

3a,23-O-Isopropylidenyl-2a,19a-dihydroxy-urs-12-en-28-oic acid, A New Pentacyclic Triterpene Isolated from *Rubus aleaefolius* as a New Cell Cycle Inhibitor

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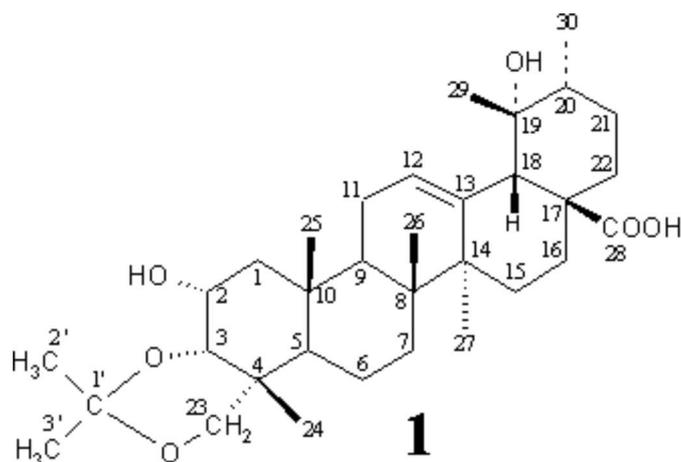
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Recently we have reported [1] six new cell cycle inhibitors belonging to plant polyphenolics from *Rubus aleaefolius* Poir. (family *Rosaceae*), the source plant of a traditional Chinese medicine Cuye Xuangouzi, which is used also as a folk medicine to cure certain cancers in partial area of China. In a continuation of that work, this communication describes a new pentacyclic triterpene, 3a,23-O-isopropylidenyl-2a,19a-dihydroxy-urs-12-en-28-oic acid (**1**), obtained from *Rubus aleaefolius* Poir., which inhibits mammalian cell cycle at G0/G1 phase.

The roots (3 kg) of *R. aleaefolius* were extracted with 60% aqueous alcohol to give an alcoholic extract (435 g) possessing potential inhibitory activity on the cell cycle of tsFT210 cells [1]. The alcoholic extract was thus suspended in water and then extracted successively with chloroform and ethyl acetate to obtain an active ethyl acetate extract (78 g). This extract was further separated by repeated solvent-extraction, silica gel column chromatography, preparative HPLC and recrystallization procedure in a bioassay-guided manner to obtain pure **1** (26.8 mg).



Compound **1**, white crystals (from MeOH), mp 196-198°C, $[\alpha]_D^{25} +31.7^\circ$ (c 0.85, MeOH), gave positive Libermann-Burchard reaction, showing typical color for triterpenoids, and its molecular formula, C₃₃H₅₂O₆ (molecular weight 544), was determined by negative HR-SIMS-MS measurement. The IR spectrum of **1** indicated the presence of OH (3449 cm⁻¹), COOH (3400-2400 br and 1702 cm⁻¹), C=C (1638 cm⁻¹), and C-O (1040 cm⁻¹) groups in **1**.

The ^1H and ^{13}C NMR spectra of **1** in CDCl_3 , analyzed by DEPT and PFG 2D NMR (^1H - ^1H COSY, HMQC, HMBC and NOESY) techniques, resembled those of known 2a,3a,19a,23-tetrahydroxy-urs-12-en-28-oic acid [2] (**2**) except for additional signals due to an extra *O*-isopropylidenyl group ($d_{\text{H}} 1.42$ s, 2'-H₃, $d_{\text{C}} 19.22$ q, C-2'; $d_{\text{H}} 1.40$ s, 3'-H₃, $d_{\text{C}} 29.38$ q, C-3'; and $d_{\text{C}} 98.67$ s, C-1') [3] in **1**, indicating that **1** is an isopropylidenyl derivative of **2**. Location of the *O*-isopropylidenyl group at C-3 and C-23 positions could be determined on the basis of HMBC correlations between 3-H and C-1' and between 23-H and C-1'. The *cis*-relation between b-axial 2-H and b-equatorial 3-H was evidenced by the chemical shift and *J* values of 2-H (d 3.88 ddd, $J=12.1, 4.1, 2.8$ Hz) and 3-H (d 3.77 d, $J=2.8$ Hz) [4]. The chemical shift and *J* values of a-axial 16-H (d 2.53 td, $J=13.4, 4.4$ Hz) established both the 19a-OH stereochemistry and the *cis*-stereochemistry of the ring-D/E junction [5]. And eventually the structure of **1** was established as 3a,23-*O*-isopropylidenyl-2a,19a-dihydroxy-urs-12-en-28-oic acid.

