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10-HYDROXY-N_b-METHYL-CORYNANTHEOL, A NEW QUATERNARY ALKALOID FROM THE STEM BARK OF *STRYCHNOS USAMBARENSIS*

J. QUETIN-LECLERCQ and L. ANGENOT

Laboratoire de Pharmacognosie, Institut de Pharmacie, Universite' de Liège, rue Fusch 5, B-4000 Liège, Belgium

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Key Word Index—*Strychnos usambarensis*; Loganiaceae; quaternary indole alkaloid; 10-hydroxy-N_b-methyl-corynantheol; HSCCC.

Abstract—A new quaternary indole alkaloid: 10-hydroxy-N_b-methyl corynantheol has been isolated from the stem bark of *S. usambarensis* by High Speed Counter Current Chromatography (HSCCC). Its IUPAC name is 2-(2-hydroxyethyl)-3-vinyl-5-methyl-1,2,3,4,6,7,12,12b-octahydro-indolo[2,3-*a*] quinolizinium-9-ol [2(*S*),3(*R*),5(*S*), 12b(*S*)].

INTRODUCTION

Extensive studies in our laboratory on leaves and roots of *Strychnos usambarensis* Gilg., the main ingredient of an African curarizing arrow poison, have resulted in the isolation of numerous alkaloids among which are 18 quaternary alkaloids [1–9]. Purification of those alkaloids was arduous because of their polarity and a possible irreversible adsorption on the solid support matrix (cellulose, silica) extensively used to purify crude fractions 15 years ago. In addition, solid supports can give rise to contamination and breakdown of samples. Progress has been made in the purification techniques with the appearance of Droplet Counter Current Chromatography (DCCC), a technique avoiding the use of a solid support matrix. Unfortunately, the slowness of the separations (two weeks or more) could lead to transformation of unstable compounds. A few years ago High Speed Counter Current Chromatography (HSCCC) [10] was introduced which gives good separations in shorter times (a few hrs, instead of weeks). We decided to test this new method in the purification of the quaternary fraction of the alkaloids extracted from the stem bark of *S. usambarensis* collected in the Ivory Coast.

RESULTS AND DISCUSSION

HSCCC was proved to be a useful and quick method in separating quaternary alkaloids. We have obtained a new compound giving a stable violet coloration with the Fast Blue Salt B reagent. The phenolic properties of this molecule were confirmed by the bathochromic effect in the UV spectrum when adding sodium hydroxide. Moreover, this UV spectrum showed the characteristic chromophore of an indole alkaloid. The FAB mass spectrum gave the molecular ion peak at *m/z* 327 [M^+], corresponding to the elemental composition $C_{20}H_{27}N_2O_2$ established by high-resolution mass measurement.

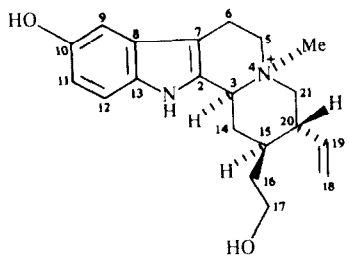
Comparison of the EI mass spectrum with those of hunterburnine α-methochloride **2** and huntrabrine methochloride **3** [11], both having the same M_r, led us to a structure close to but different from huntrabrine (Table 1). A more detailed understanding of the structure of the molecule was gained from its 400 Mz ^{1}H NMR spectrum. Resonances and coupling constants of three aromatic protons at δ6.68, 6.8 and 7.13 were consistent with a 10-hydroxy-tetrahydro-β-carboline moiety. The NMR spectrum also revealed the presence of one vinyl chain (δ 5.55 and 5.29), a hydroxymethyl group (δ4.53) [12] and a quaternary *N*-methyl group (δ3.11).

