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The Occurrence of an Isoflavene and the Corresponding Isoflavone in Licorice Root[1]

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The structures of a new isoflavone glabrone (I) and the corresponding isoflavene, glabrene (IV), isolated from the roots of *Glycyrrhiza glabra* (Leguminosae), have been determined by spectroscopic analysis and by chemical method.

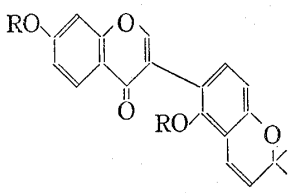
In the previous paper,[3] we reported on the structures of an isoflavan, glabridin, and a flavanone, glabrol, isolated from the roots of licorice, *Glycyrrhiza glabra* L. (Leguminosae). Further investigation led us to isolate two new isoflavonoids, glabrone (I) and glabrene (IV), along with formononetin from the same plant material.

Glabrone (I) was obtained as colorless needles, mp 224—226°, having a molecular formula, $C_{20}H_{16}O_5$ (M^+ 336). It gives a brown color with $FeCl_3$, but no coloration with the Shinoda test. The ultraviolet (UV) spectrum shows the absorptions at 247 (inf.), 252, 257 (inf.) and 301 nm, which are characteristic of isoflavones. The nuclear magnetic resonance (NMR) signal at δ 8.33 (singlet) revealed the presence of H-2 of the isoflavone skeleton in I.

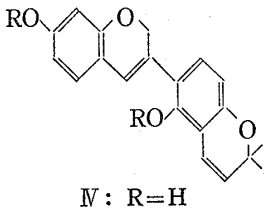
On acetylation with acetic anhydride and pyridine, I gave a diacetate (II), mp 218—220°. A bathochromic shift of UV absorption, Δ12 nm, induced by the addition of NaOAc indicated the presence of a free hydroxyl at C-7.

The NMR spectrum of I proved the presence of a chromene ring [δ 1.41 (s, $2\times CH_3$), 5.67 (d, 10 Hz), 6.80 (d, 10 Hz)], two exchangeable hydroxyls [δ 9.70 (2H, broad s)] in accordance with the formation of II, and five aromatic protons appeared as AB and ABC types of signals. Of these signals, δ 8.16 (doublet, J=8.5 Hz) was assigned to H-5, deshielded by C-4 carbonyl group. Since this proton is a component of the ABC system, the other two protons composing the AB system (*ortho*-coupling) are in B ring.

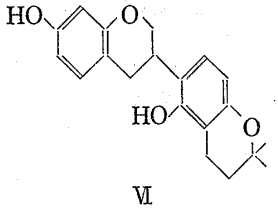
On acetylation, Hα proton of the chromene was shifted to higher field ($\Delta\delta$ 0.46 ppm) to suggest the presence of a peri hydroxyl[4] (Table I). A positive Gibbs test with I revealed that *p*-position of 2′-hydroxyl is unsubstituted. The appearance of M-31 fragment in the mass spectrum of I dimethyl ether (III) also supported the presence of 2′-hydroxyl in I.[5] These findings led the structure (I) of glabrone.



I : R=H
II : R=Ac
III : R=Me



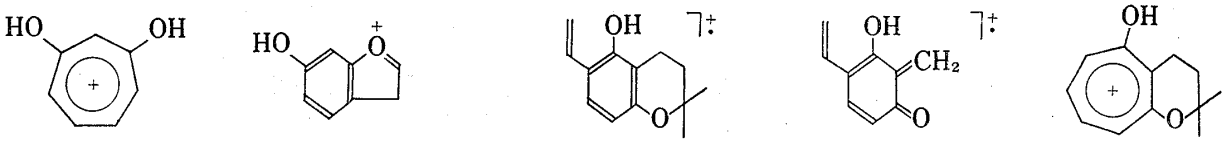
IV : R=H
V : R=Ac



VI

Chart 1

1) Part XL in the series of *Chemical Studies on the Oriental Plant Drugs*. Part XXXIX: T. Saitoh, T. Kinoshita, and S. Shibata, *Chem. Pharm. Bull.* (Tokyo), **24**, 752 (1976).
2) Location: *7-3-1 Hongo, Bunkyo-ku, Tokyo 113, Japan.*
3) cited in 1)
4) A. Arnone, G. Cardillo, L. Merlini, and R. Mondelli, *Tetrahedron Letters*, **1967**, 4201.
5) R.V.M. Campbell, S.H. Harper, and A.D. Kemp, *J. Chem. Soc.* (C), **1969**, 1787.

