Scoparic Acid A, a #-Glucuronidase Inhibitor from Scoparia dulcis

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SCOPARIC ACID A, A β-GLUCURONIDASE INHIBITOR FROM *SCOPARIA DULCIS*

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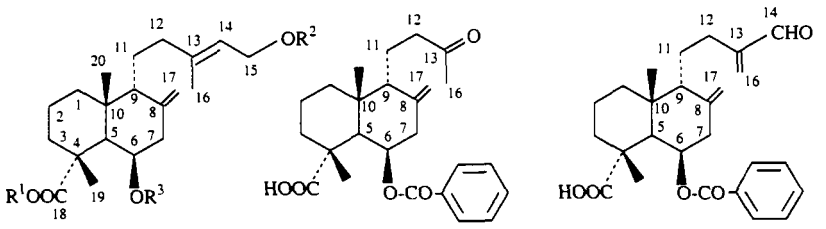
ABSTRACT.—The 70% EtOH extract of *Scoparia dulcis* showed inhibitory activity against β-glucuronidase from bovine liver. Bioassay-directed fractionation of the active extract led to the isolation of three labdane-type diterpene acids, scoparic acid A [**1**] [6-benzoyl-12-hydroxy-labda-8(17),13-dien-18-oic acid], scoparic acid B [**2**] [6-benzoyl-14,15-dinor-13-oxo-8(17)-labden-18-oic acid], and scoparic acid C [**3**] [6-benzoyl-15-nor-14-oxo-8(17)-labden-18-oic acid], the structures of which were established by spectral means, including X-ray analysis. Scoparic acid A was found to be a potent β-glucuronidase inhibitor.

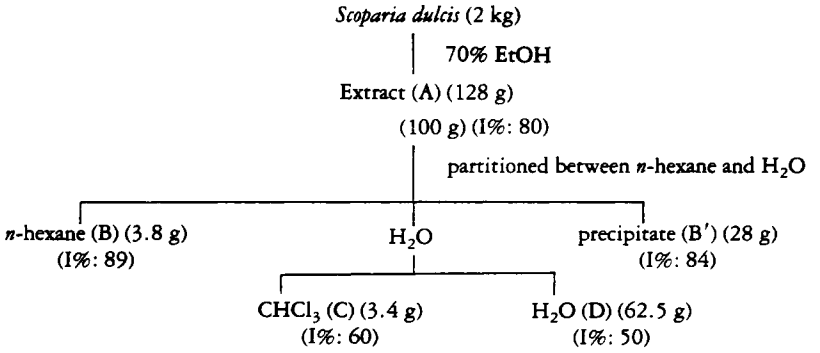
Scoparia dulcis L. (Scrophulariaceae) is a perennial herb that grows in tropical and subtropical areas and has traditionally been used as a medicament for stomach disorders, hypertension and/or hepatosis (1–3). As a result of a search for β-glucuronidase inhibitors from Paraguayan medicinal plants, a 70% EtOH extract from this plant was found to be a potent inhibitor of bovine liver β-glucuronidase. Bioassay-guided fractionation of this extract led to the isolation of a labdane-type diterpene acid named scoparic acid A [**1**] together with its congeners, scoparic acids B [**2**] and C [**3**]. In a preliminary communication (4), we reported the structure of **1** except for the configuration of the side chain attached to C-9. This paper describes the structures of **1–3** as well as their inhibitory activity against β-glucuronidase.

RESULTS AND DISCUSSION

The 70% EtOH extract of the whole plants of *S. dulcis* collected in Paraguay was fractionated as illustrated in Scheme 1, and the inhibitory activity of each fraction against β-glucuronidase was measured spectrophotometrically by the method of Nobunaga (5). The fractions B and B′ showed relatively greater activity (more than 80% inhibition at 10 μg/ml). Since fraction B′ was obtained in much higher yield than fraction B, fraction B′ was further fractionated, i.e., the $CHCl_3$-soluble part of this



1 $R^1=R^2=H$, $R^3=COC_6H_5$ **2** **3**
4 $R^1=Me$, $R^2=H$, $R^3=COC_6H_5$
5 $R^1=H$, $R^2=Ac$, $R^3=COC_6H_5$
6 $R^1=R^2=R^3=H$



SCHEME 1. Fractionation of 70% EtOH extract from *Scoparia dulcis*. I%: Inhibition % at 10 μg/ml.

fraction was separated by repeated Si gel cc, preparative tlc on Si gel, and finally hplc to give scoparic acids A [**1**], B [**2**], and C [**3**] in 0.019, 0.004, and 0.002% yield, respectively.

