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New Isoflavan and Flavanone from Licorice Root[1]

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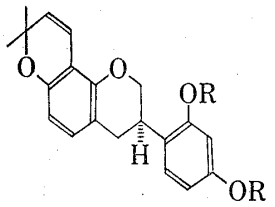
New compounds were isolated from the root of *Glycyrrhiza glabra* L. (Leguminosae) and named glabridin and glabrol, whose structures were determined to be an isoflavan (I) and a flavanone (VI), respectively.

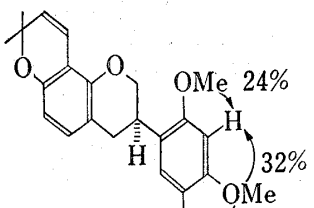
Licorice is one of the most well known and important crude drugs not only in the Orient but also in the Occident, and there are several varieties among the commercial licorice. A variety of licorice named Tohoku Kanzo (in Japanese) and produced in the northeast China is most popular in the Japanese drug market, and has been assigned to the roots of *Glycyrrhiza uralensis* Fisch. et DC., from which an isoflavan, licoricidin,[3] three coumestans, glycyrol, 5-*O*-methylglycyrol and isoglycyrol,[4] and an isoflavone, licoricone,[5] have been isolated.[6]

A comparative study has been carried out on the Russian licorice, the root of *Glycyrrhiza glabra* L., which is common in the European drug market to isolate some new constituents, an isoflavan, glabridin, and a flavanone, glabrol, with which the present paper is concerned.

Glabridin (I), $C_{20}H_{20}O_4$, mp 154—155°, $[\alpha]_D^{20}$ +8.2°, showed positive reactions with $FeCl_3$ and diazo reagent, but a negative reaction in the Shinoda test. The presence of two phenolic hydroxyls was proved by the formation of a diacetate (II), mp 164—166°, and a dimethyl ether (III), mp 109—111°.



I : R=H
II : R=Ac



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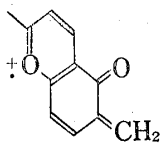

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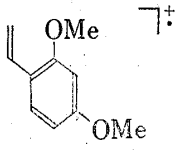

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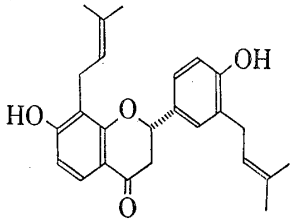
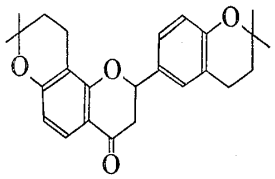
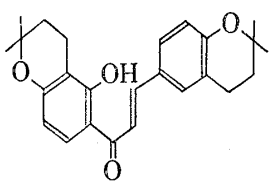


VI



VII



VIII

1) Part XXXIX in the series of Chemical Studies on the Oriental Plant Drugs. For Part XXXVIII see K. Kawai, T. Akiyama, Y. Ogihara, and S. Shibata, *Phytochem.*, **13**, 2829 (1974).
2) Location: *7-3-1, Hongo, Bunkyo-ku, Tokyo, 113, Japan.*
3) S. Shibata and T. Saitoh, *Chem. Pharm. Bull.* (Tokyo), **16**, 1932 (1968).
4) T. Saitoh and S. Shibata, *Chem. Pharm. Bull.* (Tokyo), **17**, 729 (1969).
5) M. Kaneda, T. Saitoh, Y. Iitaka, and S. Shibata, *Chem. Pharm. Bull.* (Tokyo), **21**, 1338 (1973).
6) The plant material used in this series of work was described as *G. glabra* spp. in our previous paper,[1] but now has been assigned to *G. uralensis*.

The nuclear magnetic resonance (NMR) signals (in $CDCl_3$), a broad singlet ($2\times CH_3$) at δ 1.42 and a pair of doublets at 5.53 (1H, 10.0 Hz) and 6.61 (1H, 10.0 Hz), indicated the presence of a chromene ring. The signals of five aromatic protons showed couplings of AB system (a pair of doublets with J=8.0 Hz at 6.34 and 6.76) and ABM system (a doublet with J=2.0 Hz, a quartet with J=2.0 and 8.0 Hz, and a doublet with J=8.0 Hz at 6.23, 6.31 and 6.86, respectively).

