New Steroid and Matairesinol Glycosides from Safflower (*Carthamus tinctorius* L.) Oil Cake

Akito NAGATSU,*[,a] Hui Li ZHANG,[a] Toshihiro WATANABE,[a] Nari TANIGUCHI,[a] Keiichiro HATANO,[a] Hajime MIZUKAMI,[a] and Jinsaku SAKAKIBARA[b]

Faculty of Pharmaceutical Sciences, Nagoya City University,[a] Tanabe-dori, Mizuho-ku, Nagoya 467–8603, Japan and Faculty of Pharmacy, Meijo University,[b] Yagotoyama, Tempaku-ku, Nagoya 468–8503, Japan.
Received January 7, 1998; accepted March 6, 1998

(15α,20*R*)-Dihydroxypregn-4-en-3-one 6′-*O*-acetyl-20-β-cellobioside (1) and matairesinol 4′-*O*-β-D-apiofuranosyl(1→2)-β-D-glucopyranoside (2) were isolated from safflower (*Carthamus tinctorius* L.) oil cake. The structures were confirmed by spectroscopic methods and X-ray crystal structure analysis. The ^{1}H- and ^{13}C-NMR signals of (15α,20*R*)-dihydroxypregn-4-en-3-one (1a) were assigned.

Key words *Carthamus tinctorius*; pregnan cellobioside; matairesinol glycoside; safflower oil cake

In the course of our investigation of antioxidants from oil cakes, we described the antioxidative serotonin derivatives, including three novel dimers, from safflower oil cake.[1,2] In a further investigation of the components in the oil cake, we have found new steroid and matairesinol glycosides (**1** and **2**) as major constituents. In the present paper, we report the isolation and identification of **1** and **2** (Fig. 1).

Safflower (*Carthamus tinctorius* L.) oil cake was extracted with MeOH and subjected to solvent partition. This was followed by fractionation of the AcOEt phase to give **1** (0.35%) and **2** (0.11%) together with serotonin derivatives, as described in the experimental section.

Compound **1** was isolated as a colorless amorphous powder, and formulated as $C_{35}H_{54}O_{14}$ from high-resolution (HR) FAB-MS. The IR spectrum of **1** suggested the presence of CO (1660 cm^{-1}), O–CO (1740 cm^{-1}), and OH (3400 cm^{-1}). The UV absorption at 241 nm (ε 15700) and the carbonyl carbon signal at δ_C 202.4 in the ^{13}C-NMR spectrum revealed the presence of an αβ-unsaturated ketone. The methyl signal at δ 2.05 and the carbon signal at δ_C 172.5 indicated an *O*-acetyl group. Enzymatic hydrolysis of **1** furnished aglycone and glucose. The latter was identified by GLC analysis of its silylated derivative. The aglycone was confirmed by X-ray crystal structure analysis as (15α,20*R*)-dihydroxypregn-4-en-3-one (**1a**).[3] The full NMR assignment of **1a** was achieved as shown in Table 1. The connectivity of two glucosyl moieties and an *O*-acetyl group to **1a** was determined from NMR spectra including 2D experiments (^{1}H–^{1}H correlation spectroscopy (COSY), ^{13}C–^{1}H COSY, and heteronuclear multiple bond correlation (HMBC, $^{3,4}J_{C-H}$=8.3 Hz) spectra). The HMBC correlation from an anomeric proton (δ 4.38) to the C-20 methine carbon (δ_C 75.8) indicated that one of the glucoses is connected to the 20-OH of **1a**. The other anomeric proton (δ 4.36) was correlated to the carbon signal at δ_C 81.5, which is assignable to the C-4 of the first glucose. The configurations of C-1′ and C-1″ of the glucoses were determined as β from the large coupling constants of 1′-H and 1″-H (both J=7.9 Hz) in the ^{1}H-NMR spectrum of **1**. The signals of the methylene protons at δ 4.33 and 4.61 were correlated to the carbonyl carbon signal (δ_C 172.5) in the HMBC, and had straight-forward connectivities to H-4′ (δ 3.55), which indicated that the acetyl group is at the 6′-*O* position. Thus **1** was established as the 6′-*O*-acetyl-20-β-cellobioside of **1a**. Although the 20-β-cellobioside of **1a** has already been reported from safflower,[4] its 6′-*O*-acetate (**1**) has not. The presence of **1** as one of the major components in safflower oil cake was shown by TLC of the MeOH extract.

