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TWO QUASSINOID GLYCOSIDES AND A β-CARBOLINE-1-PROPIONIC ACID FROM *HANNOA KLAINEANA*

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Key Word Index—*Hannoa klaineana*; Simaroubaceae; roots; quassinoids; 15-*O*-β-D-glucopyranosyl-21-hydroxyglaucarubolone; 15-*O*-α-D-xylofuranosyl(1 → 6)-β-D-glucopyranosyl-21-hydroxyglaucarubolone; alkaloid; β-carboline-1-propionic acid; coumarin; scopolin.

Abstract—Among the polar constituents of *Hannoa klaineana* roots, two new quassinoid glycosides, 15-*O*-β-D-glucopyranosyl-21-hydroxyglaucarubolone, 15-*O*-α-D-xylofuranosyl(1 → 6)-β-D-glucopyranosyl-21-hydroxy-glaucarubolone, an alkaloid, β-carboline-1-propionic acid and a coumarin glycoside, scopolin were isolated and their structures elucidated.

INTRODUCTION

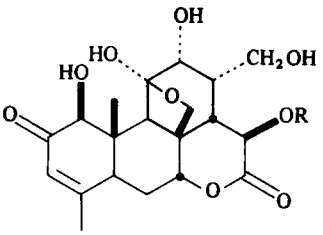
Previous studies on root samples of *Hannoa klaineana*, a tree endemic to different regions of tropical Africa, have led to the isolation and identification of two quasinoids and eight alkaloids [1, 2]. In this paper, isolation from samples of the same plant material and structural elucidation by physical and chemical methods of two new quassinoid glycosides (**1** and **4**), an alkaloid (**5**) and a coumarin glycoside (**7**) are reported.

RESULTS AND DISCUSSION

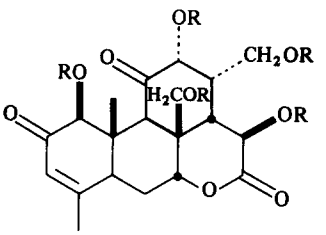
A methanolic extract of *H. klaineana* roots was fractionated by CC, firstly on silica gel then on C_8 silica gel. Further purification was achieved by prep. TLC on silica gel and led to the isolation of compounds **1**, **4**, **5** and **7**. The R_f values of these constituents and their solubilities in different solvents indicated that they were highly polar.

The colourless amorphous compound **1** was submitted to acid hydrolysis. After neutralization, one portion of the solution was demineralized by ion exchange chromatography and evaporated; TLC on silica gel and GC (TMSi derivatives) of the constituents led to the identification of glucose. The second part was purified by prep. TLC and led to **2** which exhibited a $[M]^+$ ion at *m/z* 411 $[M+H]^+$ [fast atom bombardment (FAB) mass spectrometry]. Compound **2** absorbed UV radiation at 240 nm suggesting for quassinoids the presence of an α,β-unsaturated ketone moiety. Its IR spectrum exhibited absorptions at 1720 and 1660 cm^{-1}, indicative of δ-lactone and α,β-unsaturated functions; these data confirmed the interpretation of the UV spectrum. On acetylation, **2** afforded a pentaacetate derivative (**3**) [$[M]^+$ *m/z* 620, electron impact (EI) mass spectrometry] whose structure was found to be very similar to that of glaucarubolone tetraacetate [3] except for the lack of the secondary methyl group (C-21). ^{1}H NMR of **3** showed, instead of a doublet at δ0.88, a two-proton multiplet at δ4.10 which was attributed to H-21, suggesting the presence of a primary hydroxyl function linked to C-13. ^{1}H NMR double resonance experiments unambiguously confirmed the structure attributed to **3**. These data, as well as the $[M]^+$ ion $[M+H]^+$ at *m/z* 573 (FAB mass spectrometry) confirmed the monoglucosidic nature of **1**. In order to elucidate the position of the linkage of the glucose moiety to the quassinoid, **1** was peracetylated to give an octaacetate. From comparison of the ^{1}H NMR spectra, it appeared that the H-15 signal (*d*, *J* = 10 Hz) at δ6.22 of **3** was shifted upfield in the octaacetate derivative of **1** confirming the quassinoid–glucose linkage at position C-15. The presence in the ^{1}H NMR spectrum of **1** of a one-proton doublet at δ5.41 (*J* = 7.5 Hz) supported the β-anomeric configuration of the glucose moiety. The structure was finally confirmed by comparison of the ^{13}C NMR spectrum of **1** with that of 15-*O*-β-D-glucopyranosylglaucarubolone [4] which differed structurally only in the possession of a secondary methyl group at C-21 instead of a primary hydroxyl function [5, 6].