Scoparic acid A [**1**] was isolated as a colorless amorphous powder, $[\alpha]_D$ −38.3° ($CHCl_3$), and showed ir absorptions at 3400 (OH), 1700 (CO), 1600, and 1580 (phenyl) cm^{-1} and uv absorptions at 227, 265 (sh), 270, and 277 nm. The eims of **1** indicated a molecular ion peak at *m/z* 440 and fragment ion peaks at *m/z* 422 $[M-H_2O]^+$, 394 $[M-HCOOH]^+$, 377 $[M-H_2O-COOH]^+$, 335 $[M-C_6H_5CO]^+$, 318 $[M-C_6H_5COOH]^+$, 105 $[C_6H_5CO]^+$, and 77 $[C_6H_5]^+$. Its molecular formula was deduced as $C_{27}H_{36}O_5$ by hrms and ^1H- and ^{13}C-nmr spectral data (Tables 1 and 2). This compound gave a methyl ester **4**, $C_{28}H_{38}O_5$, $[\alpha]_D$ −50.2° ($CHCl_3$), and a monoacetate **5**, $C_{29}H_{38}O_6$, $[\alpha]_D$ −4.9° ($CHCl_3$), on treatment with CH_2N_2 and with Ac_2O in pyridine, respectively. Hydrolysis of **1** with saturated KOH in MeOH/DMSO afforded benzoic acid along with a debenzoylated compound **6**, colorless prisms, mp 186–188°, $C_{20}H_{32}O_4$, $[\alpha]_D$ −4.8° ($CHCl_3$). These findings suggested that **1** was a diterpenoid

TABLE 1. ^1H-nmr Spectral Data of Compounds **1–3** in $CDCl_3$.[a]

Proton	Compound		
	1	**2**	**3**
H-5	2.48 br d (2.4)	2.45 br s	2.42 br s
H-6	5.22 br dd (2.4, 2.7)	5.49 br s	4.71 br s
H-7	2.43 br d (2.7)	2.01 br d (12.5)	1.97 m
		2.31 br d (12.5)	2.37 br d (12.5)
H-14	5.45 br t (6.7)	—	9.45 s
H-15	4.17 d (6.7)	—	—
H-16	1.68 s	2.05 s	5.91 s
			6.26 s
H-17	4.74 s	4.65 br s	4.65 br s
H-19	1.34 s	1.09 s	1.07 s
H-20	1.47 s	1.35 s	1.36 s
H-2', -6	8.01 d (7.3)	7.96 d (7.3)	7.95 d (7.3)
H-3', -5	7.42 t (7.3)	7.30 t (7.3)	7.30 t (7.3)
H-4'	7.52 t (7.3)	7.46 t (7.3)	7.46 t (7.3)

[a]Values in parentheses are *J*; s = singlet, d = doublet, t = triplet, dd = double doublet, br s = broad singlet, br d = broad doublet, br dd = broad double doublet, br t = broad triplet, m = multiplet.

TABLE 2. ^{13}C-nmr Spectral Data of Scoparic Acids A [**1**], B [**2**], and C [**3**] and Compounds **4–6** in $CDCl_3$ or Pyridine-d_5.