The infrared (IR) and ultraviolet (UV) spectra, along with the mass spectrum and the elementary analysis, suggest that the compound should be either a flavan or an isoflavan. The possibility of a flavan skeleton was excluded by the NMR spectral analysis. The signals of a hetero ring are observed at δ 4.35 (1H, quartet, 10.0 and 3.5 Hz), 4.00 (1H, triplet, 10.0 Hz), 3.3—3.4 (1H, multiplet), 2.89 (1H, doublet, 10.0 Hz) and 2.87 (1H, doublet, 6.0 Hz) as ABMXX′ system, which would be assigned to H-2 (eq, ax), H-3 (ax) and H-4 (ax, eq) protons of an isoflavan skeleton, respectively.

The mass spectrum of glabridin dimethyl ether (III) shows major ions at m/e 352 (M^+), 174, 173, 164, 151, 148 and 121. The ions, 173 (IV) and 164 (V), derived from a retro Diels-Alder fragmentation suggest that the chromene ring is attached to A ring and two hydroxyls to B ring. The location of the hydroxyls at 2′ and 4′ was revealed by the splitting pattern (ABM) of the aromatic protons in the NMR spectrum and confirmed by the observation of the nuclear Overhauser effect (NOE) in III by irradiation at two methoxyls individually.

As the optical rotatory dispersion (ORD) of glabridin in the 260—300 nm region was characterised by a positive Cotton effect, the stereochemistry at C-3 was assigned to "*R*".[7]

Glabrol (VI), $C_{25}H_{28}O_4$, mp 90°, $[\alpha]_D^{22}$ −44.2° (MeOH), shows UV absorptions at 286 and 312.5 nm. A red coloration by the Shinoda test and the resemblance of the UV spectrum to that of liquiritigenin suggest that glabrol is a 7,4′-dihydroxyflavanone derivative.

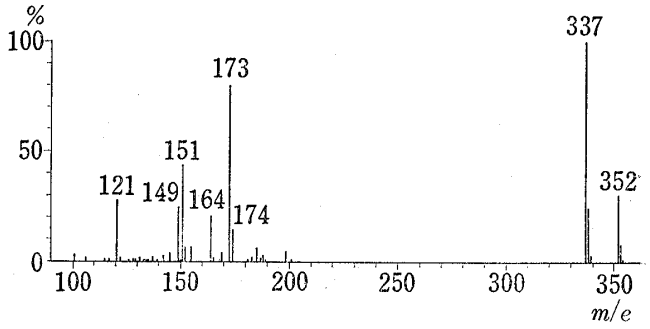


Fig. 1. Mass Spectrum of Glabridin Dimethyl Ether (III)

The NMR spectrum (in $CDCl_3$) revealed the presence of two equivalent γ,γ-dimethylallyl groupings [δ 1.70 (6H, broad singlet), 1.76 (6H, broad singlet), 3.38 (4H, doublet, 7 Hz) and 5.3 (2H, overlapped with H-2 proton)], two exchangeable hydroxyls [5.54 (1H, broad singlet) and 6.48 (1H, broad singlet)], five aromatic protons (AB and ABC systems) [6.52 (1H, doublet, 8.5 Hz) and 7.70 (1H, doublet, 8.5 Hz), 6.80 (1H, doublet, 8.5 Hz), 7.18 (1H, double doublet, 8.5 and 2 Hz) and 7.20 (1H, doublet, 2 Hz)] and a flavanone ring [2.8—2.9 (2H, multiplet) and 5.3 (1H, obscured by allylic protons)].

Reflux of a solution of glabrol in methanolic hydrochloric acid gave almost equivalent amounts of two products, cycloglabrol (VII), mp 127—129°, and isocycloglabrol (VIII), mp 135—136°.

The chalcone structure of isocycloglabrol is supported by its UV absorption (368 nm) and NMR signals at δ 7.40 (α-H, doublet, 15.0 Hz) and 7.80 (β-H, doublet, 15.0 Hz). Production of a chalcone from a flavanone was demonstrated under the same condition by liquiritigenin, producing isoliquiritigenin. A chalcone is readily formed from a flavanone by the action of acid or base when a hydroxyl group is absent at C-5 position of the flavanone skeleton.[8]

7) K. Kurosawa, W.D. Ollis, B.T. Redman, and I.O. Sutherland, *Chem. Comm.*, **1968**, 1265.
8) T.R. Seshadri, Interconversion of Flavonoid Compounds, in *The Chemistry of Flavonoid Compounds*, ed. by T.A. Geissman, Pergamon Press, Oxford. 1962. p. 159.

