Sesquiterpenoids from the Roots of *Solanum aethiopicum*

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Three new sesquiterpenoids, lubiminoic acid, epilubiminoic acid and aethione, and six known sesquiterpenoids, solavetivone, 3β-hydroxysolavetivone, 13-hydroxysolavetivone, anhydro-β-rotunol, epilubimin and lubimin, were isolated from roots of *S. aethiopicum* L. Their structures were elucidated by spectroscopic data.

Introduction

Solanum aethiopicum L., a wild relative of Solanacea, and native to Africa, is highly resistant against soil-borne pathogens such as *Fusarium oxysporum* f. sp. *melongenae*, *Verticillium dahliae* and *Ralstonia solanacearum* (formerly *Pseudomonas solanacearum*). This species is utilized as a mating source of resistant cultivars and also used as a rootstock against *Ralstonia solanacearum* (Ano *et al.*, 1991). In previous studies concerning solanaceous rootstocks which are resistant to soil-borne pathogens, Nagaoka *et al.* (1993 and 1995) have reported antifungal alkaloids, fatty acids and phenolic compounds from the roots of tomato stock. Yoshihara *et al.* (1988) have isolated antifungal sesquiterpenes and phenolic compounds from the roots of an eggplant stock, and suggested possible roles of sesquiterpenes such as solavetivone in the resistance of this stock. Sesquiterpenoids in the roots of *S. aethiopicum* L. were investigated to clarify their possible part in the resistance mechanisms of this plant to soil-borne plant pathogens.

This report describes the isolation and structure elucidation of three novel sesquiterpenoids, lubiminoic acid (**7**), epilubiminoic acid (**8**) and aethione (**9**) from the roots of *S. aethiopicum* L.. Additional two novel norsesquiterpenes isolated as a mixture were suggested to be 15-norlubiminol (**10**) and 15-norepilubiminol (**11**). Known sesquiterpenoids, solavetivone (**1**), 3β-hydroxysolavetivone (**2**), 13-hydroxysolavetivone (**3**), anhydro-β-rotunol (**4**), lubimin (**5**) and epilubimin (**6**) were also isolated.

Results and Discussion

The roots of *S. aethiopicum* L., grown in a field, were extracted with 70% ethanol. The extracts were concentrated and extracted with ethyl acetate. The ethyl acetate extract was applied to silica gel column chromatography and eluted using a solvent system of *n*-hexane-chloroform and chloroform-methanol. The eluate was further chromatographed on silica gel columns to give nine sesquiterpenoids (**1–9**) and a mixture of **10** and **11** (Fig. 1).

The ^{1}H and ^{13}C NMR and MS spectral data of compound **1–6** were identical to those of solavetivone (**1**) (Coxon *et al.*, 1974), 3β-hydroxysolavetivone (**2**) (Anderson and Gun, 1977; Uegaki *et al.*, 1981), 13-hydroxysolavetivone (**3**) (Anderson *et al.*, 1977), anhydro-β-rotunol (**4**) (Hikino *et al.*, 1971; Coxon *et al.*, 1974), lubimin (**5**) (Katsui *et al.*, 1977; Ewing, 1990) and epilubimin (**6**) (Katsui *et al.*, 1982; Ewing, 1990), which are representative vetispiranes in solanaceous plants.

Compound **7**, named lubiminoic acid, gave an M^{+} ion peak at *m/z* 252.1766 in HREIMS, which was corresponded to a molecular formula of

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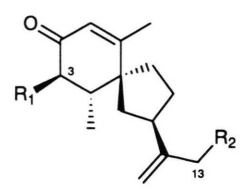
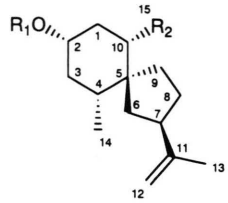
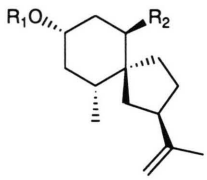


1 : R_1=H, R_2=H
2 : R_1=OH, R_2=H
3 : R_1=H, R_2=OH
4 : R_1=H, R_2=H , Δ^3



5 : R_1=H, R_2=CHO
7 : R_1=H, R_2=COOH
7a: R_1=H, R_2=COOMe
7b: R_1=Ac, R_2=COOMe
10 : R_1=H, R_2=OH



6 : R_1=H, R_2=CHO
6a: R_1=Ac, R_2=CHO
8 : R_1=H, R_2=COOH
8a: R_1=H, R_2=COOMe
8b: R_1=Ac, R_2=COOMe
11 : R_1=H, R_2=OH

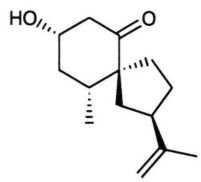


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Fig. 1. Structures of sesquiterpenoids from the roots of *S. aethiopicum*.

