the standard drugs were administered 5 minutes prior to i.p. injection of acetic acid.The number of abdominal constrictions (writhings) and stretchings with a jerk of the hind limbs were counted between 5 and 15 minutes after administering acetic acid (Koster *et al.*, 1959). The analgesic effect of the drugs was calculated by the percentage inhibition of writhing episode over that of the control group. The results were compared with that of acetylsalicylic acid (disprin; 200 mg/kg i.p.).

ii) Thermal stimulus by Eddys hot plate method

The analgesic actions were studied using Eddys hot plate method (Eddy *et al.* 1953) . Albino male mice were divided into six groups, each group containing ten mice. The temperature of the hot plate was main-

tained at 55 ±1°C. The reaction time was taken as the interval extending from the instant the mouse reached the hot plate till the animal licked its feet or jumped out of the cylinder. The average normal reaction time was about 5 seconds for selection of animals for the experiment. Reaction time was recorded at intervals of 15 minutes for 2 hours after drug administration.

Anticonvulsant activity – Strychnine antagonism by tiliacorine and d TC was examined by the method of Chen and Portman (Chen *et al.*,1952). Albino male mice were divided into fifteen groups, each group containing ten mice. The control group of animals received normal saline at a dose of 10 ml/kg i.p., convulsive responses were produced by strychnine at doses of 1.5 mg/kg (50% convulsion) and 2 mg/kg (100% convulsion). Effect of tiliacorine (1.33 to 5.30 mg/kg i.p.) was studied on strychnine induced convulsions in separate groups of mice. Strychnine induced convulsion was recorded at 5 min and 15 min respectively. Tiliacorine was administered 5 min and 15 min prior to the strychnine induced convulsion and the recovery from strychnine induced convulsion for each group of mice (n = 10) was recorded at 5 min. and 15 min respectively. Accordingly percentage of convulsion was recorded. Similar experiments were performed with d TC at doses between 0.0237 and 0.0950 mg/kg.

Statistical analysis – Values are expressed as Mean±SEM and the significance of difference of data obtained was evaluated statistically using the Students t- test.

Results

Safety evaluation – Acute toxicity tests in mice established the LD_{50} of tiliacorine and d-TC to be 10.60 mg/kg i.v. and 0.189 mg/kg i.v. respectively.

Barbiturate potentiation – Three doses of tiliacorine (1.33, 1.77, 2.65 mg/kg) potentiated the sleeping time induced by standard hypnotics viz. chlorpromazine (**CPZ**) [12.4%, 47.7% and 94.4% respectively], diazepam(**DZ**) [20.6%, 61.1% and 140.0% respectively] and pentobarbitone (**PB**) [7.3%, 50.0% and 96.4% respectively] (Fig. 1). Similarly d-TC (three doses 0.0237, 0.0316 and 0.0475 mg/kg) also potentiated the sleeping time induced by **CPZ** [16.6%, 63.2% and 110.2% respectively], **DZ** [60.3%, 123.0% and 217.5% respectively] and **PB** [12.8%, 68.6% and 103.0% respectively] (Fig. 2). Tiliacorine and d-TC did not induce sleep when administered alone.

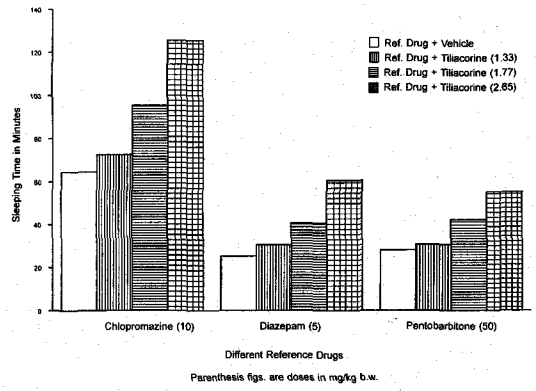


Fig. 1. Effect of Tiliacorine on Sleeping Time (Mins) Induced by Chlorpromazine (CPZ), Diazepam (DZ) & Pentobarbitone (PB) in Mice.

