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THREE FLAVONE GLYCOSIDES FROM *CITRUS SUDACHI*

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Key Word Index—*Citrus sudachi*; Rutaceae; green peel; flavones, sudachitin glycosides; sudachiin B; sudachiin C; sudachiin D; 3-hydroxy-3-methylglutaric acid.

Abstract—Three flavone glycosides, sudachiins B, C and D, were isolated from the green peel of *Citrus sudachi*. On the basis of UV, ^{1}H NMR and ^{13}C NMR spectral data sudachiins B and C were identified as sudachiin A 6″-(3-hydroxy-3-methyl)glutarate and 7-*O*-β-D-glucosyl sudachitin 6″-(3-hydroxy-3-methyl)glutarate, respectively. Sudachiin D was found to be a unique glycoside in which sudachiin A and 7-*O*-β-D-glucosylsudachitin were esterified at their 6″-positions with 3-hydroxy-3-methylglutaric acid.

INTRODUCTION

We previously reported the isolation and structure of seven flavones [1–6], sudachitin, demethoxysudachitin, dinatin, xanthomicrol, 5,7,4′-trihydroxy-6,3′-dimethoxyflavone, 7-methylsudachitin and sudachiin A (sudachitin 4′-β-D-glucoside), from green peel of *Citrus sudachi* Hort ex Shirai. In this paper we report the structure of three additional sudachitin glycosides, sudachiins B, C and D.

RESULTS AND DISCUSSION

Sudachiin A was separated from fresh green peel by polyamide column chromatography with methanol. After separating sudachiin A, three new flavone glycosides were subsequently obtained from the methanol–acetic acid eluate. Each glycoside was hydrolysed with dilute sulphuric acid to give sudachitin (5,7,4′-trihydroxy-6,8,3′-trimethoxyflavone) [1, 2] and glucose. The three glycosides are named sudachiins B, C and D, respectively.

In the UV spectra of sudachiin B in ethanol, band I (337 nm) shifted to 382 nm in the presence of sodium acetate and its intensity was markedly lower than that of the band II, suggesting that the 4′-hydroxyl group is derivatized [6]. Furthermore, the UV spectral data of sudachiin B are substantially identical with those sudachiin A (Table 1), suggesting that sudachiin B is a sudachitin 4′-glycoside. Sudachiin C is related to sudachitin 7-glycoside from a comparison of UV spectral data for sudachiin C and 7-*O*-β-D-glucosylsudachitin [6, 7].

The structures of the three new glycosides were established by ^{1}H NMR and ^{13}C NMR spectra (Tables 2 and 3). In a comparison of the NMR spectra of sudachiin A and B, the 6″-proton signals of sudachiin B at δ 4.77 and 5.14 appeared at a much lower field than those of sudachiin A, but all the proton signals of sudachiin A were found in the spectrum of sudachiin B and the chemical shift values except for the C 6″-protons are substantially identical with those of the sudachiin A spectrum. Furthermore, the chemical shift values for the carbons at the 5″- and 6″-positions on sudachiin A are also apparently different from those on sudachiin B, but all of the other corresponding carbons are substantially identical. This evidence indicates that sudachiin B is sudachiin A with a substituent at the C6″-position.

The ^{13}C NMR spectrum of sudachiin B showed the presence of a six carbon atom substituent, the signals of these carbons being a methyl (28.3), two methylene groups (46.4 and 46.7), a methine having a hydroxyl group (70.1), an ester carbonyl (171.7) and a carboxyl (174.7).

