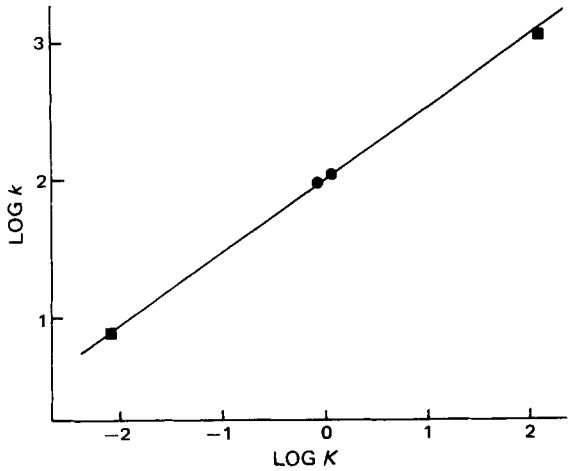

Figure 5—*Plot of log* k *against log* K *for the reactions corresponding to Scheme II for I (●) and II (■) at ionic strength 1.0* M *and 25.0°.*

Table III shows that the enthalpies of activation are higher for the forward reaction (involving Fe^{3+} and phenothiazines) than for the reverse reaction (involving Fe^{2+} and cation radicals); such low values for reactions involving radicals were found in other cases (12).

The reaction rates are independent of the acidity; it can be suggested that the reactive species, in the present conditions, is Fe^{3+}_{aq}. But in several other reactions involving Fe(III), *e.g.*, complexation (13) and redox (14), the reactive species is $FeOH^{2+}_{aq}$, which derives from the hydrolytic equilibrium:

$$Fe^{3+}_{aq} \rightleftarrows FeOH^{2+}_{aq} + H^+$$

Scheme III

with $K_7 = 1.63 \times 10^{-3}$ *M* (15). It follows that, in the present acidity range, the predominant species is Fe^{3+}_{aq}. This behavior can be ascribed to a mechanism involving a simple electron transfer rather than a hydrogen atom transfer, probably operating when $FeOH^{2+}_{aq}$ is the active species (16).

When the mechanism of the redox reaction is an electron transfer, a relationship between the free energy of activation and the overall free energy of reaction should hold. According to the Marcus (17) theory, a plot of log *k versus* log *K* is linear with a slope of about 0.50. Figure 5 shows that this expectation is satisfied in the present reactions. Such relationships have been found to be applicable in the redox reactions involving some relevant biological systems, such as cytochrome c, some catecholamines, and ascorbic acid (18).

The present findings suggest the possibility of correlating the oxidation rates of phenothiazines with thermodynamic and structural data of these systems.

REFERENCES

(1) J. S. Forrest, F. M. Forrest, and M. Berger, *Biochim. Biophys. Acta*, **29**, 441 (1958).

(2) H. Fenner, *Arch. Pharm.*, **304**, 36 (1971).

(3) *Ibid.*, **304**, 47 (1971).

(4) G. M. Nano, P. Sancin, and G. Tappi, *Pharm. Acta Helv.*, **38**, 263 (1963).

(5) M. R. Gasco, *Atti Accad. Sci. Torino*, **100**, 509 (1965–66).

(6) M. R. Gasco and R. Calvino, *ibid.*, **102**, 237 (1967–68).

(7) *Ibid.*, **102**, 1 (1967–68).

(8) J. P. Billon, *Ann. Chim. (Paris)*, **7**, 183 (1962).

(9) W. N. Latimer, "The Oxidation States of the Elements and Their Potentials in Aqueous Solution," 2nd ed., Prentice-Hall, New York, N.Y., 1952.

(10) L. B. Magnusson and J. R. Huizenga, *J. Am. Chem. Soc.*, **75**, 2242 (1953).

(11) P. Kabasakalian and J. M. Glotten, *Anal. Chem.*, **31**, 43 (1959).

(12) E. Collison, F. S. Dainton, B. Mill, S. Tazuka, and D. R. Smith, *Nature*, **198**, 26 (1963).

(13) S. Ganger and S. Stuher, *Inorg. Chem.*, **13**, 379 (1974). J. H. Espenson and S. R. Helzes, *ibid.*, **8**, 1051 (1969).

