CONSTITUENT OF THE CHINESE CRUDE DRUG "SĀNG-BÁI-PÍ" (MORUS ROOT BARKS) III[1,2]. STRUCTURE OF A NEW FLAVANONE DERIVATIVE, SANGGENON F.

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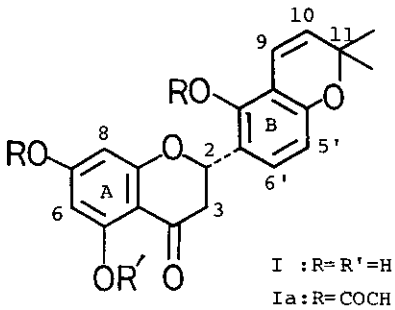
Abstract ——— From the benzene extract of the Chinese crude drug "Sāng-Bái-Pí" (Japanese name "Sōhakuhi"), the root barks of Morus sp. (Moraceae), an isoprene substituted flavanone derivative, named sanggenon F, was isolated, for which structure (I) was proposed on the basis of its spectral and chemical evidence.

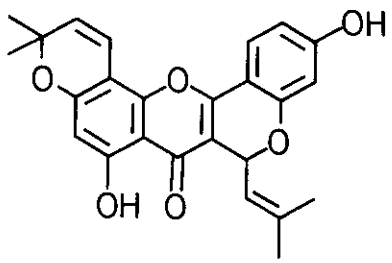
In the previous papers, we reported the isolation and structure determination of a series of flavonoids substituted with isoprenyl groups from the Chinese crude drug "Sāng-Bái-Pí" (Japanese name "Sōhakuhi") imported from the People's Republic of China[3]. Some of them seemed to be natural Diels-Alder adducts[2,3c,d] and showed significant hypotention[3c,d]. In the course of our studies, a new flavanone, named sanggenon F (I), was isolated from the benzene extract of the Chinese crude drug. In this paper, the structure determination of this new flavanone is described.

The Chinese crude drug "Sāng-Bái-Pí", the root barks of a species of Morus imported from the People's Republic of China, was cut and extracted with hexane and then with benzene. The benzene extract was fractionated sequentially by the silica-gel column chromatography and by preparative thin layer chromatography. This procedure yielded, sanggenon F (I) and cyclomorusin (II), the latter being identified as an authentic sample.

Sanggenon F (I) was obtained as colorless prisms, mp 181-184°C, $[\alpha]_D^{20}$ -24° (c= 0.032 in chloroform), $C_{20}H_{18}O_6$, m/z 354(M^+), exhibiting an intense reddish violet color with methanolic iron(III) chloride and both positive in the magnesium-hydrochloric acid test and sodium borohydride test[4]. The UV spectrum exhibited maxima at 228, 288, 319 nm, while at 227, 310, 370 nm in the presence of aluminum chloride. These findings suggested that I would be a flavanone derivative having a hydrogen bonded hydroxyl group[5]. This assumption was substantiated by the ^1H nmr spectrum of I, in which the signals were observed at δ 2.82(1H, dd, J=3.5 and 18Hz, C_3-H),



I :R=R'=H
Ia:R=$COCH_3$,R'=H
Ib:R=R'=CH_3



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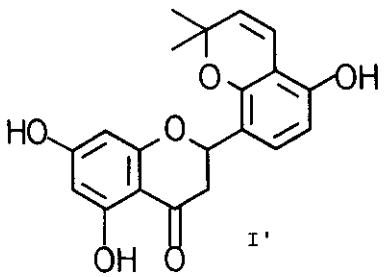
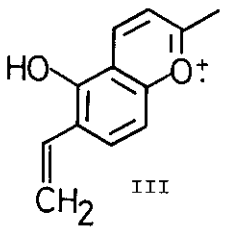
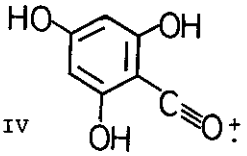


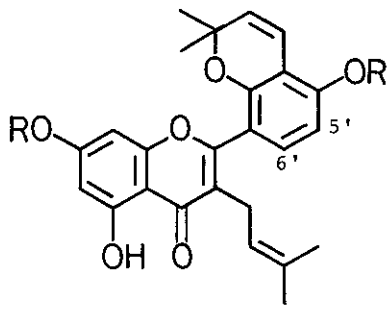
I'



