Notes

Trinorsesquiterpenoids from the Root Extract of *Pentalinon andrieuxii*

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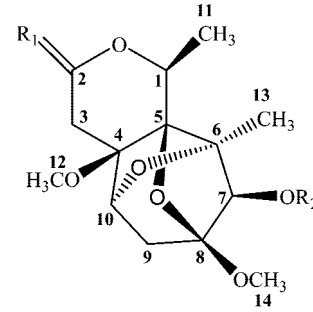
Two unusual trinorsesquiterpenoids, urechitols A (**1**) and B (**2**), were isolated from the root extract of *Pentalinon andrieuxii*, a plant used commonly in Yucatecan traditional medicine to treat leishmaniasis. The structures of **1** and **2** were identified by interpretation of their spectroscopic data and chemical correlation reactions. The relative stereochemistry of **1** was confirmed through an X-ray crystallographic study.

Pentalinon andrieuxii Muell.-Arg. (syn.: *Urechites andrieuxii*; Apocynaceae) is one of the most commonly used plants in the Yucatan Peninsula for the treatment of cutaneous eruptions (rashes) derived from leishmaniasis, an infectious disease caused by protozoan parasites of the *Leishmania* genus.[1,2] In Yucatecan traditional medicine, an infusion of the roots of *P. andrieuxii* is used to wash the lesions, and this is followed by an application of the dry-ground root on the lesion. Additionally, a masticate resulting from chewing the roots and fresh leaves of the plant is used for the treatment of snake bites, while the latex is recommended to alleviate headaches and nervous disturbances.[3,4] Recently, it has been reported that both the aqueous and organic extracts from the roots of *P. andrieuxii* exhibit activity when tested against parasites of *Leishmania mexicana* and that the metabolites responsible for the biological activity detected are of medium polarity and present in an acidic fraction.[1,2]

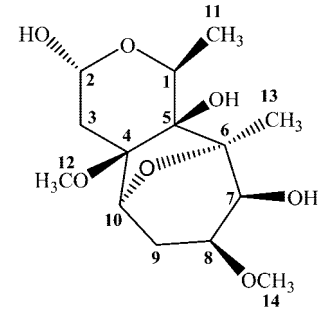
As part of our search for new leishmanicidal agents from native Yucatecan medicinal plants, we report herein the isolation and identification of the nonactive, but structurally unusual, trinorsesquiterpenoids **1** and **2**.

The methanolic root extract of *P. andrieuxii* was extracted with CH_2Cl_2 using an ultrasonic bath to obtain a dichloromethane-soluble fraction, which was subjected to an acid−base extraction. The acidic fraction was then purified using a combination of flash column chromatography and gravity column chromatography, to produce two metabolites, **1** and **2**, in pure form.

The HREIMS of the more abundant, and more polar, metabolite **1** showed a molecular ion peak at *m*/*z* 325.1284, corresponding to a molecular formula of $C_{14}H_{22}O_7Na$, which implied the presence of four unsaturation sites in the structure.[5] The IR spectrum of **1** exhibited a strong hydroxyl group absorption band at 3426 cm^{-1} and no bands in the carbonyl region, indicating that all seven oxygen atoms in the molecular formula were in either hydroxyl or ether forms in the structure.[5] Furthermore, the lack of sp^2 carbon signals in the ^{13}C NMR spectrum of **1** strongly suggested that the four unsaturation sites implied by the molecular formula corresponded to a tetracyclic structure. The nature of all carbon atoms was established through a combined analysis of the ^{13}C NMR spectrum



1	R_1= H β,	OH α	R_2= H
3	R_1= H β,	Ac α	R_2= Ac
4	R_1= H β,	OCH_3 α	R_2= H
5	R_1= O		R_2= H



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and the HSQC, DEPT-135, and DEPT-90 experiments and indicated the presence of four methyl groups, two methylenes, four methines, and four quaternary carbons (Table 1). The chemical shift values of all quaternary carbons (δ_C 80.5, 83.7, 89.9, and 104.6) and all the carbons of the methine groups (δ_C 70.4, 74.9, 79.6, and 87.9) clearly indicated that they were all bonded to oxygen. Additionally, the chemical shift values of two of the four methyl group signals (δ_C 51.9 and 52.2) also indicated their being bonded to oxygen.[6−8]

