PERGAMON

Phytochemistry 50 (1999) 637–645

PHYTOCHEMISTRY

# Quassinoids from the twigs and thorns of *Castela polyandra*

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Received 26 June 1998; accepted 18 August 1998

**Abstract**

The structures of six new $C_{20}$ quassinoids and one new $C_{19}$ quassinoid, all isolated from the twigs and thorns of *Castela polyandra*, were established by a combination of spectroscopic and single-crystal X-ray analysis. Five known quassinoids and one known sterol were also identified. 

*Keywords:* *Castela polyandra*; *Simaroubaceae*; Twigs; Thorns; Quassinoids; Bitter principles; Spectroscopy; X-ray crystallography

## 1. Introduction

The *Simaroubaceae* botanical family has played a prominent role in folk medicine (Simao, Barreiro, Das, DaSilva & Gottlieb, 1991; Grieve, 1971; Polonsky, 1968). The medicinal properties associated with these plants have been attributed to their bitter constituents, collectively called quassinoids (Kawada, Kim & Watt, 1989; Polonsky, 1985). In concert with our ongoing research program in the area of anticancer quassinoids (Moher, Reilly, Grieco, Corbett & Valeriote, 1998; Valeriote, Corbett, Grieco, Moher, Collins et al., 1998), we have focussed our efforts on the isolation of novel quassinoids from plant sources (Grieco, VanderRoest & Piñeiro-Núñez, 1995; Grieco, Moher, Seya, Huffman & Grieco, 1994), in particular, the twigs and thorns of *Castela polyandra*. We now report on the isolation and characterization of six new $C_{20}$ quassionids [1-*epi*-holacanthone (**1**), 15-*O*-acetyl-glaucarubol (**2**), 15-*O*-acetyl-$\Delta^{4,5}$-glaucarubol (**3**), 1-*epi*-5-iso-glau-carubolone (**4**), 1-*epi*-glaucarubolone (**5**), and $\Delta^{4,5}$-glaucarubol (**6**)] and one new $C_{19}$ quassinoid [15-*O*-acetyl-5(*S*)-polyandrol (**7**)] along with holacanthone (**8**), 5(*R*)-polyandrol (**9**), glaucarubolone (**10**), glaucarubol (**11**), and niloticin (**12**).

## 2. Results and discussion

The methanol extracts of the twigs and thorns of *Castela polyandra* afforded holacanthone (**8**) (Fleck & Grieco, 1992), 5(*R*)-polyandrol (**9**) (Grieco et al., 1995), glaucarubolone (**10**) (Grieco, Collins, Moher & Fleck, 1993), glaucarubol (**11**) (Khan & Shamsuddin, 1980), niloticin (**12**) (Itokawa, Kishi, Morita & Takeya, 1992) six new $C_{20}$ quassinoids (**1**–**6**) and one new $C_{19}$ quassinoid (**7**) possessing the 1,2-seco-1-nor-6(5 → 10)abeo-picrasan-2,5-olide skeleton. The structures of **8**–**12** were identified by comparison of their respective $^1$H and $^{13}$C NMR spectra with those obtained from either synthetic or natural material.

Compound **1**, mp 243–245°C, possessed a molecular formula of $C_{22}H_{28}O_9$ as indicated by mass spectrometry and elemental analysis. Examination of the $^1$H NMR data suggested a C(15) *O*-acetylated $C_{20}$ quassinoid similar to holacanthone (**8**). A comparison of the $^1$H and $^{13}$C NMR spectra of **1** with those of holacanthone suggested that both compounds were closely related, but it was inconclusive as to the exact nature of the structural differences. The structure of **1** was obtained by single-crystal X-ray analysis. Compound **1** crystallized in the space group $P2_12_12_1$ with unit cell dimensions of $a = 8.265(2)$ Å, $b = 11.317(4)$ Å, $c = 22.108(8)$ Å and $Z = 4$. The volume of the crystal was 2067.83 Å$^3$ with a density of 1.402 g cm$^{-3}$. An ORTEP view of **1** is shown in Fig. 1.

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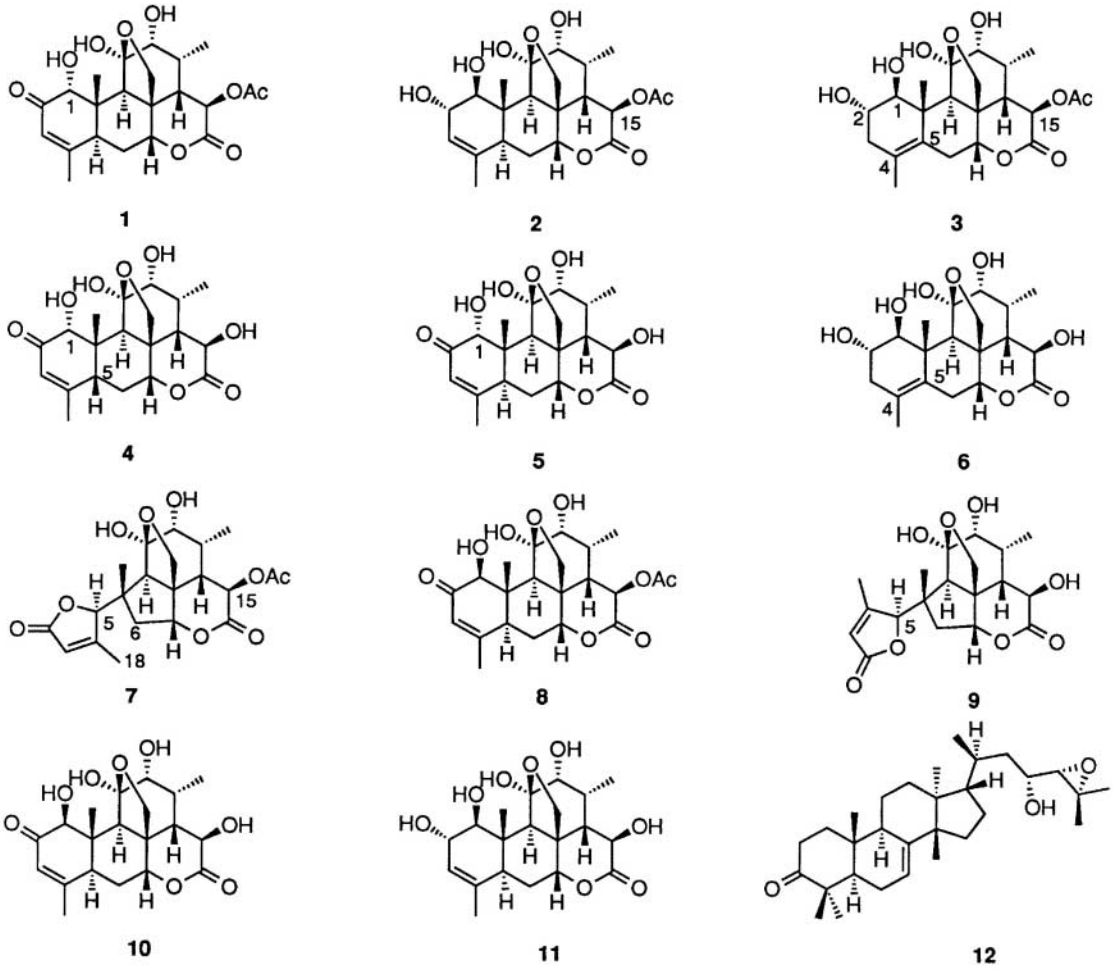
0031-9422/98 $ - see front matter 
PII: S0031-9422(98)00589-5




