

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

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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 dihydro-chalcone 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 dehydro-prenylflavanone 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 $6 \times 10^{-2}\%$ yield from the crude drug. The compound (I) showed a marked hypotensive effect (1 mg/Kg, i.v.) in rabbit.

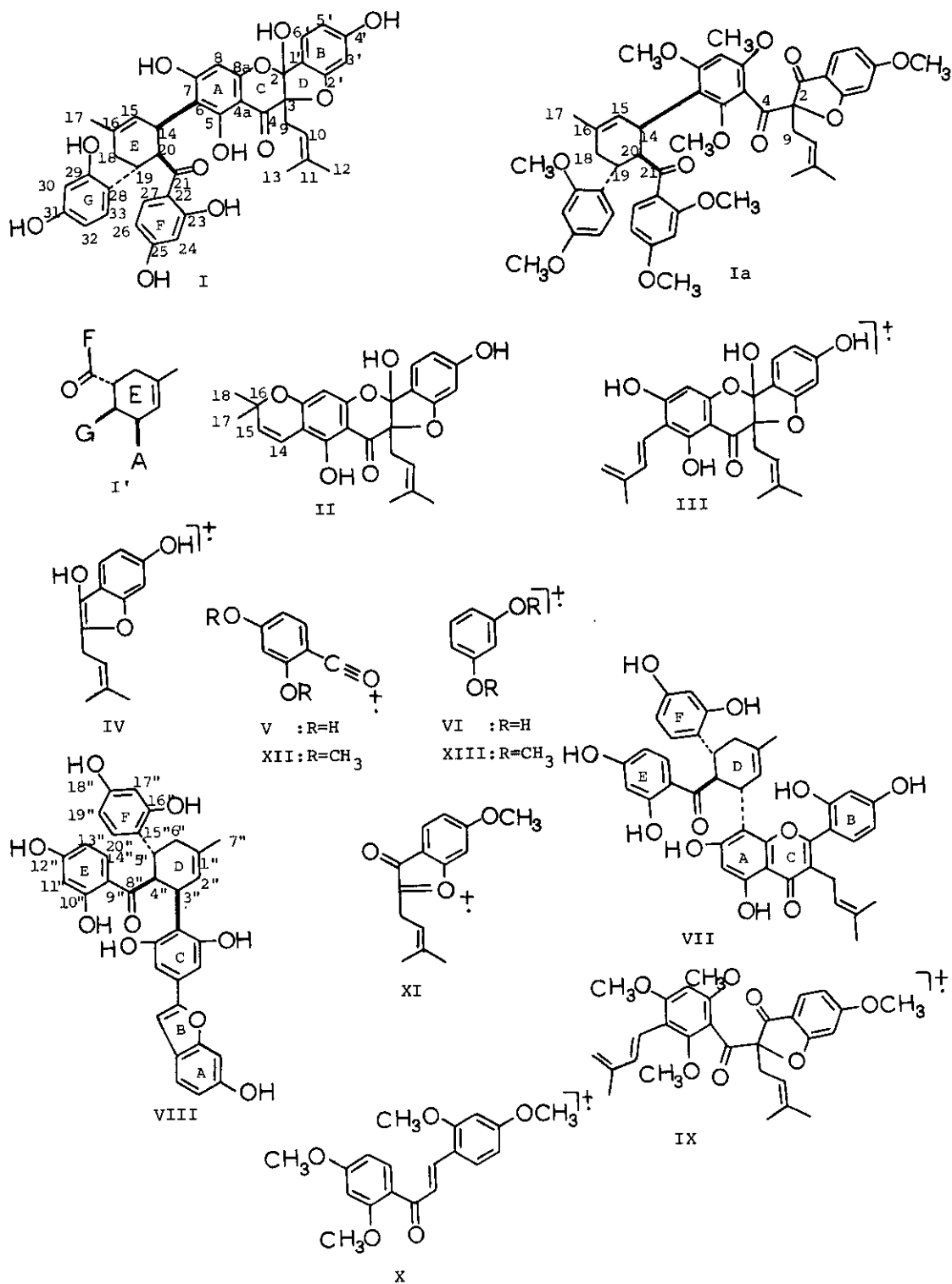


Fig. 1

Sanggenon C (I), amorphous powder, $[\alpha]_D^{16} + 304^\circ$ ($c=0.18$ in methanol), gave the FD-MS spectrum which showed the molecular ion peak at m/e 708, and the ^{13}C nmr spectrum³ indicated the presence of forty carbons [fourteen aliphatic carbons ($3\times\text{CH}_3$, $2\times\text{CH}_2$ -, $3\times\text{CH}$ -, $1\times\text{C}=\text{O}$ -, $1\times\text{C}=\text{O}$ -, $2\times\text{C}=\text{CH}$ -), twenty four aromatic carbons ($10\times\text{CH}$, $5\times\text{C}$, $9\times\text{C}=\text{O}$) and two carbonyl carbons]. The elemental analysis gave the following result: Anal. Calcd. for $\text{C}_{40}\text{H}_{36}\text{O}_{12}\cdot 2\text{H}_2\text{O}$: C, 64.85; H, 5.33. Found: C, 64.52; H, 5.38. These results suggest the composition of sanggenon C (I) to be $\text{C}_{40}\text{H}_{36}\text{O}_{12}$. 