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STRUCTURES OF PRENYLATED DIHYDROCHALCONE, GANCAONIN J AND HOMOISOFLAVANONE, GANCAONIN K FROM GLYCYRRHIZA PALLIDIFLORA[1]

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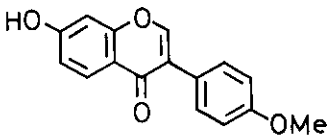
Abstract ---- A new prenylated dihydrochalcone, gancaonin J and a homoisoflavanone, gancaonin K, along with fourteen known compounds were isolated from the root of Glycyrrhiza pallidiflora MAXIM. Structures of gancaonins J and K were shown to be **1** and **2**, respectively, on the basis of spectral evidence. Gancaonin K (**2**) and 2'-O-methyllicodione (**17**) being isolated from the same material, the latter seems to be a biogenetical precursor of **2**. From the aerial parts of G. pallidiflora MAXIM., two known phenolic compounds were isolated.

We reported the structures of isoprenoid-substituted flavonoids from Xibei licorice (Glycyrrhiza species, Leguminosae, Seihoku Kanzo in Japanese) and the aerial parts of G. uralensis FISCH. et DC.[1-4] In continuation of the studies, we examined the phenolic constituents of the aerial parts and the root of G. pallidiflora. On the phenolic constituents of the root, Shibata and Saitoh reported the isolation of eight phenolic compounds.[5] In the present study we isolated two known compounds, formononetin (**3**)[6] and prunetin (**4**)[7] from the ethanol extract of the aerial parts of G. pallidiflora. On the other hand, from the ethanol extract of the root of G. pallidiflora, two new phenolic compounds gancaonins J (**1**) and K (**2**) were isolated along with fourteen known compounds, formononetin (**3**),[5,6] (±)-5,7-dihydroxy-6,8-diprenylflavanone (**5**),[8] (-)-medicarpin (**6**),[5,9] (±)-7,4'-dihydroxy-6,8-diprenylflavanone (**7**),[10] 7,4'-di-O-methyldaidzein (**8**),[11] 6,4'-dimethoxy-7,2'-dihydroxyisoflavone (**9**),[12] (±)-7-hydroxy-8-prenylflavanone (**10**),[13] afromosin (**11**),[5,14] (±)-isobavachin (**12**),[5,15] isoliquiritigenin (**13**),[5,16] liquiritigenin (**14**),[17] echinatin (**15**),[18] calycosin (**16**),[19] and 2'-O-methyllicodione (**17**).[20] The isolation



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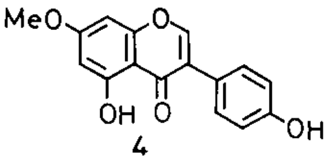

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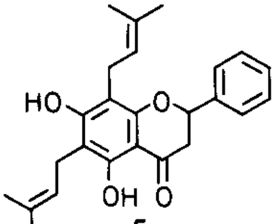

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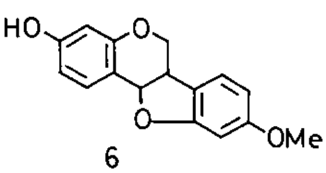

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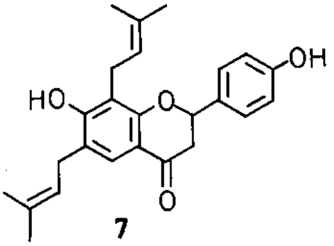

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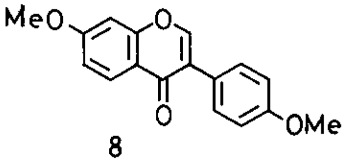

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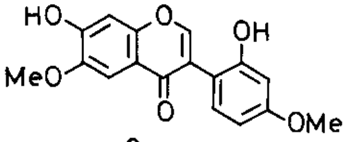

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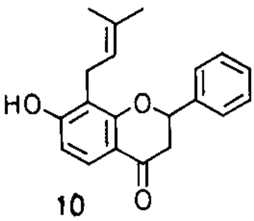

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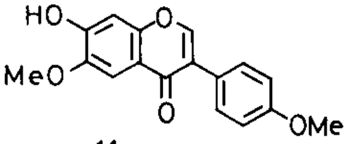

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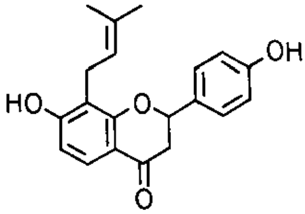

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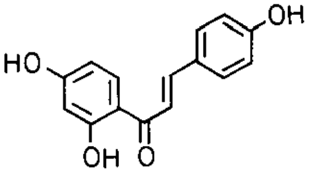

