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NOTE

Mumic acids A–E: new diterpenoids from *mumiyo*

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Abstract Five new diterpenoids belonging to labdane and isopimarane skeletons, mumic acids A–E (**1**–**5**), have been isolated from *mumiyo*. Their structures and absolute configurations were elucidated on the basis of spectroscopic data and chemical derivatization.

Keywords Mumiyo · Mumic acids A–E · Diterpenoid · Labdane

Introduction

Mumiyo, also known as *mumijo*, *mumie*, or *shilajit*, is a material often found as crusts in rock cracks or interstices in the alpine region of Central Asia. *Mumiyo* has been used as a traditional medicine in the former Soviet Union, India, and Tibet for more than 3000 years, and is currently available in numerous countries as a food supplement [1]. Although there are many claims on the activity of *mumiyo*, scientific evidences on the chemical components and bioactivity are lacking [1]. In our search for new bioactive compounds [2–11], the *mumiyo* in Kyrgyzstan was investigated, resulting in the isolation of five new diterpenoids, mumic acids A–E (**1**–**5**) and agathic acid (**6**) [12]. The structure elucidation of **1**–**5** are reported herein (Fig. 1).

Mumic acid A {**1**, $[\alpha]_D^{28}$ +9 (*c* 0.4, MeOH)} was isolated as a colorless oil, with molecular formula $C_{22}H_{32}O_6$, as determined by HRESITOFMS [*m*/*z* 415.2072 $(M + Na)^+$, Δ −2.5 mmu]. IR absorptions suggested the presence of carbonyl (1737 and 1715 cm^{-1}) and hydroxy (3420 cm^{-1}) groups. The ^1H NMR data (Table 1) of **1** suggested the presence of an oxygenated methine (δ_H 5.30, br s) and 4 methyls (δ_H 0.70, s; δ_H 1.18, s; δ_H 2.10, s; δ_H 2.12, s). The ^{13}C NMR data (Table 2) revealed 22 carbon resonances due to 3 carbonyls, 2 sp^2 quaternary carbons, 2 sp^3 quaternary carbons, 1 sp^2 methines, 3 sp^3 methines, an sp^2 methylene, 6 sp^3 methylenes, and 4 methyls. The ^1H and ^{13}C NMR data (Tables 1 and 2) of **1** showed similarities to those of agathic acid (**6**) isolated in this study, suggesting the structure of **1** as a labdane type of diterpenoid related to **6**.

Analysis of the ^1H–^1H COSY of **1** (Fig. 2) revealed 3 partial structures, **a** (C-1–C-3), **b** (C-5–C-7), and **c** (C-9, C-11 and C-12). HMBC correlations of H_3-20 to C-1, C-5, C-9, and C-10 revealed the connectivity of partial structures **a**, **b**, **c**, and C-20 through C-10. The connectivity of partial structures **a**, **b**, C-18, and C-19 through C-4 was suggested by the HMBC correlations of H_3-18 to C-3, C-4, C-5, and C-19. HMBC correlations of H_2-17 to C-7, C-8, and C-9 indicated the connectivity of partial structures **b**, **c**, and C-17 through C-8. The presence of an acetoxy group at C-3 was deduced from the HMBC correlations of H-3 and a methyl (δ_H 2.10, s) to a carbonyl (δ_C 172.4). Finally, the HMBC correlations of H_3-16 to C-12, C-13, and C-14 and

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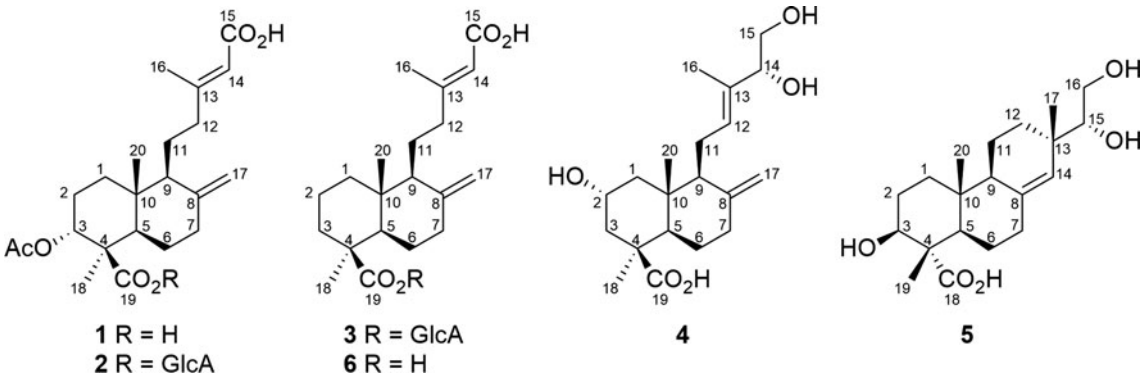

