STRUCTURE OF SANGGENON C, A NATURAL HYPOTENSIVE DIELS-ALDER ADDUCT FROM CHINESE CRUDE DRUG "SĀNG-BÁI-PÍ" (MORUS ROOT BARKS)

Taro Nomura,*,[a] Toshio Fukai,[a] Yoshio Hano,[a] and Jun Uzawa[b]

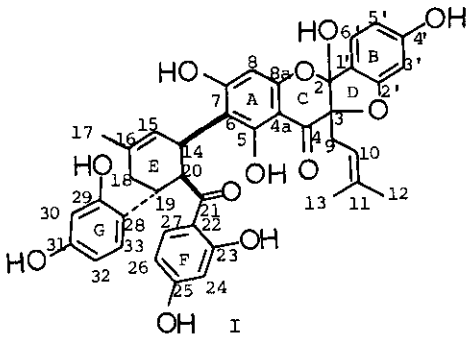
a) Faculty of Pharmaceutical Sciences, Toho University, 2-2-1, Miyama, Funabashi-shi, Chiba 274, Japan

b) The Institute of Physical and Chemical Research, Wako-shi, Saitama 351, Japan

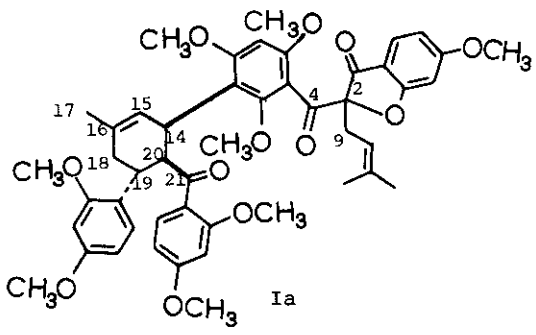
Abstract - From the methanol extract of the Chinese crude drug "Sāng-Bái-Pí" (Japanese name Sōhakuhi), the root barks of Morus sp. (Moraceae), a new flavanone derivative with a fused dihydrochalcone partial moiety was isolated and named sanggenon C. The structure was shown to be I on the basis of chemical and spectral data. Sanggenon C (I) is regarded biogenetically as a Diels-Alder adduct of a chalcone derivative and a dehydroprenylflavanone derivative. Intravenous injection of I (1 mg/Kg) produced a significant hypotension in rabbit.

In previous communication,[1] we reported that an isoprene-substituted flavanone derivative, sanggenon A, was isolated from the Chinese crude drug "Sāng-Bái-Pí" (Japanese name "Sōhakuhi"), the root barks of Morus sp. (Moraceae), and the structure was shown to be II. In this paper, we report the isolation and structure determination of a new flavanone derivative, sanggenon C (I), isolated from the methanol extract of the same drug.

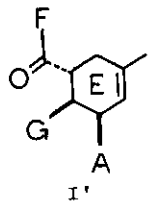
The crude drug "Sāng-Bái-Pí" (8.0 Kg) imported from the People's Republic of China was extracted successively with n-hexane, benzene, and methanol. The methanol extract was dissolved in ethyl acetate. The ethyl acetate extract was fractionated sequentially by the column and the preparative thin-layer chromatography over silica gel to give sanggenon C (I) in 6×10^{-2}% yield from the crude drug. The compound (I) showed a marked hypotensive effect (1 mg/Kg, i.v.) in rabbit.



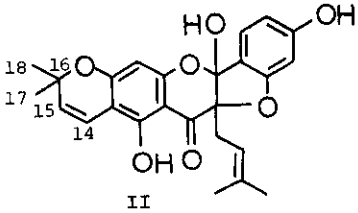
I



Ia



I'



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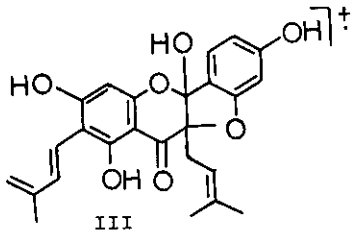

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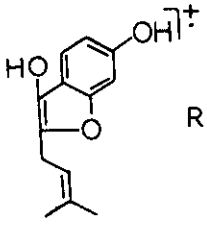

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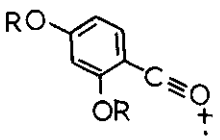
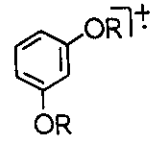


