

[Chem. Pharm. Bull.]
36(6)2061—2069(1988)

Studies on the Metabolism of Gomisin A (TJN-101). I. Oxidative Products of Gomisin A Formed by Rat Liver S9 Mix.

YUKINOBU IKEYA,*^a HEIHACHIRO TAGUCHI,^a HIROSHI MITSUHASHI,^a
HIROMI SASAKI,^b TAMAE MATSUZAKI,^b MASAKI ABURADA,^b
and EIKICHI HOSOYA^b

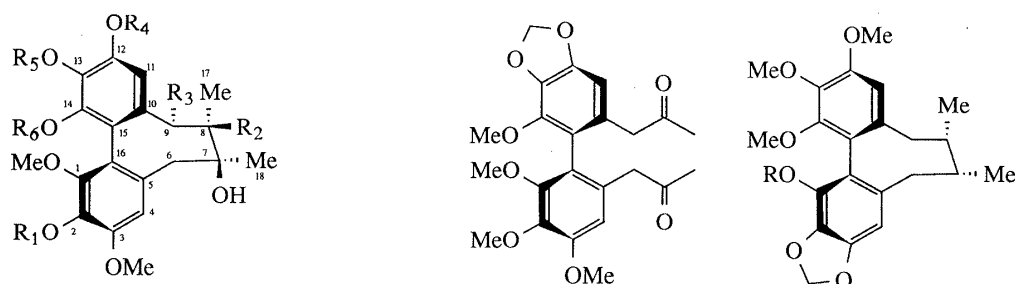
^aTsumura Laboratory,^a 3586 Yoshiwara Ami-machi, Inashiki-gun, Ibaraki 300-11, Japan
and ^bTsumura Research Institute for Pharmacology,^b 3586 Yoshiwara Ami-cho,
Inashiki-gun, Ibaraki 300-11, Japan

(Received October 22, 1987)

Gomisin A (**1**) was incubated with rat liver S9 mix., and five products were obtained from the culture solution. The major product, met B (**2**), was identified as the demethylenated derivative of **1** by direct comparison with an authentic sample. The structures of four new products, met A-II (**3**), met C (**4**), met A-I (**5**), and met A-III (**6**), were determined on the basis of chemical and spectral studies.

Keywords—gomisin A; TJN-101; *Schizandra chinensis*; metabolism; S9 mix.; met B; met A-II; met C; met A-I; met A-III

Gomisin A (TJN-101, **1**)¹⁾ is one of the components isolated from the fruits of *Schizandra chinensis* BAILL. (Schizandraceae). Pharmacological studies²⁾ have revealed that **1** shows an inhibitory effect on some chemical-induced liver injuries and antihepatotoxic effect of **1** may have partly resulted from a protective action on the liver cells. In order to clarify the metabolic fate to **1**, structure determination of its oxidative products formed by rat liver S9 mix. was carried out. Gomisin A (**1**) was incubated with S9 mix. (the 9000 × *g* supernatant fraction of rat liver homogenate), and five products, met B (**2**), met A-II (**3**), met C (**4**), met A-I (**5**), and met A-III (**6**), were obtained from the culture solution.



- 1:** $R_1 = R_6 = \text{Me}$, $R_2 = R_3 = \text{H}$, $R_4 + R_5 = \text{CH}_2$
2: $R_1 = R_6 = \text{Me}$, $R_2 = R_3 = R_4 = R_5 = \text{H}$
3: $R_1 = R_6 = \text{Me}$, $R_2 = \text{OH}$, $R_3 = \text{H}$, $R_4 + R_5 = \text{CH}_2$
4: $R_1 = R_6 = \text{Me}$, $R_2 = \text{H}$, $R_3 = \text{OH}$, $R_4 + R_5 = \text{CH}_2$
4a: $R_1 = R_6 = \text{Me}$, $R_2 = \text{H}$, $R_3 = \text{OCOCH}_3$, $R_4 + R_5 = \text{CH}_2$
5: $R_1 = \text{Me}$, $R_2 = R_3 = R_6 = \text{H}$, $R_4 + R_5 = \text{CH}_2$
5a: $R_1 = \text{Me}$, $R_2 = R_3 = \text{H}$, $R_4 + R_5 = \text{CH}_2$, $R_6 = \text{COCH}_3$
6: $R_1 = R_2 = R_3 = \text{H}$, $R_4 + R_5 = \text{CH}_2$, $R_6 = \text{Me}$
6a: $R_1 = \text{COCH}_3$, $R_2 = R_3 = \text{H}$, $R_4 + R_5 = \text{CH}_2$, $R_6 = \text{Me}$

Chart 1

A major product, met B (**2**), was identified as the demethylenated compound,³⁾ which was obtained by cleaving the methylenedioxy moiety of **1** with lead tetraacetate.

Met A-II (**3**) showed mp 163–165 °C, $[\alpha]_D + 68^\circ$ (CHCl₃), and its molecular formula was determined to be C₂₃H₂₈O₈ from the elemental analysis and the high-resolution mass spectrum (MS). Its proton nuclear magnetic resonance (¹H-NMR) spectrum in CDCl₃ (Table I) is similar to that of **1**, but shows a new hydroxyl signal (δ 3.19) and a new singlet methyl signal (δ 1.12), indicating that a hydroxyl group is attached to the C-8 carbon of **1**. Oxidation of **3** with sodium periodate gave a diketone (**3a**), C₂₃H₂₆O₈, whose infrared (IR) spectrum shows a carbonyl band (1720 cm⁻¹) and no hydroxyl band. The ¹H-NMR [δ 1.95, 2.00 (each 3H, s)] and carbon-13 nuclear magnetic resonance (¹³C-NMR) (δ 206.3, 206.4) spectra of **3a**

TABLE I. ¹H-NMR Spectral Data for **1**, **2**, **3**, **4**, **4a**, **5**, **5a**, **6**, and **6a** (δ in CDCl₃, 200 MHz)

