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## EREMOPHILANE DERIVATIVES FROM *PITTOCAULON PRAECOX*

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**Abstract** – Five new compounds; two eremophilane glucosides (**1**, **2**) and three eremophilanolides (**3**, **11**, **12**), together with several known compounds were isolated from *Pittocaulon praecox*. Their structures were elucidated by spectroscopic methods. The anti-inflammatory activity of extracts and main isolated metabolites was evaluated on 2-O-tetradecanoylphorbol-13-acetate (TPA) induced model of acute inflammation.

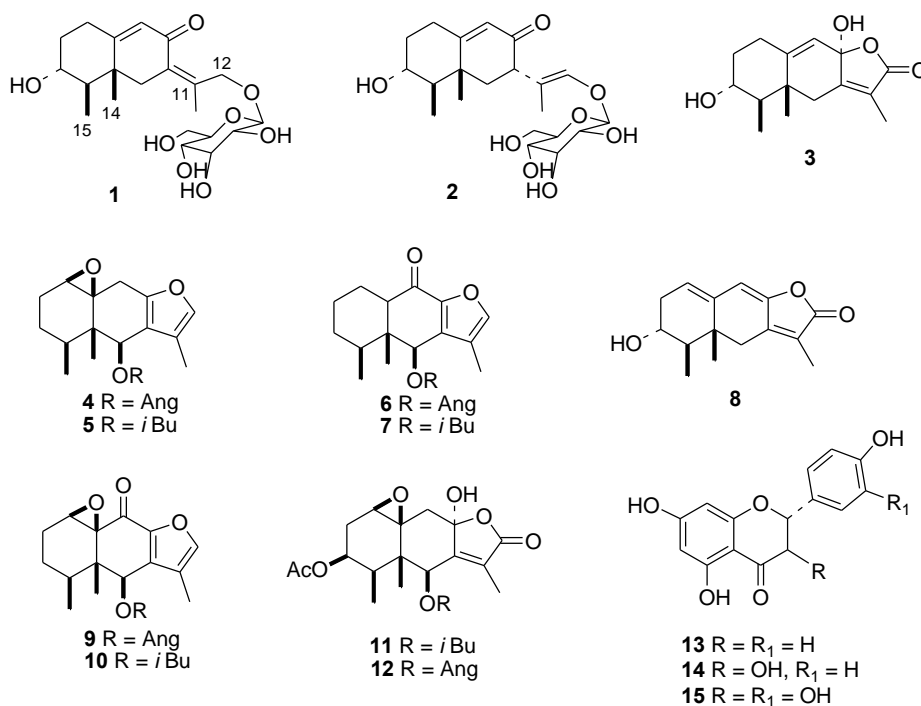
## INTRODUCTION

*Pittocaulon praecox* is one of the five species of the genus *Pittocaulon* (Asteraceae, Senecioneae, Tussilaginatae) recently segregated from the genus *Senecio*.<sup>1</sup> *P. praecox* is used in folk medicine to cure rheumatism and injuries.<sup>2</sup> Previous chemical studies showed the furanoeremophilanes as its main secondary metabolites.<sup>3,4</sup> The presence of pyrrolizidine alkaloids has also been reported.<sup>5</sup> Nevertheless as no one of the compounds already reported justified the anti-inflammatory activity attributed to *P. praecox* we undertook a new investigation in search of possible anti-inflammatory substances. Five new compounds, two glucosides of eremophilane (**1**, **2**) and three eremophilanolides (**3**, **11**, **12**), the known compounds **4-15**,<sup>4,6-14</sup> and the pyrrolizidine alkaloids senecionine and integerrimine<sup>15,16</sup> were obtained. The anti-inflammatory activity of extracts and compounds **1-7**, **9-12** was evaluated.

## RESULTS AND DISCUSSION

Compound **1** showed a molecular formula C<sub>21</sub>H<sub>32</sub>O<sub>8</sub> by HRFABMS. In the IR displayed bands at 3359,

1653 and 1605  $\text{cm}^{-1}$  and in the UV, absorptions at 204 and 248 nm suggesting the presence of hydroxyl and conjugated carbonyl groups. In the  $^{13}\text{C}$  NMR spectrum, the signal at  $\delta$  193.3 showed the presence of a carbonyl group. COSY and COLOC experiments allow to assign the signals at  $\delta$  103.2 (d), 78.3 (d), 77.9 (d), 75.0 (d), 71.7 (d), and 62.7 (t) to a sugar moiety, and those at  $\delta$  71.7 (d) and 69.9 (t) to C-3 and C-12, respectively. These experiments permitted to attribute the signal observed at  $\delta$  5.75 (d,  $J = 2.1$  Hz), in the  $^1\text{H}$  NMR spectrum, to H-9, as well as those at  $\delta$  4.87 (dd,  $J = 13.5, 1.5$  Hz) and  $\delta$  4.49 (dd,  $J = 13.5, 2.1$  Hz) to  $\text{CH}_2$ -12. Finally, the presence of three methyl group signals at  $\delta$  1.97 (d,  $J = 1.2$  Hz),  $\delta$  1.11 (d,  $J = 6.6$  Hz) and 1.05 (s) supported structure **1** (Figure 1). The sugar residue was identified as  $\beta$ -glucose by gas chromatography of the hydrolyzed product and by the coupling constant of its anomeric proton ( $\delta$  4.17, d,  $J = 7.6$  Hz). Linkage of the glucose moiety to C-12 was determined by the COLOC interaction observed between this carbon atom and the anomeric proton. The *Z* configuration of the 7(11) double bond was determined by the cross peaks of  $\text{CH}_3$ -13 with H-6 $\beta$ , observed in the NOESY experiment. In the same spectrum, the interaction between H-14 and H-3 which showed an axial-axial coupling allowed to propose the  $\alpha$ -orientation of the hydroxyl group at C-3, since on biogenetic grounds, the  $\text{CH}_3$ -14 is  $\beta$ -oriented.<sup>17</sup>