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 $\nu_{\text{max}}^{\text{Nujol}}$ 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: $\text{uv } \lambda_{\text{max}}^{\text{MeOH}}$ nm(log ϵ): 220(infl. 4.64), 230(sh 4.55), 283(4.40), 288(sh 4.39), 309(4.35); $\lambda_{\text{max}}^{\text{MeOH}+\text{AlCl}_3}$ 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

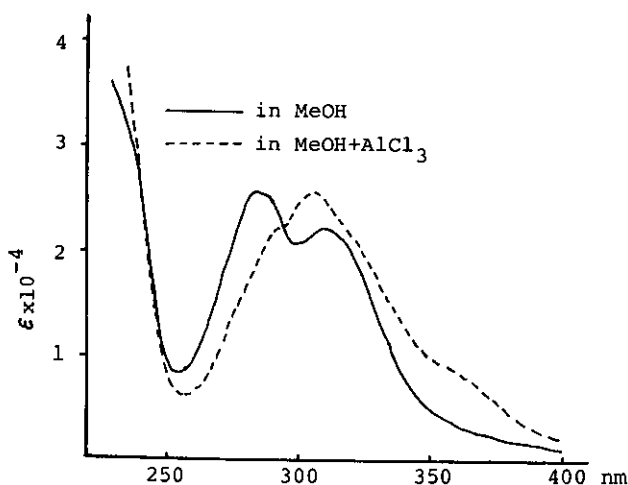


Fig. 2 The uv spectra of sanggenon C (I)

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

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: $\text{DMSO}-\text{d}_6$, C: CD_3CN , *: Assignments may be reversed.

The mass spectra of I showed the following fragments⁶: m/e 708 (M^+),⁷ 436 (III), 421 ($436-\text{CH}_3$), 353 ($421-\text{C}_5\text{H}_8$), 218 (IV), 137 (V), 110 (VI). This result suggests that sanggenon C (I) may be a Diels-Alder adduct such as kuwanon G⁸ (=albanin F⁹=moracenin B¹⁰) (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

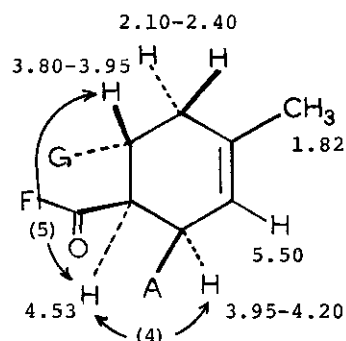


Fig. 3 (I)

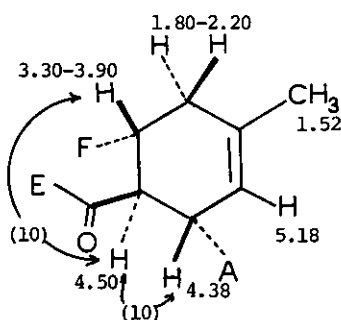


Fig. 4 (VII)

