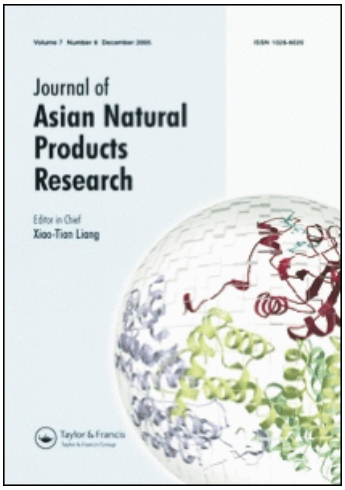

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A new triterpenoid, madhunolic acid, from the seeds of *Madhuca latifolia*

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A new triterpenoid, madhunolic acid, from the seeds of *Madhuca latifolia*

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A new triterpenoid, madhunolic acid, along with the three known constituents stigmasterol, 4-hydroxymethyl benzoate, and hydroquinone were isolated from the fruit seeds of *Madhuca latifolia*. The structure of the new compound was elucidated as 2β,3β,23-trihydroxyurs-5,12,20-trien-28-oic acid (**1**) on the basis of spectral data and chemical evidence.

Keywords: *Madhuca latifolia*; Sapotaceae; Fruit seeds; Triterpenoid; Madhunolic acid

1. Introduction

Madhuca latifolia Syn. *Madhuca latifolia* (Sapotaceae) is an important economic plant growing throughout the subtropical region of the Indo-Pak sub-continent [1]. It possesses many medicinal properties and different parts of this plant have been used in traditional medicine. In view of multifarious use of seeds, flowers, leaves and bark, systematic chemical investigations on the fruit coats and seeds were undertaken in our laboratory, which resulted in the isolation and characterisation of several new and known compounds [2–4]. The present paper reports the isolation and characterisation of a new triterpene, madhunolic acid, along with three known compounds stigmasterol [5], 4-hydroxymethyl benzoate [6], and hydroquinone [7]. 4-Hydroxymethyl benzoate and hydroquinone were isolated for the first time from this source. Assignments of the NMR data of the new compound were made by 2D NMR experiments to reveal the structure of **1** as 2β,3β,23-trihydroxyurs-5,12,20-trien-28-oic acid (figure 1).

2. Results and discussion

Compound **1** showed the molecular ion peak at *m/z* 484 in the EI-MS and at *m/z* 484.3187 in the HR-MS corresponding to the molecular formula $C_{30}H_{44}O_5$, supported by ^{13}C NMR

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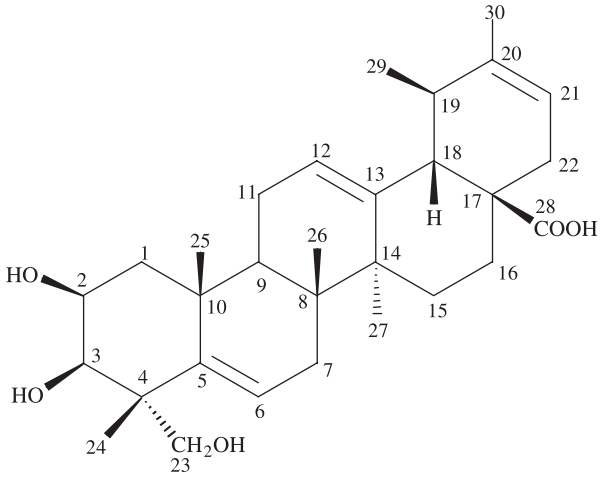

Figure 1. The structure of **1**.

spectral data. **1** was shown by its ^{1}H NMR spectrum to be a pentacyclic triterpene having four tertiary, one secondary and one olefinic methyl groups (figure 2). The nature of the carbon skeleton could be deduced as that of α amyrin from ^{1}H and ^{13}C NMR as well as MS spectral data discussed below [8–11].

