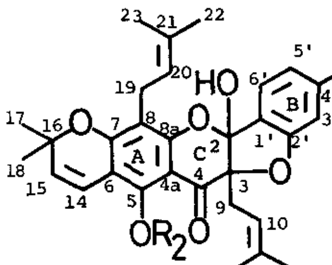
CONSTITUENTS OF THE CHINESE CRUDE DRUG "SĀNG-BÁI-PÍ" (MORUS ROOT BARK) V[1]. STRUCTURES OF THREE NEW FLAVANONES, SANGGENONS L, M, AND N

Yoshio Hano, Masato Itoh, Nanae Koyama, and Taro Nomura*
Faculty of Pharmaceutical Sciences, Toho University, 2-2-1, Miyama,
Funabashi-shi, Chiba 274, Japan

Abstract —— Three new isoprene substituted flavanones, named sanggenons L (1), M (2), and N (3) were isolated from the benzene extract of the Chinese crude drug "Sāng-Bái-Pí" (Japanese name "Sōhakuhi"), the root bark of Morus sp. (Moraceae). The structures 1, 2, and 3 were proposed for these compounds on the basis of the spectrometric and chemical evidence.

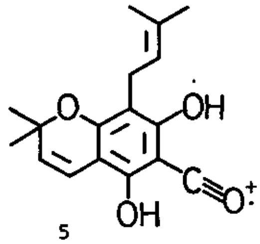
In the previous papers,[2] we reported the structure determination of a series of isoprenylated flavonoid derivatives obtained from the Chinese crude drug "Sāng-Bái-Pí" (Japanese name "Sōhakuhi") imported from the People's Republic of China. In the course of our studies, three new flavanone derivatives, sanggenons L (1), M (2), and N (3), were isolated as minor components from the benzene extract of the crude drug. This paper deals with the structural elucidation of these compounds.
The benzene extract of the crude drug was fractionated sequentially by the silica gel column chromatography, centrifugal thin layer chromatography, and preparative thin layer chromatography. This procedure yielded three new flavanone derivatives, sanggenons L (1), M (2), and N (3).
Sanggenon L (1) was obtained as amorphous powder, $[\alpha]_D^{20}$ +134° ($CHCl_3$), M^+= 504.2188, $C_{30}H_{32}O_7$, exhibiting a positive ferric chloride reaction, magnesium-hydrochloric acid test, and sodium borohydride test.[3] The ir spectrum of 1 suggested the presence of hydroxyl groups [3590, 3500, 3270 (br) cm^{-1}], aromatic rings [1620, 1610 (sh), 1580 cm^{-1}] and a chelated carbonyl group (1640 cm^{-1}), and the uv spectrum exhibited a close resemblance to that of sanggenon A (4),[2a] showing absorption maxima at 229, 273 (sh), 281, 323, 370 nm, which showed no red shift in the presence of aluminum chloride.[4] The ^1H nmr spectrum of 1 ($CDCl_3$) lacks the characteristic signals of the protons at C-2 and C-3 positions, and revealed the presence of two γ,γ- dimethylallyl groups [δ 1.59, 1.62 (each 3H, s), 3.10, 3.15 (each 1H, dd), 5.01 (1H, m); δ 1.59, 1.66 (each 3H, s), 2.82, 3.01 (each 1H, dd), 5.24 (1H, m)], 2,2-dimethylchromene ring [δ 1.40, 1.45 (each 3H, s), 5.50 (1H, d), 6.61(1H, d)], ABC type aromatic protons[δ 6.43 (1H, d), 6.48 (1H, dd), 7.30 (1H, d)], and a hydroxyl group [δ 11.46 (1H, s)]. The mass spectrum of 1 showed the significant peaks at m/z 287 (5) and 231 (6) arising from the A ring by a retro Diels-Alder fragmentation. In view of the ^1H nmr spectral data, these fragments suggest the presence of a γ,γ-dimethylallyl group, a chromene ring, and a hydroxyl group on the A ring. In the ^{13}C nmr spectrum of 1, the chemical shifts of the carbon atoms, except those of the carbon atoms at C-5, 7, and 8, were similar to those of the relevant carbon atoms of 4[2a] (Table 1). These results suggest that 1 is C-8 isoprenylated sanggenon A. The linear structure (1) for sanggenon L is supported by the changes in the chromene olefinic protons in monoacetate (1a) compared with those in the diacetate (1b) (Table 2). These changes are of the same sign and in the same order of magnitude as those observed by many investigators for similar compounds, in



1: $R_1=R_2=H$

1a: $R_1=COCH_3$, $R_2=H$

1b: $R_1=R_2=COCH_3$



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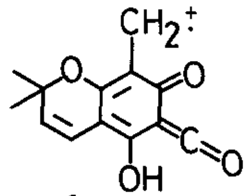

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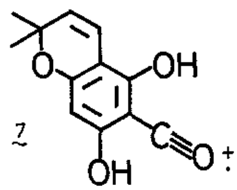

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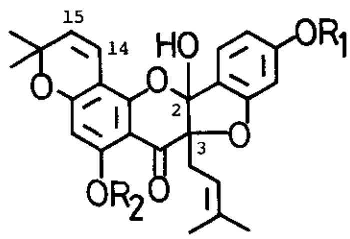
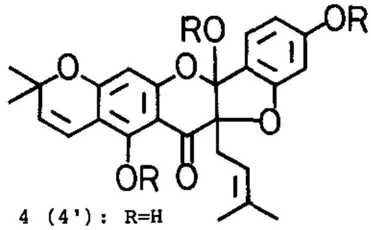