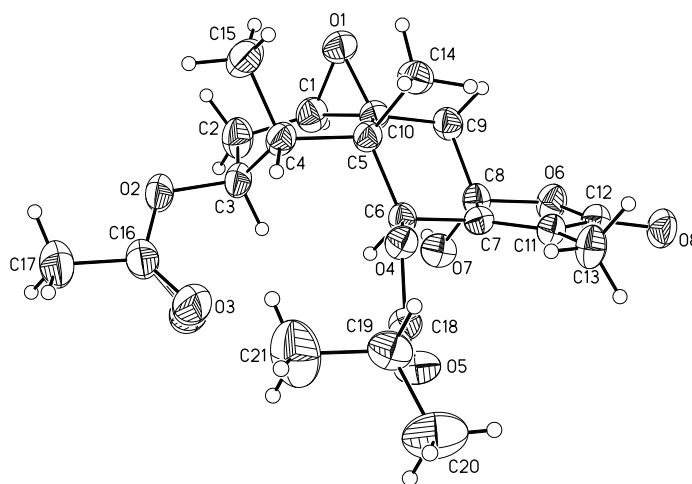


**Figure 1**

Compound **2** exhibited the same molecular formula and very similar spectroscopic data to those of compound **1**. In the  $^1\text{H}$  NMR spectrum of **2**, H-12 resonated at  $\delta$  6.30 (q,  $J = 1.5$  Hz) and its signal integrated for one hydrogen, H-7, absent in compound **1**, appeared at  $\delta$  3.03 (dd,  $J = 14.0, 5.0$  Hz), and the signals of  $\text{CH}_2$ -6 were localized at  $\delta$  1.97 (dd,  $J = 13.5, 5.0$  Hz) and  $\delta$  1.90 (dd,  $J = 14.0, 13.5$  Hz), in

accordance with structure **2**. NOESY correlations between H-7 and H-12 and CH<sub>3</sub>-14 suggested the E configuration of the C-11 double bond and the  $\beta$  orientation of H-7, the cross peak between H-3 and CH<sub>3</sub>-14, permitted to propose the  $\alpha$  orientation of the hydroxyl group. Because, the circular dichroism curve of **2** showed the same pattern as that reported for steroidal 4-en-3-ones,<sup>18</sup> its absolute stereochemistry should be 3R, 4R, 5R, 7S.

Eremophilanolid **3** presented a molecular formula C<sub>15</sub>H<sub>21</sub>O<sub>4</sub> by HRFABMS. The IR bands at 3351 and 1755 cm<sup>-1</sup> indicated the presence of hydroxyl and unsaturated  $\gamma$  lactone groups. In the <sup>1</sup>H NMR spectrum, the signal of a proton at  $\delta$  3.46 (ddd,  $J = 10.5, 10.5, 4.5$  Hz), geminal to OH, was attributed to H-3. Since this proton showed an axial-axial coupling and correlated with the CH<sub>3</sub>-14 in the NOESY experiment, the hydroxyl group should be  $\alpha$ -equatorial. At higher field, the signals of CH<sub>3</sub>-13 ( $\delta$  1.78, d,  $J = 1.5$  Hz), CH<sub>3</sub>-14 ( $\delta$  0.97 s) and CH<sub>3</sub>-15 ( $\delta$  1.10, d,  $J = 6.5$  Hz) provided an eremophilanolid profile where C-8 ( $\delta$  100.9 s) was bearing a hydroxyl group. This hydroxyl group should be  $\alpha$ -orientated by the homoallylic coupling between H-13 ( $\delta$  1.78 d,  $J = 1.5$ ) and H-6 $\alpha$  ( $\delta$  2.38, br d,  $J = 12.5$  Hz).<sup>19</sup> The orientation of H-6 $\alpha$  was determined by its NOESY interaction with H-4, while H-6 $\beta$  interacted with CH<sub>3</sub>-14 and CH<sub>3</sub>-15, in the same experiment. The signal at  $\delta$  5.66 (d,  $J = 2.0$  Hz) was assigned to the vinylic proton 9, according to structure **3**.

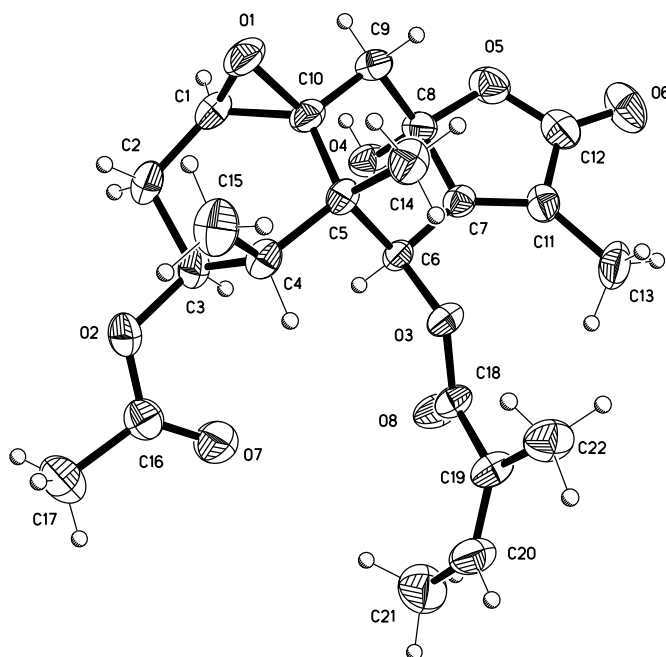


**Figure 2. ORTEP projection of 11 (crystallographic numbering)**

Eremophilanolid **11** presented a molecular formula C<sub>21</sub>H<sub>28</sub>O<sub>8</sub> by HRFABMS. The presence of hydroxyl and carbonyl groups were deduced by the absorption bands observed in the IR spectrum (3346, 1743, 1729, 1701 cm<sup>-1</sup>). In the <sup>1</sup>H NMR spectrum, the signals at  $\delta$  5.88 (d,  $J = 1.5$  Hz) and 4.86 (ddd,  $J = 11.5, 7.0, 4.0$  Hz) were assigned to the hydrogen atoms, geminal to ester functions, at C-6 and C-3, respectively, based on COSY and HMBC experiments. The ester group at C-6 was identified as isobutyrate by the