Compd.	H-4, _s H-11, _s	H-6 α (<i>J</i> =Hz)	H-6 β (<i>J</i> =Hz)	H-9 α (<i>J</i> =Hz)	H-9 β (<i>J</i> =Hz)	H-C ₁₍₈₎ -CH ₃ m (<i>J</i> =Hz)	HO ^{a)} -C ₁₍₇₎ -CH ₃ s s	OCH ₃ s	OCH ₂ O (<i>J</i> =Hz)	ArOH ^{a)} br s		
1	6.63 6.48	2.69, d (13.5)	2.35, d (13.5)	2.34, dd (14, 7)	2.60, dd (14, 2)	1.86	0.82, d (7)	1.93	1.25	3.52, 3.84, 3.91 ($\times 2$)	5.96 (2H, s)	
2	6.62 6.65	2.71, d (13.5)	2.39, d (13.5)	2.34, dd (13.5, 7)	2.60, dd (13.5, 1)	1.87	0.82, d (7)	1.87	1.27	3.28, 3.48, 3.91 ($\times 2$)	5.56 ($\times 2$)	
3	6.61 ^{b)} 6.60 ^{b)}	2.63, d (13.5)	2.36, d (13.5)	2.53, d ^{c)} (14)	2.78, d ^{c)} (14)	3.19, s ^{a)} (OH)	1.12, s	2.05	1.30	3.52, 3.85, 3.91 ($\times 2$)	5.97 (2H, s)	
3^{d)}	6.47 6.61	2.69, d ^{b)} (13.4)	2.37, d ^{b)} (13.4)	2.35 ^{b)} (13.8)	2.70 ^{b)} (13.8)	3.08, s ^{a)} (OH)	1.09, s	1.92	1.24	3.41, 3.48, 3.86, 3.88 (d, 1.3)	5.30 (d, 1.3) 5.37 (d, 1.3)	
4	6.58 6.90	2.57, d (13.5)	2.33, d (13.5)	(OH)	4.66, d (1.5)	1.97	0.78, d (7)		1.28	3.59, 3.85, 3.90 ($\times 2$)	6.00 (2H, s)	
4a	6.60 6.67	2.57, d (13.5)	2.36, d (13.5)	2.02 (3H, s) (COCH ₃)	5.56, d (1.2)	2.02	0.83, d (7)	1.58	1.28	3.59, 3.87, 3.91, 3.93 (d, 1.5)	5.99 (d, 1.5) 6.01 (d, 1.5)	
5	6.72 6.45	2.65, d (13.5)	2.36, d (13.5)	2.38, dd (14, 7)	2.57, dd (14, 2)	1.86	0.87, d (7)	1.62	1.27	3.56, 3.92 ($\times 2$)	6.00 (d, 1.5) 6.03 (d, 1.5)	5.26
5a	6.61 6.67	2.66, d (13.5)	2.34, d (13.5)	2.39, dd (14, 7)	2.65, dd (14, 2)	1.85	0.86, d (7)	1.80	1.23	3.53, 3.89, 3.91 (d, 1.5)	6.01 (d, 1.5) (3H, s) 6.04 (COCH ₃)	1.98
6^{e)}	6.63 6.49	2.68, d (13.5)	2.33, d (13.5)	2.35, dd (14, 7)	2.59, dd (14, 2)	1.87	0.82, d (7)	1.60	1.25	3.40, 3.83, 3.94	5.97 (2H, s)	5.60
6a	6.68 6.48	2.71, d (13.5)	2.39, d (13.5)	2.35, dd (14, 7.5)	2.64, dd (14, 1)	1.80	0.83, d (7)	1.82	1.26	3.33, 3.81, 3.87	5.96 (2H, s) (3H, s) (COCH ₃)	2.35

a) Hydroxy signals were confirmed by addition of D₂O. b, c) Assignments of these signals may be reversed. d) This compound was measured in C₆D₆. e) This compound was measured at 400 MHz. f) Abbreviations: d=doublet, m=multiplet, s=singlet.

show that two acetyl groups exist in **3a**. This indicates the presence of a 7,8-diol moiety in **3**.

The stereostructure of **3** was determined from the intramolecular nuclear Overhauser effect (NOE)¹⁾ in C₆D₆ (Fig. 1) and the IR spectrum in CCl₄ as follows. Irradiation of a methoxyl signal (δ 3.41) caused a 16% increase in the integrated intensity of the higher field aromatic proton signal (δ 6.47, H-4), while the lower field aromatic proton signal (δ 6.61, H-11) was unaffected by irradiation of any of the methoxyl signals. Irradiation of the higher field methyl signal (δ 1.09, C-8 methyl) caused a 15% increase in the integrated intensity of the H-11 signal, while irradiation of the lower field methyl signal (δ 1.24, C-7 methyl) did not affect the H-4 signal. These findings indicate that the C-8 methyl group and H-11 are spatially close to each other, and that the C-8 methyl group is α -oriented. This is supported by the fact that the C-8 methyl group is shielded by the aromatic ring¹⁾ and its signal (δ 1.12) appears at higher field than that of the C-7 methyl group (δ 1.30) in the ¹H-NMR spectrum of **3** in CDCl₃. The hydroxyl band in the IR spectrum (0.0025 M in CCl₄) of **1** (possessing a free hydroxyl group) appears at 3580 cm⁻¹, whereas that in the IR spectrum (0.0003 M in CCl₄) of **3** appears at 3548 cm⁻¹, indicating that two hydroxyl groups form an intramolecular hydrogen bond. These IR spectral data indicate that the 7,8-dihydroxyl group of **3** takes a *cis* configuration.

The circular dichroism (CD) spectrum of **3** shows two positive Cotton effects at 255 nm ([θ] +81000) and 241 nm ([θ] +64000) and a negative Cotton effect at 224 nm ([θ] -79000), indicating that **3** possesses the same *R*-biphenyl configuration¹⁾ as **1**.