$C_{15}H_{24}O_3$. The IR spectrum indicated the presence of a hydroxyl (3293 cm^{-1}) and a carboxyl (1669 cm^{-1}) groups. The ^1H NMR spectrum showed the signals owing to protons of an isopropenyl group at δ 1.66 (3H, s), 4.63 (1H, br.s) and 4.65 (1H, br.s), a hydroxylated methine proton at δ 3.63 (1H, m) and a methine proton attached to a carboxyl group at δ 2.36 (1H, dd), but the signal due to a formyl group was not observed (Table I). The spectral data of the methyl ester (**7a**) obtained from **7** with diazomethane and its acetate (**7b**) from **7a** with acetic anhydride and pyridine confirmed the presence of one carboxyl and one hydroxyl group. The ^{13}C NMR spectrum indicated fifteen carbon signals including a hydroxylated methine carbon (δ 69.6), an olefinic methine carbon (δ 109.0), an olefinic quarternary carbon (δ 148.2) and a carboxyl carbon (δ 178.3) (Table II). The carbon chemical shifts of **7** except at δ 36.6, 41.3, 51.4 and 178.3 were very similar to those of **5**. The ^1H and ^{13}C NMR spectral signals were assigned by means of ^1H-^1H COSY, HMQC and HMBC techniques as shown in Tables I and II, and suggested the planar structure of **7** as drawn in Fig. 2. The coupling constants of the proton signals at δ 1.12 (H-3), 1.50 (H-4), 2.04 (H-1) and 2.36 (H-10) demonstrated their conformations to be axial, axial, equatorial and axial, respectively,

Table I. ^1H-NMR spectral data for **7** and **9** (500 MHz, $CDCl_3$).

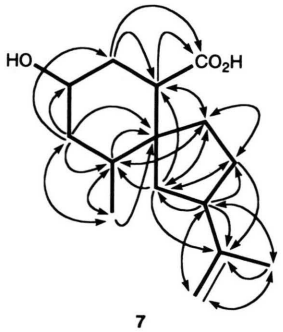
	7			**9**		
H	δ	multi.	*J* (Hz)	δ	multi.	*J* (Hz)
1 α	ca.1.67	*m*		2.66	*dd*	(13.6/10.8)
β	2.04	*dddd*	(13.1/5.2/3.5/2.4)	2.74	*ddd*	(13.6/5.3/2.1)
2 β	3.63	*m*		3.93	*m*	
3 α	1.12	*br.q*	(12.0)	1.55	*br.q*	(12.0)
β	ca.1.68	*m*		1.95	*dddd*	(12.8/4.7/2.6/2.1)
4 β	1.50	*ddq*	(12.6/2.9/7.0)	ca.1.68	*m*	
6 α	1.62	*ddd*	(13.8/7.6/1.5)	1.40	*ddd*	(13.0/7.8/1.5)
β	1.86	*dd*	(13.3/12.3)	2.50	*dd*	(13.0/11.5)
7 α	2.38	*m*		2.42	*m*	
8 α	ca.1.74[a]	*m*		ca.1.71[b]	*m*	
β	1.38[a]	*dddd*	(12.6/12.5/11.8/7.4)	1.32[b]	*dddd*	(12.5/12.1/11.8/7.0)
9 α	1.41	*ddd*	(12.6/12.3/6.1)	1.81	*ddd*	(12.7/12.7/6.1)
β	1.96	*br.dd*	(12.3/7.1)	ca.1.70	*m*	
10 β	2.36	*dd*	(13.0/2.6)			
12	4.63	*br.s*		4.67	*br.s*	
	4.65	*br.s*		4.73	*br.s*	
13	1.66	*s*		1.74	*s*	
14	0.93	*d*	(6.8)	1.07	*d*	(6.8)

[a, b] Assignment may be interchangeable.

Table. II. ^{13}C-NMR chemical shifts for **5**, **6**, **7**, **8** and **9** (125 MHz, $CDCl_3$).

C	5	6	7	8	9
1	41.7	42.5	36.6	42.4	47.4
2	69.3	66.7	69.6	66.8	68.8
3	40.3	40.0	40.8	40.2	39.9
4	41.0	37.0	41.7	34.1	36.4
5	46.8	45.8	47.6	45.8	58.7
6	33.3	32.9	41.3	35.1	34.9
7	47.4	48.1	47.5	47.8	47.4
8	32.5	30.9	32.7	31.0[a]	31.0
9	25.9	30.9	25.7	30.6[a]	30.3
10	58.3	58.6	51.4	51.1	210.6
11	147.3	147.6	148.2	147.9	147.5
12	108.8	108.5	109.0	108.4	109.0
13	21.2	21.5	21.3	21.3	20.9
14	16.4	17.1	17.1	16.9	16.1
15	204.7	205.2	178.3	178.7	

[a] Assignments may be interchangeable.