Carbon	Compound					
	1[a]	**2**[a]	**3**[a]	**4**[a]	**5**[a]	**6**[b]
C-1	37.57 (t)	38.08 (t)	38.16 (t)	39.74 (t)	37.98 (t)	38.68 (t)
C-2	17.93 (t)	18.76 (t)	18.79 (t)	18.33 (t)	18.25 (t)	19.51 (t)
C-3	39.66 (t)	40.16 (t)	40.21 (t)	39.98 (t)	40.10 (t)	40.67 (t)
C-4	46.75 (s)	48.05 (s)	48.18 (s)	47.59 (s)	47.17 (s)	49.03 (s)
C-5	42.89 (d)	43.80 (d)	44.08 (d)	43.91 (d)	43.07 (d)	45.43 (d)
C-6	73.49 (d)	74.07 (d)	74.10 (d)	73.56 (d)	73.81 (d)	70.68 (d)
C-7	36.36 (t)	37.55 (t)	37.61 (t)	36.80 (t)	36.64 (t)	41.59 (t)
C-8	143.59 (s)	144.76 (s)	144.40 (s)	143.90 (s)	143.74 (s)	146.68 (s)
C-9	57.05 (d)	56.84 (d)	57.71 (d)	57.60 (d)	57.35 (d)	58.66 (d)
C-10	38.03 (s)	38.49 (s)	38.55 (s)	38.60 (s)	38.44 (s)	39.00 (s)
C-11	23.95 (t)	20.09 (t)	24.28 (t)	24.46 (t)	24.07 (t)	25.09 (t)
C-12	37.77 (t)	41.98 (t)	26.70 (t)	38.13 (t)	38.12 (t)	38.79 (t)
C-13	138.85 (s)	209.09 (s)	150.39 (s)	139.95 (s)	142.48 (s)	137.39 (s)
C-14	123.05 (d)	—	194.82 (d)	123.49 (d)	118.31 (d)	126.02 (d)
C-15	58.63 (t)	—	—	59.47 (t)	61.45 (t)	59.02 (t)
C-16	16.11 (q)	30.13 (q)	134.23 (t)	16.60 (q)	16.74 (q)	16.55 (q)
C-17	113.11 (t)	113.10 (t)	113.14 (t)	113.55 (t)	113.76 (t)	112.07 (t)
C-18	182.37 (s)	185.07 (s)	185.30 (s)	178.70 (s)	182.23 (s)	185.01 (s)
C-19	18.99 (q)	19.78 (q)	19.73 (q)	19.42 (q)	19.30 (q)	2.01 (q)
C-20	25.31 (q)	25.51 (q)	25.53 (q)	25.73 (q)	25.72 (q)	26.10 (q)
C-21	165.71 (s)	167.10 (s)	167.35 (s)	165.98 (s)	166.06 (s)	—
C-1′	129.21 (s)	130.81 (s)	130.73 (s)	130.88 (s)	130.66 (s)	—
C-2′, -6′	128.05 (d)	129.85 (d)	129.88 (d)	129.65 (d)	129.62 (d)	—
C-3′, 5′	127.90 (d)	128.29 (d)	128.32 (d)	128.45 (d)	128.45 (d)	—
C-4′	130.33 (d)	132.81 (d)	132.89 (d)	132.82 (d)	132.89 (d)	—
CO-OCH_3	—	—	—	50.90 (q)	—	—
$OCOCH_3$	—	—	—	—	171.36 (s)	—
$OCOCH_3$	—	—	—	—	21.12 (q)	—

[a]In $CDCl_3$.
[b]In pyridine-d_6.

possessing a carboxyl group, a hydroxyl group, and a benzoyl group. In addition, the ^{1}H- and ^{13}C-nmr spectra revealed the presence of one vinyl methyl, two tertiary methyls, one hydroxymethylene, one exomethylene, and one olefinic proton, together with six sp^3 methylenes, three sp^3 methines, and four quaternary carbons (two sp^2 and two sp^3) (Tables 1 and 2). In the ^{1}H-^{1}H shift correlation (COSY) spectrum of **1**, the signal at δ 5.43 was found to correlate with the signals at δ 4.17 and 1.68. Also, the exomethylene protons at δ 4.74 were correlated with the methylene protons at δ 2.43, while the oxymethine proton at δ 5.22 was correlated with the methine proton at δ 2.48 and the methylene protons at δ 2.43. The carbon-carbon connectivities in the molecule were determined by analyses of 2D INADEQUATE and ^{1}H-^{13}C long-range COSY spectra as reported previously (4). Thus, the planar structure was elucidated to be **1**.

