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Non-quassinoid constituents from the twigs and thorns of *Castela polyandra*

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Abstract

The structures of two new constituents, a β-glycoside (**1**) and a steroid (**2**), isolated from the twigs and thorns of *Castela polyandra*, were established by a combination of spectroscopic and single-crystal X-ray analysis.

Keywords: *Castela polyandra*; Simaroubaceae; twigs; thorns; quassinoids; steroids; β-glycosides; spectroscopy; X-ray crystallography

1. Introduction

Castela polyandra is one of many plant species belonging to the botanical family Simaroubaceae which has played a prominent role in folk medicine (Simao Barreiro, Das, DaSilva & Gottliebet, 1991; Grieve, 1971; Polonsky, 1968]. We have studied extensively the root bark (Grieco, VanderRoest & Piñeiro-Núñez, 1995) and twigs (thorns) (Grieco, Haddad, Piñeiro-Núñez & Huffman, 1998) of *C. polyandra*. Over a dozen quassinoids have been isolated, identified and characterized. In a continuation of our work with this species, two new non-quassinoid constituents, a β-glycoside (**1**) and a steroid (**2**), have been isolated from the twigs and thorns. Their structures were determined by a combination of spectral analysis and single-crystal X-ray diffraction.

2. Results and discussion

The methanol extracts of the twigs and thorns of *Castela polyandra* afforded two new natural products, a β-glycoside (**1**) and a steroid (**2**). In addition, 15-*O*-acetyl-$\Delta^{4,5}$-glaucarubol (Grieco, Haddad, Piñeiro-Núñez & Huffman, 1998), glaucarubol (Khan & Shamsuddin, 1980) and 1-*epi*-glaucarubolone (Grieco, Haddad, Piñeiro-Núñez & Huffman, 1998) were isolated and identified by comparison of their respective ^{1}H and ^{13}C NMR spectra with those obtained from the authentic natural materials.

The structure of **1** was elucidated by a combination of ^{1}H and ^{13}C NMR spectroscopy, mass spectrometry, IR spectroscopy and X-ray crystallography. The mass spectrum and elemental analysis of **1** indicated a molecular formula of $C_{19}H_{28}O_8$. The requisite number of carbon atoms was present in the ^{13}C NMR spectrum. Compound **1** proved to be highly crystalline and the structure was definitively established by single-crystal X-ray analysis (Complete crystallographic data etc.). Compound **1**, mp 248–250°C, crystallized in space group $P2_12_12_1$ with unit cell dimensions of a = 6.718(2) Å, b = 12.641(4) Å, c = 22.101(7) Å and $Z = 4$. The volume of the crystal was 1876.83 Å^3 with a density of 1.361 gcm^{-3}. The absolute configuration of **1** was not determined. An ORTEP view of **1** is shown in Fig. 1.

The structure of **2** was established by a combination of IR, ^{1}H and ^{13}C NMR, mass spectrometry and X-

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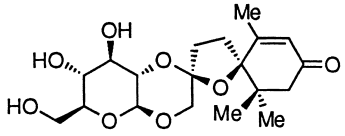

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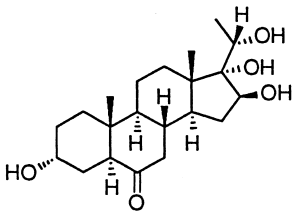

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ray crystallography. The mass spectrum, as well as the elemental analysis, indicated a molecular formula of $C_{21}H_{34}O_5$. This information, coupled with the ^1H and ^{13}C NMR data suggested that **2** was a steroid and not a C_{19} or C_{20} quassinoid. Indeed, comparison of the spectral data of **2** with those of a related steroid (Grieco, Moher, Seya, Huffman & Grieco, 1994) revealed that both the ^1H and ^{13}C NMR spectra were strikingly similar. The structure of **2**, mp 292–293°C, was unambiguously established by X-ray diffraction (Complete crystallographic data etc.). Compound **2** crystallized in space group $P2_1$ with unit cell dimensions of a = 5.980(1) Å, b = 24.252(6) Å, c = 6.428(1) Å, beta = 105.48(1) ° and $Z = 2$. The volume of the crystal was 898.35 $Å^3$ and the density was 1.355 gcm^{-3}. An ORTEP view of **2** is shown in Fig. 2.

