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ALKALOIDS FROM *STRYCHNOS USAMBARENSIS*: REVISED STRUCTURE FOR USAMBARINE

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Key Word Index—*Strychnos usambarensis*; Loganiaceae; bis-indole alkaloids; usambarine.

Abstract—Synthesis has shown an error in the structural determination of usambarine. A further examination of IR and PMR spectra indicates a revised structure for this alkaloid. Stereochemistry (3*S*, 4*R*, 15*S*, 17*S*, 20*R*) has been advanced from the CD curve and biosynthetic considerations.

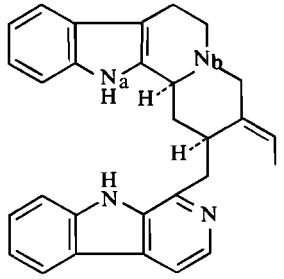
INTRODUCTION

In 1971, some new bis-indole alkaloids were isolated from the roots of *Strychnos usambarensis*: usambarensine (**1**), 3′,4′ dihydrousambarensine (**2**) and their N_b-metho derivatives [1]. Usambarine (**3**) was obtained from leaves of the same species [2]. In 1975, the structure and absolute configuration of usambarensine was proved by X-Ray analysis [3]; the structure and stereochemistry of 3′,4′ dihydrousambarensine were also established by synthesis from (±)-geissoschizoic acid [4].

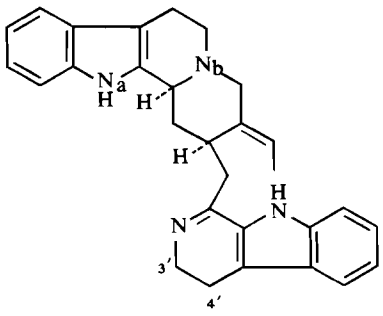
The synthesis of usambarine was then carried out. The four stereoisomers of formula (**3**) (epimeric at C-3 and C-17) were synthesized and none was identical with natural usambarine, by comparison of PMR and IR spectra: moreover none was present in the leaves of *Strychnos usambarensis*. These facts suggested that the structure (**3**) for usambarine needed reconsideration.

RESULTS AND DISCUSSION

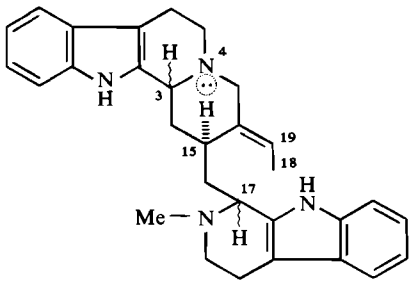
The synthetic bases were prepared by condensation of (±)-geissoschizal (or (±)-3-epi-geissoschizal) and N_b-methyltryptamine in 0.3 M H_2SO_4 at 103–105°. The



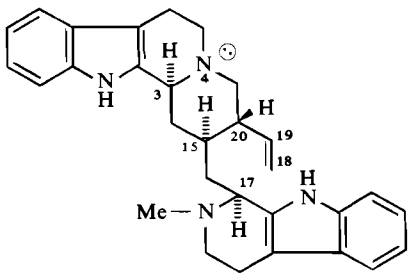
1 usambarensine



2 3′,4′-dihydrousambarensine



3 usambarine (incorrect)



4 usambarine (revised)

epimers (at C-17) were then separated by TLC. Their spectra were in agreement with structure (**3**) and were not superimposable with the IR and PMR spectra of natural usambarine.

We then extracted batches of leaves collected either in the savannah of Rwanda or in the forests of Zaïre and have isolated, amongst a dozen of other alkaloids, a base of MW 450. Accurate MS measurement has confirmed molecular formula $C_{30}H_{34}N_4$. Nevertheless, the IR and PMR spectra show some features different from the spectra of the synthetic bases, particularly for the olefinic signals. In our PMR spectrum, the complex signals between δ 4.95 and 5.46 belong to a vinyl group, as in quinine [5].

Presumably Koch and Plat [2] thought that usambarine had an ethylidene group as in usambarensine (**1**) and many other *Strychnos* alkaloids. The lack of good resolution in the NMR spectrum seriously handicapped its interpretation. The strong IR band at 918 cm^{-1} confirms a vinyl group; this band is also present on Koch's spectrum. These findings are readily accounted for in terms of structure (**4**).

