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CONSTITUENTS OF THE CHINESE CRUDE DRUG "SĀNG-BÁI-PÍ" (MORUS ROOT BARKS) IV[1]. STRUCTURES OF FOUR NEW FLAVONOIDS, SANGGENON H, I, J AND K.

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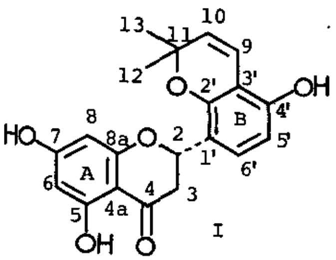
Abstract —— Two new isoprene substituted flavanone derivatives, named sanggenon H and I, and two new isoprene substituted flavone derivatives, named sanggenon J and K, were isolated from the benzene extract of the Chinese crude drug "Sāng-Bái-Pí" (Japanese name "Sōhakuhi"), the root barks of Morus sp. (Moraceae). The structures I, II, III, and IV were proposed for these compounds on the basis of the spectroscopic evidence, respectively. Photo-oxidative cyclization did not occur with sanggenon K (IV) but with sanggenon J (III).

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, four new flavonoid derivatives, sanggenon H (I), I (II), J (III), and K (IV), were isolated from the benzene extract of the crude drug. In this paper, the structure determination of these new flavonoid derivatives is described.

The benzene extract[2] of the crude drug was fractionated sequentially by the silica gel column chromatography and by preparative thin layer chromatography. This procedure yielded four new flavonoid derivatives, sanggenon H (I), I (II), J (III), and K (IV).

Sanggenon H (I) was obtained as amorphous powder, M^+ 354, $C_{20}H_{18}O_6$, $[\alpha]_D^{22}$ -31.0°, which showed positive methanolic ferric chloride(an intense reddish violet color), magnesium-hydrochloric acid, and sodium borohydride tests[3]. The UV spectra of I [λ_{max}^{EtOH} nm: 218(sh), 227, 289, 318(sh); $\lambda_{max}^{EtOH+AlCl_3}$ nm: 223, 282(infl.), 309, 372] closely resembled those of sanggenon F (V)[2f]. The ^{1}H nmr spectrum of I showed the characteristic signals for flavanone skeleton [δ 2.67(1H, dd, J=3 and 17.5), 3.13(1H, dd, J=12.5 and 17.5), 5.70(1H, dd, J=3 and 12.5)] and 2,2-dimethylchromene ring [δ 1.43(6H, s), 5.67(1H, d, J=10), 6.71(1H, d, J=10)][4]. The arrangement of substituents in the A and B rings was assumed as follows by the ^{1}H nmr spectrum of I (Table 1). The biogenetic analogy to other prenylflavonoid derivatives[4] isolated from Morus species suggests that the B ring has the 2',4'-dioxygenated pattern. The mass spectrum of I showed the significant peaks at m/z 187(VI)[2f] and 153(VII)[2f]. Hence, sanggenon H (I) is structural isomer of sanggenon F (V). In comparison of the ^{1}H nmr spectra of I and V, the signal of the C-5'-H of I was shifted to an about 0.1 ppm lower applied magnetic field than that of V (Table 1). The formula I for sanggenon H was substantiated by examination of the ^{13}C nmr spectrum using some model compounds[5] (Table 2). On the basis of the specific optical rotation (-)[6] and the CD spectrum, I has the (S)-configuration at C-2[7]. From the above results, the structure of sanggenon H could be assigned to I.

