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TRITERPENOID SAPONINS OF *MIMUSOPS ELENGI*

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Key Word Index—*Mimusops elengi*; Sapotaceae; seeds; triterpenoid saponins; Mi-glycoside 1; protobassic acid; bassic acid; mimusopic acid; mimusopsic acid; mimusopside A and B.

Abstract—In addition to characterization of taxifolin, α-spinasterol glucoside, Mi-glycoside 1, two new triterpenoid saponins mimusopside A and B were isolated from the seeds of *Mimusops elengi* and were, respectively, defined as 3-*O*-β-D-glucopyranosyl protobassic acid 28-*O*-α-L-rhamnopyranosyl (1 → 2)-α-L-arabinopyranoside and 3-*O*-β-D-glucopyranosyl 16α-hydroxy protobassic acid 28-*O*-α-L-rhamnopyranosyl(1 → 2)-α-L-arabinopyranoside by a combination of LSIMS, FABMS, ^{1}H and ^{13}C NMR and some strategic chemical transformations.

INTRODUCTION

In the preceding papers [1, 2] we reported the isolation of bassic acid, mimusopic acid, mimusopsic acid, mimusopgenone and mimugenone from the acid hydrolysate of the methanol extract of *Mimusops elengi* seeds. The isolation of bassic acid from the plant is considered as a chemical marker for the presence of saponins of protabassic acid. It is reported in the literature that sideroxyloside A, an oligosaccharide of protobassic acid isolated from *Sideroxylan cubense* showed strong cytotoxic activity on both Molt 4 cells and splenocytes [3]. The wide occurrence of the plant, coupled with the reported biological activities of the saponins of protobassic acid [3;4], prompted us to undertake a phytochemical investigation of its saponin constituents. Moreover, mimusopic acid, which possesses the novel mimusopane skeleton isolated from the seeds of the plant [1] was found to cause inhibition of HIV reverse transcriptase activity. The present paper reports the isolation and structure elucidation of two triterpene oligoglycosides mimusopside A (**1**) and mimusopside B (**2**) from the seeds of the plant.

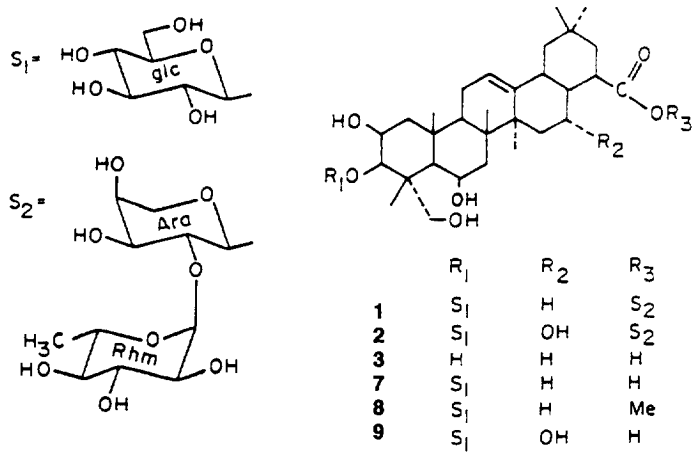
RESULTS AND DISCUSSION

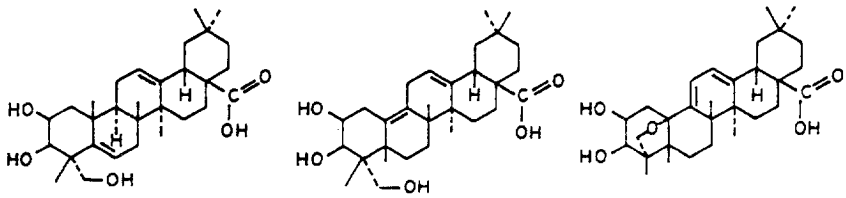
The *n*-butanol-soluble fraction of the methanol extract of the defatted seeds of the plant *M. elengi* on repeated chromatographic purification on silica gel column yielded α-spinasterol-β-D-glucoside, taxifolin (dihydroquercetin), Mi-glycoside 1 and an intimate mixture of two compounds (TLC). Preparative HPLC of the mixture using an ODS column produced two chromatographically pure compounds designated mimusopside A (**1**) and mimusopside B (**2**). Both **1** and **2** gave a positive Liebermann–Burchard test for triterpenes and Molisch test for sugars and thus appeared to be triterpene glycosides.

