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ALKALOIDS FROM *STRYCHNOS HENNINGSII*: REVISED STRUCTURES FOR HOLSTIINE AND RINDLINE, PROPOSED STRUCTURE FOR HOLSTILINE

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Key Word Index—*Strychnos henningsii*; *Strychnos holstii*; Loganiaceae; indole alkaloids; holstiine; holstiline; rindline

Abstract—Holstiine, holstiline and rindline are shown to be derivatives of *O*-demethyltsilanine (**3a**), an alkaloid with a cyclic glyoxylamide acetal structure which has been isolated from the same plant *Strychnos henningsii*. Holstiine is reformulated as *O*-demethyl-*N*-methyl-*sec*-pseudotsilanine (**5a**). Holstiline is considered to be *N*-methyl-*sec*-pseudotsilanine (**5b**). The structure of rindline is revised to that of 10-methoxy-*N*-methyl-*sec*-pseudotsilanine (**5c**).

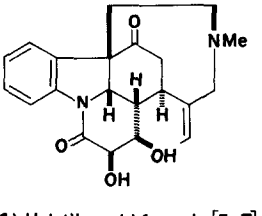
INTRODUCTION

In 1951 Bosly reported the isolation of four new alkaloids from material of *Strychnos holstii* Gilg var. *reticulata* (Burtt Davy et Honoré) Duvign. forma *condensata* Duvign. from Zaire [1]. The structures of two of these, retuline [2, 3] and condensamine [3], have been elucidated. Some structural features of a third one, holstiine, have been established [1, 4] and further work has led to a tentative structure [5] which has entered the literature [6, 7].

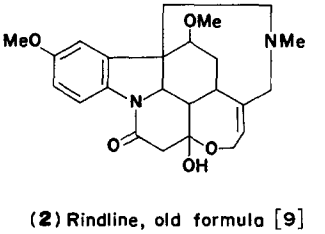
S. holstii is currently included in the species *S. henningsii* Gilg [8]; and the isolation of *O*-demethyltsilanine and related alkaloids (**3a–3d**) from *S. henningsii* has suggested that the structure **1** for holstiine needs reconsideration. A structure for holstiline, the fourth base originally obtained from *S. holstii* [1], is proposed. The structures previously put forward for rindline (**2**) and an accompanying *ar*-demethoxy base [9], alkaloids from *S. henningsii* bark of South African origin [10], are now seen to be unsatisfactory and they are brought into line with those of holstiine and holstiline.

RESULTS AND DISCUSSION

Accurate mass measurement confirms the revised molecular formula $C_{22}H_{26}N_2O_4$ for holstiine [4]. The intense IR band at 1634 cm^{-1} (Nujol) is attributed to an amide carbonyl function [4, 5] while the UV maximum at 255 nm shows the compound to be a N_a-acyldihydroindole. The IR band at 754 cm^{-1} indicates that there is no substitution in the aromatic ring of the indole moiety [4]. The presence of a second carbonyl function, indicated by an absorption band at 1658 cm^{-1} (Nujol) [4, 5]*, and of a *N*-methyl group earlier suggested comparison with vomicine and its



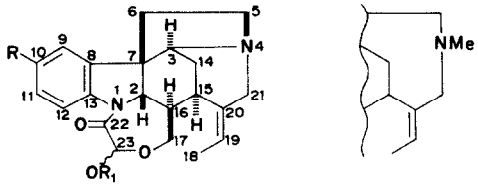
(**1**) Holstiine, old formula [5–7]



(**2**) Rindline, old formula [9]

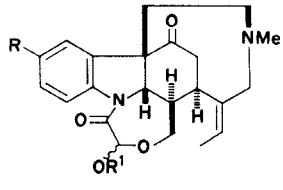
* As with other bases of the *N*-methyl-*sec*.-pseudo series [11], the IR spectrum of holstiine taken in $CHCl_3$ has only 1 carbonyl band, at 1673 cm^{-1}.

derivatives [4, cf. 11] i.e. with bases of the *N*-methyl-*sec*-pseudo series (**6b**); and the strong basicity of holstiine (p*K* 8·8) pointed to a closer similarity with derivatives in which the 7-membered ether ring is open (cf. **7**). Analysis of the NMR and MS supports these indications and the findings are readily accounted for in terms of the structure **5a**, which is related to the tsilanine type of base **3** [6].