cross peaks observed, in the HMBC spectrum, between C-1' ( $\delta$  174.9) and H-6 ( $\delta$  5.88), while H-3 ( $\delta$  4.86) correlated with the carbonyl of the acetate group ( $\delta$  169.5). In the  $^{13}\text{C}$  NMR spectrum, 21 carbon atom signals were observed, six of them being attributed to ester functions, the remaining 15 signals corresponded to an eremophilanolide profile. The presence of an epoxide ring at C-1, C-10 ( $\delta$  59.4 d, 60.7 s) was indicated by bidimensional NMR experiments. The NOESY experiment provided cross peaks of the OH with H-6, H-1 and H-9 $\alpha$ , of H-3 with H-6 and H-4 and between H-9 $\beta$  and H-14, suggesting that the OH, H-1, H-3, H-4, and H-6 should be  $\alpha$ -oriented. Structure **11** was confirmed by an X-ray analysis (Figure 2).

Compound **12** showed a molecular formula  $\text{C}_{22}\text{H}_{28}\text{O}_8$  by HRFABMS. The spectroscopic data of **11** and **12** were very similar. The  $^1\text{H}$  and  $^{13}\text{C}$  NMR spectra together with the HMBC experiment of **12** showed the presence of an ageloyloxy group ( $\delta$  6.27, qq; 2.02, dq; 1.97, quint) bonded at C-6 instead of the isobutyroyloxy observed in **11**. Structure and relative stereochemistry of compound **12** were confirmed by X-ray analysis (Figure 3).



**Figure 3.** ORTEP projection of **12** (crystallographic numbering)

Compounds **5** was previously reported in mixture,<sup>4</sup> and compound **8** was obtained as intermediary of synthesis,<sup>10</sup> therefore the spectroscopic data of both compounds are included in the experimental section. The structures of known compounds **4**, **6**, **7**, **9-10**, **12-14** were determined by comparison of their physical constants and spectroscopic features with those reported in literature. Senecionine and integerrimine were identified by comparison with authentic samples and spectroscopic data.

The anti-inflammatory activity was tested on 12-O-tetradecanoylphorbol-13-acetate (TPA) induced model of acute inflammation.<sup>20</sup> The MeOH extracts of roots and stems and the hexanic extract of stems showed mild activity with 39.9, 30.4, 34.3 % of edema inhibition, respectively, while the EtOAc extract of stems exhibited the highest activity with 66.6 % of edema inhibition. Compounds **1-7**, **9-12** were also tested and unfortunately none of them show any relevant activity. Since *P. praecox* is used to cure rheumatism in folk medicine, this effect is probably related with the presence of the flavonoids naringenine (**13**), aromadendrin (**14**), and taxifolin (**15**) whose anti-inflammatory activity has been widely reported.<sup>21,22</sup>

## EXPERIMENTAL

**General experimental procedures.** Melting points were determined on a Fisher-Jones melting point apparatus and are uncorrected. Optical rotations were determined on a Perkin-Elmer 343 polarimeter. UV and IR spectra were recorded on a Shimadzu UV 160U and a Bruker Tensor 27 spectrometer, respectively. 1D and 2D NMR spectra were obtained on an Eclipse JEOL 300 MHz, Bruker Avance 300 MHz or a Varian-Unity Inova 500 MHz spectrometer with tetramethylsilane (TMS) as internal standard. EIMS data were determined on a Bruker Daltonics Analysis 3.2 mass spectrometer. HRFABMS were performed at 10,000 resolution using electric field scans and polyethylene glycol ions (Fluka 200 and 300) as reference material. Circular dichroism was obtained on a Jasco J-720 spectropolarimeter. Column chromatography was carried out under vacuum on silica gel G 60 (Merck, Darmstadt, Germany). Flash chromatography was performed on silica gel 60 (230-400 Macherey-Nagel). TLC was carried out on Si gel 60 and preparative TLC on Si gel GF<sub>254</sub> (Merck), layer thickness 2.0 mm. GC analyses was performed on an Agilent 6890 GC system with AT5 30 m x 0.25 mm 0.1  $\mu$ m film thickness column at 100°C initial temperature raised in 10 minutes to 200°C final temperature. X-ray crystallographic analyses were realized on a Bruker Smart Apex CCD diffractometer with graphite-monochromated Mo K $\alpha$  radiation ( $\lambda = 0.71073$  Å). The structures were solved by direct methods using the program SHELXS. Non-hydrogen atoms were refined with anisotropic displacement parameters. Hydrogen atoms were included at calculated positions and were not refined.

**Plant material.** *Pittocaulon praecox* from Pedregal de San Angel Mexico D.F. and grown in Ozumba, Mexico State, Mexico, was collected in march 2007. A voucher specimen (MEXU 1236997) was deposited at the Herbario del Instituto de Biología, Universidad Nacional Autónoma de México.

