STRUCTURES OF FIVE NEW PRENYLATED FLAVONOIDS, GANCAONINS L, M, N, O, AND P FROM AERIAL PARTS OF GLYCYRRHIZA URALENSIS[1]

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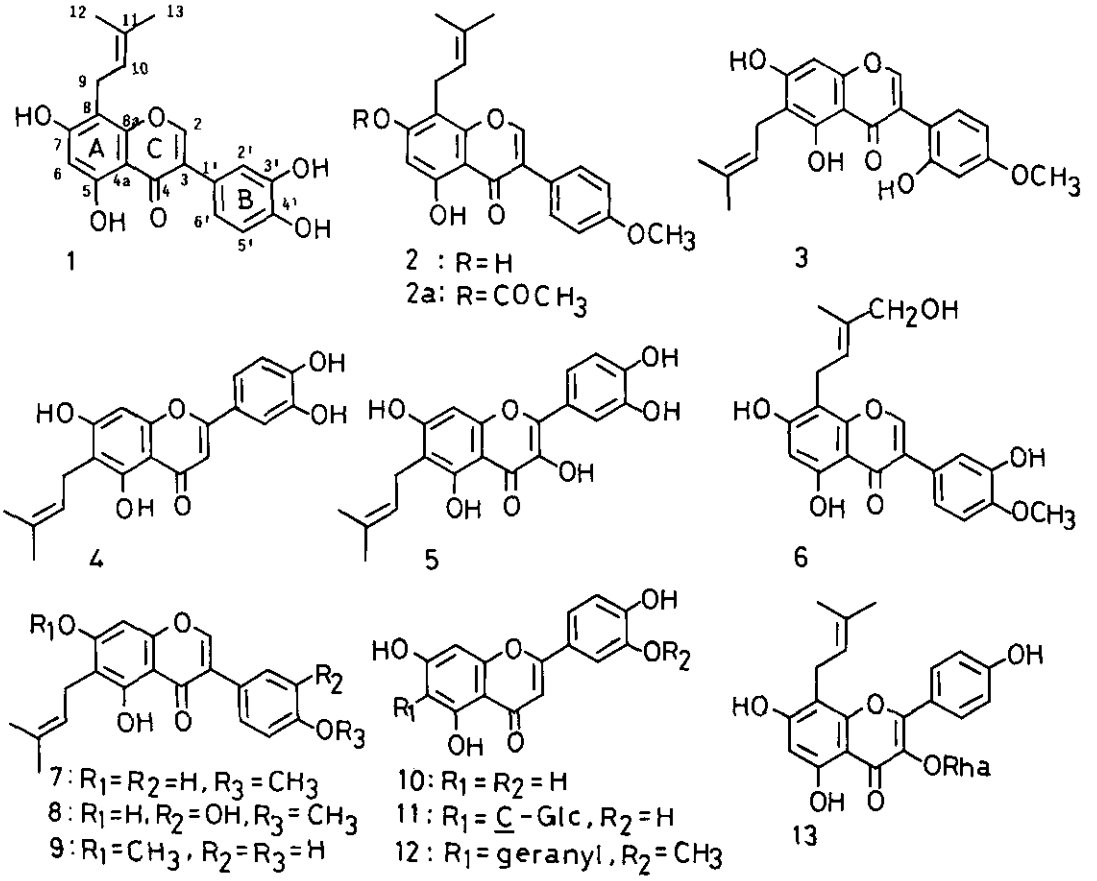
Abstract —— Five new prenylated flavonoids were isolated from the aerial parts of Glycyrrhiza uralensis FISCH. et DC. (Leguminosae), and the structures of the new compounds, gancaonins L, M, N, O, and P were elucidated as 8-prenylated 5,7,3',4'-tetrahydroxyisoflavone, 8-prenylated 5,7-dihydroxy-4'-methoxyisoflavone, 6-prenylated 4'-methoxy-5,7,2'-trihydroxyisoflavone, 6-prenylated 5,7,3',4'-tetrahydroxyflavone, and 6-prenylated 5,7,3',4'-tetrahydroxyflavonol, respectively, on the basis of spectral evidence.

In the previous papers,[1-3] we reported the structure determinations of isoprenoid-substituted flavonoids from Xibei licorice (Glycyrrhiza spp., Leguminosae, Seihoku Kanzo in Japanese) and the aerial parts of Glycyrrhiza uralensis FISCH. et DC. In continuation of the studies, we examined the phenolic constituents of the aerial parts of G. uralensis, and describe here the characterization of five new prenylated flavonoids, gancaonins L (**1**), M (**2**), N (**3**), O (**4**), and P (**5**). From the ethanol extract of the aerial parts of G. uralensis, these new flavonoids were isolated as described in "Experimental."