On the basis of the nOe difference spectral data, the methyls at C-4 and C-10 and the benzoyl group at C-6 were assigned to lie on the same side of the molecular plane and the vinyl methyl at C-13 and the hydroxymethyl at C-14 were suggested to be cis-oriented (Figure 1). However, we could not determine the relative configuration of the side chain attached to C-9 because of ambiguity of the nOe between the methylene protons at C-11 and the methyl protons at C-20 or the methine proton at C-5.

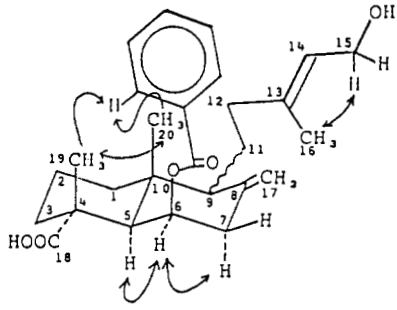


FIGURE 1. The nOe's observed in **1**.

The final structure of **1** was established by an X-ray analysis on a single crystal of compound **6** which was prepared by hydrolysis of **1**. Crystals of **6** suitable for X-ray analysis were obtained by recrystallization from aqueous MeOH, and the crystal structure was solved by the direct method. A stereoview of the molecule is illustrated in Figure 2. Thus, scoparic acid A was found to have the relative stereochemistry shown in structure **1**.

Scoparic acid B [**2**] was obtained as a colorless amorphous powder, $[\alpha]_D$ −9.8° ($CHCl_3$). Its ir spectrum exhibited bands at 3500 (OH), 1710 (CO), 1600, and 1580

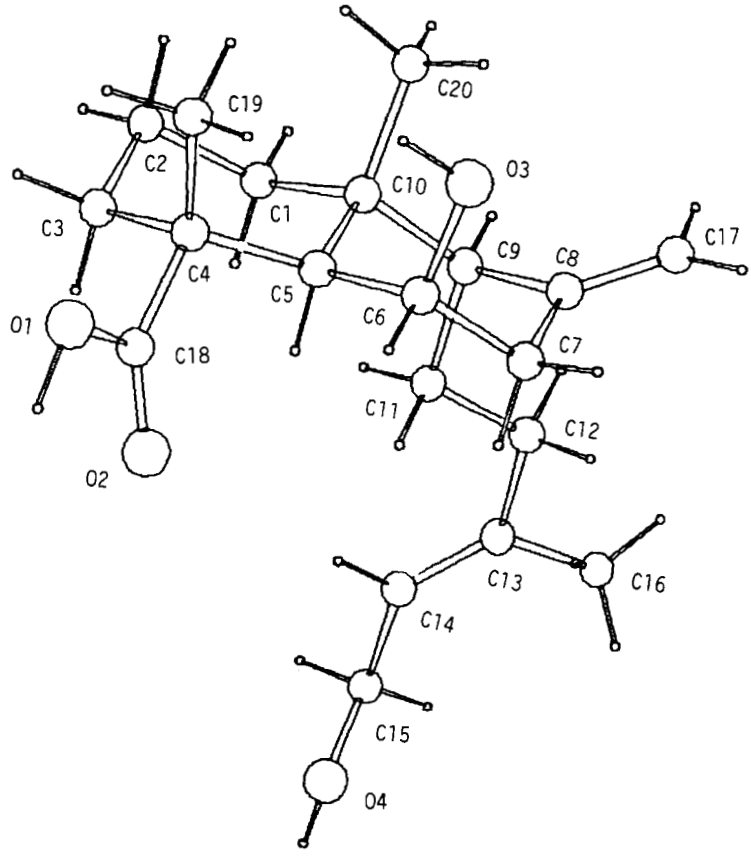


FIGURE 2. A perspective drawing of the X-ray diffraction structure of compound **6**; small circles represent hydrogen atoms.

