Structure Determination of Biliary Metabolites of Schizandrin in Rat and Dog

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After oral administration of schizandrin (1) to rat, the bile was collected and treated with β-glucuronidase and arylsulfatase. Eleven metabolites, SZ-M0 (3), SZ-M1 (4), SZ-M2 (5), SZ-M3 (6), SZ-M4 (7), SZ-M5 (8), SZ-M6 (9), SZ-M7 (10), SZ-M8 (11), SZ-M9 (12) and SZ-M10 (13) were isolated from the bile treated with the enzymes. The bile after oral administration of 1 to dog was collected and treated with enzymes in the same way, and eight metabolites, 3—8, 10 and SZ-MD2 (14) were isolated from the bile treated with enzymes. The structures of these metabolites were determined on the basis of chemical and spectral studies. The major metabolite in the bile of rat was 7 and the major metabolites in the bile of dog were 7 and 14.

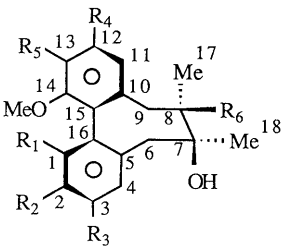
Keywords schizandrin; dibenzocyclooctadiene; lignan; metabolite; rat; dog

Schizandrin (**1**)[1] is one of the dibenzocyclooctadiene lignans isolated from the fruits of *Schisandra chinensis* BAILL. (Schisandraceae). Pharmacological studies have revealed that **1** has a restorative effect on functional depression of brain,[2] an antiulcer effect,[3] and an inhibitory effect on some chemical-induced liver injuries.[4] Cui and Wang reported on the isolation of three metabolites (**7**, **7a** and **10**) from the culture fluid with **1** and rat liver microsomal fraction.[5] We were also interested in the metabolism of this bioactive lignan (**1**), and therefore studied its *in vivo* metabolites in rat as rodent and in dog as nonrodent. We earlier reported[6] on the structure determination of biliary and urinary metabolites of the same dibenzocyclooctadiene lignan, gomisin A (**2**) in rat, and stated that **2** was hydroxylated at the C-8 position following demethylenation of the methylenedioxyl moiety or *O*-demethylation of the methoxyl groups.

After oral administration of **1** to rat, the bile was collected for 24 h. From the results of thin layer chromatography (TLC), it was clear that most of the metabolites in bile existed as their conjugated forms. Therefore, the collected bile was treated with β-glucuronidase and arylsulfatase, and eleven metabolites, SZ-M0 (**3**), SZ-M1 (**4**), SZ-M2 (**5**), SZ-M3 (**6**), SZ-M4 (**7**), SZ-M5 (**8**), SZ-M6 (**9**), SZ-M7 (**10**), SZ-M8 (**11**), SZ-M9 (**12**) and SZ-M10 (**13**) were then isolated. After oral administration of **1** to dog, bile was collected and treated with β-glucuronidase and arylsulfatase in the same way, and eight metabolites, **3**—**8**, **10** and SZ-MD2 (**14**) were isolated.

SZ-M1 and SZ-M2 were identified as gomisin T [(7*S*,-8*S*,*R*-biar)-6,7,8,9-tetrahydro-1,2,3,13,14-pentamethoxy-7,8-dimethyl-7,12-dibenzo[*a*,*c*]cyclooctenediol (**4**)] and (7*S*,8*S*,*R*-biar)-6,7,8,9-tetrahydro-1,2,3,12,14-pentamethoxy-7,8-dimethyl-7,13-dibenzo[*a*,*c*]cyclooctenediol (**5**), respectively, by direct comparison with authentic samples.[7]

SZ-M0 (**3**) and SZ-M3 (**6**) were obtained as white amorphous powder and the molecular formulae of **3** and **6** were determined to be $C_{23}H_{30}O_7$ from the high-resolution mass spectra (HR-MS). The circular dichroism (CD) spectrum of **3** shows a positive Cotton effect at 249 nm and a negative Cotton effect at 215 nm, and that of **6** shows a positive Cotton effect at 250 nm and a negative Cotton effect at 219 nm. These CD spectra indicate that



1: $R_1=R_2=R_3=R_4=R_5=OMe$, $R_6=H$
2: $R_1=R_2=R_3=OMe$, $R_4+R_5=OCH_2O$, $R_6=H$
3: $R_1=R_2=R_4=R_5=OMe$, $R_3=OH$, $R_6=H$
4: $R_1=R_2=R_3=R_5=OMe$, $R_4=OH$, $R_6=H$
5: $R_1=R_2=R_3=R_4=OMe$, $R_5=OH$, $R_6=H$
6: $R_1=R_3=R_4=R_5=OMe$, $R_2=OH$, $R_6=H$
6a: $R_1=R_3=R_4=R_5=OMe$, $R_2=OCOCH_3$, $R_6=H$
7: $R_1=R_3=R_4=R_5=OMe$, $R_2=R_6=OH$
7a: $R_1=R_2=R_3=R_4=R_5=OMe$, $R_6=OH$
7b: $R_1=R_3=R_4=R_5=OMe$, $R_2=OCOCH_3$, $R_6=OH$
8: $R_1=R_2=R_3=R_5=OMe$, $R_4=R_6=OH$
9: $R_1=R_3=R_5=OMe$, $R_2=R_4=R_6=OH$
10: $R_1=R_2=R_4=R_5=OMe$, $R_3=R_6=OH$
11: $R_1=R_4=R_5=OMe$, $R_2=R_3=R_6=OH$
11a: $R_1=R_4=R_5=OMe$, $R_2+R_3=OCH_2O$, $R_6=OH$
12: $R_1=R_3=R_4=OMe$, $R_2=R_5=R_6=OH$
13: $R_3=R_4=R_5=OMe$, $R_1=R_2=R_6=OH$
13a: $R_3=R_4=R_5=OMe$, $R_1+R_2=OCH_2O$, $R_6=OH$
14: $R_1=R_2=R_3=R_4=OMe$, $R_5=R_6=OH$
15: $R_1=R_3=OMe$, $R_2=OH$, $R_4+R_5=OCH_2O$, $R_6=H$
15a: $R_1=R_3=OMe$, $R_2=OCOCH_3$, $R_4+R_5=OCH_2O$, $R_6=H$
16: $R_1=R_2=R_3=OMe$, $R_4=R_5=R_6=OH$

Chart 1

TABLE I. ^{1}H NMR Spectral Data for Compounds **1**, **3**—**14**, and **7a** (δ in $CDCl_3$, 500 MHz)

Compound	H-4 s H-11 s	H-6α d (J=Hz)	H-6β d (J=Hz)	H-9α dd (J=Hz)	H-9β dd (J=Hz)	C-7-OH	H-8 m	C-7-Me s	C-8-Me (J=Hz)	OMe s	ArOH
1[a]	6.61 6.54	2.67 (13.5)	2.37 (13.5)	2.37 dd (14.2, 7.7)	2.66 dd (14.2, 1.8)	1.59 br s	1.87 m	1.26	0.83 d (7.3)	3.58, 3.59, 3.88, 3.89×2, 3.91	—
3	6.68 6.54	2.63 (13.5)	2.33 (13.5)	2.36 dd (14.3, 7.8)	2.71 dd (14.3, 1.9)	1.61 br s	1.86	1.23	0.82 d (7.2)	3.54, 3.56, 3.89, 3.90×2	5.74 br s
4	6.62 6.62	2.67 (13.4)	2.37 (13.4)	2.34 dd (14.2, 7.6)	2.60 dd (14.2, 1.7)	1.67 s	1.86	1.26	0.83 d (7.3)	3.55, 3.56, 3.90, 3.90, 3.92	5.70 br s
5	6.63 6.55	2.68 (13.4)	2.39 (13.4)	2.37 dd (14.3, 7.5)	2.64 dd (14.3, 1.6)	1.67 s	1.88	1.27	0.82 d (7.2)	3.43, 3.54, 3.91, 3.92×2	5.58 br s
6	6.62 6.55	2.66 (13.7)	2.35 (13.7)	2.38 dd (14.2, 7.8)	2.67 dd (14.2, 1.5)	1.57 s	1.89	1.26	0.88 d (7.3)	3.41, 3.56, 3.90, 3.90, 3.95	5.62 s
7[a]	6.63 6.68	2.76 (14.1)	2.54 (14.1)	2.42 d (13.5)	2.70 d (13.5)	2.05 s	2.05 s (OH)	1.32	1.14 s	3.41, 3.58, 3.90, 3.91, 3.96	5.73 s
8[a]	6.62 6.75	2.77 (13.9)	2.55 (13.9)	2.38 d (13.5)	2.64 d (13.5)		(OH)	1.31	1.13 s	3.55, 3.57, 3.91, 3.92, 3.93	5.71 s
9[a]	6.63 6.77	2.77 (14.1)	2.53 (14.1)	2.38 d (13.5)	2.65 d (13.5)		(OH)	1.32	1.14 s	3.39, 3.52, 3.95, 3.96	5.66 br s 5.80 br s
10[a]	6.70 6.68	2.72 (14.0)	2.50 (14.0)	2.40 d (13.5)	2.71 d (13.5)		(OH)	1.30	1.12 s	3.52, 3.59, 3.89, 3.90, 3.95	5.81 br s
11	6.71 6.69	2.71 (14.1)	2.50 (14.1)	2.42 d (13.5)	2.75 d (13.5)		(OH)	1.30	1.13	3.26, 3.53, 3.90, 3.91	5.55 br s 5.74 br s
12[a]	6.64 6.68	2.76 (14.0)	2.55 (14.0)	2.39 d (13.7)	2.70 d (13.7)		(OH)	1.32	1.12	3.38, 3.44, 3.958, 3.964	
13[a]	6.51 6.76	2.72 (14.1)	2.55 (14.1)	2.41 d (13.6)	2.65 d (13.6)		(OH)	1.32	1.16	3.60, 3.906, 3.913, 3.96	5.60 s 5.70 s
14	6.63 6.67	2.78 (13.9)	2.56 (13.9)	2.39 d (13.8)	2.68 d (13.8)	1.56 br s[b]	2.05 s[b] (OH)	1.32	1.13	3.47, 3.54, 3.91, 3.93×2	6.56 s
7a[a]	6.61 6.67	2.76 (13.9)	2.55 (13.9)	2.41 d (13.5)	2.68 d (13.5)	1.63 br s[b]	2.70 br s[b] (OH)	1.32	1.12 s	3.56, 3.61, 3.89, 3.90×2, 3.92	

a) Assignments were based on the ^{1}H–^{1}H correlation spectroscopy (COSY) and NOESY spectra. *b*) Assignments of these signals may be reversed. Abbreviations: br = broad, d = doublet, m = multiplet, s = singlet.