Mimusopside A (**1**), the major constituent, after acid hydrolysis, yielded the genuine aglycone, protobassic acid (**3**) and three acid-catalysed rearranged aglycones, bassic acid (**4**), mimusopic acid (**5**) and mimusopsic acid (**6**), identified from their mps, TLC, ^{1}H NMR and ^{13}C NMR spectral data and comparison with authentic samples [1]. The monosaccharides were characterized as D-glucose, L-arabinose and L-rhamnose in the ratio 1:1:1 by preparation of their alditol acetates and GLC comparison with authentic samples. The absolute configuration of L-arabinose unit was confirmed as described by Mahato *et al.* [5].

The liquid secondary-ion mass spectrum (LSIMS) of **1** displayed a $[M + Na]^+$ peak at *m/z* 967, suggesting the M_r to be 944. In combination with the ^{13}C NMR spectrum it suggested the molecular formula $C_{47}H_{76}O_{19}$. Of the carbons, 30 were assigned to the aglycone part and 17 to the oligosaccharide moiety (Table 1). The ^{13}C NMR data of **1** suggested the aglycone part to be protobassic acid (**3**) [4]. The spectrum also showed signals for three anomeric carbons at δ93.5, 101.2 and 105.1 and a carbonyl carbon at δ176.2. The upfield shift of one anomeric carbon signal at δ93.5 together with an absorption at 1725 cm^{-1} in its IR spectrum suggested the presence of an ester glycosidic linkage [6] in **1**.

Treatment of **1** with aqueous methanol–potassium hydroxide yielded two prosapogenins identified as Mi-glycoside 1 (**7**) [4] and its methyl ester (**8**) from their ^{1}H NMR and ^{13}C NMR data (Table 1) suggesting that D-glucose is linked to the C-3 hydroxy group of protobassic acid in **1**. The downfield shift of + 10.3 ppm for C-3 also supported a 3-*O*-glycosidic linkage in **1**. It was concluded, therefore, that the two monosaccharides other than glucose were attached through the carboxyl group of protobassic acid. The sequence and interglycosidic linkage positions due to the 28-*O*-sugar moiety





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in **1** were established as follows. Permethylation of **1** by Hakomori's method [7] yielded a permethylate which, on acid hydrolysis, yielded 2,3,4,6-tetra-*O*-methyl-D-glucose, 3,4-di-*O*-methyl-L-arabinose and 2,3,4-tri-*O*-methyl-L-rhamnose (GLC of alditol acetates). The anomeric configurations of the sugar moieties were inferred from the *J* values of the respective anomeric protons of the permethylate. From the foregoing evidence the structure of mimusopside A is proposed to be 3-*O*-β-D-glucopyranosyl protobassic acid 28-*O*-α-L-rhamnopyranosyl (1 → 2)-α-L-arabinopyranoside (**1**). The ^{13}C NMR data for **1** were assigned taking into consideration the ^{13}C NMR chemical shifts of Mi-saponin A [4], respective methyl glucosides and glycosylation shift values [8, 9].

Mimusopside B (**2**), the minor saponin showed in its LSIMS a peak at *m/z* 983 assignable to $[M + Na]^+$, suggesting the molecular weight to be 960, 16 mass units higher than that of **1**. ^{13}C NMR (Table 1) spectrum of **2** revealed that the carbohydrate moiety was identical with that of mimusopside A (**1**). On acid hydrolysis **2** yielded L-arabinose, L-rhamnose and D-glucose in the ratio 1:1:1 by GLC of their alditol acetates. When refluxed with aqueous methanol–potassium hydroxide, compound **2** furnished a prosapogenin (**9**) which was identified as 3-*O*-β-D-glucopyranoside of 16α-hydroxy protobassic acid by comparison of its physical and spectral data reported in the literature [10]. Thus, the structure of mimusopside B was unambiguously established as 3-*O*-β-D-glucopyranosyl 16α-hydroxy protobassic acid 28-*O*-α-L-rhamnopyranosyl (1 → 2)-α-L-arabinopyranoside (**2**).