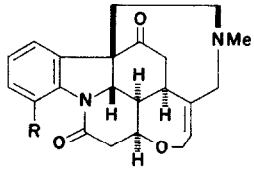


(**3a**) R = R_1 = H *O*-Demethyltsilanine
(**3b**) R = H, R_1 = Me Tsilanine
(**3c**) R = OMe, R_1 = H *O*-Demethyl-10-methoxytsilanine
(**3d**) R = OMe, R_1 = Me 10-Methoxytsilanine

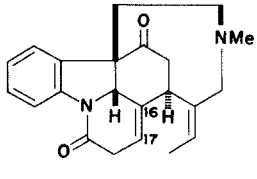
(**4**)



(**5a**) R = R_1 = H Holstiine
(**5b**) R = H, R_1 = Me Holstiline
(**5c**) R = OMe, R_1 = Me Rindline

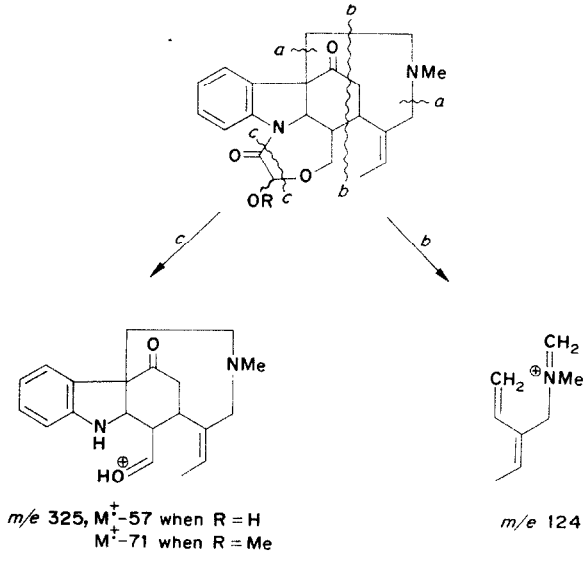


(**6a**) R = H Icajine
(**6b**) R = OH Vomicine



(**7**) Deoxy-*N*-methyl-*sec*-isopseudostrychnine

The NMR spectrum of holstiine shows a 3-hydrogen singlet at δ 1·98, belonging to the *N*-Me group. In the MS there is a peak at *m/e* 323 which is a triplet whose major component has the composition $C_{19}H_{17}NO_4$ (=M^+ − 59), corresponding to loss of C_3H_9N, i.e. part of the nitrogen bridge (scission at *a*, Scheme 1). Both these features are characteristic of bases belonging to the *N*-methyl-*sec*-pseudo series [11]. NMR signals at δ 1·63 (C-18 Me, broadened doublet) and δ *ca* 5·45 (H-19, broadened quartet) indicate the presence of an ethylidene group.



m/e 325, M^+−57 when R = H
M^+−71 when R = Me

m/e 124

Scheme 1. Cleavages *a* and *c* involve hydrogen rearrangements.

In the MS of 16,17-dihydro-16α,17α-dihydroxy-deoxy-*N*-methyl-*sec*-isopseudostrychnine [5], the main product formed on treatment of **7** with OsO_4 in pyridine, there is a prominent peak at *m/e* 124, corresponding to an ion of composition $C_8H_{14}N$. The formation of this ion is attributed to fragmentation like that indicated at *b* in Scheme 1. Occurrence of an ion of identical composition in the MS of holstiine indicates the presence of an identical structural component **4.**

Since both nitrogens in holstiine are fully substituted, the IR band at 3311 cm^{-1} must be assigned to an OH group; this group can be acetylated with Ac_2O in pyridine to yield a product having its mol. ion peak at *m/e* 424, the MW of a monoacetylholstiine. That the OH group forms part of a ketol function is suggested by the appearance in the MS

of a peak at *m/e* 325 (=$M^+ - 57$) of composition $C_{20}H_{25}N_2O_2$ which corresponds to loss of the ketol function C_2HO_2 (fragmentation *c*, Scheme 1). A further indication for the presence of the 7-membered hemiacetal ring is the position of the H-23 singlet at δ *ca* 5·35; the large paramagnetic shift is brought about by the presence of two heteroatoms on C-23 and the nearness of the amide function.

