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TWO QUASSINOIDS AND TWO COUMARINOLIGNOIDS FROM *HANNOA KLAINEANA* ROOTS

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Key Word Index *Hannoa klaineana*; Simaroubaceae; roots; quassinoids; coumarinolignoids; klaineanolide; cleomiscosin.

Abstract Two new quassinoids (klaineanolides A and B) were isolated from the root bark of *Hannoa klaineana* and their structures elucidated by X-ray diffraction and spectroscopic methods. Two coumarinolignoids (cleomiscosins A and B) were also identified.

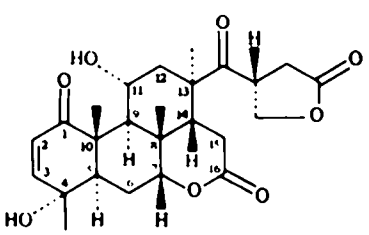
INTRODUCTION

Hannoa klaineana Pierre et Engler decoctions are used in African traditional medicine against fever and intestinal diseases [1, 2]. Previous studies on this plant have led to the isolation and the identification of alkaloids, quassinoids and coumarins [3–6]. As a continuation of our phytochemical investigations on *Hannoa klaineana* root bark, the structures of two new simarolidan quassinoids, klaineanolides A (**1**) and B (**2**), were elucidated and two coumarinolignoids, cleomiscosins A (**3**) and B (**4**), were identified. The present paper deals with the isolation, structural elucidation and identification of these compounds.

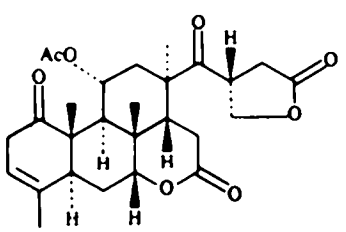
RESULTS AND DISCUSSION

An aqueous-methanolic extract of *Hannoa klaineana* root bark was fractionated by CC on silica gel. Further purifications of **1**–**4** were achieved either by crystallization, silica gel CC and/or silica gel preparative TLC. **3** and **4** were identified by UV, IR, ^{1}H NMR, MS and TLC comparisons with authentic samples [7–9].

1, $C_{25}H_{32}O_8$ (M^+ at m/z 460), showed a UV maximum at 220 nm which was related to an α,β-unsaturated ketone chromophore; its presence was confirmed in the IR spectrum by an absorption at 1660 cm^{-1}; other bands at 1765, 1720, 1690 and 1650 cm^{-1} were, respectively, indications of a γ-lactone group, a δ-lactone group, an aliphatic ketone function and an ethylene double bond. The ^{1}H NMR spectrum revealed the presence of four tertiary methyl groups and of two one-proton doublets (J = 6 Hz) in the ethylene range (δ 5.80 and 6.70 ppm) assignable to H-2 and H-3, thus confirming the attribution of the IR absorption band at 1650 cm^{-1}. Three tertiary methyl signals were attributed, respectively, as in simarolide [10–12], to Me-8, Me-10 and Me-13. Me-4 differed from that of simarolide by the presence of a hydroxy group instead of a proton; Me-4 appeared as a singlet (δ 1.52 ppm) as in guanepolide [12]. The EI mass spectrum showed a molecular ion at m/z 460; a fragment ion at m/z 442 ($M^+ - 18$) and the base peak at m/z 424, the latter being related to the loss of water molecules from two hydroxy functions in the molecular ion. In order to determine unequivocally the structure of **1** and its relative stereochemistry, it was submited to X-ray analysis, using crystals obtained from $CHCl_3$ MeOH. A stereoscopic view of the molecule is shown in Fig. 1. Interpretation of the ^{1}H NMR spectrum was achieved on the basis of X-ray diffraction results and is given in the Experimental. **2**, $C_{27}H_{34}O_8$ (M^+ at m/z 486), displayed UV absorption at 205 nm, indicating, in comparison with klaineanolide A (**1**), the lack of an α,β-unsaturated ketone function. Furthermore, unlike **1**, **2** did not present any significant IR absorption around 1650 cm^{-1} but only bands at



1



2

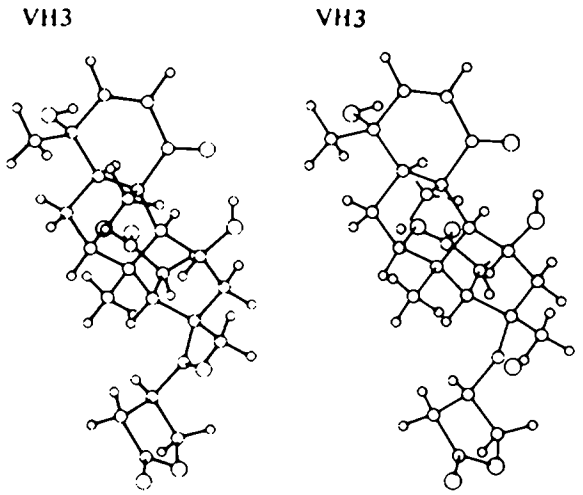


Fig. 1.