(14) J. H. Baxendale, H. R. Hardy, and L. H. Sutcliffe, *Trans. Faraday Soc.*, **47**, 963 (1951). J. H. Thomas, G. Trudel, and S. Bywater, *J. Phys. Chem.*, **64**, 51 (1960).

(15) R. M. Milburn, *J. Am. Chem. Soc.*, **79**, 537 (1957).

(16) W. L. Reynolds and R. W. Lumry, "Mechanisms of Electron Transfer," Ronald Press, New York, N.Y., 1966.

(17) R. A. Marcus, *Ann. Rev. Phys. Chem.*, **15**, 155 (1964).

(18) J. M. Yandell, D. P. Fay, and N. Sutin, *J. Am. Chem. Soc.*, **95**, 1131 (1973). J. C. Cassatt and C. P. Marini, *Biochemistry*, **13**, 5323 (1974). E. Pelizzetti, E. Mentasti, E. Pramauro, and G. Giraudi, *Inorg. Chim. Acta*, **15**, L 1 (1975). E. Pelizzetti, E. Mentasti, and E. Pramauro, *ibid.*, **15**, 2898 (1976).

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Alkaloids of *Strychnos dolichothyrsa* Gilg ex Onochie et Hepper

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Received February 23, 1977, from the *Department of Pharmacognosy, Gorlaeus Laboratories, Wassenaarseweg 76, Leiden, The Netherlands.* Accepted for publication April 22, 1977.

Abstract □ Three alkaloids of the stem bark of *Strychnos dolichothyrsa* Gilg ex Onochie et Hepper (Loganiaceae) were isolated and identified as caracurine V and its mono- and di-*N*-oxide by comparison with synthesized compounds. ^{13}C-NMR was used to confirm the structure of the *N*-oxides. The muscle relaxant activity and the toxicity of the *N*-oxides were less than those of caracurine V.

Keyphrases □ *Strychnos dolichothyrsa*—stem bark alkaloids isolated and identified, muscle relaxant activity evaluated □ Alkaloids—from stem bark of *Strychnos dolichothyrsa* isolated and identified, muscle relaxant activity evaluated □ Caracurine V and *N*-oxides—isolated from stem bark of *Strychnos dolichothyrsa*, muscle relaxant activity evaluated □ Relaxant activity, muscle—alkaloids isolated from stem bark of *Strychnos dolichothyrsa* evaluated

Extracts of the stem bark of *Strychnos dolichothyrsa* Gilg ex Onochie et Hepper showed a strong muscle relaxant effect in pharmacological screenings (1, 2), especially in the tertiary alkaloid fractions. The isolation and identification of bisnordihydrotoxiferine, one main alkaloid of this species, and some minor alkaloids derived from

Table I—Low-Field Region of the ^{13}C-NMR Spectra of Caracurine V, Strychnine, and Their *N*-Oxides[a]

Carbon	Ia	Ib			Ic	$\Delta(\delta_{Ia} - \delta_{Ic})$	III	IV	$\Delta(\delta_{III} - \delta_{IV})$
		N_b-Tertiary (A)	N_b-Oxide (B)	$\Delta(\delta_A - \delta_B)$					
C-8	133.59	133.29	130.62	(+2.67)	130.69	(+2.90)	132.62	129.35	(+3.27)
C-9	119.52	120.31	120.74	(−0.43)	121.22	(−1.70)	122.31	122.67	(−0.36)
C-10	121.70	121.76	121.76	(0)	121.95	(−0.25)	124.25	125.10	(−0.85)
C-11	128.25	128.62	129.59	(−0.97)	129.65	(−1.40)	128.62	129.95	(−1.33)
C-12	110.17	110.72	111.51	(−0.79)	111.63	(−1.46)	116.24	116.60	(−0.36)
C-13	152.34	152.10	151.92	(+0.18)	151.62	(+0.72)	142.21	141.60	(+0.61)
C-19	127.34	128.14	133.77	(−5.63)	133.78	(−6.44)	127.65	134.44	(−6.79)
C-20	141.24	140.69	135.96	(+4.73)	136.20	(+5.04)	140.27	135.05	(+5.22)
C-23	—	—	—	—	—	—	169.39	169.27	(+0.12)

[a] The chemical shifts, in parts per million, are relative to tetramethylsilane.

bisnordihydrotoxiferine were reported previously (3).

