A New Ursane Triterpene from *Monochaetum vulcanicum* that Inhibits DNA Polymerase *β* Lyase

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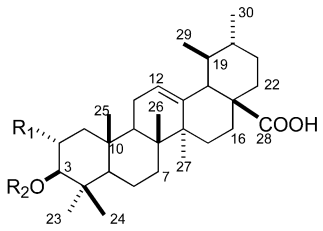
Bioassay-directed fractionation of a butanone extract of *Monochaetum vulcanicum* resulted in the isolation of a new triterpene (**1**) and four known compounds, ursolic acid (**2**), 2α-hydroxyursolic acid (**3**), 3-(*p*-coumaroyl)ursolic acid (**4**), and *β*-sitosteryl-*β*-D-galactoside (**5**). The structure of the new compound **1** was established as 3*β*-acetoxy-2α-hydroxyurs-12-en-28-oic acid on the basis of extensive 1D and 2D NMR spectroscopic interpretation and chemical derivatization. Compounds **1**−**3** and **5** exhibited polymerase *β* lyase activity.

In addition to its polymerization activity, the DNA repair enzyme DNA polymerase *β* (pol *β*) also has an intrinsic deoxyribose phosphate (dRP) lyase activity, which is important to its repair function.[1,2] This second activity constitutes a second target for the discovery of potential anticancer agents, since inhibitors of the lyase activity of pol *β* should also potentiate the cytotoxicity of DNA-damaging agents. It has already been shown by one of our groups that naturally occurring inhibitors of pol *β* can be found in nature,[3] and it is thus reasonable to suppose that specific inhibitors of the lyase activity may also exist in nature. We thus elected to begin a search for naturally occurring inhibitors of pol *β* lyase as a part of our continuing research to identify novel naturally occurring anticancer agents,[4,5] The assay system used for this purpose has been described previously.[6]

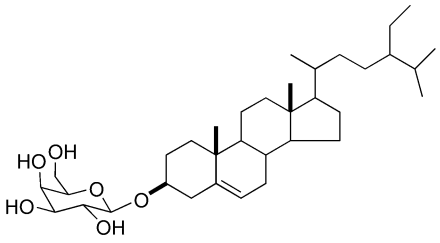
A butanone extract of the plant *Monochaetum vulcanicum* Cogn. (Melastomataceae) was selected for bioassay-guided fractionation on the basis of its strong activity at 16.2 *μ*g/mL in the pol *β* lyase assay and the absence of any reported phytochemical studies of this species. Earlier phytochemical studies of the genus *Monochaetum* resulted in the isolation of ellagitannins and flavonoid glycosides.[7]

Initial liquid−liquid partition of the crude extract of *M. vulcanicum* indicated that the activity was equally distributed between the *n*-hexane and $CHCl_3$ fractions of *n*-hexane/aqueous MeOH and $CHCl_3$/aqueous MeOH partitions, respectively. The *n*-hexane and $CHCl_3$ residues were combined on the basis of their similar nature as judged by ^{1}H NMR and TLC. The combined residue after separation by chromatography over MCI gel followed by reversed-phase PTLC yielded the new triterpenoid **1** in addition to the four known compounds **2**−**5**. The four known compounds were identified as ursolic acid (**2**),[8] 2α-hydroxyursolic acid (**3**),[9] 3-(*p*-coumaroyl)ursolic acid (**4**),[10] and *β*-sitosteryl-*β*-D-galactoside (**5**)[11] by comparison of their spectroscopic data with literature values.

Compound **1** was obtained as an optically active viscous liquid and was shown to have the molecular formula $C_{32}H_{50}O_5$ by HRFABMS, ^{13}C NMR, and APT (attached proton test) spectral data. It gave a positive Liebermann-Burchard test for triterpenoids. The IR absorption bands observed at 3450 and 1728 cm^{-1} indicated the presence of hydroxyl and carbonyl groups in its structure. The mass fragments in its EIMS at *m*/*z* 469, 454, and 436 were formed by the successive loss of COOH, AcOH, and H_2O molecules from the molecular ion and, thus, indicated the presence of carboxylic acid, acetoxy, and hydroxy groups in its structure. The ^{1}H NMR spectrum showed the presence of five methyl singlets at *δ* 0.84, 0.87, 0.89, 0.91, and 0.98, two methyl doublets at *δ* 0.82 (*J* = 6.8 Hz) and 0.88 (*J* = 7.2 Hz), an olefinic proton at *δ* 5.34 as a triplet (*J* = 2.6 Hz), an oxymethine proton at *δ* 3.22 (dt, *J* = 4.8, 11.5 Hz), a secondary acetate group [*δ* 4.46 (d, *J* = 11.3 Hz), and 2.03 (s, 3H)], eight methylenes, and five methines. The ^{13}C NMR values for all 32 carbons were assigned on the basis of APT, HMQC, and HMBC spectral data, which indicated the presence of eight sp^3 methyls, eight sp^3 methylenes, seven sp^3 methines, five sp^3 quaternary carbons, one sp^2 methine carbon, one sp^2 quaternary carbon, one ester carbonyl group, and one carboxylic acid group. A literature search suggested that the above spectral data