TABLE II. ^{13}C-NMR Spectral Data for Compounds **1**, **3**—**14**, **16**, **6a**, **7a** and **7b** (δ in $CDCl_3$, 125 MHz)

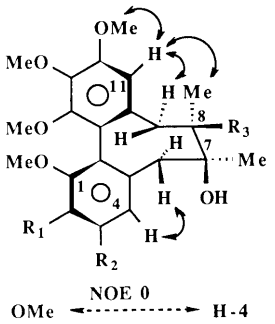
Carbon	1[a]	3	4	5	6[b]	6a[c]	7[d]	7a[d]	7b[c]	8	9[b]	10[b]	11	12	13[b]	14	15	16
1	152.0	151.0	152.1[e]	152.0	145.5	151.4	145.8	152.3	151.6	152.3	145.7	151.2[e]	152.1	145.7	141.7	152.3	145.0	152.1
2	140.8	138.4	140.9	141.0	137.0	131.8	137.6	141.4	132.3	141.1	137.7	139.0	141.6	137.8	133.6	141.6	137.0	141.6
3	152.3	148.4	152.4[e]	152.5	149.7	151.1	146.9	152.7	151.3	152.7	147.0	148.7	152.8	146.8[e]	146.5	152.9	146.6	152.8
4	110.2	112.7	110.2	110.2	109.8	110.5[e]	109.7	110.0	110.2	110.1	109.9	112.8	110.5	109.9	107.2	110.2	110.0	110.5
5	131.8	132.8	132.1	132.0	127.5	134.9	126.0	130.4	133.7	130.6	126.3	131.3	130.9	126.2	126.1	130.6	127.6	130.9
6	41.0	40.7	40.8	40.8	40.5	41.3	40.7	41.0	41.2	41.0	40.7	40.7	41.0	40.8	40.8	41.1	40.2	41.0
7	71.8	71.9	71.9	71.8	71.7	72.0	72.9	75.2	75.1	75.2	75.2	75.2	75.3	75.2	74.9	75.2	71.6	75.3
8	41.8	41.9	41.9	41.9	41.9	41.8	75.2	73.0	73.1	73.1	73.0	72.9	73.1	72.9	72.5	73.0	42.1	73.1
9	34.4	34.6	33.9	33.9	34.2	34.7	43.1	43.1	43.1	42.7	42.7	43.1	42.3	42.8	43.1	42.8	33.7	42.3
10	133.8	134.2	134.6	129.6	134.0	134.0	133.7	133.6	133.6	134.3	134.3	133.8	130.5	129.5	134.7	129.4	132.7	130.5
11	110.5	110.6	113.1	110.2	110.6	110.1[e]	110.8	110.8	110.8	113.4	113.6	110.9	114.5	110.4	112.3	110.3	106.1	114.5
12	151.9	151.9	147.8	146.3	152.1	152.2	152.4	152.3	152.5	148.2	148.4	152.3[e]	143.6	147.1[e]	152.5	146.6	148.1	143.6
13	140.3	140.3	137.8	136.3	140.5	140.5	140.8	140.7	140.8	138.2	138.4	140.7	134.7	137.0	140.9	136.8	135.1	134.7
14	151.6	151.6	150.4	150.4	151.6	151.7	151.5	151.6	151.6	150.3	150.3	151.6	145.1	145.0	150.5	145.0	141.3	145.1
15	122.8	122.5	122.5	122.5	122.5	122.4	122.1	122.4	121.9	121.6	121.4	122.3	120.2	121.4	121.3	122.5	121.6	120.2
16	124.2	123.4	123.4	123.4	123.4	123.9	123.3	124.1	123.8	124.0	123.1	123.2	123.7	123.0	118.6	123.4	123.5	123.7
17	15.9	15.8	15.8	15.8	15.9	16.0	22.6	22.6	22.7	22.6	22.5	22.5	22.6	22.5	22.5	22.6	15.8	22.6
18	29.8	29.9	29.8	29.9	29.8	29.6	26.4	26.5	26.6	26.6	26.5	26.7	26.5	26.5	26.3	26.6	30.1	26.5
C-1-OMe	60.5	60.1	60.6[f]	60.7[e]	60.2[e]	60.5	60.3	60.6	60.5	60.6[e]	60.3	60.1[f]	60.6[f]	60.4[f]	—	60.4[e]	62.2	60.6
C-2-OMe	60.8	61.0	61.0[g]	61.0	—	—	—	61.0	—	61.0	—	61.0	61.0	—	—	61.1	—	61.0
C-3-OMe	55.9[e]	—	56.0	56.0[f]	56.2[f]	56.2[f]	56.3	56.1	56.2	56.1	56.3	—	56.2	56.3[g]	56.1	56.2[f]	56.2	56.2
C-12-OMe	55.8[e]	56.0	—	56.1[f]	56.0[f]	56.0[f]	56.0	56.0	56.0	—	—	56.0	—	56.2[g]	56.3	56.1[f]	—	—
C-13-OMe	60.8	61.0	60.9[g]	—	61.0	61.0	61.1	61.0	61.0	61.0	61.1	61.0	—	—	61.2	—	—	—
C-14-OMe	60.5	60.6	60.2[f]	60.3[e]	60.8[e]	60.8	60.9	60.7	60.9	60.2[e]	60.4	60.9[f]	60.7[e]	60.6[f]	61.6	60.7[e]	59.8	80.7

a) Assinments were confirmed by the INADEQUATE spectrum. *b*) Assignments were based on the HMQC and HMBC spectra. *c*) Other signals: **6a**, δ 168.8, 22.6 ($COCH_3$); **7b**, δ 168.9, 20.6 ($COCH_3$). *d*) Assignments were based on the ^{1}H–^{13}C COSY and COLOC spectra. *e*—*g*) Assignments within any vertical column may be reversed.

3 and **6** possess an *R*-biphenyl configuration.[8] The proton magnetic resonance (^{1}H-NMR) spectra of **3** and **6** are very closely similar to that of **1** except for the functional groups on the aromatic rings as shown in Table I. Their ^{1}H-NMR spectra show that both **3** and **6** have five methoxyl groups and a phenolic hydroxyl group (δ 5.74 in **3** and δ 5.58 in **6**) on the aromatic rings, suggesting that **3** and **6** correspond to the demethylated derivative of **1**. The positions of the phenolic hydroxyl groups in **3** and **6** were determined by analyses of the ^{13}C-NMR and the

two-dimensional intramolecular nuclear Overhauser effect spectroscopy (NOESY) spectra as follows.

The higher-field protonated aromatic carbon signal at δ 110.6 in the ^{13}C-NMR spectrum of **3** (Table II) appears at the same region as the C-11 signal (δ 110.5) of **1**, and can be assignable to the C-11 signal. The other protonated aromatic carbon signal at δ 112.7 in **3** is consequently assigned to the C-4 signal. When a methoxyl group at the *ortho*-position relative to H-4 (or H-11) in the dibenzocyclooctadiene lignan is replaced by a hydroxyl group, the C-4 (or C-11) signal of the hydroxy compound shows a downfield shift of *ca.* 3 ppm.[9] From these ^{13}C-NMR spectral data, the presence of the phenolic hydroxyl group at the C-3 position in **3** was suggested. The NOESY spectrum of **3** (Fig. 1) showed an appreciable NOE between the C-8 secondary methyl signal at δ 0.82 and the aromatic proton signal at δ 6.54, indicating that the latter is assignable to the H-11 signal. Further, the NOESY spectrum showed NOE between the methoxyl signal at δ 3.90 and the H-11 signal, but no NOE between any methoxyl signal and the other aromatic proton (H-4) signal at δ 6.68. This indicates that **3** has a hydroxyl group at C-3 position. From the above results, SZ-M0 was determined to be (7*S*,8*S*,*R*-biar)-6,7,8,9-tetrahydro-1,2,-12,13,14-pentamethoxy-7,8-dimethyl-3,7-dibenzo[*a*,*c*]-cyclooctenediol (**3**).