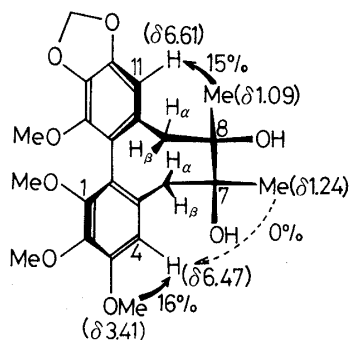
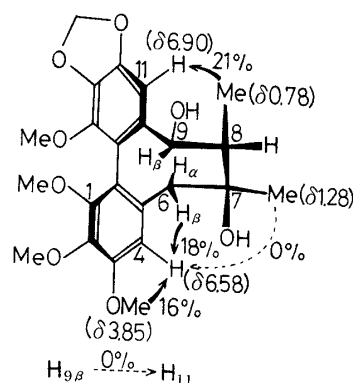
On the basis of the above results, the structure of met A-II was elucidated as **3**, possessing a twist-boat-chair conformation of the cyclooctadiene ring.

Met C (**4**) was obtained as a white amorphous powder, [α]_D +24° (CHCl₃) and its molecular formula was determined to be C₂₃H₂₈O₈ from the high-resolution MS. Its ¹H-NMR spectrum (Table I) is similar to that of **1**, but it shows a new doublet methine proton

TABLE II. ¹³C-NMR Spectral Data for **1**, **2**, **3**, **4**, **5**, **5a**, **6**, and **6a** (δ in CDCl₃; ¹³C at 50 MHz)

Carbon	1	2	3	4	5	5a	6 ^{a)}	6a	
1	152.1	151.9	152.4	151.9	150.8	151.8	145.0	151.4	
2	140.8 ^{b)}	141.1	141.1 ^{b)}	140.6 ^{b)}	141.0	140.5	137.0	131.7	
3	152.3	152.5	152.7	152.8	152.7	152.6	146.6	151.0	
4	110.4	110.6	110.1	110.3	112.0	110.6	110.0	110.5	
5	132.1	132.2	130.7	132.0	132.4	132.4	127.6	135.1	
6	40.6	40.8	40.9	39.9	40.8	40.6	40.2	40.9	
7	71.7	72.2	75.2	71.5	71.7	71.7	71.6	71.8	
8	42.1	41.8	72.9	51.7	41.9	42.0	42.1	42.0	
9	33.8	33.5	42.8	68.4	34.2	34.0	33.7	34.0	
10	132.5	130.5	132.3	135.5	133.7	133.2	132.7	132.7	
11	105.9	114.2	106.2	101.9	105.2	109.1	106.1	105.9	
12	147.9	143.4	148.2	148.6	147.5	147.7	148.1	148.2	
13	135.0	134.4	135.2	135.3	133.8	137.7	135.1	135.1	
14	141.3 ^{b)}	145.3	141.3 ^{b)}	140.9 ^{b)}	137.0	131.4	141.3	141.4	
15	121.9	120.5	121.5	119.8	119.3	122.5 ^{b)}	121.6	121.5	
16	124.2	123.9	124.0	122.6	122.0	123.5 ^{b)}	123.5	123.9	
17	15.8	15.7	22.6	11.5	15.9	15.9	15.8	15.9	
18	30.1	29.8	26.5	30.9	29.8	30.0	30.1	30.0	
OCH ₃	C-1,14	60.6, 59.6	60.7, ^{b)} 60.3 ^{b)}	60.6, 59.6	60.8, 59.7	61.2, ^{b)} —	60.7, —	60.2, 59.8	60.5, 59.8
	C-2,13	61.0, —	61.1, —	61.0, —	61.0, —	61.4, ^{b)} —	61.0, —	—, —	—, —
	C-3,12	56.0, —	56.0, —	56.0, —	56.0, —	56.1, —	56.0, —	56.2, —	56.1, —
OCH ₂ O	100.8	—	100.9	101.0	101.4	101.9	100.9	100.9	
CO-CH ₃	—	—	—	—	—	167.5, 20.4	—	168.8, 20.6	

a) This compound was measured at 100 MHz. b) Assignments within any column may be reversed.

Fig. 1. NOE in **3** (in C_6D_6)Fig. 2. NOE in **4** (in $CDCl_3$)

signal (δ 4.66, d, $J = 1.5$ Hz). On acetylation, **4** afforded a monoacetate (**4a**), $C_{25}H_{30}O_9$, mp 211–212.5 °C. The 1H -NMR spectrum of **4a** in $CDCl_3$ shows an acetoxyl signal [δ 2.02 (3H, s)] and a methine signal (δ 5.56), which is shifted downfield (0.9 ppm) compared with that of **4**. These findings indicate that **4** possesses a hydroxyl group at the C-9 carbon.

Finally, the structure of **4** was determined by measurements of NOE in $CDCl_3$ as shown in Fig. 2. Irradiation of a methoxyl signal (δ 3.85) caused a 16% increase in the integrated intensity of the higher field aromatic proton signal (δ 6.58, H-4), while the lower field aromatic proton signal (δ 6.90, H-11) was unaffected by irradiation of any methoxyl signal. Irradiation of a secondary methyl signal (δ 0.78, C-8 methyl) caused a 21% increase in the integrated intensity of the H-11 signal, while irradiation of a tertiary methyl signal (δ 1.28, C-7-methyl) did not affect the aromatic proton signals. Irradiation of the C-6 β -methylene proton signal [δ 2.33 (d, $J = 13.5$ Hz)] caused an 18% increase in the integrated intensity of the H-4 signal, while irradiation of the methine proton signal (δ 4.66, H-9 β) did not affect the H-11 signal. On the basis of these NOE data and the J values between H-9 β and H-8 ($J_{8,9\beta} = 1.5$ Hz, $\phi_{8,9} \approx 90^\circ$),¹ the structure of met C was elucidated as **4**, possessing a twist-boat-chair conformation of the cyclooctadiene ring. This structure was supported by a comparison of the ^{13}C -NMR spectrum of **4** with that of **1** (Table II).