The presence of phenolic and hydroxyl functions was confirmed by acetylation (fixation of two acetyl residues) and methylation (three methylation sites: OH (phenol),

Table 1. EIMS data of compounds **1**, **2*** and **3***

1 *m/z* %	**2** *m/z* %	**3** *m/z* %
326 (4)	326 (41)	326 (94)
312 (80)	312 (8)	312 (11)
311 (91)	311 (16)	311 (15)
295 (4)	295 (7)	295 (9)
281 (9)	—	281 (30)
—	269 (12)	—
267 (32)	—	—
265 (30)	—	—
—	255 (100)	—
—	241 (23)	—
239 (40)	239 (24)	—
201 (14)	—	201 (100)
200 (27)	—	—
186 (100)	—	186 (16)
185 (74)	—	185 (14)
184 (31)	184 (41)	184 (16)
172 (61)	—	172 (18)
160 (14)	160 (28)	160 (13)

* Data obtained from ref. [11].



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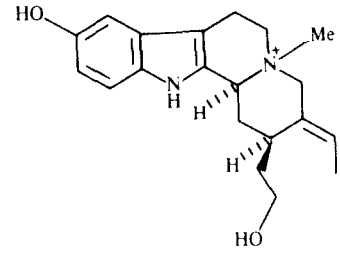

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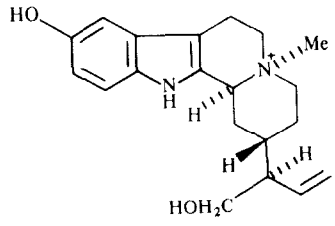

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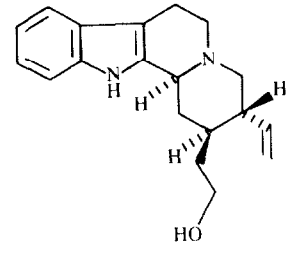

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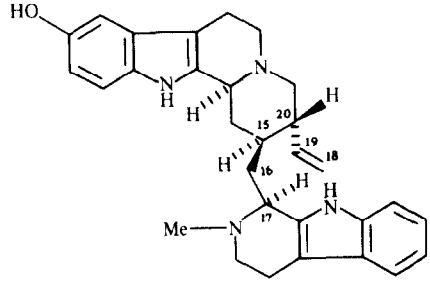

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OH (hydroxymethyl) and NH (indoline)). So we assigned to this alkaloid structure **1**. The ^{13}C NMR chemical shifts are collected in Table 2. They are fully supportive of structure **1** as compared with data for known compounds [13, 14]: among which corynantheol **4** and 10-hydroxy-usambarine **5**. Moreover, comparison of the spectra with those of 10-methoxy-N_4-methyl-corynantheol [15, 16] confirmed our proposal.

There remains to consider the stereochemistry. The CD curve, positive at 270 nm and negative at 300 nm, indicates a structure with C/D *cis* rings and a 3 α-H configuration [17]. This stereochemistry is supported not only by the relatively low field position at δ 3.11 of the quaternary N_4-methyl suggesting a *cis*-relationship between the N_4-methyl and the H-3, but also by the shielding of C-6 at δ 18.7 as compared with corynantheol where resonances occur at expected values for *trans*-quinolizidine [18].

The 15α-hydrogen configuration agrees with the biogenetic hypothesis [7]. Moreover, the chemical shift of C-15 at δ 36.1 confirmed this configuration [19]. We have assigned the 20 β-H configuration for the following reasons. Firstly, all the alkaloids found so far in *S. usambarensis* have this stereochemistry. Secondly, Wenkert [18] showed that an ethyl side chain with an α-orientation causes a shielding of the chemical shifts of C-21 and C-14 of over *ca* 3 to 4 ppm. We can assume that it would also be the case for a vinyl side chain. Table 2 shows that the shifts of C-21 and C-14 of 10-hydroxy-N_b-methyl-corynantheol are very similar to those of corynantheol which has the 20β-H configuration.