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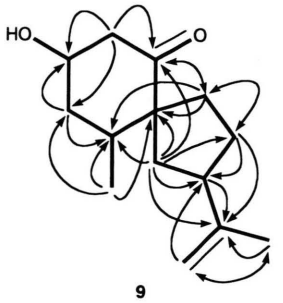

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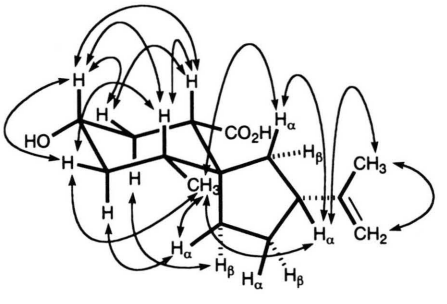
Fig. 2. Long range correlations (^{1}H to ^{13}C) for **7** and **9** in the HMBC spectra.

and also the conformation of H-2 to be axial. The NOESY spectrum showed correlations between H-14 and H-6α, between H-14 and H-7α, between H-14 and H-9α, between H-2β, H-4β and H-10β, between H-3α and H-9α, and between H-1α and H-9β. Thus the relative stereochemistry of **7** was elucidated as illustrated in Fig. 3, which was identical to that of lubimin (**5**).

Compound **8**, named epilubiminoic acid, gave an M^+ ion peak at *m/z* 252 equivalent to **7** in FI mass spectrum. The ^{1}H NMR spectrum of **8** exhibited the signals due to protons of an isopropenyl group at δ 1.70 (3H, s) and 4.68 (2H, br.s), a hydroxylated methine proton at δ 3.98 (1H, m) and a methine proton connected to a carboxyl group at δ 2.64 (1H, dd), but the signal due to a formyl group was not observed (Table I), while the signal due to a formyl group was not observed. The coupling constants (5.7 and 2.8 Hz) of the signal at δ 2.64 suggested the structure of **8** to be the epimer of **7** at C-10. In the ^{13}C NMR spectrum of **8**, two methyl, six methylene, four methine and three quarternary carbon signals were observed, and their chemical shifts were similar to those of **6** except for δ 34.1, 35.1, 51.1 and 178.7 (Table II). The ^{1}H NMR and mass spectral data of the methyl ester (**8a**) yielded from **8** and its acetate (**8b**) from **8a** confirmed the presence of one carboxyl and one hydroxyl groups. From these data, molecular formula of **8** was determined to be $C_{15}H_{24}O_3$. In order to determine the structure of **8**, epilubimin (**6**)



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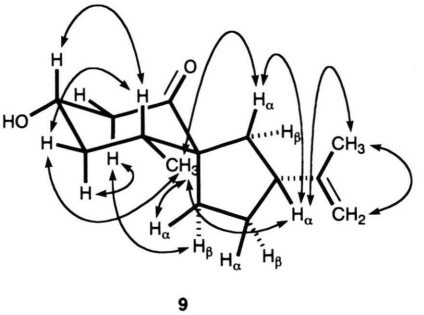

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Fig. 3. NOEs observed for **7** and **9** in NOESY spectra.

was chemically converted to **8b** through acetylation with acetic anhydride and pyridine, oxidation with sodium chlorite and methylation with diazomethane. The ^{1}H NMR and EI mass spectra of **8b** from **6** were consistent with those of **8b** from **8**.

Compound **9**, named aethione, showed an M^+ ion peak at *m/z* 222 in EI mass spectrum, and at *m/z* 222.1604, corresponded to a molecular formula of $C_{14}H_{22}O_2$ by HREIMS. The IR spectrum indicated the presence of a hydroxyl (3400 cm^{-1}) and a carbonyl (1705 cm^{-1}) groups. The ^{1}H NMR spectrum showed the signals owing to protons of an isopropenyl group at δ 1.74 (3H, s), 4.67 (1H, br.s) and 4.73 (1H, br.s), a hydroxylated methine proton at δ 3.93 (1H, m) and methylene protons neighboring a carbonyl group at δ 2.66 (1H, dd) and 2.74 (1H, ddd) (Table I). The ^{13}C NMR spectrum gave fourteen carbons consisting of two methyl, six methylene, three methine and three quarternary carbons, which contained a hydroxylated methine carbon (δ 68.8), an olefinic methine carbon (δ 109.0), an olefinic quarternary carbon (δ 147.5) and a carbonyl carbon (δ 210.6) (Table II). The ^{1}H and ^{13}C NMR spectral signals assigned by ^{1}H-^{1}H COSY, ^{13}C-^{1}H COSY, HMQC and HMBC measurements were shown in Table I and II, and suggested the planar structure of **9** as represented in Fig. 2. The coupling constants of the proton signals at δ 1.55 (H-3), 1.95 (H-3), 2.66 (H-1) and 2.74 (H-1) demonstrated their conformations to be axial, equatorial, axial and equatorial, respectively, and also the conformation of H-2 to be axial. In the NOESY investigation, NOEs were observed between H-14 and H-6α, between H-14 and H-7α, between H-14 and H-9α, between H-2β and H-4β, between H-3α and H-1α, and between H-1α and H-9β. From these data the relative stereochemistry of **9** was elucidated as illustrated in Fig. 3.