The structure of compound **2**, mp 254–256°C, followed readily from examination of all the spectral data. The molecular formula was established as $C_{22}H_{30}O_9$ by elemental analysis and mass spectrometry. This information together with the $^{13}C$ NMR data clearly indicated a C(15) acetylated $C_{20}$ quassinoid. A comparison of the $^1H$ NMR spectrum of **2** with that of glaucarubol (**11**) confirmed this assumption, since the spectra were almost identical, differing only in the presence of an acetyl group at $\delta$ 2.13 and the downfield shift of the C(15) alpha proton to $\delta$ 6.39. In order to confirm the structural assignment, **2** was submitted to basic hydrolysis under carefully controlled conditions. Thus, treatment of **2** with 1.0 N aqueous NaOH solution in the absence of oxygen afforded, after 30 min at ambient temperature, a 75% yield of crystalline glaucarubol (**11**) which was identical in all respects with a sample of natural glaucarubol.

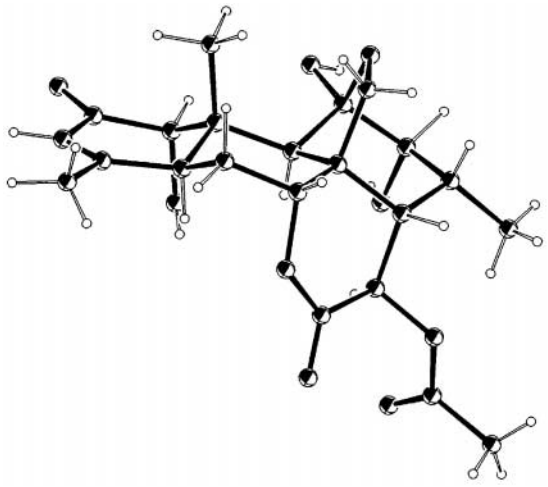
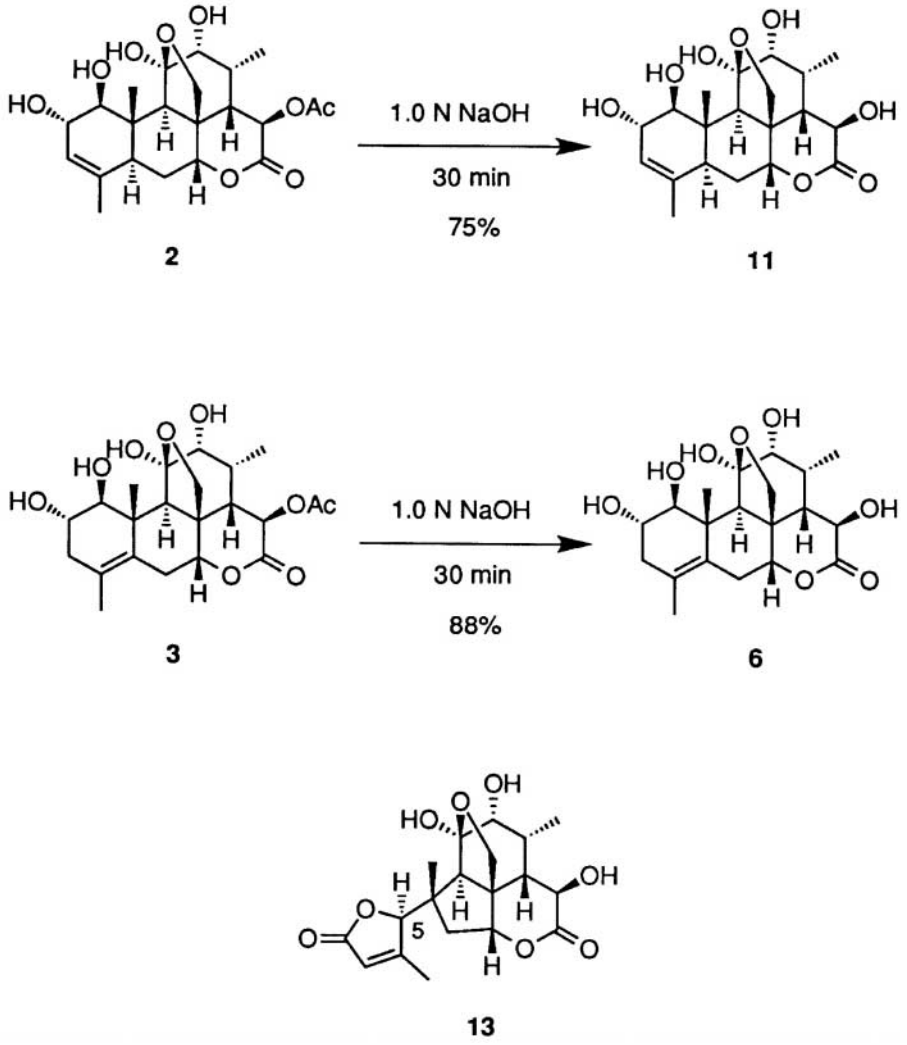


Fig. 1. An ORTEP view of **1**.

The structure of compound **3**, mp 184–186°C, was established by a combination of IR, $^1H$ and $^{13}C$ NMR spectroscopy, and mass spectrometry. Both the elemental analysis and the mass spectrum of **3** indicated a molecular formula of $C_{22}H_{30}O_9$, which, considering the presence of an acetate group (evident from the $^1H$ NMR data), clearly suggested a $C_{20}$ picrasane-like skeleton most likely acetylated at C(15). A comparison of the $^1H$ NMR data of **3** with the NMR data of hola-




canthone (**8**) revealed that both structures possessed the same functionality about the CDE ring system. This was corroborated by means of extensive $^{1}H$ NMR decoupling experiments.