The ^1H NMR spectrum of **1** showed the two three-proton singlets at δ 3.33 and 3.49, corresponding to the two methoxyl groups, in addition to two methyl group signals appearing as a doublet (δ 1.42, 6.9 Hz) and a singlet (δ 1.58); this suggested their being attached to a methine group and a quaternary carbon, respectively,

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Table 1. NMR Spectroscopic Data for Urechitol A (**1**)

position	δ_C, mult.	δ_H (J in Hz)	HMBC (C→H)
1	70.4, CH	4.18, c (6.9, 14.0)	2, 4, 5, 11
2	87.9, CH	5.28, dd (2.7, 9.6)	
3	31.8, CH_2	1.72, dd (9.7, 14.5)	2, 4
		2.36, dd (2.7, 14.0)	4,5
4	80.5, qC		
5	83.7, qC		
6	89.9, qC		
7	79.6, CH	3.59, s	5
8	104.6, qC		
9	30.9, CH_2	1.79, dd (4.1, 12.9)	8, 10
		2.42, dd (0.75, 12.8)	7, 8, 10
10	74.9, CH	4.13, d (3.9)	4, 5, 6, 8, 9
11	14.5, CH_3	1.42, d (6.9)	1, 5
12	51.9, CH_3	3.33, s	4
13	15.17, CH_3	1.58, s	5, 6, 7
14	52.2, CH_3	3.49, s	8

Chemical shift values are given in parts per million (ppm) relative to the solvent signal (7.26 and 77.0 ppm for ^1H and ^{13}C NMR spectra, respectively); coupling constants are given in Hz.

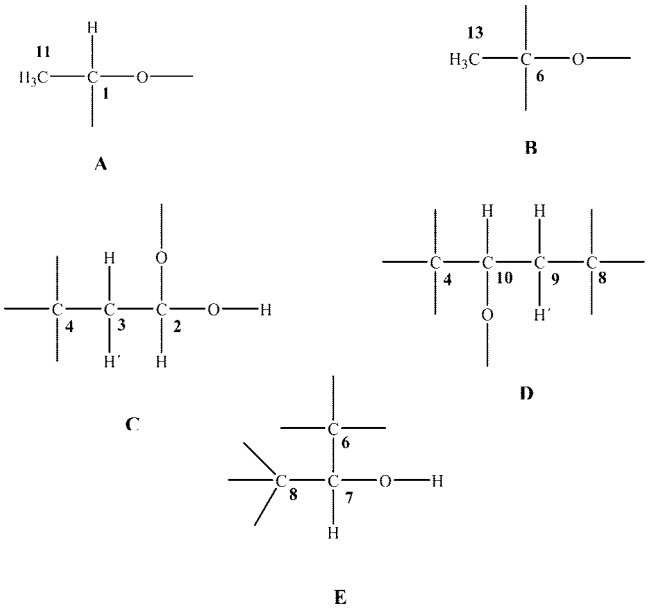

Figure 1. Structural subunits of urechitol A (**1**).

and allowed the construction of the first structural subunits **A** and **B** (Figure 1). Furthermore, a careful analysis of the $^1H-^1H$ COSY correlations allowed the construction of structural subunits **C**, **D**, and **E** (Figure 1), where the methine-proton signal at δ 5.28 in subunit **C** showed a coupling to the two protons of an isolated methylene group at δ 1.72 (m) and 2.36 (dd, J = 2.7, 14.0 Hz). The presence of a hemiacetal group in the structural subunit **C** explained the low field of both the proton (δ 5.28) and carbon (δ 87.9) signals of the methine group. In the subunit **D**, the methine proton appearing as a doublet (J = 3.9 Hz) at δ 4.13 showed a clear correlation to the protons of a second isolated methylene group at 1.79 (d, J = 12.8 Hz) and 2.42 (d, J = 12.4 Hz). Finally, the methine proton of subunit **E**, appearing as a one-proton singlet at δ 3.59, confirmed its being bonded to two quaternary carbons.