Further work on the stem bark alkaloids of *S. dolichothyrsa* showed the presence of a quite polar alkaloid as well as two chemically related minor alkaloids. This paper describes the isolation of these alkaloids and their structure elucidation based on comparisons with synthesized compounds and ^{13}C-NMR studies.

EXPERIMENTAL

Plant Material—The stem bark of *S. dolichothyrsa* Gilg ex Onochie et Hepper was collected[1] near Kribi, Cameroun, in June and July 1970.

^{13}C-NMR Spectroscopy—The spectra were recorded at 25.15 MHz in the Fourier-transform mode. The alkaloids were dissolved in deuterochloroform; for the *N*-oxides of caracurine V, 30% deuteromethanol was added. Tetramethylsilane was used as the internal standard. Both proton-noise decoupled and off-resonance spectra were recorded.

TLC—The following solvents were used in saturated tanks: A, ethyl acetate–2-propanol–25% ammonia (45:35:5); B, ethanol–ether–diethylamine (10:80:10); C, chloroform–cyclohexane–diethylamine (30:60:10); D, 1-butanol–water–acetic acid (60:15:15); E, ethyl acetate–2-propanol–25% ammonia (45:35:10); and F, methanol–2 *M* ammonia–1 *M* ammonium nitrate (70:20:10).

TLC aluminum sheets[2], precoated with silica gel 60 F_{254}, were used. A 1% ceric sulfate solution in 10% sulfuric acid was the spray reagent used to locate the alkaloids.

Isolation—Caracurine V (*Ia*) was obtained pure by column chromatography of the collected fractions as described previously (3). For the final separation, a column of 250 g of basic aluminum oxide[3] (activity III) was used with cyclohexane–chloroform (1:1), gradually changed *via* chloroform to chloroform–methanol (1:1).

Characterization of Caracurine V—From the collected fractions, 10 mg of pure caracurine V as a crystalline hydrochloride (mp >300° dec.) and 20 mg as the amorphous base were isolated. The UV spectrum of the base showed maxima at 259 and 299 nm (in methanol). The IR spectrum of the hydrochloride (potassium bromide disk) showed major peaks at 3400, 2900, 2700, 1600, 1480, 1465, 1400, 1340, 1300, 1230, 1190, 1100, 1020, 960, 920, 890, 860, and 755 cm^{-1}.

The major fragments observed in the mass spectrum of the base (160°, 70 ev) were *m/e* 585 (37), 584 (100) (M^+), 566 (10), 322 (10), 321 (10), 293 (15), 292 (14), 263 (15), 180 (29), 144 (92), 143 (54), and 137 (25). The R_f values in the TLC systems were: A, 0.24; B, 0.23; C, 0.16; and D, 0.25. A purple color was obtained with the ceric sulfate spray reagent.

Characterization of Caracurine V *N*-Oxide (*Ib*) and Caracurine V Di-*N*-oxide (Ic)—Compounds *Ib* and Ic were obtained from the column chromatography fractions as described previously (3) by preparative TLC. The UV spectra and color reactions of both alkaloids were similar to those of caracurine V. The R_f values were 0.40 and 0.18 in TLC System E and 0.45 and 0.72 in TLC System F for *Ib* and Ic, respectively. These data correspond with those found for the *N*-oxides obtained during the synthesis of caracurine V.

Synthesis of Wieland–Gumlich Aldehyde (II) and Ia–Ic—*Preparation of 23-Isonitrososstrychnine*—Strychnine (III) (50 g) was dissolved in 300 ml of ethanol (98%) in a three-necked round-bottom flask equipped with a funnel, a reflux condenser, and a stirrer. Isoamyl nitrite (90 ml) was added with stirring, and then a solution of 13.9 g of sodium in 375 ml of ethanol (98%) was added over 1 hr. The temperature was gradually increased from 60 to 90° and then kept at 90° for 4 hr.

After evaporation to dryness under reduced pressure, to remove excess isoamyl nitrite, 75 ml of water was added to the residue and it was again evaporated to dryness. The procedure was then repeated again. Finally, the residue was mixed with 450 ml of water, 80 ml of acetic acid, and 3 g of activated carbon and filtered. To the filtrate, 3 g of activated carbon was added; after filtration and addition of 37.5 ml of concentrated hydrochloric acid, the filtrate was kept at 0° for 24 hr.