Compound **1** inhibited the cell cycle progression of asynchronously cultured tsFT210 cells at the G₀/G₁ phase with the MIC value of 183.8 mmol/L.

UV I_{max} nm (log *e*) in MeOH: 206 (3.71).

IR n_{max} cm^{-1} (KBr): 3449 (OH), 3400-2400 br (COOH), 2987, 2973, 2936, 2877 (CH₃ & CH₂), 1702 (COOH), 1638 (C=C), 1459, 1380 (CH₃ & CH₂), 1200, 1150, 1040 (C-O).

Positive ESI-MS m/z : 567 [$\text{M}+\text{Na}$]⁺; negative ESI-MS m/z : 543 [$\text{M}-\text{H}$]⁻; negative HR-SIMS-MS m/z : 543.3686 (*calcd* for C₃₃H₅₁O₆ [$\text{M}-\text{H}$]⁻ 543.3690).

^1H -NMR (600 MHz, CDCl_3): d 1.28 (t, $J=12.1$ Hz, 1-H_{ax}.), 1.71 (dd, $J=ca.12.1, 4.1$ Hz, 1-Heq.), 3.88 (ddd, $J=12.1, 4.1, 2.8$ Hz, 2-H), 3.77 (d, $J=2.8$ Hz, 3-H), 1.78 (br d, $J=12.4$ Hz, 5-H), 1.27 and 1.42 (both m, 6-H₂), 1.30 (m, 7-Heq.), 1.63 (td, $J=12.6, 4.1$ Hz, 7-H_{ax}.), 1.81 (dd, $J=ca.11.1, 6.6$ Hz, 9-H), 2.06 (ddd, $J=18.7, 6.6, 3.3$ Hz, 11-Heq.), 1.99 (ddd, $J=18.7, 11.1, 3.7$ Hz, 11-H_{ax}.), 5.36 (dd, $J=3.7, 3.3$ Hz, 12-H), 1.04 (br d, $J=12.7$ Hz, 15-Heq.), 1.78 (ddd, $J=13.4, 12.7, 4.1$ Hz, 15-H_{ax}.), 2.53 (td, $J=13.4, 4.4$ Hz, 16-H_{ax}.), 1.58 (dm, $J=13.4$ Hz, 16-Heq.), 2.536 (s, 18-H), 1.39 (m, 20-H), 1.28 and 1.71 (both m, 21-H₂), 1.66 and 1.80 (both m, 22-H₂), 3.30 and 3.66 (both d, both $J=12.1$ Hz, 23-H₂), 0.71 (3H, s, 24-H₃), 0.97 (3H, s, 25-H₃), 0.72 (3H, s, 26-H₃), 1.29 (3H, s, 27-H₃), 1.21 (3H, s, 29-H₃), 0.95 (3H, d, $J=6.6$ Hz, 30-H₃), 1.42 (3H, s, 2'-H₃), 1.40 (3H, s, 3'-H₃). The above signal assignments were based on the results of PFG 2D NMR (^1H - ^1H COSY, HMQC, HMBC and NOESY) experiments.

^{13}C -NMR (150 MHz, CDCl_3): d 42.36 t (C-1), 65.33 d (C-2), 76.01 d (C-3), 36.12 s (C-4), 42.07 d (C-5), 17.76 t (C-6), 32.47 t (C-7), 40.27 s (C-8), 47.12 d (C-9), 38.12 s (C-10), 23.72 t (C-11), 129.34 d (C-12), 138.05 s (C-13), 41.15 s (C-14), 28.24 t (C-15), 25.41 t (C-16), 47.81 s (C-17), 52.92 d (C-18), 73.17 s (C-19), 41.24 d (C-20), 26.05 t (C-21), 37.56 t (C-22), 68.20 t (C-23), 16.92 q (C-24), 17.02 q (C-25), 17.24 q (C-26), 24.66 q (C-27), 184.13 s (C-28), 27.49 q (C-29), 16.25 q (C-30), 98.68 s (C-1'), 19.22 q (C-2'), 29.38 q (C-3'). The above signal assignments were based on the results of DEPT and PFG 2D NMR (^1H - ^1H COSY, HMQC, HMBC and NOESY) experiments.

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