2 (2'): $R_1=R_2=H$

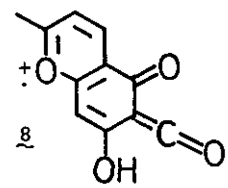
2'a: $R_1=COCH_3$, $R_2=H$

2'b: $R_1=R_2=COCH_3$



4 (4'): R=H

4b: $R=CH_3$



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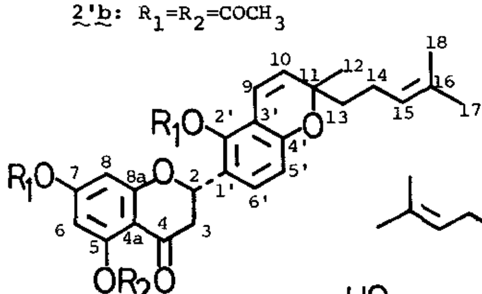
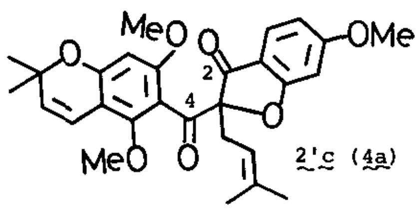


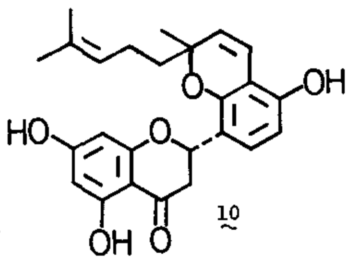
3: $R_1=R_2=H$

3a: $R_1=COCH_3$, $R_2=H$

3b: $R_1=R_2=COCH_3$



2'c (4a)



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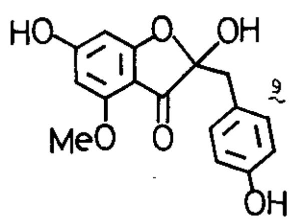

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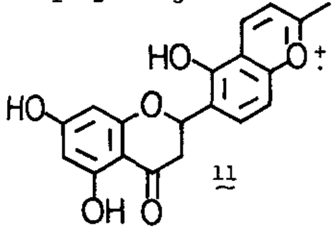

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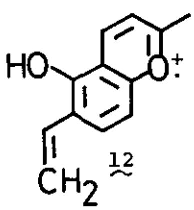

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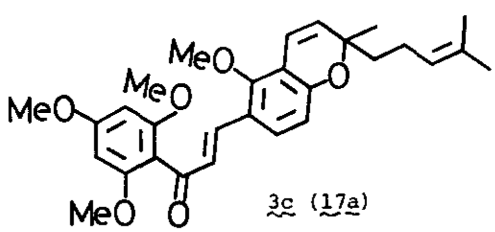

3c (17a)

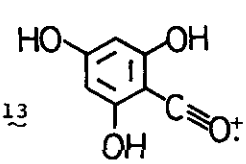

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Table 1 ^{13}C nmr Chemical Shifts (ppm)

carbon	1	4	2'	carbon	1	4	2'
C-2	102.6	102.5	102.1	C-9	32.0	32.1	32.2
C-3	92.0	92.6	92.5	C-10	118.8	118.7	118.5
C-4	189.5	188.6	188.8	C-11	136.3	136.9	137.0
C-4a	100.7	100.5	100.8	C-12	25.9	25.9	25.9
C-5	157.7	163.3	157.5	C-13	18.1	18.1	18.2
C-6	103.2	103.3	97.6	C-14	115.8	115.5	115.9
C-7	161.1	164.4	165.5	C-15	127.3	127.5	127.2
C-8	109.2	96.5	102.6	C-16	79.5	79.1	79.3
C-8a	161.9	161.4	164.4	C-17	28.5	28.5	28.5
C-1'	121.0	121.2	121.0	C-18	28.5	28.5	28.4
C-2'	159.7	159.5	161.2	C-19	21.9		
C-3'	99.5	99.6	99.6	C-20	123.3		
C-4'	161.3	161.4	161.5	C-21	131.4		
C-5'	109.9	109.9	110.0	C-22	26.0		
C-6'	125.8	125.9	125.6	C-23	18.1		

solvent; acetone-d_6

Table 2 Acetylation Shifts (ppm)

proton	1a	1b	Δ
C-14-H	6.57	6.29	+0.28
C-15-H	5.47	5.58	-0.11

measured in $CDCl_3$

Table 3 Acetylation Shifts (ppm)

proton	2'a	2'b	Δ
C-14-H	6.40	6.43	-0.03
C-15-H	5.43	5.48	-0.05

measured in $CDCl_3$

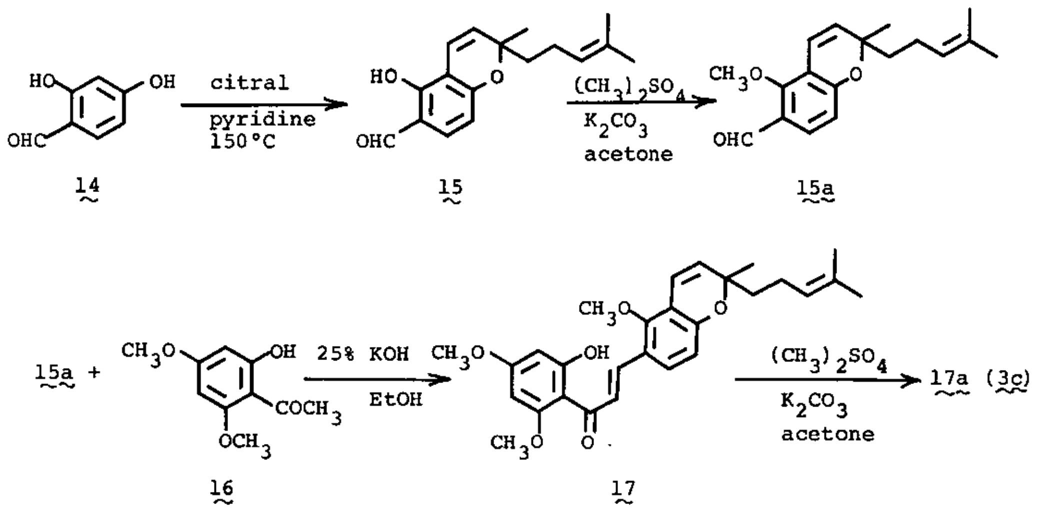

Chart 1

which the hydroxyl group is peri to C-14-H.[5] From these results, we propose the formula (1) for the structure of sanggenon L.