Compound **2** was obtained as a colorless amorphous powder, and formulated as $C_{31}H_{40}O_{15}$ from HR-FAB-MS. The IR spectrum of **2** indicated the presence of an

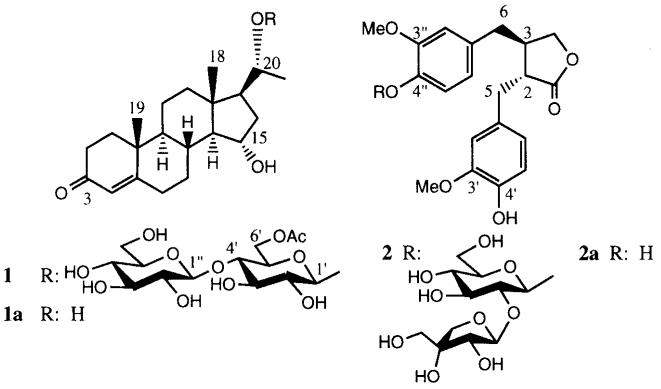

Fig. 1. Structures of **1**, **1a**, **2** and **2a**

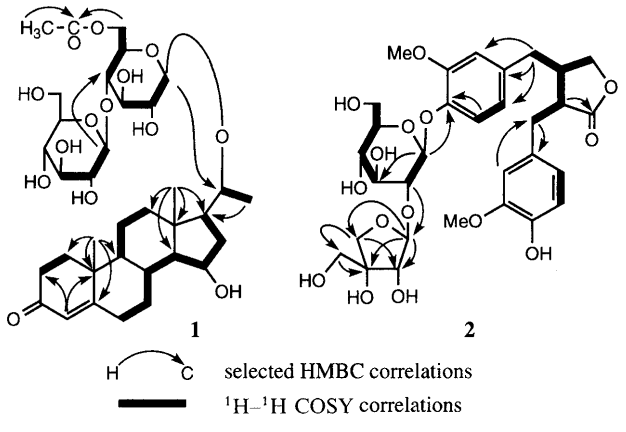

Fig. 2. ^{1}H–^{1}H COSY and Selected HMBC ($^{3,4}J_{C-H}$=8.3 Hz) Correlations of **1** and **2**

* To whom correspondence should be addressed.