Fig. 1 Structures of mumic acids A–E (**1–5**)

Table 1 ^{13}H NMR data of mumic acids A–E (**1–5**) in CD_3OD at 300 K

	1[a]	**2**[b]	**3**[b]	**4**[a]	**5**[b]
1a	1.43, m	1.41, td, 13.7, 3.7	1.11, td, 13.4, 3.7	1.05, dd, 12.3, 4.3	1.29, dt, 13.4, 3.2
1b	1.65, m	1.63, br d, 12.8	1.85, br d, 12.7	2.15, t, 12.3	1.78, m
2a	1.71, m	1.72, m	1.52, m	4.12, m	1.60, m
2b	2.25, m	2.22, br t, 14.6	1.96, m		1.65, m
3a	5.30, br s	5.33, br s	1.11, br d, 13.4	1.02, d, 12.3	4.00, dd, 11.3, 3.1
3b			2.22, td, 13.4, 3.7	2.39, t, 12.3	
5	1.75, m	1.82, dd, 12.2, 2.2	1.39, dd, 11.7, 4.4	1.35, d, 12.0	1.82, br d, 12.2
6a	1.94, m	1.93, m	1.93, m	1.83, m	1.25, m
6b	2.00, m	2.00, m	2.04, m	2.00, m	1.50, m
7a	1.98, m	1.95, m	1.93, m	1.95, br t, 13.2	2.07, dd, 14.2, 3.8
7b	2.44, m	2.44, m	2.43, dd, 10.7, 3.5	2.41, m	2.24, br t, 14.2
9	1.71, m	1.69, m	1.63, br d, 11.0	1.80, m	1.76, d, 9.2
11a	1.55, m	1.54, m	1.54, m	2.10, m	1.51, m
11b	1.72, m	1.70, m	1.72, m	2.34, m	1.64, m
12a	2.00, m	2.02, m	2.02, m	5.39, br t, 5.4	1.33, m
12b	2.30, m	2.31, ddd, 13.6, 9.8, 4.2	2.30, ddd, 13.8, 9.6, 4.3		1.50, m
14	5.66, s	5.62, s	5.60, s	3.92, dd, 7.4, 4.4	5.32, s
15a				3.44, dd, 11.3, 4.4	3.30, dd, 8.8, 2.2
15b				3.50, dd, 11.3, 7.4	
16a	2.12, s	2.13, s	2.12, s	1.64, s	3.36, dd, 11.2, 8.8
16b					3.70, dd, 11.2, 2.2
17a	4.57, s	4.56, s	4.54, s	4.52, s	0.96, s
17b	4.92, s	4.91, s	4.89, s	4.87, s	
18	1.18, s	1.25, s	1.26, s	1.26, s	
19					1.12, s
20	0.70, s	0.65, s	0.62, s	0.68, s	0.82, s
Ac	2.10, s	2.09, s			
1′		5.49, d, 7.7	5.47, d, 8.0		
2′		3.40, dd, 8.9, 7.7	3.40, dd, 9.1, 8.0		
3′		3.42, dd, 8.9, 8.7	3.42, dd, 9.3, 9.1		
4′		3.51, dd, 9.3, 8.7	3.52, dd, 9.6, 9.3		
5′		3.73, d, 9.3	3.77, d, 9.6		

