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ALKALOIDS OF *HUNTERIA ZEYLANICA*

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Key Word Index—*Hunteria zeylanica*; Apocynaceae; indole alkaloids; ^{13}C NMR.

Abstract—Twenty alkaloids were isolated from the leaves and stem bark of *Hunteria zeylanica*, collected in Kenya. They were: 3-*epi*-dihydrocorymine 3-acetate, norisocorymine, corymine, 3-*epi*-dihydrocorymine 17-acetate, picralinal, picrinine, 3-*epi*-dihydrocorymine, isositsirikine, lanceomigine, geissoschizol, gentianine, kopsinine, eburnamine, norpleiomutine, pleiocarpamine, tubotaiwine, pleiomutinine, 19′-*epi*-pleiomutinine, yohimbol and 10-hydroxy-16-*epi*-affinine.

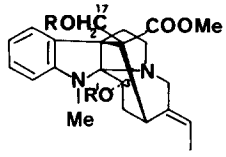
INTRODUCTION

As a continuation of our chemotaxonomic work on the genus *Hunteria* [1–10] this paper describes our results on the alkaloid content of *H. zeylanica* var. *africana* collected in Kenya. This report is prompted by a recent communication on the indole alkaloids of *H. zeylanica* from Sri Lanka [11]. Extractions were conducted in the usual fashion and the yield of alkaloids was 20 g/kg in the leaves and 13 g/kg in the stem bark. The alkaloid mixture (AM) was separated by a combination of crystallization and medium pressure liquid chromatography; when necessary, analytical samples were obtained by prep. TLC followed by crystallization.

RESULTS AND DISCUSSION

Ten alkaloids were isolated from the leaves, among which 3-*epi*-dihydrocorymine 17-acetate **4** is new. They are, by order of increasing polarity: 3-*epi*-dihydrocorymine 3-acetate **1** (6.8% of AM), norisocorymine **2** (8.7%), corymine **3** (11.1%), 3-*epi*-dihydrocorymine 17-acetate **4** (2.1%), picralinal **5** (5.7%), picrinine **6** (3.6%), 3-*epi*-dihydrocorymine **7** (37%), isositsirikine **8** (1.8%), lanceomigine **9** (7.2%) and geissoschizol **10** (0.7%). Compounds **1–3**, **6**, **7**, **9** and **10** were identified by direct comparison with reference samples available from other studies in our laboratory. Compounds **5** and **8** were identified through their spectral and physical properties (IR, UV, NMR, mass spectra, mp, $[\alpha]_D$). It is worthy of note that among these, the alkaloids of the 3-*epi* series (46% of total material) were unknown until our study of *H. congolana* (4, 5). The polar lanceomigine **9**, biogenetically related to pseudo akuammigine, was only recently identified in *Alstonia lanceolata* [13] and also in *H. congolana* [12].

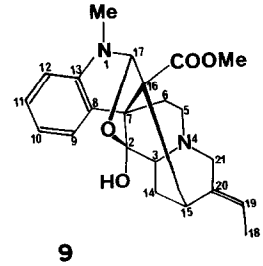
The structure of the novel 3-*epi*-dihydrocorymine 17-acetate **4** was established by spectral examination and chemical correlation. The mass spectrum of **4** showed a $M^{+\cdot}$ at *m/z* 426 ($C_{24}H_{30}O_5N_2$), 42 mu greater than dihydrocorymine, and a major fragment at *m/z* 171 typical of the corymine series. The extra acetate is located on the primary alcohol, accounting



1 R = H R′ = MeCO–

4 R = MeCO– R′ = H

7 R = H R′ = H



9

for the loss of 73 mu (–CH_2OAc). Finally, that **4** belongs to the *epi*-corymine family was proved by the selective acetylation (Ac_2O, pyridine) of 3-*epi*-dihydrocorymine **7** to **4**. Isolation of **4**, together with the previous isolation of all the other diols, monoacetates, and diacetates of the dihydrocorymine and of the 3-*epi*-dihydrocorymine series, allows the proposal of a set of rules to establish the configuration at C-3. (1) Acetates on C-17 resonate at 2.03 ± 0.02 ppm whatever the configuration at C-3, (2) acetates on C-3 are shielded at 1.85 ± 0.02 in the 3β OAc series, and (3) acetates on C-3 are deshielded at 2.15 ± 0.02 in the 3α OAc series.