Sanggenon I (II) was obtained as amorphous powder, M^+ 422, $C_{25}H_{26}O_6$, $[\alpha]_D^{22}$ -76.9°, which showed positive methanolic ferric chloride(an intense reddish violet color), magnesium-hydrochloric acid,



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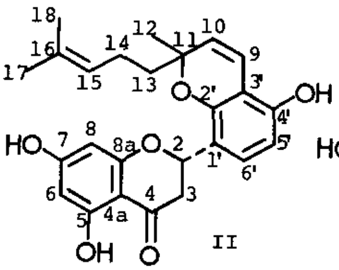

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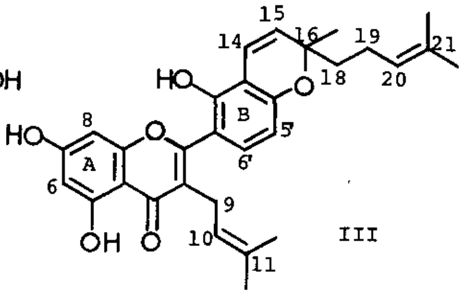

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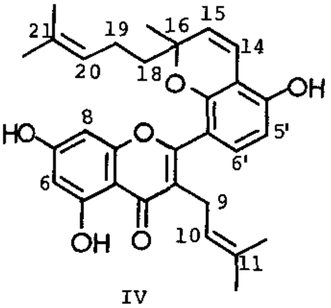

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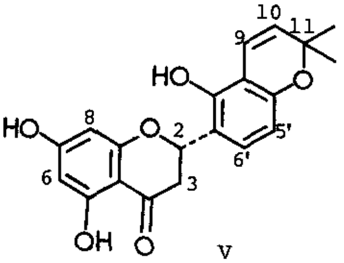

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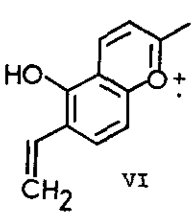

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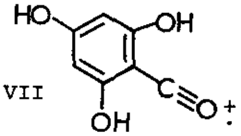

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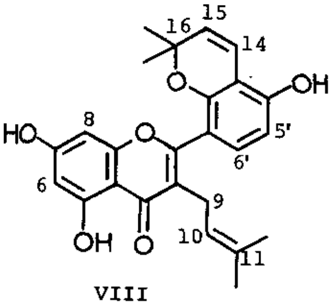

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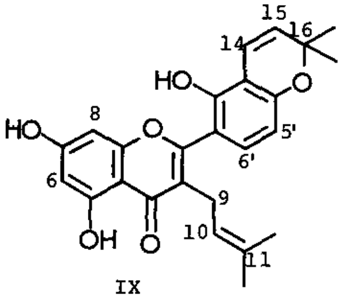

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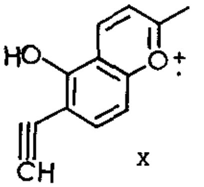

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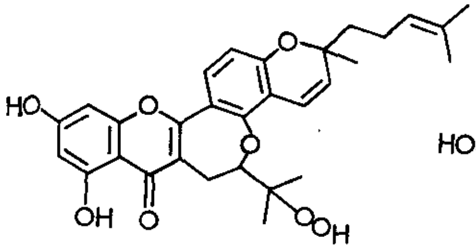

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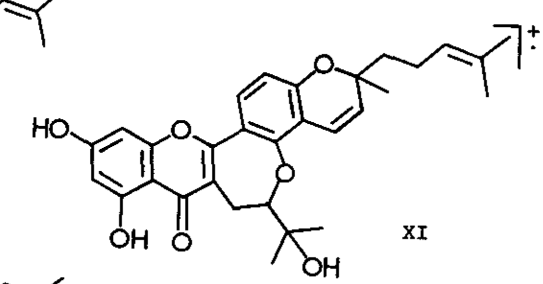

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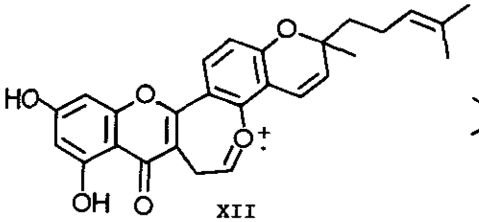

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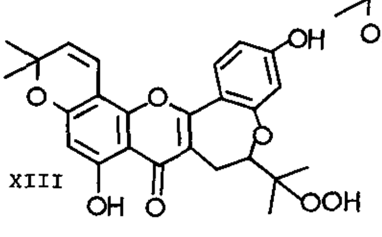