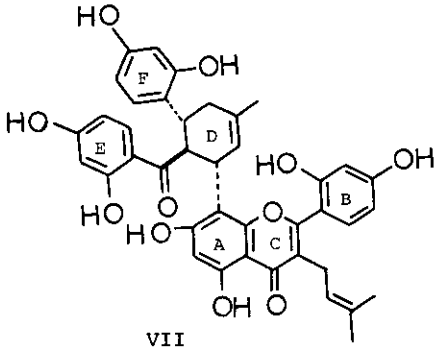
V :R=H

XII:R=CH_3



VI :R=H

XIII:R=CH_3



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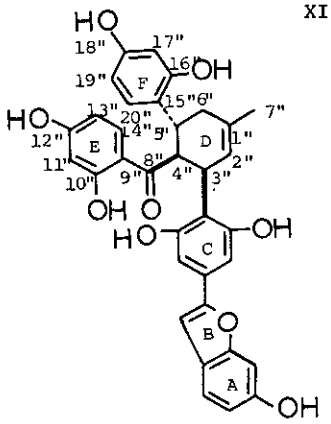

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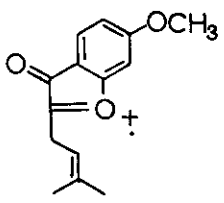

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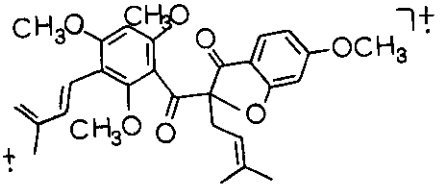
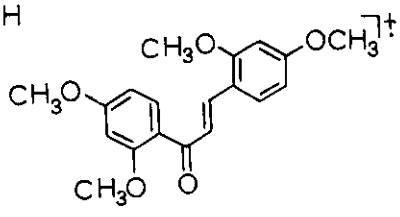


IX



X

Fig. 1

Sanggenon C (I), amorphous powder,[2] $[\alpha]_D^{16}$ + 304° (c=0.18 in methanol), gave the FD-MS spectrum which showed the molecular ion peak at m/e 708, and the ^{13}C nmr spectrum[3] indicated the presence of fourty carbons [fourteen aliphatic carbons ($3xCH_3$, $2x-CH_2-$, $3x>CH-$, $1x\geqslant C-O-$, $1x>C\overset{-O}{\underset{O}{}}$, $2x>C=CH-$), twenty four aromatic carbons (10xCH, 5xC, 9xC-O) and two carbonyl carbons]. The elemental analysis gave the following result: Anal. Calcd. for $C_{40}H_{36}O_{12}\cdot 2H_2O$: C, 64.85; H, 5.33. Found: C, 64.52; H, 5.38. These results suggest the composition of sanggenon C (I) to be $C_{40}H_{36}O_{12}$. The compound (I) showed the following color reactions: Mg-HCl test (orange), $NaBH_4$ test (violet),[4] $FeCl_3$ test (reddish violet), and showed the following spectra: ir ν_{max}^{Nujol} cm^{-1}: 3200, 1670(sh), 1660(sh), 1645(sh), 1630(br), 1600(sh), 1580(sh); ^1H nmr, δ in acetone-d_6, 12.23, 12,60 (each 1H, OH). These findings show that I is a flavanone derivative which has two hydrogen bonded hydroxyl groups. The compound (I) showed the following uv spectra: uv λ_{max}^{MeOH} nm(log ε): 220(infl. 4.64), 230(sh 4.55), 283(4.40), 288(sh 4.39), 309 (4.35); $\lambda_{max}^{MeOH+AlCl_3}$ nm(log ε): 225(4.63), 290 (sh 4.35), 305(4.40), 350 (sh 4.01), 420(3.18). The uv spectrum of I was similar to that of sanggenon A (II)[1] suggesting that I is a derivative of II. In the uv spectrum of I in the presence of $AlCl_3$, a part of the absorption at 283-288 nm showed a bathochromic shift and the absorption at 290 nm was observed as a shoulder. If the ir and the ^1H nmr spectra of I are taken into account, the absorption at 283-288 nm can be ascribed to the two conjugated carbonyl groups[10a] which are hydrogen bonded. Scherif et al. reported that $AlCl_3$-induced shift was not observed in the uv spectra when a prenyl group was located ortho to a chelated hydroxyl group.[5] These data led us to presume that one of the ortho positions of the two hydrogen bonded hydroxyl groups is substituted by a prenyl group, and another position is not. Further, the chemical shift values of the carbon atoms