Gancaonin L (**1**), colorless prisms, mp 189-192 °C, $C_{20}H_{18}O_6$, gave a dark green color with methanolic ferric chloride, and was positive to the Gibbs test. The uv spectrum of **1** exhibited maxima at 207, 266, 293 (sh), and 340 (sh) nm, and resembled the sprctra of isoflavone derivatives having an isoprenoid moiety in the A-ring.[3] Moreover the uv spectrum of **1** showed remarkable bathochromic shift upon addition of aluminum chloride, while the spectrum exhibited hypsochromic shift on addition of hydrochloric acid to the aluminum chloride solution. These results suggest the presence of the ortho-dihydroxyl groups in the structure.[4] The ^1H nmr spectrum



showed the characteristic signal at δ8.24 (C-2-H) and the signals of following protons: 1) protons in a 3,3-dimethylallyl (prenyl) group, δ1.66 (3H, br d, J = 1 Hz), 1.80 (3H, br s), 3.45 (2H, br d, J = 7 Hz), 5.25 (1H, br t, J = 7 Hz), 2) ABC type aromatic protons, δ6.88 (1H, d, J = 8 Hz), 6.95 (1H, dd, J = 2 and 8 Hz), 7.16 (1H, d, J = 2 Hz), 3) an aromatic proton, δ6.36 (1H, s), 4) a proton in a hydrogen-bonded hydroxyl group, δ13.00 (1H, s). The ^{13}C nmr spectrum of **1** (gated decoupling with NOE) was measured, and the carbon atoms were assigned as shown in Table 1 on the basis of coupling patterns as well as by comparison of the ^{13}C nmr spectrum of **1** with that of gancaonin D (**6**).[3] Two oxygenated carbon atom signals were observed at δ145.65 and 146.26 suggesting that **1** has a 3',4'-dioxygenated phenyl moiety.[5] On the other hand, our group reported that the EI mass spectrum of 8-prenylated flavonoids, except 8-prenylated flavanones, showed the intense fragment ions $(M - CH_3)^+$, $(M - C_4H_7)^+$, and $(M - C_5H_8)^+$ originated from the destruction of the prenyl group in the molecular ion, while the spectra of 6-prenylated flavonoids,

Table 1. ^{13}C Nmr data of 1 - 5 in acetone-d_6 at 30°C and gancaonin A (7)[ç]

C	1		2		3[+]	7	4		5	
2	154.39	(D)[§,a]	154.56	(D)[c]	156.42*	154.16	164.88	(Std)	146.67	(St)
3	123.90*	(Sm)	123.48*	(Std)	122.12	123.84*	104.22	(D)	136.71	(S)
4	182.02	(Sd)	181.95	(Sd)	182.28	181.67	183.13	(Sbr)	176.56	(Sd)
4a	106.33	(St)	106.28	(St)	105.93	106.17	105.29	(Std)	104.04	(St)
5	161.58	(St)[b]	161.54	(Sdd)[b]	160.42	160.77	160.23	(Std)	158.96	(Std)
6	99.48	(Dd)[b]	99.50	(Dd)[b]	112.34	112.62	112.38	(Sm)	111.81	(Sm)
7	162.23	(Sm)	162.27	(Sm)	162.96	162.69	162.42	(Std)	162.78	(Std)
8	107.22	(Sm)	107.26	(Sm)	93.95	93.95	94.10	(D)	93.91	(D)
8a	156.30	(Std)	156.32	(Std)	156.93*	156.91	156.39	(Sd)[d]	155.67	(Sd)
1'	123.80*	(Sm)	124.37*	(Std)	112.85	124.53*	124.02	(S)[d]	123.93	(Sm)
2'	117.33	(Dd)	131.11	(Dd)	157.82	131.14	114.46	(Dd)	115.76	(D)
3'	145.65	(Sddd)	114.54	(Dd)	103.48	114.62	146.46	(Sddd)	145.84	(Sddd)
4'	146.26	(Sddd)	160.69	(Sm)	162.45	160.77	149.96	(Sddd)	148.29	(Sddd)
5'	115.97	(D)	114.54		106.75	114.62	116.65	(D)	116.22	(Dd)
6'	121.56	(Dd)	131.11		131.82	131.14	120.10	(Dd)	121.43	(Dd)
9	22.07	(Td)	22.05	(Td)	22.10	22.10	22.03	(Td)	22.01	(Td)
10	123.17	(Dm)	123.13	(Dm)	123.07	123.24	123.26	(Dm)	123.24	(Dm)
11	132.03	(Sm)	132.06	(Sm)	132.84	131.72	131.61	(Sm)	131.65	(Sm)
12	17.88	(Qm)	17.89	(Qm)	17.92	17.91	17.90	(Qm)	17.91	(Qm)
13	25.83	(Qm)	25.85	(Qm)	25.86	25.83	25.87	(Qm)	25.88	(Qm)
OCH_3			55.61		55.59	55.65				

ç: measured in acetone-d_6 at 45°C. §: Capital letters refer to the pattern resulting from directly bonded proton(s) and lowercase letters to long-range ^{13}C-^{1}H coupling. +: The datum was obtained only from the complete decoupling spectrum. *: Assignment may be interchanged in each column. a: ^{1}J = 198 Hz, b: ^{1}J = 160 Hz, $^{3}J_{C6,OH5}$ = 7 Hz. c: ^{1}J = 196 Hz. d: The signal was overlapped with the part of C10 signal.

