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A LUPENE-TYPE TRITERPENE FROM *MIMUSOPS ELENGI*

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Key Word Index—*Mimusops elengi*; Sapotaceae; triterpene; 3β-hydroxy-lup-20(29)-ene-23,28-dioic acid.

Abstract—A new triterpene 3β-hydroxy-lup-20(29)-ene-23,28-dioic acid has been isolated from *Mimusops elengi*. Its structure was established through chemical and spectroscopic studies. The known triterpenes, β-amyrin, lupeol, α-taraxerol and ursolic acid were also isolated.

INTRODUCTION

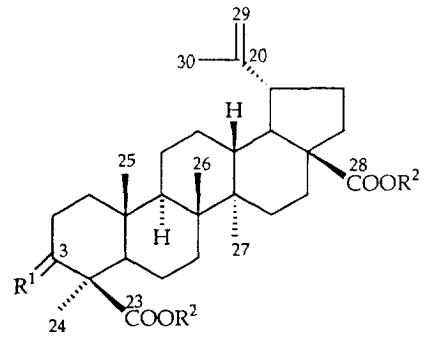
Mimusops elengi grows wild in southern India, Burma and Pakistan. Various parts of the plant are used in the indigenous system of medicine as a febrifuge, astringent, purgative and stimulant [1]. Although the presence of saponins, steroids, terpenoids, and alkaloids have been reported from *M. elengi* [2–5], but very few individual constituents have so far been isolated or characterized [5a]. We now report on the isolation and characterization of a new pentacyclic triterpene (**1**) of the lupene series from this plant. The known triterpenes β-amyrin, lupeol, α-taraxerol and ursolic acid have also been isolated from this species for the first time.

RESULTS AND DISCUSSION

Compound **1** was assigned the molecular formula $C_{30}H_{46}O_5$ by HRMS ([M]$^+$ at *m/z* 486.3307; calc. 486.3345). It gave positive Liebermann–Burchard and $CeSO_4$ tests for triterpenes and also a positive $NaHCO_3$ test for a carboxyl group(s). The IR spectrum showed the absorption for a hydroxy group (3400 cm^{-1}), a carboxyl group (2730 and 1700 cm^{-1}) and a disubstituted double bond (3075 and 1640 cm^{-1}). The formation of the dimethyl ester **1a** and a monoacetyl derivative (**1b**) indicated the presence of two carboxylic functions and one hydroxyl. Oxidation of **1a** with PCC in methylene chloride gave the keto diester **1c**, confirming the secondary nature of the hydroxyl group.

The ^{1}H NMR spectrum of **1** showed two olefinic protons at δ4.58 and 4.71, a one-proton double proton at δ3.9 (*J* = 5.1, 10.7 Hz) and five methyl groups on quaternary carbons (δ: 0.79, 0.86, 0.98, 1.30 and 1.68). The ^{13}C NMR spectrum (BB and DEPT) of **1** corroborated the presence of five methyls, nine methylenes, seven methines and five quaternary carbons, in addition to carboxylic groups (δ178.7 and 177.3) and a 1,1-disubstituted double bond (δ49.8 and δ150.0).

In the HR-mass spectrum ions at *m/z* 250.1549 ($C_{15}H_{22}O_3$), 248.1771 ($C_{16}H_{24}O_2$), 231.1472 ($C_{14}H_{21}O_3$), 234.1602 ($C_{15}H_{22}O_2$) and 219.1373 ($C_{14}H_{19}O_2$) were characteristic of a lup-20(29)-ene skeleton with a carboxy group in ring D/E and the hydroxyl and remaining carboxylic function in ring A/B [6, 7]. The ready loss of one of the carboxyl groups from the molecular ion peak gave a fragment at *m/z* 441.3327 ($C_{29}H_{45}O_3$) allowing assignment to C-17. This was supported by the chemical shifts of all the carbon atoms of rings D and E in the ^{13}C NMR spectrum, which showed complete agreement to betulinic acid [6, 8].