The protons of all methyl, methylene, and methine groups accounted for 20 of the 22 protons included in the molecular formula of **1**, suggesting that the remaining protons are part of two hydroxyl groups. This was confirmed by the treatment of **1** with a mixture of pyridine and acetic anhydride to produce the corresponding diacetylated derivative, **3**. The ^1H NMR spectrum of **3** showed the two expected singlets at δ 2.11 and 2.13, characteristic of the methyl in an acetyl group, and the downfield shift for the

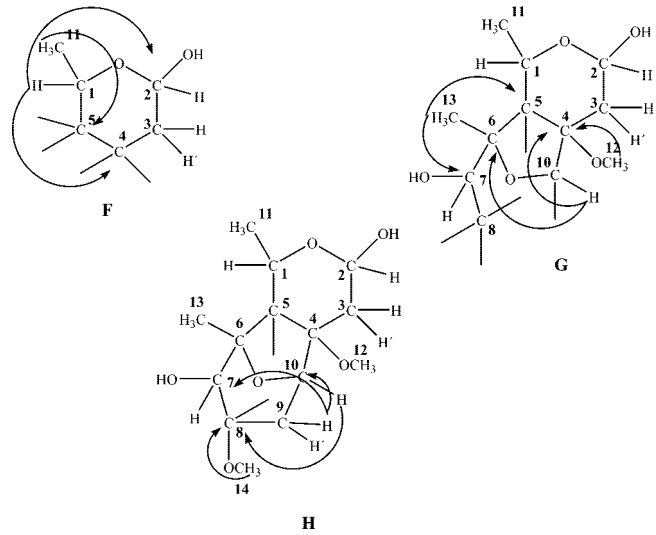

Figure 2. Connections and key HMBC correlations of urechitol A (**1**).

methine protons in structural subunits **C** (δ 5.28 to 6.13) and **E** (δ 3.59 to 4.94).

A detailed analysis of the HMBC experiment of **1** permitted the connection of the structural subunits and the construction of the gross structure; a ^3J correlation between H-1 and C-2/C-4 indicated that subunits **A** and **C** are connected through an oxygen atom. In turn, a ^3J correlation between H-11 and an unassigned quaternary carbon (C-5) suggested that C-1 and C-5 connect to complete the six-membered ring of structural subunit **F** (Figure 2). Linking of structural subunits **F** and **D** was confirmed by a ^2J correlation between H-10 and C-4. Furthermore, the ^3J correlation observed between the protons of one of the methoxyl groups (H-12) and C-4 allowed placement of the ether group at C-4. The ^3J correlation between H-10 and C-6 indicated that structural subunits **B** and **D** are connected through an oxygen atom, while the ^3J correlation between H-13 and C-7/C-5 allowed the connection of the structural subunits **B** and **E** to produce the five-membered ring subunit **G** (Figure 2). Finally, a ^2J correlation between the C-9 methylene protons and C-8/C-10, together with a ^3J correlation between the three protons of the second methoxyl group and C-8, and a ^3J correlation between H-9 and C-7, aided in the construction of tricyclic structural subunit **H** (Figure 2).

Structural subunit **H** includes six of the seven oxygen atoms of the molecular formula of **1**; the remaining oxygen atom can then be assigned to an ether linkage between C-8 and C-5 to produce the proposed tetracyclic structure for **1**. Both the structure and relative stereochemistry of **1** were confirmed unambiguously by a single-crystal X-ray diffraction experiment (Figure 3). A thorough literature search indicated that metabolite **1** is a new natural product with what appears to be a novel skeleton, and we have designated it with the common name urechitol A.