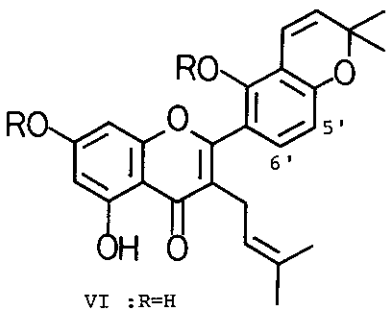
III



IV



V :R=H
Va:R=$COCH_3$



VI :R=H
VIa:R=$COCH_3$

Table 1 Chemical Shifts (ppm)

	5'-H	6'-H		5'-H	6'-H		5'-H	6'-H		5-H	6-H
I	6.39	6.86	V	6.68	7.08	VI	6.46	7.08	IX	6.48	7.25
Ia	6.71	7.20-7.25	Va	6.84	7.32	VIa	6.85	7.37	IXb	6.77	7.61
Δ	-0.32	-0.34-0.39	Δ	-0.16	-0.24	Δ	-0.39	-0.29	Δ	-0.29	-0.36
	in $CDCl_3$			in acetone-d_6			in acetone-d_6			in $CDCl_3$	

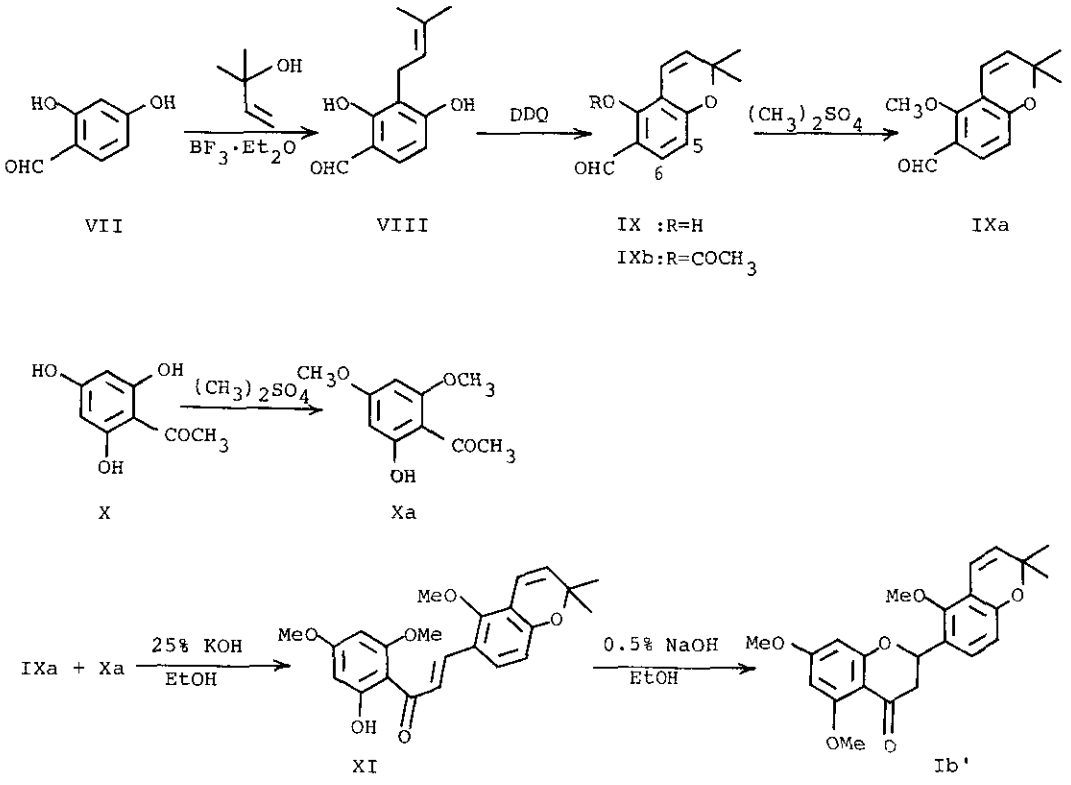


Chart 1

3.16(1H, dd, J=12 and 18Hz, C_3-H), 5.54(1H, dd, J=3.5 and 12Hz, C_2-H), 11.96(1H, s, C_5-OH). The ^{1}H nmr spectrum of I showed the two proton broad singlet at δ 6.00, which indicated that the A ring was not substituted at the 6- and the 8-position[5]. Arrangement of substituents in the B ring was deduced from the ^{1}H nmr spectrum of I: two doublet signals at δ 6.39(J=8Hz, C_5'-H) and δ6.89(J=8Hz, C_6'-H)[5] supported that the B ring of I was substituted in the 2'-, 3'-, and 4'-positions. The biogenetic analogy to other prenylflavonoids isolated from Morus species[6] suggests that B ring has the 2'-, 4'-dioxygenated function. The presence of a 2,2-dimethylchromene ring was supported by the ^{1}H nmr spectrum of I as follows: δ 1.43(6H, s), 5.63(1H, d, J=10Hz), 6.66(1H, d, J=10Hz). The mass spectrum of I showed the significant peaks at m/z 187 (III) and m/z 153 (IV)[6]. These results indicate that the structure of sanggenon F is possibly represented by I or I'

The structure (I) for sanggenon F was supported by the changes in the chemical shifts of the C_5'- and C_6'- protons in sanggenon F, compared with the chemical shifts of the relevant protons of its diacetate (Ia). The same comparison was observed

for kuwanon A (V)[6], B (VI)[6] and the compound IX (Table 1).