The precipitate that formed was filtered off and recrystallized from methanol, mp >220° dec. The UV spectrum of the product showed maxima at 234, 290, and 315 nm. The IR spectrum (potassium bromide disk) showed maxima at 3440, 3100, 2900, 1660, 1590, 1480, 1410, 1285, 1100, 1035, 980, 880, and 755 cm^{-1}. These data correspond with those of 23-isonitrososstrychnine hydrochloride according to Hymon *et al.* (4). The yield was 80%.

Preparation of II from 23-Isonitrososstrychnine—23-Isonitrososstrychnine hydrochloride (5.5 g) was added to 10 ml of thionyl chloride at 20° with stirring and left for 1 hr. Then the solution was added dropwise to 50 g of ice over 5 min and allowed to stand for 6 hr at 20° with stirring. The N_a-cyanoformyl Wieland–Gumlich aldehyde hydrochloride was filtered off and washed with 5 ml of water. On suspension in 50 ml of water, the pH was adjusted to 3 with 1 *M* sodium carbonate, the mixture was heated gently, and steam was passed through the solution until no more hydrogen cyanide could be detected in the vapors.

The solution was then cooled, the pH was brought to 9 with 1 *M* sodium carbonate, and the solution was extracted five times with ether and five times with chloroform. The ether extract gave 1.076 g of an amorphous product, and the chloroform extract gave another 80 mg of an amorphous product. From the filtrate of the N_a-cyanoformyl Wieland–Gumlich aldehyde hydrochloride, another 25 mg of II was recovered after basification to pH 9 and extraction with ether. The reaction products were identified as II by means of TLC properties, melting point (210–212°), UV and IR spectra, and comparison with data for the authentic sample of II.

The UV spectrum showed maxima at 244 and 298 nm. The IR spectrum (potassium bromide disk) showed major peaks at 3400, 2920, 2860, 1720, 1650, 1600, 1490, 1460, 1390, 1310, 1255, 1145, 1070, 1040, 940, 870, 800, and 745 cm^{-1}. The R_f values in the TLC systems were: A, 0.22; B, 0.33; C, 0.10; and D, 0.31. The color reaction with the ceric sulfate spray reagent was orange.

Preparation of Ia from II—Compound II (300 mg), together with 4 ml of pivalic acid, was heated at 120° for 8 hr in an evacuated sealed tube. After removal of pivalic acid under reduced pressure, water was added. The solution was basified with 1 *M* sodium hydroxide and extracted five times with chloroform. The products obtained were purified by column chromatography. The column, comprised of 25 g of basic aluminum oxide[3] (activity IV), was eluted with benzene–chloroform (9:1) followed by pure chloroform, chloroform–methanol (98:2), and chloroform–methanol (1:1). Pure caracurine V (60 mg) was obtained and identified by its physical data, which were in accordance with those found in the literature (5–8) and similar to those found for the alkaloid isolated from *S. dolichothyrsa*.

Compounds *Ib* and Ic were eluted from the column with chloroform–methanol (98:2) after the elution of caracurine V. Their R_f values, color reactions, and UV spectra were similar to those of the alkaloids isolated from *S. dolichothyrsa*. The color reactions and UV and IR spectra were also similar to those of caracurine V.

[1] By Dr. A. J. M. Leeuwenberg and Professor Dr. F. Sandberg. The identification of the plant material was made by Dr. A. J. M. Leeuwenberg, Laboratory of Plant taxonomy and Plant Geography, Agricultural University, Wageningen, The Netherlands, where a herbarium specimen, collection number Lg 7870, was deposited.

[2] Merck, Darmstadt, Germany.

[3] M. Woelm, Eschwege, Germany.

Table II—High-Field Region of the ^{13}C-NMR Spectra of Caracurine V, Strychnine, and Their *N*-Oxides[a]