1770 cm^{-1} (γ-lactone group), 1740 cm^{-1} (δ-lactone and ester functions) and 1700 cm^{-1} (ketone functions at C-1 and C-17). Four tertiary methyl signals and one acetoxy singlet were detected in ^1H NMR spectroscopy; H-11 (multiplet at δ4.33 ppm in **1**) and H-3 appeared as a complex two-proton multiplet at δ5.30 ppm. High resolution mass spectroscopy showed a molecular ion at *m/z* 486 and a fragment ion at *m/z* 426 indicating the loss of an acetoxy group ($M^+ - 60$). Therefore, structure **2** was attributed to klaineanolide B.

EXPERIMENTAL

UV spectra were determined in EtOH. IR spectra were measured in KBr discs. ^1H NMR spectra were recorded at 250 MHz in pyridine-d_5 using TMS as internal standard; chemical shift values were reported in δ (ppm) units. MS were obtained by direct inlet 70 eV.

Plant material. Hannoa klaineana root samples were collected in P.R. of Congo (Fulakari Falls), in June 1985 and identified by Dr P. Sita, botanist at ORSTOM Laboratory of Brazzaville. A voucher specimen has been deposited at the National Botanical Garden of Belgium (Meise).

Extraction, separation and isolation. Air dried root bark powder (3 kg) was percolated through 35 l. of MeOH–H_2O (1:1). The extract, evaporated to dryness (190 g) and absorbed on cellulose powder (800 g), was chromatographed on a silica gel (1 kg) column, eluted by $CHCl_3$, with increasing amounts of MeOH (0–50%), affording 23 fractions. Fractionation was made from the results of TLC screening on silica gel, using as mobile phases $CHCl_3$ with variable amounts of a MeOH Me_2CO (1:1) mixture. Fraction VII, also containing undulatone, eluted from the column by $CHCl_3$-MeOH (95:5) and evaporated to dryness, left a residue partially soluble in $CHCl_3$.

The insoluble fraction, purified by successive redissolution in $CHCl_3$ MeOH (1:1), afforded **3** whereas **4**, soluble in $CHCl_3$, was purified by preparative TLC on silica gel, developed with $CHCl_3$-MeOH Me_2CO (88:6:6). After elimination of **3**, fraction VII (2.7 g) was absorbed on cellulose (25 g) and chromatographed on a silica gel (75 g) column, eluted by $CHCl_3$ with increasing amounts of a MeOH Me_2CO (1:1) mixture (0–10%). A fraction, eluted with $CHCl_3$ MeOH-Me_2CO (95:2.5:2.5), afforded a residue which was further purified by preparative TLC on silica gel (mobile phase: $CHCl_3$ · MeOH-Me_2CO, 88:6:6), in order to isolate **2**, characterized by its insolubility in most organic solvents. **1** was obtained from the initial silica gel column eluted by mixtures of $CHCl_3$ and MeOH; it was eluted together with 15-desacetylundulatone, by $CHCl_3$ MeOH (92:8). These fractions were purified, at first, by silica gel column chromatography eluted with $CHCl_3$ with increasing amounts of MeOH-Me_2CO (1:1) (0–50%); **1** was eluted with $CHCl_3$-MeOH-Me_2CO (92:4:4) and was also characterized by its great insolubility.

Klaineanolide A (**1**). Parallelipiped crystals from $CHCl_3$–MeOH very slightly soluble in most organic solvents; very bitter taste. R_f 0.3 on silica gel with as mobile phase $CHCl_3$-MeOH-Me_2CO (88:6:6). Spray reagent: 1% H_2SO_4 in EtOH and heating at 110° for 5 min affording yellowish fluorescent spots at 350 nm. UV λ_{max}^{EtOH} nm: 220. ^1H NMR (pyridine-d_5) δ ppm: 1.00 (3H, *s*, Me-8); 1.10 (3H, *s*, Me-13); 1.50 (3H, *s*, Me-10); 1.52 (3H, *s*, Me-4); 2.40 (1H, *d*, J = 12.5 Hz, H-9); 4.33 (1H, *m*, H-11); 5.80 (1H, *d*, J = 6 Hz, H-3); 6.70 (1H, J = 6 Hz, H-2). MS *m/z* (relative intensity): 460 $[M]^+$ (0.5), 442 (13.5), 424 (100), 409 (13.5), 311 (80), 270 (70), 237 (60). The crystal data of klaineanolide A (**1**) are as follows: $C_{25}H_{32}O_8$, monoclinic, space group $P2_1$ with a = 7.656 (3), b = 12.469 (4), c = 11.938 (12) Å, β = 94.98 (5)°, V = 1135 (1) Å^3. Two molecules per unit cell (Z = 2) give Dx = 1.35 gcm^{-3}. The intensity data were collected on a Huber 424-511 diffractometer using graphite monochromatized MoKα radiation (λ = 0.71069 Å). 3477 reflections were measured of which 3229 with $I \geq 2.5\,\sigma(I)$ were used in the structural determination. A 16-atom fragment obtained by MULTAN80 [13] was expanded to the complete structure by SHELX 84 [14]. Refinement was carried out by SHELX 76 [15]. The R final value is 0.042. The list of atomic coordinates and molecular dimensions has been deposited at the Cambridge Crystallographic Data Centre.

