Limonoids from *Citrus sudachi*

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Received December 27, 2000; accepted February 28, 2001

Four new limonoid derivatives, 1-*O*-methylichangensin (1), sudachinoid A (2), B (3), and C (4) were isolated from the seeds of *Citrus sudachi*, together with the known compounds ichangensin, obacunone, obacunoic acid and limonin, and the structures of the new compounds were elucidated based on spectroscopic and chemical evidence.

Key words *Citrus sudachi*; Rutaceae; limonoid; sudachinoid

Citrus sudachi (Rutaceae) is an evergreen tree that is found mainly in Tokushima prefecture in Japan. The immature fruits are often used for cooking. Several limonoids and their glucosides are found in its seeds.[1)]

Recently limonoids have attracted much attention because of the marked insect antifeedant and growth regulating (IGR) activities of azadirachtin and related highly oxidized C-seco limonoids from the neem tree *Azadirachta indica* and the chinaberry tree *Melia azedarach*.[2)] Limonoids also possess anticancer activity. Inhibition of chemically induced carcinogenesis associated with the limonoid aglycones has been summarized by Lam *et al.*[3)]

In this paper, we report the isolation and structural determination of four new limonoids, 1-*O*-methylichangensin (**1**), sudachinoid A (**2**), B (**3**), and C (**4**), as well as four known limonoids (**5**—**8**) from the methanol extract of the seeds of *C. sudachi*.

Compound **1** was determined to have a molecular formula of $C_{26}H_{34}O_7$ based on its high-resolution (HR) FAB-mass spectrum, and the IR spectrum showed a lactone carbonyl band (1745 cm^{-1}) and a ketone carbonyl band (1709 cm^{-1}). The ^{1}H-NMR spectrum of **1** showed six methyl groups [δ_H 1.12, 1.20, 1.21, 1.25, 1.28, 1.33 (each 3H, s)], one methoxyl group [δ_H 3.25 (3H, s)], one furan ring [δ_H 6.33, 7.38, 7.41 (each 1H, s)], and two methine protons [δ_H 4.56, 5.60 (each 1H, s)] attached to the oxygen function. The ^{13}C-NMR spectrum (Table 1) of **1** indicated the presence of two carbonyl carbons [δ_C 168.0, 209.0], one methoxyl [δ_C 48.9], and one furan ring [δ_C 110.0, 120.7, 141.2, 142.9]. In addition, there were six methyl groups, two oxygenated methines, three quaternary carbons attached to the oxygen function, three methylenes, two methine, and three quaternary carbons. Based on the above observations, compound **1** was assumed to be a limonoid-type compound which had previously been isolated from Rutaceae plants.[2)] The ^{13}C-NMR spectrum of **1** was very similar to that of ichangensin[4)] except for the presence of one methoxy in **1**. In the HMBC spectrum, the methyl proton signal at δ_H 3.25 (OCH_3) was correlated with the carbon signal at δ_C 108.4 (C-1), and the proton signal at δ_H 1.20 (H-25) was correlated with the carbon signal at δ_C 108.4 (C-1), 53.9 (C-5), and 40.5 (C-9). These facts indicated that the methoxy group was located at C-1. In the NOESY spectrum, the proton signal at δ_H 1.72 (H-11) was correlated with the proton signals at δ_H 1.33 (H-24) and 1.20 (H-25). Thus, the relative stereochemistry of methoxyl group was elucidated as α-configuration. The structure of **1** was determined to be as shown (Fig. 1).

Compound **2** was assigned the molecular formula as $C_{26}H_{34}O_9$ based on HR-FAB-MS of its acetate (**2a**), and the IR spectrum showed a lactone carbonyl band (1749 cm^{-1}) and a ketone carbonyl band (1701 cm^{-1}). Its ^{1}H-NMR spectrum showed six methyl groups [δ_H 1.12, 1.19, 1.20, 1.25, 1.29, 1.33 (each 3H, s)], one methoxyl group [δ_H 3.26 (3H, s)], one olefinic proton [δ_H 7.30 (1H, s)], and two methines attached to the oxygen function [δ_H 4.65, 5.53 (each 1H, s)].