Table 1. UV spectral data for sudachitin and its glycosides

Compound	λ_{max} nm (log ε)						
	EtOH		EtOH–$AlCl_3$			EtOH–NaOAc	
Sudachitin	283(4.29)	350(4.24)	292(4.21)		364(4.36)	331(4.06)	400(4.18)
Sudachiin A	284(4.34)	337(4.29)	298(4.33)		354(4.35)	283(4.45)	383(4.13)
Sudachiin B	282(4.32)	337(4.30)	296(4.32)		353(4.34)	282(4.42)	382(4.12)
Sudachiin D	284(4.47)	345(4.42)	292(4.44)		356(4.49)	279(4.47)	413(4.31)
Sudachiin C	280(4.21)	348(4.35)	283(4.19)	304(4.17)	362(4.38)	266(4.24)	421(4.44)
7-*O*-Glucosylsudachitin	279(4.21)	348(4.33)	287(4.19)	303sh(4.14)	364(4.37)	266(4.29)	422(4.46)

sh: Shoulder.

Table 2. ^{1}H NMR spectral data for sudachitin glycosides in pyridine-d_5*

H	Sudachiin A	Sudachiin B	Sudachiin D		Sudachiin C	7-O-Glucosyl-sudachitin
3	6.98*s*	6.99*s*	6.99*s*	7.02*s*	7.01*s*	6.99*s*
2′	7.64*d*	7.61*d*	7.61*d*	7.67*d*	7.69*d*	7.67*d*
	(1.4)	(2.0)	(2.2)	(2.2)	(2.0)	(2.2)
5′	7.68*d*	7.74*d*	7.68*d*	7.30*d*	7.31*d*	7.32*d*
	(8.5)	(8.4)	(8.6)	(8.2)	(8.4)	(8.6)
6′	7.71*dd*	7.91*dd*	7.87*dd*	7.77*dd*	7.77*dd*	7.78*dd*
	(8.5, 1.4)	(8.4, 2.0)	(8.6, 2.2)	(8.2, 2.2)	(8.4, 2.0)	(8.5, 2.2)
3′	3.83*s*	3.78*s*	3.79*s*	3.89*s*	3.92*s*	3.86*s*
OMe	4.00*s*	4.00*s*	4.00*s*	4.21*s*	4.23*s*	4.19*s*
OMe	4.03*s*	4.10*s*	4.09*s*	4.24*s*	4.28*s*	4.21*s*
1″	5.83*d*	5.74*d*	5.73*d*	6.15*d*	6.15*d*	6.28*d*
	(7.1)	(6.8)	(7.3)	(7.3)	(7.2)	(7.7)
6″	4.59*dd*	5.14*dd*	5.02*dd*	4.99*dd*	4.99*d*	4.54*dd*
	(12.1, 2.1)	(12.1, 1.6)	(12.0, 2.2)	(12.0, 1.0)	(11.6)	(11.8, 2.6)
6‴	†	4.77*dd*	4.74*dd*	4.81*dd*	4.80*dd*	†
		(12.1, 6.8)	(12.0, 6.5)	(12.0, 5.2)	(11.6, 5.6)	
$-CH_2-$	—	3.13*d* (14.1)	2.88*d* (14.6)		2.99*d* (14.5)	—
		3.32*d* (14.1)	2.99*d* (14.6)		3.08*d* (14.5)	
$-CH_2-$	—	3.14*d* (15.3)	2.97*d* (14.5)		3.01*d* (15.0)	—
		3.20*d* (15.3)	3.04*d* (14.5)		3.07*d* (15.0)	
C-Me	—	1.74*s*	1.57*s*		1.63*s*	—
C-5 OH	13.51*s*	13.44*s*	13.45*s*	13.36*s*	13.23*s*	13.38*s*

*The *J*-values (Hz) are given in parentheses.
†Overlapped with the H-2″, H-3″ and H-4″ signals.

Additionally, the ^{1}H NMR spectrum for sudachiin B showed a singlet at δ1.74 for a methyl group and four doublets at δ3.1–3.2 (J = 14.1 and 15.1 Hz) for two isolated methylene groups. These results identify the substituent as 3-hydroxy-3-methylglutaric acid. The structure of sudachiin B is thus sudachiin A 6″-(3-hydroxy-3-methyl)glutarate. The structure of sudachiin C is also confirmed as 7-*O*-β-D-glucosylsudachitin 6″-(3-hydroxy-3-methyl)glutarate on the basis of the ^{1}H and ^{13}C NMR spectral data for sudachiin C and 7-*O*-β-D-glucosylsudachitin.

