ALKALOIDS OF *STRYCHNOS SOUBRENSIS*

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ABSTRACT.—A phytochemical investigation of the chloroform extract of the stem bark of *Strychnos soubrensis* Hutch. et Dalz. (Loganiaceae) resulted in the isolation of four alkaloids. Three of them were identified as strychnobrasiline, strychnofendlerine, and isosplendine. The fourth one is a new alkaloid for which we propose the structure 14-β-hydroxy-strychnobrasiline.

The African species of *Strychnos* has been the object of systematic research in recent years. However, whereas some species have been extensively investigated, others have received little or no attention. Therefore, so far, the knowledge of the African *Strychnos* species is not complete. In an effort to supply some of the missing information, we investigated the alkaloids of the stem bark of *Strychnos soubrensis* Hutch. et Dalz.

Strychnos soubrensis (1) (section Lanigerae) is a liana or scandent shrub, 10-50 m long, up to 40 m high, climbing in trees. It is widely distributed in the West African rain or secondary forests, often on river banks, at altitudes of 0-1000 m. The bark is pale grey to dark brown with large lenticels.

Preliminary phytochemical (2) and pharmacological (3,4) studies revealed that the small amount of alkaloids present (<0.1%) combined a weak strychnine-like (convulsant) activity with a much stronger muscle-relaxant effect.

RESULTS AND DISCUSSION

Examination of a crude alkaloidal bark extract by tlc showed four alkaloidal positive spots, two relatively large and two small, indicating that two major and two minor alkaloids were present. The minor alkaloids were identified as strychnofendlerine (figure 1) (5-8) and isosplendine (figure 1) (5,9,10) by means of spectral data by comparison (Rf, ir, uv, ms, ^{1}H-nmr) with authentic samples. One of the major alkaloids proved, by spectral data and by comparison (Rf, ^{1}H-nmr, ir, uv, ms) with an authentic sample, to be identical to strychnobrasiline (figure 2) (5,7,10,11). The second major alkaloid was not identical with any of the indole alkaloids recorded in the literature.

The uv and ^{1}H-nmr spectra of the unknown compound were similar to those of strychnobrasiline. However, inasmuch as its ms showed a molecular ion peak at *m/e* 382 (strychnobrasiline 366), it was concluded that the new compound must be a substituted strychnobrasiline derivative, the likely substituents being O(*N*-oxide) or OH(hydroxy function).

The *N*-oxide was ruled out because no significant shift was observed for N_b-CH_3 in the ^{1}H-nmr spectrum with respect to strychnobrasiline, whereas in another N-methyl *sec*-pseudo alkaloid, icajine N-oxide (12), this group was shifted downfield from δ 2.06 ppm to δ 3.27 ppm. On the other hand, the presence of a hydroxyl substituent was indicated in the ir spectrum where a strong and sharp absorption signal was observed at 3460 cm^{-1}.

Having thus established the kind of substituent, its position remained to be determined. From the uv, ^{1}H-nmr, ^{13}C-nmr data and the presence in the ms of fragments at

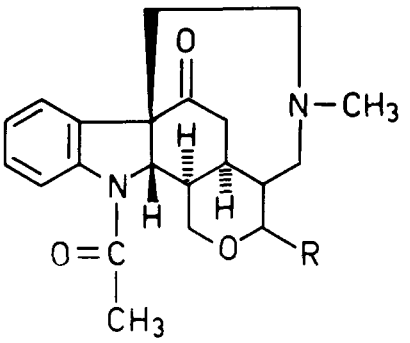

FIGURE 1a. Structure of isosplendine
R=---CH_3
1b. Structure of strychnofendlerine
R=◀ Ch_3

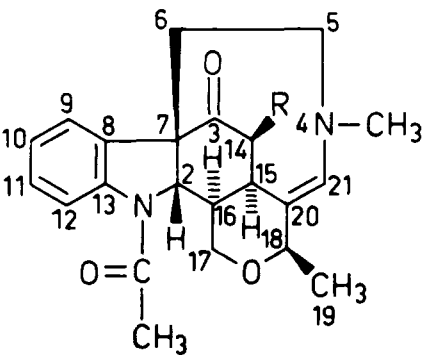

FIGURE 2a. Structure of strychnobrasiline
R=H
2b. Structure of 14-β-hydroxy-strychnobrasiline
R=OH

m/e 144, 143, 130 characteristic of unsubstituted indoles, it was concluded that substitution in the aromatic portion of the molecule was unlikely and that, therefore, the substitution was present in the aliphatic moiety.