The absolute configurations of **1** and **5** were determined by comparison of their $[\alpha]_D$ values with those in references (Coxon *et al.*, 1974; Katsui *et al.*, 1977). The absolute configurations of **2**, **3**, **4**, **6**, **7**, **8**, **9** were presumably the same as those of **1** or **5** from the point of view of biogenesis.

The mixture of compounds **10** and **11** gave an M^+ ion peak at *m/z* 224 in EI mass spectrum, which was 14 less than those of **5** or **6**. The ^{1}H NMR spectrum (in $CDCl_3$) exhibited the signals for two isopropenyl groups at δ 1.72, 1.74, 4.69 and 4.71, two methyl groups at δ 0.92 and 0.96, four oxygenated methine protons at δ 3.40, 3.67, 3.67, 4.01 and two methine protons at δ 2.40. In contrast to the ^{1}H-NMR spectrum of **5**, **6**, **7** and **8**, the proton signals corresponding to a formyl group and a methine group bound to a formyl or a carboxyl group were not detected, but instead, two other signals corresponding to oxygenated methine protons appeared at 3.40 (dd, J = 11.8 and 4.5 Hz) and 3.67 (br.t, J = 4.0 Hz). These data implied that the mixture consisted of **10** and **11**. The ^{1}H NMR spectrum measured in C_6D_6 to separate the overlapped signals showed the signals for two isopropenyl group at δ 1.67 (3H, s), 1.74 (3H, s), 4.79 (1H, br.s) and 4.82 (1H, br.s), 4.83 (1H, br.s) and 4.91 (1H, br.s), two methyl group at δ 0.72 (3H, d) and 0.73 (3H, d), four oxygenated methine protons at δ 2.94 (1H, dd), 3.19 (1H, dd), 3.22 (1H, m), 3.83 (1H, m) and two methine protons at δ 2.27 (1H, m), 2.35 (1H, m) in a pair, although the signals at δ 0–2 were complicated. The signals at δ 0.73, 1.73, 2.35, 2.94, 3.21, 4.83 and 4.91 in the ^{1}H NMR spectrum (in C_6D_6) of **10**, which was obtained from **9** by reduction with $LiAlH_4$, were identical to one of the pair of signals in the mixture. The signals at δ 2.94 (H-10) for **10** indicated the coupling constant (11.4 Hz) for di-axial coupling. Therefore the structures of **10** was suggested to be 15-norlubiminol. The structure of **11** may be 15-norepilubiminol, as the coupling constant of the signals at δ 3.22 for the mixture was 4.0 Hz.

Experimental

General experimental procedures

NMR: Bruker AMX-500 instrument, 500 MHz (^{1}H NMR) and 125 MHz (^{13}C NMR) in $CDCl_3$ (TMS as internal standard). MS: JEOL JMS-DX300 and JEOL SX102A mass spectrometer. IR: Perkin Elmer System 2000 FT-IR spectrometer. Polarimeter: JASCO DIP-350 digital polarimeter. Silica gel 60 F_{254} TLC plates (0.25 mm thickness; Merck, Darmstadt, Germany) were used for analytical and separative TLC. CC was carried out on silica gel 60 N (Kanto Chemicals, Tokyo, Japan) and silica gel 60 (Merck, Darmstadt, Germany).

Plant material

Fresh roots of *S. aethiopicum* L. grown at the Agricultural Experimental Farm of Hokkaido University were collected. The seeds of *S. aethiopicum* L. were provided by National Research Institute of Vegetables, Ornamental Plants and Tea, Mii-machi, Kurume, Japan.

Extraction and isolation

Fresh roots (6 kg) of *S. aethiopicum* L. were extracted with 70% EtOH (40 l). The extract was filtered and concentrated to 1 l under reduced pressure below 40° C. The aq. soln. was extracted with EtOAC (3 × 1 l). The EtOAC layers were pooled, dried over Na_2SO_4, and concentrated to dryness *in vacuo*.

