Phytochemistry, 1969, Vol. 8, pp. 1559 to 1563. Pergamon Press. Printed in England.

QUATERNARY ALKALOIDS FROM THE ROOT BARK OF *TILIACORA RACEMOSA*

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(*Received* 19 *November* 1968, *in revised form* 21 *February* 1969)

Abstract—Of the seven water-soluble basic constituents present in the root bark of *Tiliacora racemosa* Colebr., three have been isolated in crystalline form and designated as tiliacine, corine and mohinine. Corine showed a curare type of activity. A new tertiary base, mosine, has been isolated from the water-insoluble fraction.

INTRODUCTION

Tiliacora racemosa Colebr. (Menispermaceae) is a climber which grows abundantly in India and is reported to contain poisonous alkaloids[1] and to be efficacious for snake bite and scorpion sting.[2] Earlier investigators isolated two tertiary alkaloids, tiliacorine[3] and tiliarine,[4] from its root bark and elucidated their constitutions.[5,6] Our preliminary investigations showed that the root contained an appreciable amount of water-soluble bases besides the water-insoluble bases reported earlier. It was, therefore, of interest to reinvestigate this plant.

RESULTS

Isolation of the Alkaloids

The dried and powdered roots† (35 kg) were exhaustively extracted with aq. EtOH–AcOH and the extract concentrated under reduced pressure. The concentrate was basified with dil. NH_4OH and the precipitated base mixture was separated. It showed the presence of six Dragendorff positive spots[7] on TLC (system S_1),[8] designated alphabetically a, b, c, d, e and f (c = tiliacorine), whose R_c values are 0·69, 0·83, 1·00, 1·16, 1·27 and 1·36 respectively. The $CHCl_3$ insoluble fraction of the base mixture yielded a new alkaloid $C_{36}H_{36}O_7N_2$ named mosine (=d) which crystallized from MeOH–Et_2O, m.p. > 340°, formed a methiodide m.p. 283° and a picrate m.p. 265° and contained two methoxyl groups.

The quaternary bases in the aqueous extract were precipitated as their reineckates[9]

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† The roots were collected in the suburbs of Lucknow, U.P. (India).

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and were converted into the chlorides.[10] Paper chromatographic examination of the chloride mixture (system-S_2), indicated seven Dragendorff positive spots; six of them were provisionally designated as A, B, C, D, E and F, whose R_{csm} values are 1·70, 0·75, 0·51, 0·26, 0·14 and 0·07 respectively taking cissamine chloride[11] as the reference. Another feeble spot of R_{csm} value 2·61 detected after keeping the extract for a longer time, possibly due to an artefact, has been designated as α.

R_fs could not be determined as proper resolution of the mixture occurred only after the solvent had run off the paper.

The chloride mixture did not resolve on acid-washed Al_2O_3[12] and was separated by partition chromatography over cellulose column[11] which yielded fractions containing chromatographically pure alkaloids α, A, B, C, D, and E. Substances A, B and D could be crystallized as their iodides, perchlorates or thiocyanates and were named tiliacine, corine and mohininc respectivcly. Though substances α, C and E chlorides were obtained as chromatographically pure amorphous powders, they failed to crystallize as such or as their salts.

Tiliacine crystallized as the iodide m.p. 211–214°, thiocyanate m.p. 183–185° and perchlorate m.p. 228–230°. It analysed for $C_{19}H_{24}O_3N^+$ and contained one methoxyl group. The u.v. absorption of iodide at 280 nm (log ϵ 3·68), suggested a quaternary 1-benzyltetrahydroisoquinoline skeleton[13] and the shift of the maximum to 295 nm (log ϵ 3·88) in 0·1 N aq. NaOH indicated the presence of phenolic group(s).[14] The latter was substantiated by the positive colour tests with benzidine tetrazonium chloride[15] and $FeCl_3$[16] reagents and ν_{max}(KBr) 3155 cm^{-1} (associated OH).

