Triterpenes from *Mimusops elengi*

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Two new triterpenes, 3β,19α,23-trihydroxy-urs-12-ene (1) and 3β-(p-hydroxy-cis-cinnamoyl-oxo)urs-12-en-28-oic acid (2) have been isolated from the methanolic extract of *Mimusops elengi* along with 3β-(p-hydroxy-trans-cinnamoyl-oxo)urs-12-en-28-oic acid (3), which has been obtained for the first time from this species, and ursolic acid (4), respectively. Their structures were established through chemical and spectral studies. Compounds (3) and (4) were found to exhibit potent inhibitory activity against α-glucosidase enzyme.

*Mimusops elengi* grows wild in Southern India, Burma and Pakistan. Various parts of the plant are used in the indigenous system of medicine as a febrifuge, astringent, purgative and stimulant. Used in the indigenous system of medicine in Burma and Pakistan. Various parts of the plant are used in the indigenous system of medicine as a febrifuge, astringent, purgative and stimulant [1]. The presence of saponins, steroids, terpenoids and alkaloids have previously been reported for *M. elengi* [2]. The methanolic extract of this plant exhibited strong cytotoxicity in brine shrimp lethality test. Later, hypotensive activity in various fractions of *M. elengi* was reported by Dar et al. [3]. This prompted us to reinvestigate this plant for search of bioactive constituents. Herein we report the isolation and structure of two new triterpenes, 3β,19α,23-trihydroxy-urs-12-ene (1) and 3β-(p-hydroxy-cis-cinnamoyl-oxo)urs-12-en-28-oic acid (2), respectively, along with the trans isomer of 2 and ursolic acid 4.

**Result and Discussion**

Compound 1 was assigned the molecular formula C_{50}H_{80}O_{4} by HR-MS showing the [M]+ peak at m/z 458.3779 (calcd. 458.3759). It gave positive Liebermann-Burchard and Ce(SO_{4})_{2} tests for triterpenes. The IR spectrum showed bands at 3450 cm−1 for a hydroxyl group and a double bond at 1640 cm−1. The 1H NMR spectrum showed seven methyl signals among which six (24-, 25-, 26-, 27-, 28-, 29-Me) were singlets and one (30-Me) was a doublet. The presence of one secondary and one primary hydroxy group was inferred by signals of geminal protons at δ 3.35 (1H, dd, J = 8.9 and 3.0 Hz) along with an AB dd at δ 3.27 and δ 3.47 (1H, each, J = 8.1 Hz). Abundant ions in the mass spectra of 1 at m/z 224, 234, 219 and 201 suggested that compound 1 was a pentacyclic triterpene of the α-amyrin class with a Δ^{12} double bond [4,5]. The loss of methyl from the molecular ion forming the fragment at m/z 443 was in good agreement with its location at C-17. These observations indicated that the basic skeleton of compound 1 was an urs-12-ene possessing one hydroxyl group in ring D/E and the remaining hydroxyls in ring A/B. The presence of 18-H as a singlet at δ 2.52 allowed us to assign the hydroxyl group to C-19. It was assigned α configuration due to its characteristic anisotropic effect on the signal of 16α-H at δ 2.42 [6] which moved further downfield to δ 3.06 when the NMR spectrum was recorded in pyridine-d$_{5}$ [6]. The secondary hydroxy group was assigned to C-3 on biogenetic grounds in β and equatorial configuration based on larger coupling constants of 3α-H at δ 3.35.

The BB and DEPT $^{13}$C NMR spectra afforded resonances for 30 carbons including seven methyls, ten methylene, six methine and seven quaternary carbon atoms. The comparison of $^{13}$C NMR of 1 with the model compounds [7] suggested the presence of a hydroxymethylene group at C-4. The conclusive evidence was provided by HMBC experiments. The protons at C-3 showed a $^2$J correlation with C-4 (δ 42.0) and a $^3$J correlation with hydroxymethylene C-23 (δ 67.8). On the other hand, the geminal protons of the hydroxymethyl-
labeled group at δ 3.27 and 3.47 showed a 2J correlation with C-4 (42.0) and 3J correlation with C-3 (75.2). The 18-H at δ 2.52 showed 2J correlations with C-19 (δ 72.7) and C-17 (δ 33.6) and a further 3J correlation with 28-H3 (δ 0.88). The α stereochirality of the hydroxymethylene group at C-4 was assigned on biogenetic grounds and confirmed through chemical shifts of C-23, C-24 and C-5 which showed very close agreement to those of similar triterpenes [5,8]. Therefore 1 was assigned on the structure of 3β, 19α, 23-trihydroxy-urs-12-ene.