**Extraction and isolation.** Dried and ground root barks (1.8 kg) were extracted with MeOH at rt to obtain 250 g of extract. A portion (85 g) of this extract was submitted to reductive process<sup>23</sup> to obtain the alkaloidal residue (3.1 g). Purification of this residue by VCC (CH<sub>2</sub>Cl<sub>2</sub>-MeOH gradient system) produced senecionine (5 mg, mp 227-230 °C, MeOH),<sup>15,16</sup> and integerrimine (4.3 mg, mp 170-172 °C, MeOH).<sup>15,16</sup> The remaining MeOH extract (165 g) was fractionated through a VCC (EtOAc-MeOH gradient system)

to obtain fraction A (eluted with EtOAc), fraction B (eluted with EtOAc-MeOH 19:1, 9:1 and 4:1) and fraction C (eluted with EtOAc-MeOH 1:1 and MeOH). Fraction A (55 g) was purified by VCC eluted with hexane-EtOAc gradient system to obtain fraction D (eluted with hexane and hexane-EtOAc 19:1) and fraction E (eluted with hexane-EtOAc 9:1). Purification of fraction D (35 g) by VCC (hexane-EtOAc gradient system) afforded fractions F (eluted with hexane-EtOAc 49:1) and G (eluted with hexane-EtOAc 19:1). Fraction F, after two successive chromatographies (hexane-EtOAc 49:1), produced a mixture of compounds **4** and **5** (1.2 g), purification of 200 mg of this mixture by preparative TLC eluted with hexane-EtOAc 99:1 produced senemorin (**4**, 125 mg,  $[\alpha]_D^{25} - 18.4^\circ$ ,  $c$  0.14,  $\text{CHCl}_3$ )<sup>6</sup> and compound **5** (18 mg,  $[\alpha]_D^{25} - 34.6^\circ$ ,  $c$  0.13,  $\text{CHCl}_3$ ).<sup>4</sup> Fraction G (19.2 g), after purification by VCC eluted with hexane-EtOAc 49:1, produced compounds **6** (1.1 g,  $[\alpha]_D^{25} - 54.5^\circ$ ,  $c$  0.20,  $\text{CHCl}_3$ ),<sup>7</sup> **7** (0.7 g,  $[\alpha]_D^{25} - 38.2^\circ$ ,  $c$  0.22,  $\text{CHCl}_3$ )<sup>8</sup> and a mixture (3:2) of compounds **6** and **7** (10.8 g). Purification of fraction E (9.5 g) by flash column (hexane-EtOAc 19:1) afforded **8** (30 mg, mp 190-192 °C,  $[\alpha]_D^{25} - 94^\circ$ ,  $c$  0.25, MeOH),<sup>10</sup> **9** (75 mg,  $[\alpha]_D^{25} - 37.2^\circ$ ,  $c$  0.14,  $\text{CHCl}_3$ ),<sup>9</sup> **10** (60 mg,  $[\alpha]_D^{25} - 16.0^\circ$ ,  $c$  0.20,  $\text{CHCl}_3$ ),<sup>9</sup> a mixture (3:2) of compound **9** and **10** (5.8 g) and compound **11** (17 mg). Fraction B produced by crystallization (EtOAc-MeOH) compound **1** (5.8 g). Further purification of mother liquors of **1** (70 g) by VCC (EtOAc-MeOH in increasing gradient) produced fraction H (eluted with EtOAc-MeOH 49:1) and 5.5 g of **1**, from fractions eluted with EtOAc-MeOH 19:1, purification of this last mother liqueurs (8.5 g) by a new chromatography eluted with EtOAc-MeOH 49:1 afforded 2.8 g of **1** and 1.2 g of compound **2**. Fraction H (10 g) by flash column (EtOAc-MeOH 49:1) afforded **2** (1.2 g) and **3** (75 mg). Purification of fraction C (31 g) by VCC (EtOAc-MeOH gradient system) produced compound **1** (105 mg) and sucrose (5.5 g).

Dry and ground stem bark (1.7 kg) was extracted successively with hexane, EtOAc and MeOH. The hexane extract (122 g) was fractionated by VCC (hexane-EtOAc gradient mixture) to obtain from fractions eluted with hexane, after two successive chromatographies (hexane-EtOAc 49:1), compounds **6** (1.4 g), **7** (0.9 g) and 15.5 g of a mixture (3:2) of both compounds. From fractions eluted with hexane-EtOAc 19:1 (30 g) after purification by VCC (hexane-EtOAc 49:1) 1.2 g of **6**, 0.8 of **7** and 13.8 g of their mixture (3:2) were obtained. The EtOAc extract (76 g) was purified by VCC eluted with hexane-EtOAc in increasing gradient. Fractions eluted with hexane (8 g) were purified by flash chromatography (hexane-EtOAc 49:1) to produce compounds **6** (180 mg), **7** (140 mg) and 5.3 g of their mixture. From fractions eluted with hexane-EtOAc 17:3 (3 g) after two successive chromatographies eluted with hexane-Me<sub>2</sub>CO 19:1 and CH<sub>2</sub>Cl<sub>2</sub>, respectively, compounds **11** (75 mg) and **12** (80 mg) were obtained. Fractions eluted with hexane-EtOAc 4:1 (13 g) were purified by a new VCC (hexane-EtOAc 9:1) to obtain naringenin (**13**, 370 mg, mp 255-256 °C,  $[\alpha]_D^{25} - 21.5^\circ$ ,  $c$  0.25, MeOH).<sup>11,12</sup> Purification of fraction obtained with

hexane-EtOAc 7:3 (20 g) by VCC eluted with hexane-EtOAc 4:1, afforded **13** (60 mg), aromadendrin (**14**, 320 mg, mp 238-240 °C,  $[\alpha]_D^{25} + 11.9^\circ$ ,  $c$  0.13, EtOH),<sup>13</sup> a mixture (1:1) of **13** and **14** (3.2 g) and taxifolin (**15**, 13 mg, mp 218-220 °C,  $[\alpha]_D^{25} + 18.5^\circ$ ,  $c$  0.2, Me<sub>2</sub>CO).<sup>14</sup> Fractions eluted with EtOAc (10.5 g) were purified using EtOAc-MeOH mixture in increasing gradient to obtain compound **1** (670 mg), compound **2** (250 mg) and a mixture (2:1) of **1** and **2** (4.5 g). The MeOH extract (220 g) was fractionated with EtOAc-MeOH mixture in increasing gradient. Fractions eluted with EtOAc (20 g) produced by purification by VCC (hexane-EtOAc gradient mixture) sitosterol β-glucoside (550 mg), **13** (135 mg) and **14** (120 mg). Fractions eluted with EtOAc-MeOH 9:1 (80 g) afforded after purification by VCC column (EtOAc-MeOH 98:2) **1** (5.3g), **2** (7.8 g) and 48 g of a mixture (2:1) of both compounds.