Anti-HIV reverse transcriptase (RT) activity of mimusopic acid (**5**) was tested by Prof. Katsuhiko Ono, Laboratory of Viral Oncology, Aichi Cancer Center Research Institute, Nagoya 464, Japan by the method as described in [11]. Mimusopic acid (**5**) showed mild anti-HIV reverse transcriptase inhibitory activity compared with epigallocatechin gallate (EGC_g), a green tea compound and an efficient inhibitor of HIV-RT [12]. Thus in the presence of 2, 5 and 10 μg ml^{-1} the reverse transcriptase activity was found to be 82.6, 67.9 and 9.6%, respectively, compared with 71.0, 9.5 and 1.1% activity in presence of 0.04, 0.1 and 0.2 μg ml^{-1} of ECG_g.

EXPERIMENTAL

General. Mps: uncorr. IR: JASCO-700, KBr discs. Optical rotations: JASCO DIP-360 digital polarimeter. ^{1}H NMR and ^{13}C NMR were recorded using JEOL-FX-100 (^{1}H at 100 MHz and ^{13}C at 25.05 MHz) in $CDCl_3$ or C_5D_5N with TMS as int. standard. Prep. HPLC was performed on a Spectra Physics SP 8000B using Spherisorb S-10-ODS column (10 × 250 mm), flow rate 1 ml min^{-1} and Micromeritics 771 RI detector. GLC: (i) 3% ECNSS-M (185 × 0.6 cm) at 190° for alditol acetates and (ii) 3% OV-225 at 195° for partially methylated alditol acetates; carrier gas N_2. LSIMS: VG-XAB-SE (caesium ion) NAB as matrix with and without salt addition. FABMS: JEOL-AX-500 (Xenon) glycerol-thioglycerol as matrix. EIMS: 70 eV.

Table 1. Chemical shifts [δ_C ($\pm$ 0.1)] of mimusopside A(**1**), protobassic acid (**3**) mimusopic acid (**5**), mimusopsic acid methyl ester (**6**) Mi-glycoside methyl ester (**8**) and mimusopside (**2**)

C	1*	3*	5*	6†	8*	2*
1	46.5	47.6	29.4	29.4	47.0	46.5
2	71.0	70.8	68.5	67.3	70.4	70.8
3	83.4	73.1	74.7	78.0	83.2	83.0
4	43.8	43.6	46.6	43.8	43.7	43.9
5	49.2	49.3	41.1	50.2	49.0	49.1
6	67.9	67.2	31.0	32.1	67.5	67.5
7	41.0	41.0	30.3	29.0	40.8	41.0
8	39.5	39.4	39.4	40.3	39.2	39.3
9	49.0	49.2	133.7	149.1	48.9	48.7
10	36.9	36.8	133.7	85.0	36.7	36.8
11	24.2	24.2	29.0	120.7	24.0	24.1
12	123.5	123.3	121.7	120.3	122.5	123.4
13	144.7	143.9	144.7	139.8	143.5	144.0
14	43.0	42.9	41.6	41.2	42.6	42.7
15	28.5	28.6	27.6	27.8	28.0	36.2
16	23.8	24.0	24.0	23.8	23.7	74.2
17	47.6	47.0	47.0	45.7	47.0	49.9
18	42.0	42.2	41.5	40.6	41.9	42.0
19	46.5	46.5	47.3	46.6	46.2	47.0
20	31.0	31.0	31.0	30.7	30.7	31.0
21	34.4	34.3	34.0	34.6	34.1	34.2
22	32.9	33.3	33.1	33.8	33.1	33.1
23	65.7	65.4	67.3	73.4	65.5	65.3
24	16.7	16.8	16.0	14.2	16.5	16.8
25	18.4[a]	18.5	27.9	18.6	18.2	18.5[a]
26	19.0	18.9	24.0	20.3	18.8	18.9
27	26.3	26.3	26.2	21.4	26.3	26.4
28	176.2	180.1	179.9	178.3	177.9	180.0
29	33.2	33.2	33.1	33.0	32.9	33.1
30	23.6	23.7	23.8	23.6	23.6	23.9
$-OCH_3$	—	—	—	51.7	51.4	—
glc-1	105.1				105.7	105.3
glc-2	75.5				75.3	75.5
glc-3	78.4				78.2	78.5
glc-4	71.7				71.5	71.7
glc-5	77.9				77.8	78.0
glc-6	62.8				62.6	62.8
Ara-1	93.5					93.5
Ara-2	79.4					79.7
Ara-3	70.7					70.4
Ara-4	66.5					66.3
Ara-5	63.0					63.1
Rhm-1	101.2					101.1
Rhm-2	72.6[b]					72.3[b]
Rhm-3	72.3[b]					72.4[b]
Rhm-4	73.8					73.9
Rhm-5	70.2					69.8
Rhm-6	18.6[a]					18.6[a]