The ^{13}C-NMR spectrum (Table 1) of **2** contained resonances corresponding to three carbonyl carbons [δ_C 168.1, 169.9, 209.1], one methoxyl [δ_C 48.0], one double bond suggested to be the conjugated enone [δ_C 135.0, 151.0], two acetal carbon signals [δ_C 98.0, 108.5], and two oxygenated methine carbon signals [δ_C 55.9, 76.1]. Compound **2** also had a limonid-type framework, and its ^{13}C-NMR spectral data (Table 1) were very similar to those of **1** except for a furan ring moiety. In contrast to **1**, **2** showed signals based on an α-substituted α,β-unsaturated butyrolactol which was observed in limonexic acid.[5)] In the HMBC spectrum of **2**, the proton signal at δ_H 5.53 (H-17) was correlated with the carbon signals at δ_C 135.0 (C-18), 169.9 (C-19), and 151.0 (C-20), and the proton signal at δ_H 7.30 (H-20) was correlated with the carbon signal at δ_C 169.9 (C-19). On the other hand, the proton signal at δ_H 7.30 (H-20) showed NOE correlation with the proton signals at δ_H 1.19 (H-27) and 6.36 (H-21), the proton signal at δ_H 1.27 (H-12α) was correlated with the proton signal at δ_H 1.19 (H-27), and the proton signal at δ_H 1.92 (H-12β) was correlated with the proton signal at δ_H 5.53 (H-17). Furthermore, acetylation of **2** in the usual way gave monoacetate **2a**. Therefore, the presence of an α-substituted α,β-unsaturated butyrolactol with a 17α-configuration was determined. Thus, the structure of **2** was determined to be as shown (Fig. 1).

Compound **3** was assigned the molecular formula as $C_{25}H_{32}O_9$ based on HR-FAB-MS of its acetate (**3a**).

It was also a limonoid derivative, and its ^{1}H- and ^{13}C-NMR spectral data were very similar to those of compound **2**, except for the presence of a methoxyl group in compound **2** (Table 1). Acetylation of **3** gave monoacetate **3a**, which also supported the presence of a butyrolactol group. The 2D NMR spectral data of **3** suggested that sudachinoid B was 1-*O*-demethylsudachinoid A.

The IR spectrum of **4**, $C_{26}H_{30}O_9$, showed a lactone carbonyl band (1765 cm^{-1}) and a ketone carbonyl band (1702 cm^{-1}). The ^{1}H-NMR spectrum of **4** showed five methyl groups [δ_H 1.13, 1.24, 1.47 (each 3H, s), 1.52 (6H, s)], two double bonds [δ_H 6.00, 6.58 (each 1H, d, J=11.7 Hz); δ_H

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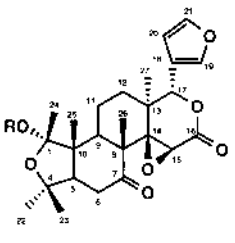
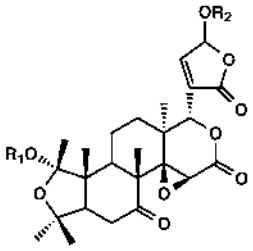
Table 1. ^{1}H- and ^{13}C-NMR Spectral Data (δ) of Compounds **1**—**4**