**Compound 1:** Colorless needles (EtOAc-MeOH); mp 204-206 °C;  $[\alpha]_D^{25} -25.4^\circ$  ( $c$  0.28, MeOH); UV (MeOH)  $\lambda_{\max}$  (log  $\epsilon$ ): 204 (4.1), 248 nm (4.2); IR (Nujol):  $\nu$  3359, 1653, 1605 cm<sup>-1</sup>; <sup>1</sup>H NMR (CD<sub>3</sub>OD, 300 MHz):  $\delta$  5.75 (1H, d,  $J = 2.1$  Hz, H-9), 4.87 (1H, dd,  $J = 13.5, 1.5$  Hz, H-12a), 4.49 (1H, dd,  $J = 13.5, 2.1$  Hz, H-12b), 4.17 (1H, d,  $J = 7.6$  Hz, H-1'), 3.80 (1H, dd,  $J = 12.0, 2.4$  Hz, H-6'a), 3.65 (1H, dd,  $J = 12.0, 5.4$  Hz, H-6'b), 3.54 (1H, ddd,  $J = 11.4, 11.4, 4.2$  Hz, H-3), 3.33 (2H, m, H-3', H-4'), 3.19 (1H, m, H-2'), 3.14 (1H, m, H-5'), 3.02 (1H, d,  $J = 13.5$  Hz, H-6β), 2.50 (1H, dddd,  $J = 15.0, 15.0, 5.1, 2.1$  Hz, H-1β), 2.36 (1H, ddd,  $J = 15.0, 4.5, 2.7$  Hz, H-1α), 2.19 (1H, br d,  $J = 13.5$  Hz, H-6α), 2.13 (1H, m, H-2β), 1.97 (3H, d,  $J = 1.2$  Hz, H-13), 1.43 (1H, m, H-2α), 1.39 (1H, m, H-4), 1.11 (3H, d,  $J = 6.6$  Hz, H-15), 1.05 (3H, s, H-14); <sup>13</sup>C NMR (CD<sub>3</sub>OD, 75.4 MHz):  $\delta$  193.3 (C, C-8), 171.2 (C, C-10), 144.5 (C, C-11), 132.2 (C, C-7), 126.3 (CH, C-9), 103.2 (CH, C-1'), 78.3 (CH, C-4')<sup>a</sup>, 77.9 (CH, C-5'), 75.0 (CH, C-2'), 71.7 (CH, C-3')<sup>a</sup>, 71.7 (CH, C-3), 69.9 (CH<sub>2</sub>, C-12), 62.7 (CH<sub>2</sub>, C-6'), 50.6 (CH, C-4), 43.8 (C, C-5), 42.4 (CH<sub>2</sub>, C-6), 36.4 (CH<sub>2</sub>, C-2), 31.7 (CH<sub>2</sub>, C-1), 17.9 (CH<sub>3</sub>, C-14), 16.5 (CH<sub>3</sub>, C-13), 11.1 (CH<sub>3</sub>, C-15).<sup>a</sup>Exchangeable signals. MS (EI, 70 eV)  $m/z$  (%): 412 (2) [M]<sup>+</sup>, 250 (100), 233 (30); HRMS (FAB<sup>+</sup>)  $m/z$ : 413.2175 (calcd. 413.2175 for C<sub>21</sub>H<sub>33</sub>O<sub>8</sub>, [M+H]<sup>+</sup>).

**Compound 2:** Colorless needles (EtOAc-MeOH); mp 133-135 °C;  $[\alpha]_D^{25} + 32.6^\circ$  ( $c$  0.17, MeOH); UV (MeOH)  $\lambda_{\max}$  (log  $\epsilon$ ): 237 nm (4.1); IR (Nujol):  $\nu$  3387, 1656, 1618 cm<sup>-1</sup>; CD (MeOH)  $\Delta\epsilon_{\lambda_{\max}}$ :  $\Delta\epsilon_{270} - 1.39$ ,  $\Delta\epsilon_{236} + 8.10$ ,  $\Delta\epsilon_{205} + 13.75$  ( $c$  3.4 x 10<sup>-5</sup> M); <sup>1</sup>H NMR (CD<sub>3</sub>OD, 500 MHz):  $\delta$  6.30 (1H, q,  $J = 1.5$  Hz, H-12), 5.73 (1H, d,  $J = 1.5$  Hz, H-9), 4.49 (1H, d,  $J = 7.5$  Hz, H-1'), 3.84 (1H, dd,  $J = 13.5, 1.5$  Hz, H-6'a), 3.66 (1H, dd,  $J = 13.5, 4.5$  Hz, H-6'b), 3.56 (1H, ddd,  $J = 11.0, 11.0, 4.5$  Hz, H-3), 3.37 (1H, m, H-5'), 3.35 (1H, m, H-2'), 3.32 (2H, m, H-3', H-4'), 3.03 (1H, dd,  $J = 14.0, 5.0$  Hz, H-7), 2.53 (1H, dddd,  $J = 14.5, 14.5, 5.0, 1.5$  Hz, H-1β), 2.33 (1H, ddd,  $J = 14.5, 4.0, 2.5$  Hz, H-1α), 2.12 (1H, m, H-2β), 1.97 (1H, dd,  $J = 13.5, 5.0$  Hz, H-6β), 1.90 (1H, dd,  $J = 14.0, 13.5$  Hz, H-6α), 1.56 (3H, d,  $J = 1.5$  Hz, H-13), 1.40 (1H, m, H-2α), 1.29 (1H, m, H-4), 1.20 (3H, s, H-14), 1.05 (3H, d,  $J = 6.5$  Hz, H-15); <sup>13</sup>C NMR (CD<sub>3</sub>OD,