1 R^1 = β-OH; α-H; R^2 = H
1a R^1 = β-OH; α-H; R^2 = Me
1b R^1 = β-OAc; α-H; R^2 = H
1c R^1 = O; R^2 = Me

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In the $^1H-^1H$ one bond COSY spectrum of **1** the carbinol methine proton at δ3.9 showed a cross-peak with two other protons thus placing it in ring A or on C-7. It was assigned to C-3 on biogenetic grounds. This was supported by the characteristic chemical shifts of C-1 and C-2 in the ^{13}C NMR spectrum. The large coupling constant allowed us to assign a β and equatorial configuration to the hydroxy group. This was also confirmed by $NaBH_4$ reduction of the keto diester **1c** back to the dimethyl ester **1a**. Evidence as to the position of the carboxylic group in ring A/B was provided by the mass spectrum of the keto diester **1c**. This showed an intense peak at *m/z* 169.0847 ($C_9H_{13}O_3$) which is also found in the mass spectrum of 3-oxo-allobetulane [9] and thus placed the carbonyl group and the carboxylic function at positions C-3 and C-4, respectively.

The key to the configuration of the isopropenyl group at C-19 and that of the carboxylic group at C-4 was provided by the physical data of the keto diester **1c** which showed complete agreement to those reported in the literature for the dimethyl ester of 3-oxo-lup-20(29)-ene-23,28-dioic acid [7]. Thus compound **1** is 3β-hydroxy-lup-20(29)-ene-23,28-dioic acid. In the $^1H-^{13}C$ long-range COSY spectrum of **1** the carbonyl carbon of the carboxylic group at δ178.70 showed a cross-peak to the protons of the methyl group at C-24, thus providing conclusive evidence for the assigned structure. Compound **1** is epimeric at C-3 with a triterpene isolated from *Scheffera octophylla* [7]. The diester obtained from the latter was reported to show two bands in the IR spectrum, one at 3630 cm^{-1} for the free hydroxyl group and one at 3510 cm^{-1} due to hydrogen bonding between OH and the ester group at C-23. No such hydrogen bonding is possible in **1a** due to the *trans* disposition of these groups, hence only a single band for a free hydroxyl group was observed at 3620 cm^{-1}.

EXPERIMENTAL

General. Mps: uncorr.; IR: $CHCl_3$; ^1H NMR (400 MHz) and ^{13}C NMR (100 MHz): $CDCl_3$ using TMS as int. standard; HRMS: 70 eV; TLC: silica gel, PF_{254}; CC: silica gel, 70–230 mesh. The DEPT, NOE and HMQC experiments were performed as reported earlier [10, 11].

Plant material. The plant material was collected from the Karachi region and was identified as *Mimusops 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.

Isolation. The shade-dried plant material (70 kg) was extracted(× 4) with MeOH at room temp. The residue from the methanolic extract was partitioned between hexane and H_2O. The hexane-soluble fr. was chromatographed over silica gel using various mixtures of hexane. $CHCl_3$ and MeOH.

3β-Hydroxy-lup-20(29)-ene-23,28-dioic acid (**1**). The fr. eluted in hexane–$CHCl_3$ (1:3) for CC contained only one major compounds on TIC. It was further purified by prep. TLC using hexane–EtOAc (3:2) and then crystallized from Me_2CO–*n*-hexane (30 mg); mp: 262–264°; $[\alpha]_D^{25}$ + 13° ($CHCl_3$; *c* 0.52); IR $\nu_{max}^{CHCl_3}$ cm^{-1}; 3400, 3075, 2730, 1700, 1640; MS *m/z* (rel. int.): 486.3307 $[M]^+$ (18) ($C_{30}H_{46}O_5$), 471.3088 (7) ($C_{29}H_{43}O_5$), 468.3188 (22) ($C_{30}H_{44}O_4$), 441.3327 (19) ($C_{29}H_{45}O_3$), 250.1549 (53) ($C_{15}H_{22}O_3$), 248.1771 (71) ($C_{16}H_{24}O_2$), 237.1472 (44), ($C_{14}H_{21}O_3$), 234.1602 (75), ($C_{15}H_{22}O_2$), 219.1373 (78) ($C_{14}H_{19}O_2$); ^1H NMR ($CDCl_3$): δ0.79, 0.86, 0.98, 1.30, 1.68 (3H, each *s*, Me), 3.9 (*dd*, *J* = 5.1 and 10.7 Hz, H-3), 4.58 and 4.71 (1H each, *m* H_2-29); ^{13}C NMR ($CDCl_3$, 100 MHz): δ39.0 (C-1), 29.4 (C-2), 84.4 (C-3), 43.0 (C-4), 44.2 (C-5), 18.3 (C-6), 33.9 (C-7), 41.5 (C-8), 49.6 (C-9), 37.0 (C-10), 23.4 (C-11), 25.3 (C-12), 38.5 (C-13), 42.8 (C-14), 30.5 (C-15), 32.2 (C-16), 56.1 (C.17), 46.8 (C-18), 49.8 (C-19), 150.0 (C-20), 29.7 (C-21), 37.0 (C-22), 178.7 (C-23), 18.1 (C-24), 18.4 (C-25), 16.1 (C-26), 14.4 (C-27), 177.3 (C-28), 109.4 (C-29), 19.0 (C-30). These assignments were made by comparison with published ^{13}C NMR data of related compounds [6–8], and confirmed in each case by $^1H-^{13}C$ correlated spectroscopy (HMQC).