The IR spectrum exhibited strong absorption bands at 3460 (OH) and 1693 cm^{-1} (C═O), while the UV spectrum showed λ_{max} at 193.6 and 205.0 nm, indicating that the system lacked conjugation. The ^{1}H NMR and ^{13}C NMR spectra showed signals for one hydroxymethyl group at δ_H 3.38 (d, J_{gem} = 10.9 Hz) and 3.50 (d, J_{gem} = 10.9 Hz); δ_C 71.3 (C-23)] and two secondary carbinylic methines at δ_H 3.86 (m, H-2α); δ_C 67.2 and δ_H 3.59 (d, 3.2 Hz, H-3α) δ_C 78.7 [12,13]. Furthermore, signals at δ_C 148.4 (C-5), 143.2 (C-13), 136.9 (C-20), 129.2 (C-12; δ_H 5.29, t, 3.7 Hz, H-12), 120.7 (C-6; δ_H 5.93, dd, 10.0, 2.9 Hz, H-6) and 120.0 (C-21; δ_H 5.23, dd, 10.2, 2.8 Hz, H-21) revealed the presence of three trisubstituted double bonds. The presence of three acylable hydroxyl groups was further confirmed by treatment of **1** with

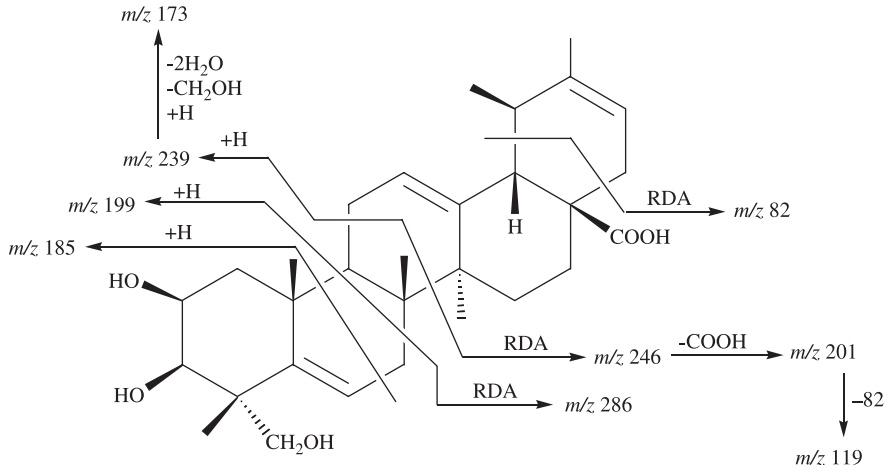

Figure 2. Diagnostic MS fragmentations of **1**.

acetic anhydride and pyridine which afforded the triacetyl derivative (**1a**), $C_{36}H_{50}O_8$ (M^+ at *m/z* 610), exhibiting three methyl singlets at δ 2.03, 1.98 and 1.93 in the ^{1}H NMR spectrum. In the mass spectrum of **1** and **1a,** the strong peaks at *m/z* 246 (RDA fragment), 201 and 119 suggested the presence of two double bonds and the carboxyl group in ring D/E. Further, a doublet at δ 2.50 ($J = 11.2$ Hz, H-18) in the ^{1}H NMR implied the α-amyrin skeleton of **1** with a double bond at C-12 and carboxylic acid moiety at C-17. The second double bond could be placed at C-20 as the ^{1}H NMR spectrum showed only one secondary methyl doublet at δ 0.92 ($J = 6.6$ Hz, H-29) instead of two doublets along with a vinyl methyl singlet at δ 1.78. This was supported by the mass fragment ion at *m/z* 82 arising from RDA fragmentation around ring E. These observations and the fragment ions at *m/z* 239 and 173 in **1** and the ion at *m/z* 364 in **1a** corresponding to *m/z* 239 in **1** suggested that the third double bond and all the hydroxyl groups are confined to ring A/B. Furthermore, fragments at *m/z* 286 due to RDA and at *m/z* 199 indicated that this double bond is at C-5 and all the hydroxyls are in ring A [13]. The chemical shifts of methylene protons of CH_2OH in **1** and CH_2OAc in **1a** were in agreement with an equatorial-CH_2OH group [15,16] i.e. C-23. The ^{1}H NMR spectrum further showed two methine protons geminal to the hydroxyl groups. The chemical shift, multiplicity and coupling constant as well as biogenetic consideration led to place one of them at C-3 and the other at C-2 both with β disposition [14]. These assignments were confirmed from the cross peaks in the ^{1}H–^{1}H COSY spectrum which showed correlation between H-2α and H-3α and from the NOESY correlation between H-23 and H-3 and between H-3 and H-2. Consequently the structure of compound **1** was elucidated as 2β,3β,23-trihydroxyurs-5,12,20-trien-28-oic acid named as madhunolic acid.