125 MHz):  $\delta$  201.9 (C, C-8), 171.7 (C, C-10), 142.9 (CH, C-12), 124.6 (CH, C-9), 114.5 (C, C-11), 103.7 (CH, C-1'), 78.1 (CH, C-4')<sup>a</sup>, 77.7 (CH, C-5'), 74.3 (CH, C-2'), 71.1 (CH, C-3')<sup>a</sup>, 71.1 (CH, C-3), 62.4 (CH<sub>2</sub>, C-6'), 51.3 (CH, C-4), 43.1 (CH<sub>2</sub>, C-6), 40.9 (C, C-5), 36.0 (CH<sub>2</sub>, C-2), 31.9 (CH<sub>2</sub>, C-1), 17.3 (CH<sub>3</sub>, C-14), 10.6 (CH<sub>3</sub>, C-13), 10.6 (CH<sub>3</sub>, C-15). <sup>a</sup>Exchangeable signals. MS (EI, 70 eV)  $m/z$  (%): 250 (55), 233 (20), 125 (100); HRMS (FAB<sup>+</sup>)  $m/z$ : 413.2175 (calcd. 413.2175 for C<sub>21</sub>H<sub>33</sub>O<sub>8</sub>, [M+H]<sup>+</sup>).

**Compound 3:** Colorless prisms (hexane-EtOAc); mp 175-177 °C;  $[\alpha]_D^{25}$  -31.2° (*c* 0.25, Me<sub>2</sub>CO); UV (MeOH)  $\lambda_{\max}$  (log  $\epsilon$ ): 226 nm (3.9); IR (KBr):  $\nu$  3351, 1755, 1651 cm<sup>-1</sup>; <sup>1</sup>H NMR, (Me<sub>2</sub>CO-*d*<sub>6</sub>, 500 MHz):  $\delta$  5.66 (1H, d, *J* = 2.0 Hz, H-9), 3.46 (1H, ddd, *J* = 10.5, 10.5, 4.5 Hz, H-3), 2.88 (1H, d, *J* = 12.5 Hz, H-6 $\beta$ ), 2.38 (1H, br d, *J* = 12.5 Hz, H-6 $\alpha$ ), 2.28 (1H, dddd, *H* = 14.5, 14.5, 5.0, 2.0 Hz, H-1 $\beta$ ), 2.13 (1H, ddd, *J* = 14.5, 7.5, 4.5 Hz, H-1 $\alpha$ ), 2.03 (1H, m, H-2 $\beta$ ), 1.78 (3H, d, *J* = 1.5 Hz, H-13), 1.41 (1H, dq, *J* = 10.5, 6.5 Hz, H-4), 1.31 (1H, m, H-2 $\alpha$ ), 1.10 (3H, d, *J* = 6.5 Hz, H-15), 0.97 (3H, s, H-14); <sup>13</sup>C NMR (Me<sub>2</sub>CO-*d*<sub>6</sub>, 125 MHz):  $\delta$  171.9 (C, C-12), 159.1 (C, C-7), 150.3 (C, C-10), 122.6 (C, C-11), 120.3 (CH, C-9), 100.9 (C, C-8), 71.1 (CH, C-3), 51.6 (CH, C-4), 46.3 (C, C-5), 37.1 (CH<sub>2</sub>, C-6), 36.9 (CH<sub>2</sub>, C-2), 31.0 (CH<sub>2</sub>, C-1), 19.1 (CH<sub>3</sub>, C-14), 11.2 (CH<sub>3</sub>, C-15), 8.2 (CH<sub>3</sub>, C-13), - MS (EI, 70 eV)  $m/z$  (%): 265 (3), 246 (35), 219 (100), 178 (50); HRMS (FAB<sup>+</sup>)  $m/z$ : 265.1440 (calcd. 265.1440 for C<sub>15</sub>H<sub>21</sub>O<sub>4</sub>, [M+H]<sup>+</sup>).

**Compound 5:** Yellow oil;  $[\alpha]_D^{25}$  -34.6° (*c* 0.13, CHCl<sub>3</sub>); IR (CHCl<sub>3</sub>):  $\nu$  1728, 1464, 1152, 964 cm<sup>-1</sup>; <sup>1</sup>H NMR (CDCl<sub>3</sub>, 300 MHz):  $\delta$  7.07 (1H, br s, H-12), 6.35 (1H, q, *J* = 1.2 Hz, H-6), 3.23 (1H, dd, *J* = 16.8, 1.8 Hz, H-9a), 3.09 (1H, d, *J* = 5.1 Hz, H-1), 2.65 (1H, hept., *J* = 7.2 Hz, H-2'), 2.18 (1H, d, *J* = 16.8 Hz, H-9b), 2.05 (1H, m, H-3a), 1.95-1.83 (2H, m, H-2a, H-3b), 1.85 (3H, d, *J* = 1.2 Hz, H-13), 1.52 (1H, ddq, *J* = 14.4, 7.2, 3.6 Hz, H-4), 1.34 (1H, m, H-2b), 1.26 (3H, s, H-14), 1.24 (6H, d, *J* = 7.2 Hz, H-3' and H-4'), 1.07 (3H, d, *J* = 7.2 Hz, H-15); <sup>13</sup>C NMR (CHCl<sub>3</sub>, 75 MHz):  $\delta$  177.0 (C, C-1'), 148.4 (C, C-8), 138.9 (CH, C-12), 119.7 (C, C-7), 116.9 (C, C-11), 69.3 (CH, C-6), 63.3 (C, C-10), 62.9 (CH, C-1), 41.0 (C, C-5), 34.4 (CH, C-4), 32.1 (CH, C-2'), 30.6 (CH<sub>2</sub>, C-9), 23.6 (CH<sub>2</sub>, C-3), 19.9 (CH<sub>2</sub>, C-2), 19.5 (CH<sub>3</sub>, C-3'), 18.7 (CH<sub>3</sub>, C-4'), 16.7 (CH<sub>3</sub>-C-14), 15.2 (CH<sub>3</sub>, C-15), 8.6 (CH<sub>3</sub>, C-13).

