Notes

Structure and Plant Growth Regulatory Activity of New Diterpenes from *Pterodon polygalaeflorus*

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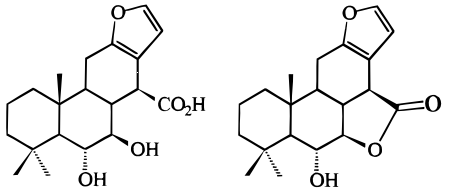
Two new furanditerpenes, vouacapane-6α,7*β*,17*β*-triol (**3**) and vouacapane-6α,7*β*,17*β*,19-tetraol (**4**), have been isolated from the seeds of *Pterodon polygalaeflorus* Benth. The selective effects of these two diterpenes and of 6α,7*β*-dihydroxyvouacapan-17*β*-oic acid (**1**) and 6α-hydroxyvouacapano-7*β*,17*β*-lactone (**2**) on the radicle growth of *Sorghum bicolor* and *Cucumis sativus* were evaluated.

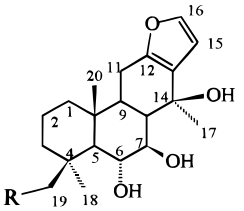
The genus *Pterodon* (Leguminosae) is comprised of five native species in Brazil: *P. abruptus* Vog., *P. apparicioi* Pedersoli, *P. emarginatus* Benth, *P. polygalaeflorus* Benth, and *P. pubescens* Benth.[1,2]

Oil from the fruits of *P. pubescens* inhibits skin penetration of *Schistosoma mansoni* cercaria, and this activity has been attributed to 14,15-dihydroxygeranylgeraniol.[2] An alcoholic infusion of the seeds of *P. polygalaeflorus* or *P. apparicioi* is traditionally used by the population of Goias and Minas Gerais states in Brazil to treat throat infections. Further chemical investigation[3] of the seed oil of *P. emarginatus* led to the isolation of 6α,7*β*-dihydroxyvouacapan-17*β*-oic acid (**1**) and 6α-hydroxyvouacapano-7*β*,17*β*-lactone (**2**). These two compounds were shown to have antiinflammatory and analgesic activity.[4] Since this discovery we have been engaged in the preparation of several derivatives of the acid **1**,[4−7] and polyols **3** and **4** were isolated during the routine isolation of quantities of **1** required for study of structure−activity relationships.

Compound **3** was isolated from *P. polygalaeflorus* Benth (Leguminosae) seeds as a white solid, mp 199−201 °C, and the molecular formula, $C_{20}H_{30}O_4$, was derived from HRMS and ^{13}C-NMR spectra. Its IR spectrum showed hydroxyl absorptions bands at 3560 (sharp, free OH) and 3425 (broad, hydrogen bonded OH) cm^{-1} and for a furan ring at 1678 and 1510 cm^{-1} (C=C).

The ^{13}C-NMR spectrum exhibited signals corresponding to four CH_3, four CH_2, seven CH, and five nonhydrogenated carbons. The presence of three hydroxyl groups was suggested by signals ($CDCl_3$) at δ 78.87 and 74.12 for CH and δ 72.46 for C and by the disappearance of three signals which were not observed in pyridine-d_5 solution ^{1}H-NMR at δ 4.02 (d, J = 1.3 Hz), 3.43 (s) and 2.18 (d, J = 4.6 Hz).





3: R=H
4: R=OH

The ^{1}H-NMR spectrum confirmed the presence of four methyl groups by the signals at δ 1.69 (s, CH_3-17), 1.54 (s, CH_3-18), 1.29 (s, CH_3-19), and 1.03 (s, CH_3-20). Each assignment was confirmed by NOE difference experiments as shown in Figure 1. Stereochemistries at C-6 and C-7 were the same for all the furanditerpenes previously isolated from *Pterodon*, and this was clear from the large NOE observed at H-6 (3.4%) when CH_3-20 was irradiated and from the absence of any significant NOE at H-7 when H-8 was irradiated. The absence of NOE at the methyl attached to carbon-14 when H-8 was irradiated confirmed the proposed stereochemistry at C-14 as having the OH group in the *β*-position (Figure 1). Full ^{1}H- and ^{13}C-NMR assignments were completed by analysis of HH-COSY, HC-HETCOR, DEPT, and NOE experiments.