(±)-Trimethylsanggenon F (Ib') was prepared according to the process shown in Chart 1. Condensation of IXa with Xa in alkaline solution gave a chalcone (XI), which was converted into Ib' in alkaline solution. The compound Ib' thus obtained was identical with sanggenon F trimethyl ether derived from natural source by the thin-layer chromatography and the ^{1}H nmr spectra.

On the basis of the specific optical rotation (-)[7] and the CD spectrum, I has the (S)-configuration at C-2[8]. From these results, sanggenon F is represented by the formula (I).

EXPERIMENTAL

All melting points were uncorrected. ^{1}H nmr spectra were measured with tetramethylsilane (TMS) as the internal reference. Chemical shifts were expressed in ppm downfield from TMS, and coupling constants (J) in Hz. Abbreviations: s=singlet, d=doublet, t=triplet, m=multiplet, br=broad, sh=shoulder. The following instruments were used for the physical data: uv spectra; Hitachi 340 UV Spectrometer: ir spectra; Hitachi 295 IR spectrometer: ^{1}H nmr spectra; JEOL FX-270, JEOL·JNM 4H-100 NMR Spectrometer, and Hitachi R-900 FT NMR Spectrometer: mass spectra (ms); JEOL·JMS OISG-2 and Hitachi RMU-6E Mass Spectrometer: optical rotation; JASCO DPI-4: CD spectra; JASCO J-20 ORD Spectrometer. For thin-layer chromatography (TLC) and preparative TLC, Wakogel B-5FM was used, and for column chromatography, Wakogel C-200.

Isolation of Sanggenon F (I) and Cyclomorusin (II)[6]

The crude drug "Sāng-Bai-Pí" (Japanese name "Sōhakuhi", 8Kg), a species of Morus (Moraceae), imported from the People's Republic of China, was finely cut and extracted with hexane and then with benzene. Evaporation of the benzene solution to dryness yield 35g of the residue. This residue (20g) was chromatographed on silica gel (300g), benzene-methanol being used as an eluent and each fraction being checked by TLC., From the fraction eluted with benzene, cyclomorusin (II, mp 231-241 °C, 38mg) was obtained by preparative TLC (hexane:acetone=3:1).

Cyclomorusin (II) obtained here was proved to be identical with an authentic specimen by TLC, UV and ^{1}H nmr spectra, and mixed melting point. Fractions eluted with benzene containing 1% methanol obtained by the silica gel column chromatography were evaporated to give the residue (2.1g). This residue (2.1g) was rechromatographed on silica gel (40g) with hexane-acetone as an eluent. The fractions eluted with hexane containing 7% acetone were evaporated to give 53mg of the residue. From this residue, sanggenon F (I, mp 181-184°c, 23mg) was obtained by preparative TLC (hexane:acetone=5:2), hexane:ether=1:1).

Sanggenon F (I)

The compound (I) was recrystallized from ether-hexane to give colorless prisms, mp 181-184 °C, $[\alpha]_D^{20}$ -24° (c=0.032 in chloroform), $FeCl_3$ test: reddish violet, Mg-HCl test: violet, $NaBH_4$ test: orange. UV λ_{max}^{EtOH} nm(log ε): 228(4.62), 288(4.33), 319(sh 3.77); $\lambda_{max}^{EtOH+AlCl_3}$ nm(log ε): 227(4.68), 310(4.32), 370(3.42). ir ν_{max}^{Nujol} cm^{-1}: 3430, 3404, 1661, 1644(sh), 1611(sh), 1607, 1592(sh). High-resolution mass spectrum: Calcd. for $C_{20}H_{18}O_6$ (M^+, m/z): 354.1103. Found: 354.1145; Calcd. for $C_{19}H_{15}O_6$ (M^+-CH_3): 339.0867. Found: 339.0909; Calcd. for $C_{12}H_{11}O_2$ (III): 187.0759. Found: 187.0770; Calcd. for $C_7H_5O_4$ (IV): 153.0187. Found: 153.0194. ^{1}H nmr (90 MHz, $CDCl_3$): 1.43(6H, s, C_{11}-CH_3x2), 2.82(1H, dd, J=3.5 and 18, C_3-H, cis), 3.16(1H, dd, J=12 and 18, C_3-H, trans), 5.54(1H, dd, J=3.5 and 12, C_2-H), 5.63(1H, d, J=10, C_{10}-H), 6.00(2H, br s, C_6- and C_8-H), 6.39(1H, d, J=8,