**Compound 8:** Yellow needles (EtOAc-MeOH); mp 190-192 °C;  $[\alpha]_D^{25}$  -94.0° (*c* 0.15, MeOH); IR (KBr):  $\nu$  3471, 1665 cm<sup>-1</sup>; <sup>1</sup>H NMR (CD<sub>3</sub>OD, 500 MHz):  $\delta$  5.86 (1H, s, H-9), 5.67 (1H, dd, *J* = 6.0, 2.5 Hz, H-1), 3.70 (1H, ddd, *J* = 10.0, 10.0, 6.0 Hz, H-3), 2.90 (1H, d, *J* = 16.5 Hz, H-6 $\beta$ ), 2.62 (1H, ddd, *J* = 19.0, 6.0, 6.0 Hz, H-2 $\beta$ ), 2.22 (1H, dd, *J* = 16.5, 1.5 Hz, H-6 $\alpha$ ), 2.10 (1H, ddd, *J* = 19.0, 10.0, 2.5 Hz, H-2 $\alpha$ ), 1.83 (3H, d, *J* = 1.5 Hz, H-13), 1.67 (1H, dq, *J* = 10.0, 7.0 Hz, H-4), 1.11 (3H, d, *J* = 7.0 Hz, H-15), 0.97 (3H, s, H-14); <sup>13</sup>C NMR (CD<sub>3</sub>OD, 125 MHz):  $\delta$  175.2 (C, C-12), 142.1 (C, C-10), 141.2 (C, C-7)<sup>a</sup>, 137.0 (C, C-8)<sup>a</sup>, 127.2 (CH, C-1), 126.4 (C, C-11), 111.8 (CH, C-9), 69.2 (CH, C-3), 47.3 (CH, C-4), 40.9 (C, C-5), 36.9 (CH<sub>2</sub>, C-2), 35.8 (CH<sub>2</sub>, C-6), 20.8 (CH<sub>3</sub>-C-14), 10.7 (CH<sub>3</sub>, C-15), 7.9 (CH<sub>3</sub>, C-13).

<sup>a</sup>Exchangeable signals.

**Compound 11:** Colorless prisms (EtOAc-MeOH); mp 258-260 °C;  $[\alpha]_D^{25}$  -46.0° (*c* 0.1.5, DMSO); UV (MeOH)  $\lambda_{\max}$  (log  $\epsilon$ ): 214 nm (3.8); IR (KBr):  $\nu$  3346, 1743, 1729, 1701  $\text{cm}^{-1}$ ; <sup>1</sup>H NMR (DMSO-*d*<sub>6</sub>, 500 MHz):  $\delta$  7.78 (s, OH), 5.88 (1H, d, *J* = 1.5, H-6), 4.86 (1H, ddd, *J* = 11.5, 7.0, 4.0 Hz, H-3), 3.19 (1H, d, *J* = 5.5, Hz, H-1), 2.70 (1H, hept, *J* = 7.0 Hz, H-2'), 2.32 (1H, d, *J* = 13.5 Hz, H-9 $\beta$ ), 2.26 (1H, ddd, *J* = 14.0, 7.0, 6.0 Hz, H-2 $\alpha$ ), 1.98 (3H, s, OAc), 1.90 (1H, br d, *J* = 13.0 Hz, H-2 $\beta$ ), 1.85 (1H, m, H-4), 1.74 (3H, d, *J* = 1.5 Hz, H-13), 1.67 (1H, d, *J* = 13.5 Hz, H-9 $\alpha$ ), 1.17 (3H, d, *J* = 7.0 Hz, H-3'), 1.14 (3H, d, *J* = 7.0 Hz, H-4'), 1.08 (3H, s, H-14), 0.91 (3H, d, *J* = 7.5 Hz, H-15); <sup>13</sup>C NMR (DMSO-*d*<sub>6</sub>, 125 MHz):  $\delta$  174.9 (C, C-1'), 170.6 (C, C-12), 169.5 (C, OAc), 154.4 (C, C-7), 123.3 (C, C-11), 101.1 (C, C-8), 71.2 (CH, C-6), 67.3 (CH, C-3), 60.7 (C, C-10), 59.4 (CH, C-1), 44.1 (C, C-5), 41.8 (CH<sub>2</sub>, C-9), 33.3 (CH, C-4), 33.1 (CH, C-2'), 24.4 (CH<sub>2</sub>, C-2), 20.8 (CH<sub>3</sub>, OAc), 18.9 (CH<sub>3</sub>, C-3'), 18.3 (CH<sub>3</sub>, C-4'), 15.0 (CH<sub>3</sub>, C-14), 9.1 (CH<sub>3</sub>, C-15), 8.2 (CH<sub>3</sub>, C-13); MS (EI, 70 eV) *m/z* (%): 408 (10) [M]<sup>+</sup>, 320 (30), 260 (30), 71 (100); HRMS (FAB<sup>+</sup>) *m/z*: 409.1865 (calcd. 409.1862 for C<sub>21</sub>H<sub>29</sub>O<sub>8</sub>, [M+H]<sup>+</sup>).