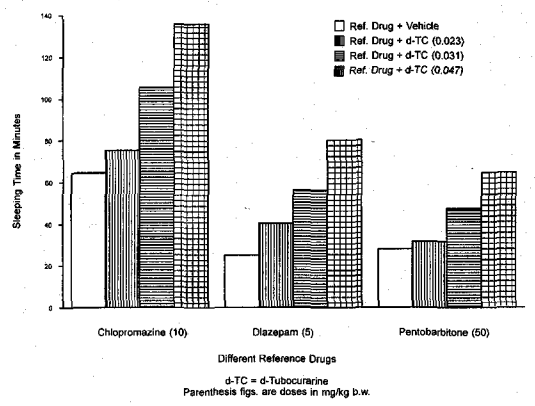


Fig. 2. Effect of d-TC on Sleeping Time (Mins) Induced by Chlorpromazine (CPZ), Diazepam (DZ) & Pentobarbitone (PB) in Mice.

Analgesic activity – Both tiliacorine and d-TC exhibited a dose- dependant and significant analgesic activity in the acetic acid induced writhing test. It was found that in combination with acetylsalicylic acid,

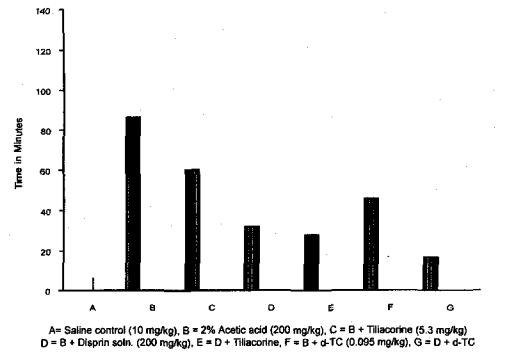


Fig. 3. Effect of Tiliacorine & d-TC on Acetic Acid Induced Writhing Episodes in Mice.

Table 1. Effect of tiliacorine, d-TC, and disprin on acetic acid induced writhing episodes in mice

Drug	Writhing episodes for 5 mins (Mean ± SEM)	Percentage of inhibitory activity
Normal saline (0.9% w/v, 10 ml / kg)	–	–
2% Acetic acid (200 mg / kg)	86.40 ± 2.48	–
Tiliacorine (5.3 mg /kg) + Acetic acid (200 mg / kg)	60.00 ± 3.40	30.56
Disprin (200 mg / kg) +2% Acetic acid (200 mg / kg)	32.00 ± 2.10	62.96
Disprin (200 mg / kg) + 2% Acetic acid (200 mg / kg) + Tiliacorine (5.3 mg /kg)	28.00 ± 1.37	67.59
d-TC (0.095 mg /kg) + 2% Acetic acid (200 mg / kg)	45.80 ± 1.71	47.00
d-TC (0.095 mg /kg) + 2% Acetic acid (200 mg / kg) + Disprin (200 mg / kg)	16.80 ± 1.30	80.55

Number of animals used for each group (n = 10)

Table 2. Effect of tiliacorine on morphine / meperidine induced analgesia in mice

Drug	Reaction time in seconds (Mean ± SEM) at different time intervals			
	15 min	30 min	60 min	120 min
Normal saline (0.9% w/v, 10 ml / kg)	–	–	–	–
Tiliacorine (5.3 mg / kg)	12.70 ± 0.98	8.00 ±0.33	6.80 ± 0.36	5.00 ±0.25
Morphine (5 mg / kg)	27.40 ± 0.30	24.50 ± 0.40	13.10 ± 1.01	5.50 ± 0.34
Meperidine (5 mg / kg)	21.66 ± 2.06	17.00 ± 1.73	8.60 ± 0.88	5.30 ± 1.05
Tiliacorine (5.3 mg / kg) + Morphine (5 mg / kg)	68.00 ± 0.59*	50.00 ± 0.77*	38.00 ± 0.77*	13.8 ± 0.46*
Tiliacorine (5.3 mg / kg) + Meperidine (5 mg / kg)	40.40 ± 0.80*	26.40 ± 0.65*	15.00 ± 0.42*	10.0 ± 0.47*

Number of animals used for each group (n = 10)
The statistical significance between means are calculated by Student s unpaired t-test.
* $P < 0.05$

tiliacorine (5.3 mg/kg i.p.) showed more significant analgesic activity in the acetic acid induced writhing episodes (Table 1, Fig. 3.). Further analgesic studies revealed that tiliacorine alone (5.3 mg/kg i.p.) produced an elevation of pain threshold after 15 min followed by progressive diminution of the activity within 120 min Morphine (5 mg/kg i.p.) and meperidine (5 mg/kg i.p.) were used as standard drugs. Tiliacorine (5.3 mg/kg i.p.) increased morphine analgesia by 2.48 times and meperidine analgesia by 1.86 times (Table 2, Fig. 4).