1 R_1 = OH; R_2 = Ac
2 R_1 = R_2 = H
3 R_1 =OH; R_2 = H
4 R_1 =H; R_2 = *p*-coumaroyl
6 R_1 = OAc; R_2 = Ac



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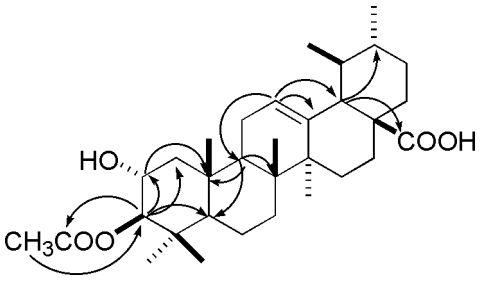

Figure 1. Selected HMBC correlations of **1**.

Table 1. IC_{50} of Polymerase β Lyase Inhibiition of Compounds Isolated from *Monochaetum vulcanicum*[a]

compound	IC_{50} (μM)
1	21.5
2	18.6
3	12.6
4	>50
5	26.5

[a] Data are the meam of three determinations.

were consistent with the presence of an urs-12-ene type of pentacyclic triterpenoid skeleton in **1**. The appearance of the two oxymethine protons indicated their presence in the A-ring of an urs-12-en-28-oic acid skeleton.[12] This was further supported by the mass fragment observed at *m/z* 198, formed by the loss of $C_{11}H_{18}O_3$ from the molecular ion, corresponding to the A-ring of **1**. The placement of the secondary hydroxy and acetoxy groups in the A-ring of **1** at the C-2 and C-3 positions was made on the basis of the key HMBC correlations: H-2/C-1, C-3, C-4, C-25 and H-3/C-1, C-2, C-4, C-23, C-24 (Figure 1). The stereochemistry of the hydroxy and acetoxy groups was assigned as rel α and β on the basis of their almost identical coupling constants with reported 2α,3β-dihydroxy- and diacetoxyurs-12-en-28-oic acid derivatives.[8,13] This was supported by the NOESY correlations of **1**, in which the oxymethine at the C-2 carbon showed correlations to the methyl protons of C-24 and C-25, whereas the acetoxymethine proton at C-3 showed a correlation to the protons of the C-23 methyl group. In addition, acetylation of compound **1** with Ac_2O−pyr afforded a product whose mp, rotation, and ^{1}H NMR data were identical with those of 2α,3β-diacetoxyurs-12-en-28-oic acid (**6**),[13] thus confirming the structure. The same product was also obtained by acetylation of **3**. On the basis of the above spectroscopic and chemical evidence, **1** was assigned as 3β-acetoxy-2α-hydroxyurs-12-en-28-oic acid (3-acetyl-2α-hydroxyursolic acid).

All the isolated compounds were tested for inhibition of DNA polymerase β lyase activity, and the results are shown in Table 1. Compounds **1**−**3** and **5** were weakly active, with IC_{50} values ranging from 12.6 to 26.5, with 2α-hydroxyursolic acid (**3**) having the greatest activity.

Experimental Section

General Experimental Procedures. Melting points were recorded with an Electrothermal digital apparatus and are uncorrected. Optical rotations were recorded on a Perkin-Elmer 241 polarimeter. IR (KBr) and UV (MeOH) spectra were measured on MIDAC M-series FTIR and Shimadzu UV-1201 spectrophotometers, respectively. NMR spectra were obtained on a JEOL Eclipse 500 spectrometer, and chemical shifts are given in ppm (δ) with TMS (tetramethylsilane) as an internal reference; coupling constants (*J*) are in Hz. The HRFAB mass spectra were obtained on a JEOL HX-110 instrument.

Plant Material. *Monochaetum vulcanicum* Cogn. (Melastomataceae) was collected in June 1964 in Costa Rica as CR-3 (B603644, leaves) and CR-4 (B603645, stems). Voucher specimens are located at the National Herbarium, Washington, DC.

Polymerase Beta Lyase Bioassay. The assay was performed at the University of Virginia as previously reported.[6]

Extract Preparation. The plant samples were dried, ground, and soaked sequentially in hexane, butanone, and MeOH. Concentrations of the individual solutions provided the dried extract. The butanone extract designated PC-9-145 was used in the present study.

