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INDOLE ALKALOIDS FROM *HANNOA KLAINEANA* ROOTS

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Key Word Index—*Hannoa klaineana*; Simaroubaceae; roots; indole; oxindole; β-carboline; canthine; *N*-oxide alkaloids.

Abstract—1-Methoxycanthin-6-one, rhyncophylline, isorhyncophylline and four new alkaloids, ethyl-β-carboline-1-propionate, ethyl-β-carboline-2*N*-oxide-1-propionate, 1-ethyl-β-carboline and 1-ethyl-β-carboline-2*N*-oxide, were isolated from three different samples of *Hannoa klaineana* roots.

INTRODUCTION

Hannoa klaineana Pierre et Engl. is a tree endemic to different regions of tropical Africa. In Zaïre (Kisangani, Kinshasa and Boma), Guinea, Angola and Popular Republic of Congo, decoctions of the roots barks are used against fever and intestinal diseases [1, 2]. Quassinoids have been isolated from the seeds of *H. klaineana* [3] but the alkaloid content of this plant has not been investigated until now, apart from the isolation of hanin, which has been tentatively identified as 1-ethyl-β-carboline, obtained by synthesis [4].

The present paper deals with the structure elucidation (UV, IR, NMR, mass spectrometry and chemical methods) of several alkaloids detected in three different samples of the plant collected in Zaïre.

RESULTS AND DISCUSSION

A standard extraction procedure of the alkaloids present in the roots of *Hannoa klaineana* and their further purification by preparative TLC on silica gel led to the isolation of seven alkaloids (**1**–**7**).

1-Methoxycanthin-6-one (**1**), rhyncophylline (**2**) and isorhyncophylline (**3**) were identified by UV, IR, ^{1}H NMR and mass spectrometry, and by direct TLC comparison with authentic samples.

The UV spectrum of **6** was related to that of β-carboline derivatives; the ^{1}H NMR spectrum (one triplet at δ 1.4 and one quartet at δ 3.2 in addition to the β-carboline signals) and mass spectrum ($[M]^+$ *m/z* 196 and $[M - C_2H_5]^+$ *m/z* 167) strongly suggested the presence of an ethyl chain in position 1 of the β-carboline skeleton. The complete identification was achieved by comparison of the spectrometric data with those of 1-ethyl-β-carboline previously obtained by synthesis [4, 5].

The UV spectrum of **4** was also very similar to those of β-carboline alkaloids. The IR, ^{1}H NMR and mass spectral data suggested a substitution of carbon 1 by an ethyl propionate chain. Alkaline hydrolysis of **4** afforded β-carboline-1-propionic acid, which was unambiguously identified by ^{1}H NMR (two triplets at δ 3.0 and 3.4 in addition to the β-carboline signals) and by mass spectrometry ($[M]^+$ *m/z* 239, $[M - COO]^+$ *m/z* 196). One NMR triplet at δ 1.2 and one quartet at δ 4.1 confirmed the ethyl esterification of the substituted propionic acid.

After storage at room temperature and more specifically upon reduction by $FeSO_4$, alkaloids **5** and **7** afforded alkaloids **4** and **6**, respectively; the *N*-oxide structure of **5** and **7** was further confirmed by UV spectroscopy (bathochromic shifts of bands at 235 and 290 nm of the β-carboline nucleus to 257 and 320 nm, respectively mass spectrometry (16 amu in addition to the $[M]^+$ of **4** and **6**), IR spectroscopy (absorption at 1190 cm^{-1}) and ^{1}H NMR spectroscopy (0.2 ppm upfield shift of the H-3 proton).

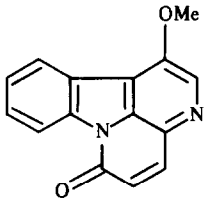
Root sample A contained 1-methoxycanthin-6-one (**1**) and traces of another alkaloid which was probably related to 1-ethyl-β-carboline (**6**). 1-Methoxycanthin-6-one (**1**), rhyncophylline (**2**) and isorhyncophylline (**3**) were identified in roots of sample B. Minor alkaloids were also detected. From root sample C, were isolated 1-methoxycanthin-6-one (**1**), ethyl-β-carboline-1-propionate (**4**), ethyl-β-carboline-2*N*-oxide-1-propionate (**5**), 1-ethyl-β-carboline (**6**) and 1-ethyl-β-carboline-2*N*-oxide (**7**). The differences of the alkaloid content between samples A, B and C were attributed to ecological factors and to the time of collection.