Dimethyl ester **1a**. Obtained from **1** by treatment with CH_2N_2 in MeOH. Amorphous; $[\alpha]_D^{25}$ + 14° ($CHCl_3$, *c* 0.19); IR $\nu_{max}^{CHCl_3}$ cm^{-1}: 3450, 3070, 1730, 1640; MS *m/z* (rel. int.): 514 $[M]^+$ (23), 499 (8), 496 (8), 496 (22), 454 (21), 264 (64), 262 (76), 251 (60), 248 (76), 233 (80).

Acetate **1b**. Obtained from **1** by treatment with Ac_2O–pyridine for 20 hr at 25°; mp 209–212° (Me_2CO–*n*-hexane); $[\alpha]_D^{25}$ + 20° ($CHCl_3$; *c* 0.17); IR $\nu_{max}^{CHCl_3}$ cm^{-1}: 3070, 1730, 1700, 1640, 1240; MS *m/z* (rel. int.): 528 $[M]^+$ (10), 513 (6), 482 (11), 486 (16), 292 (55), 248 (69), 279 (46), 234 (76), 219 (80).

Oxidation of **1a** *to keto diester* **1c**. To PCC (67 mg) in dry CH_2Cl_2 (5 ml), compound **1a** (58 mg) was added and the soln stirred at 20° for 4 hr. Standard work-up followed by CC over silica gel and elution with *n*-hexane–$CHCl_3$ (4:1) gave keto ester **1c** (34 mg); mp 131–132° (*n*-hexane); $[\alpha]_D^{25}$ + 6° ($CHCl_3$; *c* 0.2). The MS, IR and ^1H NMR data were in good agreement with those reported in ref. [7].

Reduction of **1c** *to* **1a**. To a soln of **1c** (10 mg) in 1 ml MeOH–THF (1:1) was added $NaBH_4$ (10 mg). The mixt. was stirred at 20° for 1 hr. Standard work-up followed by CC over silica gel (elution with *n*-hexane–$CHCl_3$, 7:3) provided an amorphous compound having the same R_f value and physical constants as **1a**.

Compound **2**. The fr. eluted in hexane–$CHCl_3$ (7:3) from CC showed one major spot and was crystallized from EtOH, mp: 197–198°; $[\alpha]_D^{25}$ + 99° ($CHCl_3$); MS *m/z* (rel. int.): 426 $[M]^+$ (15). The physical and spectral data identified it as β-amyrin [12].

Compound **3**. The eluent obtained from hexane–$CHCl_3$ (13:7) showed one major spot and was further purified on silica gel CC using hexane–$CHCl_3$ (3:2) as solvent system and crystallized from MeOH, mp 214–14°, $[\alpha]_D^{25}$ + 27°. MS *m/z* (rel. int.): 426 $[M]^+$ (20). The physical and spectral data identified it as lupeol [8].

Compound **4**. The fr. eluted with hexane–$CHCl_3$ (1:1) was subjected to prep. TLC using as hexane–$CHCl_3$ (3:2) solvent system. It crystallized from benzene as needles, mp 271–272°; $[\alpha]_D^{25}$ − 9.9°. MS *m/z* (rel. int.): 426 $[M]^+$

(18). It was identified as α-taraxerol on the basis of physical and spectral data [13].

Compound **5**. The material in the hexane–$CHCl_3$ (3:7) eluate was further subjected to prep. TLC using the solvent system $CHCl_3$–MeOH (49:1) and the major spot crystallized from EtOH, mp: 283–285°; $[\alpha]_D^{21}$ + 62.5–68° (MeOH). The physical and spectral data identified it as ursolic acid [14].

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