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Novel Migrated Oleanane Triterpenoid Sapogenins from *Mimusops elengi*

Sucharita Sen, Niranjan P. Sahu and Shashi B. Mahato*

Indian Institute of Chemical Biology
4, Raja S.C. Mullick Road, Jadavpur
Calcutta - 700 032, India.

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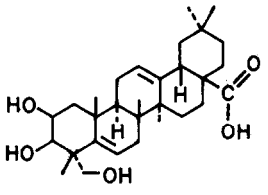
Abstract: *Two new pentacyclic triterpene acids, mimusopic and mimusopsic acids, possessing the novel migrated oleanane skeleton, mimusopane were isolated from the seeds of Mimusops elengi and were respectively defined as 2β,3β,23-trihydroxy-5,10-friedooleana-9,12-dien-28-oic acid and 2β,3β-dihydroxy (23→10)-oxido-5,10-friedooleana-9(11),12-dien-28-oic acid based on their spectroscopic properties and chemical transformations.*

Saponins of triterpenoid acids are drawing much attention in recent years for their cholesterol-lowering[1] and other biological activities[2]. The seeds of *Mimusops elengi* (Sapotaceae) widely distributed in India are used in traditional Indian medicine[3]. The previous report[4,5] of isolation from the plant of bassic acid **2** which is considered to be a chemical marker for the presence of saponins of protobassic acid **1** prompted us to take up phytochemical investigation on its saponin and sapogenin constituents.

RESULTS AND DISCUSSION

The Bu^nOH soluble fraction of MeOH extract of the defatted powdered seeds was hydrolysed with aq. MeOH-HCl under reflux for 4h. The acid hydrolysate was separated into acidic and neutral fractions by treatment with a saturated solution of $NaHCO_3$. The acidic fraction on chromatographic separation yielded three sapogenins. The medium polar sapogenin was characterized as bassic acid **2** from its physical and spectroscopic data[6,7] as well as by direct comparison with an authentic sample. The most polar sapogenin designated mimusopic acid **3** showed in its electron impact mass spectrum (EIMS) the molecular ion at m/z 486 which is identical with that of bassic acid **2**. However, the retro Diels-Alder fragments characteristic of Δ^{12}-oleanenes[8] which are observed for acid **2** were absent in the MS of acid **3**. The ^1H NMR spectrum of acid **3** exhibited a t-like signal integrating for 1H at δ 5.54 reminiscent of the presence of 12:13 double bond, which was also supported by the ^{13}C NMR spectrum which showed the signals at δ 121.7 (d) and

144.7 (s) ascribable to C-12 and C-13 respectively[9]. The spectrum also showed a two - carbon singlet assignable to a tetrasubstituted double bond. Off - resonance decoupling experiments coupled with insensitive nuclei enhancement by polarisation transfer (INEPT) studies allowed ready interpretation of carbon multiplicities. The ^{13}C data suggested the presence of a trisubstituted double bond, a tetrasubstituted double bond, two secondary and one primary hydroxyl groups, a carbonyl function, six methyls, nine methylenes, a methine and six quaternary carbons. These structural features may be accommodated in the rearranged oleanane skeletons **3** and **4**. However, Dreiding model inspection revealed that the structure **4** is very



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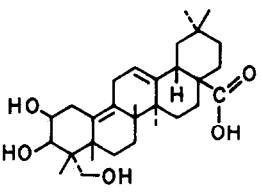

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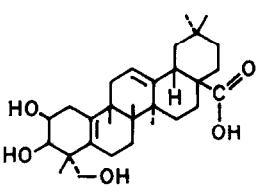

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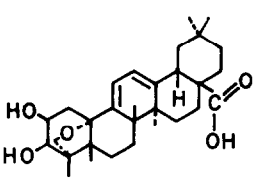