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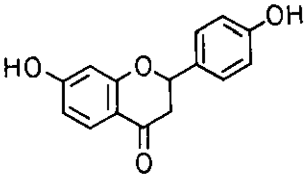

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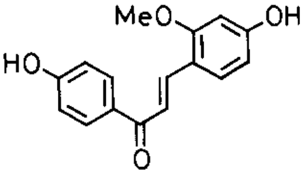

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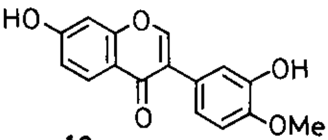

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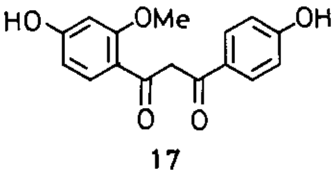

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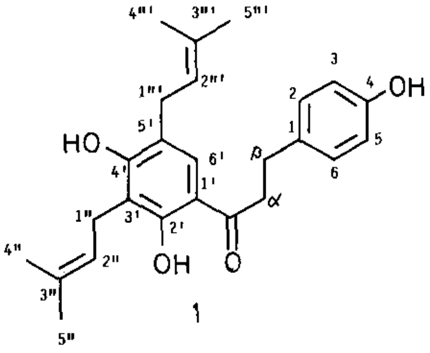
of **9** and **17** is the first example in natural product.

Gancaonin J (**1**), colorless oily substance, $C_{25}H_{30}O_4$, gave a green color with methanolic ferric chloride, and was negative to the Gibbs test. The uv spectrum of **1** resembled that of 3',5'-diprenyl-2',4'-dihydroxyacetophenone.[10] The ^1H nmr spectrum (400 MHz, acetone-d_6) showed the signals of the following protons: 1) protons in two 3,3-dimethylallyl (prenyl) groups, δ1.64, 1.73 (each 3H, br d, J = 1 Hz), 1.70, 1.77 (each 3H, br s), 3.32, 3.40 (each 2H, br d, J = 7 Hz), 5.19, 5.32 (each 1H, br t, J = 7 Hz), 2) an aromatic proton, δ7.55 (1H, s), 3) A_2B_2 type aromatic protons, δ6.76, 7.10 (each 2H, d, J = 8.5 Hz), 4) two pairs of methylene protons, δ2.92, 3.21 (each 2H, t, J = 8 Hz),[21] 5) a proton in a hydrogen-bonded hydroxyl group, δ13.08 (1H, s). The ^{13}C nmr spectrum of **1** was analyzed as shown in Table 1. The spectrum showed the signals of a carbonyl carbon atom (δ204.14) and two methylene carbon atoms (δ30.05, 40.07).[21] From these results, the formula **1** was proposed for the structure of gancaonin J.[22]

Gancaonin K (**2**), colorless needles, mp 249-252°C, $[\alpha]_D^{20}$ -27°, $C_{16}H_{12}O_5$, was negative to the methanolic ferric chloride test. The uv spectrum of **2** resembled that of licodione (**18**).[23] The ^1H nmr spectrum (400 MHz, DMSO-d_6) showed the signals of the following protons: 1) A_2B_2 type aromatic protons, δ6.86, 7.90 (each 2H, d, J = 9 Hz), 2) ABC type aromatic protons, δ 6.34 (1H, d, J = 2 Hz), 6.51 (1H, dd, J = 2 and 9 Hz), 7.62 (1H, d, J = 9 Hz), 3) a methine proton, δ 4.96 (1H, dd, J = 5 and 9 Hz), 4) a pair of methylene protons, δ 4.64 (1H, dd, J = 9 and 12 Hz), 4.68 (1H, dd, J = 5 and 12 Hz). In the ^{13}C nmr studies, the carbon atoms of **2** were assigned by the gated decoupling with NOE technique as well as by comparison of the ^{13}C nmr spectrum of **2** with the spectra of 4'-methoxy-7-hydroxyisoflavanone (**19**)[24] and echinatin (**15**)[25] (Table 1). In the spectrum of **2**, the signals of the two carbonyl carbon atoms were observed at δ187.78 and 194.27. The EI-Ms of **2** showed two fragment ions at m/z 163 (**20**, 71%) and m/z 121 (**21**, 100%). The presence of a 4-hydroxybenzoyl moiety in **2** was supported by comparison of the A_2B_2 type aromatic proton signals with the relevant proton signals of 2,4-dimethoxy-4'-hydroxydihydrochalcone (**22**) [δ6.92, 7.91 (each 2H, d, J = 8.5 Hz), in $CDCl_3$].[26] The presence of a 2- or 3-substituted 7-hydroxychromone ring was supported by comparison of the signals of ABC type aromatic, methylene, and methine protons with the relevant protons of 7,2',4'-trihydroxyisoflavanone (**23**) [δ4.09 (1H, dd, J = 5.4 and 10.7 Hz, C-3-H), 4.44 (1H, dd, J = 5.4 and 10.7 Hz, C-2-H), 4.59 (1H, t, J = 10.7 Hz, C-2-H), 6.39 (1H, d, J = 2.2 Hz, C-8-H), 6.56 (1H, dd, J = 2.2 and 8.8 Hz, C-6-H), 7.74 (1H, d, J = 8.8 Hz, C-5-H),