3. Experimental

3.1.1. General

Proton (^1H) and carbon (^{13}C) nuclear magnetic resonance spectra were recorded on Bruker AM–500 and Varian VXR–400 spectrometers. Chemical shifts are reported in parts per million (δ) relative to tetramethylsilane (δ 0.00). Elemental analyses were performed by Robertson Microlit Laboratories, Inc., Madison, NJ. Infrared (IR) spectra were recorded on a Mattson Galaxy 4020 series FTIR spectrometer. Absorption intensities are indicated as strong (s), medium (m), or weak (w). High resolution mass spectra (HRMS) were obtained on a Kratos MS 80/RFAQ spectrometer. Melting points were obtained under argon in sealed tubes utilizing a Thomas Hoover capillary melting point apparatus, and are uncorrected. Optical rotations were obtained on a Perkin-Elmer model 241 polarimeter. Thin layer chromatography (TLC) was performed using E. Merck precoated silica gel 60 F-254 (0.25 mm thickness plates). The plates were visualized by immersion in *p*-anisaldehyde solution and warming on a hot plate. E. Merck silica gel 60 (230–400 mesh) was used for flash silica gel chromatography. All chromatography solvents were reagent grade. Fraction collecting commenced after elution of the void volume of the column.

3.1.2. Plant material.

The twigs and thorns of *Castela polyandra* were procured from Baja California in April, 1993. The voucher specimens were identified by Drs. Richard Spjut and Richard Marin of World Botanical Associates, and were deposited at Universidad de Autonoma de Baja California, Ensenada, Mexico (BCMEX) and World Botanical Associates, Fallbrook, California.

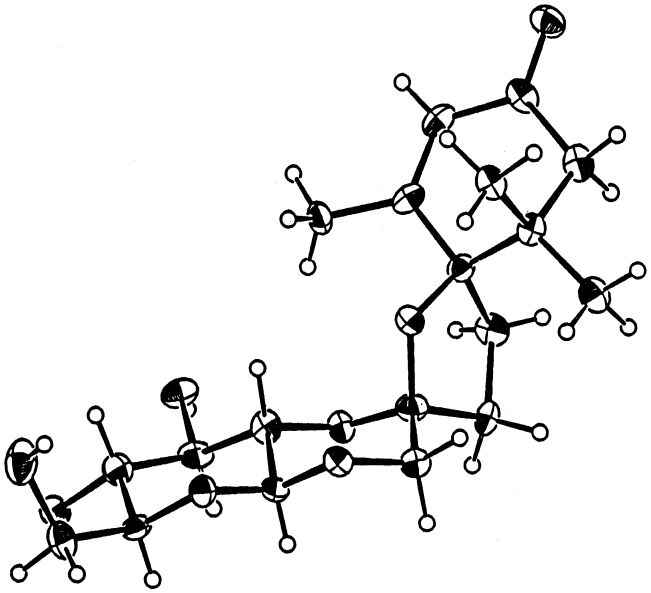

Fig. 1. An ORTEP view of **1**.

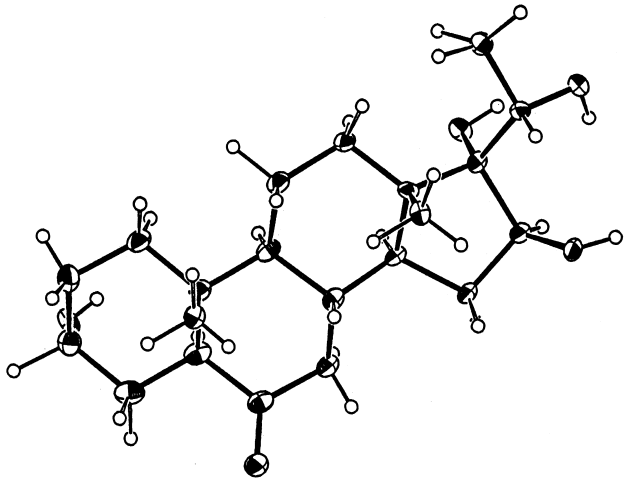

Fig. 2. An ORTEP view of **2**.