Carbon	Ia	Ib			Ic	$\Delta(\delta_{Ia} - \delta_{Ic})$	III	IV	$\Delta(\delta_{III} - \delta_{IV})$
		N_b-Tertiary (A)	N_b-Oxide (B)	$\Delta(\delta_A - \delta_B)$					
C-2	56.84	56.91	55.82	(+1.09)	55.76	(+1.08)	60.06	58.48	(+1.58)
C-3	59.94	59.94	82.63	(−22.69)	82.57	(−22.63)	60.06	82.63	(−22.57)
C-5	51.20	51.27	68.68	(−17.41)	68.68	(−17.48)	50.23	67.70	(−17.47)
C-6	40.77	40.71	37.25	(+3.46)	37.25	(+3.52)	42.83[b]	39.31	(+3.52)
C-7	55.51	55.82	56.91	(−1.09)	56.91	(−1.40)	51.93	53.26	(−1.33)
C-14	26.14	25.91	24.45	(+1.46)	24.39	(+1.75)	26.81	25.11	(+1.70)
C-15	34.03	33.85	32.46	(+1.39)	32.40	(+1.63)	31.54	30.33	(+1.21)
C-16	52.54	52.42	52.15	(+0.27)	51.99	(+0.55)	48.17	47.56	(+0.61)
C-17	98.89	98.95	98.53	(+0.42)	98.59	(+0.30)	77.53	77.29	(+0.24)
C-18	66.49	66.37	65.95	(+0.42)	65.89	(+0.60)	64.55	64.43	(+0.12)
C-21	53.32	53.03	70.50	(−17.47)	70.50	(−17.18)	52.66	70.49	(−17.83)
C-22	—	—	—	—	—	—	42.34[b]	42.22	(+0.12)

[a] The chemical shifts, in parts per million, are relative to tetramethylsilane. [b] These signals may be reversed.

Reaction of Caracurine V with Hydrogen Peroxide—Caracurine V (1 mg) was suspended in 0.5 ml of 5% hydrogen peroxide solution and left for 30 min. TLC showed the presence of about equal amounts of Ia–Ic. When the suspension was heated on a water bath, only Ic was present.

Reaction of Ib with Hydrogen Peroxide—Compound Ib (1 mg) was suspended in 0.5 ml of 5% hydrogen peroxide solution and heated on a water bath. TLC showed the presence of one main component; it had the same R_f values and a similar UV spectrum as Ic.

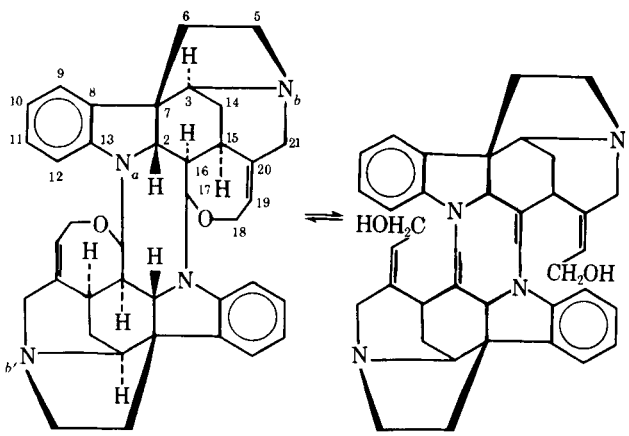
Reaction of Ib and Ic with Sulfurous Acid—The *N*-oxide (1 mg) was suspended in 0.5 ml of 5% sulfurous acid solution and left for 10 min. TLC showed the presence of one major product; it had the same R_f values, color reactions, and UV spectrum as caracurine V.

RESULTS AND DISCUSSION

A previous investigation (3) of the stem bark alkaloids of *S. dolichothyrsa* showed the presence of bisnordihydrotoxiferine and some minor alkaloids derived from this alkaloid. Further investigation on the alkaloid mixture by means of column chromatography led to the isolation of a polar alkaloid and two minor alkaloids derived from this alkaloid. Based on UV, IR, and mass spectra, the alkaloids were thought to belong to the caracurine V series. The identity of the alkaloids was established by comparison with synthesized alkaloids, Ia–Ic (Scheme I).

The synthesis of caracurine V was described by several investigators (5, 9, 10). Battersby and Hodson (9) obtained a relatively higher yield than did Bernauer *et al.* (5) by using pivalic acid instead of acetic acid for the condensation of two molecules of II (Scheme II). By this synthesis, small amounts of bisnortoxiferine also were formed. The method of Battersby and Hodson was used in this investigation; II was obtained according to the synthesis described by Hymon *et al.* (4).

The products obtained from the dimerization of II were separated by column chromatography. Caracurine V, which was obtained by this procedure, gave identical UV, IR, and mass spectra as the polar alkaloid isolated from *S. dolichothyrsa;* the spectral data were also in agreement with those reported in literature (5–8). Moreover, TLC confirmed the identification.