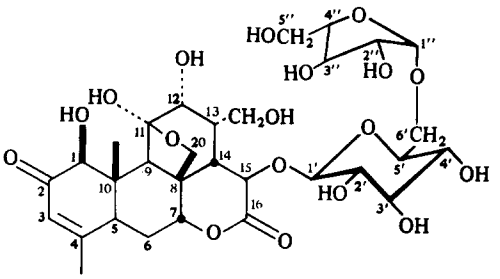
Compound **4** was hydrolysed using the same conditions as for **1**; subsequent treatments of the solubion allowed the identification of glucose, xylose and a quassinoid identical to **2** as confirmed by TLC, UV, IR, mass and ^{1}H NMR spectral data recorded on **2** and its acetylated derivative (**3**). These data and the presence of a $[M]^+$ ion at *m/z* 705 $[M+H]^+$ (FAB mass spectrometry) proved the diglycosidic nature of **4**. Moreover, this spectrum showed the presence of peaks at *m/z* 573 $[MH-132]^+$ and 411 $[MH-132-162]^+$ suggesting that the xylosyl moiety was terminal in the glycosidic chain. In order to elucidate the position of the linkage of the biose moiety to the quassinoid, **4** was peracetylated to give a decaacetate compound. In the ^{1}H NMR spectrum of this derivative, H-15 was shifted upfield in comparison with its position in **3**; this observation suggested that the aglycone–biose linkage was at position C-15. ^{1}H NMR of **4** displayed the presence of two anomeric proton doublets at δ5.40 (*J* = 7.5 Hz) and 5.94 (*J* = 3 Hz) attributed, respectively, to H-1′ (β-D-glucosyl) to H-1″ (α-D-xylosyl). ^{13}C NMR spectral data of **4** were similar to those of **1** and it was clear that it differed from **1** by the presence of an additional five carbon atoms and a downfield shift of the signal at δ63.00



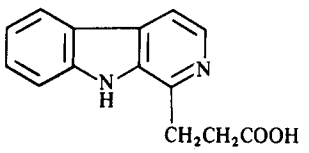
1 R = Glc
2 R = H



3 R = Ac



4



5

to 75.05. These last data indicated that C-6′ was implicated in the glucosyl–xylosyl linkage. The xylosyl moiety ^{13}C NMR spectral data of **4** were related to those of α-D-xylofuranoside [7] except for the chemical shift of C-1″ (δ110.94) and of C-5″ (δ64.92) which were probably shifted downfield because hydrogen bonds could appear between aglycone and xylosyl moieties according to a Dreiding model of **4**. Therefore, compound **4** was identified as 15-*O*-α-D-xylofuranosyl(1 → 6)-β-D-glucopyranosyl-21-hydroxyglaucarubolone.

The UV spectrum of **5** was related to that of β-carboline alkaloids [8]. The ^1H NMR (two triplets at δ2.70 and 3.40 in addition to the β-carboline signals) and EI mass spectra ($[M]^+$ at *m/z* 240) suggested the presence of a propionic acid substituent in position 1 of the β-carboline skeleton. Compound **5** was finally identified by direct comparison with an authentic sample of β-carboline-1-propionic acid (TLC and spectrometric data) isolated from *Ailanthus altissima* [9].

Compound **7** was submitted to acid hydrolysis; the solution was neutralized and then extracted with chloroform. The aqueous phase was demineralized by ion exchange chromatography; GC of the constituents (TMSi derivatives and TLC led to the identification of glucose. Preparative TLC of the chloroform residue afforded **6** which was identified as scopoletin (TLC and UV, IR, mass and ^1H NMR spectral data). These data and the presence of a $[M]^+$ ion at *m/z* 353 $[M-H]^-$ in the negative ion FAB mass spectrum confirmed the monoglucosidic nature of **7** which was, therefore, identified as scopolin [10, 11].