Sanggenon M (2) was obtained as amorphous powder, $[\alpha]_D^{20.5}$ +147° ($CHCl_3$), M^+=436.1536, $C_{25}H_{24}O_7$, exhibiting a positive ferric chloride reaction, magnesium-hydrochloric acid test and sodium borohydride test.[3] It gave the absorption bands of hydroxyl groups [3590, 3500, 3280 (br) cm^{-1}], aromatic rings (1610, 1580 cm^{-1}) and a chelated carbonyl group (1630 cm^{-1}) in the ir spectrum. The uv spectrum suggested the sanggenon A type flavanone structure,[2a] showing absorption maxima at 232, 269 (sh), 277, 319, and 375 nm. The absorption maxima at 269 (sh), 319, and 375 nm shifted in the presence of aluminum chloride to 277, 333, and 435 nm, respectively.[4] The ^1H nmr spectrum of 2 indicated the presence of a γ,γ-dimethylallyl group[δ 1.58 (6H, s), 2.77, 3.09 (each 1H, dd), 5.17 (1H, m)], 2,2-dimethylchromene ring[δ 1.37, 1.44 (each 3H, s), 5.40 (1H, d), 6.42 (1H, d)], ABC type aromatic protons[δ 6.43 (1H, d), 6.50 (1H, dd), 7.28 (1H, d)], an aromatic proton [δ 5.91 (1H, s)], and a hydroxyl group[δ 11.36 (1H, s)]. The mass spectrum of 2 showed the significant peaks at m/z 219 (7) and 203 (8).[2a] From these results, sanggenon M seems to be a structural isomer of 4, and the formula (2) was suggested. To confirm the structure, the following experiments were carried out. Sanggenon A (4) was dissolved in 0.5 M sodium carbonate solution, acidified with dilute hydrochloric acid, and extracted with ether. The ether layer was analysed with HPLC to be a 5 : 2 mixture of 2' and 4', which was purified by preparative TLC to give 2' and 4'. The ir and ^1H nmr spectra of 2' and 4' were identified with those of sanggenon M (2) and A (4), respectively.[6] The compound (2') was easily isomerized in the alkaline solution to give a 5 : 2 equilibrium mixture of 2' and 4'. The angular structure for 2' was supported by the changes in the chromene olefinic protons in monoacetate (2'a) compared with those in the diacetate (2'b) (Table 3).[2a,5] Further supporting data for the hemiketal structure of 2' was obtained by the examination of the trimethyl ether (2'c).[2c] Treatment of 2' with dimethyl sulfate and potassium carbonate in acetone gave the trimethyl ether (2'c) which was identified with the trimethyl ether (4a) obtained by the same treatment of 4. In the ^{13}C nmr spectrum of 2'c, the signals of two carbonyl carbons appeared at δ 173.0 and 194.7 attributed to C-2 and C-4 carbon, respectively. The former chemical shift value resembled that of the carbonyl carbon of the model compound (9) (δ 171.91).[7] From the above results, the structure of sanggenon M is represented by formula (2).

Sanggenon N (3) was obtained as pale yellow prisms, mp 102-105 °C, $[\alpha]_D^{20}$ -2° ($CHCl_3$), M^+=422.1736, $C_{25}H_{26}O_6$, exhibiting a positive ferric chloride reaction, magnesium-hydrochloric acid, and sodium borohydride test.[3] The ir spectrum of 3 suggested the presence of hydroxyl groups (3490 cm^{-1}), aromatic rings [1630 (sh), 1605, 1590, (sh) cm^{-1}], and a conjugated carbonyl group (1640 cm^{-1}). The uv spectrum closely resembled that of sanggenon I (10),[2g] showing absorption maxima at 213 (sh), 229, 288, 324 nm while at 228, 307, 373 nm in the presence of aluminum chloride. Treatment of 3 with acetic anhydride in pyridine at room temperature yielded a diacetate (3a) and triacetate (3b). The ^1H nmr spectrum (acetone-d_6) showed the characteristic signals of the protons at C-2 and C-3 positions [δ 5.78 (1H, dd); 2.74 (1H, dd), 3.22 (1H, dd)], and revealed the presence of a 2-methyl-2-(4-methylpent-3-enyl)chromene ring [δ 1.38, 1.58, 1.66 (each 3H, s), 2.00-2.30 (4H, m), 5.15 (1H, m), 5.72 (1H, d), 6.85 (1H, d)], ortho-coupled aromatic protons [δ 6.46 (1H, d), 7.28 (1H, d)], two aromatic protons [δ 6.00 (2H, br s)], and a hydroxyl group [δ 12.37 (1H, s)]. The mass spectrum showed the significant peaks[2g] at m/z 339 (11), 187 (12), and 153 (13). In the ^{13}C nmr spectrum of 3, the chemical shift values of the carbon atoms except those of the carbon atoms at C-2' and 4' were similar to those of the relevant carbon atoms of sanggenon I (10).[2g] Treatment of 3 with dimethyl sulfate and potassium carbonate in acetone yielded tetramethylsanggenon N (3c) which seems to be a chalcone derivative from its uv spectrum.[8] These results indicate that structure of sanggenon N is possibly represented by the formula (3).

Tetramethylsanggenon N (3c) was prepared according to the process shown in Chart 1. Condensation of 15a with 16 in alkaline solution gave a chalcone (17) which was converted into 3c. By analyzing the ir and ^{1}H nmr spectra, the compound (3c) thus obtained was identified with tetramethylsanggenon N derived from natural source. On the basis of the CD spectrum, 3 was shown to have the (S)-configuration at C-2.[9] From these results, sanggenon N is represented by the formula (3).