EXPERIMENTAL

Plant material. Bark of *Strychnos usambarensis* Gilg. collected in Ivory Coast by Prof. F. Sandberg (Uppsala) and identified by Dr Leeuwenberg. Reference specimens (voucher No. 7916) have been deposited at Wageningen (The Netherlands)

Extraction and isolation. Extraction procedures of the powdered bark followed those described earlier [20]. The fraction of polar alkaloids pptd by picric acid was solubilized in Me_2CO–MeOH–H_2O (6:2:1) and transformed into the chloride

Table 2. ^{13}C NMR spectrum of **1** compared with **4*** and **5***

C	**1** (CD_3OD 100.8 MHz)	**4** ($CDCl_3$ 22.6 MHz)	**5** ($CDCl_3$ 75.6 MHz)
2	133.4	134.5	131.1
3	66.9	60.0	59.5
5	53.8	52.9	52.8
6	18.7	21.4	21.3
7	102.7	107.0	105.3
8	128	127.0	127.6
9	103.6	117.8	100.9
10	152.6	121.0	149.9
11	113.1[a]	118.9	111.3
12	113.6[a]	110.8	111.3
13	138.5	136.1	135.5
14	35.8[b]	34.1	33.9
15	36.1	37.0	35.8
16	36.7[b]	35.9	41.8
17	69.1	61.0	58.4
18	120.7	116.9	117.7
19	136.9	139.2	139.6
20	42.4	46.8	47.1
21	60	59.9	60.8
N_4Me	50.9	—	—

[a,b] Assignments may be interchanged.
* Data obtained from refs [13, 14] respectively.

form by passage through an Amberlite IRA 400 column. This crude extract was submitted to a HSCCC separation on the Ito Multilayer-Coil Separator-Extractor (P.C. Inc.) with *n*-BuOH–aq. NaCl 0.1 M (1:1) as solvent system. 600 mg of crude extract were used for each separation with the i.d 2.6 mm tubing coil. The upper phase (*n*-BuOH) was used as stationary phase and the mobile phase (H_2O) was pumped from the head end to the tail of the column. Retention of the stationary phase was about 60% at 800 rpm and under a pressure of 1.5 kg/cm^2.

Fractions containing 10-hydroxy-N_b-methyl-corynantheol were then acidified and pptd by Mayer's reagent. The ppt. was solubilized in Me_2CO–MeOH–H_2O (6:2:1) and transformed into the chloride form by Amberlite IRA 400.

The alkaloid was then submitted to a molecular sieve separation on Fractogel® TSK 4OS with EtOH as solvent. Then the pure compound was pptd with Et_2O to afford colorless powder.

10-Hydroxy-N_b*-methyl-corynantheol* (**1**). UV λ_{max}^{MeOH} nm (log ε): 213 (4.27) 271 (3.76), 289 (3.5), 299 (3.48), 307 (3.46). λ_{max}^{MeONa} nm (log ε): 221 (4.39) 271 (3.77), 325 (3.5) CD (MeOH) $\Delta\varepsilon_{240}$: −1.03; $\Delta\varepsilon_{270}$ +0.65; $\Delta\varepsilon_{305}$: −0.11; $\Delta\varepsilon_{323}$: +0.13. I.R.: ν_{max}^{KBr} cm^{-1}: 3365, 3225, 2925, 1630, 1595, 1540, 1458, 1382, 1354, 1305, 1240, 1198, 1135, 1120, 1073, 1040, 1015, 992, 938, 905, 807. EIMS: see Table 1. High resolution mass measurements: $[M-1]^+$: $C_{20}H_{26}N_2O_2$: measured: 326.197906, calcd: 326.198880; $[M-Me]^+$: $C_{19}H_{23}N_2O_2$: measured: 311.176271, calcd: 311.175405; $C_{15}H_{15}N_2O$: measured: 239.118773, calcd: 239.117890; $C_{12}H_{13}N_2O$: measured: 201.102791, calcd: 201.102240; $C_{11}H_{10}N_2O$: measured: 186.080356, calcd 186.078764; ^1H NMR (400 MHz: CD_3OD) δ7.13 (1H, *d*, J_{ortho}, 8.7 Hz, H-12), 6.8 (1H, *d*, J_{meta}, 2.1 Hz, H-9), 6.68 (1H, *dd*, J_{ortho}, 8.7 Hz, J_{meta}, 2.1 Hz, H-11), 5.5 (1H, *m*, H-19), 5.25 (2H, *dd*, H-8), 4.62 (1H, *dd*, H-3), 4.53 (2H, *t*, H-17), 3.11 (3H, *s*, N_4^+-Me). ^{13}C-NMR: see Table 2.