The EtOAC extract residue (40 g) was subjected to CC on silica gel and eluted sequentially with $CHCl_3$/*n*-hexane (1:1 v/v), $CHCl_3$, $CHCl_3$/MeOH (19:1 v/v), (8:2 v/v), (1:1 v/v) and MeOH to yield 7 fractions (Fr. 1 – Fr. 7). The Fr. 4 (5.4 g) was further chromatographed on silica gel columns and TLC plates to give **1** (1.9 g), **2** (160 mg), **3** (22 mg), **4** (1.8 mg), **5** (110 mg), **6** (105 mg), **7** (3 mg), **8** (3 mg), **9** (4 mg) and a mixture (0.3 mg) of **10** and **11**. A bioautographic technique according to Homans and Fuchs (1970) was used with *F. oxysporum* f. sp. *melongenae* for detection of antifungal sesquiterpenoids. Anisaldehyde-H_2SO_4 was also used as a spray reagent on TLC chromatograms to detect sesquiterpenoids. The spectral data were as follows. The ^{13}C NMR spectral data of **1–4** are not shown.

Solavetivone (**1**): Colorless oil; $[\alpha]_D^{24}$ −63.25° (c 0.18, EtOH), −119.8 (c 0.19, $CHCl_3$); EIMS *m/z* (rel. int.): 218.1674 (calcd. for $C_{15}H_{22}O$, 218.1670) $[M]^+$ (80), 203 $[M\text{-}Me]^+$ (30), 108 (100); ^1H NMR ($CDCl_3$) δ: 0.99 (3H, d, *J* = 6.9 Hz, H-14), 1.76 (3H, s, H-13), 1.95 (3H, d, *J* = 1.3 Hz, H-15), 2.22 (1H, dd, *J* = 16.7, 4.5 Hz, H-3), 2.55 (1H, m, H-7), 2.66 (1H, dd, *J* = 16.7, 4.6 Hz, H-3), 4.74 (2H, br.s, H-12), 5.75 (1H, br.s, H-1); IR ν_{max} (film) cm^{-1}: 2959, 1668, 1614, 1455, 1378, 1239, 889.

3β-Hydroxysolavetivone (**2**): Colorless oil; $[\alpha]_D^{24}$ +102.9° (c 1.97, $CHCl_3$; EIMS *m/z* (rel. int.): 234.1614 (calcd. for $C_{15}H_{22}O_2$, 234.1620) $[M]^+$ (47), 176 (100); ^1H NMR ($CDCl_3$) δ: 1.22 (3H, d, *J* = 6.7 Hz, H-14), 1.77 (3H, br.s, H-13), 2.03 (3H, d, *J* = 1.2 Hz, H-15), 2.63 (1H, m, H-7), 3.56 (1H, d, *J* = 1.9 Hz, OH), 3.84 (1H, dd, *J* = 12.7, 1.9 Hz, H-3), 4.75 (2H, br.s, H-12), 5.84 (1H, br.s, H-1); IR ν_{max} (film) cm^{-1}: 3477, 2961, 1675, 1451, 1377, 1258, 1117, 1083, 889.

13-hydroxysolavetivone (**3**): Colorless oil; $[\alpha]_D^{24}$ −81.9° (c 1.35, $CHCl_3$); EIMS *m/z* (rel. int.): 234.1626 (calcd. for $C_{15}H_{22}O_2$) $[M]^+$ (14), 216 $[M\text{-}H_2O]^+$ (30), 137 (100); ^1H NMR ($CDCl_3$) δ: 1.00 (3H, d, *J* = 6.9 Hz, H-14), 1.95 (3H, s, H-15), 2.22 (1H, dd, *J* = 16.3, 4.5 Hz, H-3), 2.63 (1H, m, H-7), 2.66 (1H, dd, *J* = 16.7, 4.5 Hz, H-3), 4.14 (2H, br.s, H-13), 4.95 (1H, br.s, H-12), 5.08 (1H, br.s, H-12), 5.76 (1H, br.s, H-1); IR ν_{max} (film) cm^{-1}: 3418, 2957, 1652, 1456, 1241, 1036, 893.

Anhydro-β-rotunol (**4**): Solid; EIMS *m/z* (rel. int.): 216 $[M]^+$ (14), 201 $[M\text{-}Me]^+$ (30), 135 (100); ^1H NMR ($CDCl_3$) δ: 1.79 (3H, br.s, H-13), 2.06 (3H, d, *J* = 0.8, H-14 or 15), 2.09 (3H, d, *J* = 0.8 Hz, H-14 or 15), 2.85 (1H, m, H-7), 4.79 (2H, br.s, H-12), 6.03 (2H, br.s, H-1 and 3).