3 : R_1=OMe, R_2=OH, R_3=H

10 : R_1=OMe, R_2=OH, R_3=OH

11a: R_1+R_2=OCH_2O, R_3=OH

Fig. 1. NOE in the NOESY Spectra of **3**, **10** and **11a** (in $CDCl_3$)

In the ^{1}H-NMR spectrum of **6**, the appearance of two upfield methoxyl signals (δ 3.41 and 3.56)[10] which are shielded by the aromatic rings indicates the presence of two methoxyl groups at the C-1 and -14 positions of **6**. Further, the appearance of two upfield methoxyl signals at δ 56.0 and 56.2 in the ^{13}C-NMR spectrum of **6** indicates the presence of two methoxyl groups at the C-3 and C-12 positions of **6**. These ^{1}H- and ^{13}C-NMR data suggest that the phenolic hydroxyl group in **6** is located at the C-2 or C-13 position. The position of the phenolic hydroxyl group in **6** was determined by analyses of the ^{13}C-NMR spectra of **6** and its monoacetate (**6a**) as described below. Two dimensional (2D) NMR techniques including heteronuclear multiple quantum coherence (HMQC) and heteronuclear multiple bond connectivity (HMBC) (Fig. 2) gave the complete assignments of carbon signals in **6** as shown in Table II. The C-1—5 and C-16 shift values in **6** correspond to those in Met A-III (**15**)[6] which is one of the biliary metabolites of gomisin A(**2**) and possesses the C-2 hydroxyl group and the C-1 and -3 methoxyl groups. The C-2 shifts of **15** and its monoacetate (**15a**) show upfield

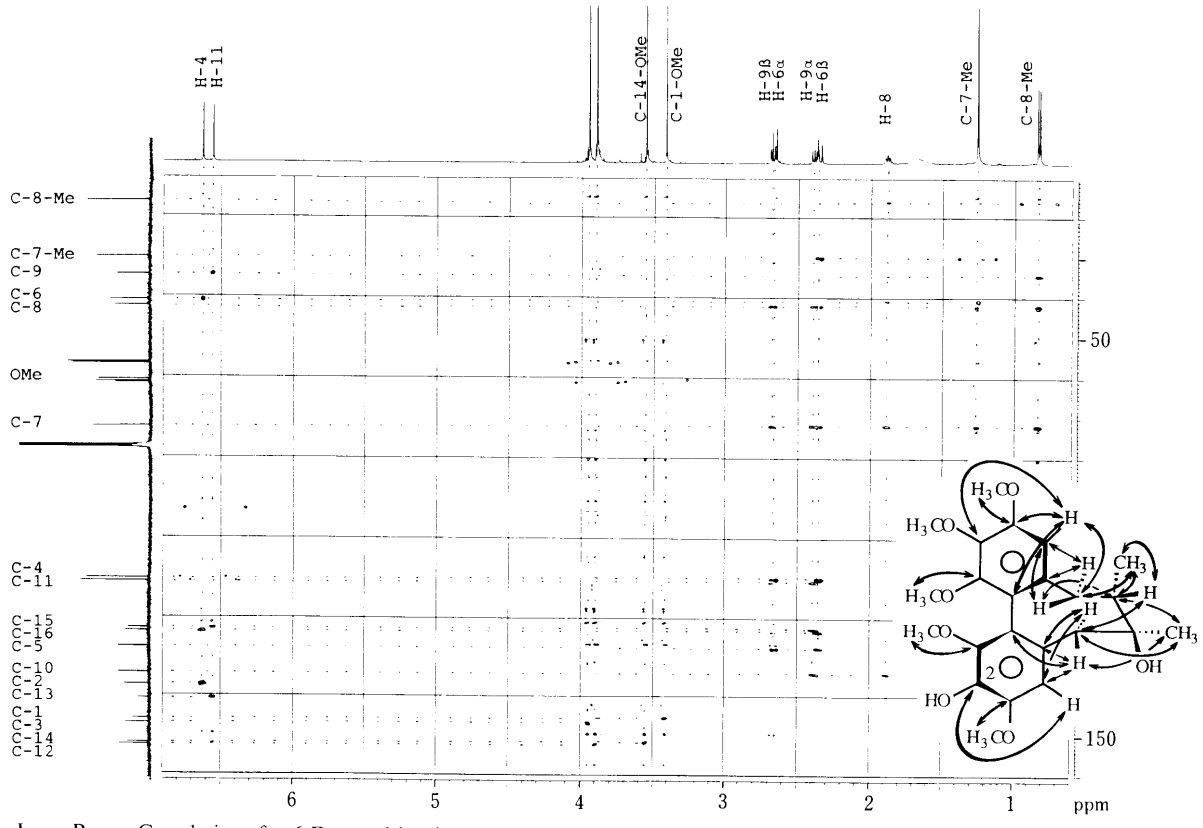


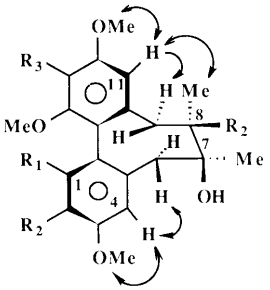
Fig. 2. Long-Range Correlations for **6** Detected by the HMBC Spectrum

shifts of 3.8 and 9.1 ppm, respectively, compared with the C-2 shift of the methyl ether (**2**) of **15**.[11] The C-2 shifts of **6** and **6a** show upfield shifts of 3.8 and 9.0 ppm, respectively, compared with the C-2 shift of **1**. The above observations indicate that the phenolic hydroxyl group in **6** is linked to C-2. Thus, SZ-M3 was determined to be (7*S*,8*S*,*R*-biar)-6,7,8,9-tetrahydro-1,3,12,13,14-pentamethoxy-7,8-dimethyl-2,7-dibenzo[*a*,*c*]cyclooctenediol (**6**).

The HR-MS of SZ-M4 (**7**), SZ-M5 (**8**), SZ-M7 (**10**) and SZ-MD2 (**14**) showed the molecular formulae of these compounds to be $C_{23}H_{30}O_8$, with one oxygen more than **6**. Their CD spectra showed positive Cotton effects around 235—255 nm and a negative Cotton effect around 215—225 nm, indicating that these compounds possess an *R*-biphenyl configuration.[8] The ^{1}H-NMR spectra of **7**, **8**, **10** and **14** (Table I) show that they have a phenolic hydroxyl and five methoxyl groups on the aromatic rings, and two tertiary methyl and two hydroxylgroups on the cyclooctadiene ring. The ^{13}C-NMR spectra of **7**, **8**, **10** and **14** (Table II) are very closely similar to that of Met F (**16**)[6] derived from gomisin A (**2**) except for the functional groups on the aromatic rings. These ^{1}H- and ^{13}C-NMR spectra suggest that **7**, **8**, **10** and **14** have a hydroxyl group at the C-8β position as well as **16**, and these compounds are isomers one another on the basis of the substitutive position of a phenolic hydroxyl group. The position of the phenolic hydroxyl group in these compounds was determined by the measurements of NOESY spectra and the ^{13}C-NMR spectral analyses as follows.

Methylation of **7** with dimethyl sulfate and potassium carbonate at 45 °C afforded **7a**, which was identified as the dimethyl ether of **16**, supporting that **7** has a hydroxyl group at the C-8β position. The NOESY spectrum of **7** (Fig. 3) showed NOEs between the methoxyl signal at δ 3.91 and the H-11 signal (δ 6.83), and between the methoxyl signal at δ 3.96 and the H-4 signal (δ 6.63), indicating the presence of two methoxyl groups at the C-3 and -12 positions in **7**. The carbon shifts in **7** and **7a** were assigned as shown in Table II by ^{1}H–^{13}C shift correlation spectroscopy (COSY) and ^{1}H–^{13}C shift correlation *via* long range coupling spectroscopy (COLOC) spectra. The C-2 shifts of **7** and its monoacetate (**7b**) show upfield shifts of 3.8 and 9.1 ppm, respectively, compared with the C-2 shift of **7a**, as in the case of the C-2 shifts of **6**, **6a** and **1**. This indicates that the phenolic hydroxyl group in **7** is linked to C-2. From the above results, SZ-M4 was determined to be (7*S*,8*R*,*R*-biar)-6,7,8,9-tetrahydro-1,3,12,13,14-pentamethoxy-7,8-dimethyl-2,7,8-dibenzo-[*a*,*c*]cyclooctenetriol (**7**).

A comparison of the ^{1}H- and ^{13}C-NMR spectra of



7 : R_1=R_3=OMe, R_2=OH

12 : R_1=OMe, R_2=R_3=OH

13 : R_1=R_2=OH, R_3=OMe

Fig. 3. NOE in the NOESY Spectra of **7**, **12** and **13** (in $CDCl_3$)

