

**3b-O-trans-Ferulyl-2a,19a-dihydroxy-urs-12-en-28-oic Acid,
A New Pentacyclic Triterpene Ester from *Rubus aleaefolius*,
Which Inhibits Mammalian Cell Cycle at G2/M phase**

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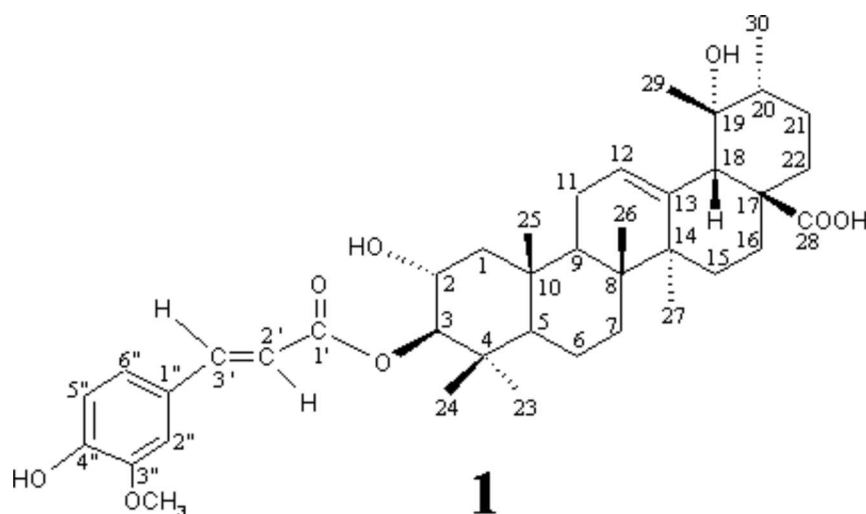
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Continuing our studies on bioactive constituents of *Rubus aleaefolius* Poir. (family *Rosaceae*) [1,2], we report here a new pentacyclic triterpene ester, 3b-O-trans-ferulyl-2a,19a-dihydroxy-urs-12-en-28-oic acid (**1**) from *Rubus aleaefolius* Poir., which inhibits mammalian cell cycle at G2/M 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 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** (26.4 mg).



Compound **1**, white crystal powder (from MeOH), mp 232-234°C, $[\alpha]_D^{25} +1.6^\circ$ (c 0.23, MeOH), gave positive reaction to the Libermann-Burchard and FeCl_3 reagents, showing typical colors for triterpenoid and phenol, respectively, and its molecular formula, $\text{C}_{40}\text{H}_{56}\text{O}_8$ (molecular weight 664), was determined by positive HR-ESI-MS measurement. It showed UV absorptions at 233 nm ($\log \epsilon$ 4.05), 299 nm (4.11) and 325 (4.25) ascribable to an α,β -unsaturated aromatic moiety and the IR spectrum indicated the presence of OH (3448 cm^{-1}), COOH ($3400\text{--}2400\text{ br}$ and 1697 cm^{-1}), conjugated ester C=O (1715 cm^{-1}), conjugated aromatic ring ($1630, 1596, 1515$), and C-O ($1268, 1175, 1034\text{ cm}^{-1}$) groups in **1**.

The ^1H NMR spectrum of **1** showed signals ascribable to a *trans*-ferulyl (d 3.90 s, 3H, OCH_3 ; 6.43 d, $J=15.9$ Hz, 2'-H; 6.81 d, $J=7.7$ Hz, 5''-H; 7.08 br d, $J=7.7$ Hz, 6''-H; 7.21 br s, 2''-H; 7.63 d, $J=15.9$ Hz, 3'-H) [3,4], a single olefin (d 5.30 br s, 12-H), six *tert*-methyl and a *sec*-methyl (d 0.93 3H, d, $J=6.6$ Hz, 30- H_3) groups. These data and the above structural information from UV and IR spectra revealed that **1** is an ursolic acid derivative with a *trans*-ferulyl group. The ^{13}C NMR spectrum of **1** resembled those of known 3b-*O-trans*-ferulyl-2a-hydroxy-urs-12-en-28-oic acid [3] (**2**) except for the remarkable downfield shift of C-19 signal and slight changes of several carbons around C-19, indicating that **1** is a 19-*O*-substituted derivative of **2**.

Eventually, detailed analyses of the ^1H and ^{13}C NMR spectra by means of DEPT and PFG 2D NMR (^1H - ^1H COSY, HMQC, HMBC and NOESY) techniques enabled us to establish the structure of **1** as 3b-*O-trans*-ferulyl-2a,19a-dihydroxy-urs-12-en-28-oic acid. Briefly, location of the *O*-ferulyl group at C-3 could be determined by the HMBC correlation between 3-H and C-1', and the *trans*-2a,3b-*O*-substitution was evident from the chemical shift and J values of 2-H (d 3.85 ddd, $J=11, 9.9, 4.4$ Hz) and 3-H (d 4.63 d, $J=9.9$ Hz) [3,4]. The chemical shift and J values of a-axial 16-H (d 2.59 td, $J=13.4, 4.3$ Hz) established both the 19a-OH stereochemistry and the *cis*-stereochemistry of the ring-D/E junction [5].