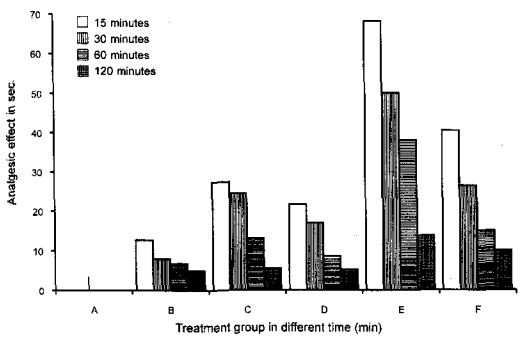


Fig. 4. Analgesic Effect of Tiliacorine, Morphine and Meperidine in Hot Plate Method in Mice.

Anticonvulsant activity – Strychnine at a dose of 1.5 and 2.0 mg/kg i.p. induced tonic type of convulsions with clonus in mice.

Degree of convulsion was measured visually. The animals were observed for a period of 5 and 15 min during the experiment. Two doses of strychnine hydrochloride (1.5 and 2.0 mg/kg i.p.) which produced 50% and 100% convulsion respectively were used in this study (Table 3). Tiliacorine at a dose of 5.3 mg/kg i.p. protected 10% of mice against strychnine (2 mg/kg) induced convulsion only at 5 min duration. On the other hand d-TC at a dose of 0.095

Table 3. Effect of tiliacorine and d-TC on strychnine induced convulsions in mice

Drug	% Convulsions (control)	% Convulsions (Pretreatment)	
		5 minutes	15 minutes
Normal saline (0.9 % w/v, 10 ml / kg)	–	–	–
Strychnine (1.5 mg / kg)	50	–	–
Strychnine (2.0 mg / kg)	100	–	–
Tiliacorine (1.33 mg /kg) + Strychnine (1.5 mg / kg)		50	50
Tiliacorine (2.65 mg /kg) + Strychnine (1.5 mg / kg)		50	50
Tiliacorine (5.30 mg /kg) + Strychnine (1.5 mg / kg)		50	50
Tiliacorine (1.33 mg /kg) + Strychnine (2.0 mg / kg)		100	100
Tiliacorine (2.65 mg /kg) + Strychnine (2.0 mg / kg)		100	100
Tiliacorine (5.30 mg /kg) + Strychnine (2.0 mg / kg)		90	100
d-TC (0.0237 mg / kg) + Strychnine (1.5 mg / kg)		50	50
d-TC (0.0475 mg / kg) + Strychnine (1.5 mg / kg)		30	30
d-TC (0.0950 mg / kg) + Strychnine (1.5 mg / kg)		00	00
d-TC (0.0237 mg / kg) + Strychnine (2.0 mg / kg)		100	100
d-TC (0.0475 mg / kg) + Strychnine (2.0 mg / kg)		100	100
d-TC (0.0950 mg / kg) + Strychnine (2.0 mg / kg)		80	100

Number of animals used for each group (n = 10)

mg/kg i.p. protected 100% of mice against convulsions induced by 1.5 mg/kg i.p. of strychnine hydrochloride over a period of both 5 and 15 min duration. It was observed that different combinations of strychnine hydrochloride and tiliacorine in both the duration did not show any significant protective action against convulsion.

Discussion

Generally sedatives and hypnotics suppress cerebral activity, blunting awareness, thereby establishing conditions favourable for sleep. These also depress the CNS beginning with the cerebral cortex and descending with increasing dose to the medullary centres, causing medullary paralysis. Tiliacorine (1.33 mg/kg) and d-TC (0.0237 mg/kg) produced drowsiness at lower doses but at higher doses (2.65 mg/kg, 0.0475 mg/kg) they prolonged the **PB** sleeping time in mice probably by their CNS depressant action. From this study it is clear that both tiliacorine and dTC themselves have no sedative action but they can potentiate the sleeping time induced by hypnotic drugs in a dose- dependant manner in mice. It was revealed from the results (Fig.1 & Fig. 2) that d- TC with different standard drugs like **CPZ**, **DZ** and **PB** exhibited more potent sleeping time than tiliacorine.