except 6-prenylated flavanones, showed the intense fragment ions $(M - C_3H_7)^+$ and $(M - C_4H_7)^+$.[6] The EI-ms of **1** showed the fragment ions at *m/z* 339 [$(M - CH_3)^+$, relative intensity 100%)], 311 [$(M - C_3H_7)^+$, 4%)], 299 [$(M - C_4H_7)^+$, 30%], and 286 [$(M - C_5H_8)^+$, 37%], suggesting that the prenyl group is located at the C-8 position. This suggestion was confirmed by the fact that the signal of the carbon atom at the C-6 position was observed in the ^{13}C nmr spectrum of **1** at δ99.48 as a doublet of doublet (^{1}J = 160 Hz, $^{3}J_{C6,OH5}$ = 7 Hz) (Table 1). The location of the prenyl group in **1** was further supported on the following evidence. In the ^{1}H nmr spectra (in acetone-d_6) of the 8-isoprenylated isoflavones having no hydroxyl group at the C-2' position (C-2'-H), the proton signal of the hydrogen-bonded hydroxyl group at the C-5 position and the signal of the proton at the C-2 position were observed at δ *ca.* 13.0 and *ca.* 8.25, respectively, while the 6-isoprenylated isoflavones (C-2'-H) showed the signals of the hydrogen-bonded hydroxyl group and of the proton at the C-2 position at δ *ca.* 13.3 and 8.15, respectively (Table 2). In the case of 8-isoprenylated 2'-hydroxyisoflavones, the signals of the hydrogen-bonded hydroxyl group at the C-5 position and of the proton at the C-2 position were

Table 2. Chemical shifts (δ) of C-5-OH and C-2-H of isoflavone in acetone-d_6

trivial name	chemichal shift of C-5-OH	C-2-H	isoprenoid group on A-ring*	OH-positions	ref.
lupiwighteone	12.98	8.26	8-C_5H_9	5,7,4'	10
wighteone	13.32	8.15	6-C_5H_9	5,7,4'	10
Δ	(0.34)	(-0.11)			
gancaonin C	12.98	8.27	8-C_5H_9O	5,7,4'	3
hydroxywighteone	13.33	8.15	6-C_5H_9O	5,7,4'	10
Δ	(0.35)	(-0.12)			
lupiwighteone hydrate	12.97	8.26	8-$C_5H_{11}O$	5,7,4'	10
wighteone hydrate	13.31	8.14	6-$C_5H_{11}O$	5,7,4'	10
Δ	(0.34)	(-0.12)			
2,3-dehydrokievitone	12.70	8.26	8-C_5H_9	5,7,2',4'	10
luteone	13.05	8.14	6-C_5H_9	5,7,2',4'	10
Δ	(0.35)	(-0.12)			
2,3-dehydrokievitone hydrate	12.68	8.26	8-$C_5H_{11}O$	5,7,2',4'	10
luteone hydrate	13.04	8.14	6-$C_5H_{11}O$	5,7,2',4'	10
Δ	(0.36)	(-0.12)			

*: C_5H_9 = prenyl, $C_5H_{11}O$ = 3-hydroxyisoamyl, C_5H_9O = 3-hydroxymethyl-2-butenyl

Table 3. Chemical shifts (δ) of C-5-OH and C-2-H of **1**, **2**, **3**, and **7** in acetone-d_6 (400 MHz)

trivial name	Chemical shift of C-5-OH	C-2-H	prenyl group on A-ring	OH-positions
biochanin A*	13.01	8.20	none	5,7,(4'-OMe)
gancaonin M (**2**)	12.95	8.28	8-prenyl	5,7,(4'-OMe)
gancaonin A (**7**)	13.29	8.15	6-prenyl	5,7,(4'-OMe)
Δ	(0.34)	(-0.13)		
gancaonin L (**1**)	13.00	8.24	8-prenyl	5,7,3',4'
gancaonin N (**3**)	13.03	8.16	6-prenyl	5,7,2',(4'-OMe)

*: commercial reagent (Aldrich Chem. Co.). The signals were observed at δ12.98 (C-5-OH) and 8.17 (C-2-H) when measured at 35°C, and at δ12.95 (C-5-OH) and 8.14 (C-2-H) when measured at 50°C in acetone-d_6.

observed at δ ca. 12.7 and ca. 8.25, respectively, while the 6-isoprenylated 2'-hydroxyisoflavones showed the signals of the hydroxyl group and of the proton at the C-2 position at δ ca. 13.0 and ca. 8.15, respectively (Table 2). The relevant proton signals of **1** were observed as shown in Table 3. From the above results, the formula **1** is represented for the structure of gancaonin L.