Ten alkaloids were separated from the stem bark alkaloid mixture, albeit in poor yield due to their polarity and instability. They are, in order of increas-

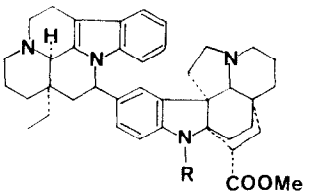
Table 1. ^{13}C NMR spectra of **20** (d_6-DMSO) and **22** ($CDCl_3$)

Position	20	22	Position	20	22
2	135.9	135.0	13	131.7	135.6
3	190.4	191.4	14	43.0	43.6
5	55.0	56.6	15	29.5	29.8
6	19.0	19.4	16	39.9	38.6
7	118.9	121.3	17	63.6	62.5
8	128.6	128.4	18	11.7	12.1
9	102.8	120.8	19	119.6	120.4
10	151.0	120.7	20	136.0	136.9
11	117.4	126.7	21	51.8	52.2
12	113.2	112.6	N(Me)	41.8	41.9

ing polarity: gentianine **11** (0.1% of AM), (−)-kopsinine **12** (1.5%) (−)-eburnamine **13** (0.6%), norpleiomutine **14** (0.6%), (+)-pleiocarpamine **15** (0.8%), (+)-tubotaiwine **16** (1.1%), pleiomutinine **17** (3.8%), 19′ *epi*-pleiomutinine **18** (0.03%), yohimbol **19** (0.03%) and 10-hydroxy-16-*epi*-affinine **20** (4.6%). All alkaloids except the novel **14** and **20** were identified by comparison with authentic samples. The bisindole **14** showed a $M^{+\cdot}$ at *m/z* 616 and its mass spectrum was reminiscent of that of pleiomutine ($M^{+\cdot}$ 630) as described by Biemann *et al.* [14]. The missing methylene was located on the kopsinine part, whose fragments were shifted 14 mu from the corresponding fragments in pleiomutine **23**; the NMR spectrum of **14** showed only one methyl singlet at 3.75 ppm (OMe), which indicated that the missing carbon was the *N*-Me of kopsinine. Confirmation of this hypothesis was obtained by methylation of **14** to pleiomutine according to a modified Eschweiler–Clarke reaction (HCHO, HOAc, $NaBH_3CN$).

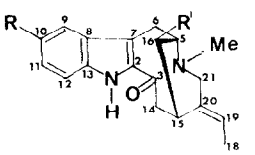
The structure of the second unknown alkaloid **20** ($M^{+\cdot}$ at *m/z* 340, $C_{20}H_{24}N_2O_3$) was elucidated mainly on the basis of the ^{13}C NMR spectrum which revealed the presence of 20 carbons: 7 quaternary carbons, 7 methines, 4 methylenes and 2 methyls (Table 1). These data were very close to those published by Wenkert *et al.* for the vobasine series [15]. Discrepancies originated in the aromatic substitution by a hydroxyl, as shown by the alkaline bathochromic UV shift typical of a phenol. ^1H NMR showed the substitution to be located either on C-10 or C-11, because of the observation of an AMX spectrum [with $J_{AM} = 8$ Hz (*ortho*), $J_{MX} = 1.5$ Hz (*meta*), $J_{AX} \simeq 0.5$ Hz (*para*)]. Application of the ^{13}C NMR rules [16] for indole substitution fixed the substituent on C-10. Other changes in the ^{13}C NMR spectra were due to the stereochemistry at C-16, our compound belonging to the *epi*-series. This was demonstrated by the preparation of the desoxy-compound **22** (16-*epi*-affinine) from 16-*epi*-vobasine (**21**) through an unexceptional set of reactions. Except for the aromatic carbons, the spectra of **20** and **22** fitted within 2 ppm, small differences probably being caused by a solvent effect.