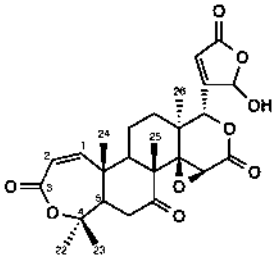
Position	**1**		**2**		**3**		**4**	
	H (*J*: Hz)	C	H (*J*: Hz)	C	H (*J*: Hz)	C	H (*J*: Hz)	C
1	—	108.4	—	108.5	—	108.3	6.58 (d, 11.7)	156.8
2	—	—	—	—	—	—	6.00 (d, 11.7)	123.1
3	—	—	—	—	—	—	—	167.2
4	—	80.0	—	80.0	—	80.0	—	84.2
5	2.68 (br d, 6.3)	53.9	2.63 (br d, 5.9)	53.5	2.66 (br d, 6.4)	53.8	2.64 (dd, 14.0, 4.6)	57.4
6	β 2.66 (br d, 6.6)	36.9	β 2.65 (br d, 7.6)	37.0	β 2.64 (br d, 7.4)	37.0	β 2.99 (t, 14.0)	39.9
	α 2.29 (dd, 6.8, 6.6)		α 2.27 (dd, 7.6, 5.9)		α 2.29 (dd, 7.4, 6.4)		α 2.31 (dd, 13.9, 4.7)	
7	—	209.0	—	209.1	—	209.0	—	207.5
8	—	50.2	—	49.7	—	49.7	—	53.2
9	3.00 (t, 8.6)	40.5	2.96 (dd, 11.2, 6.4)	40.2	2.97 (dd, 11.3, 6.3)	40.4	2.15 (s)	49.1
10	—	50.1	—	50.1	—	50.2	—	43.2
11	1.72 (2H, m)	16.5	1.74 (2H, m)	16.2	1.75 (2H, m)	16.2	1.96 (2H, br d, 7.5)	19.5
12	β 1.64 (m)	27.5	β 1.92 (m)	25.9	β 1.95 (m)	25.9	β 2.13 (br d, 2.4)	32.3
	α 1.45 (m)		α 1.27 (m)		α 1.22 (m)		α 1.67 (m)	
13	—	39.8	—	40.8	—	40.8	—	37.9
14	—	69.3	—	69.8	—	69.8	—	65.0
15	4.56 (s)	55.9	4.65 (s)	55.9	4.70 (s)	56.0	3.61 (s)	52.8
16	—	168.0	—	168.1	—	169.8	—	165.7
17	5.60 (s)	78.1	5.53 (s)	76.1	5.54 (s)	76.0	5.42 (s)	78.4
18	—	120.7	—	135.0	—	134.0	—	162.9
19	7.41 (s)	141.2	—	169.9	—	169.7	6.04 (s)	97.8
20	6.33 (s)	110.0	7.30 (s)	151.0	7.35 (s)	151.5	6.31 (s)	123.3
21	7.38 (s)	142.9	6.36 (s)	98.0	6.19 (s)	97.5	—	169.2
22	1.25 (s)	30.8	1.25 (s)	30.9	1.25 (s)	31.0	1.47 (s)	32.1
23	1.12 (s)	23.6	1.12 (s)	23.7	1.12 (s)	23.8	1.52 (s)	26.9
24	1.33 (s)	17.9	1.33 (s)	18.0	1.36 (s)	18.7	1.52 (s)	16.6
25	1.20 (s)	15.1	1.20 (s)	14.9	1.20 (s)	14.9	1.24 (s)	16.8
26	1.28 (s)	19.3	1.29 (s)	19.8	1.31 (s)	19.7	1.13 (s)	21.6
27	1.21 (s)	19.6	1.19 (s)	18.9	1.18 (s)	18.7		
OMe	3.25 (s)	48.9	3.26 (s)	48.0	—	—		



R
1 Me
5 H



R$_1$ R$_2$
2 Me H
2a Me Ac
3 H H
3a H Ac



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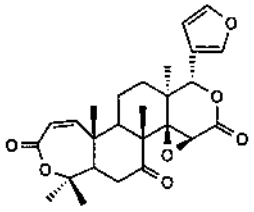

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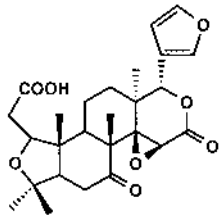

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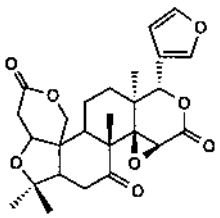

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Fig. 1. Structures of Compounds **1**—**8**