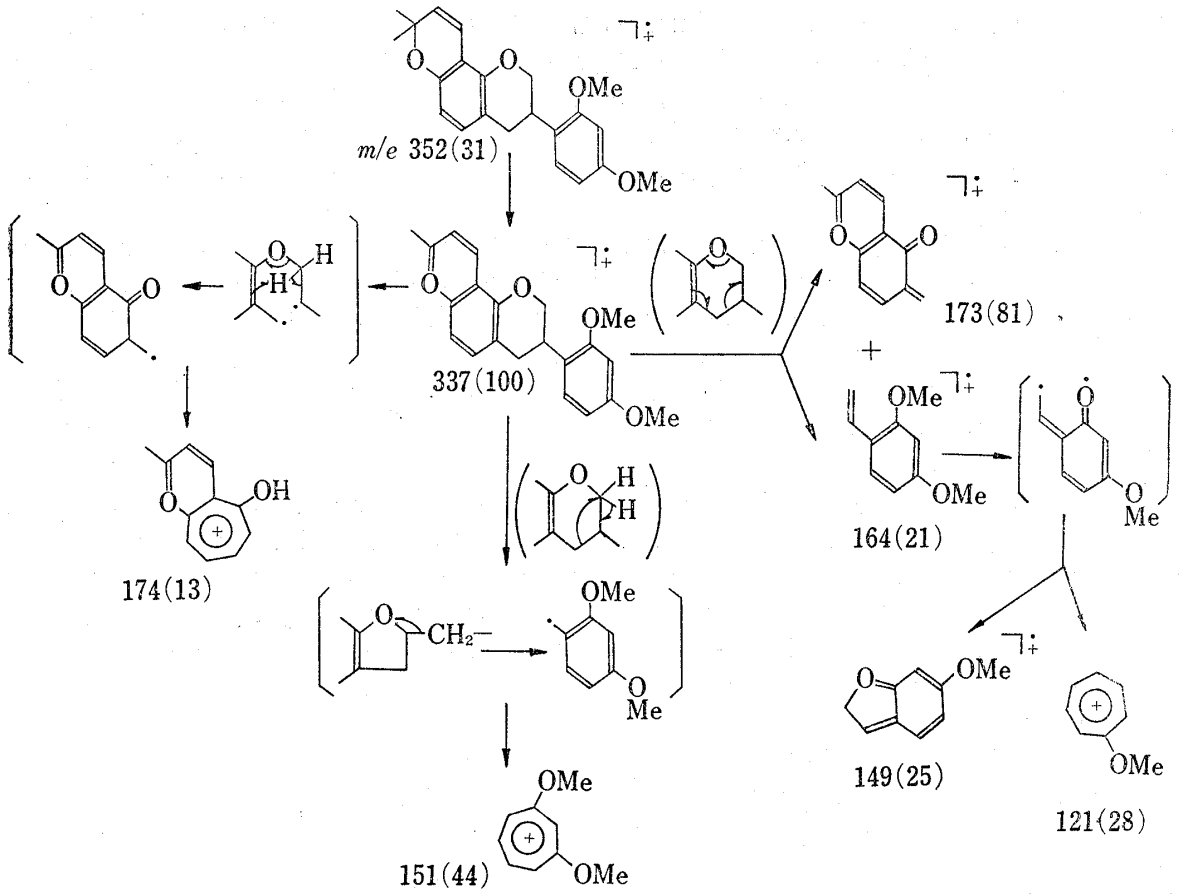


Chart 1. Mass Fragmentation of Glabridin Dimethyl Ether (III)

Formation of two 2,2-dimethylchroman rings in the both compounds indicates the location of γ,γ-dimethylallyl residues at the adjacent positions to hydroxyls.

The *S*-configuration at C-2 was revealed by its negative circular dichroism (CD) curve.[9]

Experimental

Dried and ground licorice roots (5 kg) were first percolated with *n*-hexane, and then extracted with ethyl acetate. Evaporation of ethyl acetate under reduced pressure yielded 132.5 g of a brown syrup.

Isolation of Glabridin (I) and Glabrol (VI)——The extracts were roughly separated by a silica gel chromatography. The fractions were rechromatographed on silica gel with a mixture of benzene and acetone, and finally on polyamide with methanol.