EXPERIMENTAL

Melting point was uncorrected. ^{1}H nmr and ^{13}C 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, infl. = inflection. The following instruments were used: melting points; Mitamura's micromelting point apparatus (hot-stage type), uv spectra; Hitachi 340 UV Spectrometer, ir spectra; Hitachi 295 Spectrometer, ^{1}H nmr spectra; JEOL·JNM 4H-100 NMR and JEOL GX-400 NMR Spectrometers, and Hitachi R-900 FT NMR Spectrometer, ^{13}C nmr spectra; Hitachi R-900 FT NMR Spectrometer, mass spectra (ms); JEOL·JMS OISG-2, Hitachi RMU-6E Mass Spectrometer, optical rotation; JASCO DIP-4, CD spectra; JASCO J-20 ORD Spectrometer, high-performance liquid chromatography (HPLC); Toyo Soda HLC-803, centrifugal thin-layer chromatography (TLC); Harrison Centrifugal TLC 2924. For TLC and preparative TLC, Wakogel B-5FM was used, and for centrifugal TLC, Merck Kieselgel 60 GF_{254}. For HPLC system, TSK-GEL (LS-410 AK) was used.

Isolation of Sanggenon L (1), M (2), and N (3)

The benzene extract (63 g) of the crude drug "Sāng-Bái-Pí" (Japanese name "Sōhakuhi", 18.3 Kg), a species of Morus (Moraceae), imported from the People's Republic of China, was chromatographed on silica gel (200 g) by using benzene-methanol as an eluent, each fraction being checked by TLC. The fractions eluted with benzene and benzene containing 0.5 % methanol were evaporated to give the residue (8.8 g). This residue was rechromatographed on silica gel (56 g) with hexane-acetone. The fractions eluted with hexane containing 2-3 % acetone were evaporated to give the residue (0.62 g). This residue was fractionated sequentially by centrifugal TLC (solvent system: chloroform:-acetone = 14:1) and by preparative TLC (hexane:acetone = 3:1, chloroform:ether = 8:1) to give sanggenon L (1, 10 mg) and M (2, 1 mg). The fraction eluted with hexane containing 3 % acetone was evaporated to give residue (0.62 g). This residue was fractionated sequentially by centrifugal TLC (hexane:acetone = 3:1) and by preparative TLC (chloroform:ether = 8:1, hexane:ether = 1:1, hexane:acetone = 2:1) to give sanggenon N (3, 65 mg).

Sanggenon L (1)

The compound (1) was obtained as amorphous powder, $[\alpha]_D^{20}$ +134° (c=0.478 in chloroform), $FeCl_3$ test: olive green, Mg-HCl test: orange, $NaBH_4$ test: violet. uv λ_{max}^{EtOH} nm (log ϵ): 229 (4.10), 273 (sh 4.32), 281 (4.35), 323 (3.96), 370 (3.23); $\lambda_{max}^{EtOH+AlCl_3}$ nm (log ϵ): 228 (4.11), 272 (sh 4.29), 281 (4.33), 325 (3.93), 383 (3.17). ir $\nu_{max}^{CHCl_3}$ cm^{-1}: 3590, 3500, 3270 (br), 1640, 1620, 1610 (sh), 1580. High-resolution ms: Calcd. for $C_{30}H_{32}O_7$ (M^+, m/z): 504.2146. Found: 504.2188; Calcd. for $C_{17}H_{19}O_4$ (5): 287.1282. Found: 287.1287; Calcd. for $C_{13}H_{11}O_4$ (6): 231.0656. Found: 231.0664. ^{1}H nmr (400 MHz, $CDCl_3$): δ 1.40, 1.45 (each 3H, s, C-16-CH_3), 1.59 (6H, s, C-11- and 21-CH_3), 1.62 (3H, s, C-21-CH_3), 1.66 (3H, s, C-11-CH_3), 2.82 (1H, dd, J=6.7 and 15.0, C-9-H), 3.01 (1H, dd, J=7.9 and 15.0, C-9-H), 3.10 (1H, dd, J=7.6 and 14.4, C-19-H), 3.15 (1H, dd, J= 6.4 and 14.4, C-19-H), 5.01 (1H, m, C-20-H), 5.10 (1H, s, C-2-OH), 5.24 (1H, m, C-10-H), 5.50 (1H, d, J=10.1, C-15-H), 6.43 (1H, d, J=2.1, C-3'-H), 6.48 (1H, dd, J=2.1 and 8.2, C-5'-H), 6.61 (1H, d, J=10.1, C-14-H), 7.30 (1H, d, J=8.2, C-6'-H), 11.46 (1H, s, C-5-OH).

Acetylation of 1

Sanggenon L (1, 9 mg) was acetylated with acetic anhydride (1.8 ml) and pyridine (0.6 ml) at room temperature for 3 min. The product was purified by preparative TLC (hexane:ether = 1:1) to give sanggenon L monoacetate (1a, 3 mg) and diacetate (1b, 3 mg). The compound (1a) was obtained as amorphous powder, positive to $FeCl_3$ test: olive-green. uv λ_{max}^{EtOH} nm : 227 (infl.), 272 (sh), 281, 325, 374. ir $\nu_{max}^{CHCl_3}$ cm^{-1}: 3510, 1760, 1650, 1633, 1615 (sh), 1585. ms m/z: 546 (M^+), 287, 231. ^1H nmr (90 MHz, $CDCl_3$): δ 1.39, 1.43 (each 3H, s, C-16-CH_3), 1.58 (9H, s, C-11-CH_3 and 21-CH_3 x 2), 1.64 (3H, s, C-11-CH_3), 2.27 (3H, s, C-4'-$OCOCH_3$), 2.60-3.32 (4H, m, C-9-H x 2 and 19-H x 2), 4.97 (1H, m, C-20-H), 5.13 (1H, s, C-2-OH), 5.22 (1H, m, C-10-H), 5.47 (1H, d, J=10, C-15-H), 6.57 (1H, d, J=10, C-14-H), 6.65 (1H, d, J=2, C-3'-H), 6.72 (1H, dd, J=2 and 8, C-5'-H), 7.38 (1H, d, J=8, C-6'-H), 11.40 (1H, s, C-5-OH). The compound (1b) was obtained as amorphous powder, negative to $FeCl_3$ test. uv λ_{max}^{EtOH} nm: 227 (infl.),267, 281 (infl.), 301 (infl.), 354. ir $\nu_{max}^{CHCl_3}$ cm^{-1}: 3470 (br), 1765. 1672, 1645, 1610, 1570. ms m/z: 588 (M^+), 546, 329, 287, 231. ^1H nmr (90 MHz, $CDCl_3$): δ 1.39, 1.43 (each 3H, s, C-16-CH_3), 1.58 (9H, s, C-11-CH_3 and C-16-CH_3 x 2), 1.66 (3H, s, C-11-CH_3), 2.26 (3H, s, C-4'-$OCOCH_3$), 2.38 (3H, s, C-5-$OCOCH_3$), 2.70-3.30 (4H, m, C-9-H x 2 and 19-H x 2), 4.94 (1H, m, C-20-H), 5.13 (1H, s, C-2-OH), 5.18 (1H, m, C-10-H), 5.58 (1H, d, J=10, C-15-H), 6.29 (1H, d, J=10, C-14-H), 6.50 (1H, d, J=2, C-3'-H), 6.69 (1H, dd, J=2 and 8, C-5'-H), 7.36 (1H, d, J=8, C-6'-H).