3. Experimental

3.1 General experimental procedures

Optical rotations were measured on JASCO DIP-360 digital polarimeter. UV spectrum was recorded on Hitachi-U-3200, Secomam Anthelei Junior spectrophotometers. IR spectrum was recorded on Bruker VECTOR 22 spectrophotometer. ^{1}H NMR and ^{13}C NMR, COSY, NOESY, HMQC, HMBC and *J*-resolved were performed on Bruker spectrophotometers, AVANCE 400, operating at 400 MHz for ^{1}H- and 100 MHz for ^{13}C nuclei. Chemical shifts are expressed in δ (ppm) with reference to the residual solvent signals and coupling constant J in Hz. EI-MS were measured on Varian MAT-312 mass spectrometer and Jeol JMS-600 H mass spectrometer *m/z* (rel.%) by direct prob inlet; source at 250°C and 70 eV; *m/z* (rel.%). HREI-MS were measured on a Jeol JMS-600 H mass spectrometer. The petroleum ether used was of the boiling range 60–80°C. Vacuum liquid chromatography (VLC) was carried out on silica gel 60 PF_{254} (Merck). Column chromatography (CC) was performed on silica gel 60, mesh size 70–230 (Merck, 0,063–0,200 mm). Prep. TLC was detected at 254 and 366 nm with UVKL UV lamps H. Jurgens and Co. and I_2 vapours were used for visualisation.

3.2 Plant material

The fresh fruits of *Madhuca latifolia* (40 kg) were collected in June 2000 from Karachi region. The plant was identified by Mr Sher Wali (Herbarium Incharge), Department of

Botany, University of Karachi and a voucher specimen (KUH. No. 67974) has been deposited in the Herbarium of the same university.

3.3 Extraction and isolation

The fruit coatings (10 kg) and seeds (8.5 kg) were separated manually and the seeds were extracted repeatedly (× 5) with MeOH at room temperature. The solvent of the combined extracts was removed under reduced pressure. The syrupy extract (1.084 kg) thereby obtained was partitioned between EtOAc and H_2O. The EtOAc phase was concentrated to give a gummy mass, which was treated with petroleum ether to yield petroleum ether soluble and petroleum ether insoluble fractions. The petroleum ether soluble fraction was treated with 90% aq. MeOH to furnish petroleum ether phase and 90% MeOH phase. The 90% MeOH phase was further partitioned between EtOAc and water after saturation with saline water. The organic phase was washed, dried (anhydrous Na_2SO_4), and evaporated to give a residue (10.36 g). This was separated through VLC eluting with $CHCl_3$, $CHCl_3$/MeOH, and MeOH in increasing order of polarity. The eluates were combined on the basis of TLC to afford 10 fractions. Fraction 3 (52.3 mg; eluted with 9.5:0.5 $CHCl_3$/MeOH (500 ml)) on purification over thick layer (TLC) silica gel plates (9.7:0.3 $CHCl_3$/MeOH) provided stigmasterol (10.4 mg), 4-hydroxymethyl benzoate (5.0 mg) and hydroquinone (10.5 mg). Fractions 5, 6, 7 and 8 (6.85 g, 8.5:1.5 – 8:2 $CHCl_3$/MeOH (8 L) were combined and subjected to column chromatography eluting with petroleum ether, petroleum ether/EtOAc, $CHCl_3$, $CHCl_3$/MeOH and MeOH. As a result 14 fractions were obtained. Fraction 7 (9.3:0.7 $CHCl_3$/MeOH (100 ml)) was reduced to a residue (14.7 mg) and separated into $CHCl_3$ soluble and insoluble fractions. The $CHCl_3$ insoluble fraction afforded **1** as an amorphous powder (8.0 mg; TLC 9:1 $CHCl_3$/MeOH).