[a] 400 MHz; [b]700 MHz

Table 2 ^{13}C NMR data of mumic acids A–E (**1**–**5**) in CD_3OD at 300 K

No.	**1**[a]	**2**[b]	**3**[b]	**4**[a]	**5**[b]	No.	**1**[a]	**2**[b]	**3**[b]	**4**[a]	**5**[b]
1	34.2	34.0	40.4	49.1	38.35	15	172.1	170.3	170.3	65.9	80.7
2	25.6	25.5	21.2	65.5	27.9	16	18.9	18.9	18.9	12.7	63.9
3	75.8	74.8	39.1	47.6	76.6	17	107.0	107.3	107.0	108.6	23.1
4	47.0	48.4	45.7	46.0	54.9	18	24.6	24.4	29.3	29.6	182.5
5	51.3	51.6	57.9	56.6	51.4	19	180.3	175.5	177.2	180.7	12.1
6	27.1	26.7	27.3	26.8	25.4	20	13.1	13.6	13.8	12.3	15.5
7	39.8	39.7	39.9	39.4	36.5	COMe	172.4	172.1			
8	149.3	148.9	149.3	148.9	138.6	COMe	21.2	21.1			
9	56.5	56.4	56.6	57.6	52.2	1′		95.4	95.3		
10	41.2	41.2	41.6	42.2	38.39	2′		73.8	73.8		
11	23.0	22.9	22.9	23.8	19.5	3′		78.2	78.1		
12	40.6	40.8	40.8	128.7	31.1	4′		73.3	73.2		
13	158.8	161.8	162.0	135.1	39.1	5′		78.0	77.5		
14	118.7	116.8	116.7	78.7	129.4	6′		174.0[c]	173.7[c]		

[a] 100 MHz; [b]175 MHz; [c]assigned from HMBC correlations

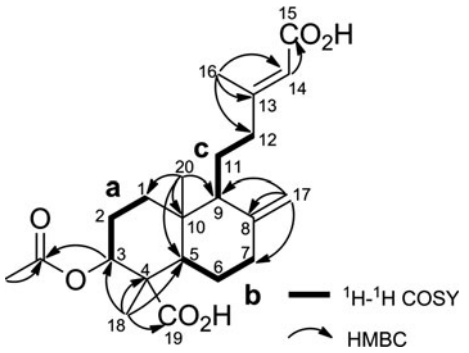

Fig. 2 Selected 2D NMR correlations for mumic acid A (**1**)

H-14 to C-15 completed the structure of **1**. Thus, **1** was deduced to be a new labdane diterpenoid with an acetoxy group at C-3 and carboxylic acids at C-4 and C-14.

The relative configuration of **1** was determined by analyses of the NOESY correlations (Fig. 3) and ^1H–^1H coupling constant data. The α-orientation of H-5, H-9, and C-18 was deduced from the NOESY correlations of H-5/H-9 and H_3-18, and those of H_3-20/H-2b and H_2-11 suggested the β-orientation of C-20. The small ^1H–^1H coupling constant value of H-3/H-2a and H-2b indicated H-3 to be β-oriented. Finally, the C-13–C-14 double bond was deduced to be of the *E* configuration based on the NOESY correlation of H-12a/H-14. Thus, the relative configuration of **1** was elucidated to be as shown in Fig. 1.

Mumic acid B {**2**, $[\alpha]_D^{23}$ −7 (*c* 0.3, MeOH)} was isolated as a colorless oil and had molecular formula $C_{28}H_{40}O_{12}$, as determined by HRESITOFMS [*m/z* 591.2438 $(M + Na)^+$, Δ +2.1 mmu]. IR absorptions suggested the presence of carbonyl (1743 and 1721 cm^{-1}) and hydroxy (3393 cm^{-1}) groups. The ^1H NMR data (Table 1) of **2** suggested the presence of a sugar moiety. Except for the signals assigned to the sugar moiety, the ^1H and ^{13}C NMR data (Tables 1 and 2) of **2** are highly similar to those of **1**, suggesting **2** to be a glycoside derivative of **1**. The sugar moiety was identified as D-glucuronic acid on the basis of HPLC analysis with chiral detector of the acid hydrolysate of **2**. The HMBC correlation of H-1′ to C-19 and C-5′ suggested the pyranose form of the sugar moiety and the connectivity of C-19 and C-1′ through an oxygen atom. The ROESY correlations for H-1′/H-3′ and H-5′, and the ^1H–^1H coupling constant value of H-1′/H-2′ (7.7 Hz) indicated the α-orientation of H-1′. Further analysis of the 2D NMR data confirmed the structure of **2** as 19-*O*-β-D-glucuronic acid-**1**.