Sanggenon M (2)

The compound (2) was obtained as amorphous powder, $[\alpha]_D^{20.5}$ +147° (c=0.373 in chloroform), positive to $FeCl_3$ test: olive-green, Mg-HCl test: orange, $NaBH_4$ test: violet. uv λ_{max}^{EtOH} nm (log ε): 232 (4.19), 269 (sh 4.40), 277 (4.43), 319 (3.99), 375 (3.40); $\lambda_{max}^{EtOH+AlCl_3}$ nm (log ε): 232 (4.19), 277 (4.44), 333 (4.05), 435 (3.34). ir $\nu_{max}^{CHCl_3}$ cm^{-1}: 3590, 3500, 3280 (br), 1630, 1610, 1580. High-resolution ms: Calcd. for $C_{25}H_{24}O_7$ (M^+, m/z): 436.1521. Found: 436.1536; Calcd. for $C_{11}H_7O_4$ (8): 203.0344. Found: 203.0369. ms m/z: 436 (M^+), 219 (7), 203. ^1H nmr (90 MHz, $CDCl_3$): δ 1.37, 1.44 (each 3H, s, C-16-CH_3), 1.58 (6H, s, C-11-CH_3 x 2), 2.77 (1H, dd, J=6 and 15, C-9-H), 3.09 (1H, dd, J=9 and 15, C-9-H), 5.17 (1H, m, C-10-H), 5.40 (1H, d, J=10, C-15-H), 5.91 (1H, s, C-6-H), 6.42 (1H, d, J=10, C-14-H), 6.43 (1H, d, J=2, C-3'-H), 6.50 (1H, dd, J=2 and 8, C-5'-H), 7.28 (1H, d, J=8, C-6'-H), 11.36 (1H, s, C-5-OH).

Alkaline Treatment of Sanggenon A (4)

A solution of 4 (10 mg) in 0.5M-Na_2CO_3 solution (30 ml) was shaken for 5 min at room temperature, acidified with 0.5N-HCl solution, extracted with ether, and followed by usual work up. After the solvent was evaporated, the product was purified by preparative TLC (hexane:ether = 1:1) to give 2' (5 mg) and 4' (2 mg). The product was also analysed to be a 5:2 mixture of 2' and 4' by HPLC with solvent system ($MeOH:H_2O$ = 7:3) as the eluent. The same alkaline treatment of 2' gave the reaction products analysed by HPLC to be a 5:2 mixture of 2' and 4'. The compound (2') was obtained as amorphous powder, $[\alpha]_D^{25}$ +3° (c=0.238 in chloroform). The ir and the ^1H nmr spectrum of 2' were identified with those of sanggenon M (2). The compound (4') was obtained as amorphous powder, $[\alpha]_D^{25}$+1° (c=0.146 in chloroform). The ir and the ^1H nmr spectrum of 4' were identified with those of sanggenon A (4).

Acetylation of 2'

The compound (2', 22 mg) was acetylated with acetic anhydride (3.6 ml) and pyridine (1.2 ml) at room temperature for 4 min. The product was purified by preparative TLC (hexane:ether = 1:1) to give monoacetate (2'a, 8 mg) and diacetate (2'b, 7 mg). The compound (2'a) was obtained as amorphous powder, positive to $FeCl_3$ test: olive-green. uv λ_{max}^{EtOH} nm (log ε): 228 (sh 4.02), 237 (infl. 3.98), 268 (sh 4.29), 277 (4.32), 319 (3.92), 375 (3.30); $\lambda_{max}^{EtOH+AlCl_3}$ nm (log ε): 231 (infl. 4.00), 275 (4.34), 333 (3.93), 417 (3.17). ir ν_{max}^{KBr} cm^{-1}: 3400 (br), 1765, 1640, 1610 (sh), 1583. ms m/z: 478 (M^+), 219, 203. ^{1}H nmr (90 MHz, $CDCl_3$): δ 1.38, 1.44 (each 3H, s, C-16-CH_3), 1.58 (6H, s, C-11-CH_3 x 2), 2.27 (3H, s, C-4'-$OCOCH_3$), 2.78 (1H, dd, J=8 and 16, C-9-H), 3.10 (1H, dd, J=9 and 16, C-9-H), 5.15 (1H, m, C-10-H), 5.43 (1H, d, J=11, C-15-H), 5.92 (1H, s, C-6-H), 6.40 (1H, d, J=11, C-14-H), 6.68 (1H, d, J=2, C-3'-H), 6.76 (1H, dd, J=2 and 8, C-5'-H), 7.45 (1H, d, J=8, C-6'-H), 11.29 (1H, s, C-5-OH). Compound (2'b) was obtained as amorphous powder, negative to $FeCl_3$ test. uv λ_{max}^{EtOH} nm (log ε): 257 (3.99), 272 (3.98), 305 (infl. 3.45), 318 (3.50), 362 (infl. 2.80) ir ν_{max}^{KBr} cm^{-1}: 3430, 1780, 1674, 1647, 1610, 1578. ms m/z: 520 (M^+), 478, 261, 219, 203. ^{1}H nmr (90 MHz, $CDCl_3$): δ 1.39, 1.44 (each 3H, s, C-16-CH_3 x 2), 1.58 (6H, s, C-11-CH_3 x 2), 2.26 (3H, s, C-4'-$OCOCH_3$), 2.36 (3H, s, C-5-$OCOCH_3$), 2.77 (1H, dd, J=7 and 16, C-9-H), 3.08 (1H, dd, J=10 and 16, C-9-H), 5.13 (1H, m, C-10-H), 5.48 (1H, d, J=11, C-15-H), 6.11 (1H, s, C-6-H), 6.43 (1H, d, J=11, C-14-H), 6.64 (1H, d, J=2, C-3'-H), 6.73 (1H, dd, J=2 and 8, C-5'-H), 7.39 (1H, d, J=8, C-6'-H).