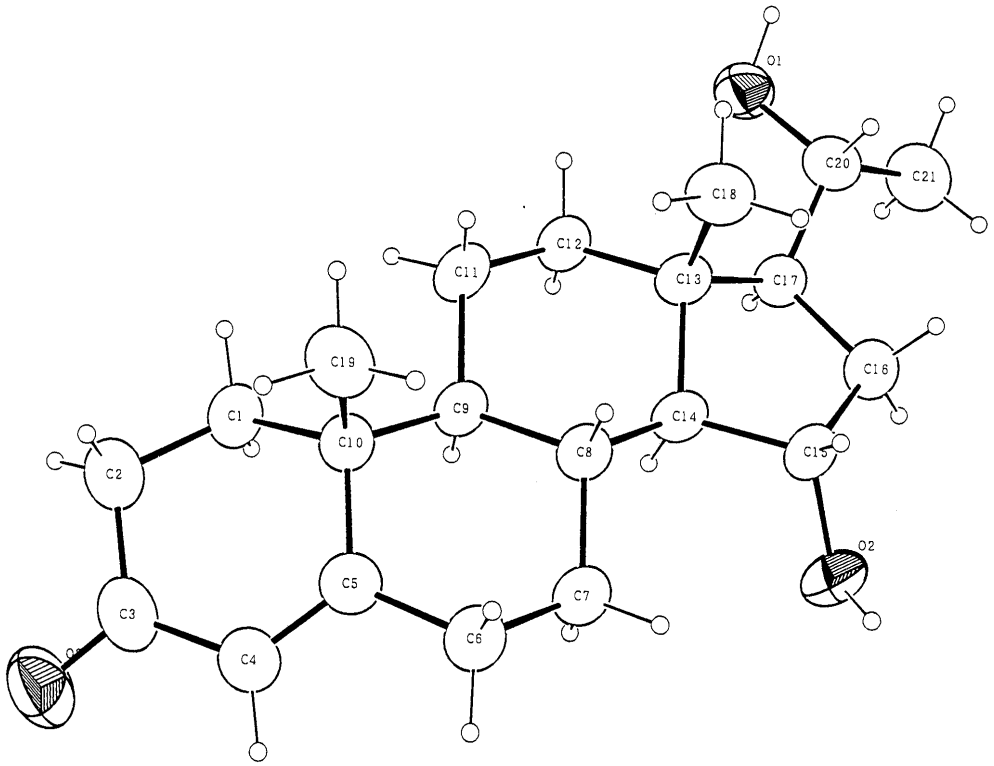

Fig. 3. Perspective View of a Molecule of **1a** with Atomic Labels and Thermal Ellipsoids at 50% Probability for Non-hydrogen Atoms

ester carbonyl group (1760 cm^{-1}). The ^{1}H-NMR spectra revealed the presence of two trisubstituted phenyl and two methoxy groups, and six methines and four methylenes attached to oxygen atoms. The sugar moieties were suggested to be glucose and an apiose from the ^{13}C-NMR spectrum. Acid hydrolysis of **2** gave the aglycone (**2a**), which was identical with the ^{1}H-NMR spectrum of matairesinol and the optical rotation was similar to that of an authentic sample.[5] The correlations observed in COSY, ^{13}C–^{1}H COSY, and HMBC experiments revealed the connectivity of the sugars and **2a** (Fig. 2). The long-range H–C correlations from the anomeric proton (δ 5.53) to the sp^2 carbon (δ_C 146.5) and the methine proton (δ 4.56) to the anomeric carbon (δ_C 110.4) showed that the glucose was connected at the C-4′ of matairesinol and the apiose was at the C-2 of the glucose moiety. The anomeric configuration of glucose was β from the coupling constant of the anomeric proton signal (δ 5.53, J=7.9 Hz) and that of apiose was also β from the ^{13}C-NMR data of the C-1 and C-2 of apiose.[6] Thus, **2** was determined as matairesinol 4′-*O*-β-D-apiofuranosyl(1→2)-β-D-glucopyranoside. This is the first example of a matairesinol glycoside possessing sugar moieties other than glucose.

Antioxidative activity of **1** and **2** was evaluated by the ferric thiocyanate method,[2] but both were inactive. Compound **2a** showed moderate activity, but the activity was reduced by the sugar on one of phenolic groups. Other biological activities such as the anti-tumor-promoting activity of **1** and **2** will be reported together with other components of safflower and cotton oil cakes in the future.

Experimental

General All melting points were determined on a Yanagimoto micro melting point apparatus and are uncorrected. The FAB-MS and HR FAB-MS were measured with a JEOL JMS DX-505 or SX-102 mass spectrometer, the IR spectra with a JASCO IRA-2 spectrometer and the UV spectra with a JASCO V-530 spectrophotometer. The ^{1}H-NMR spectra were measured with JEOL α-500 or Lambda 500 spectrometers using tetramethylsilane as an internal standard. Optical rotations were measured on a JASCO DIP-140 digital polarimeter. HPLC was carried out using a JASCO 980-PU pump, UV-970 UV detector, and RI-930 differential refractometer. TLC was carried out on precoated plates (Kieselgel 60F_{254}, 0.25 mm thick, Merck No. 5715), and spots were detected under UV light (254 nm) or by spraying with 1% $Ce(SO_4)_2$–10% H_2SO_4, followed by heating. Column chromatography was performed on Silica gel BW-200 (Fuji Davison Chemicals Co., Ltd.).