Met A-I (**5**) was obtained as a white amorphous powder, $[\alpha]_D + 37^\circ$ ($CHCl_3$), and its molecular formula was determined to be $C_{22}H_{26}O_7$ from the high-resolution MS. Its 1H -NMR spectrum (Table I) is extremely similar to that of **1**, except for the functional groups on the aromatic rings. Three methoxyl signals (δ 3.56, 3.92×2), a methylenedioxy signal [δ 6.00, 6.03 (each 1H, d, $J = 1.5$ Hz)] and a phenolic hydroxyl signal (δ 5.26) were seen in the 1H -NMR spectrum of **5** in $CDCl_3$. On acetylation, **5** afforded a monoacetate (**5a**) as a white amorphous powder, $C_{24}H_{28}O_8$. The 1H -NMR spectrum [δ 1.98 (3H, s)] and IR spectrum ($\nu_{C=O}$ 1776 cm^{-1}) of **5a** show the presence of an aromatic acetoxyl group. These data indicate that **5** corresponds to the demethylated derivative of **1**. The position of the phenolic hydroxyl group in **5** was determined by 1H - and ^{13}C -NMR spectral analysis of **5**, **5a**, and **1**. In the 1H -NMR spectrum of **5**, a higher field methoxyl signal (δ 3.56), shielded by the aromatic rings,^{5,6} is assigned to the methoxyl group at the C-1 position. The ^{13}C -NMR spectrum of **5** shows the methoxyl signal at δ 56.1, which is assignable to the methoxyl group at the C-3 position.^{4,6} In order to define the position of the hydroxyl group in **5**, the ^{13}C -NMR spectrum of **5a** was compared with that of **1**. Namely, in the ^{13}C -NMR spectrum of **5a**, the protonated aromatic carbon signal at δ 110.6 which appears at the same region as C-4 of **1** (δ 110.4), can be assigned to C-4. On the other hand, that at δ 109.1 can be assigned to C-11. The C-11 signal of **5a** shows a downfield shift of 3.2 ppm compared with the signal of **1** (δ 105.9). These findings suggest the presence of a hydroxyl group at the C-14 position (*para* position relative to H-11)⁶ in **5**. In fact, the C-14 and C-13 signals of **5a** are reasonably assigned as shown in Table

II, and show an upfield shift ($\Delta\delta -9.9$ ppm) and a downfield shift ($\Delta\delta +2.7$ ppm), respectively, compared with the signals of **1**, while other signals appear at the same regions as those of **1**. These carbon shifts are consistent with those of (+)-gomisin M₂ (**7**) possessing a hydroxyl group at the *ortho* position relative to the methylenedioxy moiety, (+)-gomisin M₂ (**7**), (+)-gomisin M₂-acetate (**7a**), and (+)-gomisin M₂-methyl ether (**7b**).⁶⁾ On the basis of the above observations as well as the carbon assignments of **5**, the position of a phenolic hydroxyl group in **5** is concluded to be at the C-14 position. Thus, the structure of met A-I was elucidated as **5**.

Met A-III (**6**) was obtained as a white amorphous powder, $[\alpha]_D +65^\circ$ (CHCl₃), and its molecular formula was determined to be the same (C₂₂H₂₆O₇) as that of met A-I (**5**) from the high-resolution MS. Its ¹H-NMR spectrum indicates that **6** is a dibenzocyclooctadiene lignan possessing the same functional groups as **5**, namely, three methoxyl groups (δ 3.40, 3.83, 3.94), a methylenedioxy moiety (δ 5.97) and a phenolic hydroxyl group (δ 5.60) on the aromatic rings, and a tertiary methyl group (C-7 methyl) attached to a carbon carrying a hydroxyl group and a secondary methyl group (C-8 methyl) on the cyclooctadiene ring. On acetylation, **6** afforded a monoacetate (**6a**) as a white amorphous powder, C₂₄H₂₈O₈. The ¹H-NMR [δ 2.35 (3H, s)] and IR ($\nu_{C=O}$ 1766 cm⁻¹) spectra of **6a** show the presence of an aromatic acetoxyl group. These facts indicate that **6** corresponds to the demethylated derivative of **1** as well as **5**. The position of a phenolic hydroxyl group in **6** was determined by ¹H-NMR (Table I) and ¹³C-NMR (Table II) analyses of **6**, **6a**, and **1**. Assignments of the carbon signals of **6** were confirmed by the NOESY spectrum (Fig. 3) and the difference NOE spectrum, and by correlation spectroscopy *via* long-range coupling (COLOC)⁷⁾ (the J_{CH} parameter was set at 6.25 Hz: three-bond correlation) (Fig. 4). In the ¹H-NMR spectrum of **6**, the existence of a higher field methoxyl signal (δ 3.40)^{5,6)} shielded by the aromatic rings indicates that **6** has a methoxyl group at the C-1 position on the aromatic rings. A higher field methyl signal (δ 56.2)^{4,6)} in the ¹³C-NMR spectrum of **6** is assigned to the methoxyl group at the C-3 position. The ¹H-¹³C shift-correlated spectrum shows a strong cross peak owing to the three-