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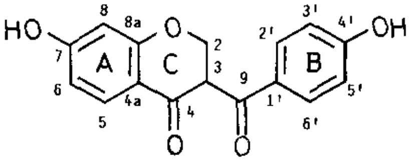

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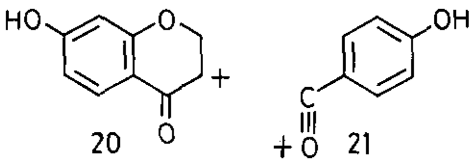

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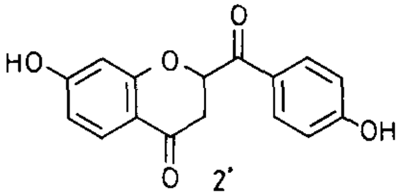
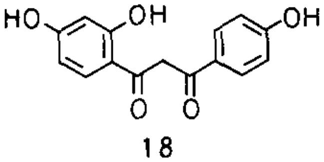


2'

Table 1. ^{13}C Nmr data of **1**, **2**, **15**, and **19** (100.4 MHz)

C	$1^{\S}$	C	2	19^{a}	C	15^{b}
1	134.66	2	68.69	72.57		
2,6	129.17	3	52.27	51.74		
3,5	115.46	4	194.27*	190.98		
4	154.17	4a	113.61	115.34		
α	40.07	5	128.73	130.17		
β	30.05	6	110.62	111.39		
C=O	204.14	7	164.69	165.13		
1'	114.37	8	102.24	103.44		
2'	161.26	8a	163.06	164.43		
3'	112.98	9	187.78*		C=O	187.6
4'	160.01	1'	128.06		1'	129.9
5'	119.03	2',6'	131.53		2',6'	131.0
6'	128.79	3',5'	115.16		3',5'	115.5
		4'	162.53		4'	162.0
solvent**	A		B	C		B

§: prenyl groups, δ17.89 (2C, C4" and C4"'), 21.88 (C1", ref. 31), 25.79 (2C, C5" and C5"'), 28.97 (C1"', ref. 31), 121.38, 121.81 (C2" and C2"'), 130.03, 135.09 (C3" and C3"'). *: Assignment may be interchanged. **: A=$CDCl_3$, B=DMSO-d_6, C=acetone-d_6. a: data from A. Pelter *et al.* (ref. 24). b: data from S. Ayabe and T. Furuya (ref. 25).



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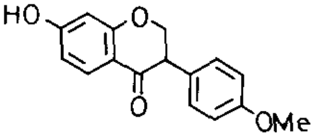

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in acetone-d_6].[27] The chemical shifts and coupling patterns of the A-ring protons of **2** were similar to those of the relevant protons of **23**, while the C-ring proton signals of **2** shifted downfield more than the relevant proton signals of **23**. These results support that the benzoyl moiety is located at the C-2 or C-3 positions of **2**. The structure **2'** was excluded with consideration of the one-bond carbon-proton coupling constants of methine carbon (δ 52.27, br d, ^1J = 128 Hz) and methylene carbon (δ 68.69, br t, ^1J = 150 Hz) of **2**. The coupling constants of methine and methylene carbons differed from those of the relevant carbons of flavanone derivatives (C2; ^1J = ca. 150 Hz, C3; ^1J = ca. 130 Hz).[28] On the other hand, one-bond carbon-proton coupling constants of α-carbons of heterocycloalkanes are larger than those of β-carbons.[29] The data supported a conclusion that the benzoyl moiety is located at the C-3 position of **2**. From the above results, the formula **2** was proposed for the structure of gancaonin K (except the stereochemistry at the C-3 position). To our knowledge gancaonin K is the first example of 9-keto-homoisoflavonoid. On the other hand, Dewik reported that 2'-methoxychalcones are biosynthetic precursors of the homoisoflavonoids.[30] Considering the above report and the fact that gancaonin K (**2**) and 2'-O-methyllicodione (**17**) were isolated from the same material, the later (**17**) seems to be a direct biogenetic precursor of the former (**2**).