The ^{13}C-nmr and the ^{1}H-nmr of both alkaloids showed considerable broadening and doubling of some peaks due to rotamers (N_a-acetyl) (13). However, in the ^{13}C-nmr spectrum of the hydroxy compound, a sharp resonance was observed at δ 73.0 ppm, which was not present in the spectrum of strychnobrasiline. The off-resonance spectra at various decoupling frequencies made it possible to establish the multiplicity of most of the resonances in the ^{13}C-nmr spectra of both alkaloids, and assignments were made for strychnobrasiline on the basis of previously reported indole alkaloids (6,14,15). It was thus evident that a triplet at ~δ 40 ppm (C-6 or C-14) in the spectrum of strychnobrasiline had disappeared and had been replaced by the doublet at δ 73.0 ppm, which would suggest that the hydroxy group was carried either on C-6 or C-14. That the hydroxy group was borne on C-14 was supported by the fact that the C-ring carbons showed considerable shifts, *i.e.*, C-3 was shifted upfield 3.5 ppm as had also been reported for other hydroxy cyclic ketones (16-18), whereas the shift for C-5 adjacent to C-6 was relatively small. In addition, an examination of the fragmentation pattern, as proposed by Iwataki and Comin (11), revealed that the fragments containing the C-6 carbon (*m/z* 130, 143, 144, 185, 186) were still evident in the hydroxy derivative, whereas those (*m/e* 136, 166, 180, 194) bearing the C-14 portion were less abundant than in strychnobrasiline. Moreover, the corresponding fragments at 16 mass units higher were not observed for C-6- containing fragments, but were apparent for those containing C-14 (*m/z* 152, 182, 196, 210).

From the foregoing observations, it was concluded that the new alkaloid was 14-hydroxy-strychnobrasiline.

The stereochemistry at C-14 could be determined from the rather low frequency of absorption of the OH-bond in the ir (3460 cm^{-1}), an indication that it was strongly hydrogen bonded. A Dreiding model showed that if the OH-group were in the β-position, it would be very close to N_b, a situation that would give rise to hydrogen bonding with the tertiary nitrogen.

The new alkaloid is thus proposed to be 14-β-hydroxy-strychnobrasiline (figure 2). The final evidence of this structure was provided by means of X-ray crystallography (details will be published separately). This is the first example of a strychnine-type alkaloid

with a substituent at C-14, although hydroxy substitution at C-15 has been reported previously (19).

CONCLUSION

With the exception of the new alkaloid, 14-β-hydroxy-strychnobrasiline, the alkaloids of *S. soubrensis* have previously been isolated from other sources.

	strychnobrasiline	strychnofendlerine	isosplendine
S. fendleri (5,6)	x	x	
S. scheffleri (7)	x	x	
S. aculeata (8)		x	
S. splendens (9)			x
S. brasiliensis (10)	x		
S. tabascana (11)	x		

S. splendens and *S. scheffleri* belong to the same section (Lanigerae) as *S. soubrensis*, and the three species are said to be closely allied to one another (1).

EXPERIMENTAL

MATERIAL.—The stem bark of *S. soubrensis* (B7499) was collected by Dr. R. Verpoorte and Dr. F. Breteler near Aboisso on the Ivory Coast during April-May 1974. Dr. A. J. M. Leeuwenberg of the Herbarium at Wageningen, The Netherlands (where a voucher specimen has been deposited) determined its identity.

EXTRACTION.—The ground material (2 kg) was moistened with 10% $NaHCO_3$ and extracted several times with chloroform. The combined chloroform extract was then concentrated under reduced pressure and shaken with 2 N HCl. The acidic extract was basified with 10% $NaHCO_3$ and re-extracted with chloroform. The organic extract was washed with water, dried over anhydrous Na_2SO_4, filtered, and evaporated to dryness under reduced pressure to yield 2 g of a pale brown residue.

ISOLATION.—The alkaloid residue (2 g) was dissolved in methanol, and the components were separated by means of repeated preparative tlc on Merck silica gel 60 PF_{254}, using the solvent system ethyl-acetate-isopropanol-ammonia 25% (9:7:2). The spots were detected under uv and by iodoplatinate reagent. The separated alkaloids were scraped off and eluted with methanol; the methanol solution was then evaporated to dryness and the residue redissolved in $CHCl_3$ for control of purity in other solvent systems.

SOLVENT SYSTEMS..—Five solvent systems were used in combination with silica tlc plates: (a) ethyl-acetate-isopropanol-ammonia 25% (9:7:1), (b) methanol-water-ammonia 25% (8:1:1), (c) chloroform-cyclohexane-diethylamine (3:6:1), (d) chloroform-methanol-ammonia 25% (15:4:1), and (e) chloroform-acetone-diethylamine (5:4:1).

IDENTIFICATIONS.—A Cary 14 Spectrophotometer recorded uv (EtOH). An SP3-200 PYE UNICAM was used for ir (KBr discs), while ^{1}H-nmr and ^{13}C-nmr were recorded on a JEOL PS-100 in FT mode.

Isosplendine (figure 1a). Uv(EtOH): 245, 275 nm; ir(KBr): 3400, 2920, 2850, 1650, 1590, 1470, 1460, 1380, 1260, 1190, 1110, 1090, 1030, 750 cm^{-1}. ^{1}H-nmr($CDCl_3$): δ 7.8-7.0 (aromatic H) 2.40(s, 3H, N_b-CH_3), 1.25(d, 3H, J=7 Hz, C-19 CH_3) ppm. Ms(70 eV) *m/z* (relative intensity): 368(M^+, 64), 353(14), 325(21), 309(21), 297(26), 295(28), 267(35), 255(35), 229(21), 225(16), 194(14), 186(30), 185(56), 168(28), 144(100), 143(42), 130(42) and identical by comparison (ir, uv, ms, ^{1}H-nmr, tlc) with an authentic sample.