Corine was characterized as crystalline iodide m.p. 156–59° and perchlorate m.p. 147–150°. Elemental analysis of these salts gave the formula $C_{19}H_{24}O_3N^+$ containing one methoxyl group. The u.v. of corine iodide was similar to that of tiliacine iodide, exhibiting absorption maxima at 283 nm (log ϵ 3·76) which shifted to 298 nm (log ϵ 3·96) in 0·1 N aq. NaOH and indicated that corine also contained a 1-benzyltetrahydroisoquinoline skeleton[13] containing phenolic groups.[14] Corine salts also gave positive colour test for phenolic group with benzidine tetrazonium chloride and $FeCl_3$ reagents and absorption bands in i.r. spectrum at ν_{max}(KBr) 3500, 3411 cm^{-1} (associated OH).

Mohinine crystallized as perchlorate m.p. 256° and iodide m.p. 242–244° and analysed for $C_{20}H_{24}O_4N^+$, including two methoxyl groups. U.v. absorption maxima of perchlorate at 231 (log ϵ 4·56), 272 (log ϵ 4·23) and 308 nm (log ϵ 3·99) indicated a 3,4,5,6-tetraoxygenated quaternary aporphine chromophore.[17] Absorption in i.r. spectrum at 3255 cm^{-1} (associated OH) and a positive colour test with benzidine tetrazonium chloride and $FeCl_3$ reagents suggested the presence of phenolic group(s).

Biological Activity

Tiliacine iodide and corine iodide were tested on cat sciatic nerve–gastrocnemius preparation. Tiliacine iodide did not show any effect on either blood pressure or myoneural

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transmission. Corine iodide, however, produced both hypotension as well as inhibition of myoneural transmission. The pharmacological effect of corine iodide was similar to that of d-tubocurarine.

EXPERIMENTAL

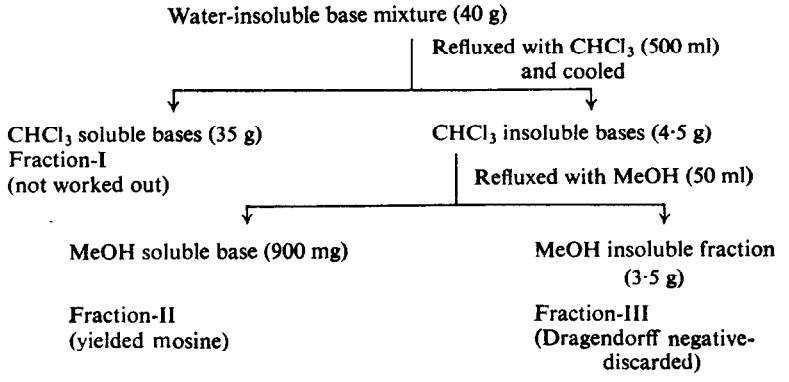
M.ps were determined on a hot stage and are uncorrected. For analysis, the substances were dried for 6 hr at 110° in high vacuum over P_2O_5. Substances for rotation and spectra were dried at 100° for 2 hr *in vacuo* over P_2O_5. Spots were visualized on chromatograms by spraying with Dragendorff's reagent.[7] TLC was carried out on Kieselgel-G (Fluka), using solvent system —S_1 [$CHCl_3$:MeOH (9:1)], with a cotton pad[8] at the top of the plate to allow continued development. Paper chromatography was carried out on Whatman No. 1 in system —S_2 [Et_2O:MeCOEt (1:5) satd. with 1% aq. HCl] and partition on cellulose columns with the solvent system —S_3 [Et_2O:MeCOEt (1:7) satd. with 1% aq. HCl].

Extraction of Roots

The dry powdered roots (35 kg) were exhaustively extracted by percolation at room temp. with 3% AcOH in 50% aq. EtOH and finally with 3% aq. AcOH. The combined extract (250 l.) was concentrated *in vacuo* below 50° to 15 l. and placed in a cold room at 8° for two days. The supernatant was separated from the resinous precipitate[18] by filtration through a layer of purified sand, basified with dil. NH_4OH to pH 9 and the precipitated base was separated by centrifuging and dried (2 kg). Extraction of the aqueous portion with $CHCl_3$ (6 × 2 l.) yielded more bases (70 g), which were combined with water-insoluble bases.