The ^{1}H and ^{13}C NMR spectra for sudachiin D showed that it contains sudachiin A, 7-*O*-β-D-glucosylsudachitin, and 3-hydroxy-3-methylglutaric acid units; all the signals except for the glutaric acid moiety are identical with those of the sudachiin A moiety in sudachiin B and the 7-*O*-β-D-glucosylsudachitin moiety in sudachiin C. The results clearly show that sudachiin D is a unique glucoside in which sudachiin A and 7-*O*-β-D-glucosylsudachitin are esterified at their 6″-positions with 3-hydroxy-3-methylglutaric acid.

The four sudachitin glycosides, isolated from *Citrus sudachi*, correlate with each other and sudachiin D consists of sudachiin A and C, or sudachiin B and 7-*O*-β-D-glucosylsudachitin as shown in Fig. 1. Therefore, the occurrence of 7-*O*-β-D-glucosylsudachitin in *C. sudachi* is also expected. Although, we have no evidence to prove that sudachiins B and C, are not in fact artifacts of sudachiin D, we believe that all three glycosides are present in sudachi peel on the basis of the following points: (1) the enzyme in sudachi peel is probably inactivated by the methanol in the extracting solvent;

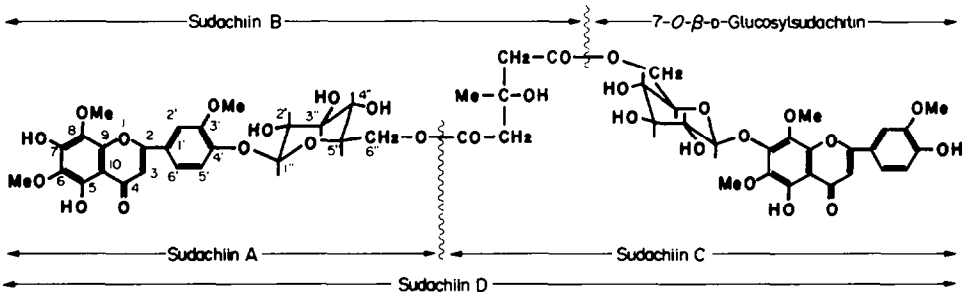

Fig. 1.

Table 3. ^{13}C NMR spectral data for sudachitin and its glycosides in pyridine-d_5

C	Sudachitin	Sudachiin A	Sudachiin B	Sudachiin D		Sudachiin C	7-*O*-Glucosyl-sudachitin
2	164.4	163.8	163.8	163.8	165.0	165.3	164.8
3	103.8	104.6	104.8	104.7	103.9	104.1	103.9
4	183.4	183.3	183.3	183.8	183.5	183.7	183.4
5	149.2	149.6–150.2*	149.6–150.2*	149.6–150.2*	149.2–	150.1	149.2
6	133.2	133.2	133.2	133.2	137.4	137.6	137.3
7	152.6	152.8	152.8	152.8	149.6–150.2*	150.1	149.6–150.2*
8	129.5	129.4	129.4	129.4	134.1	134.2	133.9
9	146.6	146.6	146.6	146.6	146.3	146.4	146.2
10	104.5	104.5	104.4	104.4	108.1	108.2	107.8
1′	123.0	125.8	126.1	126.1	122.6	122.8	122.6
2′	110.7	111.2	111.2	111.1	110.6	110.9	110.6
3′	150.0	150.5	150.7	150.6	149.6–150.2*	150.2	150.1
4′	152.6	151.3	151.2	151.1	152.8	152.9	152.9
5′	117.2	116.7	117.1	117.0	117.3	117.4	117.3
6′	121.4	120.6	120.7	120.7	121.6	121.7	121.5
OMe	56.3	56.4	56.4	56.3	56.2	56.4	56.2
OMe	60.6	60.5	60.6	60.6	61.2	61.3	61.1
OMe	61.6	61.5	61.6	61.6	62.4	62.4	62.2
1″	—	102.2	102.8	102.3	104.5	104.6	104.5
2″	—	74.9	74.8	74.7	75.7	75.7	75.9
3″	—	78.6	78.5	78.3	78.2	78.4	78.2
4″	—	71.5	71.6	71.5	71.4	71.5	71.7
5″	—	79.1	75.8	75.7	75.9	76.0	79.2
6″	—	62.6	64.8	64.7	64.4	64.5	62.7
COO–	—	—	171.7	171.6	171.6	171.8	—
COOH	—	—	174.7		—	174.8	—
$-CH_2-$	—	—	46.4	46.3		46.5	—
$-CH_2-$	—	—	46.7	46.5		46.5	—
⩾COH	—	—	70.1	69.9		70.1	—
Me	—	—	28.3	28.0		28.1	—