Isolation Safflower oil cake (600 g) was extracted with hot MeOH (1 l × 5). The extract was washed with iso octane, and after the MeOH layer was evaporated, the residue was partitioned between hexane and 80% aqueous MeOH. The 80% aqueous MeOH layer was further partitioned between EtOAc and H_2O, and the EtOAc layer was evaporated to dryness (23.4 g). The residue was subjected to SiO_2 column chromatography ($CHCl_3$–MeOH=10:1→$CHCl_3$–MeOH–H_2O=10:3:1 (lower layer) to separate serotonin derivatives,[2] followed by repeated SiO_2 column chromatography ($CHCl_3$–MeOH–H_2O=6:4:1, EtOAc–MeOH–H_2O=14:2:1) to give **1** (209 mg) as a colorless amorphous powder and the crude fraction of **2** (162.3 mg). The crude fraction of **2** was further chromatographed on SiO_2 (EtOAc–MeOH–H_2O=14:2:1) and finally purified by reversed-phase HPLC (YMC Pack AQ-314 ODS, MeOH–H_2O=4:6) to give compound **2** (66.5 mg) as a colorless amorphous powder.

(15α,20*R*)-Dihydroxypregn-4-en-3-one 6′-*O*-Acetyl-20-β-cellobioside (1) mp 145—146 °C. $[\alpha]_D^{18}$=+57.9° (c=0.60, MeOH). IR (KBr, cm^{-1}): 1660 (CO), 1740 (O–CO), 3400 (OH). UV (λ_{max}, nm (ε), MeOH): 241 (15700). HR-FAB-MS m/z: Calcd for $C_{35}H_{54}O_{14}Na$: 721.3411. Found: 721.3424. ^{1}H- and ^{13}C-NMR: Table 1.

Matairesinol 4′-*O*-β-D-Apiofuranosyl(1→2)-β-D-glucopyranoside (2) mp 115—116.5 °C. $[\alpha]_D^{18}$=−86.3° (c=0.46, MeOH). IR (KBr, cm^{-1}): 1760 (O–CO), 3400 (OH). UV (λ_{max}, nm (ε), MeOH): 229 (6900), 280 (2500). HR-FAB-MS m/z: Calcd for $C_{31}H_{40}O_{15}Na$; 675.2265. Found: 675.2225. ^{1}H- and ^{13}C-NMR: Table 2.

Enzymatic Hydrolysis of 1 The solution of **1** (56.2 mg) and naringinase

Table 1. ^{13}C- and ^{1}H-NMR Spectral Data for (15α,20*R*)-Dihydroxypregn-4-en-3-one 6′-*O*-Acetyl-20-β-cellobioside (**1**) and Its Aglycone (**1a**) (^{13}C: 125 MHz, ^{1}H: 500 MHz)