Mumic acid C {**3**, $[\alpha]_D^{30}$ +5 (*c* 0.2, MeOH)} was isolated as a colorless oil and had molecular formula $C_{26}H_{38}O_{10}$, as determined by HRESITOFMS [*m/z* 533.2398 $(M + Na)^+$, Δ +3.5 mmu]. IR absorptions suggested the presence of carbonyl (1732 and 1716 cm^{-1}) and hydroxy (3420 cm^{-1}) groups. The ^1H NMR data (Table 1) of **3** suggested the presence of a sugar moiety. The differences in the ^1H and ^{13}C NMR data (Tables 1 and 2) of **3** and **6** are reminiscent to the differences observed between **1** and **2**. Thus, **3** was assumed to be a 19-*O*-β-glucuronic acid derivative of agathic acid (**6**). Acid hydrolysis of **3** gave **6** and a sugar, which was identified as D-glucuronic acid on the basis of HPLC analysis with chiral detector.

Mumic acid D {**4**, $[\alpha]_D^{25}$ +28 (*c* 2.0, MeOH)} was isolated as a colorless oil, with molecular formula $C_{20}H_{32}O_5$, as determined by HRESITOFMS [*m/z* 375.2151 $(M + Na)^+$, Δ +0.4 mmu]. IR absorptions suggested the presence of carbonyl (1694 cm^{-1}) and hydroxy (3370 cm^{-1}) groups. The ^{13}C NMR data (Table 2) revealed 20 carbon resonances due to 1 carbonyl, 2 sp^2 quaternary carbons, 2 sp^3 quaternary

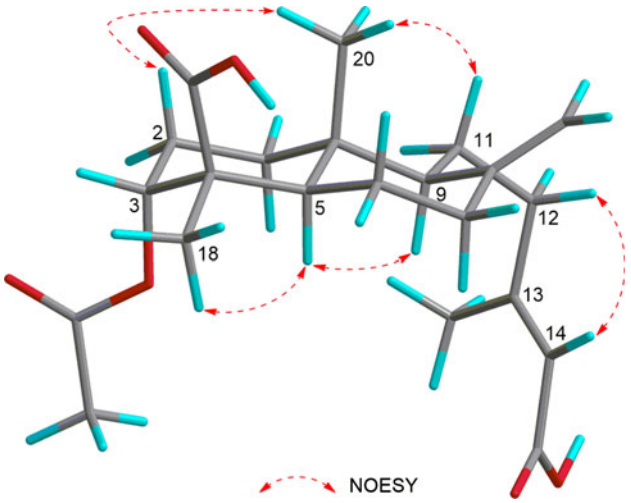

Fig. 3 Selected NOESY correlations for mumic acid A (**1**)

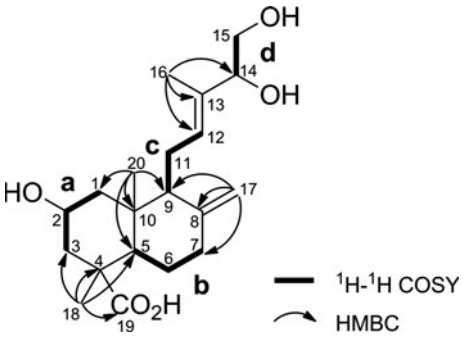

Fig. 4 Selected 2D NMR correlations for mumic acid D (**4**)

carbons, 1 sp^2 methines, 4 sp^3 methines, an sp^2 methylene, 6 sp^3 methylenes, and 3 methyls. Analysis of the 2D NMR correlations of **4** (Fig. 4) revealed the structure of **4** to be a new labdane diterpenoid with hydroxyl groups at C-2, C-14, and C-15, and carboxylic acids at C-19.

The configuration of **4** was determined as follows. The α-orientation of H-5, H-9, and C-18 was deduced from the NOESY correlations of H-5/H-9 and H_3-18, and the ROESY correlation of H_3-20/H-2 and H_2-11 suggested the β-orientation of H-2 and C-20. The double bond of C-12–C-13 was deduced to be of the *E* configuration based on the NOESY correlation of H-12/H-14. C-2 was determined to be of the *R* configuration based on the advanced Mosher's method [13]. The absolute configuration of C-14 of the terminal 1,2-diol was deduced to be *R* based on the vicinal coupling constant value of H-14/H_2-15 (5.5 Hz) and the Cotton effect (CE) signs [237 (Δε +13.7), 229 (0), and 222 (−6.8) nm] of the 2,14,15-tribenzoyl-19-methyl-derivative of **4** [14]. Thus, the structure of **4** was deduced to be (2*R*, 4*S*, 5*R*, 9*S*, 10*R*, 14*R*, *E*)-2,14,15-trihydroxy-8(17), 12-labdadien-19-oic acid.