Water-insoluble Bases

A portion (40 g) of the water-insoluble base mixture was separated according to the following scheme:



Mosine

To fraction II (900 mg) which showed only one spot on TLC, in 10 ml mixture of $CHCl_3$:MeOH (1:1) was added a slight excess of picric acid solution, heated for 15 min and then evaporated to dryness. Washing the residue with warm water yielded crude mosine-picrate (900 mg) which crystallized from MeCOEt–MeOH as yellow prisms (600 mg) m.p. 265°. (Calcd. for $C_{36}H_{36}O_7N_2.(C_6H_6O_7N_3)_2$: C, 54·04; H, 3·97; N, 10·51. Found : C, 54·14, 54·14; H, 4·05, 4·16; N, 10·54, 10·57%.) A solution of picrate (400 mg) in 100 ml of 50% aq. Me_2CO was shaken with 70 ml Amberlite IRA-400 (Cl) for 6 hr and filtered. The aq. concentrate was basified with NH_4OH and exhaustively extracted with $CHCl_3$:MeOH (4:1). The residue from the extract crystallized from MeOH–Et_2O as colourless granular clusters m.p. > 340°; $[\alpha]_D^{31}$ + 196° (c, 0·5 in pyridine); λ_{max}^{MeOH} 290 nm (log ϵ 4·09). (Calcd. for $C_{36}H_{36}O_7N_2.6H_2O$: C, 60·32; H, 6·74; N, 3·9. Found: C, 59·44; H, 6·17; N, 3·93%.) To a solution of mosine (100 mg) in 3 ml of $CHCl_3$:MeOH (1:1) was added 1 ml of MeI and kept overnight. Evaporation of solvent gave the methiodide, which crystallized from a mixture of $CHCl_3$–MeOH as colourless rhombs, m.p. 283°; $[\alpha]_D^{31}$ + 72° (c, 0·5 in pyridine); λ_{max}^{MeOH} 290 nm (log ϵ 4·01). (Calcd. for $C_{36}H_{36}O_7N_2.2CH_3I.2H_2O$: C, 49·16; H, 4·99; N, 3·01, I, 27·35; $2OCH_3$, 6·68. Found: C, 48·93, 48·85; H, 4·46, 4·44; N, 2·95, 3·06; I, 27·23, 27·55; $2OCH_3$, 6·91, 6·86%.)

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Water Soluble Bases

After separation of water-insoluble bases, the aqueous solution (20 l.) was acidified with conc. HCl to pH 2. The water-soluble quaternary bases were precipitated as the reineckates, which were filtered, washed neutral with water and dried in vacuum desiccator (720 g). The Me_2CO soluble portion of this crude reineckate was passed through a column of neutral Al_2O_3 (800 g). The eluate (7 l.) (85 mg solid per ml) was diluted with water (2 l.) and warmed to 40–50°. With vigorous stirring, Ag_2SO_4 (250 g) in hot water (6 l.) was added slowly in 4 hr and finally $BaCl_2$ (200 g) in water (700 ml) was added. The suspension was kept overnight and centrifuged. Evaporation of the clear liquid to dryness *in vacuo* below 50° yielded the water-soluble bases as their chlorides (98·6 g).

Partition Chromatography

The chloride mixture of water soluble bases (85 g) were subjected to partition chromatography in two portions on cellulose columns according to the following procedure. *Chromatography I.* A slurry of cellulose powder (2·5 kg) in solvent mixture —S_3 was poured into a column (120 in. × 2·5 in.) and the same solvent was passed through the column for four days. A MeOH solution of the chloride mixture (40 g) was intimately mixed with cellulose powder (100 g), dried *in vacuo* and placed on this column. Elution was carried out with the same solvent system at the rate of 100 ml per hour. In about 2 months 194 fractions of 800 ml each were collected. The bases remaining on the column were washed down with MeOH (Table 1). *Chromatography II.* Another portion of the chloride mixture (45 g) was partitioned over cellulose powder (3 kg) in a similar manner as in chromatography I and in 2½ months 211 fractions of 800 ml were collected (Table 1).