Lubimin (**5**): Colorless oil; $[\alpha]_D^{20}$ +36.3° (c 2.49, EtOH); EIMS *m/z* (rel. int.): 236.1775 (calcd. for $C_{15}H_{24}O_2$, 236.1776) $[M]^+$ (83), 218 $[M\text{-}H_2O]^+$ (30), 107 (100); ^1H NMR ($CDCl_3$) δ: 0.95 (3H, d, *J* = 6.9 Hz, H-14), 1.70 (3H, s, H-13), 1.97 (1H, dddd, *J* = 13.1, 5.2, 3.6, 2.5 Hz, H-1β), 2.26 (1H, ddd, *J* = 12.7, 3.3, 3.3 Hz, H-10β), 2.43 (1H, m, H-7α), 3.70 (1H, m, H-2β), 4.68 (2H, br.s, H-12), 9.81 (1H, d, *J* = 3.3 Hz, H-15); IR ν_{max} (film) cm^{-1}: 3401, 2946, 2872, 1717, 1645, 1455, 1377, 1124, 1044, 887. ^{13}C NMR: see Table II.

Epilubimin (**6**): Colorless oil; $[\alpha]_D^{20}$ −21.0° (c 1.63, $CHCl_3$); EIMS *m/z* (rel. int.): 236.1771 (calcd. for $C_{15}H_{24}O_2$, 236.1776) $[M]^+$ (33), 218 $[M\text{-}H_2O]^+$ (21), 107 (100); ^1H NMR ($CDCl_3$) δ: 0.95 (3H, d, *J* = 6.7 Hz, H-14), 1.73 (3H, s, H-13), 2.03 (1H, ddd, *J* = 13.0, 6.8, 1.8 Hz, H-6α), 2.27 (1H, dddd, *J* = 13.1, 5.0, 2.5, 2.5 Hz, H-1β), 2.43 (1H, m, H-7α), 2.44 (1H, dd, *J* = 5.3 and 2.5 Hz, H-10α), 3.74 (1H, m, H-2β), 4.67 (2H, br.s, H-12), 9.86 (1H, br.s, H-15); IR ν_{max} (film) cm^{-1}: 3391, 2953, 2864, 1716, 1645, 1456, 1377, 1115, 1028, 887. ^{13}C NMR: see Table II.

Lubiminoic acid (**7**): White solid; $[\alpha]_D^{20}$ +24.4° (c 0.09, $CHCl_3$). FDMS *m/z* (rel. int.): 252 $[M]^+$ (100); EIMS *m/z* (rel. int.): 252.1766 (calcd. for $C_{15}H_{24}O_3$, 252.1726) $[M]^+$ (29), 234 $[M\text{-}H_2O]^+$ (100), 219 (25); IR ν_{max} (film) cm^{-1}: 3293, 2947, 1669. ^1H NMR: see Table I. ^{13}C NMR: see Table II.

Epilubiminoic acid (**8**): White solid; $[\alpha]_D^{20}$ −20.0° (c 0.5, $CHCl_3$); FDMS *m/z* (rel. int.): 252 $[M]^+$ (100); EIMS *m/z* (rel. int.): 234.1607 (calcd. for $C_{15}H_{22}O_2$, 234.1620) $[M\text{-}H_2O]^+$ (2), 83 (100); ^1H NMR ($CDCl_3$) δ: 0.92 (3H, d, *J* = 6.9 Hz, H-14), 1.70 (3H, *s*, H-13), 1.90 (1H, ddd, *J* = 13.3, 7.3, 1.9 Hz, H-6α), 2.14 (1H, dddd, *J* = 13.4, 5.0, 2.0, 2.0 Hz, H-1β), 2.34 (1H, m, H-4β), 2.36 (1H, m, H-7α), 2.64 (1H, dd, *J* = 5.7, 2.8 Hz, H-10α), 3.98 (1H, m, H-2β), 4.68 (2H, br.s, H-12); IR ν_{max} (film) cm^{-1}: 3400, 2926, 1700. ^{13}C NMR: see Table II.