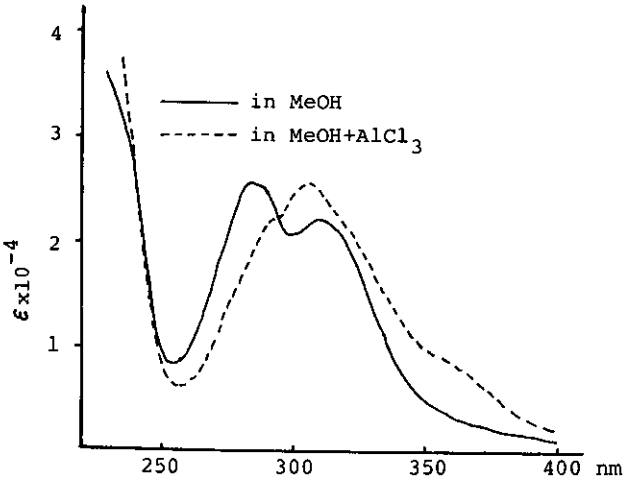


Fig. 2 The uv spectra of sanggenon C (I)

of the flavanone skeleton of I were similar to those of II except the signals of carbon atoms (C-6 and C-7) which were affected by the additional substituent effect (Table 1). These results, together with the fact that sanggenon A (II) coexists with sanggenon C (I), suggest that both I and II have the same flavanone skeletal structure.

Table 1 ^{13}C nmr chemical shifts

	II	I	I		II	I	I	VII	VIII
C-2	92.6	92.0	90.4	C-14(3")	115.5	35.8	33.1	(38.5)[c]	33.6
C-3	102.5	102.4	101.7	C-15(2")	127.5	122.8	121.4	123.2	121.5
C-4	188.6	188.4	187.2	C-16(1")	79.1	135.0	132.6	132.8	133.4
C-4a	100.5	99.9	98.8	C-17(7")	28.5	23.7	23.3	22.5	23.4
C-5	163.3	163.9	163.3	C-18(6")	28.5	33.8	32.8	(37.9)[c]	34.5
C-6	103.3	109.0	107.5	C-19(5")		32.8	33.1	(38.5)[c]	33.3
C-7	164.4	167.6	167.0	C-20(4")		48.3	47.2	45.8	47.1
C-8	96.5	96.5	94.1	C-21(8")		208.8	206.2	208.1	207.5
C-8a	161.4	162.0	160.1	C-22(9")		114.0	113.8	114.0	113.4
C-1'	121.2	122.2	122.4	C-23(10")		165.9[*1]	164.0[*1]	164.2	164.6
C-2'	161.4	161.2	159.5	C-24(11")		103.8	102.6	102.6	102.7
C-3'	99.6	99.5	98.3	C-25(12")		166.8[*1]	164.3[*1]	164.2	164.6
C-4'	159.5	161.2	159.8	C-26(13")		107.6[*3]	105.9[*3]	107.2	106.4[*1]
C-5'	109.9	109.7	108.7	C-27(14")		129.0	128.2	130.8	128.1
C-6'	125.9	125.6	124.9	C-28(15")		121.3	119.5	120.7	122.3
C-9	32.1	32.0	30.9	C-29(16")		156.5[*2]	155.5[*2]	155.8	155.3[*2]
C-10	118.7	118.6	117.5	C-30(17")		103.5	102.2	102.0	101.5
C-11	136.9	136.2	135.3	C-31(18")		157.8[*2]	155.8[*2]	155.8	155.9[*2]
C-12	25.9	25.9	25.5	C-32(19")		108.7[*3]	107.5[*3]	106.8	108.1[*1]
C-13	18.1	18.1	17.7	C-33(20")		133.7	132.6	132.4	133.4
solvent	a	a	b		a	a	b	b	b

a: acetone-d_6, b: DMSO-d_6, C: CD_3CN , *: Assignments may be reversed.