With respect to the functionality in ring A, the IR and $^{13}C$ NMR data revealed the absence of an additional carbonyl. In addition, the $^{13}C$ NMR spectrum indicated the presence of two olefinic carbons at $\delta$ 127.77 and 129.26 which were determined to be quaternary by a DEPT experiment. From this data it followed that compound **3** possessed a double bond between C(4) and C(5). The DEPT experiment was also consistent with the presence of one methylene and two methine protons in ring A. One of the two methine protons appeared in the $^{1}H$ NMR spectrum as a doublet ($\delta$ 3.90, $J = 9.6$ Hz) and the other as a multiplet ($\delta$ 4.22), suggesting a vicinal diaxial relationship between the protons located at C(1) and C(2). The proposed structure for compound **3** is consistent with all the spectral data. The complete assignments for the $^{1}H$ and $^{13}C$ NMR resonances were established by $^{13}C$-$^{1}H$ COSY experiments (HETCOR) in pyridine-$d_5$ and $CDCl_3$. The *trans* diequatorial arrangement of the hydroxyls located at C(1) and C(2) coupled with unsaturation between C(4) and C(5) in ring A has been observed in quassinoids on two previous occasions (Itokawa, Qin, Morita, Takeya & Iitaka, 1993; Kubo & Chaudhuri, 1992).

The structure of **4**, mp 251–252°C, was elucidated by a combination of $^{1}H$ and $^{13}C$ NMR spectroscopy, mass spectrometry, and single-crystal X-ray analysis. The mass spectrum of **4** indicated a molecular formula of $C_{20}H_{26}O_8$. The NMR data suggested a $C_{20}$ quassinoid related to glaucarubolone (**10**). Single-crystal X-ray analysis unambiguously established the structure of **4** as shown, in which the C(1) and C(5) positions were epimeric with those of glaucarubolone (**10**). Compound **4** crystallized in the space group $P2_12_12_1$ with unit cell dimensions of $a = 7.412(3)$ Å, $b = 12.664(6)$ Å, $c = 22.312(10)$ Å and $Z = 4$. The volume of the crystal was 2094.22 Å$^3$ with a density of 1.419 gcm$^{-3}$. An ORTEP view of **4** is shown in Fig. 2. This type of structural arrangement in $C_{20}$ quassinoids wherein the AB rings are *cis*-fused and the hydroxyl

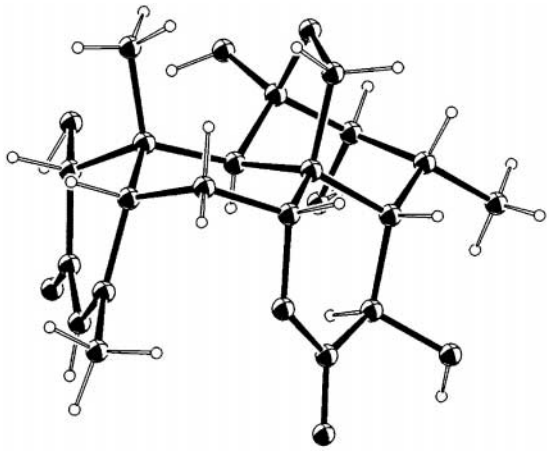


Fig. 2. An ORTEP view of **4**.

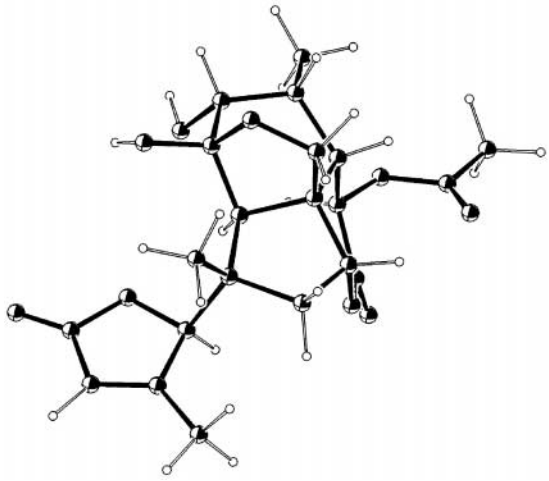


Fig. 4. An ORTEP view of **7**.

group at C(1) is alpha oriented has not previously been observed.

Compound **5**, mp 276–278°C, possessing the molecular formula $C_{20}H_{26}O_8$, as determined by its mass spectrum, was readily identified as a $C_{20}$ quassinoid isomeric with glaucarubolone (**10**) from its detailed $^1H$ and $^{13}C$ NMR spectra. The structure was determined by single-crystal X-ray analysis. Compound **5** crystallized in space group $P2_12_12_1$ with unit cell dimensions of $a = 7.103(1)$ Å, $b = 13.274(2)$ Å, $c = 19.117(3)$ Å and $Z = 4$. The volume of the crystal was 1802.44 Å$^3$ with a density of 1.453 g cm$^{-3}$. An ORTEP view of **5** is shown in Fig. 3.

The structure of compound **6**, mp 178–180°C, was determined by a combination of spectral analysis and chemical correlation. The molecular formula, $C_{20}H_{28}O_8$, was obtained from the mass spectrum and elemental analysis. The $^1H$ NMR spectrum suggested a $C_{20}$ quassinoid isomeric with that of glaucarubol (**11**). Furthermore, comparison of the $^1H$ NMR spectrum of **6** with the $^1H$ NMR spectrum of **3** suggested that **6** was the C(15) deacetylated version of **3**. In order to probe this issue, compound **3** was submitted to hydrolysis. Brief (30 min) treatment of **3** with deoxygenated 1.0 N NaOH solution afforded (88%) semisynthetic **6** which was identical in all respects with **6** isolated from the plant material.

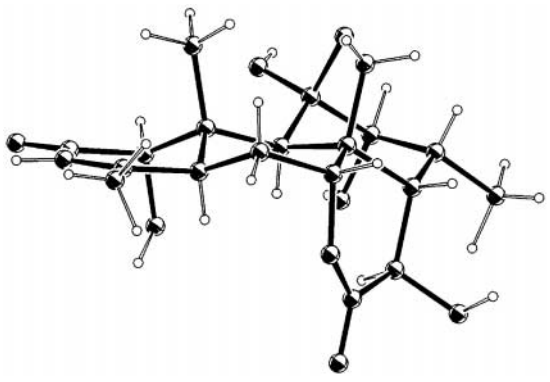


Fig. 3. An ORTEP view of **5**.