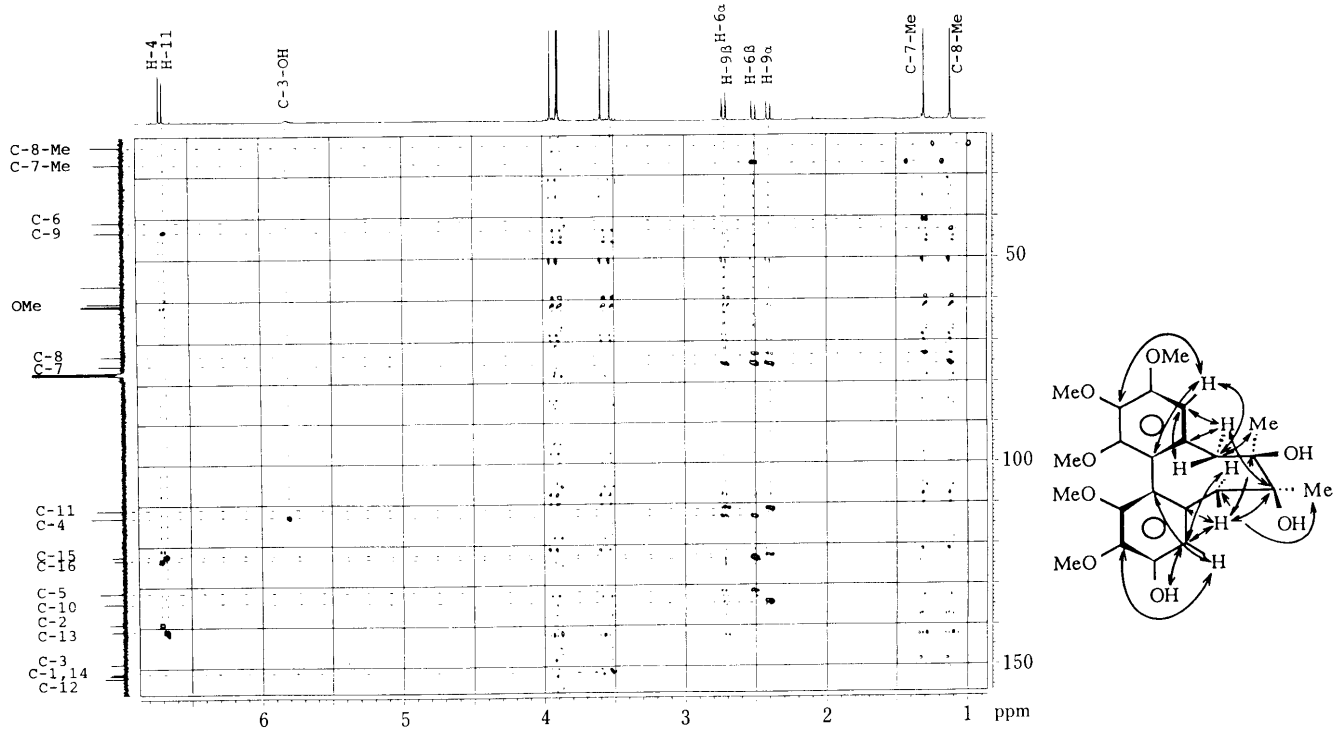


Fig. 4. Long-Range Correlations for **10** Detected by the HMBC Spectrum

SZ-M7 (**10**) with those of **7** suggested **10** to be the isomer of **7** by the position of phenolic hydroxyl group. The NOESY spectrum of **10** (Fig. 1) showed appreciable NOE between the H-11 signal (δ 6.68) and the methoxyl signal at δ 3.90, but no NOE between the H-4 signal (δ 6.70) and any methoxyl signals, indicating the presence of a phenolic hydroxyl group at the C-3 position (*ortho*-position relative to H-4) in **10**. This was further supported by the appearance of a cross peak between the phenolic hydroxyl signal at δ 5.81 and the C-4 signal at δ 112.8 in the HMBC spectrum of **10** (Fig. 4). Thus, SZ-M7 was determined to be (7*S*,8*R*,*R*-biar)-6,7,8,9-tetrahydro-1,2,-12,13,14-pentamethoxy-7,8-dimethyl-3,7,8-dibenzo[*a*,*c*]-cyclooctenetriol (**10**).

The ^{1}H-NMR spectrum of SZ-M5 (**8**) is similar to that of **7** except for the H-11 shift value. The NOESY spectrum of **8** (Fig. 5) showed appreciable NOE between the H-4 signal (δ 6.62) and the methoxyl signal at δ 3.92, but no NOE between the H-11 signal (δ 6.75) and any methoxyl signals, indicating the presence of a phenolic hydroxyl group at the C-12 position (*ortho*-position relative to H-11) in **8**. Thus, SZ-M5 was determined to be (7*S*,8*R*,*R*-biar)-6,7,8,9-tetrahydro-1,2,3,13,14-pentamethoxy-7,8-dimethyl-7,8,12-dibenzo[*a*,*c*]cyclooctenetriol (**8**)

The aromatic carbon shift values in the ^{13}C-NMR spectrum of SZ-MD2 (**14**) are very similar to those of SZ-M2 (**5**) possessing a phenolic hydroxyl group at the C-13 position as shown in Table II. This suggests the presence of a phenolic hydroxyl group at the C-13 position in **14**. Methylation of Met F (**16**)[6] with dimethyl sulfate and potassium carbonate at room temperature afforded the 12,13-*O*-dimethyl ether (**7a**), the 13-*O*-methyl ether (**8**), and **14**, which was identified as SZ-MD2. From the above observations, SZ-MD2 was determined to be (7*S*,8*R*,*R*-biar)-6,7,8,9-tetrahydro-1,2,3,12,14-pentamethoxy-7,8-dimethyl-7,8,13-dibenzo[*a*,*c*]cyclooctenetriol (**14**).

The HR-MS of SZ-M6 (**9**), SZ-M8 (**11**), SZ-M9 (**12**) and SZ-M10 (**13**) showed the molecular formulae of these compounds to be the same $C_{22}H_{28}O_8$. Their CD spectra show positive Cotton effects around 235—255 nm and a negative Cotton effect around 215—225 nm, indicating that these compounds possess an *R*-biphenyl configuration.

The ^{1}H-NMR spectrum (Table I) of SZ-M9 (**12**) shows that **12** has four methoxyl groups on the aromatic rings and two tertiary methyl groups on the cyclooctadiene ring. On methylation with dimethyl sulfate and potassium carbonate, **12** afforded **7a**. From this fact, **12** was assumed to be the compound which two methoxyl groups in **7a** were replaced by two hydroxyl groups. The positions of two phenolic hydroxyl groups in **12** were determined by the measurement of the NOESY spectrum and ^{13}C-NMR spectral analyses as follows. The appearance of two up-field methoxy signals (δ 3.38 and 3.44) in the ^{1}H-NMR

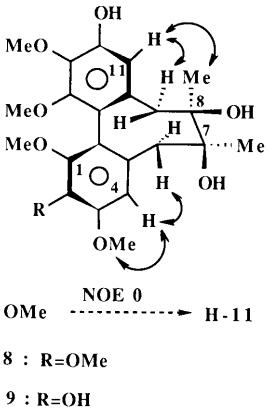


Fig. 5. NOE in the NOESY Spectra of **8** and **9** (in $CDCl_3$)

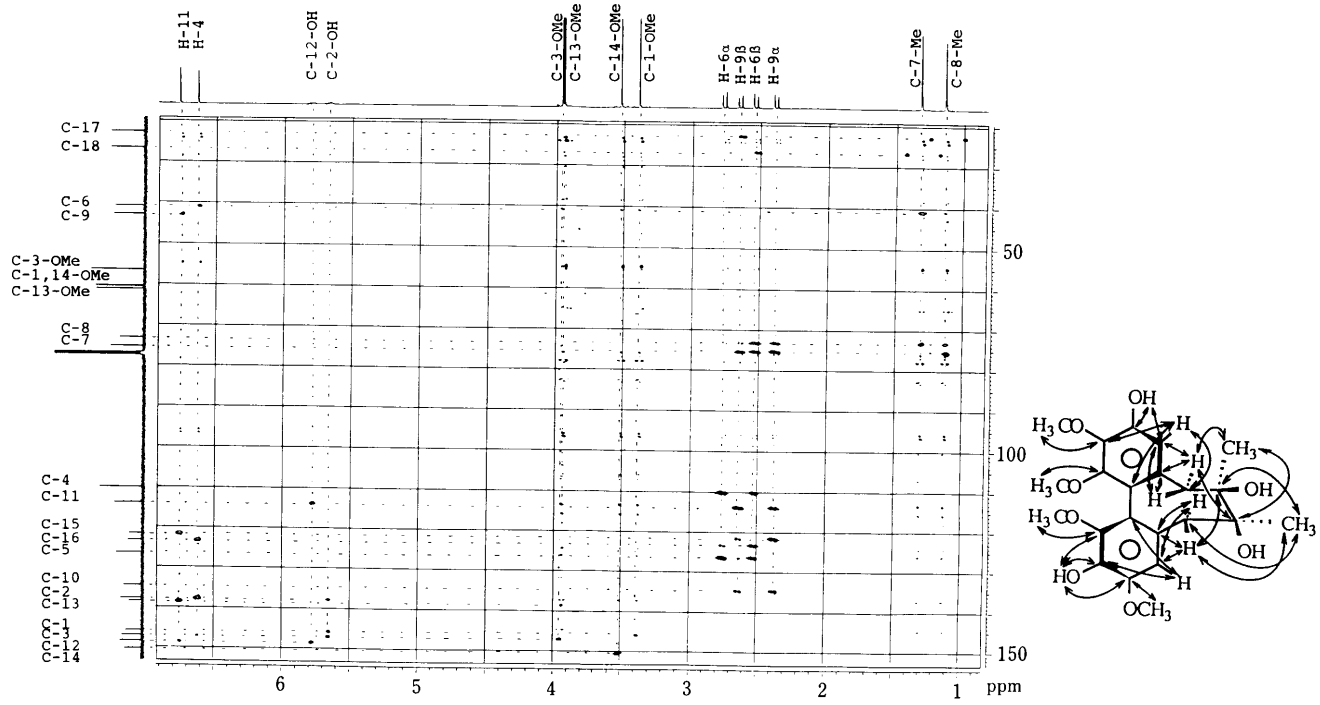


Fig. 6. Long-Range Correlations for **9** Detected by the HMBC Spectrum

spectrum of **12** showed the presence of two methoxyl groups at the C-1 and C-14 positions of **12**. The NOESY spectrum of **12** (Fig. 3) showed NOEs between the H-4 (δ 6.64) and the methoxyl signal at δ 3.958, and the H-11 (6.68) and the methoxyl signal at δ 3.964, indicating the presence of two methoxyl groups at the C-3 and -12 positions. The C-1—5 and C-16 shift values in the ^{13}C-NMR spectrum of **12** are in agreement with those of **7** possessing a hydroxyl group at the C-2 position. Further, the C-10—14 and -15 shift values of **12** agree with those of **14** possessing a hydroxyl group at the C-13 position. Thus, SZ-M9 was determined to be (7*S*,8*R*,*R*-biar)-6,7,8,9-tetrahydro-1,3,12,14-tetramethoxy-7,8-dimethyl-2,7,8,13-dibenzo[*a*,*c*]cyclooctene-tetraol (**12**).