EXPERIMENTAL

UV spectra were determined in EtOH or MeOH. IR spectra were measured using KBr discs. ^1H and ^{13}C NMR spectra were recorded, respectively, at 250 and 62.89 MHz in pyridine-d_5, $CDCl_3$ or MeO^2H using TMS as int. standard; chemical shift values are reported in δ (ppm) units. EIMS were obtained by direct inlet at 70 eV.

Plant material. Quassinoid and scopolin isolation. Roots of *H. klaineana* Pierre et Engl. were collected in Zaïre (Lukula-Luki-Bas Zaïre) in November 1982 and identified by Dr. Breyne of the Kinshasa Herbarium. A voucher specimen has been deposited at the INERA Herbarium of Kinshasa (sample 1). *Alkaloid isolation.* Roots of *H. klaineana* were collected in the Popular Republic of Congo (Fulakari falls) in April 1983 and identified by Dr. P. Sita, a botanist at ORSTOM (Office des Recherches Scientifiques et Techniques Outre-Mer), Laboratory of Brazzaville. A voucher specimen has been deposited at the

National Botanical Garden of Belgium (Meise) (sample 2).

Extraction and separation. Air-dried roots (500 g) were first defatted in petrol and then extracted with MeOH–H_2O (1:1). After removal of MeOH, the aq. phase was concd (50 ml) and washed with 3 × 50 ml of $CHCl_3$. The $CHCl_3$ soln was discarded. CC of the aq. residue (28 g) on silica gel eluted with $CHCl_3$–MeOH–H_2O (70:30:3) afforded seven fractions. Reversed-phase CC on Lichroprep® RP-8 column (Merck) of fractions 3–7 followed by prep. TLC led to the isolation of **1** (120 mg), **4** (70 mg) and **7** (5 mg) from sample 1, and an additional constituent, **5** (65 mg) from sample 2.

15-O-β-D-*Glucopyranosyl*-21-*hydroxyglaucarubolone* (**1**). Colourless amorphous compound. R_f 0.31 on silica gel; solvent A: $CHCl_3$–MeOH–H_2O (70:30:3). UV λ_{max}^{MeOH} nm: 240. IR ν_{max}^{KBr} cm^{-1}: 3400, 1720, 1660. ^{1}H NMR (pyridine-d_5): δ1.54 (3H, *s*, Me-10), 1.77 (3H, *s*, Me-4), 2.04–2.16 (2H, *m*, H-6a, H-6e), 2.97 (2H, *m*, H-13, H-14), 3.16 (1H, *d*, *J* = 11.5 Hz, H-5), 3.28 (1H, *s*, H-9), 3.80–4.49 (11H, complex *m*, H-12, H-20, H-21, H-6′a, H-6′e, H-5′, H-4′, H-3′, H-2′), 4.63 (1H, *m*, H-7), 4.90 (1H, *s*, H-1), 5.41 (1H, *d*, *J* = 7.5 Hz, H-1′), 5.67 (1H, *d*, *J* = 9.5 Hz, H-15), 6.09 (1H, *s*, H-3). ^{13}C NMR (pyridine-d_5) (broad band decoupled): δ10.56 (Me-10), 22.33 (Me-4), 26.54 (C-6), 41.22 (C-13), 42.32 (C-9), 45.49 (C-10), 46.20 (C-5), 46.79 (C-8), 47.95 (C-14), 61.59 (C-21), 63.00 (C-6′), 71.16 (C-20), 71.84 (C-4′), 75.63 (C-7), 76.93 (C-2′), 77.21 (C-5′), 78.38 (C-12, C-3′), 79.22 (C-15), 84.40 (C-1), 104.15 (C-1′), 110.49 (C-11), 126.07 (C-3), 162.50 (C-4), 170.82 (C-16), 197.23 (C-2). FABMS *m/z* (rel. int.): 573 $[M+H]^+$ (4), 545 (42), 411 (73), 393 (40), 315 (73), 163 (5.5), 115 (100), 55 (91).