Methylation of 2' [Formation of 2'c (4a)]

A mixture of 2' (18 mg), dimethyl sulfate (0.05 ml), and potassium carbonate (5 g) in acetone (30 ml) was refluxed for 1 h. The product was purified by preparative TLC (hexane:acetane = 3:1) to give 2'c (2 mg), which was identified with 4a obtained from 4 by comparing the ir spectrum of 2'c with that of 4a.

Methylation of 4 [Formation of 4a (2'c) and 4b]

A mixture of 4 (18 mg), dimethyl sulfate (0.15 ml), and potassium carbonate (5 g) in acetone (30 ml) was kept at 40-45°C for 30 min. The product was purified by preparative TLC (benzene; hexane: acetone = 3:1; benzene:ether = 5:1) to give 4a (7 mg) and 4b (4 mg). The compound 4a was obtained as amorphous powder, $[\alpha]_D^{16}$ +1° (c=0.241 in chloroform), negative to Mg-HCl test, and negative to $FeCl_3$ test. uv λ_{max}^{EtOH} nm (log ε): 236 (4.38), 276 (4.42), 324 (3.78), 391 (infl. 3.36). ir ν_{max}^{KBr} cm^{-1}: 1745, 1730, 1680, 1635, 1605, 1565, 1557. ms m/z: 478 (M^+), 410, 381, 246. ^{1}H nmr (100 MHz, $CDCl_3$): δ 1.44 (6H, s, C-16-CH_3 x 2), 1.53 (6H, s, C-11-CH_3 x 2), 3.07 (2H, br d, J=7, C-9-H x 2), 3.56., 3.78, 3.81 (each 3H, s, OCH_3), 5.09 (1H, m, C-10-H), 5.58 (1H, d, J=10, C-15-H), 6.11 (1H, s, C-8-H), 6.37 (1H, d, J=2, C-3'-H), 6.48 (1H, dd, J=2 and 8, C-5'-H), 6.59 (1H, d, J=10, C-14-H), 7.37 (1H, d, J=8, C-6'-H). ^{13}C nmr ($CDCl_3$):δ17.9 (C-13), 25.8 (C-12), 28.5 (C-17 and 18), 55.4, 55.6, 62.1 (OCH_3), 78.3 (C-16), 89.0 (C-3), 99.5 (C-8), 101.0 (C-3'), 105.0 (C-5'), 109.8 (C-6), 110.3 (C-4a), 115.8 (C-14), 116.5 (C-10), 121.7 (C-1'), 127.8 (C-6'), 128.9 (C-15), 137.3 (C-11), 157.9 (C-2' and 4'), 161.7 (C-5), 161.9 (C-8a), 164.0 (C-7), 173.0 (C-2), 194.7 (C-4). The compound (4b) was obtained as amorphous powder, positive to Mg-HCl test: orange, and negative to $FeCl_3$ test. uv λ_{max}^{EtOH} nm (log ε): 232 (4.27), 255 (infl. 4.26), 271 (4.36), 290 (infl. 4.20), 300 (infl. 4.04), 338 (3.48). ir ν_{max}^{KBr} cm^{-1}: 1680. 1625, 1600, 1562. ms m/z: 478 (M^+), 246, 231, 203. ^{1}H nmr (100 MHz, $CDCl_3$):δ 1.36, 1.40 (each 3H, s, C-16-CH_3 x 2), 1.54 (3H, s, C-11-CH_3), 1.60 (3H, s, C-11-CH_3), 2.70-2.92 (2H, m, C-9-H x 2), 3.72, 3.73, 3.79 (each 3H, s, OCH_3), 5.30 (1H, m, C-10-H), 5.48 (1H, d, J=10, C-15-H), 6.00 (1H, s, C-8-H), 6.41 (1H, d, J=2, C-3'-H), 6.42 (1H, dd, J=2 and 8, C-5'-H), 6.49 (1H, d, J=10, C-14-H), 7.21 (1H, d, J=8, C-6'-H). ^{13}C nmr ($CDCl_3$):δ18.0 (C-13), 25.9 (C-12), 28.3 (C-17 and 18), 31.6 (C-9), 53.6, 55.6, 62.4 (OCH_3), 77.9 (C-16), 91.2 (C-3), 97.5 (C-8), 101.2 (C-3'), 104.4 (C-2), 108.2 (C-5'), 108.6 (C-6), 110.0 (C-4a), 116.1 (C-14), 117.9 (C-10), 121.5 (C-1'), 125.0 (C-6'), 128.6 (C-15), 135.7 (C-11), 157.3 (C-2'), 159.8 (C-4'), 160.7 (C-5), 162.4 (C-7 and 8a), 183.5 (C-4).