Ethyl-β-carboline-1-propionate (**4**), 1-ethyl-β-carboline (**6**) and the corresponding *N*-oxide structures have been isolated for the first time from plant material. Furthermore, ethyl-β-carboline-1-propionate (**4**) and β-carboline-1-propionic acid might be implicated in the biosynthesis of canthine alkaloids which were hypothetically derived from tryptamine and 3-formylpropionic acid [6]. Finally, this is the first report of oxindole alkaloids in the Simaroubaceae. Such alkaloids are generally found in the Rubiaceae and the occurrence of **2** and **3** in *H. klaineana* confirms the chemotaxonomic relationships between the two botanical families.

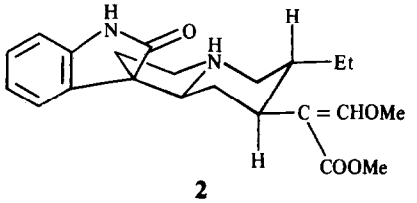
EXPERIMENTAL

UV spectra were determined in EtOH. IR spectra were measured in KBr discs. ^{1}H NMR spectra were recorded at 80 MHz in $CDCl_3$ or Me_2CO-d_6 using TMS as int. standard; chemical shift values are reported in δ (ppm) units. MS were obtained by direct inlet, 70 eV.

Plant material. Roots of sample A were collected at the INERA Station, Luki (Lukula, Zaïre) in April 1982 and identified by Prof. Penge O'nokoko (Kinshasa University). A voucher specimen has



1



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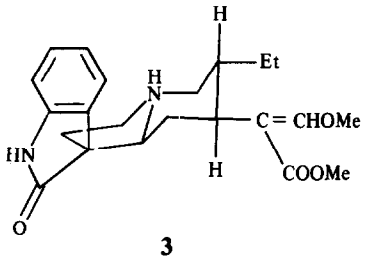

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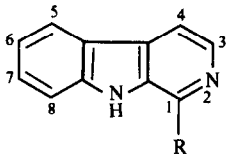
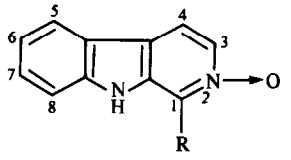


4 R = $CH_2CH_2COOCH_2CH_3$ (1′ 2′ 3′ 4′ 5′)
R = CH_2CH_3 (1′ 2′)



5 R = $CH_2CH_2COOCH_2CH_3$ (1′ 2′ 3′ 4′ 5′)
7 R = CH_2CH_3 (1′ 2′)

been deposited at the INERA Herbarium of Kinshasa. Roots of sample B had the same origin as A but were collected in August 1982. Roots of sample C were collected around Kisangani in September 1982 and identified by Dr. J. Lejoly (Botanical Institute, Free University of Brussels). A voucher specimen has been deposited at the Botanical Institute of the Free University of Brussels.

Extraction and separation. Air-dried roots were extracted with EtOH. The EtOH-soluble residue was suspended in 0.1 M HCl, extracted with $CHCl_3$ and re-extracted by the same solvent after being made alkaline (pH 9) by the addition of NH_4OH. Further purification of the $CHCl_3$ extracts was performed by prep. TLC on silica gel (solvent A: toluene–Me_2CO–EtOH–NH_4OH, 50:30:4:1.5). UV at 254 and 360 nm and Dragendorff–iodoplatinate reagents were used for alkaloid detection.

Ethyl-β-carboline-1-propionate (**4**). Whitish yellow needles from EtOH. R_f = 0.58 (solvent A). UV λ_{max}^{EtOH} nm: 235, 243, 252, 290, 350. IR ν_{max}^{KBr} cm^{-1}: 3320, 1720, 1620. ^{1}H NMR ($CDCl_3$): δ 1.2 (3H, *t*, *J* = 7.5 Hz, H-5′), 3.0 (2H, *t*, *J* = 6.5 Hz, H-1′), 3.4 (2H, *t*, *J* = 6.5 Hz, H-2′), 4.1 (2H, *q*, *J* = 7.5 Hz, H-4′), 7.3 (1H, *m*, H-8), 7.5 (2H, *m*, H-6 and H-7), 7.8 (1H, *d*, *J* = 5 Hz, H-4), 8.1 (1H, *d*, *J* = 8 Hz, H-5), 8.4 (1H, *d*, *J* = 5 Hz, H-3), 9.3 (1H, *s*, H–N<). MS *m/z* (rel. int.): 268 $[M]^+$ (39), 223 (22), 222 (40), 221 (24), 220 (29), 196 (22), 195 (100), 194 (54), 192 (21), 167 (9), 140 (14).