Ia: caracurine V
Ib: N_b^+-O^-
Ic: N_b^+-O^-, N_b^+-O^-

bisnortoxiferine

Scheme I

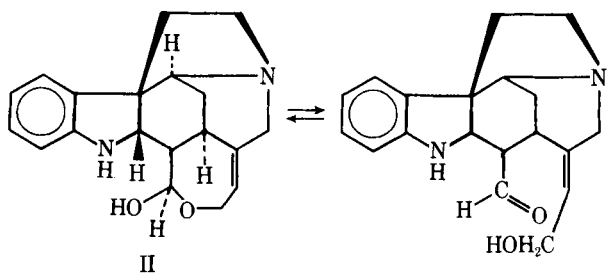
Two by-products, obtained by the synthesis of caracurine V, were isolated after the column chromatographic separation. Their UV spectra, TLC behavior, and color reactions corresponded with the two minor alkaloids isolated from *S. dolichothyrsa.* Although the spectra and color reactions were very similar to those of caracurine V, the two compounds had a more polar character on TLC. Mass spectrometry failed to give an M^+ peak for the most polar compound. For the other compound, a very small peak was observed at *m/e* 584 and an even smaller peak was observed *m/e* 600.

Treatment of both compounds with sulfurous acid yielded caracurine V according to TLC and color reactions. Oxidation of caracurine V with 5% hydrogen peroxide for 30 min yielded two main products which, on TLC, corresponded with the unknown compounds. Heating caracurine V with hydrogen peroxide solution yielded the most polar compound.

Based on these facts, the two compounds were identified as Ib and Ic (Scheme I), analogous to the occurrence of the *N*-oxides of bisnordihydrotoxiferine (3). Further evidence for these structures was obtained by means of ^{13}C-NMR spectroscopy, which, in contrast to UV, IR, and proton NMR spectroscopy, showed distinct and readily interpretable differences in the spectra.

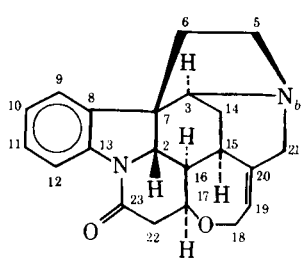
The chemical shifts recorded for Ia–Ic are summarized in Tables I and II. The assignments were made with the aid of off-resonance decoupled spectra and by comparison of the spectra with the chemical shifts recorded for a number of *Strychnos* alkaloids (11). To permit direct comparison of the shifts resulting from the *N*-oxidation of caracurine V and strychnine (III), the data for strychnine and strychnine *N*-oxide (IV) are also summarized in Tables I and II.

The carbons adjacent to the nitrogen, which is oxidized, were affected most (Table I). The shifts of C-3, C-5, and C-21, as observed for strychnine, were 22.57, 17.47, and 17.83 ppm, respectively, downfield upon *N*-oxidation. The shifts observed for caracurine V upon *N*-oxidation for those three carbons were very similar: 22.63, 17.48, and 17.18 ppm, respectively. The other carbons in caracurine V di-*N*-oxide that shifted



II

Scheme II



III: strychnine
IV: N_b^+-O^-

Table III—Muscle Relaxant Effect of Caracurine V and Its *N*-Oxides in Mice[a]

Compound	Dose, mg/kg	Screen Grip Test
Ia	6	–
	10	++
	13	++++(lethal)
Ib	10	–
	18	++
	25	++(lethal)
Ic	25	–
	40	+++
	60	++++(lethal)

[a] The activity was determined with the screen grip test as it was used in the screening of *Strychnos* plant material (1, 2). The loss of screen grip is rated as follows: ++++, the mouse falls off as the screen is tilted to a 45° angle; +++, the mouse falls off as the screen is tilted to a 90° angle; ++, the mouse falls off as the screen is inverted; and +, the mouse falls off at the first gentle shake.

considerably were C-20 and C-19, 5.04 ppm downfield and 6.44 ppm upfield, respectively. These shifts agree with those observed for strychnine *N*-oxide. The shifts of the signals of the other carbons, although not as large as those mentioned, are also in good agreement for both caracurine V and strychnine (Tables I and II).