Glabridin was recrystallised from benzene to form colorless plates (2.0 g), mp 154—155°, $[\alpha]_D^{20}$: +8.2°. (c=2.07, $CHCl_3$) It gave a brown color and a yellowish brown color with $FeCl_3$ and diazo reagent, respectively. UV λ_{max}^{EtOH} nm (log ε): 281.5 (4.18), 288 (4.13 inf.), 312 (3.47 sh.). IR ν_{max}^{KBr} cm^{-1}: 3270, 2940, 1611, 1587, 1526, 1480. NMR $\delta_{ppm}^{CDCl_3}$: 1.42 (3H×2, s, CH_3–2″), 2.87 (1H, d, 6.0, H-4eq), 2.89 (1H, d, 10.0, H-4ax), 3.3—3.4 (1H, m, H-3ax), 4.00 (1H, t, 10, H-2ax), 4.35 (1H, q, 10 and 3.5, H-2eq), 5.53 (1H, d, 10, H-3″), 5.80 (2H, broad s, OH-2′ and 4′, disappeared by the addition of D_2O), 6.23 (1H, d, 2, H-3′), 6.31 (1H, d.d, 8 and 2, H-5′), 6.34 (1H, d, 8, H-6), 6.61 (1H, d, 10, H-4″), 6.76 (1H, d, 8, H-5), 6.86 (1H, d, 8, H-6′). Mass Spectrum *m/e*: 324 (M^+). *Anal.* Calcd. for $C_{20}H_{20}O_4$: C, 74.05; H, 6.22. Found: C, 74.33; H, 6.23.

Grabrol was recrystallised from dil. MeOH to give colorless needles (2.2 g), mp 90°, $[\alpha]_D^{22}$: −44.2°. (c=0.91, MeOH) It gave a dark brown color with $FeCl_3$ and no coloration with the diazo reagent. UV λ_{max}^{EtOH} nm (log ε): 281 (4.35), 312.5 (4.06). IR ν_{max}^{KBr} cm^{-1}: 3360, 2920, 1649, 1586, 1505, 1442. NMR $\delta_{ppm}^{CDCl_3}$: 1.70 (3H×2, broad s, CH_3 of dimethylallyl), 1.76 (3H×2, broad s, CH_3 of dimethylallyl), 2.8—2.9 (2H, m, H-3), 3.38 (4H, d, 7, benzylic of dimethylallyl), 5.3 (2H, m, H-2 and olefinic of dimethylallyl), 5.54 (1H, s, OH-4′), 6.48 (1H, s, OH-7, exchangeable with D_2O), 6.52 (1H, d, 8.5, H-6), 6.80 (1H, d, 8.5, H-5′), 7.18 (1H, d.d, 8.5 and

9) W. Gaffield, *Tetrahedron*, **26**, 4093 (1970).

2, H-6′), 7.20 (1H, d, 2, H-2′), 7.70 (1H, d, 8.5, H-5). Mass Spectrum *m/e*: 392.1971 (M^+) (Calcd. for $C_{25}H_{28}O_4$: 392.1985). CD ($c=0.01$, MeOH): $[\theta]_{320}$ 0; $[\theta]_{300}$ −25900 (max); $[\theta]_{261}$ 0.

Glabridin Diacetate (II)——A mixture of glabridin (50 mg) in pyridine (1.5 ml) and acetic anhydride (1 ml) was allowed to stand overnight at room temperature, and then poured dropwise into ice water. The solid was filtered by suction and dried. Recrystallisation from ethanol gave glabridin diacetate as colorless prisms (40 mg), mp 164—166°, UV λ_{max}^{EtOH} nm (log ε): 280 (4.10), 291 (4.03 inf.), 312.5 (3.47 sh.). IR $\nu_{max}^{CHCl_3}$ cm^{-1}: 1767, 1615, 1586, 1503, 1483. NMR $\delta_{ppm}^{CDCl_3}$: 1.42 (3H×2, s, CH_3-2″), 2.28 (3H, s, $COCH_3$), 2.30 (3H, s, $COCH_3$), 2.88 (2H, broad d, 8, H-4ax and H-4eq), 3.3 (1H, m, H-3ax), 3.97 (1H, t, 10, H-2ax), 4.31 (1H, q, 10 and 3.5, H-2eq), 5.54 (1H, d, 10, H-3″), 6.35 (1H, d, 8, H-6), 6.60 (1H, d, 10, H-4″), 6.78 (1H, d, 8, H-5), 6.89 (1H, d, 2, H-3′), 6.95 (1H, d.d, 8 and 2, H-5′), 7.17 (1H, d, 8, H-6′). *Anal.* Calcd. for $C_{24}H_{24}O_6$: C, 70.57; H, 5.92. Found: C, 70.30; H, 5.90.