Gancaonin M (**2**), colorless prisms, mp 139-141 °C, $C_{21}H_{20}O_5$, gave a dark brown color with methanolic ferric chloride, and was negative to the Gibbs test. The uv spectrum of **2** resembled the spectra of **1** and gancaonin A (**7**),[3] and showed a remarkable bathochromic shift upon addition of aluminum chloride. The ^1H nmr spectrum exhibited the signals of following protons: 1) protons in a prenyl group, δ1.66, 1.81 (each 3H, br d, J = 1 Hz), 3.45 (2H, br d, J = 7 Hz), 5.25 (1H, br t, J = 7 Hz), 2)

protons in a methoxyl group, δ 3.84 (3H, s), 3) an olefin proton, δ 8.28 (1H, s), 4) A_2B_2 type aromatic protons, δ 7.00 (2H, d, J = 9 Hz), 7.56 (2H, d, J = 9 Hz), 5) an aromatic proton, δ 6.37 (1H, s), 6) a proton in a hydrogen-bonded hydroxyl group, δ 12.95 (1H, s). The EI-ms of **2** showed the fragment ions at m/z 337 [$(M - CH_3)^+$, 100%], 309 [$(M - C_3H_7)^+$, 5%)], 297 [$(M - C_4H_7)^+$, 31%], 284 [$(M - C_5H_8)^+$, 48%]. Comparison of the ^{13}C nmr spectra between **2** and **1** revealed that the chemical shifts of all the carbon atoms in the A- and C-ring of **2** were in good agreement with those of the relevant carbon atoms of **1**. The location of the methoxyl group was confirmed by the acetylation shift. In the ^1H nmr spectrum of gancaonin M monoacetate (**2a**), the proton signal at the C-6 position showed a remarkable downfield shift (-0.25 ppm). The location of the prenyl group was supported by the chemical shifts of C-5 hydroxyl group and C-2 proton (Table 3). From these results, the structure of gancaonin M is represented by the formula **2**.

Gancaonin N (**3**), colorless prisms, mp 159-162 °C, $C_{21}H_{20}O_6$, gave a reddish violet color with methanolic ferric chloride, and was positive to the Gibbs test. The uv spectrum of **3** resembled the spectra of **1** and **2**. The ^1H nmr spectrum (in $CDCl_3$) showed the signals of the following protons: 1) protons in a prenyl group, δ 1.78 (3H, br d, J = 1 Hz), 1.85 (3H, br s), 3.48 (2H, br d, J = 7.5 Hz), 5.28 (1H, br t, J = 7.5 Hz), 2) protons in a methoxyl group, δ 3.82 (3H, s), 3) an olefin proton, δ 7.95 (1H, s), 4) ABC type aromatic protons, δ 6.56 (1H, dd, J = 2.5 and 8 Hz), 6.65 (1H, d, J = 2.5 Hz), 7.05 (1H, d, J = 8 Hz), 5) an aromatic proton, δ 6.46 (1H, s), 6) a proton in a hydrogen-bonded hydroxyl group, δ 12.66 (1H, s). The above results suggest that **3** is an isoflavone derivative having a prenyl group in the A-ring. The EI-ms of **3** showed the intense fragment ions at m/z 325 [$(M - C_3H_7)^+$, 100%] and 313[$(M - C_4H_7)^+$, 72%], while the weak ones at m/z 353 [$(M - CH_3)^+$, 16%] and 300 [$(M - C_5H_8)^+$, 4%]. This fragmentation pattern of the prenyl group is characteristic for the 6-prenylated flavonoids,[6] and the similar results were observed in the spectra of 6-prenylated isoflavones, such as gancaonins A (**7**),[3] B (**8**),[3] and G (**9**).[1] Comparison of the ^{13}C nmr spectra between **3** and **7** revealed that the chemical shifts of carbon atoms at the C-6 and C-8 positions of **3** were in good agreement with those of the relevant carbon atoms of **7** (Table 1). While the chemical shifts of carbon atoms at the C-6 and C-8 positions of **3** were inconsistent with those of the relevant carbon atoms of **1** and **2** (Table 1). The 2'-hydroxy-4'-methoxyphenyl partial structure for the B-ring was confirmed from the following evidences (1-3).

1. The ^1H nmr spectrum of **3** showed the ABC type aromatic proton signals being

assignable to the B-ring protons.

2. The ^{13}C nmr spectrum of **3** exhibited the signals of the oxygenated carbon atoms in the B-ring at δ157.82 and 162.45 suggesting that the B-ring is no o/p-oxygenations.[5]

3. The nuclear Overhauser effect (NOE) measurement (in $CDCl_3$) was carried out as follows: When the methoxy signal was irradiated, the proton signal at the C-3' (δ6.65, d, J = 2.5 Hz) increased by 20% in area and the proton signal at the C-5' (δ6.56, J = 2.5 and 8 Hz) increased by 10%.

The location of the prenyl group was further supported by the chemical shifts of C-5 hydroxyl group and C-2 proton (Table 3). From the above results, the structure of gancaonin N is represented by the formula **3**.