3.3.1 2β,3β,23-Trihydroxyurs-5,12,20-trien-28-oic acid (1). Amorphous powder (8.0 mg). $[\alpha]_D^{27}$ + 35.5 (*c* 0.023, MeOH). UV (MeOH) λ_{max} nm (log ε): 193.6 (4.70), 205.0 (3.68). IR (KBr) ν_{max} cm^{-1}: 3460 (OH), 2925–2857 (C—H), 1693 (C═O), 1637 (C═C), 1445, 1377, 1222, 1156, 1035, 768, 672. ^{1}H NMR (400 MHz, CD_3OD) and ^{13}C NMR (100 MHz, CD_3OD): see table 1. EI-MS *m/z*: 484 (29, M^+), 440 (30, M^+-COO), 286 (28), 246 (35), 239 (43), 237 (36), 219 (33, 237-H_2O), 201 (63), 199 (37), 185 (51), 173 (71), 119 (100), 82 (44). HR-MS *m/z*: 484.3187 (Calcd for $C_{30}H_{44}O_5$, 484.3188), 440.3248 ($C_{29}H_{44}O_3$), 237.1513 ($C_{14}H_{21}O_3$), 201.1598 ($C_{15}H_{21}$), 173.1279 ($C_{13}H_{17}$).

3.3.2 Acetylation of 1. Compound **1** (4.0 mg) was dissolved in pyridine (0.5 ml) to which acetic anhydride (1 ml) was added and left overnight at room temperature. The reaction mixture was poured over crushed ice and extracted with EtOAc. After usual work-up of the EtOAc phase and purification by column chromatography **1a** was obtained as an amorphous powder (2.3 mg). ^{1}H NMR (400 MHz, $CDCl_3$): see table 1. MS *m/z*: 610 (49, M^+), 566 (16, M^+-COO), 493 (33, M^+-44-CH_2OCOCH_3), 451 (38, 493–42), 369 (40, 451–82 (C_6H_{10})), 364 (47, RDA), 282 (40, RDA-42), 246 (63, RDA), 239 (45, 282–42-H), 201 (85, 246–45), 197 (51, 282-(2 × 42)-H), 173 (92, 364-(2 × AcOH)-CH_2OCOCH_3), 119 (100, 201–82), 82 (57, RDA). HR-MS *m/z*: 610.3591 (Calcd for $C_{36}H_{50}O_8$, 610.3506), 566.3647 ($C_{35}H_{50}O_6$), 173.1422 ($C_{13}H_{17}$).

Table 1. ^{1}H NMR and ^{13}C NMR data of compounds **1** and **1a** (δ in ppm, J in Hz).

	1		***1a***
C	δ_C	δ_H *(m, Hz)*	δ_H *(m, Hz)*
1	42.2	Not obs.	–
2	67.2	3.86 m	4.16 m
3	78.7	3.59 d (3.6)	4.29 d (4.2)
4	44.4	–	–
5	148.4	–	–
6	120.7[†]	5.93 dd (10.0, 2.9)	5.94 dd (10.2, 3.0)
7	39.1[†]	Not obs.	–
8	38.5	–	–
9	44.1	Not obs.	–
10	33.7	–	–
11	24.7	Not obs.	–
12	129.2	5.29 t (3.7)	5.34 t (3.9)
13	143.2	–	–
14	42.7	–	–
15	27.7	Not obs.	–
16	29.5	Not obs.	–
17	47.5	–	–
18	55.1	2.50 d (11.2)	2.50 d (11.2)
19	43.0	Not obs.	–
20	136.9	–	–
21	120.0[†]	5.23 dd (10.2, 2.8)	5.20 dd (10.0, 3.0)
22	39.0[†]	Not obs.	–
23	71.3	3.38 d (10.9) 3.50 d (10.9)	3.73 d (10.4) 4.04 d (10.5)
24	17.5	0.77 s	0.73 s
25	27.0	1.19 s	1.18 s
26	18.3	1.01 s	1.06 s
27	24.9	1.28 s	1.29 s
28	178.8	–	–
29	16.5	0.92 d (6.6)	0.92 d (6.6)
30	19.1	1.78 s	1.78 s

Signal assignments based on ^{1}H–^{1}H COSY, HMQC, HMBC and NOESY experiments.
[†] Values are interchangeable.

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