Compound **1** inhibited the cell cycle progression of synchronously cultured tsFT210 cells [1] at the G2/M phase with the MIC value of 37.6 mmol/L. Although several 3-*O*-ferulyl derivatives of ursolic acid have hitherto been reported [3,4], the present result provides a new pentacyclic triterpene ester, **1**, as a new cell cycle inhibitor, which may possess potential anticancer effect.

UV λ_{max} nm (log ϵ) in MeOH: 202 (4.32), 213 (4.21), 233 (4.05), 299 sh (4.11), 325 (4.25).

IR ν_{max} cm^{-1} (KBr): 3448 (OH), 3400-2400 br (COOH), 2970, 2937, 2884 (CH_3 & CH_2), 1715 (conjugated ester C=O), 1697 (COOH), 1630 (C=C), 1596, 1515 (aromatic ring), 1452, 1379 (CH_3 & CH_2), 1268, 1175, 1126, 1034 (C-O).

Positive ESI-MS m/z : 665 $[\text{M}+\text{H}]^+$, 687 $[\text{M}+\text{Na}]^+$; negative ESI-MS m/z : 663 $[\text{M}-\text{H}]^-$; positive HR-ESI-MS m/z : 665.4053 (*calcd* for $\text{C}_{40}\text{H}_{57}\text{O}_8$ $[\text{M}+\text{H}]^+$ 665.4048), 687.3858 (*calcd* for $\text{C}_{40}\text{H}_{56}\text{O}_8\text{Na}$ $[\text{M}+\text{Na}]^+$ 687.3867).

^1H -NMR (600 MHz, CD_3OD): d 1.06 (dd, *ca.* $J=12.6, 11$ Hz, 1-H_{ax.}), 2.02 (dd, $J=12.6, 4.4$ Hz, 1-H_{eq.}), 3.85 (ddd, $J=11, 9.9, 4.4$ Hz, 2-H), 4.64 (d, $J=9.9$ Hz, 3-H), 1.02 (br d, $J=12.1$ Hz, 5-H), 1.58 and 1.63 (both m, 6- H_2), 1.35 (br d, *ca.* $J=13.2$ Hz, 7-H_{eq.}), 1.62 (br t, $J=13.2$ Hz, 7-H_{ax.}), 1.81 (m, 9-H), 2.00 and 2.04 (both AB type, 11- H_2), 5.30 (br s, 12-H), 1.02 (br d, $J=12.1$ Hz, 15-H_{eq.}), 1.82 (overlapped with 9-H, 15-H_{ax.}), 2.59 (td, $J=13.7, 4.3$ Hz, 16-H_{ax.}), 1.52 (dm, $J=13.7$ Hz, 16-H_{eq.}), 2.51 (br s, 18-H), 1.35 (m, 20-H), 1.23 and 1.73 (m and AB type, 21- H_2), 1.73 (AB type, 22-H_{eq.}), 1.62 (br t, $J=13.2$ Hz, 22-H_{ax.}), 0.90 (3H, s, 23- H_3), 0.96 (3H, s, 24- H_3), 1.062 (3H, s, 25- H_3), 0.81 (3H, s, 26- H_3), 1.97 (3H, s, 27- H_3), 1.20 (3H, s, 29- H_3), 0.93 (3H, d, $J=6.6$ Hz, 30- H_3), 6.43 (d, $J=16$ Hz, 2'-H), 7.63 (d, $J=16$ Hz, 3'-H), 7.21 (br s, 2''-H), 6.81 (d, $J=7.7$ Hz, 5''-H), 7.08 (br d, $J=7.7$ Hz, 6''-H), 3.90 (3H, s, 3''- OCH_3). 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 48.55 t (C-1), 67.65 d (C-2), 85.64 d (C-3), 40.65 s (C-4), 56.46 d (C-5), 19.58 t (C-6), 34.03 t (C-7), 42.68 s (C-8), 8.71 d (C-9), 39.00 s (C-10), 24.75 t (C-11), 129.14 d (C-12), 140.16 s (C-13), 41.14 s (C-14), 29.59 t (C-15), 26.60 t (C-16), 48.72 s (C-17), 55.09 d (C-18), 73.57 s (C-19), 43.09 d (C-20), 27.30 t (C-21), 39.22 t (C-22), 29.25 t (C-23), 18.32 q (C-24), 16.60 q (C-25), 17.07 q (C-26), 24.83 q (C-27), 182.29 s (C-28), 27.05 q (C-29), 17.50 q (C-30), 169.61 s (C-1'), 116.16 d (C-2'), 146.50 d (C-3'), 127.90 s (C-1''), 111.58 d (C-2''), 149.40 s (C-3''), 150.53 s (C-4''), 116.49 d (C-5''), 124.04 d (C-6''), 56.51 q (OCH_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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