3.1.3. Extraction and isolation

Dried, ground twigs (1229 g) were soaked in MeOH (2.5 L). After 3 days, the plant material was drained and rinsed with MeOH (3 × 1.0 L). This process was repeated a total of 8 times. The combined MeOH extracts and washings were concentrated in vacuo to afford a greenish brown residue, which was diluted with 20% MeOH–CH_2Cl_2 (1.5 L), stirred for 24 h, and filtered through a pad of silica gel (500 g), washing well with 20% MeOH–CH_2Cl_2 (2.5 L). The filtrate and washings were concentrated in vacuo to afford a greenish brown oil (ca. 47 g) which was chromatographed on 1500 g of flash silica gel (10% MeOH–CH_2Cl_2, 200 ml frs): frs 18–23 (fraction I) afforded 3.7 g of a brownish-white solid and frs 24–25 (fraction II) afforded 2.1 g of a brown residue.

Fraction I (3.7 g) was purified on 180 g of flash silica gel (7% MeOH in Et_2O–CH_2Cl_2 (1:1), 25 mL frs) giving fraction IA (frs 14–31) and fraction IB (frs 32–73). Fraction IA provided 66 mg of a yellow residue, which upon further chromatography (100 g of flash silica gel, 7% MeOH in EtOAc–Et_2O–CH_2Cl_2 (1:2:2), 15 mL frs) afforded 21 mg of 15–*O*–acetyl–$\Delta^{4,5}$–glaucarubol as a crystalline compound, mp 184–186°C (Grieco, Haddad, Piñeiro-Núñez & Huffman, 1998 183–186°C).

Fraction IB (168 mg) was chromatographed on 160 g of flash silica gel (7% MeOH in Et_2O–CH_2Cl_2 (1:1), 20 ml frs) to afford 84 mg of a yellow oil (frs 54–101). Further purification (100 g of flash silica gel, 50% Me_2CO–CH_2Cl_2, 10 ml frs) gave two fractions: frs 36–50 (fraction IB_1) and frs 51–65 (fraction IB_2). Fraction IB_1 afforded 32 mg of a white solid, which upon recrystallization from 50% EtOAc–CH_2Cl_2 gave 19 mg of **1** as colorless prisms. Purification of the mother liquor by preparative TLC (1 plate, 0.5 mm thickness, 30% Me_2CO–Et_2O) afforded another 6 mg of **1** as a white solid. Fraction IB_2 provided 18 mg of an impure white solid, which was purified by preparative TLC (2 plates, 30% Me_2CO–Et_2O) affording 5 mg of **2** as a white solid.

Fraction II (2.1 g) crystallized from Me_2CO, giving 28 mg of glaucarubol as fine needles, mp 285–287°C ([6] 285°C). The mother liquor was purified on 200 g of flash silica gel (15% MeOH in Et_2O–CH_2Cl_2 (1:1), 25 mL frs) to provide 493 mg of a yellow oil. Further chromatography on 180 g of flash silica gel (10% MeOH in Et_2O–CH_2Cl_2 (1:1), 25 mL frs) afforded two fractions: fraction IIA (frs 7–16) and fraction IIB (frs 17–32).

Fraction IIA (85 mg) was purified on 80 g of flash silica gel (10% MeOH–Et_2O, 10 mL frs) to give 32 mg of a yellow solid which was purified further on 60 g of flash silica gel (10% MeOH–Et_2O, 5 mL frs) to provide 24 mg of a white solid (frs 9–15). Additional purification using preparative TLC (2 plates, 0.5 mm thickness, 10% MeOH–Et_2O) afforded 14 mg of crystalline 1-*epi*-glaucarubolone, mp 277–278°C (Grieco, Haddad, Piñeiro-Núñez & Huffman, 1998 276–278°C).

Fraction IIB (216 mg) was purified by chromatography on 200 g of flash silica gel (10% MeOH–Et_2O, 25 ml frs) to provide 33 mg of a yellow residue. Additional purification (100 g of flash silica gel, 10% MeOH–CH_2Cl_2, 15 ml frs) afforded 9 mg of **2** as a white solid (frs 25–31).