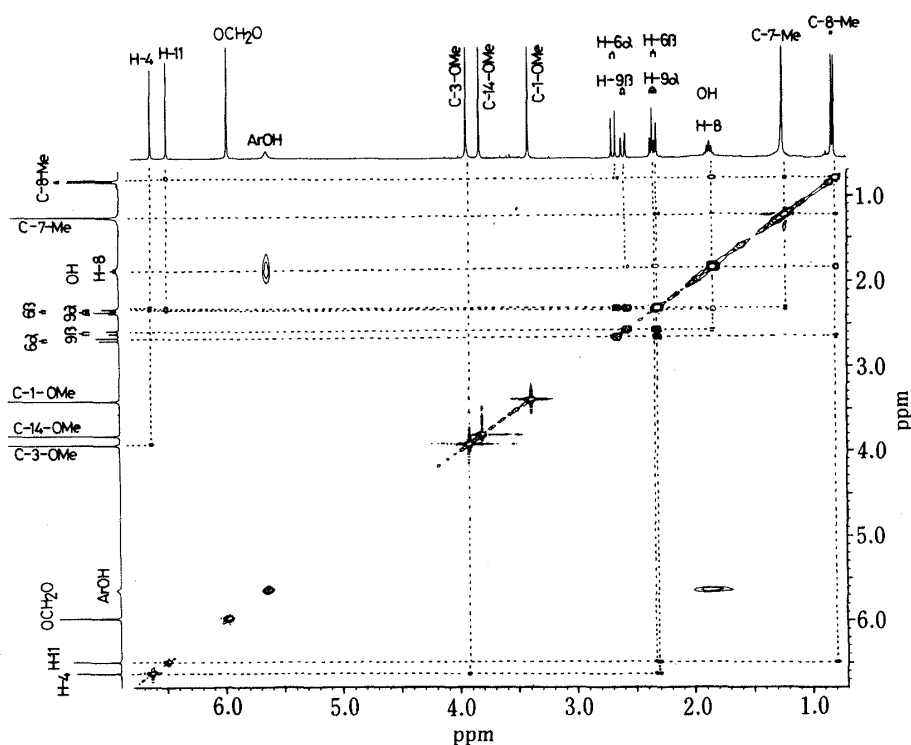


Fig. 3. NOESY Spectrum of **6** in CDCl₃

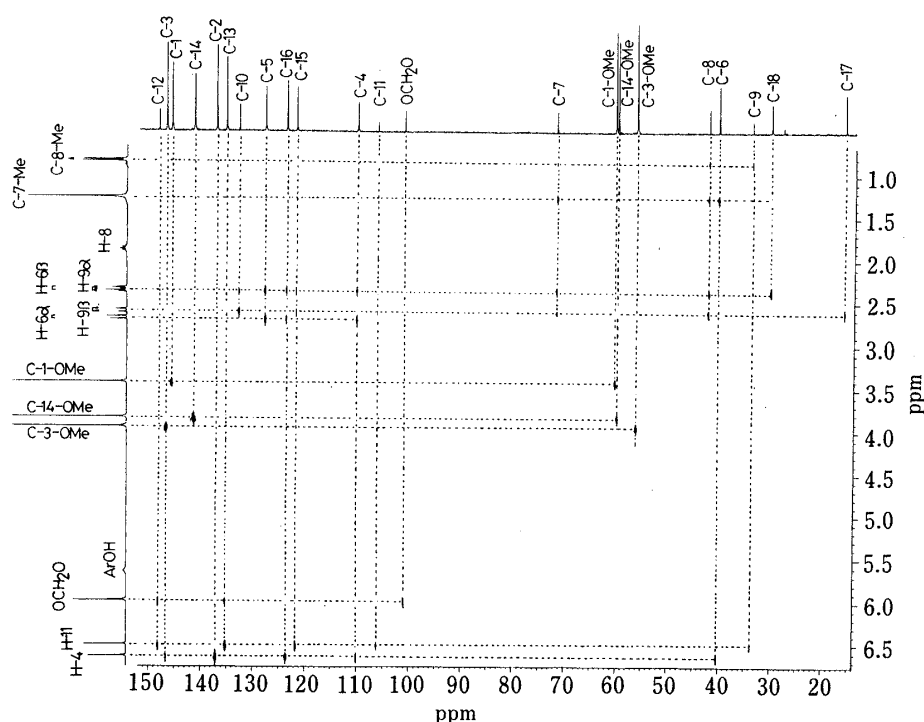


Fig. 4. Contour Map of the ^1H - ^{13}C Shift-Correlated Spectrum of **6** obtained by COLOC (in CDCl_3)

The $J_{\text{CH para}}$ meter was set at 6.25 Hz.

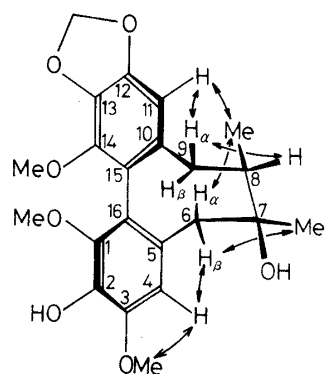


Fig. 5. NOE in the NOESY Spectrum of **6**

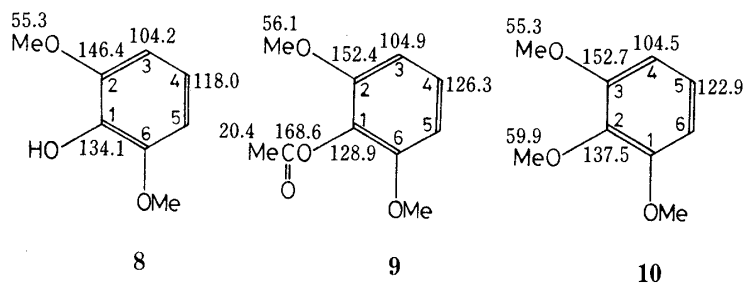


Fig. 6. ^{13}C -NMR Data for **8**–**10** in CDCl_3

Data for **8** and **10** are those reported by Wenkert *et al.*⁸⁾

bond correlation between the H-4 signal (δ 6.63) and the quaternary aromatic carbon signal at δ 137.0, which is assignable to the C-2 signal. The C-2 shifts of **6** and **6a** show upfield shifts of 3.8 and 9.1 ppm, respectively, compared with the C-2 shift of **1**. These shift values correspond with those in model compounds [2,6-dimethoxyphenol (**8**),⁸⁾ 2,6-dimethoxyphenylacetate (**9**),

1,2,3-trimethoxybenzene (**10**)^{8]} as shown in Fig. 6. Namely, the C-1 shifts of **8** and **9** show upfield shifts of 3.4 and 8.6 ppm respectively, compared with the C-2 shift of **10**. The C-1, C-3, and C-5 signals of **6** are also reasonably assigned as shown in Table II, and show upfield shifts of 6.5, 5.7, and 4.5 ppm, respectively, while other signals appear at the same regions as those of **1**. The above observations indicate that the phenolic hydroxyl group in **6** is linked to C-2. The NOESY spectral data and the J values of the C-9 methylene and C-8 methine protons ($J_{8,9\beta} = 2$ Hz, $\phi_{8,9\beta} \approx 90^\circ$; $J_{8,9\alpha} = 7$ Hz, $\phi_{8,9\alpha} \approx 150^\circ$)¹⁾ indicate that **6** possesses a twist-boat-chair conformation of the cyclooctadiene ring. Thus, the structure of met A-III was elucidated as **6**.