Mahajan and Monteiro[3] previously proposed structure **3** for a compound isolated from *P. emarginatus*. The structure was proposed on the basis of elemental

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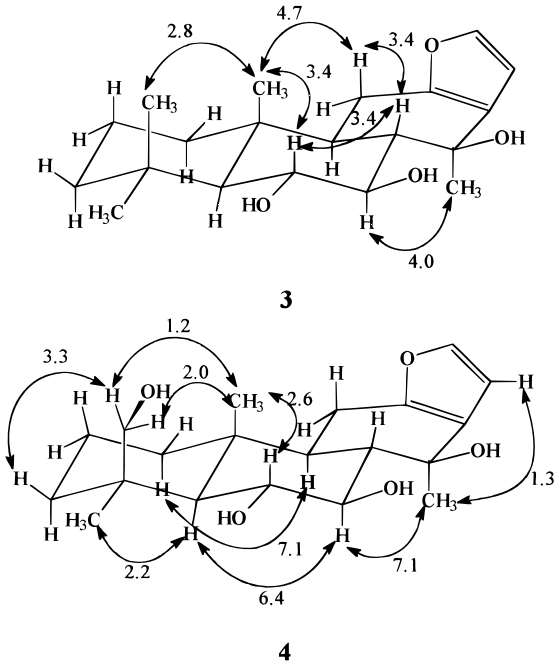

Figure 1. Major NOE data for compounds **3** and **4**.

analysis, IR, and degradation studies, but no NMR data were reported. The published melting point for compound **3** (218−222 °C) was higher than the value (199−201 °C) found by us. Also, the IR data reported[3] showed only one stretching band for OH at 3413 cm^{-1}, while our data for compound **3** revealed two distinct OH stretching bands at 3560 and 3425 cm^{-1}. In view of these differences in spectroscopic and physical data we believe that the compound isolated by Mahajan and Monteiro[3] could be an isomer of triol **3**.

Furanditerpene (**4**)[8] analyzed for $C_{20}H_{30}O_5$ by HRMS. Its IR spectrum showed a broad band at 3150−3550 cm^{-1}, with maxima at 3450, 3400, and 3280 cm^{-1}. The ^{1}H-NMR ($CDCl_3$) spectrum was very similar to that of compound **3**, but only three methyl signals were present (δ 1.47, 1.25, and 1.08). However, four hydroxyl groups were observed in $CDCl_3$ at δ 4.15 (s), 3.90 (d, J = 3.0 Hz), 3.79 (s) and 2.24 (bt, J_1 = 3.1 Hz, J_2 = 2.8 Hz) that were absent in pyridine-d_5. Also, hydrogens H-19 and H-19′ appeared as a pair of double doublets at δ 4.27 (J_1 = 10.3 Hz, J_2 = 3.1 Hz) and δ 3.39 (J_1 = 10.3 Hz, J_2 = 2.8 Hz). In pyridine-d_5, these signals appeared at δ 4.00 and 3.90 respectively.

The NOE observed at H-19 (2.0%) and H-19′ (1.2%) when the methyl at C-10 was irradiated and at H-5 (2.2%) when CH_3-18 was irradiated confirmed the β-(hydroxymethyl) stereochemistry at C-4 (Figure 1). ^{1}H-NMR and ^{13}C-NMR assignments presented were made on the basis of HH-COSY, HC-HETCOR, DEPT, and NOE experiments.