(phenyl) cm^{-1}, and the uv spectrum showed absorption maxima at 230, 266, and 278 nm. The eims revealed a molecular ion peak at *m/z* 412 corresponding to $C_{25}H_{32}O_5$ and fragment ion peaks at *m/z* 122, 105, and 77 suggesting the presence of a benzoyl group in **2**. The ^1H- and ^{13}C-nmr spectral data (Tables 1 and 2) confirmed this and indicated the close resemblance of **1** and **2**. However, signals due to a vinyl methyl, a hydroxymethyl, and an olefinic proton were missing in the ^1H-nmr spectrum of **2**, and instead it presented a signal corresponding to an acetoxy group (δ 2.14). Furthermore, the ^{13}C-nmr spectrum of **2** indicated the presence of a ketone (δ 209.09, s) and an acetoxymethyl (δ 30.13, q) that were not observed in that of **1**. These spectral data suggested the planar structure of scoparic acid B to be **2**. The ^1H-^1H COSY and ^1H-^{13}C COSY spectra of scoparic acid B confirmed the structure of **2**.

Scoparic acid C [**3**] was isolated as a colorless amorphous powder, $C_{26}H_{32}O_5$, $[\alpha]_D$ $-13.9°$ ($CHCl_3$). It showed ir absorption bands at 3500 (OH), 1710, 1700 (CO), 1605, and 1590 (phenyl) cm^{-1} and uv absorption maxima at 226, 267, and 270 nm. In the eims of **3**, the molecular ion peak appeared at *m/z* 424 and significant fragment ion peaks were observed at *m/z* 122, 105, and 77 suggesting the presence of a benzoyl group in **3**. The ^1H- and ^{13}C-nmr spectral data were similar to those of **1** and **2** as listed in Tables 1 and 2. Compound **3**, however, showed signals assigned to exomethylene protons (δ_H 5.91 and 6.26; δ_C 134.23, t) and aldehyde (δ_H 9.45; δ_C 194.82, d) instead of signals due to the vinyl methyl and hydroxymethyl protons in **1**. In the ^1H-^1H COSY spectrum of **3** the signals at δ 5.91 and 6.26 were correlated with the signal at δ 1.98 assignable to H-12. These spectral data suggested the planar structure of scoparic acid C to be **3**.

The relative stereochemistry of **2** and **3** was elucidated on the basis of nOe difference spectral data and coupling constants of H-5, H-6, and H-7 (Table 1). In the nOe difference spectra of both **2** and **3**, the signal intensity of H-2′ and H-6′ was increased on irradiation at the Me-19 or Me-20 signal. On the other hand, no increase of signal intensity of H-11 was observed on irradiation at the Me-20 signal. These experimental results suggest that the orientations of Me-19, Me-20, benzoyl group at C-6, and the side chain at C-9 in **2** and **3** are the same as those in **1**. Therefore the relative stereostructures of scoparic acid B and scoparic acid C were assigned to be **2** and **3**, respectively.

The inhibitory activities of **1**–**3** against bovine liver β-glucuronidase were examined (6,7). As illustrated in Table 3, scoparic acid A showed potent inhibitory activity (50% inhibition at 6.8×10^{-6} M). This potency was one-fourth that of the well-known β-glucuronidase inhibitor, glucosaccharo-1:4-lactone (8).

The inhibition mechanism of **1** was studied by kinetic analysis with double-reciprocal plotting. The Lineweaver-Burk plots indicated that the inhibition of β-glucuronidase by **1** was non-competitive with *p*-nitrophenyl-β-D-glucuronide as substrate. The Km value found for **1** was 0.0143 M.

TABLE 3. Inhibitory Activities on β-Glucuronidase of Compounds Isolated from *Scoparia dulcis*.

Compound	Ic_{50} (M)
Scoparic acid A [**1**]	6.8×10^{-6}
Scoparic acid B [**2**]	—[a]
Scoparic acid C [**3**]	1.0×10^{-5}
Glucosaccharo-1:4-lactone . . .	1.8×10^{-6}

[a]$Ic_{50} > 10^{-4}$ M.

To confirm the specificity of scoparic acid A inhibition against β-glucuronidase, the inhibitory activity of **1** was examined in the presence of bovine serum albumin (BSA). BSA decreased the degree of enzyme inhibition by **1** slightly (5–10%). Yet in the presence of a large amount of BSA (one-hundredfold), **1** still inhibited considerably, suggesting that scoparic acid A inhibits the activity of β-glucuronidase by binding specifically with the enzyme.