The structure of **7**, mp 244–246°C, was established by a combination of IR, $^1H$ and $^{13}C$ NMR spectroscopy, mass spectrometry and X-ray crystallography. The mass spectrum and the elemental analysis of **7** indicated a molecular formula of $C_{21}H_{26}O_9$. This information, coupled with the $^1H$ and $^{13}C$ NMR data, suggested that **7** was a C(15) *O*-acetylated $C_{19}$ quassinoid. Comparison of the $^1H$ and $^{13}C$ NMR spectra of **7** with the NMR spectra of 5(*R*)-polyandrol (**9**) (Grieco et al., 1995) revealed that both the proton and carbon spectra were strikingly similar. That C(15) possessed an acetoxy group was indicated by the presence of a three-proton singlet at $\delta$ 2.10, as well as by the chemical shift of the C(15) proton, which appeared at $\delta$ 6.25, substantially deshielded with respect to the C(15) proton of 5(*R*)-polyandrol. More informative were the chemical shifts for the C(6$\alpha$) proton ($\delta$ 2.43) and the C(18) methyl group ($\delta$ 1.95) which were shielded relative to those in 5(*R*)-polyandrol ($\delta$ 2.96 and $\delta$ 2.45, respectively). These observations suggested that **7** possessed the *S* configuration at C(5), opposite to that of **9**. The structure of **7** was unambiguously established by single-crystal X-ray analysis. Compound **7** crystallized in space group $P2_12_12_1$ with cell dimensions of $a = 10.261(1)$ Å, $b = 25.237(4)$ Å, $c = 7.276(1)$ Å and $Z = 4$. The volume of the crystal was 1884.05 Å$^3$ with a density of 1.489 g cm$^{-3}$. An ORTEP view of **7** is shown in Fig. 4. We have previously isolated and characterized 5(*S*)-polyandrol (**13**) from the stem of *Castela polyandra* (Piñeiro-Núñez, 1996).

## 3. Experimental

### 3.1. General

Proton ($^1$H) 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 ($\delta$) relative to tetramethylsilane ($\delta$ 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 a *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 are reagent grade. Fraction collecting commenced after elution of the void volume of the column.

### 3.2. Plant material

The twigs and thorns of *Castela polyandra* were procured from Baja California in April 15, 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.

### 3.3. 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 sludge, 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 flash silica gel (1500 g) (10% MeOH–$CH_2Cl_2$, 200 ml frs) to provide seven fractions.

Fraction I (7.0 g) was purified by chromatography on 400 g of flash silica gel (40% $Et_2O$–hexane, 25 ml frs). Frs 93–109 were combined and concentrated *in vacuo* to provide a brownish-yellow oil (721 mg); frs 153–185 (eluted with 10% MeOH–$Et_2O$) were combined, concentrated *in vacuo* (2.1 g of a brown oil), and added to fraction II. The brownish-yellow oil (721 mg) was purified by chromatography on 150 g of flash silica gel (10% $Et_2O$-$CH_2Cl_2$, 15 ml frs). Frs 26–46 were combined and concentrated *in vacuo* to provide 334 g of a white solid which was recrystallized from petroleum ether to give niloticin (**12**) as needle-shaped crystals, mp 143–144°C (ref. (Itokawa et al., 1992) 139–141°C). Niloticin (**1**) was identified by comparison of its spectroscopic data (IR, MS, $^{13}$C NMR) with those previously reported in the literature (Itokawa et al., 1992).

Fraction II (8.66 g) was purified by chromatography on 800 g of flash silica gel (10% MeOH–$Et_2O$, 25 ml frs). Frs 105–131 were concentrated *in vacuo* to 1.68 g of a yellowish-white solid which was crystallized from 50% MeOH–$Et_2O$ to give 812 mg of crystalline holacanthone (**8**), mp 246–248°C (ref. Fleck & Grieco, 1992 245–247°C). Holacanthone (**8**) was identified by comparison of its spectroscopic data (IR, MS, $^1$H and $^{13}$C NMR) with those previously reported in the literature (Fleck & Grieco, 1992). The mother liquor (*ca.* 700 mg) was purified by chromatography on 140 g of flash silica gel (7% MeOH–$CH_2Cl_2$, 15 ml frs). Frs 34–55 were combined and concentrated *in vacuo* to provide 260 mg of a white solid, which upon recrystallization from MeOH afforded two types of crystals easily separable from each other: 220 mg of holacanthone (**8**) as small needles, and 32 mg of **7** as prisms.

Fraction III (3.4 g) was purified by chromatography on 400 g of flash silica gel ($Et_2O$, 25 ml frs). After 110 frs, the solvent was switched to 15% MeOH–$Et_2O$. Frs 136–156 were combined and concentrated *in vacuo* to give 25 mg of crude holacanthone (**8**) which was combined and purified with fraction II; frs 157–189 provided a yellow oil (45 mg), which was further purified (70 g of flash silica gel, 10% MeOH–$CH_2Cl_2$, 5 ml frs) to give 5 mg of pure holacanthone (**8**) as a white solid (frs 6–7).

Fraction IV (3.35 g) was purified by chromatography on 400 g of flash silica gel (10% MeOH–$Et_2O$, 25 ml frs) to provide four fractions: frs 44–63 afforded 215 mg of a yellow oil (fraction IVA); frs 81–88 provided 63 mg of a yellow oil (fraction IVB); frs 89–117 provided 744 mg of a yellow oil (fraction IVC); and frs 118–146 afforded 83 mg of a yellow oil (fraction IVD).

Fraction IVA (215 mg) was purified by chromatography (120 g of flash silica gel, MeOH–$Et_2O$–$CH_2Cl_2$ (1:4:5), 15 ml frs) to provide 34 mg of a yellow oil (frs 20–30) which was purified further on 60 g of flash silica gel (5% MeOH in $Et_2O$–$CH_2Cl_2$ (2:3), 10 ml frs). Frs 19–33 were concentrated *in vacuo* to afford 29 mg

of a white solid which was purified on 60 g of flash silica gel (5% MeOH in EtOAc–$Et_2O$–$CH_2Cl_2$ (1:2:2), 5 ml frs) to provide two fractions: frs 22–26 (fraction $IVA_1$) afforded 8 mg of 5(*R*)-polyandrol (**9**) as a white solid, mp 191–192°C ((Grieco et al., 1995) 190–192°C) which was identified by comparison of its spectroscopic data (IR, MS, $^1H$ and $^{13}C$ NMR) with those previously reported (Grieco et al., 1995) in the literature; frs 27–60 (fraction $IVA_2$) afforded 18 mg of a white solid which was further purified by preparative TLC (2 plates, 0.5 mm thickness, 30% $Me_2CO$–$Et_2O$) to give 10 mg of a white solid. Subsequent recrystallization from $CH_2Cl_2$ gave 7 mg of **1** as prisms.