The mass spectra of I showed the following fragments[6]: m/e 708 (M^+),[7] 436 (III), 421 (436-CH_3), 353 (421-C_5H_8), 218 (IV), 137 (V), 110 (VI). This result suggests that sanggenon C (I) may be a Diels-Alder adduct such as kuwanon G[8] (=albanin F[9] =moracenin B[10]) (VII) regarded as a cycloaddition product with the chalcone and the dehydroprenylflavanone derivative. This was substantiated by detailed analysis of the ^1H nmr spectrum (acetone-d_6) using sequential decoupling and by comparison of the ^1H nmr spectra of sanggenon A (II) and other Diels-Alder adducts obtained from Morus species.[8-13] The chemical shifts (δ) and coupling constants (Hz) of protons of the relevant cyclohexene ring

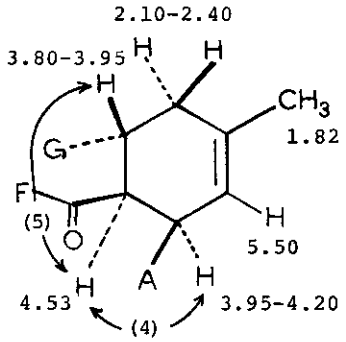


Fig. 3 (I)

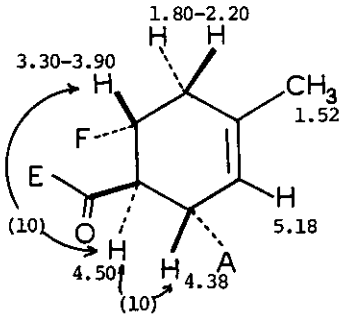


Fig. 4 (VII)

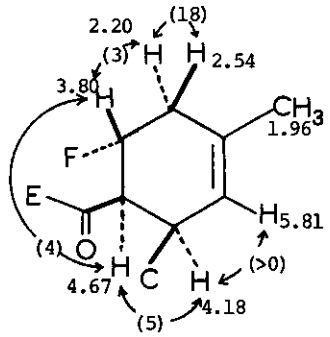


Fig. 5 (VIII)

are shown in Fig. 3, while the remaining protons are summarized as follows: protons in flavanone moiety, 5.73 (1H, s, C_8-H), 6.44 (1H, d, J=2, C_3,-H), 6.45 (1H, dd, J=2 and 8, C_5,-H), 7.28 (1H, d, J=8, C_6,-H); aromatic protons in a 2,4-dihydroxybenzoyl moiety, 6.17 or 6.30 (1H, d, J=2, C_{24}-H), 6.33 (1H, dd, J=2 and 8, C_{26}-H), 8.36 (1H, d, J=8, C_{27}-H); aromatic protons in a 2,4-dihydroxyphenyl moiety, 6.17 or 6.30 (1H, d, J=2, C_{30}-H), 6.25 (1H, dd, J=2 and 8, C_{32}-H), 6.90 (1H, d, J=8, C_{33}-H); γ,γ-dimethylallyl moiety, 1.47, 1.55 (each 3H, s, C_{11}-CH_3), 2.68 (1H, dd, J=6 and 14, C_9-H), 3.08 (1H, dd, J=9 and 14, C_9-H), 5.15 (1H, m, C_{10}-H). As the methylene protons of γ,γ-dimethylallyl group appear to be nonequivalent, it is suggested that the γ,γ-dimethylallyl group is located at the asymmetric center.[1] All these results indicate that the structure of sanggenon C is possibly represented by I or I' (except stereochemistry).

Detailed comparative examination of the ^{1}H nmr spectra of I, kuwanon G[8] (VII) and of mulberrofuran C[12b] (VIII), revealed that the chemical shifts and coupling constants of protons of the relevant cyclohexene ring of I resembled those of VIII better than those of VII (Figs. 3-5). In the ^{13}C nmr spectrum of I, the chemical shifts of the carbon atoms of the relevant cyclohexene ring of I more closely resembled those of VIII than those of VII (Table 1). These results suggest that sanggenon C (I) and mulberrofuran C (VIII) have the same disposition concerning the location of the dihydroxyphenyl and dihydroxybenzoyl moiety on the cyclohexene ring, and have the same relative configuration.