Mumic acid E {**5**, $[\alpha]_D^{25}$ +9 (*c* 0.3, MeOH)} was isolated as a colorless oil, with molecular formula $C_{20}H_{32}O_5$, as

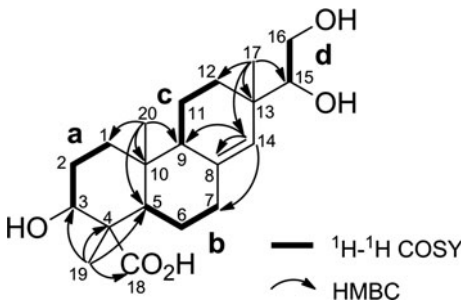

Fig. 5 Selected 2D NMR correlations for mumic acid E (**5**)

determined by HRESITOFMS [*m/z* 375.2131 $(M + Na)^+$, Δ −1.6 mmu]. IR absorptions suggested the presence of carbonyl (1710 cm^{-1}) and hydroxy (3370 cm^{-1}) groups. The ^{13}C NMR data (Table 2) revealed 20 carbon resonances due to 1 carbonyl, 1 sp^2 quaternary carbons, 3 sp^3 quaternary carbons, 1 sp^2 methines, 4 sp^3 methines, 7 sp^3 methylenes, and 3 methyls. Analysis of the ^1H–^1H COSY of **5** (Fig. 5) revealed 4 partial structures, **a** (C-1–C-3), **b** (C-5–C-7), **c** (C-9, C-11 and C-12), and **d** (C-14 and C-15). HMBC correlations of H_3-20 to C-1, C-5, C-9, and C-10 revealed the connectivity of partial structures **a**, **b**, **c**, and C-20 through C-10. The connectivity of partial structures **a**, **b**, C-18, and C-19 through C-4 was suggested by the HMBC correlations of H_3-19 to C-3, C-4, C-5, and C-18. HMBC correlations of H-14 to C-7, C-8, and C-9 indicated the connectivity of partial structures **b**, **c**, and C-17 through C-8. Finally, the HMBC correlations of H_3-17 to C-12, C-13, C-14, and C-15 revealed the connectivity of **c**, **d**, C-14, and C-17 through C-13.

The configuration of **5** was determined as follows. The β-orientation of C-17, C-19, and C-20 was deduced from the ROESY correlations of H_3-20/H_3-17 and H_3-19. The ^1H–^1H coupling constant value of H-3/H-2a and H-2b (11.3 and 3.1 Hz) indicated H-3 to be α-oriented. C-3 was determined to be of *S* configuration based on the advanced Mosher's method [13]. The vicinal coupling constant value of H-14/H-15a and H-15b (Hz, respectively) and the CE signs in the CD spectrum [238 (Δε −12.5), 229 (0), and 221 (+6.9) nm] of the 3,15,16-tribenzoyl-18-methyl-derivative of **5** [14] indicated the absolute configuration of C-15 of the terminal 1,2-diol to be *R*. Thus, **5** was deduced to be a new isopimarane diterpenoid with hydroxyl group at C-3, C-15, and C-16, a carboxylic acid at C-18, and C-8–C-14 double bond.

Compounds **1**–**5** were tested for cytotoxic activity against the HL-60 (human promyelocytic leukemia) cell line, LPS-induced NO production inhibitory activity on the RAW264.7 (murine leukemic monocyte macrophage) cell line, melanin-production inhibitory activity on the B16F10 (murine melanoma) cell line, lipid-droplet accumulation inhibitory activity on the MC3T3-G2/PA6 (mouse pre-

adipocyte) cell line, and vasorelaxant activity on rat aortic artery. All compounds gave negative results for these bioactivity assays.