The ^{1}H-NMR spectrum of SZ-M6 (**9**) is similar to that of **7** except for the H-11 shift value and the lack of one methoxyl signal. The NOESY spectrum of **9** (Fig. 5) showed NOE between the H-4 signal (δ 6.63) and the methoxyl signal at δ 3.96, but no NOE between the H-4 (δ 6.77) and any methoxyl signals, indicating that one of the two phenolic hydroxyl groups in **9** is linked to C-12. The HMBC spectrum of **9** (Fig. 6) showed cross peaks between the phenolic hydroxyl signal at δ 5.66 and the C-1, -2 and -3 signals, and between the phenolic hydroxyl signal at δ 5.80 and the C-11 and -12 signals. This showed the presence of two phenolic hydroxyl groups at the C-2 and -12 positions of **9**. Thus, SZ-M6 was determined to be (7*S*,8*R*,*R*-biar)-6,7,8,9-tetrahydro-1,3,13,14-tetramethoxy-7,8-dimethyl-2,7,8,12-dibenzo[*a*,*c*]cyclooctenetetraol (**9**).

The ^{1}H-NMR spectrum of SZ-M8 (**11**) showed two phenolic hydroxyl signals (δ 5.55 and 5.74) and four methoxyl signals (δ 3.26, 3.53, 3.90 and 3.91) on the aromatic rings and two tertiary methyl signals as well as **9**. The ^{13}C-NMR spectrum (Table II) of **11** is similar to that of **9** except for the aromatic carbon signals. The MS of **11** afforded a base peak at *m*/*z* 418 $[M-2H]^+$, suggesting the presence of a catechol moiety[12] in **11**. Methylenation of **11** with methylene iodide and potassium carbonate afforded **11a**, $C_{23}H_{30}O_8$. The ^{1}H-NMR spectrum of **11a** showed a methylenedioxyl signal at δ 5.99 and 6.00 (each 1H, d, J=1.5 Hz) and no phenolic hydroxyl signal. This fact supported the presence of a catechol moiety in **11**. The NOESY spectrum of **11a** (Fig. 1) showed NOE between the H-11 signal (δ 6.67) and the methoxyl signal at δ 3.89, but no NOE between the H-4 signal (δ 6.56) and any methoxyl signals, indicating that the methylenedioxyl moiety in **11a** is located at the C-2 and -3 positions. Consequently, two phenolic hydroxyl groups in **11** are linked to C-2 and -3. From these observations, SZ-M8 was determined to be (7*S*,8*R*,*R*-biar)-6,7,8,9-tetrahydro-1,12,13,14-tetramethoxy-7,8-dimethyl-2,3,7,8-dibenzo[*a*,*c*]cyclooctenetetraol (**11**).

The ^{1}H-NMR spectrum of SZ-M10 (**13**) (Table I) showed two phenolic hydroxyl (δ 5.60 and 5.70) and four methoxyl (δ 3.26, 3.53, 3.90 and 3.91) signals on the aromatic rings and two tertiary methyl signals as well as **11**. The NOESY spectrum of **13** indicated the presence of two methoxyl groups at the C-3 and -12 positions in **13** as shown in Fig. 3. Methylenation of **13** with methylene iodide and potassium carbonate afforded **13a** whose ^{1}H-NMR spectrum showed a methylenedioxyl signal at δ 5.90 and 6.01 (each 1H, d, J=1.5 Hz) and no phenolic hydroxyl signal. This fact indicates the presence of a catechol moiety in **13**. From these observations, two phenolic hydroxyl groups in **13** were assumed to be linked to C-1 and -2, or to C-13 and -14. The HMBC spectrum of **13** (Fig. 7) showed the cross peaks between the phenolic

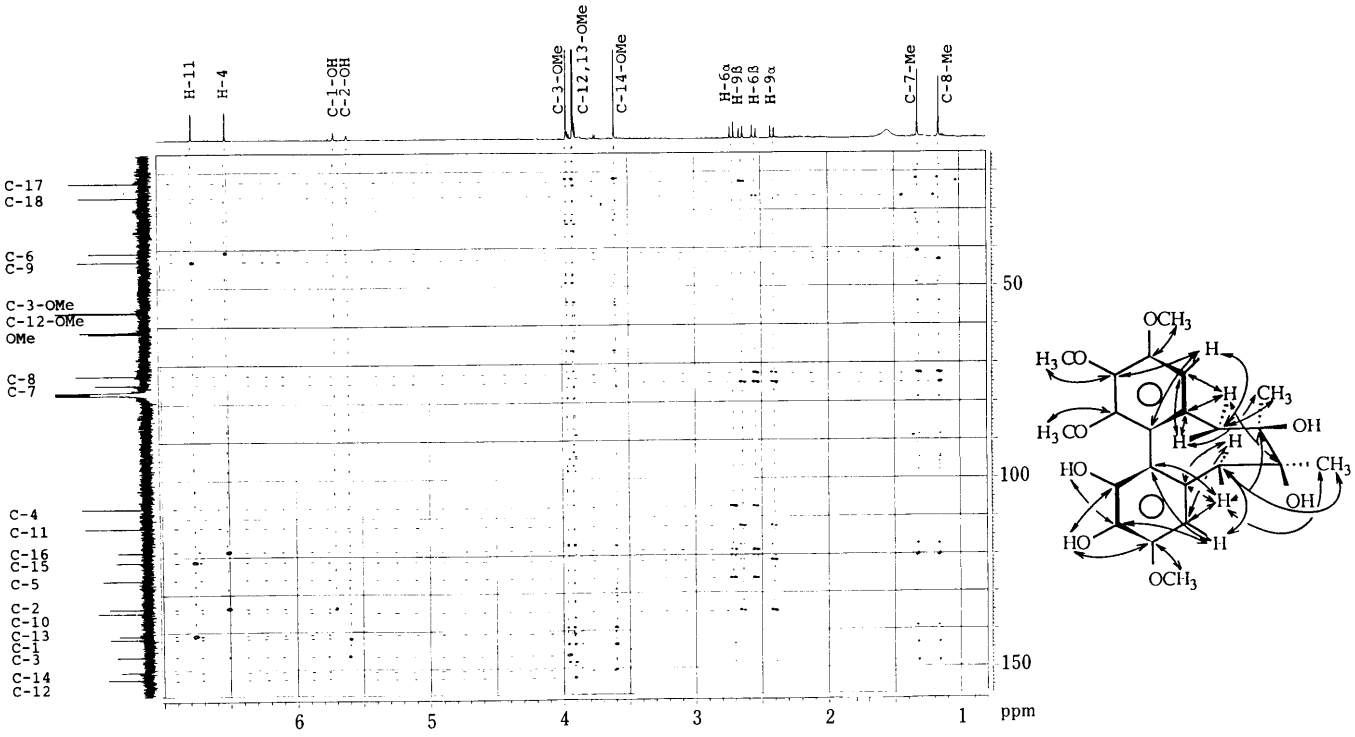


Fig. 7. Long-Range Correlations for **13** Detected by the HMBC Spectrum

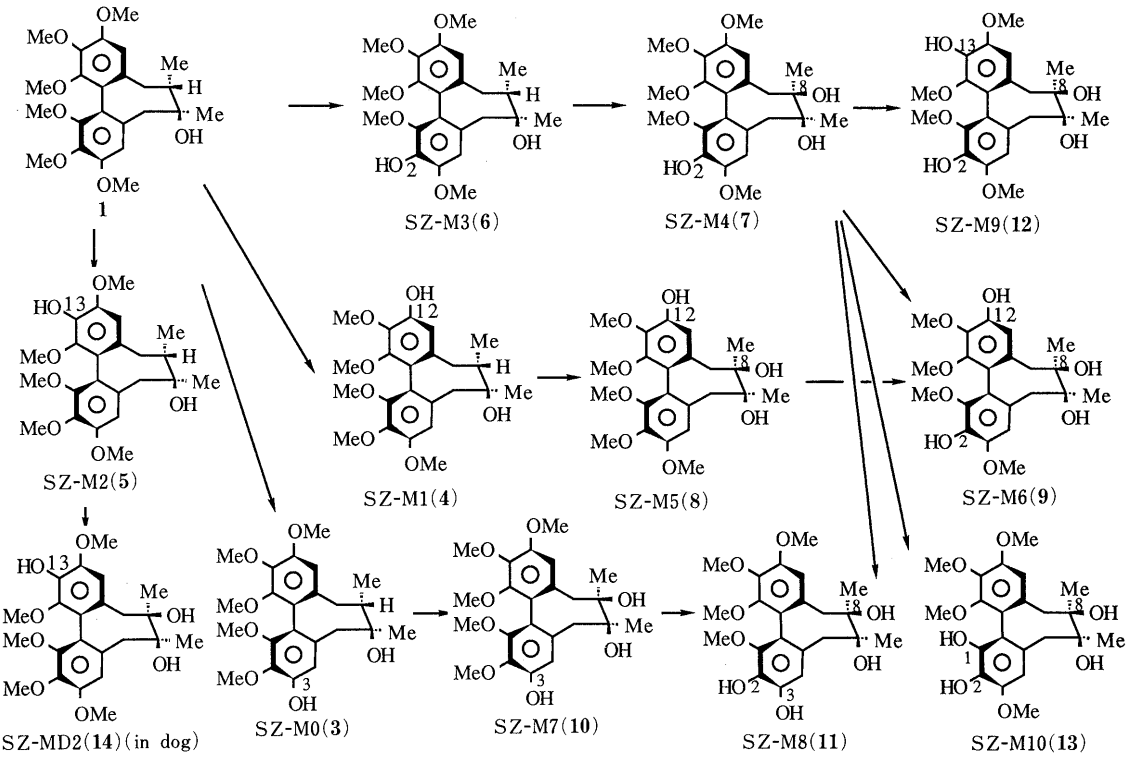


Chart 2. Biliary Metabolites of **1** in Rat

hydroxyl signal at δ 5.60 and the C-1 and -3 signals, indicating the presence of the C-2 hydroxyl group in **13**. Further, the appearance of a cross peak between the phenolic hydroxyl signal at δ 5.70 and the C-2 signal showed the presence of the C-1 hydroxyl group in **13**. Thus, SZ-M10 was determined to be (7*S*,8*R*,*R*-biar)-6,7,8,9-tetrahydro-3,12,13,14-tetramethoxy-7,8-dimethyl-1,2,7,8-dibenzo[*a*,*c*]cyclooctenetetraol (**13**).