Gancaonin O (**4**), yellow needles, mp 245-248 °C, $C_{20}H_{18}O_6$, gave a dark green color with methanolic ferric chloride test, and was positive to the magnesium-hydrochloric acid test. The uv spectrum of **4** was similar to the spectra of luteolin (**10**),[4] isoorientin (**11**),[4] and canniflavone-2 (**12**).[7] Moreover the uv spectrum of **4** showed a bathochromic shift upon addition of aluminum chloride, while the spectrum showed a hypsochromic shift on addition of hydrochloric acid to the aluminum chloride added solution, suggesting the presence of ortho-dihydroxyl groups in the structure of **4**.[4] The ^1H nmr spectrum showed the signals of the following protons: 1) protons in a prenyl group, δ1.65 (3H, br d, J = 1 Hz), 1.78 (3H, br s), 3.35 (2H, br d, J = 7 Hz), 5.28 (1H, br t, J = 7 Hz), 2) ABC type aromatic protons, δ7.00 (1H, d, J = 8 Hz), 7.45 (1H, dd, J = 2 and 8 Hz), 7.49 (1H, d, J = 2 Hz), 3) olefin and/or aromatic proton(s), δ6.58, 6.59 (each 1H, s), 4) proton in a hydrogen-bonded hydroxyl group, δ13.28 (1H, s). In the ^{13}C nmr spectrum of **4** (gated decoupling with NOE), the carbon atoms were assigned as shown in Table 1 from coupling patterns of the signals as well as by comparison of the ^{13}C nmr spectrum of **4** with those of **3**, **10**,[5] and **12**.[7] Presence of a 3',4'-dioxygenated phenyl moiety in **4** was suggested by the chemical shifts of two oxygenated carbon atoms in the B-ring at δ146.46 and 149.96.[5] This suggestion was further supported by the presence of the ABC type aromatic proton signals in the ^1H nmr spectrum of **4**. The EI-ms of **4** showed the intense fragment ions at m/z 311 [$(M - C_3H_7)^+$, 100%], 299 [$(M - C_4H_7)^+$, 84%], while exhibited the weak ions at m/z 339 [$(M - CH_3)^+$, 19%], 286 [$(M - C_5H_8)^+$, 5%]. In the ^{13}C nmr spectrum of **4**, the signals of carbon atoms at the C-6 and C-8 positions were observed as a multiplet of singlet and a doublet (^1J = 164 Hz), respectively. Moreover the chemical shifts of carbon atoms at the C-6 and C-8 positions (C6:

δ112.38, C8: δ94.10) were similar to those of the relevant signals of **12** (C6: δ111.8, C8: δ94.0 in DMSO-d_6).[7] From the above results, the structure of gancaonin O is represented by the formula **4**.

Gancaonin P (**5**), yellow needles, mp 238-240 °C, $C_{20}H_{18}O_7$, gave a green color with methanolic ferric chloride, and was positive both to the magnesium-hydrochloric acid test and to the zirconium oxychloride test. The uv spectrum of **5** was similar to the spectra of flavonol derivatives.[4] The ^1H nmr spectrum showed the signals of the following protons: 1) protons in a prenyl group, δ1.65 (3H, br s), 1.79 (3H, br s), 3.37 (2H, br d, J = 7 Hz), 5.28 (1H, br t, J = 7 Hz), 2) ABC type aromatic protons, δ6.99 (1H, d, J = 8.5 Hz), 7.82 (1H, d, J = 2 Hz), 7.69 (1H, dd, J = 2 and 8.5 Hz), 3) an aromatic proton, δ6.59 (1H, s), 4) proton in a hydrogen-bonded hydroxyl group, δ12.41 (1H, s). The EI-ms of **5** showed the intense fragment ions at m/z 327 [$(M - C_3H_7)^+$, 86%], 315 [$(M - C_4H_7)^+$, 100%], while exhibited the weak ions at m/z 355 [$(M - CH_3)^+$, 18%], 302 [$(M - C_5H_8)^+$, 6%]. In the ^{13}C nmr spectrum of **5**, the signals of the carbon atoms at the C-6, C-8, and C-8a positions were observed as a multiplet of singlet (δ111.81), a doublet (δ93.91, ^1J = 164 Hz), and a doublet (δ155.67, $^2J_{C8a,H8}$ = 4 Hz), respectively (Table 1). While in the ^{13}C nmr spectrum of 8-prenylated flavonol, ikarisoside A (**13**), the signals of carbon atoms at the C-6, C-8, and C-8a positions were observed as a doublet of doublet (δ98.42, ^1J = 160 Hz, $^3J_{C6,OH5}$ = 7 Hz), a multiplet of singlet (δ106.0), and a triplet (δ153.59, $^3J_{C8a,H9}$ = 4 Hz), respectively.[8] These results reveal the location of prenyl group at the C-6 position. A 3',4'-dioxygenated phenyl structure for the B-ring was confirmed by the chemical shifts of two oxygenated carbon atoms in the B-ring at δ145.84 and 148.29 (Table 1),[5] and by the presence of the ABC type aromatic proton signals. From the above results, the structure of gancaonin P is represented by the formula **5**.