21-*Hydroxyglaucarubolone* (**2**). Colourless amorphous compound. R_f 0.55 on silica gel; solvent A. UV λ_{max}^{EtOH} nm: 240. IR ν_{max}^{KBr} cm^{-1}: 3400, 1720, 1660. ^{1}H NMR (pyridine-d_5): δ1.30 (3H, *s*, Me-10), 1.52 (3H, *s*, Me-4), 1.70 (2H, *m*, H-6a, H-6e), 2.40 (1H, *dd*, *J* = 5, 12.5 Hz, H-14), 2.74 (1H, *m*, H-13), 2.91 (1H, *d*, *J* = 12.5 Hz, H-5), 3.20 (1H, *s*, H-9). 3.62, 3.90 (1H, *d*, *J* = 7.5 Hz, H-20), 3.84 (1H, *s*, H-7), 4.10 (1H, *s*, H-1), 4.30 (2H, *m*, H-21), 4.46 (1H, *s*, H-12), 5.32 (1H, *d*, *J* = 11 Hz, H-15), 5.86 (1H, *s*, H-3). FABMS *m/z* (rel. int.): 411 $[M+H]^+$ (54), 393 (20.5), 375 (14), 357 (10.5), 301 (40), 283 (50.5), 207 (9.5), 131 (10.5), 115 (100).

Pentaacetate compound (**3**). Colourless amorphous compound. R_f 0.50 on silica gel; solvent B: $CHCl_3$–MeOH (19:1). UV λ_{max}^{EtOH} nm: 240. IR ν_{max}^{KBr} cm^{-1}: 2900, 1750, 1720, 1670, 1620. ^{1}H NMR ($CDCl_3$): δ1.44 (3H, *s*, Me-10), 1.98 (3H, *s*, Me-4), 2.10–2.35 (15H, *m*, OAc-5), 2.60 (1H, *m*, H-13), 2.94 (1H, *dd*, *J* = 5, 10 Hz, H-14), 3.14 (1H, *d*, *J* = 12.5 Hz, H-5), 3.56 (1H, *s*, H-9), 3.90, 4.66 (1H, *d*, *J* = 12.5 Hz, H-20), 4.10 (2H, *m*, H-21), 4.76 (1H, *s*, H-7), 5.19 (1H, *s*, H-12), 5.20 (1H, *s*, H-1), 6.06 (1H, *s*, H-3), 6.22 (1H, *d*, *J* = 10 Hz, H-15). EIMS *m/z* (rel. int.): 620 $[M]^+$ (7), 619 (18.0), 578 (24), 577 (79), 560 (10), 500 (11), 479 (7), 134 (52), 94 (100).

15-O-α-D-*Xylofuranosyl*(1 → 6)-β-D-*glucopyranosyl*-21-*hydroxyglaucarubolone* (**4**). Colourless amorphous compound. R_f 0.28 on silica gel; solvent A. UV λ_{max}^{MeOH} nm: 240. IR ν_{max}^{KBr} cm^{-1}: 3400, 1720, 1660. ^{1}H NMR (pyridine-d_5): δ1.53 (3H, *s*, Me-10), 1.76 (3H, *s*, Me-4), 2.03–2.18 (2H, *m*, H-6a, H-6e), 2.96 (1H, *m*, H-13), 3.15 (1H, *d*, *J* = 11.5 Hz, H-5), 3.81–4.50 (11H, *m*, H-6′a, H-6′e, H-5′, H-4′, H-3′, H-2′, H-5″a, H-5″e, H-4″, H-3″, H-2″), 4.70 (1H, *s*, H-7), 4.72 (1H, *m*, H-12), 4.84 (1H, *s*, H-1), 5.40 (1H, *d*, *J* = 7.5 Hz, H-1′), 5.62 (1H, *d*, *J* = 9.5 Hz, H-15), 5.93 (1H, *d*, *J* = 3 Hz, H-1″), 6.09 (1H, *s*, H-3). ^{13}C NMR (pyridine-d_5) (broad band decoupled): δ10.60 (Me-10), 22.34 (Me-4), 26.61 (C-6), 41.21 (C-13), 42.37 (C-9), 45.54 (C-10), 46.28 (C-5), 46.92 (C-8), 47.47 (C-14), 61.68 (C-21), 64.92 (C-5″), 71.17 (C-20), 71.84 (C-4), 75.05 (C-6′), 75.42 (C-7), 76.54 (C-3″), 76.98 (C-2′), 77.03 (C-2″), 77.22 (C-5′), 77.48 (C-3′), 78.46 (C-4″), 79.24 (C-12, C-3′), 80.01 (C-15), 84.46 (C-1), 103.91 (C-1′), 110.57 (C-11), 110.94 (C-1″), 126.13 (C-3), 162.47 (C-4), 170.62 (C-16), 197.29 (C-2). FABMS *m/z* (rel. int.): 705 $[M+H]^+$ (4.0), 573 (1.85), 411 (85), 395 (40), 393 (33.5), 377 (20), 315 (83.5), 299 (50), 286 (55), 267 (18.5), 223 (13), 207 (18.5), 167 (4), 131 (13), 115 (68.5), 29 (100).