Position	**1** (in CD_3OD)			**1a** (in C_5D_5N)		
	^{13}C	^{1}H		^{13}C	^{1}H	
	δ_C (ppm) (mult.)	δ (ppm)	mult., *J* (Hz)	δ_C (ppm) (mult.)	δ (ppm)	mult., *J* (Hz)
1	36.9 (t)	1.73	m	35.8 (t)	1.45—1.56	m
		2.11	m		1.72—1.82	m
2	34.1 (t)	2.30[b]	m	34.3 (t)	2.36—2.45	m
		2.51[c]	m			
3	202.4 (s)			198.6 (s)		
4	123.9 (d)	5.74	s	123.7 (d)	5.85	s
5	175.6 (s)			171.3 (s)		
6	33.6 (t)[a]	2.30[b]	m	33.1 (t)[a]	1.42[a]	m
		2.51[c]	m		2.19[a]	ddd, 2.6, 3.6, 14.0
7	34.8 (t)[a]	1.22	m	32.9 (t)[a]	2.32[a]	ddd, 1.8, 3.4, 14.0
		2.30[b]	m		2.57[a]	m
8	36.6 (d)	1.82	m	35.6 (d)	1.68—1.78	m
9	55.8 (d)	1.00	dt-like, 3.7, 10.3	54.5 (d)	0.93	br dd, 10.4, 16.5
10	40.1 (s)			38.8 (s)		
11	22.0 (t)	1.45	m	21.1 (t)	1.38—1.56	m
		1.55	m			
12	40.4 (t)	1.22	m	40.5 (t)	1.50	m
		2.30[b]	m		2.63	m
13	44.3 (s)			43.4 (s)		
14	63.3 (d)	1.09	dd, 9.4, 10.3	62.6 (d)	1.42	dd, 9.1, 10.7
15	74.3 (d)	3.95	m	73.3 (d)	4.26	ddd, 4.5, 9.1, 13.5
16	39.0 (t)	1.56	m	39.3 (t)	1.90—2.02[b]	m
		1.88	m			
17	55.0 (d)	1.78	dt-like, 3.7, 10.3	56.5 (d)	1.90—2.02[b]	m
18	13.2 (q)	0.86	s	13.9 (q)	1.01	s
19	17.9 (q)	1.28	s	17.2 (q)	1.06	s
20	75.8 (d)	3.82	m	69.3 (d)	3.88	qd, 6.1, 8.5
21	19.0 (q)	1.14	d, 6.1	24.3 (q)	1.31	d, 6.1
1′	100.9 (d)	4.38	d, 7.9			
2′	74.9 (d)	3.22	m			
3′	76.4 (d)	3.55[d]	m			
4′	81.5 (d)	3.55[d]	m			
5′	73.6 (d)	3.63	m			
6′	64.2 (t)	4.33	dd, 5.3, 11.7			
		4.61	br d, 11.7			
1″	104.9 (d)	4.36	d, 7.9			
2″	74.9 (d)	3.25	dd, 7.9, 9.2			
3″	77.9 (d)	3.39	m			
4″	71.5 (d)	3.35	m			
5″	78.2 (d)	3.33	m			
6″	62.6 (t)	3.69	dd, 6.1, 11.9			
		3.92	dd, 1.8, 11.9			
$\underline{CH_3}CO$	20.9 (q)	2.09	s			
$CH_3\underline{C}O$	172.5 (s)					

a) The assignments may be interchanged in the same virtical column. *b*—*d*) The signals are overwraped with each other in the same virtical column.

(100 mg, from *Penicillium decumbens*, Sigma Co., Ltd., St. Louis, U.S.A.) in acetate buffer (pH 4.0, 2.7 ml) and EtOH (7 ml) was stirred at 40 °C for 8 d. The solvent was removed under reduced pressure, and the residue was chromatographed on SiO_2 to give aglycone **1a** (17.2 mg) and a sugar fraction. The sugar fraction was silylated and subjected to GLC analysis as described previously,[2] and only silylated glucose was detected.