**Compound 12:** Colorless prisms (EtOAc); mp 210-212 °C;  $[\alpha]_D^{25}$  -111.1° (*c* 0.27, CHCl<sub>3</sub>); UV (MeOH)  $\lambda_{\max}$  (log  $\epsilon$ ): 213.5 nm (3.6); IR (KBr):  $\nu$  3364, 1732, 1709  $\text{cm}^{-1}$ ; <sup>1</sup>H NMR (Me<sub>2</sub>CO-*d*<sub>6</sub>, 500 MHz):  $\delta$  6.68 (s, OH), 6.27 (1H, qq *J* = 7.5, 1.5 Hz, H-3'), 6.11 (1H, q, *J* = 1.5 Hz, H-6), 5.04 (1H, ddd, *J* = 11.5, 7.0, 4.5 Hz, H-3), 3.24 (1H, d, *J* = 6.0 Hz, H-1), 2.39 (1H, d, *J* = 13.0 Hz, H-9 $\beta$ ), 2.36 (1H, ddd, *J* = 12.5, 7.0, 6.0 Hz, H-2 $\alpha$ ), 2.02 (1H, dq, *J* = 7.5, 1.5 Hz, H-4'), 1.97 (3H, quint, *J* = 1.5 Hz, H-5'), 1.99-1.95 (1H, m, H-2 $\beta$ ), 1.96 (3H, s, OAc), 1.97-1.95 (1H, m, H-4), 1.79 (1H, d, *J* = 19.0 Hz, H-9 $\alpha$ ), 1.75 (3H, d, *J* = 1.5 Hz, H-13), 1.23 (3H, s, H-14), 1.00 (3H, d, *J* = 7.5 Hz, H-15); <sup>13</sup>C NMR (Me<sub>2</sub>CO-*d*<sub>6</sub>, 125 MHz):  $\delta$  171.3 (C, C-12), 170.3 (C, OAc), 166.6 (C, C-1') 155.1 (C, C-7), 141.2 (CH, C-3'), 127.7 (C, C-2'), 124.9 (C, C-11), 101.9 (C, C-8), 72.3 (CH, C-6), 68.4 (CH, C-3), 61.7 (C, C-10), 60.9 (CH, C-1), 45.4 (C, C-5), 43.2 (CH<sub>2</sub>, C-9), 35.0 (CH, C-4), 25.7 (CH<sub>2</sub>, C-2), 20.9 (CH<sub>3</sub>, OAc)<sup>a</sup>, 20.6 (CH<sub>3</sub>, C-5')<sup>a</sup>, 16.0 (CH<sub>3</sub>, C-4'), 16.0 (CH<sub>3</sub>, C-14), 9.5 (CH<sub>3</sub>, C-15), 8.0 (CH<sub>3</sub>, C-13). <sup>a</sup>Exchangeable signals. MS (EI, 70 eV) *m/z* (%): 420 (2), 320 (10), 83 (100); HRMS (FAB<sup>+</sup>) *m/z*: 421.1869 (calcd. 421.1862 for C<sub>22</sub>H<sub>29</sub>O<sub>8</sub>, [M+H]<sup>+</sup>).

**Acid Hydrolysis of Compounds 1 and 2:** Compounds **1** and **2** (100 mg each) were separately heated at 70 °C for 0.5 h in 10% HCl. The reaction mixtures were extracted with CH<sub>2</sub>Cl<sub>2</sub> and the aqueous layers were evaporated, silylated with HMDS:TMCS:pyridine (3:1:9) and analyzed by GC to identify glucose as the sugar present in both compounds **1** and **2**.

**Crystal data of 11:** C<sub>21</sub>H<sub>28</sub>O<sub>8</sub>, *M<sub>r</sub>* 408.43, orthorhombic, space group *P* 2<sub>1</sub> 2<sub>1</sub> 2<sub>1</sub>, *a* = 8.4002 (6) Å,  $\alpha$  = 90°, *b* = 11.0620 (8) Å,  $\beta$  = 90°, *c* = 22.1000 (16) Å;  $\gamma$  = 90°, *V* = 2053.6(3) Å<sup>3</sup>, *Z* = 4, *D<sub>c</sub>* = 1.321 Mg/m<sup>3</sup>, *F*(000) = 872; crystal dimensions / shape / color 0.34 x 0.16 x 0.08 mm / prism / colorless. Reflections collected 22413, independent reflections 3770. Number of parameters refined 274; final *R* indices

[I>2 $\sigma$ (I)]  $RI = 0.0356$ ,  $wR^2 = 0.0783$ ;  $R$  indices (all data)  $R = 0.0465$ ,  $wR^2 = 0.0815$ .

**Crystal data of 12:**  $C_{22}H_{28}O_8$ ,  $M_r$  420.44, orthorhombic, space group  $P 2_1 2_1 2_1$ ,  $a = 9.664$  (1) Å,  $\alpha = 90^\circ$ ,  $b = 11.878$  (2) Å,  $\beta = 90^\circ$ ,  $c = 18.889$  (2) Å;  $\gamma = 90^\circ$ ,  $V = 2168.1$ (4) Å<sup>3</sup>,  $Z = 4$ ,  $D_c = 1.288$  Mg/m<sup>3</sup>,  $F(000) = 896$ ; crystal dimensions / shape / color 0.484 x 0.382 x 0.298 mm / block / colorless. Reflections collected 21590, independent reflections 2917. Number of parameters refined 338; final  $R$  indices [I>2 $\sigma$ (I)]  $RI = 0.0553$ ,  $wR^2 = 0.1302$ ;  $R$  indices (all data)  $R = 0.0653$ ,  $wR^2 = 0.1371$ .

Crystallographic data for **11** (CCDC-715671) and **12** (CCDC-715672) are available free of charge via the Internet at <http://www.ccdc.cam.ac.uk/conts/retrieving.html> (or from the CCDC, 12 Union Road, Cambridge CB2 1EZ, U.K.; fax: + 441223336033; [deposit@ccdc.cam.ac.uk](mailto:deposit@ccdc.cam.ac.uk)).

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