Extraction and Isolation. The crude extract (0.45 g) was suspended in aqueous MeOH (MeOH−H_2O, 9:1, 50 mL) and extracted with hexane (3 × 50 mL). The aqueous layer was then diluted to 70% MeOH (v/v) with H_2O and extracted with $CHCl_3$ (3 × 50 mL). The aqueous layer was concentrated, and the residue obtained was suspended in H_2O (25 mL) and extracted with BuOH (2 × 25 mL). The hexane and $CHCl_3$ extracts were found to be equally active and were combined on the basis of their similar nature on TLC and their similar ^{1}H NMR spectra. The combined residue (0.35 g) was fractionated over MCI gel using MeOH−H_2O (75:25 to 100:0) to furnish 11 fractions (A−K), of which fractions C, E, and H−K were selected for further fractionation on the basis of their activity and their ^{1}H NMR spectra. Fraction C on reversed-phase preparative TLC (MeOH−H_2O, 80:20) yielded ursolic acid (**2**, 1.8 mg). Similarly, fraction E on reversed-phase preparative TLC (MeOH−H_2O, 85:15) afforded 2α-hydroxyursolic acid (**3**, 2.6 mg). Fractions I and J on reversed-phase preparative TLC with mobile phase MeOH−H_2O (90:10) afforded 3-(*p*-coumaroyl)ursolic acid (**4**, 2.2 mg) and β-sitosteryl-β-D-galactoside (**5**, 2.8 mg), respectively. Fraction K on reversed-phase preparative TLC (MeOH−H_2O, 90:10) yielded the new triterpene **1** (1.5 mg). The four known compounds **2**−**5** were identified by comparison of their spectral data with literature values.[8−11]

3β-Acetoxy-2α-hydroxyurs-12-en-28-oic acid (1): colorless oil; $[\alpha]_D$ +56.2° (*c* 0.64, $CHCl_3$); UV (MeOH) λ_{max} 216 nm (ϵ 14 200); IR ν_{max} 3450, 2955, 1728, 1435, 1110, 1050 cm^{-1}; ^{1}H NMR, δ 0.82 (d, J = 6.8, H-29), 0.84 (s, 3H, H-26), 0.87 (s, 3H, H-25), 0.88 (d, J = 7.2, H-30), 0.89 (s, 3H, H-23), 0.91 (s, 3H, H-24), 0.98 (s, 3H, H-27), 1.04 (1H, m, H-5), 1.22 (1H, m, H-7), 1.30 (2H, m, H-6 and H-16), 1.32 (1H, m, H-15), 1.36 (1H, m, H-21), 1.38 (1H, m, H-19), 1.40 (1H, m, H-11), 1.51 (1H, m, H-9), 1.52 (1H, m, H-6), 1.54 (1H, m, H-22), 1.60 (1H, m, H-21), 1.62 (1H, m, H-16), 1.64 (2H, m, H-7 and H-1), 1.98 (1H, m, H-15), 2.02 (1H, m, H-22), 2.03 (3H, 3-OAc), s 2.04 (1H, m, H-19), 2.10 (1H, m, H-11), 2.16 (1H, m, H-1), 2.27 (1H, d J = 11.2, H-18), 3.22 (1H, dt, J = 4.8, 11.5, H-2), 3.63 (1H, m, 2-OH), 4.46 (1H, d, J = 11.3, H-3), and 5.34 (1H, t, J = 2.6); ^{13}C NMR, δ 50.3, 68.3, 80.4, 38.4, 55.5, 18.3, 33.3, 40.2, 47.8, 39.2, 24.8, 125.6, 138.2, 42.4, 28.2, 24.6, 49.8, 53.2, 39.6[a], 39.4[a], 31.8, 37.9, 28.3, 17.5[b], 16.9[b], 17.0[b], 23.6, 179.1, 21.3, 17.3[b] for carbons 1−30, respectively (values having the same superscript are interchangeable), 21.3 (3-Ac), and 170.8 (3-Ac); EIMS *m/z* (rel int) 514 [M$^+$] (18), 469 (32), 454 (24), 436 (12), 376 (13), 335 (15), 334 (23), 323 (15), 248 (100), 235 (10), 226 (32), 198 (21), 181 (21), 180 (16), 121 (14), 61 (24); HRFABMS *m/z* 437.3431 [M − AcOH − H_2O]$^+$ (calcd for $C_{30}H_{45}O_2$, 437.3420).

Acetylation of 3β-Acetoxy-2α-hydroxyurs-12-en-28-oic Acid (1). Compound **1** (0.8 mg) was treated with Ac_2O−pyr (1:1, 0.5 mL) at room temperature for 10 h. Concentration of the mixture under vacuum followed by reversed-phase preparative TLC (MeOH−H_2O, 98:2) gave **6** (0.6 mg), which had ^{1}H NMR and MS data identical with those of 2α,3β-diacetyloxyurs-12-en-28-oic acid.[13]

Acetylation of 2α-Hydroxyursolic Acid (3). Acetylation of 2α-hydroxyursolic acid (**3**, 1.5 mg) as described above gave a product (1.2 mg) that was identical with **6**.

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