Experimental section

General experimental procedures

Optical rotations were measured on a JASCO DIP-1000 polarimeter. UV spectra were recorded on a Shimadzu UVmini-1240 spectrophotometer and IR spectra on a JASCO FT/IR-4100 spectrophotometer. CD spectra were recorded on a JASCO J-820 polarimeter. High-resolution ESI MS were obtained on an LTQ Orbitrap XL (Thermo Scientific). ^{1}H and 2D NMR spectra were measured on a 400- or 700-MHz spectrometer at 300 K, while ^{13}C NMR spectra were measured on a 125- or 175-MHz spectrometer. The residual CD_3OD chemical shift used as an internal standard are δ_H 3.31 and δ_C 49.0 and for $CDCl_3$ are δ_H 7.26 and δ_C 77.0. Standard pulse sequences were used for the 2D NMR experiments.

Material

The natural *mumiyo* samples were collected from Kyrgys. To 1 kg of natural *mumiyo*, 4 L of water was added, and the mixture was then stirred and heated to boiling for about 40 min. The mixture was cooled, and the precipitates were separated from the supernatant. The precipitates were then extracted with water repeatedly to obtain the water extract. The supernatant and the water extract were combined and subjected to centrifugation for 10 min. The supernatant was collected and concentrated by an evaporator. The concentrated solution was again subjected to centrifugation, and the resulting supernatant was concentrated by an evaporator (until a water content of 30 %). The final concentrated solution (50 g) was cooled and then packed as commercial *mumiyo*. The *mumiyo* sample used in this study is stored at the Department of Pharmacognosy, Hoshi University, as sample HOSHI12001.

Extraction and isolation

Mumiyo (50 g) was dissolved in water successively partitioned with EtOAc and *n*-BuOH, and a part of the *n*-BuOH-soluble materials (540 mg) were further separated with silica gel column chromatography ($CHCl_3$/MeOH, 1:0 → 0:1). The fractions eluted with $CHCl_3$/MeOH (20:1) were combined and subjected to ODS silica gel column chromatography (H_2O/MeOH, 3:2 → 0:1) to obtain **1** (7.0 mg, 0.22 %).

The rest of the *n*-BuOH-soluble materials (7.5 g) were subjected to ODS silica gel column chromatography (H_2O/MeOH, 4:1 → 0:1). Further separation of the fraction eluted with H_2O/MeOH (4:1) using silica gel column chromatography ($CHCl_3$/MeOH, 1:0 → 0:1, with 0.1 % HCO_2H) yielded **5** (24.8 mg, 0.053 %), and separation of the fraction eluted with H_2O/MeOH (1:1) using silica gel column chromatography ($CHCl_3$/MeOH, 1:0 → 0:1, with 0.1 % HCO_2H) and ODS HPLC (C-18 Capcell Pak MG-III, 250 × 10 mm; 53 % MeOH aq. with 0.1 % HCO_2H; flow rate 2.0 mL/min; UV detection at 210 nm) yielded **4** (11.7 mg, 0.025 %).

On the other hand, a part of the water-soluble materials (1.6 g) were separated by DIAION HP-20 column chromatography (H_2O/MeOH, 1:0 → 3:2) and the fraction eluted with H_2O/MeOH (3:2) was subjected to ODS silica gel column chromatography (H_2O/MeOH, 7:3 → 3:7) to obtain **2** (9.9 mg, 0.099 %) and **3** (15.0 mg, 0.15 %).

Mumic acid A (1): colorless oil; $[\alpha]_D^{28}$ +9 (*c* 0.4, MeOH); IR (ZnSe) ν_{max} 3420, 2943, 1737, and 1715 cm^{-1}; ^{1}H NMR data (Table 1) and ^{13}C NMR data (Table 2); ESIMS *m/z* 415 $(M + Na)^+$; HRESIMS *m/z* 415.2072 $(M + Na)^+$; calcd. for $C_{22}H_{32}O_6Na$, 415.2097).

Mumic acid B (2): colorless oil; $[\alpha]_D^{23}$ −7 (*c* 0.3, MeOH); IR (ZnSe) ν_{max} 3393, 2943, 1743, and 1721 cm^{-1}; ^{1}H NMR data (Table 1) and ^{13}C NMR data (Table 2); ESIMS *m/z* 591 $(M + Na)^+$; HRESIMS *m/z* 591.2438 $(M + Na)^+$; calcd. for $C_{28}H_{40}O_{12}Na$, 591.2417).