In his revision of the Apocynaceae, Pichon [18] had divided *H. zeylanica* into 3 varieties: var. *zeylanica* and var. *salicifolia*, both common to South East Asia, and var. *africana* encountered in Kenya and Tanzania. The alkaloidal content of *H. zeylanica* var. *africana* as described here is markedly different from the one of *H. zeylanica* collected in Sri Lanka, although in this case the variety is unsure; it is also different from the content of *H. corymbosa* (a synonym for *H. zeylanica*) as described by G. F. Smith [19] and in which only corymine was isolated.



14 R = H

23 R = Me



20 R = OH R' = CH_2OH

21 R = H R' = COOMe

22 R = H R' = CH_2OH

EXPERIMENTAL

General. Mps are uncorr. Rotations were determined in $CHCl_3$ and, unless otherwise stated, NMR were measured in $CDCl_3$ solns; chemical shifts are given in δ with TMS as the int. standard. ^{13}C NMR spectra were obtained at 15 MHz. ^1H NMR spectra at 250 MHz and ^{13}C NMR spectra at 50 MHz were obtained on a Cameca 250 spectrometer. Chromatographic columns were packed with Si (Merck H60) and prep. TLC plates were Merck 60F-254. Colour reactions (CR) were obtained by spraying plates with a soln of Ce (IV) $(NH_4)_2SO_4$.

Extraction and isolation of alkaloids. Dried ground leaves (610 g) were wetted with 360 ml 50% NH_4OH and lixiviated by means of 18 l. EtOAc. The lixiviate was extracted with 2% H_2SO_4 (10 l.), and the aq. phase made alkaline with NH_4OH and extracted with $CHCl_3$ (6 l.). The $CHCl_3$ layer was dried (Na_2SO_4) and evapd *in vacuo* to give 12.3 g of crude alkaloid mixture (20 g/kg). Direct crystallization from MeOH yielded 2.7 g of 3-*epi*-dihydrocorymine **7**. Mother liquors

(9.5 g) were purified by HPLC using 1 kg of Si gel eluted in 30-ml fractions with $CHCl_3$ (2.5 l.), $CHCl_3$–MeOH (49:1, 6 l.; 19:1, 7 l.; 17:3, 5 l.) and MeOH (1 l.). Tubes were analysed by TLC and pooled according to their composition. Fractions 150–162 yielded 3-*epi*-dihydrocorymine 3-acetate **1** (656 mg), fractions 167–175 yielded norisocorymine **2** (557 mg), fractions 186–232 yielded corymine **3** (1.08 g), fractions 241–254 yielded 3-*epi*-dihydrocorymine 17-acetate **4** (217 mg), fractions 298–355 yielded picralinal **5** (602 mg), fractions 360–370 yielded picrinine **6** (380 mg), fractions 380–440 yielded 3-*epi*-dihydrocorymine **7** (1.85 g), fractions 585–605 yielded laceomigine **9** (158 mg), and fractions 661–670 yielded geissoschizol **10** (41 mg). Additional amounts of these alkaloids were obtained by prep. TLC of the other fractions, together with 50 mg of isositsirikine **8** from fractions 474–528.

In an analogous fashion, the dried ground stem bark (1.64 kg) yielded 21.5 g of crude alkaloid mixture (13.4 g/kg), which was submitted to HPLC as above (30-ml fractions). Solvents were $CHCl_3$ (3 l.), $CHCl_3$–MeOH (99:1, 3 l.; 49:1, 6 l.; 19:1, 3 l.; 9:1, 3 l.; 4:1, 3 l.; 7:3, 3 l.; 1:1, 3 l.) then MeOH (3 l.). Gentianine **11** (128 mg) was isolated from tubes 201–215, kopsinine **12** (640 mg) from tubes 407–435, pleiocarpamine **15** (310 mg) from tubes 436–470, tubotaiwine **16** (118 mg) from tubes 471–489, eburnamine **13** (350 mg) from tubes 490–520, norpleiomutine **14** (268 mg) from tubes 521–545, pleiomutinine **17** and 19′-*epi*-pleiomutine **18** (550 mg) from tubes 610–670, 10-hydroxy-16-*epi*-affinine **20** was found in fractions 671–720 (1.6 g) together with yohimbol **19**.