*In C_5D_5N.

†In $CDCl_3$.

The data of **5** and **6** are cited from ref. [1]. glc = glucose, Ara = arabinose, Rhm = rhamnose.

[a,b]May be interchanged in each vertical column.

Isolation of saponins. Air-dried powdered seeds (2 kg) of *M. elengi* were defatted with petrol (60–80°) and then extracted at ambient temp. with MeOH. The MeOH extract was conc. under reduced pressure and partitioned between *n*-BuOH and H_2O. The *n*-BuOH extract was washed with H_2O and evapd to dryness under reduced pressure to give a dark brown mass (20 g), which was chromatographed on a column of silica gel. Eluations being carried out with various ratios of $CHCl_3$–MeOH mixture. $CHCl_3$–MeOH (19:1, 9:1) eluents on further purification gave α-spinasterol glucoside (0.5 g), taxifolin (1.2 g) and Mi-glycoside 1 (0.4 g) which were identified by IR, NMR and TLC comparison with authentic samples. Fractions eluted with $CHCl_3$–MeOH (17:3) yielded an intimate mixture of two compounds, as revealed by TLC. This, on HPLC purification (mobile phase: MeOH–H_2O (70:30), yielded mimusopside A (150 mg) and mimusopside B (72 mg).

Mimusopside A (**1**). Amorphous powder, mp 245–48° (dec.), $[\alpha]_D$ – 20.9° (MeOH; *c*, 0.22); IR ν_{max} cm^{-1} 3300–3500, 1725; LSIMS (positive) *m/z* 967 $[M + Na]^+$; ^1H NMR (C_5D_5N): δ0.90, 0.99, 1.23, 1.56, 1.80 and 2.08 (all *s*, 6 × Me), 1.65 (3H, *d*, *J* = 5.8 Hz rhamnose –Me), 5.07 (1H, *d*, *J* = 7.6 Hz, glucose, H-1), 5.59 (1H, *t*-like, H-12), 5.95 (1H, *br s*, rhamnose H-1) and 6.29 (1H, *s*, arabinose H-1); ^{13}C NMR (Table 1). Found: C, 59.68; H, 8.21; $C_{47}H_{76}O_{19}$ requires C, 59.73; H, 8.11%.

Acid hydrolysis of mimusopside A (**1**). A soln of **1** (160 mg) in 2 N HCl–MeOH (aq.) was heated at 95° for 3 hr. Usual work-up followed by chromatographic purification on a silica gel column yielded **3** (10 mg), **4** (30 mg), **5** (20 mg) and **6** (5 mg).