$C_{5'}$-H), 6.66(1H, d, J=10, C_9-H), 6.86(1H, d, J=8, $C_{6'}$-H), 11.96(1H, s, C_5-OH). CD spectrum : $[\theta]_{333}$ +3218; $[\theta]_{290}$ -13677; $[\theta]_{250}$ +2575 (c 0.011%, EtOH).

Sanggenon F Diacetate (Ia)

Sanggenon F (I, 1.3mg) was acetylated with acetic anhydride (0.5ml) and pyridine (0.05ml) at room temperature for 3 min. The product was purified by preparative TLC (hexane:acetone=2:1) to give an amorphous powder (Ia, 0.8mg) which showed only one spot on TLC. The compound (Ia) was positive to $FeCl_3$ test (reddish violet). ms m/z: 438(M^+), 423, 381, 187. ^1H nmr (90 MHz, $CDCl_3$): δ 1.41(6H, s, C_{11}-CH_3x2), 2.25, 2.28(each 3H, s, $OCOCH_3$), 2.81(1H, dd, J=3.5 and 18, C_3-H, cis), 3.26(1H, dd, J=12 and 18, C_3-H, trans), 5.36(1H, dd, J=3.5 and 12, C_2-H), 5.64(1H, d, J=10, C_{10}-H), 6.19(1H, d, J=2, C_6-H), 6.24(1H, d, J=2, C_8-H), 6.30(1H, d, J=10, C_9-H), 7.20-7.25(1H, $C_{6'}$-H, overlapping with the signal of the solvent), 11.98(1H, s, C_5-OH).

Sanggenon F Trimethyl ether (Ib)

A mixture of sanggenon F (I, 9mg), dimethyl sulfate (0.5ml) and potassium carbonate (5g) in acetone (30ml) was refluxed for 3 h, and filtered. The filtrate was evaporated. Water was added to the residue which was then extracted with chloroform. The chloroform layer was treated as usual and finally evaporated. The residue was purified by preparative TLC (benzene:ether=5:2) to give the amorphous powder (Ib, 5mg) which showed only one spot on TLC. $FeCl_3$ test: nagative. ms m/z: 396 (M^+), 381, 365, 201. $[\alpha]_D^{22}$ -19° (c=0.21 in chloroform). ^1H nmr (270 MHz, $CDCl_3$): δ 1.44, 1.45 (each 3H, s, C_{11}-CH_3), 2.69(1H, dd, J=3 and 16.5, C_3-H, cis), 3.09(1H, dd, J=13.5 and 16.5, C_3-H, trans), 3.78, 3.81, 3.91(each 3H, s, OCH_3), 5.65(1H, dd, J=3 and 13.5, C_2-H), 5.68(1H, d, J=10, C_{10}-H), 6.10(1H, d, J=2, C_6-H), 6.12(1H, d, J=2, C_8-H), 6.57(1H, d, J=10, C_9-H), 6.67(1H, d, J=8, $C_{5'}$-H), 7.28(1H, d, J=8, $C_{6'}$-H).

Isoprenylation of VII

A mixture of VII (10g), boron trifluoride-etherate (3ml), 1,1-dimethyl-2-propen-1-ol (4.9g), and dioxane (70ml) was kept at room temperature for 2 h. The reaction mixture was extracted with ether and the extracts were subjected to the usual work-up and evaporated. The resulting oil (20g) was chromatographed on silica gel (250g), and the elution with benzene gave a crystalline solid. Recrystallization from hexane-ether afforded colorless needles (VIII, 540mg)[9], mp 125°C. ms m/z: 206(M^+), 191, 163, 151. ^1H nmr (100 mHz, $CDCl_3$): δ 1.66, 1.77(each 3H, s), 3.33(2H, br d, J=8), 5.25(1H, br t, J=8), 6.30(1H, d, J=8), 7.30(1H, d, J=8), 9.88(1H, s), 12.08(1H, s).