Mumic acid C (3): colorless oil; $[\alpha]_D^{30}$ +5 (*c* 0.2, MeOH); IR (ZnSe) ν_{max} 3420, 1732, and 1716 cm^{-1}; ^{1}H NMR data (Table 1) and ^{13}C NMR data (Table 2); ESIMS *m/z* 533 $(M + Na)^+$; HRESIMS *m/z* 533.2398 $(M + Na)^+$; calcd. for $C_{26}H_{38}O_{10}Na$, 533.2363).

Mumic acid D (4): colorless oil; $[\alpha]_D^{25}$ +28 (*c* 2.0, MeOH); IR (ZnSe) ν_{max} 3370, 2942, and 1694 cm^{-1}; ^{1}H NMR data (Table 1) and ^{13}C NMR data (Table 2); ESIMS *m/z* 375 $(M + Na)^+$; HRESIMS *m/z* 375.2151 $(M + Na)^+$; calcd. for $C_{20}H_{32}O_5Na$, 375.2147).

Mumic acid E (5): colorless oil; $[\alpha]_D^{25}$ +9 (*c* 0.3, MeOH); IR (ZnSe) ν_{max} 3370, 2938, and 1710 cm^{-1}; ^{1}H NMR data (Table 1) and ^{13}C NMR data (Table 2); ESIMS *m/z* 375 $(M + Na)^+$; HRESIMS *m/z* 375.2131 $(M + Na)^+$; calcd. for $C_{20}H_{32}O_5Na$, 375.2147).

Acid hydrolysis of **2** and **3**

2 (2.0 mg) was treated with 2 M aqueous HCl (400 μL) at 100 °C for 1 h. After neutralization with 2 M aqueous NaOH, the mixture was extracted with $CHCl_3$. The aqueous layer was submitted to HPLC analysis (GL science NH2 column φ 4.6 × 250 mm, eluent: 70 % aqueous MeCN, flow rate 1.0 mL/min, JASCO OR-1590 chiral detector). Retention times of authentic L- and D-glucuronic acid were as follows: L (4.9 min with negative intensity) and D (4.9 min with positive intensity). The retention time of

glucuronic acid in the aqueous layer of hydrolysate of **2** was 4.9 min, with positive intensity. **3** (1.0 mg) was subjected to a similar treatment as **3**, and the retention time of glucose in the aqueous layer of hydrolysate of **3** was 4.9 min, with positive intensity.

Synthesis of 2,14,15-tri-*O*-acyl-19-methyl-**4** and 3,14,15-tri-*O*-acyl-18-methyl-**5**

To a solution of **4** (0.8 mg in 100 μL MeOH), 20 μL of TMS-diazomethane (10 % in *n*-hexane) was added and left at room temperature. After 10 min, the reaction mixture was dried under an N_2 stream, and the resulting residue (0.8 mg) was dissolved in 150 μL of CH_2Cl_2. To the CH_2Cl_2 solution, a catalytic amount of 4-(dimethylamino)pyridine and 2 μL of triethylamine were added, and the mixture was then separated into three containers (50 μL each). Into the container, (*R*)-MTPA chloride, (*S*)-MTPA chloride, or benzoyl chloride was added, and the solutions were allowed to stand at room temperature overnight. The residue obtained under an N_2 stream was subjected to SiO_2 column chromatography ($CHCl_3$) to obtain the tri-(*S*)-MTPA, tri-(*R*)-MTPA, and tri-benzoyl derivatives of 19-methyl-**4**. The same procedure was used to obtain tri-(*S*)-MTPA, tri-(*R*)-MTPA, and tri-benzoyl derivatives of 19-methyl-**5**.

*2,14,15-tri-O-[(R)-MTPA]-19-methyl-**4***

^{1}H NMR ($CDCl_3$, 400 MHz) δ 1.11 (dd, 12.3, 4.3; H-1a), 2.20 (t, 12.3; H-1b), 5.57 (m; H-2), 1.30 (d, 12.3; H-3a), 2.55 (t, 12.3; H-3b), 1.86 (m; H-7a), 2.38 (m; H-7b), 2.08 (m, H_2-11), 5.51 (br t, 5.4; H-12), 5.60 (dd, 7.4, 4.4; H-14), 4.27 (dd, 11.3, 7.4; H-15a), 4.49 (dd, 11.3, 4.4; H-15b), 1.63 (s; H_3-16), 4.38 (s; H-17a), 4.84 (s; H-17b), 1.29 (s; H_3-18), 0.62 (s; H_3-20), and 3.68 (s; 19-OMe).