Protobassic acid (**3**). Needles from MeOH mp > 300°, $[\alpha]_D$ + 14.2° (pyridine); *c*, 0.22); EIMS (*m/z*): 504 $[M]^+$, 486 $[M - H_2O]^+$, 301, 248, 203; ^1H NMR (C_5D_5N): δ0.92, 1.00, 1.28, 1.40, 1.90, 2.20 (all *s*, 6 × Me), 5.05 (1H, *brs*, H-6α), 5.50 (1H, *brs*, H-12); ^{13}C NMR (Table 1).

Bassic acid (**4**). Crystallized from MeOH, mp 286–288°. EIMS (*m/z*): 486 $[M]^+$, 407 , 248, 238, 203 and 189; ^1H NMR (C_5D_5N): δ0.96 (*s*, Me), 1.00 (*s*, Me), 1.20 (*s*, 2 × Me), 1.72 (2 × Me), 3.31 (1H, *br d*, *J* = 9 Hz, 18-H), 4.04, 4.24, (2H, each *d*, *J* = 10 Hz, 23-H_2), 4.25 (1H, *d*, *J* = 3.5 Hz, 3α-H), 4.54 (1H, *d*, *J* = 3.5 Hz, 2α-H), 5.62 (1H, *t*-like, H-12) and 5.92 (1H, *t*-like H-6).

Mimusopic acid (**5**). Crystallized from MeOH, mp 290–292°, $[\alpha]_D$ + 48.2° (pyridine, *c*, 0.12); EIMS (*m/z*): 486 $[M]^+$, 468, 450, 437, 420, 409, 251, 235, 203 and 189; ^1H NMR (C_5D_5N): δ0.92, 1.00, 1.14, 1.22, 1.58, 1.62 (each *s*, 6 × Me), 2.90 (2H, *t*-like, 11-H_2), 2.39 (1H, *dd*, *J* = 10 Hz, 4 Hz, 18-H), 3.96 (2H, *brs*, 23-H), 4.36 (1H, *m*, $W_{1/2}$ = 13.5 Hz, 2-H). 4.81 (1H, *d*, *J* = 3.5 Hz, 3-H) and 5.52 (1H, *t*-like, 12-H); ^{13}C NMR (Table 1).

Mimusopsic acid (**6**). Crystallized from MeOH, mp 234–236°, $[\alpha]_D$ + 20° (MeOH; *c* 0.12); UV λ_{max}^{MeOH} nm: 284; ^1H NMR (C_5D_5N): δ0.87, 0.94, 1.20, 1.32, 1.36, 1.48 (each *s*, 6 × Me), 2.76 (1H), 3.5 (2H, *br*, *s*, 23-H), 3.90 (1H, *d*, *J* = 6 Hz, 3-H), 4.42 (1H, *m*, $W_{1/2}$ = 14 Hz, 2-H), 5.84 and 6.15 (each 1H, each *d*, *J* = 7 Hz, 11-H and 12-H); ^{13}C NMR (Table 1).

The aq. layer was worked up as described [5] and analysed by GLC (column (i)], D-glucose, L-arabinose and L-rhamnose were identified as their alditol acetates using authentic samples.

Alkaline hydrolysis of mimusopside A (**1**). Compound **1** (150 mg) was refluxed with 10% KOH in aq. MeOH (25 ml) for 4 hr. Worked up as usual. The residue was chromatographed over a column of silica gel to yield Mi-glycoside 1 (**7**) and its methyl ester (**8**). Compound **7** was identified on TLC with an authentic sample and comparison of its ^{13}C NMR with the data reported in literature [4].

Methyl ester of Mi-glycoside (**8**). Crystallized from MeOH, mp 250–252° $[\alpha]_D$ + 13.4° (MeOH; *c* 0.12) IR ν_{max} cm^{-1}: 3200–3400, 1730. FABMS (positive) *m/z* 703 $[M + Na]^+$; ^{13}C NMR (Table 1).