6.31 (s)], two methine protons attached to the oxygen function [δ_H 3.61, 5.42 (each 1H, s)], and one hemiacetal proton [δ_H 6.04 (s)]. The ^{13}C-NMR spectrum of **4** contained resonances corresponding to four carbonyl carbons [δ_C 165.7, 167.2, 169.2, 207.5], two double bonds [δ_C 123.1, 123.3, 156.8, 162.9], one hemiacetal carbon signal at δ_C 97.8, and two oxygenated methine carbon signals [δ_C 52.8, 78.4]. A comparison of the ^{13}C-NMR spectral data of **4** and obacunone[6] (**6**) showed almost the same chemical shift values, except for a furan ring moiety in **6**. In the HMBC spectrum of **4**, the proton signals at δ_H 6.04 (H-19) and 6.31 (H-20) were correlated with the carbon signal at δ_C 169.2 (C-21). This fact and the chemical shifts of C-20 and C-18 [δ_C 123.3 (d), 162.9 (s)] confirmed the presence of a β-substituted α,β-unsaturated butyrolactol. Moreover, the proton signal at δ_H 5.42 (H-17) was correlated with the signals at δ_C 37.9 (C-13), 65.0 (C-14), 162.9 (C-18), 123.3 (C-20), and 21.6 (C-26). Thus, the unsaturated butyrolactol moiety was located at C-17. The structure of **4** was determined to be as shown. Compounds **5**, **6**, **7**, and **8** were identified to be ichangensin, obacunone, obacunoic acid, and limonin, respectively.

Experimental

NMR experiments were run on a Bruker ARX-400 instrument. ^{1}H-NMR, 400 MHz; ^{13}C-NMR, 100 MHz, both used tetramethylsilane as internal standard. MS were obtained on a JEOL JMSD-300 instrument. Chromatography column, Silica gel 60 (Merck), TOYOPEARL (HW-40c, TOSOH) and Sephadex LH-20 (Pharmacia); HPLC, JASCO Gulliver Series, PU-986/987 (pump), RI930 and UV970 (detector). Column type, LiChrosorb Si 60 HPLC (Hibar RT 250-25, 20.0×250 mm, Kanto Chemical Co., Inc.), ODS (Hibar RT 250-25, RP-18 (7 μm), Kanto Chemical Co., Inc.). IR spectra were recorded on a 1720 Infrared Fourier Transform spectrometer (Perkin-Elmer), UV spectra on a UV 2100 UV-VIS recording spectrometer (Shimadzu). Optical rotation were measured with a JASCO DIP-370 digital polarimeter.

Plant Material The seeds of *Citrus sudachi* HORT. ex TANAKA were collected in 1999 from Kamiyama, Tokushima prefecture in Japan.

Extraction and Isolation The seeds (4.3 kg) of *Citrus sudachi* were crushed and extracted with MeOH (15 l×3) at 60 °C for 6 h. The MeOH extracts were concentrated *in vacuo* to give a residue (900 g), which was partitioned between EtOAc and H_2O. The EtOAc layer was concentrated to give a residue (286 g), which was chromatographed on silica gel column [1.2 kg, 11×100 cm]. The column was eluted with solvent of increasing polarity (hexane–EtOAc, EtOAc, EtOAc–MeOH, and MeOH) to give 14 major fractions (frs. 1—14).