Fraction IVB (63 mg) was purified by chromatography on 120 g of flash silica gel (7% MeOH in $Et_2O$–$CH_2Cl_2$ (2:3), 20 ml frs) providing 56 mg of a yellow solid (frs 16–49) which was recrystallized from MeOH to give 6 mg of **2** as tiny needle-shaped crystals. The mother liquor was purified by chromatography on 70 g flash silica gel (7% MeOH–$CH_2Cl_2$, 10 ml frs) to provide 24 mg of a yellowish-white solid (frs 47–52) which upon recrystallization from MeOH afforded a second crop of crystalline **2** (7 mg). Further purification of the mother liquor (*ca* 14 mg) on 40 g of flash silica gel (MeOH–$Et_2O$–$CH_2Cl_2$ (1:4:5), 5 ml frs) provided 9 mg of 5(***R***)-polyandrol (**9**) as a white solid (frs 24–43).

Fraction IVC (744 mg) was purified on 240 g of flash silica gel (5% MeOH–$CH_2Cl_2$, 25 ml frs) to provide three fractions: frs 11–31 (fraction $IVC_1$) afforded 151 mg of pure glaucarubolone (**10**), mp 256–258°C (ref. Grieco et al., 1993, 255–258°C) which was identified by comparison of its spectroscopic data (IR, MS, $^1H$ and $^{13}C$ NMR) with those previously reported (Grieco et al., 1993) in the literature; frs 60–93 (fraction $IVC_2$) afforded 158 mg of **3** as a white solid; frs 94–120 (fraction $IVC_3$) provided 210 mg of impure **3** which upon further purification (140 g of flash silica gel, 6% MeOH–$CH_2Cl_2$, 10 ml frs) afforded 91 mg of pure **3** (frs 31–53) as a white solid.

Fraction IVD (83 mg) was purified by chromatography on 70 g of flash silica gel (5% MeOH–$CH_2Cl_2$, 10 ml frs). Frs 38–63 (fraction $IVD_1$) provided 36 mg of **4** as a white solid which was further purified (70 g of flash silica gel, 8% MeOH–$CH_2Cl_2$, 10 ml frs) to give 32 mg of fairly pure material (frs 14–21). Additional purification (50 g of flash silica gel, MeOH–$Et_2O$–$CH_2Cl_2$ (2:9:9), 10 ml frs) provided 29 mg of **4** (frs 20–35) as a white solid. Frs 84–97 (fraction $IVD_2$) provided 31 mg of **3** as a white solid.

Fraction V (3.7 g) was purified on 180 g of flash silica gel (7% MeOH–$CH_2Cl_2$, 25 ml frs) giving rise (frs 14–31) to 66 mg of a yellow oil which upon further purification (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 **3** as a white solid.

Fraction VI (2.1 g) was recrystallized from $Me_2CO$ giving rise to 28 mg of glaucarubol (**11**) as fine needles, mp 285–287°C (ref. Khan & Shamsuddin, 1980 285°C). Glaucarubol (**11**) was identified by comparison of its spectroscopic data (IR, MS, $^1H$ and $^{13}C$ NMR) with those previously reported (Khan & Shamsuddin, 1980) in the literature. 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 purification on 180 g of flash silica gel (10% MeOH in $Et_2O$–$CH_2Cl_2$ (1:1), 25 ml frs) afforded fraction VIA (frs 7–16). Fraction VIA (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 further purified (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 was accomplished by preparative TLC (2 plates, 0.5 mm thickness, 10% MeOH–$Et_2O$) giving rise to 14 mg of **5** as a white solid.

Fraction VII (2.8 g) was chromatographed on 350 g of flash silica gel (15% MeOH–$CH_2Cl_2$, 25 ml frs), giving rise to two fractions: fraction VIIA (frs 41–47) and fraction VIIB (frs 48–57). Fraction VIIA (378 mg) was purified on 140 g of flash silica gel (15% MeOH in $Et_2O$–$CH_2CL_2$ (1:1), 20 ml frs) to afford 122 mg of a yellow solid (frs 17–29) which was further purified on 100 g of flash silica gel (15% MeOH–$Et_2O$, 15 ml frs) to give 64 mg of a white solid (frs 18–29). Additional purification on 100 g of flash silica gel (5% MeOH in $Me_2CO$–$CH_2Cl_2$ (1:1), 20 ml frs) provided 18 mg of **6** as a white solid.

Fraction VIIB (624 mg) was purified on 300 g of flash silica gel (15% MeOH in $Et_2O$–$CH_2Cl_2$ (1:1), 25 ml frs) to provide 465 mg of a yellow solid which was further purified on 250 g of flash silica gel (15% MeOH in $Et_2O$–$CH_2Cl_2$ (1:1), 25 ml frs) affording two fractions: fraction $VIIB_1$ (frs 35–47) and fraction $VIIB_2$ (frs 48–49). Fraction $VIIB_1$ (139 mg) was purified on 130 g of flash silica gel (5% MeOH in $Me_2CO$-$CH_2Cl_2$ (1:1), 15 ml frs) to provide 21 mg of **6** as a white solid (frs 20–24). Fraction $VIIB_2$ (186 mg) was purified on 150 g of flash silica gel (15% MeOH in $Et_2O$–$CH_2Cl_2$ (1:1), 15 ml frs) affording 9 mg of pure **6** as a white solid (frs 8–11), as well as 77 mg (frs 12–28) of a mixture of **6** and another compound (inseparable by column chromatography).