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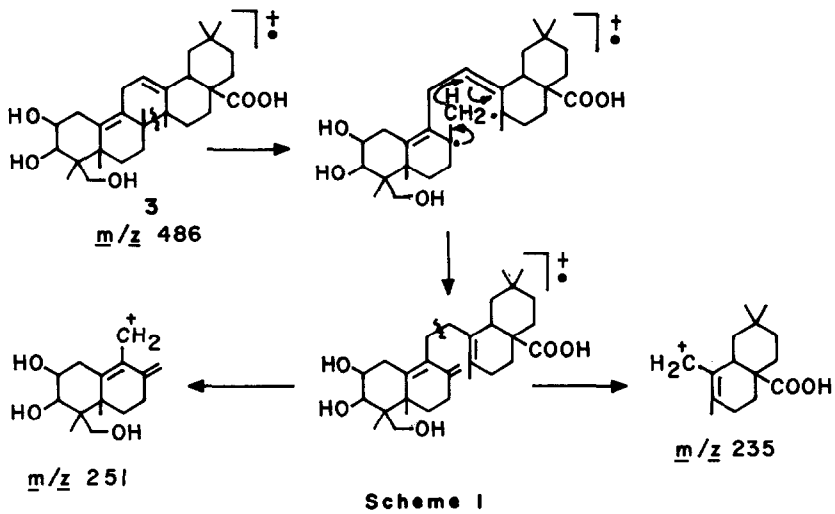
much unstable and its mechanistic formation is difficult to rationalize. The mass spectral data of mimusopic acid are found to be compatible with the structure **3** and the formation of the base peak at m/z 251 may be rationalized as shown in scheme **1**. The assignments of ^{13}C signals of the acid **3** (see experimental) was made by known chemical shift rules[10,11] and comparison with those of model triterpenes possessing similar carbon atoms. The reversal of conformations of the C-2 and C-3 hydroxyl groups in acid **3** in comparison to bassic acid **2** was apparent from the ^{1}H NMR spectrum of the former. While bassic acid exhibits in its ^{1}H NMR spectrum the C-2 and C-3 carbinyl protons at δ4.57 (1H,q,J 3.5 Hz) and 4.34 (1H,d,J 3.7 Hz) respectively[7], the acid **3** shows the same protons at δ4.38 (1H, m,$W_{\frac{1}{2}}$ 14 Hz) and 4.81 (1H,d,J 3.5 Hz) indicating equatorial and axial conformations of its C-2 and C-3 hydroxyl groups. Such changes are not unexpected in view of the known conformational preferences in oleanane vis-a-vis friedelane derivatives. Consequently, the structure of mimusopic acid **3** may be suggested as 2β,3β,23-trihydroxy-5,10-friedooleana-9,12-dien-28-oic acid.

The least polar acid designated mimusopsic acid **5** displayed in its EIMS the molecular ion peak at m/z 484. The ^{1}H NMR spectrum of acid **5** showed the presence of two trisubstituted double bonds. That these two trisubstituted double bonds form a homoannular diene system was evident from its UV absorption maximum at 284 nm (ϵ8100) characteristic of such a system. Moreover, transformation of mimusopic acid **3** to mimusopsic acid **5** was disclosed by TLC examination of the MeOH solution of the former kept standing for 24h which showed partial formation of mimusopsic acid **5**. As a matter of fact acid **3** could be converted to



Scheme I

acid 5 in about 10% yield by heating a solution of the former in MeOH under reflux for 4h. This phenomenon as well as co-occurrence of acids 2,3 and 5 strongly indicated that both 3 and 5 could be artefacts formed from bassic acid during acid hydrolysis of the saponin fraction. In fact, when a solution of bassic acid 2 in MeOH-HCl (aq) was boiled under reflux for 4h and worked up as usual for the isolation of the products, acid 3 (40%), 5 (10%) and unconverted acid 2 (50%) were obtained. ^{1}H NMR and ^{13}C NMR (see experimental) data are quite in conformity with the structure shown. It may be mentioned that while the conformations of C-2 and C-3 hydroxyls of acid 3 are opposite to those of bassic acid 2 the conformations of the same hydroxyls of acid 5 should be the same as those of acid 3 considering the pathway envisaged for the formation of acid 5 from 3 (vide infra). The C-3 carbinyl proton of acid 5 appears upfield (δ3.91,1H,d, J 6 Hz) than that of C-2 carbinyl proton which appears atδ4.44 (1H,m,$W_{\frac{1}{2}}$ 14 Hz). The upfield shift of the C-3 carbinyl proton signal of acid 5 may be ascribed to the shielding effect of the 23⟶10 oxide ring. The assignments of the ^{13}C signals were straightforward using known chemical shift rules as well as by comparison with those of model triterpenes with similar carbon atoms. The upfield shift of 5.8,0.9 and 5.5 ppm of C-9,C-12 and C-13 respectively and downfield shift of 4.6 ppm of C-11 in comparison to those of saikogenin B[12] [3β,16β,28-trihydroxy-oleana-9(11),12-diene] are understandable if one considers the desk top molecular modeller (DTMM) generated model of acid 5 (Figure 1).