Octaacetate derivative of **1**. Colourless amorphous compound. R_f 0.45 on silica gel; solvent B. UV λ_{max}^{EtOH} nm: 240. IR ν_{max}^{KBr} cm^{-1}: 2900, 1750, 1720, 1680, 1620. ^{1}H NMR ($CDCl_3$): δ1.40 (3H, *s*, Me-10), 1.98 (3H, *s*, Me-4), 2.0–2.15 (24H, *m*, OAc-8), 2.65 (1H, *m*, H-13), 3.25 (2H, *m*, H-5, H-9), 3.68 (1H, *m*, H-14), 3.88–5.20 (15H, complex *m*, H-1, H-7, H-12, H-15, H-20, H-21 and glucosyl protons), 6.08 (1H, *s*, H-3).

Decaacetate compound of **4**. Colourless amorphous compound. R_f 0.40 on silica gel; solvent B. UV λ_{max}^{EtOH} nm: 240. IR ν_{max}^{KBr} cm^{-1}: 2900, 1750, 1720, 1680, 1620. ^{1}H NMR ($CDCl_3$): δ1.40 (3H, *s*, Me-10), 1.98 (3H, *s*, Me-4), 2.0–2.15 (30H, *m*, OAc-10), 2.65 (1H, *m*, H-13), 3.25 (2H, *m*, H-9), 3.65–5.20 (21H, complex *m*, H-1, H-7, H-12, H-15, H-20, H-21 and xylosyl–glucosyl protons), 6.08 (1H, *s*, H-3).

Acid hydrolysis of **1** *and* **4**. A soln of **1** or **4** (30 mg) in 0.5 M H_2SO_4–MeOH (1:1) was refluxed for 2 hr and then neutralized with 1 M NaOH. The mixture was divided into two parts. One was concd and purified by prep. TLC (solvent A) to give **2** (15 mg); the second was passed through an ion exchange resin ANGMI-615 (J. T. Baker) and sugars were identified by comparison with authentic samples of glucose and xylose (TLC and GC of their TMSi derivatives).

Hydrolysis of **7**. A soln of **7** (3 mg) was refluxed in 0.5 M H_2SO_4–MeOH (1:1) soln for 2 hr and then neutralized with 1 M NaOH. After extraction of the aq. phase with 3 × 5 ml $CHCl_3$ and subsequent evaporation, the residue was chromatographed by prep. TLC to give 1.7 mg **2**. The aq. soln was demineralized by ion exchange resin ANGMI-615 (J. T. Baker) to give glucose which was identified by TLC and GC of its TMSi derivative.

Acetylation of **2**. Ac_2O (1 ml) was added to a soln of **2** (20 mg) in pyridine (1 ml) and left at room temp. for 24 hr. After decomposition of excess Ac_2O by the addition of H_2O followed by evaporation of the soln, the residue was purified by prep. TLC (solvent B) to give **3** (15 mg).

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