Permethylation of **1**. Compound **1** (50 mg) was methylated with NaH (100 mg), MeI (2 ml) in DMSO (5 ml) by Hakomori's method and the product obtained after usual work up was purified by CC to provide the permethylate as a powder (30 mg); ^1H NMR ($CDCl_3$): δ4.06 (1H, *d*, *J* = 6 Hz, arabinose H-1), 4.50 (1H, *d*, *J* = 7.5 Hz, glucose H-1) and 5.2 (1H, *brs*, rhamnose H-1). Hydrolysis of the permethylate with 2 M HCl in aq. MeOH (5 ml) at 95° for 3 hr yielded, after usual work up, alditol acetates of partly methylated sugars which were identified as 2,3,4,6-tetra-*O*-methyl-D-glucose (R_t 1.00), 3,4-di-*O*-L-arabinose (R_t 1.32) and 2,3,4-tri-*O*-methyl-L-rhamnose (R_t 0.35) by comparison of the R_t values (GLC) reported in lit. [13].

Mimusopside B (**2**). Powder from MeOH–$CHCl_3$ mp 214–16°, $[\alpha]_D$ − 31.8° (MeOH, *c* 0.17); LSIMS (positive) *m/z* 983 $[M + Na]^+$; ^1H NMR (C_5D_5N): 0.98, 1.14, 1.60, 1.72, 1.98, 2.13 (all *s*, 6 × me) 1.64 (3H, *d*, *J* = 6 Hz rhamnose Me), 5.04 (1H, *d*, *J* = 7.8 Hz glucose H-1), 5.68 (1H, *t*-like, H-12), 6.01 (1H, *br s*, rhamnose H-1) and 6.34 (1H, *s*, arabinose H-1); ^{13}C NMR (Table 1).

Alkaline hydrolysis of **2**. A soln of **2** (70 mg) in 10% KOH in aq. MeOH was refluxed for 3 hr and worked up as usual. Purification of the product on silica gel provided **9** (25 mg), crystallized from MeOH, mp 280–282°, $[\alpha]_D$ + 7.5° (MeOH, *c*, 0.12). FABMS (positive) 683 $[M + Na]^+$; ^1H NMR (C_5D_5N): δ1.00, 1.18, 1.58, 1.74, 1.89, 2.10 (all *s*, 6 × Me), 5.04 (1H, *d*, *J* = 7.5 Hz, glucose H-1), 5.70 (1H, *br s*, H-12).

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REFERENCES

1. Sen, S., Sahu, N. P. and Mahato, S. B. (1993) *Tetrahedron* **49**, 9031.
2. Sen, S., Sahu, N. P. and Mahato, S. B. (1995) *Phytochemistry* **38**, 205.
3. Jiang, Y., Weniger, B., Beji, L., Italiano, L., Beck, J. P. and Anton, R. (1993) *Planta Medica V* **59** (Suppl.), P.A 680.
4. Toyota, M., Msonthi, J. D. and Hostettmann, K. (1990) *Phytochemistry* **29**, 2849.
5. Mahato, S. B., Sahu, N. P., Roy, S. K. and Sen, S. (1991) *Tetrahedron* **47**, 5215.
6. Sahu, N. P. and Mahato, S. B. (1994) *Phytochemistry* **37**, 1425.
7. Hakomori, S. (1964) *J. Biochem.* **55**, 205.
8. Seo, S., Tomita, Y., Tori, K. and Yoshimura, Y. (1978) *J. Am. Chem. Soc.* **100**, 3331.
9. Kasai, R., Okihara, O., Asakawa, J., Mizutani, K. and Tanaka, O. (1979) *Tetrahedron* **35**, 1427.
10. Nigam, S. K., Li, X. C, Wang, D. Z., Misra, G. and Yang, C. R. (1992) *Phytochemistry* **31**, 3169.
11. Nakane, H., Arisawa, M., Fujita, A., Koshimura, S. and Ono, K. (1991) *FEBS Letters* **286**, 83.
12. Nakane, H. and Ono, K. (1990) *Biochemistry* **29**, 2841.
13. Jenson, P. E., Kene, L., Liedgren, H., Lindberg, B. and Lonngren, J. (1976) *Chem. Commun. Univ. Stockholm* **8**, 23.