Glycoside 1: R_f 0.19 (5% MeOH–EtOAc), 0.30 (10% MeOH–CH_2Cl_2); FTIR (KBr): 3530 (s), 3437 (s), 2959 (m), 2918 (m), 1644 (s), 1441 (w), 1379 (m), 1290 (m), 1126 (s), 1036 (s), 883 (m) cm^{-1}; 500 MHz ^1H NMR (pyridine-d_5) δ 5.84 (s, 1H), 4.74 (d, 1H, J = 7.5 Hz), 4.54 (dd, 1H, J = 12.0, 2.1 Hz), 4.38 (dd, 1H, J = 12.0, 5.2 Hz), 4.20 (q, 1H, J = 6.0 Hz), 4.18 (d, 1H, J = 11.8 Hz), 4.14 (m, 2H), 4.04 (m, 1H), 3.93 (d, 1H, J = 11.9 Hz), 2.50 (d, 1H, J = 17.0 Hz), 2.30 (d, 1H, J = 17.0 Hz), 2.28 (d, 3H, J = 1.0 Hz), 2.17 (m, 1H), 1.94 (m, 2H), 1.75 (m, 1H), 1.08 (s, 3H), 0.84 (s, 3H); 100 MHz ^{13}C NMR (pyridine-d_5) δ 196.7, 166.7, 125.4, 105.0, 99.2, 92.9, 80.3, 74.8, 74.3, 71.4, 70.8, 62.1, 50.0, 41.5, 34.9, 29.2, 24.8, 22.9, 21.0; High-resolution MS (CI) calcd for $C_{19}H_{29}O_8$ (M + 1) *m/z* 385.1863, found 385.1866. An analytical sample was prepared by recrystallization from 50% EtOAc–CH_2Cl_2, mp 248–250°C; $[\alpha]_D^{25}$-36.0° (c. 2.15, pyridine-d_5). Anal. calcd for $C_{19}H_{28}O_8$: C, 59.36; H, 7.34. Found: C, 59.21; H, 7.29.

(−)−[3α,16β,17α,20(S)]-3,16,17,20-Tetrahydroxypregnane-6-one 2: R_f 0.33 (5% MeOH–EtOAc), 0.28 (10% MeOH–CH_2Cl_2); FTIR (KBr): 3484 (br s), 2946 (s), 1690 (s), 1389 (m), 1264 (m), 1040 (s); 500 MHz ^1H NMR (pyridine-d_5) δ 5.39 (m, 4H), 4.72 (q, 1H, J = 6.4 Hz), 4.55 (dd, 1H, J = 7.8, 5.4 Hz), 4.33 (br s, 1H), 3.12 (dd, 1H, J = 14.2, 4.0 Hz), 2.43 (dd, 1H, J = 12.5, 4.0 Hz), 2.20 (m, 4H), 2.04 (t, 2H, J = 12.4 Hz), 1.92 (m, 3H), 1.65 (m, 3H), 1.53 (d, 3H, J = 6.4 Hz), 1.38 (m, 4H), 1.06 (s, 3H), 0.80 (s, 3H); 100 MHz ^{13}C NMR (pyridine-d_5) δ 211.8, 86.0, 81.9, 67.7, 64.3, 53.5, 51.9, 49.2, 46.9, 45.4, 41.4, 37.5, 34.9, 32.0, 32.0, 28.8, 28.7, 20.5, 18.0, 13.8, 12.4; High-resolution MS (CI) calcd for $C_{21}H_{35}O_5$ (M + 1) *m/z* 367.2486, found 367.2473. An analytical sample was prepared by recrystallization from 50% MeOH–Me_2CO, mp 292–293°C, $[\alpha]_D^{25}$-20.0° (c. 0.37, pyridine-d_5). Anal. calcd for $C_{21}H_{34}O_5$: C, 68.82; H, 9.35. Found: C, 68.67; H, 9.31.

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Complete crystallographic data for **1** (report No. 95108) and **2** (report No. 95110) can be obtained from Dr. John C. Huffman, Molecular Structure Center, Indiana University, Bloomington, IN 47405.

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