The structures of biliary metabolites of **1** in rat and dog are represented as shown in Chart 2. Most of these metabolites are assumed to exist in bile as their conjugated forms (sulfates or glucuronides). The major biliary metabolite of **1** in rat was identified as **7**. On the other hand, **7** and **14** were found as the major biliary metabolites of **1** in dog. These facts suggest that the metabolism of **1** is variant by species. Further, from the results of the above preliminary studies, the metabolic fate of **1** after oral administration in rat and dog is suggested as described below. The *O*-demethylated derivatives of **1** (such as **3**, **4**, **5** and **6**) are first formed. Next, the C-8-hydroxylated compounds of the *O*-demethylated derivatives, **7**, **8**, **10** and **14** are formed, and part of these compounds are further *O*-demethylated to give **9**, **11**, **12** and **13**. The metabolism of **1** is similar to that of gomisin A (**2**) from the viewpoint of *O*-demethylation and C-8-hydroxylation except for the demethylenation of the methylenedioxyl moiety in **2**.[6] Detailed metabolic studies of **1** using these authentic samples are being undertaken by our co-workers.[13]

Experimental

All the melting points were taken on a Yanagimoto micromelting point apparatus and are uncorrected. Optical rotations were measured on a JASCO DIP-360 polarimeter and CD spectra with a JASCO J-600 spectropolarimeter. UV spectra were taken with a Hitachi U-3200 spectrophotometer and IR spectra with a Hitachi 270-30 infrared spectrophotometer. ^{1}H- and ^{13}C-NMR spectra were taken on a Bruker AM-500 and a JEOL JNM FX-200 spectrometer with tetramethylsilane (TMS) as an internal standard. ^{1}H–^{1}H COSY, ^{1}H–^{13}C COSY, NOESY, HMBC, and COLOC spectra were obtained and processed with the standard Bruker software. EI-MS and HR-MS were obtained with Kratos Concept 1H and 1S mass spectrometer. For silica gel column chromatography, Kieselgel 60 (Merck) was used. Kieselgel 60 F_{254} (Merck precoated plate) was used for preparative thin layer chromatography (prep. TLC) and spots were detected under UV (254 nm). For preparative high-pressure liquid chromatography (prep. HPLC), a Hitachi L-6250 intelligent pump with L-4000 UV detector was used.

Isolation of Biliary Metabolites in Rat Male SD strain rats weighing 250—280 g (n=160) were orally given **1** suspended in 0.5% sodium carboxymethyl cellulose (CMC-Na) at 50 mg/kg. The common bile duct was immediately cannulated under ether anesthesia to collect the bile (2.7 l) for 24 h. The bile was dissolved in 2.1 l, of 0.1 M acetate buffer (pH 5.0), β-glucuronidase (from *Helix pomatia*, type H-1, Sigma Chemical Company) (5×10^6 units) was added and this solution was incubated at 37 °C for 18 h. The incubated solution was chromatographed on Sepabeads SP207 (Mitsubishi Chemical Industries, Limited) (1.5 l), developing with H_2O (1 l), 20% MeOH (6 l) and then MeOH (6 l). The MeOH eluate was concentrated to give a residue (20.03 g), which was chromatographed on silica gel (4.5 cm i.d. × 28 cm) with a mixture of hexane–EtOAc–MeOH. The details of this chromatography are given in Table III.

Fraction 2 (267 mg) in Table III was purified by prep. TLC [hexane–EtOAc (1 : 2)], and the zones with *Rf* 0.65, 0.55 and 0.48 were extracted with $CHCl_3$–MeOH (4 : 1) and concentrated. The extracts of the zones with *Rf* 0.65, 0.55 and 0.48 were further purified by prep. TLC using hexane–ether–EtOH (4 : 5 : 1) or $CHCl_3$–MeOH (19 : 1) to give **3** (1.5 mg) and **4** (4.0 mg), **5** (16.7 mg), and **6** (10.1 mg), respectively. Fraction **3** (141 mg) was purified by prep. TLC [hexane–EtOAc (1 : 3)], and the zones with *Rf* 0.50 and 0.46 were extracted with $CHCl_3$–MeOH (4 : 1) and concentrated. The extracts of the zones with *Rf* 0.50 and 0.46 were further purified by prep. TLC using $CHCl_3$–EtOH (19 : 1) to give **10** (5.0 mg) and **8** (2.0 mg), respectively. Fraction **4** (778 mg) was purified by prep. TLC [hexane–EtOAc (1 : 3)], and the zones with *Rf* 0.30 and

TABLE III. Silica Gel Column Chromatography of Biliary Metabolite Fraction in Rat

Fraction No.	Solvent	Volume (ml)	Yield (mg)
1	Hexane–EtOAc (1:1)	600	1692
2	Hexane–EtOAc (2:3)	600	267
3	Hexane–EtOAc (1:4)	300	141
4	Hexane–EtOAc (1:4)	600	778
5	EtOAc	400	101
6	EtOAc–MeOH (9:1)	500	136
7	EtOAc–MeOH (4:1)	900	721

0.20 were extracted with $CHCl_3$–MeOH (4:1) and concentrated. The extract of the zone with *Rf* 0.30 was further purified by prep. TLC [hexane–acetone (3:2), *Rf* 0.28] to give **7** (263 mg). The extract of the zone with *Rf* 0.20 was purified by prep. HPLC [column, YMC-Pack S-343 ODS (20 mm i.d. × 250 mm); mobile phase, CH_3CN–MeOH–H_2O (1:1:4); flow rate, 5 ml/min; detection, UV 275 nm; t_R, 72 min] to give **9** (9.0 mg). Fraction **5** (101 mg) was purified by prep. TLC [hexane–EtOAc (1:3)] and the zone with *Rf* 0.20 was extracted with $CHCl_3$–MeOH (4:1) and concentrated. The residue was purified by prep. TLC [$CHCl_3$–EtOH (19:1)], and the zones with *Rf* 0.38 and 0.27 were extracted with $CHCl_3$–MeOH (4:1) and concentrated. The extract of the zones with *Rf* 0.38 and 0.27 were further purified by prep. TLC [hexane–ether–EtOH (4:5:1)] to give **12** (3.5 mg), **13** (1.5 mg) and **11** (7 mg), respectively.

SZ-M0 (3) White amorphous powder, $[\alpha]_D^{25}$ +71° (c=0.059, $CHCl_3$). IR ν_{max}^{KBr} cm^{-1}: 3440 (OH),1588 (aromatic ring). UV λ_{max}^{EtOH} nm (log ε): 216 (4.53), 252 (4.01), 289 (sh 3.22). CD (c=0.0122, MeOH) $[\theta]^{25}$ (nm): −86200 (215), +40300sh (236), +72100 (249), +9600sh (276). EI-MS m/z (%): 418(M^+, 100), 400 (67), 315 (34), 149 (55), 71 (56), 57 (84). HR-MS, Calcd for $C_{23}H_{30}O_7$ (M^+): 418.1991. Found: 418.1984.

SZ-M1 (4) White amorphous powder, $[\alpha]_D^{24}$ +55.8° (c=0.233, $CHCl_3$). IR ν_{max}^{KBr} cm^{-1}: 3420 (OH), 1584 (aromatic ring). EI-MS m/z (%): 418 (M^+,100), 400 (23), 375 (18), 344 (30), 316 (35). HR-MS, Calcd for $C_{23}H_{30}O_7$ (M^+): 418.1991. Found: 418.1981.

SZ-M2 (5) White amorphous powder, $[\alpha]_D^{24}$ +69.6° (c=0.330, $CHCl_3$). IR ν_{max}^{KBr} cm^{-1}: 3432 (OH), 1598 (aromatic ring). EI-MS m/z (%): 418 (M^+, 100), 400 (32), 347 (28), 334 (21), 316 (50). HR-MS, Calcd for $C_{23}H_{30}O_7$ (M^+): 418.1991. Found: 418.1995.

SZ-M3 (6) White amorphous powder, $[\alpha]_D^{24}$ +74.3° (c=0.160, $CHCl_3$). IR ν_{max}^{KBr} cm^{-1}: 3440 (OH), 1598 (aromatic ring). UV λ_{max}^{EtOH} nm (log ε): 217 (4.56), 254 (sh 4.02), 287 (sh 3.46). CD (c=0.0110, MeOH) $[\theta]^{25}$ (nm): −89600 (219), +70300 (238), +74100 (250). EI-MS m/z (%): 418 (M^+, 100), 400 (44), 343 (22), 315 (40), 107 (23). HR-MS, Calcd for $C_{23}H_{30}O_7$ (M^+): 418.1991. Found: 418.2011.

SZ-M4 (7) Colorless prisms, mp 157—159 °C, $[\alpha]_D^{24}$ +110° (c=1.773, $CHCl_3$). IR ν_{max}^{KBr} cm^{-1}: 3516, 3268 (OH), 1600 (aromatic ring). UV λ_{max}^{EtOH} nm (log ε): 219 (4.66), 256 (sh 4.12), 289 (sh 3.57). CD (c=0.0121, MeOH) $[\theta]^{26}$ (nm): −118000 (219), +106000 (238), +10600 (251). EI-MS m/z (%): 434 (M^+, 49), 416 (33), 391 (65), 348 (56), 347 (100), 316 (89). HR-MS, Calcd for $C_{23}H_{30}O_8$ (M^+): 434.1941. Found: 434.1913.