### *3.3.1. 1-Epi-holacanthone (**1**)*

$R_f$ 0.44 (5% MeOH–EtOAc), 0.40 (10% MeOH–$CH_2Cl_2$); FT-IR (KBr): 3560 (*s*), 3486 (*s*), 3337 (*s*), 2967 (*m*), 2944 (*m*), 2897 (*m*), 1759 (*s*), 1730 (*s*), 1670 (*s*), 1385 (*m*), 1219 (*s*), 1049 (*s*), 910 (*m*) $cm^{-1}$; 500 MHz $^1H$ NMR (pyridine-$d_5$) $\delta$ 6.50 (*br s*, 1H), 6.09 (*s*, 1H), 5.10 (*s*, 1H), 4.78 (*s*, 1H), 4.26 (*d*, 1H, $J = 8.5$ Hz), 4.14 (*s*, 1H), 4.04 (*d*, 1H, $J = 2.9$ Hz), 3.86

(*d*, 1H, $J = 8.5$ Hz), 3.66 (*d*, 1H, $J = 12.8$ Hz), 2.56 (*m*, 2H), 2.19 (*ddd*, 1H, $J = 14.5, 4.5, 2.6$ Hz), 2.08 (*s*, 3H), 2.01 (*ddd*, 1H, $J = 15.0, 13.1, 1.9$ Hz), 1.74 (*s*, 3H), 1.67 (*s*, 3H), 1.28 (*d*, 3H, $J = 6.7$ Hz); 100 MHz $^{13}$C NMR (pyridine-$d_5$) $\delta$ 198.93, 169.69, 168.05, 160.68, 125.24, 112.24, 79.64, 78.62, 76.93, 71.98, 70.70, 47.24, 45.81, 41.89, 36.34, 36.06, 33.11, 26.11, 22.13, 20.84, 15.15, 14.27; High-resolution MS (CI) calcd for $C_{22}H_{29}O_9$ (M + 1) *m*/*z* 437.1812, found 437.1803. An analytical sample was prepared by recrystallization from $CH_2Cl_2$, mp 243–245°C; $[\alpha]_D^{25} + 23.2°$ (*c* 0.41, pyridine-$d_5$). Anal. calcd for $C_{22}H_{28}O_9$: C, 60.54; H, 6.47. Found: C, 60.27; H, 6.31.

### 3.3.2. 15-O-Acetyl-glaucarubol (**2**)

$R_f$ 0.38 (10% MeOH–$CH_2Cl_2$), 0.35 (5% MeOH–EtOAc); FT-IR (KBr): 3468 (*s*), 3408 (*s*), 3254 (*br m*), 2947 (*m*), 2895 (*m*), 2722 (*br m*), 2581 (*br m*), 1740 (*s*), 1520 (*w*), 1393 (*m*), 1229 (*s*), 1049 (*s*), 960 (*m*) cm$^{-1}$; 500 MHz $^1$H NMR (pyridine-$d_5$) $\delta$ 6.39 (*br d*, 1H, $J = 12.4$ Hz), 5.74 (*s*, 1H), 4.57 (*br d*, 1H, $J = 5.8$ Hz), 4.17 (*d*, 1H, $J = 8.8$ Hz), 4.02 (*d*, 1H, $J = 3.5$ Hz), 3.85 (ABq, 2H, $J = 8.2$ Hz, $\Delta\nu_{AB} = 14.1$ Hz), 3.16 (*s*, 1H), 2.58 (*m*, 2H) 2.54 (*dd*, 1H, $J = 12.2, 6.1$ Hz), 2.13 (*s*, 3H), 1.98 (*ddd*, 1H, $J = 14.7, 3.8, 2.5$ Hz), 1.88 (*ddd*, 1H, $J = 15.4, 12.9, 2.5$ Hz), 1.67 (*s*, 3H), 1.54 (*s*, 3H), 1.28 (*d*, 3H, $J = 7.2$ Hz); 100 MHz $^{13}$C NMR (pyridine-$d_5$) $\delta$ 169.71, 168.06, 134.63, 126.82, 110.77, 83.33, 79.76, 78.83, 72.43, 71.34, 70.15, 47.77, 45.64, 45.21, 41.69, 41.43, 32.65, 25.62, 20.90, 20.87, 15.06, 10.74; High-resolution MS (CI) calcd for $C_{22}H_{30}O_9$ (M) *m*/*z* 438.1889, found 438.1879. An analytical sample was prepared by recrystallization from MeOH, mp 254–256°C; $[\alpha]_D^{25} + 34.6°$ (*c* 1.00, pyridine-$d_5$). Anal. calcd for $C_{22}H_{30}O_9$: C, 60.26; H, 6.90. Found: C, 60.09; H, 6.85.

### 3.3.3. 15-O-Acetyl-$\Delta^{4,5}$-glaucarubol (**3**)

R*f* 0.19 (EtOAc), 0.45 (10% MeOH-$CHCl_3$); FTIR ($CHCl_3$) 3428 (*m*), 3009 (*w*), 2928 (*m*), 1740 (*s*), 1605 (*w*), 1456 (*w*), 1377 (*m*), 1235 (*s*), 1196 (*m*), 1063 (*s*), 999 (*m*), 957 (*w*), 899 (*w*) cm$^{-1}$; 400 MHz $^1$H NMR ($CDCl_3$) $\delta$ 9.00 (*br s*, 1H), 6.60 (*br s*, 1H), 5.67 (*d*, 1H, $J = 11.2$ Hz), 4.44 (*br s*, 1H), 4.12 (*d*, 1H, $J = 8.4$ Hz), 3.93–3.83 (*m*, 1H), 3.78 (*d*, 1H, $J = 10.0$ Hz), 3.69 (*d*, 1H, $J = 8.8$ Hz), 3.52 (*d*, 1H, $J = 3.6$ Hz), 2.84 (*dd*, 1H, $J = 15.2, 3.2$ Hz), 2.53 (*s*, 1H), 2.44–2.28 (*m*, 3H), 2.18 (*dd*, 1H, $J = 11.8, 6.2$ Hz), 2.11 (*s*, 3H), 2.30–1.95 (*m*, 1H), 1.66 (*br s*, 3H), 1.42 (*s*, 3H), 1.01 (*d*, 3H, $J = 6.8$ Hz); 400 MHz $^1$H NMR (pyridine-$d_5$) $\delta$ 10.2 (*br s*, 1H), 9.50 (*br s*, 1H), 7.23 (*d*, 1H, $J = 4.8$ Hz), 6.48 (*br s*, 1H), 6.30 (*d*, 1H, $J = 11.2$ Hz), 4.72–4.68 (*m*, 1H), 4.37 (*d*, 1H, $J = 8.8$ Hz), 4.22–4.12 (*m*, 1H), 4.03 (t, 1H, $J = 4.6$ Hz), 3.90 (*d*, 1H, $J = 9.6$ Hz), 3.87 (*d*, 1H, $J = 8.8$ Hz), 3.08 (*s*, 1H), 2.89 (*dd*, 1H, $J = 15.0, 3.4$ Hz), 2.64–2.53 (*m*, 2H), 2.51–2.41 (*m*, 2H), 2.07 (*s*, 3H), 2.30 (*dd*, 1H, $J = 16.8, 10.3$ Hz), 1.90 (*s*, 3H), 1.69 (*s*, 3H), 1.25 (*d*, 3H, $J = 7.6$ Hz); 100 MHz $^{13}$C NMR ($CDCl_3$) $\delta$ 170.26, 167.33, 128.66, 127.08, 109.56, 82.07, 78.93, 78.87, 71.36, 69.64, 66.81, 48.11, 47.95, 45.74, 43.58, 40.43, 31.73, 27.42, 21.11, 19.39, 17.92, 14.37; High-resolution MS (CI) calcd for $C_{22}H_{31}O_9$ (M + 1) *m*/*e* 439.1960, found 439.1968. An analytically pure sample was prepared *via* crystallization from EtOAc, mp 183–186°C; $[\alpha]_D^{25} + 114.7°$ (*c* 0.6, methanol). Anal. calcd for $C_{22}H_{30}O_9$: C, 60.26; H, 6.90. Found: C, 60.50; H, 6.84.