EXPERIMENTAL

Abbreviations: s = singlet, d = doublet, dd = double doublet, t = triplet, m = multiplet, br = broad, sh = shoulder, infl = inflection. The general procedures followed as described in our previous paper[1,9] and the instruments used are described in the paper.[1]

Isolation of Gancaonins L (**1**), M (**2**), N (**3**), O (**4**), and P (**5**) from the Aerial Parts of Glycyrrhiza uralensis

The ethanol extract (590 g)[3] was obtained from the dried aerial parts (6 Kg) of Glycyrrhiza uralensis FISCH. et DC. (Leguminosae). A part of the extract (280 g) was chromatographed on Amberlite XAD-2 (1 l), successively with H_2O (1.5 l), and methanol (1.5 l) as an eluent. The H_2O

eluted fraction was lyophillized to give a residue (101 g), and the methanol eluted fraction was evaporated to give a residue (48 g). The residue (48 g) eluted with methanol was rechromatographed on Amberlite XAD-2 (1 l), successively with n-hexane (1 l), benzene (3 l), benzene-acetone=4:1 (2 l), benzene-acetone=3:2 (2 l), acetone (2 l), and methanol (1 l). The benzene eluted fraction was evaporated to give a residue (25 g). The residue (25 g) was chromatographed on silica gel (200 g), succesively with n-hexane-benzene=1:1 (fractions 1-6), benzene (fr. 7-20), benzene-acetone=1:1 (fr. 21-22), and acetone (fr. 23-25) as an eluent, each fraction (eluted volume of 500 ml) being monitored by tlc. The fraction 4 (0.2 g of residue) was fractionated by HPLC (solvent: n-hexane-ethyl acetate=4:1, column: Senshu Pak SSC-Silica 4251-N, 5 μ, 1 cm ϕ x25 cm, detector: 280 nm) to give gancaonin N (**3**, 3 mg). The fraction 7 (1 g) was fractionated by preparative tlc (silica gel, solvent system, n-hexane-acetone=2:1, chloroform-acetone=6:1) to give gancaonin M (**2**, 20 mg). The benzene-acetone=4:1 eluted fraction on the Amberlite XAD-2 was evaporated to give a residue (10 g). This residue (10 g) was chromatographed on silica gel (200 g), successively, with chloroform (fr. 1-5, eluted volume of 300 ml each), chloroform-acetone=9:1 (fr. 6-9), chloroform-acetone=1:1 (fr, 10-19), and acetone (fr. 20-24) as an eluent. The fractions 5-6 (0.7 g) was chromatographed on Sephadex LH-20 (2 cm ϕ x25 cm) with methanol as an eluent to give gancaonin P (**5**, 20 mg). The fraction 7 (0.5 g) was fractionated by Sephadex LH-20 chromatography (2 cm ϕ x25 cm) with methanol as an eluent, and then by preparative tlc (silica gel, chloroform-acetone=2:1) to give gancaonin L (**1**, 23 mg). The fraction 11 (0.4 g) was chromatographed on Sephadex LH-20 (2 cm ϕ x25 cm) with methanol as an eluent to give gancaonin O (**4**, 15 mg).

Gancaonin L (**1**)

Gancaonin L (**1**) was recrystallized from acetone-n-hexane to give colorless prisms, mp 189-192 °C. $FeCl_3$ test: dark green. Gibbs test: positive. Uv λ_{max}^{MeOH} nm (log ε): 207 (4.54), 266 (4.50), 293 (sh 4.05), 340 (sh 3.57). Uv $\lambda_{max}^{MeOH+AlCl_3}$: 209 (4.55), 277 (4.49), 390 (3.55). Uv $\lambda_{max}^{MeOH+AlCl_3+HCl}$: 212 (4.59), 279 (4.53), 370 (3.68). Ir ν_{max}^{KBr} cm^{-1}: 3280, 1620, 1610 (sh), 1500. EI-Ms (probe), 70 eV, m/z (rel. int.): 355 $[M+1]^+$ (21 %), 354 $[M]^+$ (87), 339 (100), 311 (4), 299 (30), 298 (4), 286 (37), 165 (7), 134 (6). HR-Ms, m/z: 354.1080 $[M]^+$ ($C_{20}H_{18}O_6$ requires: 354.1104), 339.0847 $[M - CH_3]^+$ ($C_{19}H_{15}O_6$ requres: 339.0869), 299.0551 $[M - C_4H_7]^+$ ($C_{16}H_{11}O_6$ requires: 299.0556), 286.0482 $[M - C_5H_8]^+$ ($C_{15}H_{10}O_6$ requires: 286.0477). ^1H Nmr (acetone-d_6): δ 1.66 (3H, br d, J = 1 Hz, C-11-CH_3), 1.80 (3H, br s, C-11-CH_3), 3.45 (2H, br d, J = 7 Hz, C-9-Hx2), 5.25 (1H, br t, J = 7 Hz, C-10-H), 6.36 (1H, s, C-6-H), 6.88 (1H, d, J = 8 Hz, C-5'-H), 6.95 (1H, dd, J = 2 and 8 Hz, C-6'-H), 7.16 (1H, d, J = 2 Hz, C-2'-H), 8.24 (1H, s, C-2-H), 13.00 (1H, s, C-5-OH).