Glabridin Dimethyl Ether (III)——Glabridin (202 mg) was methylated with CH_2N_2 in the usual manner. After removal of the solvent the residue was recrystallised from EtOH to give glabridin dimethyl ether as colorless needles, mp 109—111° (150 mg). ORD ($c=1.02$, $CHCl_3$). $[\alpha]_D^{28}$: +11.8°. UV λ_{max}^{EtOH} nm (log ε): 280 (4.13), 290 (3.99 inf.), 312.5 (3.44 sh.). IR $\nu_{max}^{CHCl_3}$ cm^{-1}: 2980, 1615, 1588, 1507, 1482. $\delta_{ppm}^{C_6H_6\text{-benzene}}$: 1.35 (3H×2, s, CH_3-2″), 2.76 (2H, m, H-4ax and H-4eq), 3.25 (3H, s, OMe-2′), 3.39 (3H, s, OMe-4′), 3.6 (1H, m, H-3ax), 3.93 (1H, t, 10, H-2ax), 4.37 (1H, d.q, 10, 3.5 and 1, H-2eq), 5.35 (1H, d, 10, H-3″), 6.29 (1H, d.d, 8 and 2, H-5′), 6.39 (1H, d, 2, H-3′), 6.61 (1H, d, 8, H-6), 6.74 (1H, d, 8, H-5), 6.84 (1H, d, 8, H-6′), 6.97 (1H, d, 10, H-4″). *Anal.* Calcd. for $C_{22}H_{24}O_4$: C, 74.97; H, 6.86. Found: C, 74.72; H, 6.84. ORD ($c=0.007$, $CHCl_3$) $[\phi]_{265}$ −4570, $[\phi]_{270}$ −5840, $[\phi]_{275}$ −6900, $[\phi]_{280}$ −10300 $[\phi]_{285}$ −6580, $[\phi]_{290}$ −3190, $[\phi]_{295}$ 0, $[\phi]_{300}$ +960, $[\phi]_{303}$ +1490, $[\phi]_{310}$ +530. CD ($c=0.007$, $CHCl_3$) $[\theta]_{323}$ 0; $[\theta]_{290}$ +190000 (max); $[\theta]_{270}$ 0.

Cycloglabrol (VII) and Isocycloglabrol (VIII)——A mixture of glabrol, methanol and hydrochloric acid was refluxed for 4 hr, and the solvent was removed under reduced pressure. The products were separated by preparative thin-layer chromatography on silica gel.

Cycloglabrol was crystallised from MeOH in colorless needles, mp 127—129°. UV λ_{max}^{EtOH} nm (log ε): 278.5 (4.19) and 307.5 (3.88 sh.), IR $\nu_{max}^{CHCl_3}$ cm^{-1}: 2920, 1666, 1602, 1582, 1500. NMR $\delta_{ppm}^{CDCl_3}$: 1.36 (3H×4, broad s, CH_3 of chromans), 1.8 (4H, m, CH_2 of chromans), 2.8 (6H, m, benzylic CH_2 of chromans and H-3), 5.34 (1H, d.d, 12 and 4, H-2), 6.46 (1H, d, 8, H-6), 6.78 (1H, d, 8, H-5′), 7.13 (1H, broad s, H-2′), 7.16 (1H, d, 8, H-6′), 7.68 (1H, d, 8, H-5). Mass Spectrum *m/e* 392 (M^+). *Anal.* Calcd. for $C_{25}H_{28}O_4$: C, 76.50; H, 7.19. Found: C, 76.28; H, 7.12.

Isocycloglabrol was crystallised from MeOH to give yellow needles, mp 135—136°, λ_{max}^{EtOH} nm (log ε): 315 (4.00 inf.) and 368 (4.46), $\nu_{max}^{CHCl_3}$ cm^{-1}: 3300, 2920, 1628, 1590, 1565, 1492, $\delta_{ppm}^{CDCl_3}$: 1.36 (3H×4, broad s, CH_3 of chromans), 1.80 (4H, t, 7, CH_2 of chromans), 2.80 (4H, m, benzylic CH_2 of chromans), 6.34 (1H, d, 8, H-5′), 6.76 (1H, d, 8, H-5), 7.2—7.3 (2H, H-2 and H-6), 7.40 (1H, d, 15, Hα), 7.66 (1H, d, 8, H-6′), 7.80 (1H, d, 15, Hβ), 13.92 (1H, s, chelated OH-2′, exchangeable with D_2O). Mass Spectrum *m/e*: 392 (M^+). *Anal.* Calcd. for $C_{25}H_{28}O_4$: C, 76.50; H, 7.19. Found: C, 76.26; H, 7.16.

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