### 3.3.4. 1-Epi-5-iso-glaucarubolone (**4**)

$R_f$ 0.25 (5% MeOH–EtOAc), 0.38 (10% MeOH–$CH_2Cl_2$); FTIR (KBr): 3598 (*s*), 3432 (*s*), 3376 (*s*), 3194 (*s*), 2928 (*m*), 2903 (*m*), 1736 (*s*), 1670 (*s*), 1435 (*m*), 1196 (*s*), 1063 (*s*), 1011 (*s*) cm$^{-1}$; 400 MHz $^1$H NMR (pyridine-$d_5$) $\delta$ 6.71 (*br s*, 1H), 6.02 (*d*, 1H, $J = 1.2$ Hz), 5.14 (*d*, 1H, $J = 11.2$ Hz), 4.52 (*dd*, 1H, $J = 2.8, 2.4$ Hz), 4.29 (*s*, 1H), 4.14 (*d*, 1H, $J = 8.8$ Hz), 3.99 (*d*, 1H, $J = 3.6$ Hz), 3.80 (*d*, 1H, $J = 8.8$ Hz), 3.03 (*s*, 1H), 2.56 (*m*, 2H), 2.47 (*ddd*, 1H, $J = 15.6, 3.2, 2.8$ Hz), 2.38 (*ddd*, 1H, $J = 14.8, 5.2, 3.2$ Hz), 2.17 (*dd*, 1H, $J = 11.2, 5.6$ Hz), 2.15 (*s*, 3H), 2.00 (*s*, 3H), 1.60 (*d*, 3H, $J = 7.2$ Hz); 100 MHz $^{13}$C NMR (pyridine-$d_5$) $\delta$ 197.18, 172.70, 162.82, 124.11, 110.85, 83.26, 80.17, 77.57, 71.87, 68.27, 49.33, 47.30, 46.71, 42.12, 37.80, 32.80, 25.54, 23.20, 22.93, 16.30; High-resolution MS (CI) calcd for $C_{20}H_{27}O_8$ (M + 1) *m*/*z* 395.1706, found 395.1720. An analytical sample was prepared by recrystallization from 50% EtOAc–$CH_2Cl_2$, mp 251–252°C; $[\alpha]_D^{25} - 28.3°$ (*c* 2.76, pyridine-$d_5$). Anal. calcd for $C_{20}H_{26}O_8$: C, 60.90; H, 6.64. Found: C, 60.75; H, 6.55.

### 3.3.5. 1-Epi-glaucarubolone (**5**)

$R_f$ 0.20 (10% MeOH–$CH_2Cl_2$), 0.33 (5% MeOH–EtOAc); FTIR (KBr): 3401 (*br s*), 2957 (*w*), 2878 (*w*), 1707 (*s*), 1659 (*m*), 1439 (*w*), 1385 (*w*), 1240 (*m*), 1117 (*m*), 1040 (*m*), 1007 (*m*) cm$^{-1}$; 500 MHz $^1$H NMR (pyridine-$d_5$) $\delta$ 6.08 (*br s*, 1H), 5.61 (*d*, 1H, $J = 10.8$ Hz), 5.10 (*s*, 1H), 4.68 (*s*, 1H), 4.23 (*d*, 1H, $J = 8.3$ Hz), 4.11 (*d*, 1H, $J = 3.6$ Hz), 4.08 (*s*, 1H), 3.87 (*d*, 1H, $J = 8.3$ Hz), 3.66 (*d*, 1H, $J = 12.4$ Hz), 2.70 (*m*, 1H), 2.34 (*dd*, 1H, $J = 10.7, 6.2$ Hz), 2.22 (*d*, 1H, $J = 5.5$ Hz), 2.00 (*ddd*, 1H, $J = 14.4, 12.3, 2.1$ Hz), 1.74 (*s*, 3H), 1.70 (*d*, 3H, $J = 7.3$ Hz), 1.66 (*s*, 3H); 100 MHz $^{13}$C NMR (pyridine-$d_5$) $\delta$ 199.04, 174.06, 160.89, 125.25, 112.37, 80.22, 78.41, 77.05, 72.26, 68.40, 49.64, 46.74, 41.84, 36.72, 36.16, 33.49, 26.50, 22.17, 16.20, 14.33; High-resolution MS (CI) calcd for $C_{20}H_{27}O_8$ (M + 1) *m*/*z* 395.1706, found 395.1689. An analytical sample was prepared by recrystallization from 50% EtOH-EtOAc, mp 276–278°C; $[\alpha]_D^{25} + 34.5°$ (*c* 0.40, pyridine-$d_5$). Anal. calcd for $C_{20}H_{26}O_8$: C, 60.90; H, 6.64. Found: C, 60.69; H, 6.54.