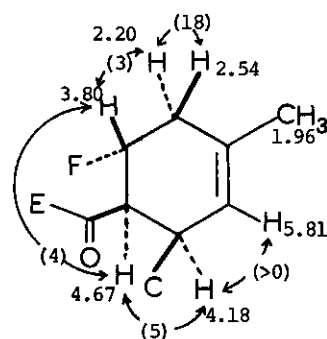


Fig. 5 (VIII)

are shown in Fig. 3, while the remaining protons are summarized as follows: protons in flavanone moiety, 5.73 (1H, s, C₈-H), 6.44 (1H, d, J=2, C₃-H), 6.45 (1H, dd, J=2 and 8, C₅-H), 7.28 (1H, d, J=8, C₆-H); aromatic protons in a 2,4-dihydroxybenzoyl moiety, 6.17 or 6.30 (1H, d, J=2, C₂₄-H), 6.33 (1H, dd, J=2 and 8, C₂₆-H), 8.36 (1H, d, J=8, C₂₇-H); aromatic protons in a 2,4-dihydroxyphenyl moiety, 6.17 or 6.30 (1H, d, J=2, C₃₀-H), 6.25 (1H, dd, J=2 and 8, C₃₂-H), 6.90 (1H, d, J=8, C₃₃-H); *r,r*-dimethylallyl moiety, 1.47, 1.55 (each 3H, s, C₁₁-CH₃), 2.68 (1H, dd, J=6 and 14, C₉-H), 3.08 (1H, dd, J=9 and 14, C₉-H), 5.15 (1H, m, C₁₀-H). As the methylene protons of *r,r*-dimethylallyl group appear to be nonequivalent, it is suggested that the *r,r*-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 ¹H nmr spectra of I, kuwanon G⁸ (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 ¹³C 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, FeCl₃ test (negative),

$[\alpha]_D^{20} + 218^\circ$ ($c=0.15$ in chloroform), showed the following spectra: ir $\nu_{\max}^{\text{Nujol}} \text{ cm}^{-1}$: 1735, 1675, 1615(sh), 1585, 1560(sh); uv $\lambda_{\max}^{\text{EtOH}} \text{ nm}(\log \epsilon)$: 228(4.59), 273(4.24), 305(4.14), 328(infl. 3.99); $\lambda_{\max}^{\text{EtOH+AlCl}_3}$: 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, $\text{C}_{11}\text{-CH}_3$), 1.73(3H, s, $\text{C}_{15}\text{-CH}_3$), 2.25 (2H, m, $\text{C}_{18}\text{-Hx2}$), 3.02 (2H, m, $\text{C}_9\text{-Hx2}$), 3.40-3.90 ($\text{OCH}_3 \times 8$), 4.00 (1H, m, $\text{C}_{19}\text{-H}$), 4.50 (1H, m, $\text{C}_{14}\text{-H}$), 4.60 (1H, m, $\text{C}_{20}\text{-H}$), 5.10 (1H, m, $\text{C}_{10}\text{-H}$), 5.35 (1H, br s, $\text{C}_{15}\text{-H}$), 5.98 (1H, s, $\text{C}_8\text{-H}$), 6.00-6.40 (6H, m), 6.48 (1H, dd, $J=2$ and 8, $\text{C}_5\text{-H}$, $\text{C}_{26}\text{-H}$, or $\text{C}_{32}\text{-H}$), 6.94 (2H, d, $J=8$, $\text{C}_6\text{-H}$ and $\text{C}_{33}\text{-H}$), 7.34 (1H, d, $J=8$, $\text{C}_{20}\text{-H}$); mass spectrum, m/e 820 (M^+), 752 ($\text{M}^+ - \text{C}_5\text{H}_8$), 587 ($752 - \text{C}_9\text{H}_9\text{O}_3$), 492 (IX), 424 ($492 - \text{C}_5\text{H}_8$), 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.

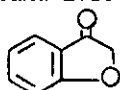
It is also interesting that sanggenon C (I) is obtained only from the extracts of Chinese crude drug "Sāng-Bái-Pí", and could not be detected in the extracts of Japanese cultivated mulberry root barks.

ACKNOWLEDGEMENT We are grateful to Prof. S. Sakai, Faculty of Pharmaceutical Sciences, Chiba University, for mass spectrum measurement, and also grateful to Dr. Y. Momose, Faculty of Medicine, Toyama Medical and Pharmaceutical University, for observation of hypotensive action, and also grateful to Dr. K. Fukushima, Research Institute for Chemobiodynamics, Chiba University, for FD-MS measurement.

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Received, 29th August, 1981