Klaineanolide B (**2**). Needles from $CHCl_3$ MeOH Me_2CO, very slightly soluble in most organic solvents; very bitter taste. R_f 0.7 on silica gel with $CHCl_3$ MeOH-Me_2CO (88:6:6) as mobile phase. Spray reagent see **1**, affording yellowish fluorescent spot at 350 nm. UV λ_{max}^{EtOH} nm: 205. ^1H NMR (pyridine-d_5) δ ppm: 0.95 (6H, *s*, Me-8 and Me-13); 1.20 (3H, *s*, Me-10); 1.50 (3H, *s*, Me-4); 1.94 (3H, *s*, AcO-11); 3.40 (1H, *d*, J = 18 Hz, H-5); 5.30 (2H, *m*, H-3 and H-11). MS *m/z* (relative intensity): 486 $[M]^+$ (60), 426 (95), 398 (80), 383 (70), 363 (25), 313 (35), 295 (35), 285 (55), 267 (40), 253 (35), 225 (100).

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NEOLIGNANS FROM THE FRUITS OF *LICARIA ARMENIACA**

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Key Word Index *Licaria armeniaca*; Lauraceae; fruits; benzofuranoid neolignans; bicyclo[3.2.1]octanoid neolignans.

Abstract—Fruits of *Licaria armeniaca* contain, besides eight known lignoids, three novel neolignans: (1*S*,5*R*,6*S*,7*R*,8*R*)-8-acetoxy-1-allyl-3,5-dimethoxy-7-methyl-6-(3′-methoxy-4′,5′-methylenedioxyphenyl)-4-oxobicyclo[3.2.1]oct-2-ene; (1*S*,5*R*,6*S*,7*R*)-1-allyl-3-methoxy-7-methyl-6-(3′-methoxy-4′,5′-methylenedioxyphenyl)-4,8-dioxobicyclo[3.2.1]oct-2-ene and (1*S*,5*R*,6*S*,7*R*)-1-allyl-3-methoxy-7-methyl-6-(3′,4′,5′-trimethoxyphenyl)-4,8-dioxobicyclo[3.2.1]oct-2-ene.

INTRODUCTION

Previous work on the trunk wood of *Licaria armeniaca* (Ness) Kosterm. led to the isolation of 6,7-dimethoxycoumarin [2, 3], the oxoaporphine alkaloid tri-*O*-methylmoscatoline [3], the furofuran lignan magnolin **1a** [3], the hexahydrobenzofuran neolignans armenin-A and -B [2] as well as the bicyclo[3.2.1]octanoid neolignan **2a** [3]. Work on the fruits and fruit calyces of the same species reported in the present paper yielded, besides **1a** and **2a**, and additional furofuran lignan **1b**, the additional bicyclooctanoid neolignans **2b**, **3a** and **3b**, and the tetrahydrobenzofuranoid neolignans **4a**, **4b**, **5a**, **5b** and **6**. The biogenetic nomenclature and numbering of neolignans follow the rules outlined in a review [4].

RESULTS AND DISCUSSION

Compounds **1a** and **1b** are known constituents of a *Magnolia* [5] and a *Piper* [6] species, respectively. Compound **2a** [3, 7] served as a model in the structural elucidation of the novel compound **2b**. The spectral differences can all be attributed to the presence of a 3,4-methylenedioxyphenyl group in **2a** versus a 3-methoxy-4,5-methylenedioxyphenyl group in **2b**. The chiroptical data of compounds **2a** and **2b** are also closely comparable. Compound **3a** had been obtained previously by partial synthesis [8]. It served as a model for the structural elucidation of the new compound **3b**. Again the spectral differences could all be attributed to diverse substitution of the aromatic parts of the molecules, a 3-methoxy-4,5-methylenedioxyphenyl group in **3a** and a 3,4,5-trimethoxyphenyl group in **3b**. And again both isolates **3a** [8] and **3b** gave nearly superimposable ORD and CD curves. All other compounds have been isolated previously from other sources: **4a** from *Licaria camara* [9], *Aniba terminalis* [10] and another *Aniba* species [11]; **4b** from *A. williamsii* [8, 12, 13]; **5a** from *A. terminalis* [10] and another *Aniba* species [11]; **5b** from *A. williamsii* [8, 12, 13] and **6** from *A. williamsii* [12]. Stereochemical assignments of the neolignans were based on published data [14–16].

Most of these neolignans have been isolated previously from *A. williamsii*. This species was designated *A. simulans* in all the original papers. The revision of the name has been reported [17].

* Part 82 in the series "The Chemistry of Brazilian Lauraceae". For Part 81 see ref. [1]. Taken from part of the doctorate thesis presented by J.M.B.-F. to the Universidade de São Paulo (1986).

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