With icajine (**6a**) the signals for H-9 and H-12 are found at δ 7·78 and δ 8·07 because of deshielding by the C-3 and C-22 carbonyl functions, respectively, and the H-10 and H-11 signals occur as a 2-hydrogen multiplet at δ 6·92–7·32. However, in holstiine H-9 is less deshielded and its signal forms part of a 3-hydrogen multiplet at δ 6·84–7·60 which includes the signals for H-10 and H-11 as well. On the other hand, H-12 in holstiine appears to be deshielded even more and its signal is observed at δ 8·23.*

The doublet at δ 4·84 (*J* 10 Hz) in the holstiine spectrum is assigned to H-2 and the large coupling constant suggests that H-2 and H-16 are *trans* to each other as in the tsilanine series [6] and in the great majority of *Strychnos* alkaloids. Dreiding models show that the downfield position is due to deshielding by either the OH group or the ether oxygen, depending on the configuration adopted by the hemiacetal ring. Correlation with bases of the isoretuline-retuline type [6] is not possible as the appropriate derivatives in the *N*-methyl-*sec*-pseudo series are not known.

The spectral properties of holstiline (**5b**) show that it must be *O*-methylholstiine, and the following features indicate clearly the presence of the methoxyl function: the mol. ion peak at *m/e* 396 is 14 m.u. higher than for holstiine and a peak at *m/e* 365 (=$M^+ - 31$) corresponds with the loss of OMe, while in the NMR spectrum the OMe group appears as a singlet at δ 3·61. The presence in the MS of an intense peak at *m/e* 124 (Scheme 1) is also compatible with the proposed structure **5b**. Holstiine and holstiline are thus the analogues in the *N*-methyl-*sec*-pseudo series of *O*-demethyltsilanine and tsilanine, respectively.

Spiteller-Friedmann and Spiteller [9] investigated the structures of rindline and an accompanying minor base which according to the MS was an *ar*-demethoxyrindline. Comparison of the MS of this *ar*-demethoxy base with that of holstiline shows that the major features of the two spectra are identical† and this in turn suggests that rindline is an *ar*-methoxyholstiline. Further assignments in the NMR spectrum of rindline (see Experimental) are consistent with this suggestion. Thus, signals at δ 1·95 [9] and at δ 1·65 and *ca* 5·45 are similar to those found in the spectra of holstiine and holstiline; they are indicative of a *N*-Me [9] and an ethylidene group, respectively, and together with the prominent peak in the MS at *m/e* 124 [9], show that rindline has the part structure **4**. Like holstiine and holstiline, rindline has an IR spectrum with a single intense carbonyl absorption band, at 1640 cm^{-1} (KBr), considered to be due to coincidence of the bands for carbonyl functions at C-22 and C-3, i.e. the base belongs to the *N*-methyl-*sec*-pseudo series. Signals in the rindline NMR spectrum at δ 3·59 [9], 5·08 and 5·37 correspond with signals in the holstiline spectrum and point to the occurrence of an identical structural feature, the cyclic methyl acetal. All these indications, along with the location of the *ar*-methoxyl group at C-10 [9], are in agreement with the reformulation of rindline as 10-methoxyholstiline, i.e. 10-methoxy-*N*-methyl-*sec*-pseudotsilanine (**5c**).

EXPERIMENTAL

The MS were determined with an AEI MS9 high-resolution instrument having a direct inlet system and operating at 70 eV. The 60-MHz NMR spectrum of holstiline was taken in $CDCl_3$. The 90-MHz NMR spectrum of holstiine was recorded on a Perkin–Elmer R32 instrument; because of poor solubility in $CDCl_3$ less than 2 mg compound could be used and the spectrum was derived from a CAT summation after 148 sweeps. δ values are relative to TMS (δ 0·00), which was used as internal standard.