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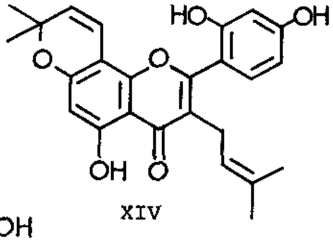

XIV

Table 1 ^{1}H nmr spectra (δ in acetone-d_6)

	I	II	V
2-H	5.70(1H,dd,J=3, 12.5)	5.68(1H,dd,J=3, 14)	5.72(1H,dd,J=3, 12.5)
3-H	2.67(1H,dd,J=3, 17.5)	2.71(1H,dd,J=3, 17)	2.76(1H,dd,J=3, 17.5)
	3.13(1H,dd,J=12.5, 17.5)	3.16(1H,dd,J=14, 17)	3.16(1H,dd,J=12.5, 17.5)
6-H	5.97(1H,s)	5.96(1H,d,J=2)	5.99(1H,d,J=2)
8-H	5.97(1H,s)	5.97(1H,d,J=2)	6.01(1H,d,J=2)
5'-H	6.52(1H,d,J=8)	6.50(1H,d,J=8)	6.42(1H,d,J=8)
6'-H	7.22(1H,d,J=8)	7.21(1H,d,J=8)	7.19(1H,d,J=8)
9-H	6.71(1H,d,J=10)	6.75(1H,d,J=10)	6.76(1H,d,J=10)
10-H	5.68(1H,d,J=10)	5.67(1H,d,J=10)	5.65(1H,d,J=10)
11-CH_3	1.43(6H,s)	1.38(3H,s)	1.39(6H,s)
5-OH	12.99(1H,s)	12.21(1H,s)	12.31(1H,s)
13-H 14-H		2.00-2.20(4H,m)	
15-H		5.13(1H,m)	
16-CH_3		1.56, 1.64(each 3H,s)	

	III	IV	VIII
6-H	6.21(1H,d,J=2)	6.26(1H,d,J=2)	6.26(1H,d,J=2)
8-H	6.30(1H,d,J=2)	6.31(1H,d,J=2)	6.32(1H,d,J=2)
5'-H	6.44(1H,d,J=8)	6.58(1H,d,J=8)	6.68(1H,d,J=9)
6'-H	7.08(1H,d,J=8)	7.09(1H,d,J=8)	7.08(1H,d,J=9)
9-H	3.13(2H,br d,J=7)	3.10(2H,br d,J=7)	3.11(2H,br d,J=8)
10-H	4.95-5.20(1H,m)	5.00-5.20(1H,m)	5.13(1H,m)
11-CH_3	1.40or1.42, 1.57(each 3H,s)	1.44or1.47, 1.57(each 3H,s)	1.45, 1.58(each 3H,s)
14-H	6.80(1H,d,J=10)	6.78(1H,d,J=10)	6.75(1H,d,J=10)
15-H	5.70(1H,d,J=10)	5.70(1H,d,J=10)	5.69(1H,d,J=10)
16-CH_3	1.28(3H,s)	1.35(3H,s)	1.40(6H,s)
18-H 19-H	2.10-2.35(4H,m)	2.00-2.30(4H,m)	
20-H	4.95-5.20(1H,m)	5.00-5.20(1H,m)	
21-CH_3	1.40or1.42, 1.65(each 3H,s)	1.44or1.47, 1.60(each 3H,s)	
5-OH	12.99(1H,s)	13.14(1H,s)	13.13(1H,s)

	IX
6-H	6.26(1H,d,J=2)
8-H	6.31(1H,d,J=2)
5'-H	6.46(1H,d,J=9)
6'-H	7.08(1H,d,J=9)
9-H	3.12(2H,br d,J=6)
10-H	5.11(1H,br t,J=6)
11-CH_3	1.42, 1.58(each 3H,s)
14-H	6.77(1H,d,J=10)
15-H	5.72(1H,d,J=10)
16-CH_3	1.47(6H,s)
5-OH	13.07(1H,s)