**Compound (2)** was assigned the molecular formula C35H53O5 on the basis of the HR-FAB MS [m/z 601.3875 (M-H)+ (C35H53O5 requires 601.3879). It showed positive Liebermann-Burchard and Ce(SO4)2 tests for triterpenes. The IR spectrum showed the absorption for a hydroxy group (3400 cm⁻¹), an ester and acid carbonyls (1710 and 1700 cm⁻¹), phenylconjugated double bond (1625 cm⁻¹), an aromatic ring (1605 and 1595 cm⁻¹), a cis double bond (727 cm⁻¹) and a 1,4-disubstituted benzene (833 cm⁻¹). The UV spectrum showed maxima at 310 nm. The formation of monoacetyl derivative showed an olefinic proton at δ 5.20, a one-proton double doublet at δ 4.6 (J = 5.2, 11.2 Hz), a cis-disubstituted double bond at δ 5.8 and 6.8 (Jα,β = 12.7 Hz), a 1,4-disubstituted phenyl group at δ 6.7 and 7.6 (Jortho = 8.6 Hz), five methyl groups on quaternary carbons (δ: 0.79, 0.86, 0.89, 0.97 and 1.07), two sec. methyls at δ 0.92 and 0.95 (J = 6.5 and 6.6 Hz) and the signal at δ 2.2 (1H, d, J=11.0 Hz, 18-H). The 13C NMR spectrum (BB and DEPT) revealed the presence of seven methyls, nine methylene, thirteen methine and ten quaternary carbons, a carboxylic group (δ 177.5), an ester carbonyl (δ 167.70), a trisubstituted double bond (δ 125.24 and 138.07) and a disubstituted double bond (δ 144.62 and 115.60).

In the EI-MS the compound showed the base peak at m/z 147 representing the loss of a p-hydroxycinnamoyl moiety from the molecular ion peak. The ions at m/z 133 and m/z 248 originated through retro Diels-Alder cleavage of ring C. The ion at m/z 203 was due to the further loss of carboxylic moiety from this ion which allowed us to assign the carboxyl group to ring D/E and the [HO–C₆H₄–CH=CH–COO–] residue to ring A/B [9]. The ready loss of the carboxyl group from the molecular ion peak gave a fragment at m/z 557 allowing its assignment to C-17. This was supported by the chemical shifts of all the carbon atoms of rings D and E in the 13C NMR spectrum which were in close agreement to those of jacoumaric acid [10].

The ester moiety was assigned to C-3 on biogenetic grounds. This was confirmed by the HMBC spectrum which showed correlations of 3-H (δ 4.6) to C-23 (δ 27.98), C-24 (δ 17.21) and C-1' (δ 167.7). The values of coupling constants (J=11.2 Hz and 5.2 Hz) allowed us to assign the α or axial configuration to 3-H. The two olefinic protons at C-2' (δH₆ 5.8) and C-3' (δH₆ 6.8) exhibited vicinal coupling with each other. The coupling constant for H₆ and H₅ showed that the nature of double bond was cis. The C-2''/6'' (δ 7.6) protons showed couplings with protons at C-3''/C-5'' (δ 6.7). This confirmed that the α,β-unsaturated ester and the hydroxyl group are 1,4-disubstituted to the phenyl residue. The compound 2 was, therefore, assigned the structure 3β-(p-hydroxy-cis-cinnamoyl)urs-12-en-28-oic acid, which is a (Z) isomer of compound previously reported from *Tripetaleia peniculata*.
Plat material

The plant material was collected from the Karachi region and was identified as *Minusops elengi* by Prof. M. Qaiser, Department of Botany, University of Karachi. A voucher specimen is deposited in the herbarium of the Department of Botany, University of Karachi.

Extraction and isolation

The fresh and undried whole plant material (70 kg) was cut into small pieces and extracted four times with methanol (100 liters, 96 h each). The combined methanolic extract was evaporated under reduced pressure to yield a gummy residue. It was partitioned between hexane and water for one hour with constant shake and heat. The hexane soluble fraction was evaporated under reduced pressure to give residue. The hexane insoluble part was extracted with chloroform to obtain chloroform fraction. The chloroform fraction (30.0 g) was subjected to column chromatography over silica gel and eluted with increasing polarities of mixtures of hexane, CHCl₃ and MeOH. This operation provided four main fractions through hexane, hexane-CHCl₃ (7:3), hexane-chloroform (1:1), CHCl₃, CHCl₃-MeOH (9.5:0.5) and CHCl₃-MeOH (9:1). The fraction which was eluted with CHCl₃ was a mixture of three major and four minor spots. It was chromatographed over silica gel and eluted with hexane-CHCl₃ (7:2, 1:1, 3:7). The fraction obtained with hexane-CHCl₃ (2:8) from CC afforded 4 (50 mg). The physical and spectral data identified it as ursolic acid [10]. The fraction which was obtained from CHCl₃ showed two major compounds. It was purified by PTLC employing CHCl₃ + few drops of MeOH providing 2 (50 mg) and 3 (45 mg). The general spectral features of compound 3 identified it as 3/3-(/?-hydroxy-trans-cinnamoyloxy)urs-12-en-28-oic acid [11]. The fraction which was eluted with CHCl₃-MeOH (9:5:0.5) was partially pure and further purified by CC affording 1 (25 mg).