S. dulcis has been used for hepatosis in Paraguay. The inhibitory effect of scoparic acid A on β-glucuronidase may play an important role in the pharmacological action of this herb.

EXPERIMENTAL

GENERAL EXPERIMENTAL PROCEDURES.—Melting points were determined on a Yanagimoto micro melting point apparatus and are uncorrected. Optical rotations were measured in $CHCl_3$ solutions on a JASCO DIP-140 polarimeter. The uv spectra were taken with a Hitachi 220s double beam spectrophotometer, and ir spectra were recorded on a Hitachi 260-10 infrared spectrometer. Mass spectra were obtained on a JEOL JMS-D-200MS spectrometer operating at 70 eV. ^{1}H- and ^{13}C-nmr spectra were recorded in $CDCl_3$, using TMS as internal standard, employing a JNM-GX 400 instrument operating at 400 MHz, and chemical shifts are given in δ (ppm) values. Two-dimensional ^{1}H-^{1}H, ^{1}H-^{13}C, and ^{1}H-^{13}C long-range shift correlation spectra and INADEQUATE were measured on a JEOL JNM-GX 400 instrument in $CDCl_3$.

PLANT MATERIAL.—Plant materials were collected near Asuncion in Paraguay in 1985. Voucher specimens were deposited in the Herbal Garden of the Faculty of Pharmaceutical Sciences, Toyama Medical and Pharmaceutical University.

EXTRACTION AND ISOLATION OF DITERPENOIDS.—Dried Typychá kuratũ (*S. dulcis*) (2 kg) was ground into powder and extracted with hot 70% EtOH three times (7.5 liters). After filtration, the extract was concentrated in vacuo and continuously freeze-dried to give a brownish powder (128 g). The extract was separated as shown in Scheme 1. A part of the fraction (B′) (13.7 g) was extracted with $CHCl_3$, and the $CHCl_3$-soluble part (9.0 g) was chromatographed on a Si gel column using a $CHCl_3$/MeOH stepwise gradient (1:0→4:1). The fraction eluted with $CHCl_3$-MeOH (9:1) (1 g) was chromatographed on a Si gel column using eluents of *n*-hexane/EtOAc. The fraction eluted with *n*-hexane–EtOAc (2:1) (140 mg) was then subjected to plc using C_6H_6-Me_2CO (2:1) as eluent to yield **2** (40 mg) and **3** (14 mg). The fraction eluted with *n*-hexane–EtOAc (1:1) (613 mg) was further purified by hplc to give **1**. The hplc was performed under the following conditions: column, TSKgel silica-60 (21.5 mm × 30 cm); mobile phase, $CHCl_3$-MeOH (99:1→9:1); flow rate, 90 μl/min; pump, Shimadzu LC 5A; column temperature, room temperature; detector, Shimadzu SPD 2A; wave length for detection, 254 nm.

Scoparic acid A [**1**].—Colorless amorphous powder: $[\alpha]^{26}_D$ −38.3° (*c* = 1.00, $CHCl_3$); uv λ max (EtOH) (log ε) 227 (4.05), 265 sh (2.80), 270 (2.87), 277 (2.77) nm; ir ν max ($CHCl_3$) 3400, 1700, 1600, 1580 cm^{-1}; ^{1}H nmr see Table 1; ^{13}C nmr see Table 2; eims *m/z* (rel. int. %) $[M]^+$ 440 (0.1), 422 (1), 394 (0.5), 377 (0.5), 335 (7), 318 (11), 173 (52), 159 (48), 122 (45), 105 (100), 77 (100); hrms *m/z* $[M+1]^+$ 441.2670 (calcd for $C_{27}H_{37}O_5$, 441.2631).