The spectrum of Ib obviously results from the addition of the spectra of Ia and Ic. Whether or not the *N*-oxides are artifacts is difficult to say. The *N*-oxides are readily formed, for example, in chloroform solution (3). However, on TLC of a freshly prepared 1% acetic acid in water extract of the stem bark of *S. dolichothyrsa*, the *N*-oxides were observed. The *N*-oxides were tested in the pharmacological screening as described in investigations of African *Strychnos* species (1, 2). Both compounds proved to be less toxic and less active as muscle relaxants than caracurine V (Table III).

REFERENCES

(1) F. Sandberg, E. Lunnell, and K. J. Ryrberg, *Acta Pharm. Suec.*, **6**, 79 (1969).

(2) F. Sandberg, R. Verpoorte, and A. Cronlund, *ibid.*, **8**, 341 (1971).

(3) R. Verpoorte and A. Baerheim Svendsen, *Lloydia*, **39**, 357 (1976).

(4) J. R. Hymon, H. Schmid, P. Karrer, A. Boller, H. Els, P. Fahrni, and A. Fürst, *Helv. Chim. Acta*, **52**, 1564 (1969).

(5) K. Bernauer, F. Berlage, W. v. Philipsborn, H. Schmid, and P. Karrer, *ibid.*, **41**, 2293 (1958).

(6) A. R. Battersby, D. A. Yeowell, L. M. Jackman, H. D. Schroeder, M. Hesse, H. Hiltebrand, W. v. Philipsborn, H. Schmid, and P. Karrer, *Proc. Chem. Soc.*, **1961**, 413.

(7) M. Hesse, "Indolakaloide, Teil 1 und 2, Fortschritte der Massenspektrometrie," vol. 1, Verlag Chemie, Weinheim, West Germany, 1974.

(8) H. Asmis, H. Schmid, and P. Karrer, *Helv. Chim. Acta*, **37**, 1983 (1954).

(9) A. R. Battersby and H. F. Hodson, *J. Chem. Soc.*, **1960**, 736.

(10) H. A. Hiltebrand, Dissertation, Zurich, Switzerland, 1964.

(11) R. Verpoorte, P. J. Hylands, and N. G. Bisset, *Org. Magn. Reson.*, **9**, 567 (1977).

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Light-Scattering Studies on Bile Acid Salts I: Pattern of Self-Association of Sodium Cholate, Sodium Glycocholate, and Sodium Taurocholate in Aqueous Electrolyte Solutions

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Abstract □ The pattern of association of the trihydroxy bile salts in aqueous electrolyte solutions was investigated utilizing the light-scattering technique. The turbidity of the bile salts sodium cholate, sodium taurocholate, and sodium glycocholate was determined over the concentration range of 0–25 mg/ml at 25°. For sodium cholate, the concentration of the supporting electrolyte was varied from 0.15 to 0.5 *M*. For all bile salts in 0.15 *M* electrolyte, the turbidity was determined in sodium fluoride, sodium chloride, sodium bromide, and sodium iodide. Comparison of the light-scattering data with a monomer–micellar model showed that qualitative agreement was obtained; however, quantitative agreement could not be achieved. Further examination of the data showed that the light-scattering results were in good agreement with a model that includes dimers, trimers, and a higher aggregate containing approximately eight monomeric units.

Keyphrases □ Sodium cholate—pattern of association in aqueous electrolyte solutions, light-scattering study □ Sodium glycocholate—pattern of association in aqueous electrolyte solutions, light-scattering study □ Sodium taurocholate—pattern of association in aqueous electrolyte solutions, light-scattering study □ Association—sodium cholate, glycocholate, and taurocholate, pattern in aqueous electrolyte solutions, light-scattering study □ Light scattering—study of pattern of association of sodium cholate, glycocholate, and taurocholate in aqueous electrolyte solutions □ Bile acid salts—pattern of association in aqueous electrolyte solutions, light-scattering study

The mechanism by which bile salts solubilize various solutes such as steroids, fat-soluble vitamins, and drugs has often been described following the model for detergent micelles (1). In this model, it is assumed that a reversible equilibrium exists between monomeric species and micelles. The polydispersity of the micelle is assumed to be quite small. The micelle size is dependent on the nature of the bile salt, *i.e.*, the number of hydroxyl groups and conjugation, as well as on temperature, pH, ionic strength, *etc.* (2).