Experimental

All melting points were determined on a Yanagimoto micromelting point apparatus (a hot-stage type) and are uncorrected. The ultraviolet (UV) spectra were recorded with a Hitachi U-3200 spectrophotometer and the IR spectra with a Hitachi 270-30 infrared spectrophotometer. The ¹H-NMR and ¹³C-NMR spectra were recorded with Bruker AM-400 and JEOL JNM-FX-200 NMR spectrometers using tetramethylsilane (TMS) as an internal standard. The specific rotations were measured with a JASCO DIP-360 digital polarimeter and the mass spectra with a JEOL JMS-DX 300 mass spectrometer. The CD spectrum was recorded with a JASCO J-40. For silica gel column chromatography, Kieselgel 60 (Merck) was used. Kieselgel 60 F₂₅₄ (Merck precoated plate) was used for preparative thin layer chromatography (prep. TLC) and spots were detected under UV (254 nm).

Preparation of S9 Mix. (the 9000 × *g* Supernatant Fraction of Rat Liver Homogenate) and Oxidation of **1 by S9 Mix.**—Male Wistar rats weighing about 200 g were given phenobarbital (80 mg/kg/d, i.p., 3 d) for the induction of drug-metabolizing enzymes. About 24 h after the last administration of phenobarbital, the livers were quickly excised and perfused with 1.15% potassium chloride solution (in 0.1 M phosphate buffer). Next, the livers were homogenized in an amount of 1.15% potassium chloride solution (in 0.1 M phosphate buffer) equivalent to three times the liver weight. After centrifugation of the homogenate at 9000 × *g*, the supernatant fraction (1800 ml) was collected. The supernatant fraction and 40 mM reduced nicotinamide adenine dinucleotide phosphate (NADPH) solution (600 ml) (S9 mix.) were added to 5 mM compound **1** solution (in 2% Tween 80) (600 ml). The mixture was incubated at 37 °C for 24 h and then extracted with ether (3000 ml) twice. The ethereal extract was concentrated to give a crude product (13.76 g), which was chromatographed on silica gel (3 cm i.d. × 20 cm) with a mixture of hexane–EtOAc. The details of this chromatography are given in Table III.

Isolation of **1**—Fraction (Fr.) 2 (784 mg) in Table III was purified by prep. TLC [benzene–ether (3 : 2), *R_f* 0.65] to give **1** (724 mg) as colorless needles, mp 93–94 °C. Compound **1** was identified as gomisin A¹⁾ by direct comparison (¹H-NMR and mixed melting point) with an authentic sample.

Isolation of **2, **3**, **4**, **5**, **6****—Fr. 4 (152 mg) was separated by prep. TLC [hexane–EtOAc (1 : 2), *R_f* 0.45] to give a mixture of **2** and **3**. The mixture was purified by prep. TLC [hexane–acetone (3 : 2)] to give **2** (*R_f* 0.56, 63.2 mg) and **3** (*R_f* 0.68, 27 mg). Repeated prep. TLC [i) hexane–EtOAc (1 : 2), *R_f* 0.35; ii) benzene–ether (3 : 7), *R_f* 0.1] of Fr. 5 (200 mg) gave **4** (10.4 mg). Fr. 3 (40 mg) was separated by prep. TLC [hexane–EtOAc (1 : 2)] to give crude **5** (*R_f* 0.5) and crude **6** (*R_f* 0.45). Crude **5** was purified by prep. TLC [benzene–ether (1 : 2), *R_f* 0.35] to give **5** (10 mg). Crude **6** was purified by prep. TLC [benzene–ether (1 : 2), *R_f* 0.28] to give **6** (5 mg).

Met B (2**)**—Met B was obtained as pale brown prisms (from ether), mp 194–196 °C, $[\alpha]_D^{23} + 92^\circ$ ($c = 0.41$, CHCl₃). IR $\nu_{\text{max}}^{\text{KBr}}$ cm^{−1}: 3524, 3384 (OH), 1616, 1592 (aromatic ring). MS m/z (%): 404 (M^+ , 100), 386 (10), 361 (6.8), 333 (14), 329 (13). High-resolution MS, Calcd for C₂₂H₂₈O₇ (M^+): 404.1835. Found: 404.1880. This was identified as the demethylenated derivative (**2**)³⁾ of **1** by direct comparison (IR, ¹H-NMR, $[\alpha]_D$ and mixed melting point).