*2,14,15-tri-O-[(S)-MTPA]-19-methyl-**4***

^{1}H NMR ($CDCl_3$, 400 MHz) δ 1.20 (dd, 12.3, 4.3; H-1a), 2.22 (t, 12.3; H-1b), 5.57 (m; H-2), 1.21 (d, 12.3; H-3a), 2.49 (t, 12.3; H-3b), 1.86 (m; H-7a), 2.38 (m; H-7b), 2.08 (m, H_2-11), 5.34 (br t, 5.4; H-12), 5.50 (dd, 7.4, 4.4; H-14), 4.27 (dd, 11.3, 7.4; H-15a), 4.54 (dd, 11.3, 4.4; H-15b), 1.53 (s; H_3-16), 4.36 (s; H-17a), 4.85 (s; H-17b), 1.28 (s; H_3-18), 0.63 (s; H_3-20), and 3.67 (s; 19-OMe).

*2,14,15-tri-O-benzoyl-19-methyl-**4***

UV (MeOH) λ_{max} (log ε) 229 (4.75) nm; CD (MeOH) λ_{max} (Δε) 237 (+13.7), 229 (0), and 222 (−6.8) nm. ^{1}H NMR (CD_3OD, 400 MHz) 5.50 (m; H-2), 5.58 (br t, 5.4; H-12), 5.66 (t, 5.5; H-14), 4.53 (d, 5.5; H_2-15), 1.81 (s; H_3-16), 4.50 (s; H-17a), 4.82 (s; H-17b), 1.29 (s; H_3-18), 0.65 (s; H_3-20), and 3.66 (s; 19-OMe).

*3,14,15-tri-O-[(R)-MTPA]-18-methyl-**5***

^{1}H NMR ($CDCl_3$, 400 MHz) δ 1.43 (dd, 13.4, 3.2; H-1a), 1.78 (m; H-1b), 1.72 (m; H-2a), 2.01 (m; H-2b), 5.46 (dd, 11.3, 3.1; H-3), 1.80 (m; H-5), 1.10 (m; H-6a), 1.50 (m; H-6b), 1.93 (m; H-7a), 2.14 (br d, 14.2; H-7b), 1.73 (m; H-9), 5.21 (s; H-14), 5.22 (d, 8.8; H-15), 4.19 (dd, 11.2, 8.8; H-16a), 4.80 (br d, 11.2; H-16b), 1.00 (s; H_3-17), 1.17 (s; H_3-19), 0.80 (s; H_3-20), and 3.58 (s; 18-OMe).

*3,14,15-tri-O-[(S)-MTPA]-18-methyl-**5***

^{1}H NMR ($CDCl_3$, 400 MHz) δ 1.42 (dd, 13.4, 3.2; H-1a), 1.76 (m; H-1b), 1.60 (m; H-2a), 1.93 (m; H-2b), 5.46 (dd, 11.3, 3.1; H-3), 1.82 (m; H-5), 1.12 (m; H-6a), 1.51 (m; H-6b), 1.95 (m; H-7a), 2.19 (br d, 14.2; H-7b), 1.73 (m; H-9), 5.26 (s; H-14), 5.22 (d, 8.8; H-15), 4.07 (dd, 11.2, 8.8; H-16a), 4.81 (br d, 11.2; H-16b), 1.05 (s; H_3-17), 1.19 (s; H_3-19), 0.80 (s; H_3-20), and 3.66 (s; 18-OMe).

*3,14,15-tri-O-benzoyl-18-methyl-**5***

UV (MeOH) λ_{max} (log ε) 228 (4.84) nm; CD (MeOH) λ_{max} (Δε) 238 (−12.5), 229 (0), and 221 (+6.9) nm. ^{1}H NMR (CD_3OD, 400 MHz) 5.39 (br d 11.0; H-3), 5.50 (s; H-14), 5.35 (dd, 8.4, 2.2; H-14), 4.47 (dd, 11.0, 8.4; H-15a), 4.77 (br d, 11.0; H-15b), 0.94 (s; H_3-17), 1.09 (s; H_3-19), 0.78 (s; H_3-20), and 3.67 (s; 18-OMe).

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