Methylation. To 0.5 mg of 10-hydroxy-N_b-methyl-corynantheol in MeOH was added 1 ml CH_3I and 2 mg NaH. After standing 1 hr at room temp., the solvents were removed by evapn to yield a trimethylated derivative with a molecular ion peak at *m/z* 369 (FABMS).

Acetylation. To 1 mg of 10-hydroxy-N_b-methyl-corynantheol was added 1 ml pyridine and 0.2 ml Ac_2O. After standing 5 hr at room temp., reagents were removed by evapn to afford the diacetylated compound with the molecular ion peak at *m/z* 412 (FABMS).

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STUDIES ON THE ALKALOIDS OF *RHAZYA STRICTA*

ATTA-UR-RAHMAN* and KHURSHID ZAMAN

H.E.J. Research Institute of Chemistry, University of Karachi, Karachi-32, Pakistan

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Key Word Index—*Rhazya stricta*, Apocynaceae, dimeric indole alkaloids, secamine, decarbomethoxytetrahydrosecamine, 1,2-dehydroaspidospermidine *N*-oxide.

Abstract—Two new dimeric indole alkaloids, 16*S*,16′-decarbomethoxytetrahydrosecamine (**1**) and 16*R*,16′-decarbomethoxytetrahydrosecamine (**2**) have been isolated from the roots of *Rhazya stricta*. The structures of the two isomers have been established through spectroscopic studies. The ^{13}C NMR spectrum of 1,2-dehydroaspidospermidine-*N*-oxide (**3**) is also presented.

INTRODUCTION

Rhazya stricta (Apocynaceae) is widely distributed in Pakistan and has been used for the treatment of various ailments [1–6]. The anticancer activity of some of its alkaloids is also reported [7–9]. We have previously reported a number of new alkaloids from the plant [10–13] including didemethoxycarbonyltetrahydrosecamine from the roots [13] which was found to be cytotoxic against Eagles KB carcinoma of the nasopharynx in cell cultures [8]. '16-Decarbomethoxytetrahydrosecamine' was previously reported from the roots of *Amsonia tabernaemontana* and from the rootbark of *Aspidosperma excelsum* but it was not determined whether the isolated compound possessed 16*S* or 16*R*-stereochemistry [14, 15]. In this communication we report the isolation and structure of both the isomeric biologically interesting dimeric alkaloids 16*S*,16′-decarbomethoxytetrahydrosecamine (**1**) and 16*R*,16′-decarbomethoxytetrahydrosecamine (**2**), together with ^{13}C NMR studies on 1,2-dehydroaspidospermidine-*N*-oxide (**3**).

RESULTS AND DISCUSSION

The alkaloids 16*S*,16′-decarbomethoxytetrahydrosecamine (**1**) $[\alpha]_D = 0°$ and 16*R*,16′-decarbomethoxytetrahydrosecamine (**2**) $[\alpha]_D = 0°$, were obtained after separation of the alkaloidal fractions on the basis of differential basicities and preparative TLC. The UV spectra of both were identical to those reported for secamines [6, 8, 13, 14] showing λ_{max}(MeOH) 220, 283, 291. They also possessed virtually identical IR spectra and gave absorptions at 3650 (NH), 2900, 1720 (satd ester C=O), 1600, 1340 and 1010 cm^{-1}.

The mass spectrum of each afforded the molecular ion peak at *m/z* 622 with the exact masses of (**1**) and (**2**) at 622.4576 ($C_{40}H_{54}N_4O_2$) and 622.4273 ($C_{40}H_{54}N_4O_2$)

*In our earlier publication on the structure of didemethoxycarbonyltetrahydrosecamine, we have assigned the C-16′H at δ4.04. An examination of the spectra of the repurified material leads us to reverse the assignments as follow: δ4.01 (C-16H), δ5.33 (C-16′H) [13].