*3.3.6. $\Delta^{4,5}$-Glaucarubol (**6**)*

$R_f$ 0.13 (5% MeOH–EtOAc), 0.18 (10% MeOH–$CH_2Cl_2$); FTIR (KBr): 3408 (*br s*), 2994 (*w*), 2924 (*s*), 1719 (*s*), 1649 (*w*), 1383 (*m*), 1248 (*m*), 1065 (*s*) $cm^{-1}$; 400 MHz $^1H$ NMR (pyridine-$d_5$) $\delta$ 10.12 (*br s*, 1H), 9.32 (*br s*, 1H), 7.76 (*d*, 1H, $J = 4.4$ Hz), 6.93 (*d*, 1H, $J = 4.0$ Hz), 6.46 (*br s*, 1H), 5.35 (*dd*, 1H, $J = 10.4$, 2.4 Hz), 4.60 (*s*, 1H), 4.34 (*d*, 1H, $J = 8.4$ Hz), 4.17 (*m*, 1H), 4.09 (*s*, 1H), 3.93 (*d*, 1H, $J = 10.0$ Hz), 3.88 (*d*, 1H, $J = 8.8$ Hz), 2.99 (*s*, 1H), 2.91 (*dd*, 1H, $J = 15.2$, 3.6 Hz), 2.68 (*m*, 1H), 2.60 (*br d*, 1H, $J = 14.8$ Hz), 2.42 (ABq, 2H, $J_{AB} = 16.8$ Hz, $\Delta\nu_{AB} = 46.4$ Hz), 2.36 (*dd*, 1H, $J = 16.4$, 9.6 Hz), 2.28 (*dd*, 1H, $J = 10.4$, 6.4 Hz), 1.89 (*s*, 3H), 1.74 (*s*, 3H), 1.66 (*d*, 3H, $J = 7.6$ Hz); 100 MHz $^{13}C$ NMR (pyridine-$d_5$) $\delta$ 173.77, 129.61, 127.50, 111.14, 83.53, 80.20, 79.47, 71.84, 68.57, 67.01, 49.92, 49.55, 48.05, 44.28, 41.49, 32.94, 28.44, 19.82, 18.58, 16.15; High-resolution MS (CI) calcd for $C_{20}H_{29}O_8$ (M + 1) $m/z$ 397.1863, found 397.1875. An analytical sample was prepared by crystallization from EtOAc, mp 177–180°C; $[\alpha]_D^{25}$ + 86.9° (*c* 0.88, pyridine-$d_5$). Anal. calcd for $C_{20}H_{28}O_8$: C, 60.59; H, 7.12. Found: C, 60.33; H, 7.09.

*3.3.7. 15-O-acetyl-5(S)-polyandrol (7)*

$R_f$ 0.31 (5% MeOH–$CH_2Cl_2$), 0.44 (5% MeOH–EtOAc); FTIR (KBr): 3564 (*m*), 3503 (*s*), 3130 (*w*), 2976 (*m*), 2941 (*m*), 2910 (*m*), 1730 (*s*), 1643 (*w*), 1371 (*s*), 1217 (*s*), 1024 (*s*) $cm^{-1}$; 400 MHz $^1H$ NMR (pyridine-$d_5$) $\delta$ 6.50 (*br s*, 1H), 8.18 (*br s*, 1H), 6.25 (*br d*, 1H, $J = 10.0$ Hz), 5.90 (*d*, 1H, $J = 1.6$ Hz), 4.87 (*d*, 1H, $J = 4.8$ Hz), 4.75 (*s*, 1H), 4.06 (*br s*, 1H), 3.91 (ABq, 2H, $J = 8.8$ Hz, $\Delta\nu_{AB} = 15.4$ Hz), 3.56 (*s*, 1H), 2.70 (*br dd*, 1H, $J = 10.8$, 6.0 Hz), 2.50 (*br m*, 1H), 2.43 (*d*, 1H, $J = 15.6$ Hz), 2.24 (*br dd*, 1H, $J = 16.0$, 4.8 Hz), 2.10 (*s*, 3H), 1.95 (*s*, 3H), 1.51 (*s*, 3H), 1.28 (*d*, 3H, $J = 7.2$ Hz); 100 MHz $^{13}C$ NMR (pyridine-$d_5$) $\delta$ 172.44, 169.96, 167.84, 167.70, 120.67, 111.91, 91.69, 83.82, 80.48, 71.88, 70.36, 56.53, 47.28, 45.59, 45.04, 43.01, 34.04, 20.96, 20.15, 16.42, 14.67; High-resolution MS (CI) calcd. for $C_{21}H_{27}O_9$ (M + 1) $m/z$ 423.1655, found 423.1665. An analytical sample was prepared by recrystallization from MeOH, mp 244–246°C; $[\alpha]_D^{25} - 61.6°$ (*c* 1.02, pyridine-$d_5$). Anal. calcd for $C_{21}H_{26}O_9$: C, 59.71; H, 6.20. Found: C, 59.57; H, 6.18.

*3.3.8. Hydrolysis of **2***

Compound **2** (5 mg, 0.0115 mmol) was hydrolyzed at ambient temperature with 55 mL of a 1.0 N NaOH solution (previously purged with nitrogen and evacuated several times with argon to ensure the absence of oxygen). After 30 min, the cloudy reaction mixture was neutralized with 50% HCl solution (10 mL) and diluted with pyridine (0.5 mL). The product was isolated by extraction with $Et_2O$ (3×10 mL). The combined organic extracts were dried over anhydrous magnesium sulfate, filtered and concentrated *in vacuo*. The resulting solid was chromatographed on 20 g of flash silica gel (10% MeOH–$CH_2Cl_2$, 5 ml frs). Frs 14–21 were combined and concentrated *in vacuo* to provide 3.4 mg (75%) of glaucarubol (**11**) as a crystalline solid which was identical in all respects with a sample of natural **11**.

*3.3.9. Hydrolysis of **3***

Compound **3** (30 mg, 0.065 mmol) was hydrolyzed at ambient temperature with 33 mL of a 1.0 N NaOH solution (previously purged with nitrogen and evacuated several times with argon to ensure the absence of oxygen). After 30 min, the bright yellow solution was treated with 50% HCl solution (60 mL) and diluted with pyridine (1.0 mL). The product was isolated by extraction with $Et_2O$ (3×20 mL). The combined organic extracts were dried over anhydrous magnesium sulfate, filtered and concentrated *in vacuo*. The resulting solid was chromatographed on 50 g of flash silica gel (12% MeOH–$CH_2Cl_2$, 10 ml frs). Frs 11–21 were combined and concentrated *in vacuo* to afford 24 mg (88%) of **6** as a crystalline material which was identical in all respects with a sample of natural **6**.

## Acknowledgements

This investigation was supported by Public Health Service Research Grant CA 28865 from the National Cancer Institute. Plant material was collected in collaboration with the Universidad de Autonoma de Baja California, Ensenada, Mexico. We express appreciation to Graciela Guerra Rivas (Jefe, Farmacologia) for permission to collect samples of *Castela polyandra* in Baja California.

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