Gancaonin M (**2**)

Gancaonin M (**2**) was recrystallized from ether-n-hexane to give colorless prisms, mp 139-141 °C. $FeCl_3$ test: dark brown. Gibbs test: negative. Uv λ_{max}^{MeOH} nm (log ε): 208 (4.51), 265 (4.61), 333 (3.62). Uv $\lambda_{max}^{MeOH+AlCl_3}$: 210 (4.58), 277 (4.62), 391 (3.64). Uv $\lambda_{max}^{MeOH+NaOAc}$: 212 (4.64), 269 (4.54), 334 (3.78). EI-Ms, m/z (rel. int.): 353 $[M+1]^+$ (22%), 352 $[M]^+$ (94), 337 (100), 309 (5), 297 (31), 284 (48), 165 (6), 155 (8), 148 (14), 132 (9). HR-Ms, m/z: 352.1294 $[M]^+$ ($C_{21}H_{20}O_5$ requires: 352.1311), 337.1063 $[M - CH_3]^+$ ($C_{20}H_{17}O_5$ requires: 337.1076), 297.0774 $[M - C_4H_7]^+$ ($C_{17}H_{13}O_5$ requires: 297.0763), 284.0701 $[M - C_5H_8]^+$ ($C_{16}H_{12}O_5$ requires: 284.0685). ^1H Nmr (acetone-d_6): δ 1.66, 1.81 (each 3H, br d, J =1 Hz, C-11-CH_3), 3.45 (2H, br d, J = 7 Hz, C-9-Hx2), 3.84 (3H, s, OCH_3), 5.25 (1H, br t, J = 7 Hz, C-10-H), 6.37 (1H, s, C-6-H), 7.00 (2H, d, J = 9 Hz, C-3'-H and C-5'-H), 7.56 (2H, d, J = 9 Hz, C-2'-H and C-6'-H), 8.28 (1H, s, C-2-H), 9.65 (1H, br s, OH), 12.95 (1H, s, C-5-OH).

Gancaonin M Monoacetate (**2a**)

A mixture of **2** (3 mg), acetic anhydride (0.1 ml), and pyridine (0.1 ml) was kept at room temperature for 10 min and treated as usual. Gancaonin M monoacetate (**2a**, 1 mg) was obtained as colorless prisms, mp 104-106 °C (from acetone-n-hexane). $FeCl_3$ test: reddish violet. EI-Ms, m/z (rel. int.): 395 $[M+1]^+$ (20%), 394 $[M]^+$ (75), 352 (56), 351 (64), 337 (78), 335 (21), 297 (100), 284 (41), 135 (28), 132 (21). ^1H Nmr(acetone-d_6):δ1.67 (3H, br d, J = 1 Hz, C-11-CH_3), 1.81 (3H, br s, C-11-CH_3), 2.35 (3H, s, OAc), 3.42 (2H, br d, J = 7 Hz, C-9-Hx2), 3.85 (3H, s, OCH_3), 5.14 (1H, br t, J = 7 Hz, C-10-H), 6.62 (1H, s, C-6-H), 7.02 (2H, d, J = 9 Hz, C-3'-H and C-5'-H), 7.59 (2H, d, J = 9 Hz, C-2'-H and C-6'-H), 8.43 (1H, s, C-2-H), 12.87 (1H, s, C-5-OH).

Gancaonin N (**3**)

Gancaonin N (**3**) was recrystallized from acetone-benzene-n-hexane to give colorless prisms, mp 159-162 °C. $FeCl_3$ test: reddish violet. Gibbs test: positive. Uv λ_{max}^{MeOH} nm (log ε): 206 (4.45), 213 (sh 4.43), 265 (4.27), 290 (sh 4.00), 330 (infl 3.49). Uv $\lambda_{max}^{MeOH+NaOAc}$: 271 (4.24), 338 (3.78). Uv λ_{max}^{EtOH}: 206 (4.42), 266 (4.27), 290 (sh 4.00), 300 (infl 3.50). $\lambda_{max}^{EtOH+AlCl_3}$ (after 30 min): 208 (4.45), 278 (4.29), 310 (sh 3.82), 378 (3.37). EI-Ms, m/z (rel. int.): 369 $[M+1]^+$ (23%), 368 $[M]^+$ (96), 353 (16), 339 (5), 325 (100), 300 (4), 313 (72), 312 (17), 295 (5), 269 (2), 165 (19), 143 (13). HR-Ms m/z: 368.1255 $[M]^+$ ($C_{21}H_{20}O_6$ requires 368.1259), 325.0717 $[M - C_3H_7]^+$ ($C_{18}H_{13}O_6$ requires 325.0712), 313.0716 $[M - C_4H_7]^+$ ($C_{17}H_{13}O_6$ requires 313.0712). ^1H Nmr (acetone-d_6): δ1.65 (3H, br d, J = 1 Hz, C-11-CH_3), 1.78 (3H, br s, C-11-CH_3), 3.38 (2H, br d, J = 7 Hz, C-9-Hx2), 3.80 (3H, s, OCH_3), 5.27 (1H, br t, J = 7 Hz, C-10-H), 6.53 (1H, dd, J = 2.5 and ca. 8 Hz, C-5'-H, overlapping with the signals at 6.54), 6.54 (1H, s, C-8-H), 6.54 (1H, d, J = 2.5 Hz, C-3'-H, overlapping with the signals at 6.53), 7.21 (1H, d, J = 8 Hz, C-6'-H), 8.16 (1H, s, C-2-H), 13.03 (1H, s, C-5-OH). ^1H Nmr ($CDCl_3$): δ1.78 (3H, br d, J = 1 Hz, C-11-CH_3), 1.85 (3H, br s, C-11-CH_3), 3.48 (2H, br d, J = 7.5 Hz, C-9-Hx2), 3.82 (3H, s, OCH_3), 5.28 (1H, br t, J = 7.5 Hz, C-10-H), 6.46 (1H, s, C-8-H), 6.56 (1H, dd, J = 2.5 and 8 Hz, C-5'-H), 6.65 (1H, d, J = 2.5 Hz, C-3'-H), 7.05 (1H, d, J = 8 Hz, C-6'-H), 7.95 (1H, s, C-2-H), 12.66 (1H, s, C-5-OH).