Sanggenon N (3)

The compound (3) was recrystallized from hexane-ether (5:1) to give pale yellow prisms, mp 102-105°C, $[\alpha]_D^{20}$ -2° (c=0.349 in chloroform), positive to $FeCl_3$ test: brown, Mg-HCl test: violet,

$NaBH_4$ test: reddish orange. uv λ_{max}^{EtOH} nm (log ε): 213 (sh 4.55), 229 (4.70), 288 (4.40), 324 (sh 3.82); $\lambda_{max}^{EtOH+AlCl_3}$ nm (log ε): 228 (4.72), 307 (4.38), 373 (3.50). ir ν_{max}^{KBr} cm^{-1}: 3490, 1640, 1630 (sh), 1605, 1590 (sh). High-resolution ms: Calcd. for $C_{25}H_{26}O_6$ (M^+, m/z): 422.1735. Found: 422.1736. ms m/z: 422 (M^+), 339 (11), 187 (12), and 153 (13). ^1H nmr (100 MHz, acetone-d_6): δ 1.38 (3H, s, C-11-CH_3), 1.58, 1.66 (each 3H, s, C-16-CH_3), 2.00–2.30 (4H, m, C-13-H x 2 and 14-H x 2), 2.74 (1H, dd, J=3 and 17, C-3-H), 3.22 (1H, dd, J=13 and 17, C-3-H), 5.15 (1H, m, C-15-H), 5.72 (1H, d, J=11, C-10-H), 5.78 (1H, dd, J=3 and 13, C-2-H), 6.00(2H, br s, C-6-H and 8-H), 6.46 (1H, d, J=9, C-5'-H), 6.85 (1H, d, J=11, C-9-H), 7.28 (1H, d, J=9, C-6'-H), 12.37 (1H, s, C-5-OH). ^{13}C nmr (acetone-d_6): δ 17.6 (C-18), 23.3 (C-14), 25.8 (C-17), 26.4 (C-12), 41.6 (C-13), 42.4 (C-3), 75.8 (C-2), 78.7 (C-11), 96.0 (C-8), 96.9 (C-6), 103.1 (C-4a), 109.3 (C-5'), 111.1 (C-3'), 117.8 (C-9), 119.1 (C-1'), 125.0 (C-15), 128.0 (C-10), 129.2 (C-6'), 131.9 (C-16), 150.9 (C-4'), 155.2 (C-2'), 164.5 (C-8a), 165.3 (C-5), 167.4 (C-7), 197.4 (C-4). CD spectrum: $[\theta]_{333}$ +300, $[\theta]_{286}$ −3900, $[\theta]_{255}$ +600.

Acetylation of 3 (Formation of 3a and 3b)

Sanggenon N (3, 10 mg) was acetylated with acetic anhydride (1.2 ml) and pyridine (0.4 ml) at room temperature for 2 min. The product was purified by preparative TLC (hexane:ether = 1:1) to give diacetate (3a, 7 mg) and triacetate (3b, 2 mg). The compound (3a) was obtained as amorphous powder, positive to $FeCl_3$ test: brown. uv λ_{max}^{EtOH} nm (log ε): 209 (sh 4.66), 227 (4.89), 272 (4.32), 320 (3.83), 342 (sh 3.70); $\lambda_{max}^{EtOH+AlCl_3}$ nm (log ε): 208 (sh 4.64), 226 (4.92), 300 (4.34), 391 (3.81). ir $\nu_{max}^{CHCl_3}$ cm^{-1}: 1770, 1650, 1630, 1620 (sh), 1580. ms m/z: 506 (M^+), 423, 381, 187. ^1H nmr (90 MHz, acetone-d_6): δ 1.39 (3H, s, C-11-CH_3), 1.56, 1.63 (each 3H, s, C-16-CH_3), 1.90–2.20 (4H, m, C-13-H x 2 and 14-H x 2), 2.25, 2.31 (each 3H, s, $OCOCH_3$), 2.72 (1H, dd, J=3 and 17, C-3-H), 3.33 (1H, dd, J=13 and 17, C-3-H), 5.09 (1H, m, C-15-H), 5.69 (1H, dd, J=3 and 13, C-2-H), 5.78 (1H, d, J=11, C-10-H), 6.22 (1H, d, J=2, C-6-H), 6.25 (1H, d, J=2, C-8-H), 6.40 (1H, d, J=11, C-9-H), 6.76 (1H, d, J=8, C-5'-H), 7.43 (1H, d, J=8, C-6'-H). The compound (3b) was obtained as amorphous powder, negative to $FeCl_3$ test. uv λ_{max}^{EtOH} nm (log ε): 229 (5.04), 260 (4.30), 311 (3.93). ir $\nu_{max}^{CHCl_3}$ cm^{-1}: 1770, 1690, 1620, 1580. ms m/z: 548 (M^+), 465, 423, 381, 187. ^1H nmr (90 MHz, acetone-d_6): δ 1.39 (3H, s, C-11-CH_3), 1.56, 1.63 (each 3H, s, C-16-CH_3), 1.95–2.15 (4H, m, C-13-H x 2 and 14-H x 2), 2.27, 2.28, 2.33(each 3H, s, $OCOCH_3$), 3.18 (1H, dd, J=13 and 17, C-3-H), 5.08 (1H, m, C-15-H), 5.66 (1H, dd, J=3 and 13, C-2-H), 5.77 (1H, d, J=11, C-10-H), 6.41 (1H d, J=11, C-9-H), 6.55 (1H, d, J=2, C-6-H), 6.70 (1H, d, J=2, C-8-H), 6.75 (1H, d, J=9, C-5'-H), 7.43 (1H, d, J=9, C-6'-H).