The difference of 2 mass units between 3 and 5 indicated the presence of either a lactone ring or an oxido linkage in the latter. However, the presence of a lactone function was excluded by its IR spectrum. The 23⟶10 oxido linkage in

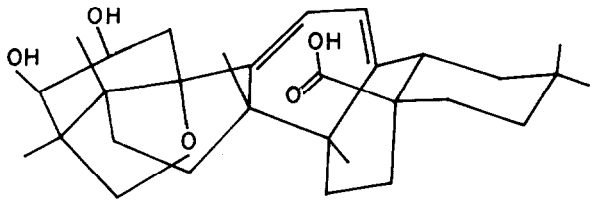
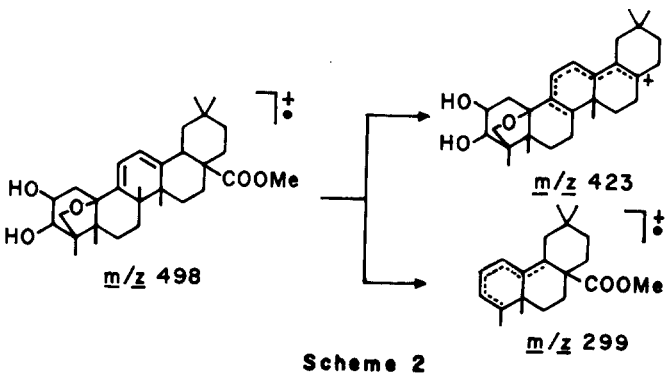


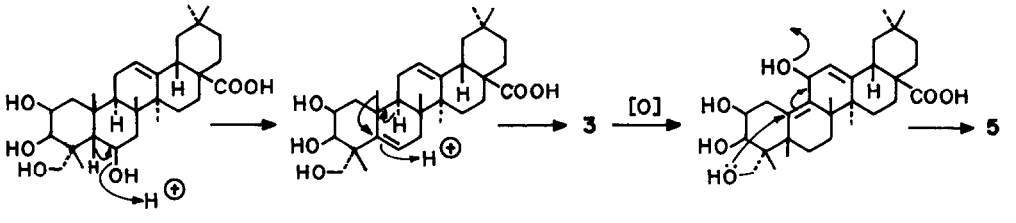
Figure I

acid 5 was ascertained by (i) its inactivity to form trityl derivative with trityl chloride and (ii) the characteristic ^{13}C signals of C-23 (δ73.4,t) and C-10 (δ85.0, s) the latter being the only quaternary carbon atom containing an oxygen atom in the molecule. The FABMS of acid 5 and its methyl ester showed significant peaks at m/z 423 and 299 whose formation may be rationalized as shown in scheme 2.



Scheme 2

The mechanism of formation of mimusopsic acid 5 from protobassic acid 1 is shown in scheme 3. Although transformation of protobassic acid 1 to bassic acid 2 is known to occur by facile dehydration of 6β(axial)-hydroxyl group under acidic condition which is under strong steric 1,3-diaxial interactions with 4β-,8β- and 10β-axial methyls, further conversions of acid 2 to acid 3 and 5 are unprecedented. Inspection of Dreiding model reveals that 1,3-diaxial interaction exists in acid 2 involving 10β-,4β-methyls and 2β-hydroxyl group. Protonation of the 5,6-double bond followed by migration of the 10β-methyl to 5β-position resulting in reversal of conformation of the A-ring substituents release the strains and lead to the formation of mimusopic acid 3 with the novel 5,10-friedooleanane skeleton. However, because of the diallylic nature of 11-CH_2 of acid 3 it is prone to aerial oxidation presumably leading to the intermediate formation of 11-hydroxy compound 6. Elimination of the 11-hydroxy of acid 6, migration of the 9:10 double bond to 9:11 position and formation of 23⟶10-oxido ring furnish the novel 2β,3β-dihydroxy-(23⟶10)-oxido-5,10-friedooleana-9(11),12-dien-28-oic acid(mimusopsic acid) 5.