Strychnofendlerine (figure 1b). Uv(EtOH): 245, 277 nm; ir(KBr): 3400, 2920, 2850, 1660, 1590, 1500, 1460, 1350, 1260, 1210, 1190, 1110, 1090, 750 cm^{-1}. ^{1}H-nmr($CDCl_3$: δ 7.9-7.0 (romatic H) 2.40(s, 3H), 2.01(s, 3H, 1.17(d, 3H, J=7 Hz C-19 CH_3) ppm. Ms(70 eV) *m/z* (relative intensity: 368 (M^+, 68), 353(14), 325(25), 309(26), 297(21), 295(16), 267(37), 255(41), 229(21), 225(16), 194(14), 186(37), 185(59), 168(42), 144(100), 143(42), 130(31) and identical by comparison (ir, uv, ms, ^{1}H-nmr, tlc) with an authentic sample.

Strychnobrasiline (figure 2a). Uv(EtOH): 250, 277(sh) nm; ir(KBr): 2980, 2920, 2870, 1660, 1650, 1590, 1480, 1380, 1360, 1320, 1290, 1260, 1170, 1110, 1090, 1040, 970, 860, 820, 760 cm^{-1}. ^{1}H-nmr($CDCl_3$): δ .95 (d, J=8 Hz, H-12), 7.53 (d, J=8 Hz, H-12), 7.32-6.97 (m, 3H, H-9, H-10, H-11), 6.01 (s, 1H, H-21), 5.26 (d, J=8 Hz, H-2), 4.64 (d, J=8 Hz, H-2), 2.34 (s, 3H, N_a-acetyl), 2.22 (s, 3H, N_b-methyl), 1.37 (d, 3H, J=6.2 Hz, C-19 CH_3) ppm; ms(70 eV 175°) *m/z* (relative intensity):

366(M^+, 96), 351(10), 194(33), 186(24), 185(14), 180(22), 166(33), 152(22), 144(100), 143(30), 138(20), 136(20), 130(21), 122(31), 110(24), 108(23), 96(23), and identical by comparison (ir, uv, ms, ^{1}H-nmr, tlc) with an authentic sample.

14-β-hydroxy-strychnobrasiline (figure 2b). Uv(EtOH): 245, 277(sh) nm; ir(KBr): 3460, 2980, 2920, 2860, 1660, 1630, 1590, 1470, 1380, 1350, 1300, 1285, 1260, 1230, 1210, 1190, 1090, 1060, 1000, 970, 940, 865, 805, 755, 730 cm^{-1}. ^{1}H-nmr($CDCl_3$): δ 7.94 (d, J=8 Hz, H-12), 7.56 (d, J=8 Hz, H-12), 7.3-6.9 (m, 3H, H-9, H-10, H-11), 6.09 (s, 1H, H-2), 5.26 (d, J=8 Hz, H-2), 4.64 (d, J=8 Hz, H-2), 3.9 (d, J=7 Hz), 2.33 (s, 6H, N_a-acetyl, N_b-methyl), 1.37 (d, 3H,, J=6.2 Hz, C-19 Ch_3) ppm; ms(70 eV 200°) *m/z* (relative intensity): 382(M^+, 100), 367(21), 354(9), 339(9), 325(6), 323(12), 311(4), 296(5), 251(4), 255(6), 227(5), 210(6), 202(12), 196(9), 194(5), 186(17), 185(9), 182(7), 180(9), 170(10), 168(12), 166(9), 152(37), 144(83), 143(33), 130(38), 122(19), 110(34), 108(38), 96(28). ^{13}C-nmr: see table 1.

TABLE 1. ^{13}C-nmr (CD_3OD): shifts in δ ppm downfield from TMS.

Carbon	Strychnobrasiline	14-β-hydroxy-strychnobrasiline
2	63.2	63.9
3	189.6	186.1
5	53.5	54.1
6	41.0	40.9
7	54.8	57.3
8	134.1	135.0
9	124.7[a]	125.0
10	124.8[a]	125.0
11	128.0	128.2
12	119.1	119.3
13	141.4	141.0
14	40.4	73.0
15	42.3[a]	45.8[a]
16	41.0[a]	42.3[a]
17	68.4	68.5
18	77.0	76.9
19	16.8	16.8
21	134.1	133.9
C=O	169.5	169.9
CH_3	23.3	22.9
N-CH_3	41.8	43.0

[a]Values may be interchanged.

ACKNOWLEDGMENTS

We are grateful to Prof. J. Comin, Prof. M. Koch, and Mlle. Caprasse for the supply of authentic samples of strychnobrasiline, isosplendine, and strychnofendlerine, respectively.

This research was supported by a postgraduate scholarship to F. C. Ohiri by the University of Nigeria, Nsukka.

Received 2 August 1982

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