Conversion VIII to IX

A mixture of VIII (212mg) and 2,3-dicyano-5,6-dichloro-p-benzoquinone (DDQ, 273mg) in dry ether (7ml) was kept at room temperature overnight. The reaction mixture was filtered and the filtrate was concentrated. The residue was purified by preparative TLC (benzene) to give a crystalline solid (IX). Recrystallization from water-ethanol afforded colorless needles (IX, 160mg), mp 70°C. ms m/z: 204(M^+), 189. ^1H nmr (100 MHz, $CDCl_3$): δ 1.43(6H, s), 5.57(1H, d, J=10), 6.48(1H, d, J=9), 6.76(1H, d, J=10), 7.25(1H, d, J=9), 9.73(1H, s), 11.83(1H, s).

Methylation of IX (Formation of IXa)

A mixture of IX (55mg), dimethyl sulfate (0.3ml), and potassium carbonate(5g) in acetone (40ml) was refluxed for 1 h, and treated as mentioned above. The reaction products were purified by preparative TLC (benzene) to give the amorphous powder (IXa, 52mg), which showed only one spot on TLC. ms m/z: 218(M^+), 203, 160. ^1H nmr (100 MHz, $CDCl_3$): δ 1.43(6H, s), 3.90(3H, s), 5.60(1H, d, J=10), 6.53 (1H, d, J=9), 6.54(1H, d, J=10), 7.51(1H, d, J=9), 10.02(1H, s).

Acetylation of X (Formation of IXb)

Compound IX (18mg) was acetylated with acetic anhydride (2.5ml) and pyridine (1ml) at room temperature for 2 h. The products were purified by preparative TLC (benzene) to give the amorphous

powder(IXb, 12mg), which showed only one spot on TLC. ms m/z: 246(M^+), 231. ^{1}H nmr (100 MHz, $CDCl_3$): δ 1.46(6H, s), 2.39(3H, s), 5.72(1H, d, J=10), 6.38(1H, d, J=10), 6.77(1H, d, J=9), 7.61 (1H, d, J=9), 9.72(1H, s).

Methylation of X (Formation of Xa)[10]

A mixture of X (200mg), dimethyl sulfate (0.3ml), and potassium carbonate (5g) in acetone (40ml) was refluxed for 30 min. The reaction mixture was treated as usual, and purified by preparative TLC (chloroform) to give a crystalline solid (Xa). Recrystallization from hexane-ether afforded colorless prisms (Xa, 50mg), mp 79-80°C, ms m/z: 196(M^+), 181. ^{1}H nmr (100 MHz, $CDCl_3$): δ 2.59, 3.78, 3.83(each 3H, s), 5.89(1H, d, J=2), 6.03(1H, d, J=2), 14.32(1H, s).

Condensation of IXa and Xa (Formation of XI)

To a mixture of IXa (30mg) and Xa (33mg) in ethanol (2ml), 25% aqueous potassium hydroxide(0.5ml) was added, and the mixture was allowed to stand overnight at room temperature. The reaction products were purified by preparative TLC (ether:hexane=1:1) to give XI. Crystallization from methanol gave yellow prisms (XI, 26mg), mp 141-142°C. UV λ_{max}^{EtOH} nm(log ε): 296(3.32), 372(3.62); $\lambda_{max}^{EtOH+AlCl_3}$: 306 (3.17), 415(3.68). ms m/z: 396(M^+), 381, 365. ^{1}H nmr (100 MHz, $CDCl_3$): δ 1.44(6H, s), 3.78, 3.81, 3.90(each 3H, s), 5.66(1H, d, J=10), 5.94(1H, d, J=2), 6.10(1H, d, J=2), 6.62(1H, d, J=10), 6.63 (1H, d, J=8), 7.44(1H, d, J=8), 7.83(1H, d, J=15), 8.05(1H, d, J=15), 14.75(1H, s).

Conversion of XI to Ib'

A mixture of XI (14 mg), ethanol (5ml), and 0.5% aqueous sodium hydroxide solution (2ml) was refluxed for 5 h and then allowed to stand for 48 h at room temperature. The reaction products were purified by preparative TLC (hexane:ether=2:1) to give an amorphous powder (Ib'), which showed only one spot on TLC. The ^{1}H nmr spectrum (270 MHz, $CDCl_3$) of Ib' was found to be superimposable over that of sanggenon F trimetyl ether (Ib).

ACKNOWLEDGEMENT We are grateful to Prof. S. Sakai, Faculty of Pharmaceutical Sciences, Chiba University, for mass spectral measurement, and also grateful to Dr. M. Takase, Research Laboratory of Zen-Yaku Kogyo Co., Ltd., for ^{1}H nmr spectral measurement (270 MHz), and also grateful to Dr. I. Yokoe, Faculty of Pharmaceutical Sciences, Josai University, for CD spectral measurement.

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Received, 23rd December, 1982