Aethione (**9**): Colorless oil; $[\alpha]_D^{20}$ +38.8° (c 0.13, $CHCl_3$); FIMS: *m/z* (rel. int.): 222 $[M]^+$ (100); EIMS *m/z* (rel. int.): 222.1604 (calcd. for $C_{14}H_{22}O_2$, 222.1620) $[M]^+$ (9), 204 $[M\text{-}H_2O]^+$ (10), 40 (100); ^1H NMR (C_6D_6) δ: 0.96 (3H, d, *J* = 6.6 Hz, H-14), 1.74 (3H, s, H-13), 2.26 (1H, m, H-7α), 2.33 (1H, dd, *J* = 13.6, 10.6 Hz, H-1α), 2.56 (1H, ddd, *J* = 13.6, 5.2, 2.3 Hz, H-1β), 2.76 (1H, dd, *J* = 13.4, 11.4 Hz, H-6β), 3.33 (1H, m, H-2β), 4.81 (1H, br.s, H-12), 4.90 (1H, br.s, H-12); IR ν_{max} (film) cm^{-1}: 3400, 1705. 1640, 890. ^1H NMR ($CDCl_3$): see Table I. ^{13}C NMR: see Table II.

Mixture of **10** *and* **11**: Colorless oil; EIMS *m/z* (rel. int.): 224 $[M]^+$ (2), 206 $[M\text{-}H_2O]^+$ (17); ^1H NMR ($CDCl_3$) δ: 0.92 (3H, d, *J* = 7.0 Hz, H-14), 0.96 (3H, d, *J* = 6.9 Hz, H-14), 1.73 (3H, s, H-13), 1.74 (3H, s, H-13), 2.40 (2H, m, H-7α), 3.40 (1H, dd, *J* = 11.8, 4.5 Hz, H-10β), 3.67 (1H, br.t, *J* = 4.0 Hz, H-10α), 3.67 (1H, m, H-2β), 4.01 (1H, m, H-2β), 4.69 (3H, br.s, H-12), 4.71 (1H, br.s, H-12); ^1H NMR (C_6D_6) δ: 0.72 (3H, d, *J* = 6.9 Hz, H-14), 0.73 (3H, d, *J* = 6.8 Hz, H-14), 1.67 (3H, s, H-13), 1.74 (3H, s, H-13), 2.27 (1H, m, H-7α), 2.35 (1H, m, H-7α), 2.94 (1H, dd, *J* = 11.8, 4.5 Hz, H-10β), 3.19 (1H, br.t, *J* = 4.0 Hz, H-10α), 3.22 (1H, m, H-2β), 3.83 (1H, m, H-2β), 4.79 (1H, br.s, H-12), 4.82 (1H, br.s, H-12), 4.83 (1H, br.s, H-12), 4.91 (1H, br.s, H-12).

Chemical conversions

Methylation and acetylation of **7**: Treatment of **7** (1 mg) with CH_2N_2 gave **7a** (1 mg), and acetylation of **7a** (1 mg) with Ac_2O (0.2 ml) and C_5H_5N (0.2 ml) gave **7b** (1.1 mg). Data for **7a**: FIMS *m/z* (rel. int.): 266.1866 (calcd. for $C_{16}H_{26}O_3$, 266.1882) $[M]^+$ (7), 248 $[M\text{-}H_2O]^+$ (49), 107 (100): 248 $[M\text{-}H_2O]^+$ (14), 41 (100); ^1H NMR ($CDCl_3$) δ: 0.93 (3H, d, *J* = 6.8 Hz, H-14), 1.67 (3H, s, H-13), 1.78 (1H, dd, *J* = 13.4, 12.4 Hz, H-6β), 1.96 (1H, dd, *J* = 13.3, 7.6 Hz, H-9β), 1.99 (1H, dddd, *J* = 13.0, 5.6, 3.3, 2.3 Hz, H-1β), 2.34 (1H, dd, *J* = 13.1, 3.5 Hz, H-10β), 2.34 (1H, m, H-7α), 3.62 (1H, m, H-2β), 3.66 (3H, s, H-OMe), 4.64 (1H, br.s, H-12), 4.65 (1H, br.s, H-12). Data for **7b**: EIMS *m/z* (rel. int.): 248.1765 (calcd. for $C_{16}H_{24}O_2$, 248.1776) $[M\text{-}AcOH]^+$ (14), 44 (100); ^1H NMR ($CDCl_3$) δ: 0.93 (3H, d, *J* = 6.8 Hz, H-14), 1.67 (3H, s, H-13), 1.78 (1H, ddd, *J* = 12.8. 12.8, 11.8 Hz, H-1α), 1.80 (1H, dd, *J* = 13.4, 12.1 Hz, H-6β), 1.96 (1H, dd, *J* = 13.4, 7.7 Hz, H-9β), 2.01 (1H, dddd, *J* = 12.8. 5.6, 3.3, 2.3 Hz, H-1β), 2.02 (3H, s, H-Ac), 2.34 (1H, m, H-7α), 2.39 (1H, dd, *J* = 13.1, 3.2 Hz, H-10β), 3.65 (3H, s, H-OMe), 4.65 (2H, br.s, H-12), 4.70 (1H, m, H-2β).