Holstiine (**5a**). Previously determined properties, see Refs. [1] and [4]; IR: ν_{max}^{Nujol} 3311, 1658, 1634, 1585, 1258, 1144, 1111, and 754 cm^{-1}; NMR: δ 1·63 (3H, *d*, *J ca* 7 Hz, broadened by homoallylic coupling with the 2 H-21; =CH–Me), 1·98 (3H, *s*; N-Me), 4·84 (1H, *d*, *J* 10 Hz; H-2), *ca* 5·35 (1H, *s*; H-23), *ca* 5·45 (1H, *q*, broadened by allylic coupling with H-15 and 2 H-21;

* That H-9 in holstiine is less deshielded than in other *N*-methyl-*sec*-pseudo bases appears to be due to a slight alteration in the angle between the dihydroindole ring system and the ring with the C-3 carbonyl function. The downfield position of the H-12 signal, which is also seen in the spectrum of tsilanine (**3b**) [6], is probably a consequence of the 7-membered ether ring being open, since in the spectrum of deoxy-*N*-methyl-*sec*-isopseudostrychnine (**7**) the H-12 signal is also at δ 8·23 [5].

† Presumably the *ar*-demethoxyrindline was in fact holstiline; however, since only the MS of a sample of the base contaminated with a little rindline is available [9], a definite identification of the compound is not possible.

=CH–Me), 6·84–7·60 (3H, *m*; H-9, H-10, H-11), 8·13–8·33 (1H, *m*; H-12); MS: *m/e* 382 (M^+, $C_{22}H_{26}N_2O_4$; 100%), 337 (16), 325 (16), 323 (9), 249 (18), 248 (19), 144 (33), 143 (27), 130 (33), 124 (73), 58 (42), and 57 (80).

Accurate mass measurements: found: M^+ 382·1896, $C_{22}H_{26}N_2O_4$ requires M^+ 382·1892; found 337·1906, $C_{21}H_{25}N_2O_2$ requires 337·1916; found 325·1915, $C_{20}H_{25}N_2O_2$ requires 325·1916; found 323·1155, $C_{19}H_{17}NO_4$ requires 323·1157; found 124·1128, $C_8H_{14}N$ requires 124·1126.

Holstiline (**5b**). Previously determined properties, see Ref. [1]; $[\alpha]_D^{20°}$ +202° ±2° (*c* 0·25, $CHCl_3$); UV: λ_{max}^{EtOH} 259 (log ε 4·12), 271 (sh; 4·03), 283 (sh; 3·83), 293 (3·69) nm, λ_{min}^{EtOH} 231 (log ε 4·00) nm; IR: ν_{max}^{KBr} 1665, 1592, 1345, 1272, 1205, 1112, 1064 and 770 cm^{-1}; NMR: δ 1·62 (3H, *d*, *J* 6·5 Hz, split by homoallylic coupling (*J'* 1·5 Hz) with the 2 H-21; =CH–Me), 1·94 (3H, *s*; N–Me), 3·60 (3H, *s*; O–Me), 5·11 (1H, *s*; H-23), 5·40 (1H, *d*, *J* 10 Hz; H-2), 5·45 (1H, *q*, *J* 6·5 Hz, broadened by allylic coupling with H-15 and the 2 H-21; =CH–Me), 6·87–7·55 (3H, *m*; H-9, H-10, H-11), 8·11–8·34 (1H, *m*; H-12); MS: *m/e* 396 (M^+, $C_{23}H_{28}N_2O_4$; 100%), 381 (4), 368 (7), 365 (13), 337 (12), 309 (8), 249 (24), 195 (13), 154 (13), 144 (23), 143 (23), 130 (19), 124 (70), 58 (37) and 57 (70).

Accurate mass measurements: found: M^+ 396·2047, $C_{23}H_{28}N_2O_4$ requires M^+ 396·2049; found 365·1855, $C_{22}H_{25}N_2O_3$ requires 365·1865; found 337·1920, $C_{21}H_{25}N_2O_2$ requires 337·1916; found 337·1324, $C_{20}H_{19}NO_4$ requires 337·1314; found 124·1124, $C_8H_{14}N$ requires 124·1126.

Rindline (**5c**). Previously determined properties, see Refs. [9] and [10]; NMR: δ 1·65 (3H, broadened *d*, *J* 7 Hz; =CH–Me), 1·95 (3H, *s*; N–Me), 3·59 (3H, *s*; O–Me), 3·78 (3H, *s*; aromatic O–Me), 5·08 (1H, *s*; H-23), 5·37 (1H, *d*, *J* 10 Hz; H-2), *ca* 5·45 (ill-defined *q* (?), *J* 7 Hz; =CH–Me), 6·75 (1H, *dd*, *J* 8·5 Hz, *J'* 2·5 Hz; H-11), 7·01 (1H, *d*, *J* 2·5 Hz; H-9), 8·08 (1H, *d*, *J* 8·5 Hz; H-12).