The antiinflammatory activity of compound **1** and some other furanditerpenes has been reported,[4] but no report on the plant growth regulatory activity of this class of compound is available. Allelopathic activity of monoterpenes is widely reported,[9] but little is known about the effect of diterpenes on plant growth.[10] Compounds **1**−**4** were then submitted to a plant growth bioassay[11] to evaluate their effect on the radicle growth of *Sorghum bicolor* and *Cucumis sativus*. The experiments were carried out at two concentrations (100 and 1000 ppm) of each compound since it is known that some

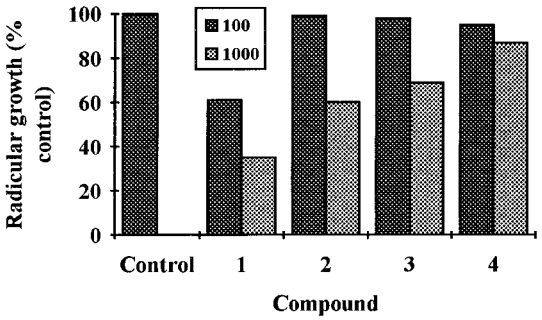

Figure 2. Effect of compounds **1**−**4** on the radicular growth of *S. bicolor* at 100 and 1000 ppm after 3 days of incubation at 25 °C.

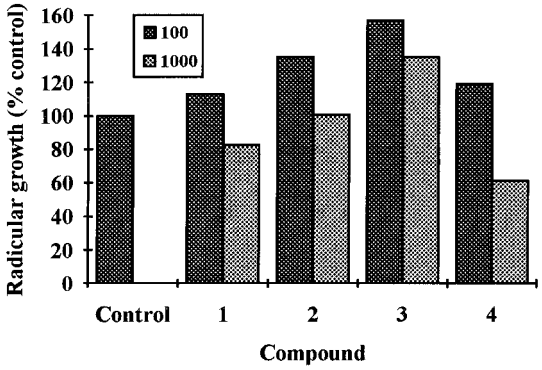

Figure 3. Effect of compounds **1**−**4** on the radicular growth of *C. sativus* at 100 and 1000 ppm after 3 days of incubation at 25 °C.

compounds exhibit both stimulatory and inhibitory effects on seedling growth depending on the concentration.[10] Germination rate was not affected by any of these compounds. Diterpene **1** showed a 39% inhibitory effect on the radicular growth of *S. bicolor* at 100 ppm, and this effect was even higher (65%) at 1000 ppm. The other three compounds (**2**−**4**) showed a small inhibitory effect at 100 ppm, which increased to 13−40% at 1000 ppm (Figure 2). All compounds showed a stimulatory effect on radicular growth at 100 ppm for *C. sativus*. This effect decreased at 1000 ppm for compounds **2** and **3**, while compounds **1** and **4** were significantly inhibitory (Figure 3).

Experimental Section

General Experimental Procedures. IR spectra were recorded with a Perkin-Elmer 881 double-beam grating spectrophotometer. NMR spectra were recorded with a Bruker WH 400 spectrometer (400 MHz), using TMS as an internal standard. Mass spectra were recorded on a V. G. Analytical ZAB-IF spectrometer. Flash chromatography was performed using Crosfield Sorbil C60 (40−60 μm), and the solvents used were purified according to Perrin and Armarego.[12] The melting points were corrected.

Plant Material. Seeds of *P. polygalaeflorus* Benth were collected in Três Marias-MG in September 1992. A voucher specimen (no. 10714) is deposited at the Herbarium of Department of Biology of Federal University of Viçosa.

Extraction and Isolation. Seeds were ground and extracted in a Soxhlet apparatus with hexane for 40 h. The hexane extract was submitted to column chromatography on silica gel,[3] eluting with hexane:ether (1:10), to afford compounds **3** (R_f = 0.40) and **4** (R_f = 0.22).