Methylation of **1**, under mild conditions (K_2CO_3 and CH_3I), produced a monomethylated derivative (**4**), which, in its ^1H NMR spectrum, showed a third three-proton singlet in the methoxyl group region (δ 3.32) and the expected high-field displacement (δ 5.28 to 4.82) of the H-2 signal. Similarly, treatment of **1** with PCC led to the formation of an unexpected monoxidized derivative (**5**); the absence of the H-2 signal and the presence of the H-7 methine proton signal in the ^1H NMR spectrum of the oxidized product, together with the absorption bands at 1736 (lactone carbonyl) and 3477 cm^{-1} (hydroxyl group) in its IR spectrum, indicated that, under these conditions, only the hemiacetal hydroxyl group is oxidized.

The IR spectrum of metabolite **2** showed, as in the case of **1**, the presence of a strong hydroxyl group absorption at 3436 cm^{-1} and the absence of absorption bands in the carbonyl region.

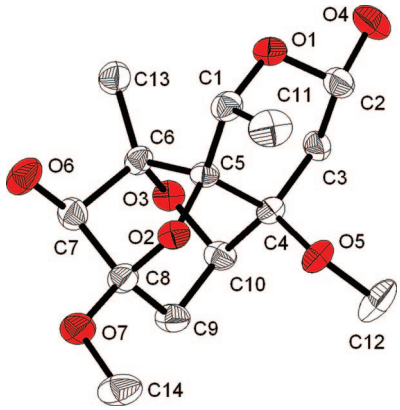

Figure 3. Single-crystal X-ray structure and relative stereochemistry of urechitol A (**1**).

Similarly, the ^{1}H NMR spectrum of **2** showed two methoxyl group singlets at δ 3.45 and 3.49, in addition to two methyl group signals at δ 1.27 (d, $J = 6.0$ Hz) and 1.42 (s), which suggested a structure similar to **1**. However, the observed molecular ion peak at *m/z* 304 in the HREIMS of **2**, corresponding to a formula of $C_{14}H_{24}O_7$, indicated one less unsaturation site in the structure of **2**. The presence of a new methine proton at δ 3.82 in the ^{1}H NMR spectrum of **2**, together with the absence of the C-8 signal at δ 104.6 observed in the ^{13}C NMR of **1**, and the presence of a methine-carbon signal at 78.1 in that of **2**, confirmed the proposed tricyclic structure for the new metabolite. Being that **2** is also a new natural product and taking into account its structural similarity to **1**, we have given it the trivial name urechitol B.

The extraction of the roots of *P. andrieuxii* with ethanol showed the presence of both **1** and **2** in the corresponding crude ethanolic extract, thus ruling out the possibility of the methoxyl groups in **1** and **2** originating from using methanol as the extraction solvent.

Existing phytochemical knowledge on the genus *Pentalinon* is limited, with there being a single reference on the isolation and identification of cardenolides and pyrrolizidine alkaloids having antitumor and hepatotoxic activity.[9] This is the first report on the isolation and identification of secondary metabolites from *P. andrieuxii* and the first report of the novel "campechane" trinorsesquiterpenoid skeleton.