Methylation and acetylation of **8**: Treatment of **8** (1.5 mg) with CH_2N_2 gave **8a** (1.5 mg), and acetylation of **8a** (1.5 mg) with Ac_2O (0.2 ml) and C_5H_5N (0.2 ml) gave **8b** (1.7 mg). Data for **8a**: FIMS *m/z* (rel. int.): 266 $[M]^+$ (100); EIMS *m/z* (rel. int.): 266.1884 (calcd. for $C_{16}H_{26}O_3$, 266.1882) $[M]^+$ (12), 248 $[M\text{-}H_2O]^+$ (18), 107 (100); ^1H NMR ($CDCl_3$) δ: 0.92 (3H, d, *J* = 7.0 Hz, H-14), 1.71 (3H, s, H-13), 1.83 (1H, ddd, *J* = 13.4, 7.3, 1.9 Hz, H-6α), 2.06 (1H, dddd, *J* = 13.4, 5.0, 2.0, 2.0 Hz, H-1β), 2.35 (1H, m, H-4β), 2.36 (1H, m, H-7α), 2.59 (1H, dd, *J* = 5.5, 2.7 Hz, H-10α), 3.65 (3H, s, H-OMe), 3.97 (1H, m, H-2β), 4.67 (2H, br.s, H-12). Data for **8b**: FIMS *m/z* (rel. int.): 308 $[M]^+$ (100); EIMS *m/z* (rel. int.): 248.1767 (calcd. for $C_{16}H_{24}O_2$, 248.1776) $[M\text{-}AcOH]^+$ (24), 43 (100); ^1H NMR ($CDCl_3$) δ: 0.92 (3H, d, *J* = 6.9 Hz, H-14), 1.70 (3H, s, H-13), 2.01 (3H, s, H-Ac), 2.05 (1H, br.d, *J* = 13.0 Hz, H-1β), 2.32 (1H, m, H-4β), 2.36 (1H, m, H-7α), 2.64 (1H, dd, *J* = 4.6, 4.6 Hz, H-10α), 3.67 (3H, s, H-OMe), 4.67 (2H, br.s, H-12), 5.04 (1H, m, H-2β).

Acetylation and oxidation of **6**: Treatment of **6** (0.5 mg) with Ac_2O (0.2 ml) and C_5H_5N (0.2 ml) gave **6a** (0.6 mg). The aq. soln. (0.03 ml) of NaH_2PO_4 (0.016 mmol) and $NaClO_2$ (0.024 mmol) was added slowly to soln. (0.05 ml) of **6a** (0.7 mg, 0.0025 mmol) in *t*-BuOH (0.04 ml) and 2-methyl-2-butene (0.01 ml), and reacted at room temp. for 1 hr. The reacted soln. was acidified and extracted with Et_2O. The Et_2O extract was methylated with CH_2N_2 followed by TLC separation gave **8b** (0.5 mg). Data for **6a**: EIMS *m/z* (rel. int.): 278 $[M]^+$ (1), 260 $[M\text{-}H_2O]^+$ (1), 218 $[M\text{-}AcOH]^+$ (34),

43 (100); ^{1}H NMR ($CDCl_3$) δ: 0.95 (3H, d, J = 6.7 Hz, H-14), 1.72 (3H, s, H-13), 2.02 (3H, s, H_3-Ac), 2.22 (1H, m, H-1β), 2.43 (1H, m, H-7α), 2.50 (1H, dd, J = 4.1 and 4.1 Hz, H-10α), 4.69 (2H, br.s, H-12), 4.82 (1H, m, H-2β), 9.86 (1H, br.s, H-15). The ^{1}H-NMR and EI mass spectra of **8b** were identical to **8b** from **8a**.

Reduction of **9** *to* **10**: The soln. of **9** (2 mg, 0.009 mmol) in 0.5 ml Et_2O was treated with $LiAlH_4$ (0.68 mg, 0.018 mmol) and separated with TLC to yield **10** (0.2 mg). Data for **10**: FIMS m/z (rel. int.: 224 $[M]^+$ (100); EIMS m/z (rel. int.): 206.1695 (calcd. for $C_{14}H_{22}O$, 206.1670) $[M-H_2O]^+$ (8), 188 (47), 57 (100); ^{1}H NMR (C_6D_6) δ: 0.73 (3H, d, J = 6.8 Hz, H-14), 1.73 (3H, s, H-13), 2.35 (1H, m, H-7α), 2.94 (1H, dd, J = 11.4, 4.1 Hz, H-10β), 3.21 (1H, m, H-2β), 4.83 (1H, br.s, H-12), 4.91 (1H, br.s, H-12).

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