SZ-M5 (8) White amorphous powder, $[\alpha]_D^{24}$ +85.9° (c=0.185, $CHCl_3$). IR ν_{max}^{KBr} cm^{-1}: 3448 (OH), 1588 (aromatic ring). UV λ_{max}^{EtOH} nm (log ε): 218 (4.59), 253 (4.10), 290 (sh 3.39). CD (c=0.0105, MeOH) $[\theta]^{27}$ (nm): −93200 (215), +46600sh (236), +88100 (251). EI-MS m/z (%): 434 (M^+, 63), 416 (85), 400 (70), 391 (93), 316 (77), 315 (100). HR-MS, Calcd for $C_{23}H_{30}O_8$ (M^+): 434.1941. Found: 434.1946.

SZ-M6 (9) White amorphous powder, $[\alpha]_D^{25}$ +125° (c=0.295, $CHCl_3$). IR ν_{max}^{KBr} cm^{-1}: 3428 (OH), 1612, 1590 (aromatic ring). UV λ_{max}^{EtOH} nm (log ε): 218 (4.58), 252 (4.05), 289 (sh 3.47). CD (c=0.0108, MeOH) $[\theta]^{26}$ (nm): −76600 (218), +50500sh (239), +79100 (252), +19600sh (274). EI-MS m/z (%): 420 (M^+, 61), 377 (63), 334 (26), 333 (47), 302 (41), 301 (100). HR-MS, Calcd for $C_{22}H_{28}O_8$ (M^+): 420.1783. Found: 420.1783.

SZ-M7 (10) Colorless prisms, mp 195.5—198 °C, $[\alpha]_D^{25}$ +110° (c=0.375, $CHCl_3$). IR ν_{max}^{KBr} cm^{-1}: 3504, 3304 (OH), 1586 (aromatic ring). UV λ_{max}^{EtOH} nm (log ε): 217 (4.61), 253 (sh 4.12), 289 (sh 3.40). CD (c=0.0133, MeOH) $[\theta]^{28}$ (nm): −97300 (215), +38400 (235), +95800 (251), +15300sh (275). EI-MS m/z (%): 434 (M^+, 52), 416 (60), 391 (100), 348 (28), 347 (57), 315 (31). HR-MS, Calcd for $C_{23}H_{30}O_8$ (M^+): 434.1941. Found: 434.1913.

SZ-M8 (11) Yellow amorphous powder, $[\alpha]_D^{24}$ +118° (c=0.345, $CHCl_3$). IR ν_{max}^{KBr} cm^{-1}: 3448 (OH), 1598 (aromatic ring). UV λ_{max}^{EtOH} nm (log ε): 215 (4.51), 255 (sh 3.97), 291 (sh 3.43). CD (c=0.0108, MeOH) $[\theta]^{27}$ (nm): −71600 (220), +53800sh (241), +61800 (251), +13700sh (279). EI-MS m/z (%): 420 (M^+, 37), 418 (100), 377 (57), 343 (69), 318 (77), 301 (77). HR-MS, Calcd for $C_{22}H_{28}O_8$ (M^+): 420.1783. Found: 420.1782.

SZ-M9 (12) White amorphous powder, $[\alpha]_D^{24}$ +99.9° (c=0.0102, $CHCl_3$). IR ν_{max}^{KBr} cm^{-1}: 3420 (OH), 1612 (aromatic ring). UV λ_{max}^{EtOH} nm (log ε): 217 (4.67), 255 (sh 4.14), 279 (3.80). CD (c=0.0150, MeOH) $[\theta]^{26}$ (nm): −101000 (220), +91500 (240), +71500sh (251), +20900 (278). EI-MS m/z (%): 420 (M^+, 56), 418 (23), 402 (96), 377 (65), 333 (61), 301 (100). HR-MS, Calcd for $C_{22}H_{28}O_8$ (M^+): 420.1783. Found: 420.1771.

SZ-M10 (13) Pale yellow powder, $[\alpha]_D^{24}$ +110° (c=0.020, $CHCl_3$). IR ν_{max}^{KBr} cm^{-1}: 3444 (OH), 1622, 1594 (aromatic ring). UV λ_{max}^{EtOH} nm (log ε): 218 (4.47), 256 (sh 3.97), 288 (3.40). CD (c=0.0121, MeOH) $[\theta]^{28}$ (nm): −71300 (207), −57200sh (215), +58500 (241), +59500 (251), +7100sh (275), −2600 (291). EI-MS m/z (%): 420 (M^+, 33), 377 (62), 376 (100), 333 (86), 301 (49), 287 (47). HR-MS, Calcd for $C_{22}H_{28}O_8$ (M^+): 420.1783. Found: 420.1801.

Acetylation of 6 A solution of **6** (4.5 mg) in a mixture of pyridine (0.25 ml) and Ac_2O (0.15 ml) was allowed to stand at room temperature overnight, then diluted with ether. The ethereal solution was washed with 1 N HCl, 5% $NaHCO_3$, then H_2O, dried over Na_2SO_4, and concentrated. The residue was purified by prep. TLC [hexane–acetone (3:2)] to give **3a** (4.0 mg) as colorless prisms (from ether–hexane), mp 182—183, $[\alpha]_D^{25}$ +82.8° (c=0.175, $CHCl_3$). IR ν_{max}^{KBr} cm^{-1}: 3576, 3488 (OH), 1760 (C=O), 1598 (aromatic ring). EI-MS m/z (%): 460 (M^+, 47), 419 (26), 418 (100), 316 (10), 315 (20), 287 (9.9), 43 (27). HR-MS, Calcd for $C_{25}H_{32}O_8$ (M^+): 460.2097. Found: 460.2097. ^{1}H-NMR (δ in $CDCl_3$): 0.84 (3H, d, J=7.2 Hz, C-8-Me), 1.26 (3H, s, C-7-Me), 1.90 (1H, m, H-8), 2.33 (3H, s, OAc), 2.39 (1H, d, J=14.3, 7.7 Hz, H-9α), 2.41 (1H, d, J=13.4 Hz, H-6β), 2.69 (1H, d, J=13.4 Hz, H-6α), 2.71 (1H, dd, J=14.3, 1.9 Hz, H-9β), 3.34 (3H, s), 3.57 (3H, s), 3.87 (6H, s), 3.89 (3H, s) (5 × OMe), 6.53 (1H, s, H-11), 6.68 (1H, s, H-4).

Methylation of Met F (16) Dimethyl sulfate (0.02 ml) and potassium carbonate (40 mg) were added to a solution of **16** (3.5 mg) in dry acetone (2 ml). The reaction mixture was stirred at 45 °C for 3 h, then diluted with ether. The ethereal solution was washed with H_2O, dried over Na_2SO_4, and concentrated. The residue was purified by prep. TLC [hexane–acetone (3:2)] to give the dimethyl ether of **16** (3.0 mg) as colorless prisms (from ether–hexane), mp 161—162.5 °C, $[\alpha]_D^{24}$ +131° (c=0.071, $CHCl_3$). IR ν_{max}^{KBr} cm^{-1}: 3520 (OH), 1598 (aromatic ring). EI-MS m/z (%): 448 (M^+, 64), 430 (42), 405 (81), 362 (51), 361 (100), 330 (95). HR-MS, Calcd for $C_{24}H_{32}O_8$ (M^+): 448.2097. Found: 448.2099.

Methylation of 7 Dimethyl sulfate (0.05 ml) and potassium carbonate (80 mg) were added to a solution of **7** (12 mg) in dry acetone (2 ml). The reaction mixture was stirred at 45 °C for 3 h, then treated as described for methylation of **16** to give **7a** (11 mg) as colorless prisms (from ether–hexane), mp 161—162.5 °C, $[\alpha]_D^{24}$ +110° (c=0.167, $CHCl_3$). HR-MS, Calcd for $C_{24}H_{32}O_8$(M^+): 448.2097. Found: 448.2101. Compound **7a** was identified as the dimethyl ether of **16** by direct comparison (IR, ^{1}H-NMR, MS, $[\alpha]_D$ and mixed melting point).

Acetylation of 7 A solution of **7** (29 mg) in a mixture of pyridine (0.4 ml) and Ac_2O (0.2 ml) was allowed to stand at room temperature overnight. The reaction mixture was treated as described for acetylation of **6** to give **7b** (24 mg) as white amorphous powder. $[\alpha]_D^{26}$ +93.7° (c=0.223, $CHCl_3$). IR ν_{max}^{KBr} cm^{-1}: 3520 (OH), 1766 (C=O), 1598 (aromatic ring). EI-MS m/z (%): 476 (M^+, 34), 434 (19), 433 (71), 391 (100), 347 (30), 316 (38). HR-MS, Calcd for $C_{25}H_{32}O_9$ (M^+): 476.2045. Found: 476.2036. ^{1}H-NMR (δ in $CDCl_3$): 1.14 (3H, s, C-8-Me), 1.33 (3H, s, C-7-Me), 2.34 (3H, s, OAc), 2.42 (1H, d, J=13.6 Hz, H-9α), 2.59 (1H, d, J=13.9 Hz, H-6β), 2.74 (1H, d, J=13.6 Hz, H-9β), 2.79 (1H, d, J=13.9 Hz, H-6α), 3.33 (3H, s), 3.59 (3H, s), 3.88 (6H, s), 3.90 (3H, s) (5 × OMe), 6.67 and 6.68 (each 1H, s, H-4 and -11).