Further supporting data for the structure I was obtained by the examination of the octamethyl ether (Ia). Treatment of I with dimethyl sulfate and potassium carbonate in acetone (reflux 6 h) gave the octamethyl ether (Ia, 50% yield) as an amorphous powder. Octamethyl ether (Ia), M^+ 820, $FeCl_3$ test (negative),

$[\alpha]_D^{20}$ + 218° (c=0.15 in chloroform), showed the following spectra: ir ν_{max}^{Nujol} cm^{-1}: 1735, 1675, 1615(sh), 1585, 1560(sh); uv λ_{max}^{EtOH} nm(log ε): 228(4.59), 273(4.24), 305(4.14), 328(infl. 3.99); $\lambda_{max}^{EtOH+AlCl_3}$: 230(4.59), 275(4.23), 306(4.14), 330 (infl. 3.97); ^1H nmr, δ in $CDCl_3$, 1.50, 1.57 (each 3H, s, C_{11}-CH_3), 1.73(3H, s, C_{15}-CH_3), 2.25 (2H, m, C_{18}-Hx2), 3.02 (2H, m, C_9-Hx2), 3.40-3.90 (OCH_3x8), 4.00 (1H, m, C_{19}-H), 4.50 (1H, m, C_{14}-H), 4.60 (1H, m, C_{20}-H), 5.10 (1H, m, C_{10}-H), 5.35 (1H, br s, C_{15}-H), 5.98 (1H, s, C_8-H), 6.00-6.40 (6H, m), 6.48 (1H, dd, J=2 and 8, C_5,-H, C_{26}-H, or C_{32}-H), 6.94 (2H, d, J=8, C_6,-H and C_{33}-H), 7.34 (1H, d, J=8, C_{20}-H); mass spectrum,[6] m/e 820 (M^+), 752 (M^+-C_5H_8), 587 (752-$C_9H_9O_3$), 492 (IX), 424 (492-C_5H_8), 328 (X), 231 (XI), 165 (XII), 138 (XIII). In the ^{13}C nmr spectrum of Ia, the signals of three carbonyl carbons appeared at δ 173.0 (C-2), 194.9 (C-4), and 201.3 (C-21).[3,14] These results suggest that octamethyl ether (Ia) does not have a hemiketal partial structure but a triketone structure (Ia). The compound (Ia, 30 mg) was pyrolyzed at 280°C. The reaction products were purified by preparative tlc to give 2,4,2',4'-tetramethoxychalcone (XIV, 2 mg) which was identified with authentic sample. This result suggests that sanggenon C (I) is a Diels-Alder adduct of a chalcone derivative and dehydroprenylflavanone derivative. The disposition concerning the location of the dihydroxyphenyl and dihydroxybenzoyl moiety on the cyclohexene ring of Ia was supported by the following long-range selective ^1H decoupling (LSPD) technique: when the signals at δ 2.25 (18-Hx2) were weakly irradiated, the signal at δ 201.3 (C-21) remained unchanged. The irradiation of the signals at δ 4.52 (14-H and 20-H) increased the area (ca. + 50%) of the signal at δ 201.3 (C-21). These findings suggest that the dihydroxybenzoyl moiety is located at C-20 position, and not at C-19 position. The similar results were reported on the case of kuwanon G (VI).[8b] The presence of γ,γ-dimethylallyl group at C-3 position of Ia was also supported by the LSPD technique and the ir spectrum of Ia as follows: the irradiation of the signals at δ 3.02 (9-Hx2) increased the area (ca. + 80%) of the signal at δ 194.9 (C-4). In the ir spectrum of Ia, the absorption bands at 1735 cm^{-1} can be ascribed to a five-membered conjugated carbonyl group.[16] From the above results, the structure of sanggenon C octamethyl ether (Ia) is possibly represented by Ia, so that we propose the formula (I) for the structure of sanggenon C.

Sanggenon C (I) is optically active and is the first example of a natural product which is considered to be formed by a Diels-Alder type of enzymatic reaction process of a chalcone derivative and a dehydroprenylflavanone derivative.

It is also interesting that sanggenon C (I) is obtained only from the extracts of Chinese crude drug "Sāng-Bái-Pí", and could not be detected in the extracts of Japanese cultivated mulberry root barks.

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2 Although only one spot was detected on tlc, this compound could not be isolated as crystalline form.

3 Assignments of the carbon atoms were performed by comparison of the ^{13}C nmr spectra of model compounds, sanggenon A (II)[1] and the other Diels-Alder adducts obtained from Morus root barks.[8-13]

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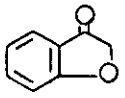

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