TABLE 1. PARTITION CHROMATOGRAPHY* OF QUATERNARY BASE CHLORIDE

Chromatography-I (40 g chloride on 2·5 kg cellulose powder)			Chromatography-II (45 g chloride on 3·0 kg cellulose powder)			Paper chromatographic control
	Fractions	Wt. in g		Fractions	Wt. in g	
I_1	1–5	0·62	II_1	1–5	0·90	Dragendorff negative
I_2	6	1·38	II_2	6	1·71	α†
I_3	7–8	0·62	II_3	7–11	1·38	A
I_4	9–14	0·76	II_4	12–18	2·78	Dragendorff negative
I_5	15–41	9·03	II_5	19–33	5·80	A
I_6	42–43	0·70	II_6	34–35	0·82	A+B‡
I_7	44–56	3·35	II_7	36–48	3·59	B
I_8	57–60	0·77	II_8	49–55	1·11	B+C‡
I_9	61–82	2·80	II_9	56–81	3·22	C†
I_{10}	83–99	1·09	II_{10}	82–94	2·01	C+D‡
I_{11}	100–149	9·50	II_{11}	95–177	19·15	D
I_{12}	150–157	1·55	II_{12}	178–181	0·83	D+E‡
I_{13}	158–194	7·73	II_{13}	182–211	6·72	E†
I_{14}	Methanol-washed portion	12·98	II_{14}	Methanol-washed portion	10·25	E+F‡

* Eluant: Et_2O:MeCOEt (1:7) satd. with 1% aq. HCl.
† Could not be crystallized as such or as their iodide, thiocyanate or perchlorate.
‡ Mixtures were not further separated.

Characterisation of Isolated Bases

Tiliacine. Iodide: The residue from fractions I_3 and II_3, I_5 and II_5 (16·83 g), failed to crystallize. Treatment of its conc. solution with an excess of satd. aq. KI afforded the iodide as a brown solid which crystallized (3·8 g) from MeOH–AcOEt. An analytical sample was prepared by decolourizing its MeOH solution with animal charcoal and recrystallization as colourless rhombs, m.p. 211–214°; $[\alpha]_D^{31}$ —18° (c, 1·0 in MeOH); λ_{max}^{EtOH} 280 nm (log ε 3·68); (in 0·1, N NaOH) λ_{max} 295 nm (log ε 3·88). (Calcd. for $C_{19}H_{24}O_3N^+.I^-$: C, 51·67; H, 5·48; N, 3·17; I, 28·77; 1-OCH_3, 3·4. Found: C, 51·4; H, 5·4; N, 3·2; I, 28·8, 28·9; 1-OCH_3 3·6%.) It did not absorb H_2 in 30 min during microhydrogenation in $HCONMe_2$ with PtO_2 as catalyst.
Thiocyanate: To a solution of iodide (150 mg) in MeOH (3 ml) was added an excess of aq. satd. NH_4CNS. Concentration of the solution yielded crystalline thiocyanate which was recrystallized from MeOH–AcOEt as colourless rhombs (100 mg) m.p. 183–185°; $[\alpha]_D^{38}$ −32° (c, 1·0 in MeOH); λ_{max}^{EtOH} 280 nm (log ε 3·74). (Calcd.

for $C_{19}H_{24}O_3N^+$, SCN^-: C, 64·48; H, 6·49; N, 7·52; S, 8·61; 1-OCH_3, 8·33. Found: C, 63·81; H, 6·35, N, 7·12 7·19; S, 8·88, 8·74; 1-OCH_3 8·36, 8·05%.)