Methylenation of 11 Methylene iodide (20 mg) and potassium carbonate (30 mg) were added to a solution of **11** (12 mg) in dry acetone (1 ml). The reaction mixture was stirred at 45 °C for 3 h, then diluted with ether. The ethereal solution was washed with H_2O, dried over Na_2SO_4, and concentrated. The residue was purified by prep. TLC

TABLE IV. Silica Gel Column Chromatography of Biliary Metabolite Fraction in Dog

Fraction No.	Solvent	Volume (ml)	Yield (mg)
1	Hexane–EtOAc (3:2)	400	349
2	Hexane–EtOAc (2:3)	200	36
3	EtOAc	50	21
4	EtOAc	200	81
5	EtOAc–MeOH (9:1)	150	13
6	EtOAc–MeOH (4:1)	200	883

[$CHCl_3$–MeOH (19:1)] to give **11a** as white amorphous powder, IR ν_{max}^{KBr} cm^{-1}: 3460 (OH), 1620, 1598 (aromatic ring). EI-MS *m/z* (%): 432 (M^+, 60), 389 (93), 346 (60), 345 (87), 315 (56), 314 (100). HR-MS, Calcd for $C_{23}H_{28}O_8$ (M^+): 432.1784. Found: 432.1789. ^{1}H-NMR (δ in $CDCl_3$): 1.11 (3H, s, C-8-Me), 1.29 (3H, s, C-7-Me), 2.40 (1H, d, J=13.5 Hz, H-9α), 2.50 (1H, d, J=14.0 Hz, H-6β), 2.70 (1H, d, J=14.0 Hz, H-6α), 2.72 (1H, d, J=13.5 Hz, H-9β), 3.56 (3H, s), 3.83 (3H, s), 3.89 (6H, s) (4×OMe), 5.99 and 6.00 (each 1H, d, J=1.5 Hz, OCH_2O), 6.56 (1H, s, H-4), 6.67 (1H, s, H-11).

Methylation of 12 Dimethyl sulfate (0.02 ml) and potassium carbonate (30 mg) were added to a solution of **12** (1.1 mg) in dry acetone (1.5 ml). The reaction mixture was stirred at 45 °C for 3 h, then treated as described for methylation of **17** to give white amorphous powder (1.0 mg), $[\alpha]_D^{24}$ +133° (c=0.072, $CHCl_3$). HR-MS, Calcd for $C_{24}H_{32}O_8$ (M^+): 448.2097. Found: 448.2103. This compound was identified as **7a** by direct comparison (MS, ^{1}H-NMR, $[\alpha]_D$ and TLC).

Methylenation of 13 Methylene iodide (0.1 ml) and potassium carbonate (30 mg) were added to a solution of **13** (1 mg) in dry acetone (1 ml). The reaction mixture was stirred at 45 °C for 3 h, then diluted with ether. The ethereal solution was washed with H_2O, dried over Na_2SO_4, and concentrated. The residue was purified by prep. TLC [hexane–EtOAc (1:3)] to give **13a** as white amorphous powder, IR ν_{max}^{KBr} cm^{-1}: 3508 (OH), 1598 (aromatic ring). EI-MS *m/z* (%): 432 (M^+, 75), 389 (80), 346 (50), 345 (100), 314 (87), 287 (22). HR-MS, Calcd for $C_{23}H_{28}O_8$ (M^+): 432.1784. Found: 432.1784. ^{1}H-NMR (δ in $CDCl_3$): 1.13 (3H, s, C-8-Me), 1.32 (3H, s, C-7-Me), 2.43 (1H, d, J=13.6 Hz, H-9α), 2.56 (1H, d, J=14.1 Hz, H-6β), 2.70 (1H, d, J=13.6 Hz, H-9β), 2.76 (1H, d, J=14.1 Hz, H-6α), 3.71 (3H, s), 3.89 (6H, s), 3.97 (3H, s) (4×OMe), 5.90 and 6.01 (each 1H, d, J=1.5 Hz, OCH_2O), 6.49 (1H, s, H-4), 6.69 (1H, s, H-11).

Isolation of Biliary Metabolites in Dog Male beagles weighing 12 kg (n=3) were anesthetized with pentobarbital-Na. Compound **1** suspended in 0.5% CMC-Na was given to the beagles at 20 mg/kg *via* the gastric intraluminal canula. The common bile duct was immediately cannulated to collect the bile (0.2 l) for 12 h. The bile was dissolved in 0.6 l of 0.1 M acetate buffer (pH 5.0), β-glucuronidase (from *Helix pomatia*, type H-1) (2×10^6 units) was added and this solution was incubated at 37 °C for 18 h. The incubated solution was chromatographed on Sepabeads SP207 (1.5 l), developing with H_2O (4 l), 20% MeOH (3 l) and then MeOH (2 l). The MeOH eluate was concentrated to give a residue (3.04 g), which was chromatographed on silica gel (3 cm i.d.×35 cm) with a mixture of hexane–EtOAc–MeOH. The details of this chromatography are given in Table IV.

Fraction 2 (36 mg) in Table IV was purified by prep. TLC [hexane–EtOAc (1:3)] to give **5** (*Rf* 0.66, 6.1 mg) and a mixture of **3** and **4** (*Rf* 0.73, 4.5 mg). The mixture of **3** and **4** was purified by prep. TLC [$CHCl_3$–MeOH (19:1)] to give **3** (*Rf* 0.70, 2.0 mg) and **4** (*Rf* 0.80, 1.5 mg). Fraction 3 (21 mg) was purified by prep. TLC [hexane–EtOAc (1:3), *Rf* 0.60] to give **6** (6.1 mg). Fraction 4 (81 mg) was purified by prep. TLC [hexane–EtOAc (1:3)], and the zones with *Rf* 0.46, 0.33 and 0.30 were extracted with $CHCl_3$–MeOH (4:1) and concentrated. The extract of the zone with *Rf* 0.46 was further purified by prep. TLC [$CHCl_3$–MeOH (19:1)] to give **8** (*Rf* 0.54, 3.1 mg) and **10** (*Rf* 0.50, 7.1 mg). The extracts of the zones with *Rf* 0.33 and 0.30 were purified by prep. TLC [$CHCl_3$–MeOH (19:1)] to give **14** (13.1 mg) and **7** (11.4 mg), respectively. Fraction 5 (13 mg) was purified by prep. TLC [i) hexane–EtOAc (1:3); ii) $CHCl_3$–MeOH (19:1)] to give **7** (0.4 mg, total 11.8 mg). Compounds **3**, **4**, **5**, **6**, **7**, **8** and **10** were identified as SZ-M0, SZ-M1, SZ-M2, SZ-M3, SZ-M4, SZ-M5 and SZ-M7, respectively, by direct comparison (MS, ^{1}H-NMR, IR and $[\alpha]_D$) with authentic samples obtained from the bile of rat.

SZ-MD2 (14) Colorless prisms (from ether–hexane), mp 201.5—203 °C, $[\alpha]_D^{25}$ +110° (c=0.453, $CHCl_3$). IR ν_{max}^{KBr} cm^{-1}: 3480, 3232 (OH), 1598 (aromatic ring). UV λ_{max}^{EtOH} nm ($\log\varepsilon$): 218 (4.57), 257 (sh 4.05), 291 (sh 3.43). CD (c=0.0134, MeOH) $[\theta]^{27}$ (nm): −104000 (218), +93900 (238), +104000 (251), +21900sh (274). EI-MS *m/z* (%): 434 (M^+, 71), 391 (100), 348 (70), 347 (88), 315 (26), 302 (42), 287 (46). HR-MS, Calcd for $C_{23}H_{30}O_8$ (M^+): 434.1941. Found: 434.1940.

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References and Notes

1) *a*) N. K. Kochetkov, A. Khorlin, O. S. Chizhov, V. I. Sheichenko, *Tetrahedron Lett.*, **1961**, 730; *b*) Y. Ikeya, H. Taguchi, I. Yosioka, and H. Kobayashi, *Chem. Pharm. Bull.*, **27**, 1383 (1979).
2) Y. Kessoku, A. Ishige, M. Yuzurihara, K. Sekiguchi, K. Goto, S. Iizuka, A. Sugimoto, M. Aburada, T. Oyama, *Jpn. J. Pharmacol.*, **58**, Suppl. I, 255 (1992).
3) S. Maeda, K. Sudo, M. Aburada, Y. Ikeya, H. Taguchi, I. Yosioka, M. Harada, *Yakugaku Zasshi*, **101**, 1030 (1981).
4) S. Maeda, K. Sudo, Y. Miyamoto, S. Takeda, M. Shinbo, M. Aburada, Y. Ikeya, H. Taguchi, M. Harada, *Yakugaku Zasshi*, **102**, 579 (1982).
5) Y.-y. Cui, M.-z. Wang, *Acta Pharmaceutica Sinica*, **27**, 57 (1992).
6) Y. Ikeya, H. Mitsuhashi, H. Sasaki, Y. Matsuzaki, T. Matsuzaki, E. Hosoya, *Chem. Pharm. Bull.*, **38**, 136 (1990).
7) Y. Ikeya, H. Kanatani, M. Hakozaki, H. Taguchi, H. Mitsuhashi, *Chem. Pharm. Bull.*, **36**, 3974 (1988).
8) Y. Ikeya, H. Taguchi, I. Yosioka, H. Kobayashi, *Chem. Pharm. Bull.*, **27**, 1383 (1979).
9) Y. Ikeya, H. Taguchi, H. Sasaki, K. Nakajima, I. Yosioka, *Chem. Pharm. Bull.*, **28**, 2414 (1980).
10) A. F. A. Wallis, *Tetrahedron Lett.*, **1968**, 5287.
11) Y. Ikeya, H. Taguchi, H. Mitsuhashi, H. Sasaki, T. Matsuzaki, M. Aburada, E. Hosoya, *Chem. Pharm. Bull.*, **36**, 2061 (1988).
12) R. T. Aplin, W. T. Pike, *Chem. Ind.* (London), **1966**, 2009.
13) H. Ono, Y. Matsuzaki, Y. Wakui, S. Takeda, Y. Ikeya, S. Amagaya, M. Maruno, *J. Chromatogr.*, submitted.