Met A-II (3**)**—Met A-II was obtained as colorless prisms (from EtOH), mp 163–165 °C, $[\alpha]_D^{24} + 68^\circ$ ($c =$

TABLE III. Silica Gel Column Chromatography of Metabolites of **1**

Fraction No.	Solvent	Volume (ml)	Yield (mg)
1	Hexane–EtOAc (60 : 40)	360	3992
2	Hexane–EtOAc (40 : 60)	200	784
3	Hexane–EtOAc (40 : 60)	120	40
4	Hexane–EtOAc (40 : 60)	360	152
5	Hexane–EtOAc (10 : 90)	160	200
6	Hexane–EtOAc (10 : 90)	100	8
7	EtOAc	200	—

0.366, CHCl_3). IR $\nu_{\text{max}}^{\text{KBr}}$ cm^{-1} : 3348 (OH), 1620, 1598 (aromatic ring). $\nu_{\text{max}}^{\text{CCl}_4}$ cm^{-1} : 3548 (OH). UV $\lambda_{\text{max}}^{\text{EtOH}}$ nm (log ϵ): 221 (4.55), 256 (4.01), 293 (sh 3.37). MS m/z (%): 432 (M^+ , 74), 414 ($\text{M}^+ - \text{H}_2\text{O}$, 17), 389 (75), 345 (100), 315 (98). High-resolution MS, Calcd for $\text{C}_{23}\text{H}_{28}\text{O}_8$ (M^+): 432.1784. Found: 432.1785. CD ($c=0.0102$, MeOH) $[\theta]^{24}$: -79000 (224), +64000 (241), +81000 (255). Anal. Calcd for $\text{C}_{23}\text{H}_{28}\text{O}_8 \cdot 1/2\text{H}_2\text{O}$: C, 62.57; H, 6.62. Found: C, 62.58; H, 6.67.

Met C (4)—Met C was obtained as a white amorphous powder, $[\alpha]_{\text{D}}^{24} + 24^\circ$ ($c=0.25$, CHCl_3). IR $\nu_{\text{max}}^{\text{KBr}}$ cm^{-1} : 3444 (OH), 1620, 1596 (aromatic ring). UV $\lambda_{\text{max}}^{\text{EtOH}}$ nm (log ϵ): 218 (4.52), 254 (sh 3.84), 288 (sh 3.30). MS m/z (%): 432 (M^+ , 33), 414 (15), 360 (26), 343 (25), 83 (100). High-resolution MS, Calcd for $\text{C}_{23}\text{H}_{28}\text{O}_8$ (M^+): 432.1784. Found: 432.1725.

Met A-I (5)—Met A-I was obtained as a white amorphous powder, $[\alpha]_{\text{D}}^{24} + 37^\circ$ ($c=0.27$, CHCl_3). IR $\nu_{\text{max}}^{\text{KBr}}$ cm^{-1} : 3416 (OH), 1630, 1596 (aromatic ring). UV $\lambda_{\text{max}}^{\text{EtOH}}$ nm (log ϵ): 218 (4.51), 254 (sh 3.95), 288 (sh 3.28). MS m/z (%): 402 (M^+ , 100), 331 (9), 327 (25), 318 (14), 299 (41). High-resolution MS, Calcd for $\text{C}_{22}\text{H}_{26}\text{O}_7$ (M^+): 402.1679. Found: 402.1629.

Met A-III (6)—Met A-III was obtained as a white amorphous powder, $[\alpha]_{\text{D}}^{24} + 65^\circ$ ($c=0.34$, CHCl_3). IR $\nu_{\text{max}}^{\text{KBr}}$ cm^{-1} : 3448 (OH), 1616 (aromatic ring). UV $\lambda_{\text{max}}^{\text{EtOH}}$ nm (log ϵ): 218 (4.50), 259 (sh 3.90), 280 (3.60). High-resolution MS, Calcd for $\text{C}_{22}\text{H}_{26}\text{O}_7$ (M^+): 402.1579. Found: 402.1591. MS m/z (%): 402 (M^+ , 100), 331 (9), 327 (31), 318 (16), 299 (50).

Oxidation of 3 with Sodium Periodate—A solution of sodium periodate (14 mg) in 1 N H_2SO_4 (0.6 ml) was added to a solution of **3** (12 mg) in EtOH (0.8 ml). The mixture was stirred at room temperature for 3 h, diluted with H_2O and extracted with ether. The ethereal extract was washed with H_2O , dried over Na_2SO_4 , and concentrated to dryness. The residue was purified by prep. TLC [hexane-acetone (3:2)] to give **3a** (9 mg) as a pale yellow oil, $[\alpha]_{\text{D}}^{26} + 11^\circ$ ($c=0.45$, CHCl_3). IR $\nu_{\text{max}}^{\text{KBr}}$ cm^{-1} : 1720 (C=O), 1616, 1600 (aromatic ring). MS m/z (%): 430 (M^+ , 52), 402 (8.1), 287 (9.1), 369 (11), 355 (27), 330 (100). High-resolution MS, Calcd for $\text{C}_{23}\text{H}_{26}\text{O}_8$ (M^+): 430.1628. Found: 430.1661. $^1\text{H-NMR}$ (in CDCl_3 , 200 MHz) δ : 1.95, 2.00 (each 3H, s, $2 \times \text{CH}_3$), 3.28 (2H, d, $J=1$ Hz, ArCH_2-), 3.35 (2H, s, ArCH_2-), 3.60 (3H, s, OCH_3), 3.85 (3H, s, OCH_3), 3.88 (6H, s, $2 \times \text{OCH}_3$), 5.98 (2H, s, $-\text{OCH}_2\text{O}-$), 6.48, 6.56 (each 1H, s, $2 \times$ aromatic H). $^{13}\text{C-NMR}$ (in CDCl_3 , 50 MHz) δ : 29.6, 29.7 (each q, $2 \times \text{CH}_3$), 47.5, 47.7 (each t, $2 \times \text{ArCH}_2-$), 55.9, 59.2, 60.6, 60.9 (each q, $4 \times \text{OCH}_3$), 101.0 (t, OCH_2O), 104.7, 109.1 (each d, $2 \times$ aromatic C), 121.7, 123.0, 128.7, 130.0, 135.0, 141.0, 141.2, 148.9, 151.7, 152.9 (each s, $10 \times$ aromatic C), 206.3, 206.4 (each s, $2 \times \text{C=O}$).