Vouacapane-6α,7β,17β-triol (3): white solid; mp 199−201 °C (lit.[3] mp 218−220 °C); IR (KBr) ν_{max} 3560, 3425, 3000, 2925, 1875, 1678, 1510, 1468, 1370, 1280, 1150, 1090, 910, and 725 cm^{-1}; ^{1}H-NMR (pyridine-d_5, 400 MHz) δ 1.03 (s, 20-CH_3), 1.03 (btd, $J_1 \cong J_2 = 12.8$ Hz, $J_3 = 3.5$ Hz, H-1α), 1.04 (d, $J = 12.2$ Hz, H-5), 1.29 (s, 19-CH_3), 1.35 (btd, $J_1 \cong J_2 = 12.8$ Hz, $J_3 = 4.1$ Hz, H-1β), 1.54 (s, 18-CH_3), 1.59−1.71 (m, H-3α, H-3β), 1.63−1.67 (m, H-9), 1.69 (s, 17-CH_3), 2.26 (dd, $J_1 = 12.5$ Hz, $J_2 = 10.1$ Hz, H-8), 2.47 (dd, $J_1 = 16.6$ Hz, $J_2 = 10.5$ Hz, H-11β), 2.67 (dd, $J_1 = 16.6$ Hz, $J_2 = 6.5$ Hz, H-11α), 4.02−4.09 (m, H-6 and H-7), 6.71 (d, 1.8 Hz, H-15), 7.47 (d, 1.8 Hz, H-16); ^{1}H-NMR ($CDCl_3$, 400 MHz) δ 0.95 (dt, $J_1 \cong J_2 = 12.7$ Hz, $J_3 = 4.0$ Hz, H-1α), 0.99 (s, 20-CH_3), 1.03 (d, $J = 11.1$ Hz, H-5), 1.08 (s, 19-CH_3), 1.18 (s, 18-CH_3), 1.23 (ddt, $J_1 \cong J_2 = 12.7$ Hz, $J_3 = 4.1$ Hz, H-3β), 1.37−1.43 (m, H-9), 1.40−1.68 (m, H-1β, H-2α, H-2β, H-3α), 1.45 (s, 17-CH_3), 1.96 (dd, $J_1 = 12.4$ Hz, $J_2 = 10.4$ Hz, H-8), 2.18 (d, $J = 4.6$ Hz, 6-OH), 2.41 (dd, $J_1 = 16.7$ Hz, $J_2 = 10.6$ Hz, H-11β), 2.58 (dd, $J_1 = 16.7$ Hz, $J_2 = 6.6$ Hz, H-11α), 3.43 (s, 14-OH), 3.67 (bt, $J_1 \cong J_2 = 9.1$ Hz, H-7), 3.82 (ddd, $J_1 = 11.1$ Hz, $J_2 = 9.1$ Hz, $J_3 = 4.6$ Hz, H-6), 4.02 (d, $J = 1.3$ Hz, 7-OH), 6.40 (d, 1.9 Hz, H-15), 7.23 (d, 1.9 Hz, H-16); ^{13}C-NMR (pyridine-d_5, 100 MHz) δ 149.17 (C-12), 141.70 (C-16), 125.51 (C-13), 108.41 (C-15), 78.87 (C-7), 74.12 (C-6), 72.46 (C-14), 55.76 (C-5), 49.04 (C-8), 47.38 (C-9), 44.26 (C-3), 40.18 (C-1), 38.78 (C-10), 37.30 (C-18), 33.90 (C-4), 26.64 (C-17), 22.96 (C-19), 22.91 (C-11), 19.00 (C-2), 16.17 (C-20); HRMS (70 eV) *m/z* 334.2135 (M^+, $C_{20}H_{30}O_4$, requires 334.2136, 2), 319 (79), 301 (100), 283 (31), 173 (9), 145 (22), 131 (7), 109 (26), 81 (16), 69 (20), 43 (25).