Perchlorate: A solution of iodide (100 mg) in methanol (2 ml) was treated with an aq. satd. solution of $NaClO_4$. Removal of MeOH under reduced pressure yielded crystalline perchlorate. For purification it was recrystallized from aq. MeOH as light brown cubes (75 mg) m.p. 228–230°. (Calcd. for $C_{19}H_{24}O_3N^+.ClO_4^-$: C, 55·13; H, 5·80; N, 3·38. Found: C, 54·83; H, 5·82; N, 3·16%.)

Corine. Iodide: Amorphous chloride contained in Fractions I_7 and II_7 (6·94 g) was converted into the iodide (2 g) by the usual procedure (KI). The crude iodide was purified by recrystallization from EtOH as colourless rhombs, m.p. 156–159°; $[\alpha]_D^{31}$ −10·6 (c, 1·0 in MeOH); λ_{max}^{EtOH} 283 nm (log ε 3·76): (in 0·1 N-NaOH) λ_{max} 298 nm (log ε 3·96). (Calcd. for $C_{19}H_{24}O_3N^+.I^-.H_2O$: C, 49·66; H, 5·70; N, 3·04; I, 27·65; 1-OCH_3, 6·75. Found: C, 49·70, 49·54; H, 5·61, 5·64; N, 2·65, 2·53; I, 27·03, 1-OCH_3, 7·03, 6·96%.)

Perchlorate: Corine perchlorate was prepared from iodide in the usual way by the addition of aq. $NaClO_4$. It crystallized from cyclohexane as brown granules m.p. 147–150°; $[\alpha]_D^{31}$ −70° (c, 1·0 in Me_2CO); $\lambda_{max}^{50\%MeOH}$ 280 nm (log ε 3·81). (Calcd. for $C_{19}H_{24}O_3N^+.ClO_4^-.H_2O$; C, 52·81; H, 6·06; N, 3·24. Found: C, 52·20; H, 5·62; N, 2·70, 2·70%.)

Mohinine. Perchlorate: Fractions 100–110 of II_{11} (1·05 g) were converted into perchlorate by treating its aq. concd. solution with a satd. solution of $NaClO_4$. The amorphous precipitate crystallized (260 mg) from aq. MeOH, m.p. 256°; $[\alpha]_D^{32}$ +122° (c, 1·0 in pyridine); λ_{max}^{EtOH} 231 nm (log ε 4·56), 272 (4·23), 308 (3·99). (Calcd. for $C_{20}H_{24}O_4N^+.ClO_4^-$: C, 54·34; H, 5·47; N, 3·17; Cl, 8·04; 2-OCH_3, 6·8. Found: C, 53·9; H, 5·1; N, 3·2; Cl, 8·3; 2-OCH_3, 7·0%.) It did not absorb H_2 in 30 min during microhydrogenation in $HCONMe_2$ with PtO_2 as catalyst.

Iodide: To a solution of perchlorate (50 mg) in (3 ml) aq. 50% MeOH was added an excess of aq. satd. solution of KI. Removal of MeOH under reduced pressure yielded the iodide as a resinous mass which crystallized from MeOH as light brown granules, m.p. 242–244°; λ_{max}^{EtOH} 228 (log ε 4·78), 270 (4·07), 330 nm (4·10). (Calcd. for $C_{20}H_{24}O_4N^+.I^-$: C, 51·16; H, 5·15; N, 2·98. Found: C, 50·74; 51·00; H, 5·32, 5·34; N, 3·06, 2·85%.)

Acknowledgements—The authors thank Dr. L. Ram Chandra Row, Andhra University, for the supply of the sample of tiliacorine, Dr. M. Barbier, Institut de Chimie des substances Naturelles, Paris, for assistance in microanalysis, Dr. A. C. Roy of Process Development Division, C.D.R.I., Lucknow, for providing facilities in the extraction of the plant material and Dr. K. P. Bhargava, Pharmacology Department, King George's Medical College, Lucknow, for the evaluation of biological activity. One of us (T.K.P.) is grateful to the Council of Scientific and Industrial Research, New Delhi (India), for a Junior Research Fellowship.