1a: Colorless prisms ($CHCl_3$–hexane), mp 229.5—231 °C. $[\alpha]_D^{18} = +149°$ ($c=0.22$, $CHCl_3$). [lit.[3] mp 222 °C. $[\alpha]_D^{27} = +110°$ ($CHCl_3$)]. ^{1}H- and ^{13}C-NMR: Table 1.[7]

X-Ray Structural Analysis of 1a[8] Crystal data: $C_{21}H_{32}O_3$, M.W. = 332.5, orthorhombic, space group $P2_12_12_1$, $a=7.938$ (1) Å, $b=12.365$ (1) Å, $c=18.431$ (2) Å, $V=1807.0$ (7) Å^3, $Z=4$. $D_c=1.222$ g/cm^3. The diffraction intensities were collected from a crystal with dimensions $0.18\times0.24\times0.24$ mm on an Enraf–Nonius CAD4 diffractometer with graphite-monochromated MoK_α radiation ($\lambda=0.71073$ Å). Of a total of 1855 reflections observed within a 2θ range of 4—50°, 1382 were unique. The structure was solved by the direct method and refined by the difference Fourier and least-squares techniques.[9] The final cycle of full-matrix least-squares refinement was based on 1382 observed reflections ($F_0 > 3.0\sigma(F_0)$) and 219 evaluable parameters. The final *R* value was 0.046 ($R_w=0.044$). The atomic coordinates are shown in Table 3.

Acid Hydrolysis of 2 Compound **2** (11.4 mg) in 10% H_2SO_4 (5 ml) was stirred at 100 °C for 2 h. The resulting mixture was extracted with $CHCl_3$. The $CHCl_3$ layer was washed with brine, dried over Na_2SO_4, and evaporated under reduced pressure. The residue was chromatographed on SiO_2 to give matairesinol (**2a**, 4.4 mg). The ^{1}H-NMR specrum of **2a** is superimposable on the authentic matairesinol.

2a: $[\alpha]_D^{14} = -39.8°$ ($c=0.22$, EtOH). [lit.[5] $[\alpha]_D^{8} = -40°$ (EtOH)].

Acknowledgments The authors thank Prof. S. Nishibe of Health Sciences University of Hokkaido for donating matairesinol. The authors also thank Ms. S. Kato and Ms. K. Takahashi of this faculty for measurement of NMR and FAB-MS spectra. This work was supported

Table 2. ^{13}C- and ^{1}H-NMR Spectral Data for Matairesinol 4′-*O*-*β*-D-Apiofuranosyl(1→2)-*β*-D-glucopyranoside (**2**) (^{13}C: 125 MHz, ^{1}H: 500 MHz, in pyridine-d_5)

	^{13}C	^{1}H			^{13}C	^{1}H	
	δ_C (ppm) (mult.)	δ (ppm)	mult., J (Hz)		δ_C (ppm) (mult.)	δ (ppm)	mult., J (Hz)
1	179.0 (s)			Me	56.0 (q)	3.77	s
2	46.8 (d)	2.73	m		56.0 (q)	3.82	s
3	41.6 (d)	2.55	m	Glc			
4	71.4 (t)	3.82	t-like, 7.9	1	100.8 (d)	5.53	d, 7.9
		4.04	dd, 8.0, 8.5	2	76.8 (d)	4.56	t-like, 8.5
5	37.9 (t)	2.40	dd, 8.5, 13.7	3	78.8 (d)	4.00	m
		2.65	dd, 5.5, 13.7	4	71.6 (d)	4.20	t-like, 8.6
6	34.9 (t)	3.00	dd, 6.7, 14.0	5	79.1 (d)	4.35	t-like, 9.1
		3.12	dd, 5.5, 14.0	6	62.3 (t)	4.46	br d, 12.0
1′	133.0 (d)					4.25	dd, 12.0, 5.5
2′	113.5 (d)	6.70	d, 1.8	Api			
3′	149.4 (s)			1	110.4 (d)	6.62	br s
4′	146.5 (s)			2	78.1 (d)	4.84	br s
5′	116.5 (d)	7.41	d, 7.9	3	81.0 (s)		
6′	121.1 (d)	6.58	dd, 1.8, 7.9	4	75.8 (t)	4.42	d, 9.7
1″	129.5 (s)					4.93	d, 9.7
2″	113.7 (d)	7.00	d, 1.8	5	66.4 (t)	4.18	d, 11.0
3″	147.1 (s)					4.24	d, 11.0
4″	148.8 (s)						
5″	116.6 (d)	7.18	d, 7.9				
6″	122.8 (d)	6.89	dd, 1.8, 7.9				

in part by a Grant-in-Aid for Scientific Research from the Ministry of Education, Science, Sports and Culture of Japan.