Vouacapane-6α,7β,17β,19-tetraol (4): white solid; mp 181−183 °C; IR (KBr) ν_{max} 3500−3200, 3010, 2950, 2860, 1650, 1518, 1480, 1350, 1250, 1080, 1030, 1010, 900, and 690 cm^{-1}; ^{1}H-NMR (pyridine-d_5, 400 MHz) δ 0.98−1.02 (m, H-1α), 0.99 (s, 20-CH_3), 1.17 (dt, $J_1 \cong J_2 = 12.8$ Hz, $J_3 = 4.1$ Hz, H-3α), 1.35−1.40 (m, H-2α), 1.46−1.48 (m, H-2β), 1.43 (d, $J = 11.1$ Hz, H-5), 1.55 (bd, $J = 12.6$ Hz, H-1β), 1.63 (ddd, $J_1 = 12.0$ Hz, $J_2 = 10.6$ Hz, $J_3 = 6.5$ Hz, H-9), 1.71 (s, 18-CH_3), 1.73 (s, 17-CH_3), 1.94 (bd, $J = 13.4$ Hz, H-3β), 2.36 (dd, $J_1 = 12.0$ Hz, $J_2 = 10.5$ Hz, H-8), 2.39 (dd, $J_1 = 16.6$ Hz, $J_2 = 10.6$ Hz, H-11β), 2.62 (dd, $J_1 = 16.6$ Hz, $J_2 = 6.5$ Hz, H-11α), 3.90 (d, $J = 10.5$ Hz, H-19′), 4.00 (d, $J = 10.5$ Hz, H-19), 4.16 (dd, $J_1 = 10.5$ Hz, $J_2 = 8.7$ Hz, H-7), 4.37 (dd, $J_1 = 11.1$ Hz, $J_2 = 8.7$ Hz, H-6), 6.81 (d, 1.7 Hz, H-15), 7.54 (d, 1.7 Hz, H-16); ^{1}H-NMR ($CDCl_3$, 400 MHz) δ 1.00−1.08 (m, H-3α), 1.08 (s, 20-CH_3), 1.13 (d, $J = 11.3$ Hz, H-5), 1.18−1.25 (m, H-2α), 1.25 (s, 18-CH_3), 1.39−1.45 (m, H-1α and H-9), 1.45−1.55 (m, H-1β, H-2β), 1.47 (s, 17-CH_3), 1.72 (dm, H-3β), 2.02 (dd, $J_1 = 12.5$ Hz, $J_2 = 10.5$ Hz, H-8), 2.24 (bt, $J_1 = 3.1$ Hz, $J_2 = 2.8$ Hz, 19-OH), 2.42 (dd, $J_1 = 16.6$ Hz, $J_2 = 10.6$ Hz, H-11β), 2.60 (dd, $J_1 = 16.6$ Hz, $J_2 = 6.5$ Hz, H-11α), 3.39 (dd, $J_1 = 10.3$ Hz, $J_2 = 2.8$ Hz, H-19′), 3.69 (dd, $J_1 = 10.5$ Hz, $J_2 = 9.7$ Hz, H-7), 3.79 (s, 7-OH), 3.90 (d, $J = 3.0$ Hz, 6-OH), 4.04 (ddd, $J_1 = 11.3$ Hz, $J_2 = 9.7$ Hz, $J_3 = 3.0$ Hz, H-6), 4.15 (s, 14-OH), 4.27 (dd, $J_1 = 10.3$ Hz, $J_2 = 3.1$ Hz, H-19), 6.44 (d, $J = 1.9$ Hz, H-15), 7.24 (d, $J = 1.9$ Hz, H-16); ^{13}C-NMR (pyridine-d_5, 100 MHz) δ 148.94 (C-12), 141.72 (C-16), 125.71 (C-13), 108.73 (C-15), 78.36 (C-7), 74.21 (C-6), 72.39 (C-14), 66.49 (C-19), 56.08 (C-5), 49.20 (C-8), 47.59 (C-9), 40.26 (C-1), 39.29 (C-4), 39.25 (C-3), 38.16 (C-10), 32.15 (C-18), 26.94 (C-17), 23.03 (C-11), 18.82 (C-2), 16.98 (C-20); HRMS *m/z* 350.2084 (M^+, $C_{20}H_{30}O_5$, requires 350.2085, 3), 335 (100), 317 (72), 299 (16), 269 (16), 131 (10), 109 (27), 81 (13), 43 (29).

Bioassays. Bioassays were carried out according to the method of Einhelling *et al.*[10] with seeds of *S. bicolor* and *C. sativus*. CH_2Cl_2 solutions of compounds **1**−**4** were prepared at concentrations of 100 and 1000 ppm.

Assays were conducted in 100 × 15 mm glass Petri dishes lined with one sheet of Whatman no. 1 filter paper and sealed with Parafilm. To each dish was added 2 mL of each solution, and the solvent was evaporated before addition of 2 mL of water followed by 20 seeds of one of the two species. Assays were carried out at 25 °C under fluorescent light (8 × 40 W) in an incubator for 3 days. Radicle length was measured, and total germination was recorded. Seeds were considered to have germinated if a radicle protruded at least 1 mm. A control experiment was carried out under the same conditions described, using only water. Each bioassay was replicated four times in a complete randomized design.

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