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Scheme 3

Mimusopic acid and mimusopsic acid represent the first members of a hitherto unknown 5,10-friedooleanane skeleton[13].

It is noteworthy that Kitagawa *et al.*[14] have demonstrated that bassic acid **2** is an artefact derived from protobassic acid **1** during acid hydrolysis of saponins containing protobassic acid as the aglycone. Saponins containing protobassic acid as the aglycone have been isolated from a number of plants by several groups of workers[7,15,16]. However, isolation of only bassic acid as the acid rearranged product from the acid hydrolysate of the saponins has so far been reported. Considerable attention is being given in recent years for evaluation of antitumor activity of triterpene acids and encouraging results in this regard have been published[17-19]. The new skeleton triterpenoid acids **3** and **5** easily available from protobassic acid containing saponins are of much interest to study their structure activity relationship.

EXPERIMENTAL

The plant material was identified at Indian Botanic Garden, Howrah and a voucher specimen was deposited at the herbarium of IICB. All melting points were measured on a capillary melting point apparatus and are uncorrected. TLC was carried out on Silica gel G (BDH) plates with solvent $CHCl_3$-MeOH-EtOAc (10:1:1) and the spots were visualized by spraying Liebermann-Burchard reagent and warming the plates to 120°C. Optical rotations were measured on a JASCO DIP-360 digital polarimeter. IR spectra were taken on a JASCO-700 instrument in KBr discs. UV spectra were recorded on a Shimadzu model, UV-260 spectrophotometer. ^1H and ^{13}C NMR spectra were recorded on JEOL-FX-100 and JEOL-GX-400 spectrometers (operating at 25.05 and 100 MHz respectively for ^{13}C) in $CDCl_3$ or C_5D_5N with tetramethylsilane as internal standard. Fast atom bombardment mass spectra were obtained on a VG ZAB-SE mass spectrometer using NBA as matrix. Electron impact mass spectra were recorded on a JEOL-AX-500 mass spectrometer by direct inlet at 70 eV.

The air dried powdered seeds of *M. elengi* (1 kg) was first defatted with petroleum ether (60-80°C) and then exhaustively extracted with MeOH. The

methanolic extract on removal of the solvent under reduced pressure yielded a viscous dark brown mass (105 g). The extract was partitioned between Bu^nOH and H_2O. The organic layer was further washed with water for complete removal of inorganic impurities and free sugars and the solvent removed under reduced pressure to yield a brown residue (74 g). It was hydrolysed with aq. MeOH-HCl under reflux for 4h.

Isolation of bassic acid (2), mimusopic acid (3) and mimusopsic acid (5). - The acid hydrolysate (74 g) was separated into acidic and neutral fractions by treatment with a saturated solution of $NaHCO_3$. The acidic fraction (46 g) was chromatographed over a column of silica gel (500 g) with petroleum ether (40-60°C), petroleum ether - $CHCl_3$ (1:1),(3:1),$CHCl_3$ and $CHCl_3$-MeOH (97:3,95:5) as successive eluents. The $CHCl_3$-MeOH eluents on further chromatographic separation yielded bassic acid **(2)** (400 mg), mp 288-290°C, M^+ 486 (lit.[6] mp 289-291°C), mimusopic acid **(3)** (250 mg) and mimusopsic acid **(5)** (15 mg).