References and Notes

1) Zhang H. L., Nagatsu A., Sakakibara J., *Chem. Pharm. Bull.*, **44**, 874—876 (1996).
2) Zhang H. L., Nagatsu A., Sakakibara J., Okuyama H., *Chem. Pharm. Bull.*, **45**, 1910—1914 (1997).
3) Palter R., Lundin R. E., Fuller G., *Phytochemistry*, **11**, 819—822 (1972).
4) Palter R., Haddon W. F., Lundin R. E., *Phytochemistry*, **11**, 2327—2328 (1972).

Table 3. Positional Parameters and Their Estimated Standard Deviations for **1a**

Atoms	x	y	z	Beq (A^2)
O1	−0.5124(4)	0.2796(3)	0.7092(2)	4.19(8)
O2	0.2041(4)	0.3562(3)	0.6207(2)	4.25(8)
O3	0.2010(5)	−0.2215(3)	0.3326(2)	5.22(9)
C1	−0.0895(6)	−0.1205(4)	0.4665(3)	4.1(1)
C2	−0.0116(7)	−0.2141(4)	0.4229(3)	4.7(1)
C3	0.1518(6)	−0.1815(4)	0.3892(2)	3.8(1)
C4	0.2523(6)	−0.1035(4)	0.4289(3)	3.7(1)
C5	0.2021(6)	−0.0534(4)	0.4901(2)	3.2(1)
C6	0.3204(6)	0.0196(4)	0.5305(3)	3.6(1)
C7	0.2397(5)	0.1256(4)	0.5546(2)	3.5(1)
C8	0.0766(5)	0.1059(3)	0.5968(2)	2.80(9)
C9	−0.0446(5)	0.0375(3)	0.5499(2)	2.87(9)
C10	0.0298(5)	−0.0715(3)	0.5232(2)	2.99(9)
C11	−0.2180(5)	0.0235(4)	0.5864(3)	3.8(1)
C12	−0.2963(5)	0.1306(4)	0.6110(2)	3.2(1)
C13	−0.1772(5)	0.1955(3)	0.6591(2)	2.79(9)
C14	−0.0110(5)	0.2116(3)	0.6172(2)	2.75(9)
C15	0.0840(5)	0.2963(4)	0.6621(2)	3.10(9)
C16	−0.0572(6)	0.3694(4)	0.6909(2)	3.5(1)
C17	−0.2271(5)	0.3149(3)	0.6723(2)	2.73(9)
C18	−0.1513(6)	0.1372(4)	0.7326(2)	3.8(1)
C19	0.0545(7)	−0.1517(4)	0.5876(3)	4.1(1)
C20	−0.3623(6)	0.3372(4)	0.7293(2)	3.5(1)
C21	−0.4039(7)	0.4568(4)	0.7374(3)	4.9(1)

5) Inagaki I., Hisada S., Nishibe S., *Phytochemistry*, **10**, 211—213 (1971).
6) Kitagawa I., Sakagami M., Hashiguchi F., Zhou J. L., Yoshikawa M., Ren J., *Chem. Pharm. Bull.*, **37**, 551—553 (1989).
7) The NMR data, except for the methyl proton signals, have not been reported previously.
8) Anisotropic thermal parameters of non-H atoms, atomic coordinates, bond distances, and bond angles are deposited in the Cambridge Crystallographic Data Centre (CCDC).
9) The programs used for solution and refinement were locally modified versions of Burla, Camalli, Cascarano, Giacovazzo, Polidori, Spagna, and Viterbo's SIR92 and Busing and Levy's ORFLS, and Johnson's ORTEP II for ploting.