Methylation of 3 (Formation of 3c)

A mixture of 3 (13 mg), dimethyl sulfate (0.3 ml), and potassium carbonate (5 g) in acetone (30 ml) was refluxed for 2 h, and treated as usual. The product was purified by preparative TLC (hexane:acetone = 3:1, benzene:ether = 3:1) to give the amorphous powder (3c, 8 mg). uv λ_{max}^{EtOH} nm: 240 (sh), 289, 343. ir $\nu_{max}^{CHCl_3}$ cm^{-1}: 1660 (sh), 1640 (sh), 1600, 1590, 1570 (sh). ms m/z: 478 (M^+), 447, 395, 195. ^1H nmr (90 MHz, acetone-d_6): δ 1.38 (3H, s, C-9-CH_3), 1.54, 1.61 (each 3H, s, C-14-CH_3), 1.90–2.20 (4H, m, C-11-H x 2 and 12-H x 2), 3.65 (3H, s, OCH_3), 3.73 (6H, s, OCH_3 x 2), 3.84 (3H, s, OCH_3), 5.08 (1H, m, C-13-H), 5.73 (1H, d, J=11, C-8-H), 6.28 (2H, s, C-3'-H and 5'-H), 6.59 (1H, d, J=8, C-5-H), 6.60 (1H, d, J=11, C-7-H), 6.75 (1H, d, J=16, C-α-H), 7.43 (1H, d, J=16, C-β-H), 7.50 (1H, d, J=8, C-6-H).

6-Hydroxy-2-methyl-2-(4-methylpent-3-enyl)chromene-7-al (15)[10]

A mixture of 2,4-dihydroxybenzaldehyde (14, 2.2 g), citral (2 ml), and dry pyridine (1.3 ml) was heated and stirred at 150°C for 6 h. More citral (1 ml) was introduced and the mixture was heated under reflux for a further 4 h. The mixture was evaporated under reduced pressure, and the residue was chromatographed on silica gel. From the fraction eluted with benzene, 15 (844 mg) was obtained by preparative TLC (hexane:benzene = 1:1). The compound (15) showed the following spectral data: uv λ_{max}^{EtOH} nm (logε): 256 (sh 4.23), 260 (sh 4.24), 274 (4.30), 302 (3.97), 314 (infl. 3.93); $\lambda_{max}^{EtOH+AlCl_3}$ nm (logε): 260 (infl. 4.19), 272 (4.27), 286 (infl. 4.10), 319 (3.93), 330 (3.92), 386 (2.85). ir $\nu_{max}^{CHCl_3}$ cm^{-1}: 1640, 1620, 1580. ms m/z: 272 (M^+), 257, 189. ^{1}H nmr (90 MHz, acetone-d_6):δ 1.44, 1.57, 1.66 (each 3H, s), 1.95–2.30 (4H, m), 5.13 (1H, m,), 5.73 (1H, d, J=10), 6.49 (1H, d, J=8), 6.76 (1H, d, J=10), 7.53 (1H, d, J=8), 9.84 (1H, s), 12.03 (1H, s).

Methylation of 15 (Formation of 15a)

A mixture of 15 (106 mg), dimethyl sulfate (0.11 ml) and potassium carbonate (5 g) in acetone (30 ml) was refluxed for 15 min and treated as usual. The product was purified by preparative TLC (hexane:benzene = 1:1) to give 15a (93 mg). The compound (15a) showed the following spectral data. uv λ_{max}^{EtOH} nm (log ε): 234 (4.29), 264 (4.56), 299 (3.92). ir $\nu_{max}^{CHCl_3}$ cm^{-1}: 1670, 1640, 1590, 1570. ms m/z: 286 (M^+), 203, 160. ^{1}H nmr (90 MHz, acetone-d_6):δ 1.42, 1.54, 1.63 (each 3H, s), 1.90–2.30 (4H, m), 3.93 (3H, s), 5.11 (1H, m), 5.83 (1H, d, J=10), 6.69 (1H, d, J=8), 6.73 (1H, d, J=10), 7.63 (1H, d, J=8), 10.26 (1H, s).

Condensation of 15a and 16 (Formation of 17)

To a mixture of 15a (87 mg) and 16 (60 mg) in ethanol (8 ml) was added 25 % aqueous potassium hydroxide (8 ml). The mixture was heated at 60°C for 3 h, and allowed to stand for 29 h. The reaction mixture was treated as usual, and purified by preparative TLC (hexane:ether = 3:1; benzene) to give 17 (36 mg). The compound (17) showed the following spectral data. uv λ_{max}^{EtOH} nm (log ε): 233 (sh 3.99), 300 (3.87), 377 (4.21); $\lambda_{max}^{EtOH+AlCl_3}$ nm (log ε): 223 (sh 4.15), 246 (sh 3.89), 315 (sh 3.70), 337 (sh 3.71), 419 (4.25). ir $\nu_{max}^{CHCl_3}$ cm^{-1}: 1620, 1590, 1560. ms m/z: 464 (M^+), 381, 181. ^{1}H nmr(90 MHz, acetone-d_6):δ1.38 (3H, s, C-9-CH_3), 1.53, 1.61 (each 3H, s, C-14-CH_3), 1.70–2.25 (4H, m, C-11-H x 2 and 12-H x 2), 3.79, 3.83, 3.96 (3H, s, OCH_3), 5.08 (1H, m, C-13-H), 5.77 (1H, d, J=10, C-8-H), 6.06 (2H, s, C-3'-H and 5'-H), 6.62 (1H, d, J=9, C-5-H), 6.67 (1H, d, J=10, C-7-H), 7.56 (1H, d, J=9, C-6-H), 7.94 (2H, s, C-α-H and β-H), 14.40 (1H, s, C-2'-OH).

Methylation of 17 [Formation of sanggenon N-tetramethyl ether (3c)]

A mixture of 17 (15 mg), dimethyl sulfate (0.1 ml), and potassium carbonate (5 g) in acetone (30 ml) was refluxed for 2 h and treated as usual. The product was purified by preparative TLC (hexane: ether = 2:1) to give the amorphous powder (17a, 10 mg). The compound (17a) obtained here was identified with sanggenon N tetramethyl ether (3c) by comparing the ^{1}H nmr and ir spectra of 17a with those of 3c.

ACKNOWLEDGEMENT We are grateful to Prof. S. Sakai, Faculty of Pharmaceutical Sciences, Chiba University, for high-resolution mass spectral and CD spectral measurements, and also grateful to Dr. J. Uzawa, the Institute of Physical and Chemical Research, for ^{1}H nmr spectral measurement (400 MHz).

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Received, 20th April, 1984