Ethyl-β-carboline-2N-oxide-1-propionate (**5**). Whitish yellow needles from EtOH. R_f = 0.29 (solvent A). UV λ_{max}^{EtOH} nm: 257, 320. IR ν_{max}^{KBr} 3320, 1720, 1620, 1190. ^{1}H NMR ($CDCl_3$): δ 1.2 (3H, *t*, *J* = 7.5 Hz, H-5′), 3.0 (2H, *t*, *J* = 6.5 Hz, H-1′), 3.4 (2H, *t*, *J* = 6.5 Hz, H-2′), 4.1 (2H, *q*, *J* = 7.5 Hz, H-4′), 7.3 (1H, *m*, H-8), 7.5 (2H, *m*, H-6 and H-7), 7.8 (1H, *d*, *J* = 6.5 Hz, H-4), 8.0 (1H, *d*, *J* = 8 Hz, H-5), 8.2 (1H, *d*, *J* = 6.5 Hz, H-3), 9.6 (1H, *s*, H–N<). MS *m/z* (rel. int.): 284 $[M]^+$ (21), 268 (29), 239 (10), 222 (25), 221 (15), 211 (19), 196 (23), 195 (100), 168 (11), 167 (9).

β-Carboline-1-propionic acid. Whitish yellow needles from MeOH–$CHCl_3$. R_f = 0.10 (solvent A). UV λ_{max}^{EtOH} nm: 234, 240, 250, 290, 350. IR ν_{max}^{KBr} cm^{-1}: 3300, 1660, 1620, 1520, 1550. ^{1}H NMR (Me_2CO-d_6): δ 3.0 (2H, *t*, *J* = 7.5 Hz, H-1′), 3.4 (2H, *t*, *J* = 7.5 Hz, H-2′), 7.3 (1H, *m*, H-8), 7.5 (2H, *m*, H-6 and H-7), 7.9 (1H, *d*, *J* = 5 Hz, H-4), 8.2 (1H, *d*, *J* = 8 Hz, H-5), 8.3 (1H, *d*, *J* = 5 Hz, H-3), 10.8 (1H, *s*, H–N<). MS *m/z* (rel. int.): 239 $[M]^+$ (26), 222 (8), 221 (9), 196 (18), 195 (100), 194 (12), 193 (19), 167 (8).

1-Ethyl-β-carboline (**6**). Whitish yellow needles from MeOH–$CHCl_3$. R_f = 0.52 (solvent A). UV λ_{max}^{EtOH} nm: 235, 242, 252, 290, 350. IR ν_{max}^{KBr} cm^{-1}: 3400, 3060, 1620, 1600, 1550, 1500. ^{1}H NMR ($CDCl_3$): δ 1.4 (3H, *t*, *J* = 7 Hz, H-2′), 3.2 (2H, *q*, *J* = 7 Hz, H-1′), 7.3 (1H, *m*, H-8), 7.5 (2H, *m*, H-6 and H-7), 7.8 (1H, *d*, *J* = 5 Hz, H-4), 8.1 (1H, *d*, *J* = 8 Hz, H-5), 8.4 (1H, *d*, *J* = 5 Hz, H-3), 9.4 (1H, *s*, H–N<). MS *m/z* (rel. int.): 196 $[M]^+$ (77), 195 (100), 180 (8), 168 (29).

1-Ethyl-β-carboline-2N-oxide (**7**). Whitish yellow needles from MeOH–$CHCl_3$. R_f = 0.31 (solvent A). UV λ_{max}^{EtOH} nm: 257, 320. IR ν_{max}^{KBr} cm^{-1}: 3400, 3060, 1620, 1550, 1500, 1190. ^{1}H NMR ($CDCl_3$): δ 1.4 (3H, *t*, *J* = 7 Hz, H-2′), 3.4 (2H, *q*, *J* = 7 Hz, H-1′), 7.3 (1H, *m*, H-8), 7.5 (2H, *m*, H-6 and H-7), 7.7 (1H, *d*, *J* = 6.5 Hz, H-4), 8.0 (1H, *d*, *J* = 7.5 Hz, H-5), 8.2 (1H, *d*, *J* = 6.5 Hz, H-3), 11.1 (1H, *s*, H–N<). MS: *m/z* (rel. int.): 212 $[M]^+$ (35), 195 (100), 196 (22), 167 (9).

Hydrolysis of **4**. A soln of **4** (30 mg) in MeOH (5 ml) was diluted with an ammoniacal buffer soln (pH 10) (5 ml) and refluxed for 3 hr. After evapn of MeOH, the aq. phase was extracted with $CHCl_3$.

Reduction of N-*oxide alkaloids* (**5** and **7**). A MeOH soln (2 ml)

of alkaloid (2 mg) was diluted with 2 ml 25% aq. $FeSO_4$ soln and refluxed for 1 hr. After evapn of MeOH, the aq. soln was made alkaline with NH_4OH and extracted with $CHCl_3$. Reaction products were identified by TLC and UV spectrometry.

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