Table 2 ^{13}C nmr spectra

	I	II		I	II
C-2	74.9	74.9	C-9	117.8	118.2
C-3	42.8	42.7	C-10	127.6	127.8
C-4	197.5	197.5	C-11	77.0	79.5
C-4a	103.1	103.2	C-12	27.9	25.8
C-5	165.3	165.3	C-13	27.9	41.9
C-6	96.7	96.8	C-14		23.6
C-7	167.5	167.4	C-15		125.0
C-8	95.9	95.8	C-16		131.5
C-8a	164.8	164.8	C-17		25.8
C-1'	118.6	118.3	C-18		17.7
C-2'	151.6	151.9			
C-3'	110.0	110.0			
C-4'	154.3	154.3			
C-5'	108.6	108.4			
C-6'	129.5	128.4			

solvent: acetone-d_6

and sodium borohydride tests[3]. The UV spectra of II closely resemble those of sanggenon F (V)[2f] and H (I). The ^{1}H nmr spectrum of II showed the characteristic signals for a 2-methyl-2-(4-methylpent-3-enyl)chromene ring[4] as follows: δ 1.38(3H, s), 1.56, 1.64(each 3H, s), 2.00-2.20(4H, m), 5.13 (1H, m), 5.67(1H, d, J=10), 6.75(1H, d, J=10). Other proton signals are shown in Table 1 and resemble those of relevant protons of I. In the ^{13}C nmr spectrum of II, the chemical shift values of the carbon atoms of the flavanone skeleton were similar to those of the relevant carbon atoms of I (Table 2). The configuration at C-2 was supported by the CD spectrum of II[7]. From the above results, the structure of sanggenon I could be assigned to II.

Sanggenon J (III), M^+ 488, $[\alpha]_D^{21}$ -16.9°, which showed positive methanolic ferric chloride(a brown color) and magnesium-hydrochloric acid tests. The UV spectra of III [λ_{max}^{EtOH} nm: 209, 235, 261, 282(sh), 320; $\lambda_{max}^{EtOH+AlCl_3}$ nm: 209, 220(sh), 270, 300(sh), 370] closely resembled those of kuwanon A (VIII)[4] and B (IX)[4]. The ^{1}H nmr spectrum of III showed the characteristic signals for γ,γ-dimethylallyl group and a 2-methyl-2-(4-methylpent-3-enyl)chromene ring as follows: δ 1.28(3H, s), 1.40, 1.42, 1.57, 1.65(each 3H, s), 2.10-2.35(4H, m), 3.13(2H, br d, J=7), 4.95-5.20(2H, m), 5.70 (1H, d, J=10), 6.80(1H, d, J=10)[4]. Comparative examination of the ^{1}H nmr spectra of III, VIII[4], and IX[4], revealed that the chemical shift and coupling constants of protons of flavone skeleton of III resemble those of IX better than those of VIII (Table 1). The mass spectrum of III showed the significant peaks at m/z 405, 337, 185(X), and 153(VII). From these results, the structure of sanggenon J could be assigned to III.

Sanggenon K (IV) was obtained as amorphous powder, M^+ 488, $C_{30}H_{32}O_6$, $[\alpha]_D^{16}$ -21.4°, which showed positive methanolic ferric chloride(a brown color) and magnesium-hydrochloric acid tests. The UV spectrum of IV closely resembled those of sanggenon J (III), and the mass spectrum of IV also showed the similar fragmentation species to those of III. In comparison of the ^{1}H nmr spectra of III and IV, the signal of the C-5'-H of IV was shifted to an about 0.2 ppm lower applied magnetic field than that of III, and the other proton signals of IV closely resemble the relevant protons of III (Table 1). Hence, the structure of sanggenon K could be assigned to IV.