Fraction 5 (4.9 g) was chromatographed on silica gel with $CHCl_3$–MeOH (95 : 5) to give 4 fractions (frs. 5.1—5.4). Fraction 5.3 (4.6 g) was chromatographed on TOYOPEARL ($CHCl_3$–MeOH, 1 : 1) to give 6 fractions (frs. 5.3.1—5.3.6). Fraction 5.3.4 was chromatographed by a medium-pressure liquid chromatography (MPLC) on a silica column eluting with $CHCl_3$–MeOH (98 : 2) to give 673 mg of 1-*o*-Methylichangensin (**1**), and other 5 fractions (frs. 5.3.4.1—5.3.4.5). Fraction 5.3.4.3 was separated by HPLC (hexane–AcOEt, 2 : 3, 3 : 2) give 4 fractions (frs. 5.3.4.3.1—5.3.4.3.4). Fractions were monitored by TLC (silica gel, $CHCl_3$–MeOH, 95 : 5) and those giving identical spots were combined and separated by ODS (MeOH–H_2O, 8 : 2) to give 12.5 mg of Sudachinoid A (**2**), and other 2 fractions. Fraction 5.3.4.4 was separated by ODS (MeOH–H_2O, 8 : 2) to give 12.0 mg of Sudachinoid B (**3**), and other 3 fractions. Fraction 7 (13.0 g) was chromatographed on silica gel with $CHCl_3$–MeOH (99 : 1—8 : 2) to give 5 fractions (frs. 7.1—7.5). Fraction 7.1 was chromatographed on Sephadex LH-20 with MeOH to give 3 fractions. (frs. 7.1.1—7.1.3). Fraction 7.1.2 was chromatographed on using HPLC ($CHCl_3$–MeOH, 97 : 3) to give 3 fractions. (frs. 7.1.2.1—7.1.2.3). Fraction 7.1.2.3 was chromatographed on using GPC (MeOH) to give 3.8 mg of Sudachinoid C (**4**). Fraction 7.1.2.1 was chromatographed using GPC ($CHCl_3$) to give 196 mg of obacunone (**6**). Fraction 7.2 was separated by LH-20 (MeOH), HPLC ($CHCl_3$–MeOH, 97 : 3) and ODS (MeOH–H_2O, 9 : 1) to give **1** (211 mg), 90 mg of ichangensin (**5**), 32 mg of **6**. Fraction 11 was chromatographed by MPLC eluting with $CHCl_3$–MeOH (97 : 3) to give 384 mg of isoobacunoic acid (**7**), 492 mg of limonin (**8**).

Comopound **1**: Colorless needle, $[\alpha]_D^{25}$ +20.0° (c=1.2, MeOH). IR ν_{max}^{KBr} cm^{-1}: 3441, 2964, 1745, 1709, 1631, 1454, 1385, 1272, 1155, 1038, 925, 877, 755, 607. UV λ_{max}^{MeOH} nm (log ε): 207 (3.67). ^{1}H-NMR ($CDCl_3$): see Table 1. ^{13}C-NMR ($CDCl_3$): see Table 1. HR-FAB-MS m/z: 481.2235 $[M+Na]^+$. $C_{26}H_{34}O_7Na$ required 481.2202.

Comopound **2**: Colorless oil, $[\alpha]_D^{25}$ +51.1° (c=0.6, MeOH). IR ν_{max}^{KBr} cm^{-1}: 3445, 2925, 1749, 1701, 1635, 1541, 1457, 1120, 1027. UV λ_{max}^{MeOH} nm (log ε): 206 (3.95). ^{1}H-NMR ($CDCl_3$): see Table 1. ^{13}C-NMR ($CDCl_3$): see Table 1. HR-FAB-MS (**2a**) m/z: 555.2115 $[M+Na]^+$. $C_{28}H_{36}O_{10}Na$ required 555.2217.

Comopound **3**: Colorless oil, $[\alpha]_D^{25}$ +18.5° (c=0.7, MeOH). IR ν_{max}^{KBr} cm^{+1}: 3435, 1782, 1623, 1455, 1202, 1127, 1037, 908, 757. UV λ_{max}^{MeOH} nm (log ε): 207 (3.91). ^{1}H-NMR ($CDCl_3$): see Table 1. ^{13}C-NMR ($CDCl_3$): see Table 1. HR-FAB-MS (**3a**) m/z: 541.2092 $[M+Na]^+$. $C_{27}H_{34}O_{10}Na$ required 541.2050.

Comopound **4**: Colorless oil, $[\alpha]_D^{25}$ −31.7° (c=0.4, MeOH). IR ν_{max}^{KBr} cm^{-1}: 3422, 2926, 2362, 1765, 1702, 1458, 1395, 1282, 1121, 1044. UV λ_{max}^{MeOH} nm (log ε): 201 (4.52). ^{1}H-NMR ($CDCl_3$): see Table 1. ^{13}C-NMR: ($CDCl_3$): see Table 1. HR-FAB-MS m/z: 509.1762 $[M+Na]^+$. $C_{26}H_{30}O_9Na$ required 509.1788.

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