It has been proposed, on the basis of CD curves, that a positive Cotton effect in the 270–290 nm region can be due to a $C_3H\alpha$ configuration. In the case of bis-indole dimers, the Cotton effect will be very intense if $C_{17}H$ has the same configuration as C_3H, as in ochrolifuanine B [6]. The fact that usambarine has a CD curve identical to this last alkaloid demonstrates that both have the same configuration (3*S*, 17*S*).

In Koch's IR spectrum as well as in ours, the IR absorption between 2780 and 2840 cm^{-1} (Bohlmann bands) indicates a structure with CD *trans* rings. This assumption is confirmed by the absence of low field shift of H-3 in the PMR spectrum [6].

Finally, examination of molecular stereomodels has shown that the α-configuration for $C_{15}H$ agrees with biosynthetic considerations.

The β-configuration for $C_{20}H$ is based on comparison with the stereochemistry observed in biogenetic precursors such as vincoside and strictosidine and in two other alkaloids (strychnofoline and strychnopentamine) isolated from leaves of *Strychnos usambarensis* [7, 8].

EXPERIMENTAL

Plant material. The leaves of *Strychnos usambarensis* were collected in Rwanda by L.A. (voucher specimen Angenot 22. Herbarium of the National Botanical Garden of Belgium). Another batch was collected in Zaïre by Z. Bacq and P. Duvigneaud (voucher specimen Duvigneaud 786 B. Herbarium of Brussels). Specimens are also kept in the herbarium of the Pharmaceutical Institute, University of Liège.

Isolation. The dried plant material from Rwanda (1 kg) was extracted with cold MeOH, the extract concentrated and the resulting soln made acid. This acidic soln (A) was then extracted with Et_2O to remove non-alkaloidal products. The remaining soln was basified with Na_2CO_3 and extracted successively with Et_2O and $CHCl_3$, giving 3.6 g crude alkaloids. The acidic soln (A) was extracted with successive fractions of $CHCl_3$, to give only 2 alkaloids. These combined extracts were shaken with 2% HOAc and concentrated. The residue was discarded and the acidic soln, after basification with Na_2CO_3, was extracted with Et_2O. The 2 alkaloids (500 mg) were recovered by evaporation of the solvent and usambarine was separated by PLC. This alkaloid was also present in the same amount in the leaves collected in Zaïre.

Usambarine (**4**): powder, mp 215°. $C_{30}H_{34}N_4$ (meas. 450.2794; calc. 450.2783). UV λ_{max}^{MeOH} nm (log ε): 226 (4.81), 275 (4.17), 282 (4.19), 290 (4.1). IR $\bar{\nu}_{max}^{KBr}$ cm^{-1}: 2840, 2795, 2780 (Bohlmann bands), 918 (vinyl), 740 (*O*-disubstituted C_6H_6). MS: *m/e* (%) 450 [M^+] (27), 435 (2), 406 (3), 265 (6), 251 (10), 250 (7), 249 (9), 223 (3), 199 (16), 185 (100), 171 (12), 169 (9), 156 (7), 144 (10), 143 (4), 130 (3). PMR ($CDCl_3$): δ = 5.46–4.95 (3H vinyl group, C-18 and C-19). δ = 2.42 (3H, *s*, N-Me) δ = 7.74 and 6.40 (each 1 H, *br m* disappearing on deuteration; NH). CD (MeOH) $\textcircled{H}_{276}$ +14.520 and $\textcircled{H}_{245}$ = 0. TLC: R_f in EtOAc–*i*-PrOH–NH_4OH (48:2:1) 0.90.

Synthesis. Geissoschizal (3*S*, 15*S*) and 3-epi-geissoschizal (3*R*, 15*S*) were obtained as described previously [9]. 0.1 mM geissoschizal and 0.2 mM *N*-Me-tryptamine in H_2SO_4 0.3 M were refluxed at 100° for 3 hr ander N_2. After cooling the mixture was diluted with ice water, made alkaline with 5% aq. K_2CO_3 and extracted with 3 portions of $CHCl_3$. The combined organic extracts were dried and evaporated. Separation of the mixture by PLC with Me_2CO–MeOH (92:8) × 3 provides **3(a)** (R_f 0.29) and **3(b)** (R_f 0.22).