Acetylation of 4—A solution of **4** (4 mg) in a mixture of pyridine (0.2 ml) and Ac_2O (0.15 ml) was allowed to stand at room temperature overnight, then diluted with H_2O and extracted with ether. The ethereal extract 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-EtOAc (3:2)] to give **4a** (3.5 mg) as colorless needles (from MeOH), mp 211–212.5°C. IR $\nu_{\text{max}}^{\text{KBr}}$ cm^{-1} : 3504 (OH), 1744 (C=O), 1622, 1596 (aromatic ring). MS m/z (%): 474 (M^+ , 17), 456 ($\text{M}^+ - \text{H}_2\text{O}$, 26), 414 ($\text{M}^+ - \text{CH}_3\text{COOH}$, 62), 396 (21), 343 (75), 236 (100). High-resolution MS, Calcd for $\text{C}_{25}\text{H}_{30}\text{O}_9$ (M^+): 474.1890. Found: 474.1853.

Acetylation of 5—A solution of **5** (7 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 the acetylation of **4** to give **5a** (4.7 mg) as a white amorphous powder. IR $\nu_{\text{max}}^{\text{KBr}}$ cm^{-1} : 3540 (OH), 1776 (C=O), 1598 (aromatic ring). MS m/z (%): 444 (M^+ , 100), 402 (96), 331 (20), 327 (32), 300 (30). High-resolution MS, Calcd for $\text{C}_{24}\text{H}_{28}\text{O}_8$ (M^+): 444.1784. Found: 444.1806.

Acetylation of 6—A solution of **6** (5 mg) in a mixture of pyridine (0.25 ml) and Ac_2O (0.15 ml) was allowed to stand at room temperature overnight. The reaction mixture was treated as described for the acetylation of **4** to give **6a** (4.2 mg) as a white amorphous powder, $[\alpha]_{\text{D}}^{24} + 43^\circ$ ($c=0.39$, CHCl_3). IR $\nu_{\text{max}}^{\text{KBr}}$ cm^{-1} : 3496 (OH), 1766 (C=O), 1610 (aromatic ring). MS m/z (%): 444 (M^+ , 7), 426 (42), 384 ($\text{M}^+ - \text{CH}_3\text{COOH}$, 100), 295 (15), 217 (13).

Preparation of 9—A solution of 2,6-dimethoxyphenol (0.290 g, Kokusan Chemical Works Ltd., Tokyo) in a mixture of pyridine (1 ml) and Ac_2O (0.5 ml) was allowed to stand at room temperature overnight, then diluted with H_2O and extracted with ether. The ethereal extract was washed with 1 N HCl, 5% NaHCO_3 , then with H_2O , dried over Na_2SO_4 and concentrated. The residue was purified by prep. TLC [hexane-EtOAc (3:1)] to give **9** (0.278 g) as a colorless oil. FD-MS m/z : 196 (M^+). $^1\text{H-NMR}$ (in CDCl_3 , 200 MHz) δ : 2.34 (3H, s, COCH_3), 3.82 (6H, s, $2 \times \text{OCH}_3$), 6.61 (2H, d, $J=8$ Hz, H-3, H-5), 7.13 (1H, dd, $J=8, 8$ Hz, H-4). $^{13}\text{C-NMR}$ (in CDCl_3 , 50 MHz): Fig. 6.

Acknowledgements The authors wish to express their gratitude to Dr. M. R. Wälichli, Bruker Japan Co., Ltd., for measurements of the ^1H - ^{13}C COSY, the NOESY, and the difference NOE spectra and for his kind advice on the NMR spectral data. Thanks are also due to Prof. T. Inoue and Dr. N. Sakurai, Hoshi University, for measurements of the CD spectra, Mr. K. Sato, Meiji College of Pharmacy, for elemental analysis, Dr. K. Niitsu, Tsumura Laboratory, for measurements of NOE, and Mr. I. Sakakibara, Tsumura Laboratory, for measurements of MS.

References and Notes

- 1) Y. Ikeya, H. Taguchi, I. Yosioka, and H. Kobayashi, *Chem. Pharm. Bull.*, **27**, 1383 (1979).

-
- 2) a) S. Maeda, K. Sudo, M. Aburada, Y. Ikeya, H. Taguchi, I. Yosioka, and M. Harada, *Yakugaku Zasshi*, **101**, 1030 (1981); b) S. Maeda, K. Sudo, Y. Miyamoto, S. Takeda, M. Shinbo, M. Aburada, Y. Ikeya, H. Taguchi, and M. Harada, *ibid.*, **102**, 579 (1982); c) Y. Kiso, M. Tohkin, H. Hikino, Y. Ikeya, and H. Taguchi, *Planta Medica*, **1985**, 291; d) S. Maeda, S. Takeda, Y. Miyamoto, M. Aburada, and M. Harada, *Jpn. J. Pharmacol.*, **38**, 347 (1985); e) S. Takeda, S. Maemura, K. Sudo, Y. Kase, I. Arai, Y. Ohkura, S. Funo, Y. Fujii, M. Aburada, and E. Hosoya, *Folia Pharmacol. Japon.*, **87**, 169 (1986); f) S. Takeda, Y. Kase, I. Arai, M. Hasegawa, Y. Sekiguchi, S. Funo, M. Aburada, E. Hosoya, Y. Mizoguchi, and S. Morisawa, *ibid.*, **88**, 321 (1986).
 - 3) Y. Ikeya, H. Taguchi, and I. Yosioka, *Chem. Pharm. Bull.*, **29**, 2893 (1981).
 - 4) Y. Ikeya, H. Taguchi, H. Sasaki, K. Nakajima, and I. Yosioka, *Chem. Pharm. Bull.*, **28**, 2414 (1980).
 - 5) Y. Ikeya, H. Taguchi, and I. Yosioka, *Chem. Pharm. Bull.*, **28**, 2422 (1980).
 - 6) Y. Ikeya, H. Taguchi, and I. Yosioka, *Chem. Pharm. Bull.*, **30**, 132 (1982).
 - 7) H. Kessler, C. Griesinger, J. Zarbock, and H. R. Loosli, *J. Magn. Reson.*, **57**, 331 (1984).
 - 8) E. Wenkert, H. E. Gottlieb, O. T. Gottlieb, M. O. S. Pereira, and M. P. Formig, *Phytochemistry*, **15**, 1547 (1976).