*Hydroxylation of deoxy-*N*-methyl-*sec*-isopseudostrychnine* (**7**). To a soln in pyridine (4·5 ml) of **7** (300 mg), obtained from icajine (**6a**) by the method of Boit [12], was added OsO_4 (150 mg). After leaving the rn mixture in the dark for 24 hr, the complex was decomposed by addn of 40% aq $NaHSO_3$ soln (0·6 ml), H_2O (3·9 ml), and pyridine (0·9 ml), followed by stirring for 35 min [13]. Basification with NH_4OH soln, followed by extraction into CH_2Cl_2, drying over anhydrous Na_2SO_4, and taking to dryness gave a mixture from which prep. TLC (solvent system CH_2Cl_2–MeOH, 90:10) enabled separation of the main component 16,17-*dihydro*-16α,17α-*dihydroxy-deoxy*-N-*methyl*-sec-*isopseudostrychnine*, crystallizing as hexagonal plates (41 mg) in MeOH, mp 251–253° (decomp.); $[\alpha]_D$ +130° (*c* 0·35, MeOH); UV: λ_{max}^{EtOH} 253 (log ε 4·21), 282·5 (3·71) and 290 (3·60) nm; IR: ν_{max}^{Nujol} 3475, 3320, 1665 and 759 cm^{-1}; NMR: δ CF_3CO_2H 1·90 (3H, *d*, *J* 6·5 Hz; =CH–Me), 3·23 (3H, *s*; N–Me), 6·25 (1H, *q*, *J* 6·5 Hz; =CH–Me), 7·15–7·75 (2H, *m*; H-10, H-11), 7·90–8·35 (2H, *m*; H-9, H-12); MS: *m/e* 382 (M^+, $C_{22}H_{26}N_2O_4$; 100%), 365 (35), 347 (8), 339 (10), 337 (19), 325 (4), 318 (14), 306 (7), 290 (19), 144 (46), 143 (32), 130 (55) and 124 (61).

Accurate mass measurement: found: M^+ 382·1896, $C_{22}H_{26}N_2O_4$ M^+ 382·1892.

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REFERENCES

1. Bosly, J. (1951) *J. Pharm. Belg.* [n.s.] **6,** 150, 243; (1949) *Bull. Séanc. Inst. R. Colon. Belge* **20,** 593.
2. Bisset, N. G. (1965) *Chem. Ind. (Lond.)* 1036; Wenkert, E. and Sklar R. (1966) *J. Org. Chem.* **31,** 2689; Hymon, J. R. and Schmid, H. (1966) *Helv. Chim. Acta* **49,** 2067.
3. Occolowitz, J. J., Biemann, K. and Bosly, J. (1965) *Farmaco, (Pavia) Ed. Sci.* **20,** 751.
4. Janot, M. M., Goutarel, R. and Bosly, J. (1951) *Compt. Rend.* **232,** 853.
5. Bisset, N. G. (1968) Ph.D. Thesis, University of London, pp. 73–74, 125, 127, 167–170.
6. Sarfati, R., Païs, M. and Jarreau, F. X. (1970) *Phytochemistry* **9,** 1107.
7. Koch, M., Plat, M., Das, B. C., Fellion, E. and Le Men, J. (1969) *Ann. Pharm. Franc.* **27,** 229; Bisset, N. G. and Phillipson, J. D. (1971) *Lloydia* **34,** 1, 31.
8. Leeuwenberg, A. J. M. (1969) *Meded. Landbouwhogesch. Wageningen* **69** (1), 126.
9. Spiteller-Friedmann, M. and Spiteller, G. (1968) *Ann. Chem.* **711,** 205.
10. Grossert, J. S., Hugo, J. M., Klemperer, M. E. Von and Warren, F. L. (1965) *J. Chem. Soc.* 2812; cf. Rindl, M. M. and Sapiro, M. L. (1935/36) *Trans. R. Soc. S. Africa* **23,** 361.
11. Bisset, N. G., Das, B. C. and Parello, J. (1973) *Tetrahedron* **29,** 4137.
12. Boit, H. G. (1950) *Chem. Ber.* **83,** 217.
13. Baran, J. S. (1960) *J. Org. Chem.* **25,** 257.