Final proof for the discrimination of III from IV was obtained by the photooxidative cyclization[4]. When a solution of III in chloroform was irradiated with a high-pressure mercury lamp for 3 h, sanggenon J hydroperoxide (IIIa) was obtained. The mass spectrum of IIIa showed the following significant peaks at m/z 504(XI)[4], 445(XII)[4]. The similar fragmentation species were detected in the mass spectrum of morusin hydroperoxide (XIII)[4]. On the other hand, this photooxidation did not occur in sanggenon K (IV). The structural requirement in the photooxidative cyclization of morusin (XIV)[4] leads us to a conclusion that sanggenon J (III) has the hydroxyl group at the 2'-position and the γ,γ-dimethylallyl group at the 3-position. We thus propose the formula (III) for sanggenon J and the (IV) for sanggenon K.

EXPERIMENTAL

^{1}H nmr spectra were measured with tetramethylsilane (TMS) as an internal reference. Chemical shifts were expressed in ppm down field 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 for the physical data: UV spectra; Hitachi 340 UV Spectrometer, ir spectra; Hitachi 295 Spectrometer, ^{1}H nmr spectra; JEOL·JNM 4H-100 NMR Spectrometer and Hitachi R-900 FT NMR Spectrometer, mass spectra; 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 H (I), I (II), J (III), and K (IV)

The benzene extract[2f] (20g) of the crude drug "Sāng-Bái-Pí" (Japanese name "Sōhakuhi"), a species of Morus (Moraceae), imported from the People's Republic of China, was chromatographed on silica gel (300g) using benzene-methanol as an eluent, each fraction being checked by TLC. The fraction eluted with benzene containing 1% methanol were evaporated to give the residue (2.1g). This residue (2.1g) was rechromatographed on silica gel (40g) using hexane-acetone as an eluent. The fraction eluted with hexane containing 5% acetone were evaporated to give 84mg of residue. From this residue, sanggenon H (I , amorphous powder, 20mg), I (II, amorphous powder, 15mg), J (III, amorphous powder, 5mg), K (IV, amorphous powder, 5mg) were obtained by using preparative TLC (hexane:acetone=5:2, chloroform:ether=7:1).

Sanggenon H (I)

The compound (I) was obtained as amorphous powder, $[\alpha]_D^{22}$ -31.0° (c=0.026 in chloroform), FeCl$_3$ test: reddish violet, Mg-HCl test: violet, NaBH$_4$ test: orange. UV λ_{max}^{EtOH} nm(log ε): 218(sh 4.51), 227(4.54), 289(4.31), 318(sh 3.80); $\lambda_{max}^{EtOH+AlCl_3}$: 223(4.62), 282(infl. 3.99), 309(4.36), 374 (3.52). ir ν_{max}^{Nujol} cm^{-1}: 3270(br), 1639, 1603, 1595. High-resolution mass spectrum: Calcd. for $C_{20}H_{18}O_6$ (M^+, m/z): 354.1102. Found: 354.1131; Calcd. for $C_{19}H_{15}O_6$ (M^+-CH_3): 339.0867. Found: 339.0867; Calcd. for $C_{12}H_{11}O_2$ (VI): 187.0758. Found 187.0755. EI-MS m/z: 354 (M^+), 339, 187 (VI), 153 (VII). CD spectrum (EtOH): $[\theta]_{330}$ +1517, $[\theta]_{284}$ -8597, $[\theta]_{250}$ +2023.