Gancaonin O (**4**)

Gancaonin O (**4**) was recrystallized from n-hexane-acetone to give yellow needles, mp 245-248 °C. $FeCl_3$ test: dark green. Mg-HCl test: reddish orange. Zn-HCl test: violet. Uv λ_{max}^{MeOH} nm (log ε): 214 (4.53), 254 (4.17), 273 (4.20), 345 (4.32). Uv $\lambda_{max}^{MeOH+AlCl_3}$: 215 (4.66), 278 (4.27), 419 (4.36). Uv $\lambda_{max}^{MeOH+AlCl_3+HCl}$: 215 (4.66), 268 (4.13), 285 (4.19), 295 (sh 4.15), 364 (4.30). Uv $\lambda_{max}^{MeOH+NaOAc}$: 215 (4.62), 240 (4.20), 273 (4.20), 366 (4.19). Ir ν_{max}^{KBr} cm^{-1}: 3450 (br), 3250 (br), 1655, 1620, 1600 (sh), 1495. EI-Ms, m/z (rel. int.): 355 $[M+1]^+$ (17%), 354 $[M]^+$ (66), 339 (19), 311 (100), 299 (84), 298 (5), 286 (5), 165 (8). HR-Ms, m/z: 354.1065 $[M]^+$ ($C_{20}H_{18}O_6$ requires 354.1104), 311.0559 $[M - C_3H_7]^+$ ($C_{17}H_{11}O_6$ requires 311.0555), 299.0568 $[M - C_4H_7]^+$ ($C_{16}H_{11}O_6$ requires 299.0555). ^1H Nmr (acetone-d_6): δ1.65 (3H, br d, J = 1 Hz, C-11-CH_3), 1.78 (3H, br s, C-11-CH_3), 3.35 (2H, br d, J = 7 Hz, C-9-H), 5.28 (1H, br t, J = 7 Hz, C-10-H), 6.58 (1H, s, C-3-H or C-8-H), 6.59 (1H, s, C-8-H or C-3-H), 7.00 (1H, d, J = 8 Hz, C-5'-H), 7.45 (1H, dd, J = 2 and 8 Hz, C-6'-H), 7.49 (1H, d, J = 2 Hz, C-2'-H), 8.95 (3H, br s, OHx3), 13.28 (1H, s, C-5-OH).

Gancaonin P (**5**)

Gancaonin P (**5**) was recrystallized from acetone to give yellow needles, mp 238-240 °C. $FeCl_3$ test:

green. Mg-HCl test: dark red. Zn-HCl test: negative. $ZrOCl_2$-citric acid test: positive. Uv λ_{max}^{MeOH} nm (log ε): 208 (4.44), 231 (sh 4.15), 257 (4.18), 272 (sh 4.03), 295 (sh 3.72), 372 (4.20). Uv $\lambda_{max}^{MeOH+AlCl_3}$: 209 (4.42), 270 (4.26), 370 (sh 3.70), 442 (4.34). Uv $\lambda_{max}^{MeOH+AlCl_3+HCl}$: 209 (4.42), 266 (4.27), 366 (sh 3.76), 439 (4.34). Uv $\lambda_{max}^{MeOH+NaOAc}$: 213 (4.66), 262 (4.14), 384 (4.15). Ir ν_{max}^{KBr} cm^{-1}: 3600, 3500, 3300, 1650, 1615, 1605, 1595, 1510 (sh), 1480. EI-Ms, m/z (rel. int.): 371 $[M+1]^+$ (19%), 370 $[M]^+$ (80), 355 (18), 327 (86), 315 (100), 314 (15), 302 (6), 137 (12). HR-Ms, m/z: 370.1037 $[M]^+$ ($C_{20}H_{18}O_7$ requires 370.1052), 327.0512 $[M - C_3H_7]^+$ ($C_{17}H_{11}O_7$ requires 327.0505), 315.0496 $[M - C_4H_7]^+$ ($C_{16}H_{11}O_7$ requires 315.0505), 314.0406 $[M - C_4H_8]^+$ ($C_{16}H_{10}O_7$ requires 314.0426). ^1H Nmr (acetone-d_6): δ1.65, 1.79 (each 3H, br s, C-11-CH_3), 3.37 (2H, br d, J = 7 Hz, C-9-Hx2), 5.28 (1H, br t, J = 7 Hz, C-10-H), 6.59 (1H, s, C-8-H), 6.99 (1H, d, J = 8.5 Hz, C-5'-H), 7.69 (1H, dd, J = 2 and 8.5 Hz, C-6'-H), 7.82 (1H, d, J = 2 Hz, C-2'-H), 12.41 (1H, s, C-5-OH).

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