METHYLATION OF **1**.—Scoparic acid A [**1**] (100 mg) was treated overnight with CH_2N_2 to give a methyl ester **4** (100 mg): $[\alpha]^{23}_D$ −50.2° (*c* = 0.36, $CHCl_3$); uv λ max (EtOH) (log ε) 230 (4.10), 273 (2.78), 280 (2.63) nm; ir ν max ($CHCl_3$) 3500, 1710, 1600, 1580 cm^{-1}; ^{1}H nmr ($CDCl_3$) δ 1.33 (3H, s, H-19), 1.47 (3H, s, H-20), 1.69 (3H, s, H-16), 2.38 (2H, br d, *J* = 2.7, H-7), 2.41 (1H, d, *J* = 2.4, H-5), 3.69 (3H, s, OMe), 4.17 (2H, d, *J* = 6.7, H-15), 4.72 (2H, s, H-17), 5.22 (1H, br d, *J* = 2.4, H-6), 5.43 (1H, dd, *J* = 5.8, 6.76, H-14), 7.43 (2H, t, *J* = 7.3, H-3′, -5′), 7.55 (1H, t, *J* = 7.3, H-4′), 8.01 (2H, d, *J* = 7.3, H-2′, -6′); ^{13}C nmr see Table 2; eims *m/z* (rel. int. %) $[M]^+$ 454 (1), 436 (1), 349 (2), 332 (1), 122 (1), 105 (100), 77 (100); elemental analysis C 73.11, H 8.05; calcd for $C_{28}H_{38}O_5 \cdot \frac{1}{2}H_2O$, C72.90, H 8.28.

ACETYLATION OF **1**.—Scoparic acid A [**1**] (100 mg) was treated overnight with Ac_2O and pyridine at room temperature, and the reaction mixture was worked up as usual to give a monoacetate **5** (120 mg) as amorphous powder, $[\alpha]^{26}_D$ −4.9° (*c* = 0.74, $CHCl_3$); uv λ max (EtOH) (log ε) 227 (4.00), 270 (2.82), 277 (2.72) nm; ir ν max ($CHCl_3$) 1710, 1600, 1580 cm^{-1}; ^{1}H nmr ($CDCl_3$) δ 1.34 (3H, s, H-19), 1.48 (3H, s, H-20), 1.72 (3H, s, H-16), 2.07 (3H, s, OAc), 2.44 (2H, m, H-5, -7), 4.60 (2H, d, *J* = 7.1, H-15), 4.73 (2H, s, H-17), 5.36 (2H, m, H-6, -14), 7.27 (2H, t, *J* = 7.6, H-3′, -5′), 7.47 (1H, t, *J* = 7.6, H-4′), 8.02 (2H, d, *J* = 7.6, H-2′, -6′); ^{13}C nmr see Table 2; eims *m/z* (rel. int. %) $[M-HOAc+H]^+$

423 (3), 377 (5), 360 (8), 122 (27), 105 (100), 77 (40); hrms *m/z* $[M]^+$ 482.2482 (calcd for $C_{29}H_{38}O_6$, 482.2666).

DEBENZOYLATION OF **1**.—Saturated KOH in MeOH solution (3 ml) and DMSO (1 ml) were added to a solution of scoparic acid A [**1**] (50 mg) in MeOH, and the mixed solution was heated at 120° for 12 h in a sealed tube, then allowed to cool. Four ml of H_2O was added and the whole was acidified with 1 N HCl. The reaction mixture was extracted with EtOAc, and the EtOAc layer was concentrated in vacuo. The residue was chromatographed on a Si gel column with $CHCl_3$ to give benzoic acid (7 mg) and debenzoylated compound **6** (20 mg), colorless prisms (aqueous MeOH): mp 186–188°; $[\alpha]^{23}D$ −4.8° (*c* = 1.21, $CHCl_3$); ir *v* max ($CHCl_3$) 3450, 1710, 1600 cm^{-1}; ^1H nmr (pyridine-d_6) δ 1.27 (3H, s, H-19), 1.56 (3H, s, H-20), 1.68 (3H, s, H-16), 2.37, 2.53 (1H each, br d, *J* = 13.1, H-7), 2.55 (1H, br s, H-5), 4.16 (2H, d, *J* = 6.9, H-15), 4.68 (1H, br s, H-6), 4.88, 4.93 (1H each, br s, H-17), 5.80 (1H, t, *J* = 6.9, H-14); ^{13}C nmr see Table 2; eims *m/z* (rel. int. %) $[M]^+$ 336 (1), 300 (2), 285 (2), 255 (2), 239 (4), 173 (5); hrms *m/z* $[M]^+$ 336.2324 (calcd for $C_{20}H_{38}O_4$, 336.2299).