The same procedure was applied to 3-epi-geissoschizal and N_b-Me-tryptamine and also gave an amorphous mixture. Separation was made by PLC with $CHCl_3$–MeOH 9:1, × 2 to afford **3(c)** (R_f 0.57) and **3(d)** (R_f 0.49).

Synthetic base **3(a)** amorphous powder. Stereochemistry 3*S*, 4*S*, 15*S*, 17 unsettled. UV λ_{max}^{EtOH} nm: 229, 275, 282 and 290. IR $\bar{\nu}_{max}^{CHCl_3}$ cm^{-1}: 3450, 2940, 1446, 1318, 1302, 1192, 1110, 1010.

MS: *m/e* (%) 450 [M^+] (58), 406 (6), 265 (30), 263 (22), 262 (25), 252 (100), 251 (50), 249 (70), 235 (11), 223 (25), 199 (58), 185 (56), 171 (22), 169 (22), 156 (16), 144 (34), 143 (58). PMR ($CDCl_3$): δ = 5.37 (1H, *q*, H-19; J_1 = 14 Hz, J_2 = 7 Hz), 4.35 (1H, *m*, H-3 α *cis*), 2.52 (3H, *s*, N–Me), 1.62 (3H, *d*, Me-18; *J* = 7 Hz).

Synthetic base **3(b)** amorphous powder. Stereochemistry 3*S*, 4*S*, 15*S*, epimer of **3(a)** at C_{17}. UV λ_{max}^{EtOH} nm: 232, 275, 283 and 291 nm. IR $\tilde{\nu}_{max}^{CHCl_3}$ cm^{-1}: 3480, 2935, 1450, 1318, 1302, 1192, 1110, 1010 cm^{-1}. MS: *m/e* (%) 450 [M^+] (48), 406 (5), 265 (24), 263 (18), 262 (12), 252 (100), 251 (43), 250 (45), 249 (56), 235 (10), 223 (17), 199 (72), 185 (72), 171 (26), 169 (24), 156 (18), 144 (38), 143 (90). PMR ($CDCl_3$): δ = 5.45 (1H, *q*, H-19) 4.2 (1H, *m*, H-3 α cis), 2.44 (3H, *s*, N-Me), 1.65 (3H, *d*, Me-19).

Synthetic base **3(c)** amorphous powder. Stereochemistry 3*R*, 4*S*, 15*S*, 17 unsettled. UV λ_{max}^{EtOH} nm: 228, 276, 283 and 291. IR $\tilde{\nu}_{max}^{CHCl_3}$ cm^{-1}: 3430, 2930, 2800 (Bohlmann bands), 1455, 1380, 1324, 1276, 1165, 1105, 1010. MS: *m/e* (%) 450 [M^+] (68), 406 (6), 265 (10), 263 (11), 262 (17), 251 (32), 250 (100), 249 (75), 235 (15), 223 (11), 199 (8), 185 (36), 171 (7), 169 (10), 155 (9), 144 (10), 143 (13). PMR ($CDCl_3$): δ = 5.40 (1H, *q*, H-19), 2.54 (3H, *s*, N-Me), 1.73 (3H, *d*, Me-18).

Synthetic base **3(d)** amorphous powder. Stereochemistry 3*R*, 4*S*, 15*S*, epimeric of **3(c)** on C-17. UV λ_{max}^{EtOH} nm: 228, 276, 282 and 290. IR $\tilde{\nu}_{max}^{CHCl_3}$ cm^{-1}: 3500, 2935, 2800 (Bohlmann bands), 1455, 1375, 1322, 1276, 1160, 1105, 1010. MS: *m/e* (%) 450 [M^+] (47), 406 (5), 265 (10), 263 (10), 262 (17), 251 (33), 250 (100), 249 (87), 235 (15), 223 (11), 199 (10), 185 (36), 171 (7), 169 (10), 155 (9), 144 (11), 143 (15). PMR ($CDCl_3$): δ = 5.30 (1H, *q*, H-19), 2.52 (3H, *s*, N-Me), 1.50 (3H, *d*, Me-18).

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