Experimental Section

General Experimental Procedures. Analytical TLC was carried out using aluminum-backed silica gel ($60F_{254}$) plates (E.M. Merck, 0.2 mm thickness). Chromatographic purifications were performed using E.M. Merck silica gel (70−230 mesh). The various components in the chromatograms were visualized using a solution of phosphomolibdic acid (20 g) and ceric sulfate (2.5 g) in 500 mL of sulfuric acid (5%). The optical rotation was measured in $CHCl_3$ using a Perkin-Elmer 341 polarimeter. IR spectra were recorded in $CHCl_3$ (film) using an FT-Nicolet Magna Protégé 460 spectrophotometer. ^{1}H NMR (400 MHz) and ^{13}C NMR (100 MHz) spectra were obtained in $CDCl_3$ on a Bruker Avance 400 spectrometer using the residual $CHCl_3$ signal (7.26 and 77.00 ppm for ^{1}H and ^{13}C, respectively) as reference. GC analyses were run on a Hewlett-Packard 5890 gas chromatograph [GC conditions: split injection of 1 μL of sample; ZB-50 column (30 m × 0.32 mm i.d.), flow rate 1 mL/min (Nitrogen); oven temperature program $T_1 =$ 180 °C (2 min), $T_2 =$ 270 °C (5 min), gradient 10 °C/min, injector 300° and detector (FID) 300 °C]. Mass spectra were performed with a JEOL-JMS-SX102 and ESI-HRMS (electro-spray ionization mass with the Waters Q-TOF microsystem) using 0.1% phosphoric acid in a 1:1 water/acetonitrile mixture as reference.

Plant Material. The roots of *P. andrieuxii* were collected in March 2003 from a field located 3.5 k northeast of the city of Campeche, on the road to Chiná, Campeche, México. The plant was identified by taxonomist Paulino Simá-Polanco, and a voucher specimen has been deposited in the herbarium of the Unidad de Recursos Naturales of the Centro de Investigación Científica de Yucatán (CICY) under the collection number PSimá-2245 and folio number 46178.

Extraction and Isolation. The plant material was washed with tap water and dried, first for a week at room temperature and then for 72 h in an oven at 55 °C. The dry roots (1.02 kg) were cut, ground, and extracted four times with MeOH (2 L), at room temperature. The extracts were combined and the solvent was removed under reduced pressure to produce 136 g of crude methanolic extract. Sonication (ultrasound bath Cole-Parmer 8853) of the crude methanolic extract with CH_2Cl_2 (800 mL) for 5 h yielded 40.5 g of a medium- to low-polarity fraction, which was then subjected to an acid−base extraction to produce the corresponding acidic (2.15 g), basic (3.39 g), and neutral (11.68 g) fractions. Successive column chromatography (EtOAc/Hx/MeOH, 60:36:4, and CH_2Cl_2/MeOH, 97:3) purifications of the acid fraction led to the isolation of **1** (28 mg) and **2** (4 mg) in pure form.

Urechitol A (1): colorless needles (hexane/acetone); mp 156−158 °C; $[\alpha]^{27}_D$ −20 (*c* 0.01, $CHCl_3$); IR ($CHCl_3$, film) ν_{max} 3426 (OH), 2939 and 2832 (C−H), 1081 and 1045 (C−O−C) cm^{-1}; ^{1}H NMR ($CDCl_3$, 400 MHz) and ^{13}C NMR ($CDCl_3$, 100 MHz), see Table 1; HRESIMS *m/z* 325.1284 [M$^+$] (calcd for $C_{14}H_{22}O_7Na$, 325.1263); TLC R_f 0.30 in CH_2Cl_2/MeOH, 94:6; GC $t_R = 9.06$ min.

X-ray Crystallography of Urechitol A (1).[10] Intensity data were collected at 293 K for **1** with an Oxford Diffraction Xcalibur 3 system using ω-scans and Mo Kα radiation ($\lambda = 0.71073$ Å).[11] CCD data were extracted and integrated using Crysalis RED.[12] Crystal data for **1**: $C_{14}H_{22}O_7$; colorless prism, orthorhombic, space group $P2_12_12_1$, $a =$ 10.0030(5) Å, $b =$ 11.4956(6) Å, $c =$ 13.1084(7) Å, $V =$ 1507.34(14) Å^3, $Z = 4$, $\mu = 0.107$ mm^{-1}, 15 696 reflections measured, 3081 unique ($R_{int} = 0.0819$), which were used in all calculations. The structures were solved using direct methods and refined by full-matrix least-squares calculations on F^2 using SHELXTL 5.1.[13] Non-H atoms were refined with anisotropic displacement parameters. Hydrogen atoms were constrained to parent sites, using a riding model.