New alkaloids. 3-Epi-*dihydrocorymine* 17-*acetate* **4**. (CR pink then purple); mp: 260° (Me_2CO); $[\alpha]_D$ −76° (*c* 0.7); UV: λ_{max}^{MeOH} nm (log ϵ): 217 (4.33), 260 (4.35), 317 (3.88), $\lambda_{max}^{MeOH+HCl}$ nm: 215, 249, 304; IR ($CHCl_3$) cm^{-1}: 3550, 1740, 1730, 1600, 1210; MS *m/z* (rel. int.): 426 (100), 409, 384, 354, 353, 171 (95); ^1H NMR ($CDCl_3$–CD_3OD) δ 7.60 (*t*, 7 Hz), 7.05 (*d*, 7 Hz), 6.55 (*t*, 7 Hz), 6.25 (*d*, 7 Hz), 5.40 (*q*, 7 Hz), 4.85 (*d*, 12 Hz), 3.80 (*s*, 3H), 2.90 (*s*, 3H), 2.03 (*s*, 3H), 1.80 (*d*, 7 Hz, 3H).

Norpleiomutine **14**. (CR yellow), amorphous; $[\alpha]_D$ −65° (*c* 0.5); UV λ_{max}^{MeOH} nm (log ϵ): 212 (4.67), 230 (4.57), 253 (4.15), 285 (4.05), 292 (4.05); IR ($CHCl_3$) cm^{-1}: 3360, 1730, 1610, 1450, 1200; MS *m/z* (rel. int.): 616 (100), 587, 546, 431, 364 (6), 336, 308, 280, 252 (90), 208, 185, 156, 124 (80), 109 (90); ^1H NMR ($CDCl_3$) δ 7.50–6.50 (*m*, 7H), 4.95 (*dd*, 8, 4 Hz), 4.0 (*br s*), 3.75 (*s*, 3H), 0.85 (*t*, 7 Hz, 3H).

10-*Hydroxy*-16-epi-*affinine* **20**. (CR yellow then purple); amorphous, $[\alpha]_D$ −122° (*c* 0.46); UV λ_{max}^{MeOH} nm (log ϵ): 212 (4.46), 228 (*sh* 4.29), 277 (*sh*), 328 (4.17); $\lambda_{max}^{MeOH+NaOH}$ nm: 215, 233, 282, 337; IR (Nujol) cm^{-1}: 3350, 1620, 1520; MS *m/z* (rel. int.): 340 (1), 322 (40), 152 (100); ^1H NMR (250 MHz, DMSO) δ 11.5 (*s*), 9.05 (*br s*), 7.35 (*d*, 8 Hz), 7.05 (*d*, 1.5 Hz), 6.95 (*dd*, 8, 1.5 Hz), 5.48 (*q*, 7 Hz), 2.42 (*s*, 3H), 1.62 (*d*, 7 Hz, 3H).

Acetylation of 3-epi-*dihydrocorymine* (**7**→**4**). Dihydrocorymine (100 mg, 0.25 mmol) was dissolved in pyridine (1 ml), and 25 μl of Ac_2O (0.25 mmol) were added. After 24 hr at room temp. solvents were removed *in vacuo* and the crude mixture purified by prep. TLC. The product (23 mg) was identical in all respects to **4** (mp, MS, IR, NMR).

Methylation of norpleiomutine **14**. Norpleiomutine (12 mg) was dissolved in a mixture of 1 ml 40% HCHO and 5 drops of HOAc. $NaBH_3CN$ (30 mg) was added slowly over 30 min. The reaction mixture was then poured into H_2O, neutralized with $KHCO_3$ and extracted with $CHCl_3$. After drying and evaporation, 9.5 mg of an oil was obtained which gave one spot on TLC and whose spectra mostly fit those described for pleiomutine [4] {$[\alpha]_D$ −95°, (*c* 0.5); lit. −111° (*c* 2.0)}.

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