EXPERIMENTAL

Abbreviations: s=singlet, d=doublet, dd=double doublet, t= triplet, m=multiplet, br=broad, sh=shoulder. The general procedures followed as described in our previous papers[1-4] and the instruments used are described in the papers.[1-4]

Plant Materials

The aerial parts and roots of Glycyrrhiza pallidiflora MAXIM. (Leguminosae) were collected in Shenyang, Liaoning Province, China in September, 1987. The material was identified by Prof. P.-Y. Zhang, Institute of Forestry and Soil, Liaoning Province Shenyang Academy of Science. The sample has been deposited in the herbarium of Heilongjiang Institute of Drug Control.

Isolation of Phenolic Compounds from the Aerial Parts of Glycyrrhiza pallidiflora

The dried aerial parts of G. pallidiflora (6 Kg) were exhaustively extracted six times with ethanol (each 20 l) at room temperature (each 3 days). Evaporation of the extract to dryness yielded 230 g of a residue. This residue (115 g) was chromatographed on Amberlite XAD-2 (500 ml), successively, with H_2O (2 l), methanol (2 l), and benzene (2 l) as an eluent. The methanol solution was washed with n-hexane, and then the methanol layer was evaporated to give a residue (3.8 g). This residue (3.8 g) was chromatographed on silica gel (200 g), successively, with benzene (fractions 1-26) and benzene-methanol=99:1 (fr. 27-54) as an eluent, each fraction (eluted volume 500 ml) being monitored by tlc. The fractions 27-42 (0.2 g of residue) were purified by preparative tlc (solvent system, chloroform-methanol=8:1, silica gel) to give formononetin [**3**, mp 264-267°C (recrystallized from benzene-methanol), 1 mg][6] and prunetin [**4**, mp 240-245°C (benzene-methanol), 2 mg].[7] The physical and spectral data of **3** and **4** were identified with the relevant published data.

Isolation of Phenolic Compounds from the Root of Glycyrrhiza pallidiflora

The dried root of *G. pallidiflora* (3 Kg) was exhaustively extracted four times with ethanol (each 20 l) at room temperature (each 3 days). Evaporation of the extract to dryness yielded 190 g of the residue. This residue (190 g) was chromatographed on Amberlite XAD-2 (800 ml), successively, with H_2O (3.5 l), methanol (4 l), and benzene (1 l) as an eluent. The methanol solution was evaporated to give a residue (60 g). This residue (60 g) was chromatographed on silica gel (330 g), successively, with benzene (fr. 1-21), benzene-methanol=99.5:0.5 (fr. 22-29), benzene-methanol=99:1 (fr. 30-72), and benzene-methanol=97:3 (fr. 73-85) as an eluent, each fraction (eluted volume of 500 ml) being monitored by tlc. The fraction 2 (0.3 g) was purified by preparative tlc (*n*-hexane-benzene=1:1) to give (±)-5,7-dihydroxy-6,8-diprenylflavanone [**5**, mp 101-102°C (*n*-hexane-acetone), 4 mg].[8] The fraction 3 (0.2 g) was fractionated by hplc (solvent: *n*-hexane-ethyl acetate=4:1, column: Senshu Pak SSC-silica 4251-N, 5 μ, 1 cmφ x25 cm, detector: 280 nm) to give gancaonin J (**1**, 50 mg). The fractions 4 and 5 (1.3 g) were purified by preparative tlc (*n*-hexane-acetone=5:1), followed by recrystallization from benzene to give (-)-medicarpin [**6**, mp 134-137°C (benzene), $[\alpha]_D^{20}$ -245° (C=1.05, methanol), 300 mg].[9] The fraction 6 (3.5 g) was purified by preparative tlc (chloroform-acetone=6:1, *n*-hexane-ethyl acetate=4:1), and then by hplc as described above to give (±)-7,4'-dihydroxy-6,8-diprenylflavanone [**7**, mp 158-161°C (*n*-hexane-ethyl acetate), 4 mg],[10] 7,4'-di-*O*-methyldaidzein [**8**, mp 162-164°C (*n*-hexane-acetone), 11 mg],[11] 6,4'-dimethoxy-7,2'-dihydroxyisoflavone [**9**, mp 195-198°C (acetone), 7 mg],[12] (±)-7-hydroxy-8-prenylflavanone [**10**, mp 141-144°C (*n*-hexane-ethyl acetate), 8 mg],[13] and afromosin [**11**, mp 231-233°C (benzene-acetone), 25 mg].[14] The fractions 9 and 10 (0.3 g) were purified by preparative tlc (chloroform-*n*-hexane-acetone=2:1:1) to give formononetin [**3**, mp 261-264°C (methanol), 60 mg].[6] The fraction 29 (0.35 g) was purified by preparitive tlc (benzene-ethyl acetate=4:1) to give (±)-isobavachin [**12**, mp 200-206°C (acetone-*n*-hexane), 8 mg].[15] The fraction 30 (0.4 g) was purified by preparative tlc (benzene-ethyl acetate=4:1) to give isoliquiritigenin [**13**, mp 191-194°C (benzene-acetone), 40 mg].[16] The fractions 43-46 (0.4 g) were purified by preparative tlc (ether) to give liquiritigenin [**14**, mp 194-197°C (benzene-acetone), 50 mg].[17] The fractions 50-54 (1 g) were purified by preparative tlc (acetone-chloroform) to give echinatin [**15**, mp 214-217°C (acetone-acetonitrile=1:3), 25 mg].[18] The fraction 55 (0.2 g) was purified by preparative tlc (chloroform-acetone=2:1) to give calycosin [**16**, mp 256-259°C (acetone), 37 mg].[19] The fraction 56 (0.4 g) was purified by preparative tlc (chloroform-acetone=2:1) to give gancaonin K (**2**, 6 mg) and 2'-*O*-methyllicodione [**17**, mp 187-189°C (acetone-benzene), 40 mg].[20] Identification of the known compounds (except **9**) was carried out by comparison of the physical and spectral data of these compounds with the relevant published data. The compound **9** was identified by direct comparisom of the ^{1}H nmr spectra.