It was found that tiliacorine potentiated the analgesic action of morphine and meperidine. In the present study the analgesic action of morphine was more potentiated by tiliacorine in mice than meperidine. Tiliacorine alone did not exhibit analgesic activity. The analgesic activity of tiliacorine was further supported by its anti-stretching activity in mice. Tiliacorine alone has no action on writhing episode in mice. So it can be considered as a non narcotic analgesic agent. Moreover, the narcotic analgesic potentiation action of tiliacorine may be considered to be due to its CNS depressant action.

The convulsant action of strychnine is due to interference with post synaptic inhibition that is mediated by glycine. The main site of action of strychnine is on the spinal cord and convulsion occurs after removal of the rest of the nervous system. The appearance of some induced convulsive state is related to a decrease in gamma amino butyric acid (**GABA**) content of brain (Ramanjaneyulu *et al.*, 1984, Sutinski *et al.* 1964, Killan *et al.* 1960). A definite correlation of a decreased **GABA** level with a state of convulsion and *vice-versa* has not been ascertained. Different dose combinations of strychnine with tiliacorine and d-TC with strychnine indicated that tiliacorine has less potent anti-convulsive activity. Though the exact mechanism is not clear, excessive liberation of **GABA** from the neural junction by tiliacorine in this action is a possibility.

The study reveals that tiliacorine possesses hypnosis potentiating, analgesic and mild anticonvulsive activities and it is less potent than d-TC in these actions.

Acknowledgements

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

References

Chen, G., Portman, R., Titration of central-nervous system depression. *A. M. A. Arch. Neurol. Psychiat.*, **68**, 498-505 (1952).

Eddy, N. B., Leimbach, D., Synthetic analgesics. II. Dithienyl-butenyl and dithienyl butylamines. *J. Pharmac. Exp. Ther.*, **107**, 385 (1953).

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

Guinaudeau, H., Freyer, A. J., Shamma, M., Mitra, S. K., Ray, A. K., Mukherjee, B., The structure of (+)-tiliarine. *J. Nat. Prod.*, **48**, 651-653 (1985).

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

Killan, D. F., Dasgupta, S. R., Willam, E. K., *Inhibition in the Nervous System and Gamma Amino Butyric Acid* edited by Eugen R., Pergamon Press, Oxford, p. 302, 1960.

Kirtikar, K.R., Basu, B. D., Indian Medicinal Plants, Bishen Sing Mahendra Pal Sing, Dehradun, India, p. 32, 1984.

Koster, R., Anderson, M., De Beer E. J., Acetic acid for analgesic screening. *Fed. Proc.*, **18**, 412 (1959).

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

Litchifield, J. T. (Jr.), Wilcoxon F. A., Simplified method of evaluating dose-effect exxperiments. *J. Pharmac. Exp. Ther.*, **96**, 99-113 (1949).

Ramanjaneyulu , R., Ticku, M. K., Interactions of pentamethylenetetrazole and tetrazole analogues with the picrotoxinin site of the benzodiazepine-GABA receptor-ionophore. *Eur. J. Pharmacol.*, **987**, 337 (1984).

Ray, A. K., Mukhopadhyay, G., Mitra, S.K., Guha, K. P., Mukherjee, B., Rahaman. A., Nelofar, A., (±) - N - methyltiliamosine, an alkaloid from *Tiliacora racemosa*. *Phytochemistry*, **28**, 675-676 (1989).

Ray, A. K., Mukhopadhyay, G., Mitra, S.K., Guha, K. P., Mukherjee,B., Rahaman. A., Nelofar, A. A, diphenylbisbenzylisoquinoline alkaloid from *Tiliacora racemosa*. *Phytochemistry*, **29**, 1020-1022 (1990).

Sutinski, I. A., Priyatkina, T. N., Effect of certain drugs on gama amino butyric acid content of central nervous system. *Fed. Proc.*, **23**, 879 (1964).

Turner, R. A., *Screening Method in Pharmacology* Vol. 1, Academic Press, New York and London, p. 114, 1965.

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

Wu, W.-N., Beal, J. L., Leu, R. P., Doskotch, R. W., Alkaloids of *Thalictrum*. XXII. Isolation of alkaloids with hypotensive and antimicrobial activity from *Thalictrum revolutum*. *Lloydia*, **40**, 384-394 (1977).

(Accepted June 28, 1999)