Urechitol B (2): colorless oil; IR ($CHCl_3$, film) ν_{max} 3436 (OH), 2934 (C−H), 1060 (C−O−C) cm^{-1}; ^{1}H NMR ($CDCl_3$, 400 MHz) δ 1.27 (3H, d, $J = 6.0$ Hz, H-11), 1.42 (3H, s, H-13), 1.86 (1H, dd, $J =$ 9.0, 16.0 Hz, H-9a), 2.21 (1H, ddd, $J = 7.0$, 14.0, 1.6 Hz, H-3a), 2.35 (1H, dd, $J = 5.5$, 16.0 Hz, H-3b), 2.59 (1H, dd, $J = 5.5$, 15.8 Hz, H-9b), 3.20 (1H, d, $J = 8.3$ Hz, H-7), 3.45 (3H, s, H-12), 3.49 (3H, s, H-14), 3.82 (1H, bddd, $J = 2.1$, 8.2, 17.0 Hz, H-8), 3.90 (1H, dd, $J =$ 1.5, 4.4 Hz, H-10), 4.07 (1H, q, $J = 6.0$ Hz, H-1), 5.15 (1H, dd, $J =$ 9.0, 5.6 Hz, H-2); ^{13}C NMR ($CDCl_3$, 100 MHz) δ 15.4 (q, C-11), 20.6 (q, C-13), 32.08 (t, C-9), 32.13 (t, C-3), 53.4 (q, C-12), 57.8 (q, C-14), 67.4 (d, C-1), 78.1 (d, C-8), 78.3 (d, C-10), 80.9 (s, C-4), 81.87 (d, C-7), 81.97 (s, C-5), 84.4 (s, C-6), 89.4 (d, C-2); HRESIMS *m/z* 327.1455 [M$^+$] (calcd for $C_{14}H_{24}O_7Na$, 327.1420); TLC R_f 0.37 in CH_2Cl_2/MeOH, 94:6; GC $t_R = 11.25$ min.

Acetylation of Urechitol A (1). A fraction containing **1** as the main component (50 mg) was combined with acetic anhydride (2 mL) and pyridine (1 mL) and allowed to stir overnight at room temperature. The reaction mixture was poured over water (50 mL), and the resulting suspension was extracted with EtOAc (3×, 1:1). The organic layer was successively washed (2×, 1:1) with 5% HCl, 5% $NaHCO_3$, and brine and then dried over anhydrous sodium sulfate. Evaporation of the solvent under reduced pressure yielded 42 mg (65.72%) of crude acetylated product, which was purified by column chromatography (CH_2Cl_2/MeOH, 98:2) to produce 30 mg (46.9%) of a pure acetylated derivative (**3**): colorless needles; IR ($CHCl_3$, film) ν_{max} 2986 and 2934 (C−H), 1736 (C=O) cm^{-1}; ^{1}H NMR ($CDCl_3$, 400 MHz) δ 1.47 (3H, s, H-13), 1.51 (3H, d, $J = 6.9$ Hz, H-11), 1.84 (1H, d, $J = 4.0$ Hz, H-3a), 1.87 (1H, dd, $J = 2.3$, 4.2 Hz, H-9a), 2.11 (3H, s, CH_3COO), 2.14 (3H, s, CH_3COO), 2.35 (1H, dd, $J = 2.9$, 14.2 Hz, H-3b), 2.40 (1H, dd, $J = 0.6$, 12.9 Hz, H-9b), 3.34 (3H, s, H-12), 3.46 (3H, s, H-14), 4.16 (1H, d, $J = 4.0$ Hz, H-10), 4.19 (1H, d, $J = 6.9$ Hz, H-1), 4.94 (1H, s, H-7), 6.13 (1H, dd, $J = 2.8$, 10.0 Hz, H-2); LREIMS *m/z* 386 [M$^+$], 327 [M$^+$ + H$^+$ − CH_3COOH], 267 [M$^+$ + H$^+$ − 2 × CH_3COOH]; TLC R_f 0.45 in CH_2Cl_2/MeOH, 98:2; GC $t_R = 11.11$ min.