Sanggenon I (II)

The compound (II) was obtained as amorphous powder, $[\alpha]_D^{22}$ -76.9° (c=0.021 in chloroform), FeCl$_3$ test: reddish violet, Mg-HCl test: violet, NaBH$_4$ test: orange. UV λ_{max}^{EtOH} nm (log ε): 214(4.40), 228(4.43), 289(4.19), 319(sh 3.71); $\lambda_{max}^{EtOH+AlCl_3}$: 205(4.41), 225(4.49), 282(infl. 3.86), 309(4.24), 364(3.45). ir ν_{max}^{Nujol} cm^{-1}: 3280(br), 1657(sh), 1642, 1610(sh), 1602. High-resolution mass spectrum: Calcd. for $C_{25}H_{26}O_6$ (M^+, m/z): 422.1727. Found: 422.1726; Calcd. for $C_{25}H_{24}O_5$ (M^+-H_2O): 404.1622. Found: 404.1623; Calcd. for $C_{19}H_{15}O_6$ (M^+-C_6H_{11}): 339.0867. Found: 339.0855; Calcd. for $C_{12}H_{11}O_2$ (VI): 187.0758. Found: 187.0755. EI-MS m/z: 422 (M^+), 339, 187 (VI), 153 (VII). CD spectrum (EtOH): $[\theta]_{325}$ +639, $[\theta]_{285}$ -4475, $[\theta]_{250}$ +895.

Sanggenon J (III)

The compound (III) was obtained as amorphous powder, $[\alpha]_D^{21}$ -16.9° (c=0.030 in chloroform), FeCl$_3$ test: brown, Mg-HCl test: orange, UV λ_{max}^{EtOH} nm(log ε): 209(4.52), 235(4.47), 261(4.35), 282(sh 4.12), 320(3.99); $\lambda_{max}^{EtOH+AlCl_3}$: 209(4.66), 220(sh 4.55), 270(4.47), 300(sh 4.09), 370(4.03). ir ν_{max}^{Nujol} cm^{-1} : 3380(br), 3310, 1661(sh), 1654, 1623, 1590. EI-MS m/z: 488 (M^+), 405 (M^+-C_6H_{11}), 337 (405-C_5H_8), 185 (X), 153 (VII).

Sanggenon K (IV)

The compound (IV) was obtained as amorphous powder, $[\alpha]_D^{16}$ -21.4° (c=0.042 in chloroform), FeCl$_3$ test: brown, Mg-HCl test: orange, UV λ_{max}^{EtOH} nm(log ε): 208(4.52), 228(4.42), 260(4.31), 282(4.05), 320(3.93); $\lambda_{max}^{EtOH+AlCl_3}$: 208(4.61), 270(4.40), 286(sh 4.05), 315(3.90), 373(3.90). ir ν_{max}^{Nujol} cm^{-1} : 3320(br), 1663(sh), 1653, 1623(sh), 1617, 1560. High-resolution mass spectrum: Calcd. for $C_{30}H_{32}O_6$ (M^+, m/z): 488.2196. Found: 488.2175; Calcd. for $C_{27}H_{25}O_6$ (M^+-C_3H_7): 445.1649. Found: 445.1644; Calcd. for $C_{24}H_{21}O_6$ (M^+-C_6H_{11}): 405.1336. Found: 405.1363; Calcd. for $C_{19}H_{13}O_6$ (M^+-C_6H_{11}-C_5H_8): 337.0711. Found: 337.0723; Calcd. for $C_{12}H_9O_2$ (X): 185.0602. Found: 185.0612. EI-MS m/z: 488 (M^+), 405, 337, 185 (X), 153 (VII).

Photooxidation of Sanggenon J (III) and K (IV)

a) Sanggenon J (III): A solution of III (1mg) in chloroform (0.2ml) in a Pyrex test tube with a

100W high-pressure mercury lamp for 3 h. After evaporation, the residue was purified by preparative TLC to give sanggenon J hydroperoxide (IIIa, amorphous powder). IIIa showed the following mass spectrum: EI-MS m/z: 520 (M^+), 504 (M^+-O, XI), 445 (M^+-O-C_3H_7O, XII), 421 (base peak, M^+-O -C_6H_{11}), 403 (M^+-O-C_6H_{11}-H_2O).

b) Sanggenon K (IV): A solution of IV (0.5mg) in chloroform (0.2ml) in a Pyrex test tube with a 100W high-pressure mercury lamp for 3 h. TLC analysis revealed that the starting material was recovered unchanged.

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