Scoparic acid B [**2**].—Colorless amorphous powder: $[\alpha]^{23}D$ −9.8° (*c* = 0.63, $CHCl_3$); uv λ max (EtOH) (log ε) 230 (4.03), 266 (3.05), 278 (2.89) nm; ir *v* max ($CHCl_3$) 3500, 1710, 1600, 1580 cm^{-1}; ^1H nmr see Table 1; ^{13}C nmr see Table 2; eims *m/z* (rel. int. %) $[M]^+$ 412 (2), 394 (1), 366 (2), 341 (1), 307 (20), 122 (100), 105 (100), 77 (100); hrms *m/z* $[M - C_6H_5COOH]^+$ 290.1839 (calcd for $C_{18}H_{26}O_3$, 290.1878).

Scoparic acid C [**3**].—Colorless amorphous powder: $[\alpha]^{22}D$ −13.9° (*c* = 0.69, $CHCl_3$); uv λ max (EtOH) (log ε) 226 (4.08), 267 (2.96), 270 (2.89) nm; ir *v* max ($CHCl_3$) 3500, 1710, 1700, 1605, 1590 cm^{-1}; ^1H nmr see Table 1; ^{13}C nmr see Table 2; eims *m/z* (rel. int. %) $[M]^+$ 424 (1), 122 (100), 105 (100), 77 (100); hrms *m/z* $[M - C_6H_5COOH]^+$ 302.1863 (calcd for $C_{19}H_{26}O_3$, 302.1881).

X-RAY CRYSTAL STRUCTURE ANALYSIS OF DEBENZOYLATED COMPOUND **6**[1].—Crystal data.—$C_{20}H_{32}O_4$, MW = 336.47, orthorhombic, space group $P2_12_12_1$, *a* = 22.33 (1) Å, *b* = 11.247 (2) Å, *c* = 7.574 (2) Å, V = 1902.2 $Å^3$, Z = 4, Dc = 1.175 g/cm^3; crystal size = 0.30 × 0.30 × 0.40 mm. F(000) = 736; (CuKα) = 6.05 cm^{-1}. The final R factors were R = 0.0495, Rw = 0.0689; S = 1.51. A total of 1473 reflections were collected on a Rigaku AFC-5Ru diffractometer with an ω–2θ scan mode [$|Fo| > 3\sigma(|Fo|)$, θ < 60°] using graphite monochromated CuKα radiation (λ = ± 1.5418). The structure was solved by direct method and refined by block diagonal least-squares method on a FALCOM M380 computer in the Data Processing Center of Kyoto University, using KPPXRAY Programs.

DETERMINATION OF β-GLUCURONIDASE ACTIVITY.—β-Glucuronidase activity was determined by measuring the absorbancy at 405 nm of *p*-nitrophenol formed from the substrate by the method of Nobunaga (5) with following modification. The reaction mixture containing 0.9 ml of 0.1 M acetate buffer (pH 5.0), 0.03 ml of 0.1 M *p*-nitrophenyl-β-D-glucuronide, and 0.1 ml of adequately diluted enzyme solution was incubated at 37° for 20 min. After addition of 0.25 ml of 0.2 M Na_2CO_3 to stop the reaction, the absorbancy at 405 nm was measured. The inhibitory activity was determined as described for the assay of enzyme activity except that test material dissolved in 0.1 M acetate buffer (pH 5.0) was mixed with substrate and enzyme. The inhibitory activity (%) was calculated as follows: [(E − S)/E] × 100 where E is the activity of enzyme without test material and S is the activity of enzyme with test material.

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[1]Atomic coordinates for this compound have been deposited with the Cambridge Crystallographic Data Centre and can be obtained on request from Dr. Olga Kennard, University Chemical Laboratory, 12 Union Road, Cambridge CB2 1EZ, UK.

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