Conversion of bassic acid (2) to mimusopic acid (3) and mimusopsic acid (5). - Bassic acid **(2)** (50mg) (no λ_{max} at 284 nm) refluxed with 6% MeOH-HCl (aq) (25 ml) for 4h, cooled, worked up as usual and the residue on chromatographic separation and crystallization from MeOH afforded unconverted bassic acid **(2)** (30 mg), mimusopic acid (8 mg) (no λ_{max} at 284 nm) and mimusopsic acid (2 mg) (λ_{max}^{MeOH} 284 nm).

Conversion of mimusopic acid (3) to mimusopsic acid (5). - Mimusopic acid (40 mg) was dissolved in MeOH (10 ml) and refluxed for 4h at water bath temperature. MeOH was distilled off, the residue was chromatographed over silica gel and two homogeneous compounds thus obtained on crystallization from MeOH yielded mimusopic acid **(3)** (20 mg) (no λ_{max} at 284 nm) and mimusopsic acid **(5)** (2.5 mg) (λ_{max}^{MeOH} 284 nm).

Mimusopic acid (3). - It was crystallized from MeOH, mp 292-294°C, $[\alpha]_D^+$ 48.57° (c 0.21 in pyridine); IR ν_{max} cm^{-1} 3426, 2934, 1705, 1459, 1367, 1228, 1163, 1035, 955, 855; ^1H NMR (99.6 MHz) (C_5D_5N) δ 0.92, 1.00, 1.16, 1.20, 1.60, 1.64 (each 3H, s, 6X-CH_3), 2.92 (2H, t-like, 11-H), 2.39(1H, dd, J 10 Hz, 4 Hz, 18-H), 3.96 (2H, br s, 23-H), 4.38 (1H, m, $W_{\frac{1}{2}}$ 14 Hz, 2-H), 4.81 (1H, d, J 3.5 Hz, 3-H) and 5.54 (1H, t-like, 12-H); ^{13}C NMR (25.05 MHz) (C_5D_5N) δ 179.9 (C-28), 144.7 (C-13), 133.7 (C-10), 133.7 (C-9), 121.7 (C-12), 74.7 (C-3), 68.5 (C-2), 67.3 (C-23), 47.3 (C-19), 47.0 (C-17), 46.6 (C-4), 41.6 (C-14), 41.5 (C-18), 41.1 (C-5), 39.4 (C-8), 34.3 (C-21), 33.1 (C-22), 33.1 (C-29), 31.0 (C-6), 31.0 (C-20), 30.3 (C-7), 29.4 (C-1), 29.0 (C-11), 27.9 (C-25), 27.6 (C-15), 26.2 (C-27), 24.0 (C-26), 24.0 (C-16), 23.8 (C-30) and 16.0 (C-24); EIMS m/z (rel.int.) : 486 $[M]^+$ (38), 468 $[M-H_2O]^+$ (31), 450 $[M-2H_2O]^+$ (6), 437 $[M-CH_2OH-H_2O]^+$ (72), 420 (12), 409 (11), 370 (19), 307 (8), 251 (100), 235 (20), 203 (54) and 189 (50). (Found : C, 74.12; H, 9.49; $C_{30}H_{46}O_5$ requires C, 74.03; H, 9.53%).

Mimusopic acid methyl ester (7). - Mimusopic acid **(3)** (100 mg) dissolved in MeOH was treated with an ethereal solution of CH_2N_2. The reaction mixture was kept overnight at 0°C and worked up as usual to give a residue which on crystallization from MeOH yielded fine needles (25 mg) of mimusopic acid methyl ester **(7)**, mp 260-262°C, $[\alpha]_D$ + 66.5° (c, 0.15 in $CHCl_3$); ^1H NMR (99.6 MHz) ($CDCl_3$) δ 0.84, 0.88, 0.92, 1.00, 1.06, 1.24 (each 3H, s, 6X-CH_3), 3.64 (3H, s, $COOCH_3$), 3.92 (2H, m, 2-H and 3-H) and 5.4 (1H, t-like, 12-H); EIMS m/z (rel.int.) 500 $[M]^+$ (78), 482 (40), 451 (61), 441 (15), 409 (15), 384 (25), 368 (9), 323 (9), 251 (100), 203 (78), 189 (98) and 175 (42.5). (Found : C, 74.32; H, 9.69; $C_{31}H_{48}O_5$ requires C, 74.36; H, 9.66%).