Gancaonin J (**1**)

Compound **1** was obtained as colorless oily substance. $FeCl_3$ test: green. Gibbs test: negative. Uv λ_{max}^{MeOH} nm (log ε): 207 (5.49), 223 (5.50), 286 (5.24), 330 (4.84). Uv $\lambda_{max}^{MeOH+AcONa}$: 262 (4.95), 285 (4.99), 349 (5.33). Ir $\nu_{max}^{CHCl_3}$ cm^{-1}: 3600, 3400, 1630, 1600 (sh), 1520. EI-Ms (probe) 70 eV: *m/z* (rel. int.): 395 $[M+1]^+$ (29%), 394 $[M]^+$ (99), 379 (5), 351 (9), 339 (27), 338 (26), 323 (41), 273 (27), 231 (26), 217 (32), 177 (11), 161 (38), 107 (100). High-resolution Ms (HR-Ms), m/z: 394.2152 $[M]^+$ ($C_{25}H_{30}O_4$ requires: 394.2144), 107.0466 (C_7H_7O requires: 107.0496).

Gancaonin K (2)

Compound 2 was recrystallized from benzene-acetone to give colorless needles, mp 249-252°C, $[\alpha]_D^{20}$ -27°(c=0.033, methanol). $FeCl_3$ test: negative. Uv λ_{max}^{MeOH} nm (log ε): 204 (4.24), 212 (4.26), 226 (sh 4.04), 284 (4.25), 370 (3.40). Uv $\lambda_{max}^{MeOH+AcONa}$: 210 (4.68), 256 (sh 3.84), 290 (4.04), 338 (4.33). Ir ν_{max}^{KBr} cm^{-1}: 3340, 1665, 1640, 1600, 1470. EI-Ms (probe) 70 eV: m/z (rel. int.): 285 $[M+1]^+$ (8%), 284 $[M]^+$ (43), 164 (13), 163 (71), 121 (100). HR-Ms m/z: 284.0703 $[M]^+$ ($C_{16}H_{12}O_5$ requires 284.0685).

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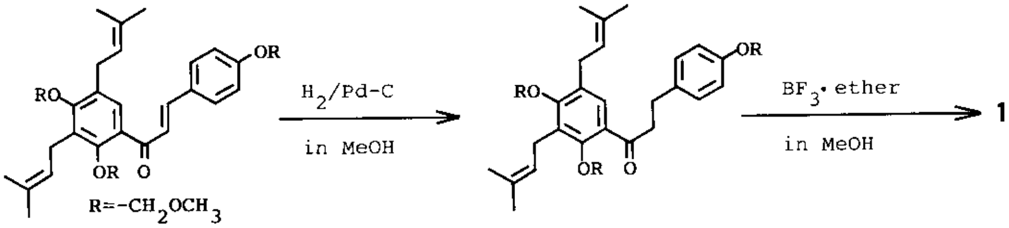

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