Methylation of Urechitol A (1). A mixture of CH_3I (1 mL), K_2CO_3 (200 mg), and a fraction containing **1** as the main component (52 mg) in 3 mL of acetone was left to stir overnight at room temperature and then diluted with 15 mL of water. The resulting solution was extracted with ethyl acetate (2×, 4:1) and the organic layer dried over anhydrous sodium sulfate. Evaporation of the solvent produced 46 mg (84.5%) of crude methylated product, which was purified by column chromatography (hexane/acetone, 6:4) to yield 20 mg (36.8%) of a pure

methylated derivative (**4**): colorless needles; IR ($CHCl_3$, film) ν_{max} 3482 (OH), 2934 (C−H) cm^{-1}; ^1H NMR ($CDCl_3$, 300 MHz) δ 1.40 (3H, d, $J = 6.9$ Hz, H-11), 1.55 (3H, s, H-13), 1.71 (1H, d, $J = 4.2$ Hz, H-3a), 1.75 (1H, dd, $J = 3.1$, 4.6 Hz, H-9a), 2.27 (1H, dd, $J = 3.3$, 14.5 Hz, H-3b), 2.42 (1H, dd, $J = 1.2$, 12.9 Hz, H-9b), 3.32 (3H, s, H-12), 3.45 (3H, s, H-14), 3.49 (3H, s, OCH_3), 3.57 (1H, s, H-7), 4.13 (1H, dd, $J = 1.2$, 4.5 Hz, H-10), 4.17 (1H, d, $J = 9.2$, H-1) 4.82 (1H, dd, $J = 3.1$, 9.4 Hz, H-2); LREIMS *m/z* 316 [M^+], 299 [$M^+ + H^+ - H_2O$], 285 [$M^+ - CH_3O$]; TLC R_f 0.42 in hexane/acetone, 6:4; GC t_R= 7.87 min.

Oxidation of Urechitol A (1). A 200 mg portion of pyridinium chlorochromate (PCC Corey's reagent) was added to a solution of a fraction containing **1** as the main component (64 mg) in CH_2Cl_2 (3 mL), and the mixture was allowed to stir overnight at room temperature. The reaction mixture was passed through a silica gel bed (70−230 mesh; 2 cm diameter, 3 cm high), and the adsorbent was washed with CH_2Cl_2/MeOH (99:1) to produce 46 mg (72.4%) of crude oxidized product, which was then purified by column chromatography (hexane/acetone, 1:1) to yield 27 mg (42.5%) of the oxidized derivative (**5**) in pure form: colorless needles; IR ($CHCl_3$, film) ν_{max} 3477 (OH), 2945 (C−H), 1736 (C═O) cm^{-1}; ^1H NMR ($CDCl_3$, 300 MHz) δ 1.47 (3H, s, H-13), 1.49 (3H, d, $J = 6.9$ Hz, H-11), 1.76 (1H, dd, $J = 4.0$, 13.0 Hz, H-9a), 2.47 (1H, dd, $J = 1.2$, 12.9 Hz, H-9b), 2.69 (1H, d, $J = 17.4$ Hz, H-3a), 3.08 (1H, d, $J = 17.4$ Hz, H-3b), 3.34 (3H, s, H-12), 3.53 (3H, s, H-14), 3.62 (1H, s, H-7), 4.28 (1H, dd, $J = 1.0$, 4.3 Hz, H-10), 4.62 (1H, c, $J = 6.7$ Hz, H-1); TLC R_f 0.47 in hexane/acetone, 1:1; GC t_R = 10.40 min.

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(10) The crystal structure of urechitol A (**1**) has been deposited at the Cambridge Crystallographic Data Centre and allocated the deposition number CCDC 708823.
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