Mimusopsic acid (5). - It was crystallized from MeOH, mp 234-236°C, $[\alpha]_D$ + 20° (c, 0.12 in MeOH); λ_{max} (MeOH) 284 nm (ε8100), ^1H NMR (99.6 MHz) (C_5D_5N) δ 0.88, 0.96, 1.22, 1.32, 1.38, 1.48 (each 3H, s, 6X-CH_3), 2.76 (1H), 3.5 (2H, br s, 23-H), 3.91 (1H, d, J 6 Hz, 3-H), 4.44 (1H, m, $W_{\frac{1}{2}}$ = 14 Hz, 2-H), 5.84 and 6.16 (each 1H, each d, J 6 Hz, 11-H and 12-H); FAB-MS m/z (rel.int.) 485 $[M+H]^+$ (100), 467 (13), 439 (20), 423 (12), 367 (12), 351 (10), 285 (32), 256 (15), 253 (18) and 239 (20). (Found : C, 74.30; H, 9.20; $C_{30}H_{44}O_5$ requires C, 74.34; H, 9.15%).

Mimusopsic acid methyl ester (8). - Mimusopsic acid **(5)** (10 mg) dissolved in MeOH (15 ml) was treated with ethereal solution of CH_2N_2. The reaction mixture was kept overnight at 0°C and worked up as usual to give a residue which on crystallization from MeOH yielded needles of mimusopsic acid methyl ester **(8)**, mp 194-196°C; $[\alpha]_D$ + 37.14 (c, 0.1 in MeOH), λ_{max} (MeOH) 284 nm; ^1H NMR (99.6 MHz) ($CDCl_3$) δ 0.88, 0.94, 1.02, 1.04, 1.12, 1.24 (each 3H, s, 6X-CH_3), 2.48 (1H, m), 3.06 (2H, br d, J 10 Hz), 3.45 (2H, br s, 23-H), 3.64 (3H, s, $COOCH_3$), 3.7 (1H, d, signal overlapped with -CH_3 of $COOCH_3$, 3-H), 4.16 (1H, m, 2-H), 5.68 and 5.96 (each 1H, each d, J 6 Hz, 11-H and 12-H); ^{13}C NMR (100 MHz) ($CDCl_3$) δ 178.3 (C-28), 149.1 (C-9), 139.8 (C-13), 120.7 (C-11), 120.3 (C-12), 85.0 (C-10), 78.0 (C-3), 73.4 (C-23), 67.3 (C-2), 51.7 (-OCH_3), 50.2 (C-5), 45.7 (C-17), 46.6 (C-19), 43.8 (C-4), 41.2 (C-14), 40.6 (C-18), 40.3 (C-8), 34.6 (C-21), 33.8 (C-22), 33.0 (C-29), 32.1 (C-6), 30.7 (C-20), 29.0 (C-7), 27.8 (C-15), 24.9 (C-1), 23.8 (C-16), 23.6 (C-30), 21.4 (C-27), 20.3 (C-26), 18.6 (C-25) and 14.2 (C-24); EIMS m/z (rel. int.) 498 (M^+, 100), 480 (11), 468 (5), 439 (25), 423 (55), 393 (10), 349 (12), 321 (10), 299 (25), 239 (22), 213 (12), 201 (38) and 189 (50). (Found : C, 74.62; H, 9.34; $C_{31}H_{46}O_5$ requires C, 74.66; H, 9.30%).

Acknowledgements. - We thank Prof. K. Yamasaki, Hiroshima University, School of Medicine, Japan for the ^{13}C NMR spectrum of mimusopsic acid and Dr. B. Pramanik, Schering Research, Bloomfield, USA, for the FABMS. Thanks are also due to Dr.(Mrs) N. Ghosal, IICB, for preparing DTMM structure of mimusopsic acid. One of us (S.S) is thankful to CSIR for financial assistance.

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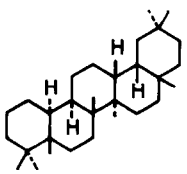
13. We recommend the name mimusopane for the new carbon skeleton present in mimusopic acid and mimusopsic acid as shown below.



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