**Compound 1**: Colourless crystals, m.p. 200 °C (dec). – IR (KBr): ν = 1640, 3450 cm⁻¹. – ¹H NMR (500 MHz, CDCl₃ + few drops of CD₂OD): δ = 0.84 (s, 3H, 26-H), 0.88 (s, 3H, 28-H), 0.92 (d, 3H, J = 6.7Hz, 30-H), 0.95 (s, 3H, 25-H), 0.96 (s, 3H, 24-H), 1.2. (s, 3H, 29-H), 1.30 (s, 3H, 27-H), 2.42 (ddd, 1H, J₁ = J₂ = 13.4 Hz, J₃ = 4.4 Hz, 16-Ha), 2.52 (s,1H, 18-H), 3.27 (dd, 1H, J = 8.1 Hz, 23-Ha), 3.35 (dd,1H, J = 8.9, 3.0 Hz, 3-Ha), 3.47 (dd, 1H, J = 8.1 Hz, 23-Hb), 5.1 (t, 1H, J = 3.5 Hz, 12-H). – ¹³C NMR (125 MHz, CDCl₃ + few drops of CD₂OD): δ = 12.68 (C-24), 15.6 (C-30), 16.7 (C-...
Compound 2: Colourless crystals, m.p. 164 °C. – UV/vis (CHCl3): λmax (lg ε) = 310 nm (3.96) – IR (KBr): ν = 3400, 1710, 1700, 1625, 1605, 1595, 727, 833 cm⁻¹. – 1H NMR (500 MHz, CDCl3): δ 7.29 (s, 3H, 26-H), 5.92 (d, 3H, 25-H), 1.06 (s, 3H, 27-H), 2.21 (d, 1H, 29-H), 0.95 (d, 3H, 23-H), 0.96 (d, 3H, 24-H), 0.89 (s, 3H, 25-H), 0.96 (d, 3H, J = 6.6 Hz, 30-H), 1.06 (s, H, 27-H), 2.21 (d, 1H, J = 11.4 Hz, 18-H), 2.31 (s, 3H, CH₃-CO), 4.5 (dd, 1H, J = 5.3 Hz, 11.2 Hz, 3-H), 5.2 (brs, 1H, 12-H), 5.92 (d, 1H, J = 12.7 Hz, 2'-H), 6.85 (d, 1H, J = 12.7 Hz, 3''-H), 6.99 (d, 2H, J = 8.6 Hz, 3'''-H, 5''-H), 7.65 (d, 2H, J = 8.6 Hz, 2''-H, 6''-H). – MS (EI, 70 eV): m/z (%) = 644 [M⁺], 438 [M-acetyl cinnamoyl-oxyl]+ (8), 396 (15), 248 [retro-Diels-Alder fragment] (45), 203 [retro-Diels-Alder fragment-COOH] (20), 206 (36.0), 190 (18), 189 (15), 133 (28), 107 (11.2), 95 (17.3), 91 (28.7).

Methylation of 2a: Compound 2a (20 mg) was treated with freshly prepared CH₃N₂ in MeOH. After the usual workup the pure monomethyl ester 2b was obtained as a gummy residue. – 1H NMR (500 MHz, CDCl3): δ = 0.79 (s, 3H, 26-H), 0.85 (s, 3H, 24-H), 0.88 (s, 3H, 23-H), 0.91 (d, 3H, J = 6.5 Hz, 29-H), 0.97 (d, 3H, J = 7.0 Hz, 30-H), 0.95 (s, 3H, 25-H), 1.06 (s, 3H, 27-H), 2.20 (d, 1H, J = 11.4 Hz, 18-H), 2.29 (s, 3H, CH₃-CO), 3.57 (3H, s, CO₂CH₃), 4.50 (dd, 1H, J = 5.3 Hz, 11.0 Hz, 3-H), 5.21 (t, 1H, J = 3.7 Hz, 12-H), 5.9 (d, 1H, J = 12.7 Hz, 2'-H), 6.86 (d, 1H, J = 12.7 Hz, 3''-H), 6.98 (d, 2H, J = 8.6 Hz, 3'''-H, 5''-H), 7.64 (d, 2H, J = 8.6 Hz, 2''-H, 6''-H). – MS (EI, 70 eV): m/z (%) = 658 [M⁺], 469 [M-p-acetoxyccinamoylmoiety] (5), 452 [M-p-acetoxyccinamic acid] (5.7), 396 (17.2), 262 [retro-Diels-Alder fragment]+ (20), 203 [retro-Diels-Alder fragment, COOMe]+ (32.0), 206 (36.7), 190 (20.8), 189 (17.8), 133 (29.0), 107 (19.0), 91 (25).

Assay of α-glucosidase inhibition

Inhibitory activities of compounds 3 and 4 against α-glucosidase type VI (Sigma G6136) were observed spectrophotometrically at pH 6.8 and at 37 °C using 0.7 mM p-nitrophenyl α-D-glucopyranoside (PNP-G) as a substrate and 0.017 units/ml enzyme, in 50 mM sodium phosphate buffer containing 100 mM NaCl. 1-Deoxynojirimycin (0.3 mM) was used as a positive control. The IC5₀ value of deoxyojiririmycin was found to be the same as given by N. Asano et al. [13]. The absorption increase at 400 nm due to the hydrolysis of PNP-G by α-glucosidase was monitored continuously with a spectrophotometer ( Molecular Devices USA) [14].