*Overlapped with the carbon signals for pyridine-d_5.

(2) sudachiin D is relatively stable; and (3) sudachiin A is obtained from the peel in larger quantities than sudachiins B and C.

EXPERIMENTAL

All mps uncorr. ^1H (400 MHz) and ^{13}C (100.60 MHz) NMR spectra were recorded on a Bruker 400 spectrometer using TMS as an int. standard (δ value). SIM spectra were taken on a Hitachi M-80B spectrometer. CC was performed using polyamide C-200 (Wako Pure Chemical Industries, Ltd.).

Isolation of sudachiins B, C and D. Fresh green peel (8 kg) of *C. sudachi* collected at Tokushima Prefecture in August were extracted with MeOH (*ca* 20 l.) for several days. The MeOH extract was treated in the same way as described for the separation of sudachiin A [6]. The extract was chromatographed on a polyamide column eluting with MeOH and then MeOH–HOAc (100:1). Sudachiin A was isolated from the eluate of the former.

The MeOH–HOAc eluant was extracted with MeCOEt satd with H_2O (MeCOEt–H_2O). The concd extract was dissolved in MeOH (about 50 ml) and allowed to stand in the cold for a week giving ppt D. The mother liquor was concd, dissolved in MeCOEt–H_2O (*ca* 30 ml), and again allowed to ppt in the cold. The ppt was separated, dissolved in a small amount of MeOH and Et_2O added to give ppt B which was separated by filtration. The mother liquor and filtrate obtained above were combined, concd, dissolved in H_2O and washed with Et_2O. The aq. layer was concd and the residue dissolved in MeOH. To the MeOH soln was added an equal amount of Et_2O to give a ppt which was filtered off and the filtrate concd. The residue was divided into two main fractions by polyamide CC with MeCOEt–H_2O (1000 ml) and HOAc (2 ml) as eluent. The second fraction was concd and the residue dissolved in a small amount of MeCOEt–H_2O was allowed to stand at room temp. to give ppt C.

Ppt B was redissolved in MeCOEt–H_2O (1:1), allowed to stand at room temp. to reppt and recryst. from MeCOEt to give sudachiin B (150 mg) as yellow needles, mp 190–192°; $[\alpha]_D = -82.1°$ (*c* 0.28; 80% aq. MeOH); SIMS *m/z* 667 (MH^+: $C_{30}H_{34}O_{17}+H$). Ppt C was recryst. from aq. MeOH to give sudachiin C (100 mg) as yellow needles, mp 144–146°; $[\alpha]_D = +61.1°$ (*c* 0.20; 80% aq. MeOH); SIMS *m/z* 667 (MH^+:$C_{30}H_{34}O_{17}+H$). Ppt D was recryst. from MeOH to give sudachiin D (100 mg) as yellow prisms, mp 158–160°; $[\alpha]_D = -12.0°$ (*c* 2.38; pyridine-d_5); SIMS *m/z* 1171 (MH^+: $C_{54}H_{58}O_{29}+H$).

Sudachiins B, C and D when hydrolysed with 5% sulphuric acid all gave sudachitin and glucose.

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