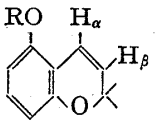


VII VIII IX X XI

Fig. 1. Mass Fragments of Tetrahydroglabrene (VI)

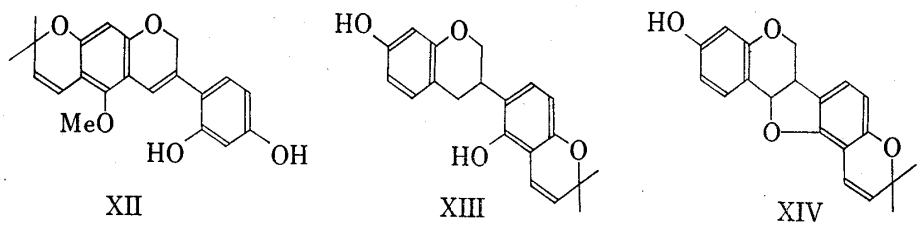
Glabrene (IV), $C_{20}H_{18}O_4$, was isolated as colorless plates, mp 198—202° (decomp.) and gave a positive Gibbs test, but negative Shinoda test. It is very unstable, especially in a solution, and no significant spot was observed on thin-layer chromatography (TLC) after standing only a few hours in solution, *e.g.* in acetone. It shows no C=O absorption in the infrared (IR) spectrum and UV absorption maxima at 248, 281, 297 and 324 nm, but no shift by the addition of shift reagents, such as $AlCl_3$ and NaOAc. The NMR spectrum revealed the presence of a chromene and two hydroxyls, besides six proton signals in the 6—7 ppm region. A broad singlet at δ 4.97 (2H) is a characteristic signal in the spectrum of IV. As in the case of glabrone, a shift of Hα proton of the chromene was observed on acetylation (Table I).

Table I. NMR Shifts of Chromene Protons induced by Acetylation of a Peri Hydroxyl

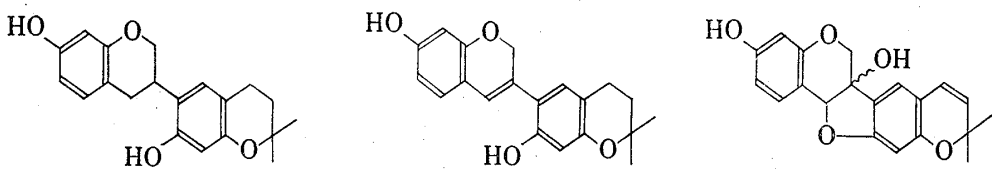


Compound	Proton	R			$\Delta\delta$	Solvent
		H	Me	Ac		
Glabrone (I)	Hα		6.57	6.27	+0.30	a
		6.80		6.34	+0.46	b
	Hβ		5.60	5.66	−0.06	a
		5.67		5.75	−0.08	b
Glabrene (IV)	Hα	6.70		6.37	+0.33	c
		6.67		6.42	+0.25	b
	Hβ	5.55		5.67	−0.12	c
		5.63		5.78	−0.15	b

a: $CDCl_3$, b: d_6-acetone, c: $CDCl_3$ and d_6-acetone (85: 15)



XII XIII XIV



XV XVI XVII

Chart 2

On catalytic hydrogenation, IV yielded tetrahydroglabrene (VI), mp 187—189°, whose, NMR spectrum revealed that it is an isoflavan derivative, that is, H-4 (2 protons equivalent) H-3, H-2*ax* and H-2*eq* appeared at δ 2.98 (d, 10), 3.3 (m), 3.91 (t, 10) and 4.21 (d.q, 10, 3 and 1), respectively. The mass spectrum is in accord with the structure (VI), giving the fragments (VII) (*m/e* 123) and (VIII) (*m/e* 135), derived from A ring, (IX) (*m/e* 204), (X) (*m/e* 148) and (XI) (*m/e* 191) from B ring.

On the basis of these results, the structure of glabrene is represented by IV.

Isoflavene is not common in plant products, and only one example, neorauflavene (XII),[6] is known in literatures. Probably isoflavenes would be biosynthesized *via* pterocarpans, but the latter has not been found in the extracts of this drug so far. It is of interest, however, from the biogenetic viewpoint to find the corresponding isoflavone, co-existing with an isoflavene.

It should be noted that an isoflavan, phaseollinisoflavan[7] (XIII), having corresponding substitutions to glabrene has been isolated as a phytoalexin from *Phaseolus vulgaris* infected with tobacco necrosis virus, along with the corresponding pterocarpan, phaseollin[8] (XIV).

An isomer (XV) of tetrahydroglabrene and the corresponding isoflavene (XVI) have been reported[9] as the derivatives of tuberosin (XVII), isolated from *Pueraria tuberosa*.

Experimental

Dried and ground licorice roots (5 kg) were first percolated with *n*-hexane, and then extracted with ethyl acetate at room temperature. After removal of ethyl acetate, the residue was chromatographed on silica gel with chloroform and acetone to give a crude mixture of glabrone and glabrene. The mixture was rechromatographed on polyamide with methanol, affording glabrone (60 mg) and glabrene (400 mg) as a crystalline form.

Glabrone (I)——Glabrone was recrystallised from acetone to form colorless needles, mp 224—226°. λ_{max}^{EtOH} nm (log ε): 243 (4.49 inf.), 252 (4.54), 257 (4.52 inf.) and 301 (4.19 sh.). $\lambda_{max}^{EtOH+NaOAc}$ nm: 264, 330. ν_{max}^{KBr} cm^{-1}: 3220, 2980, 1615, 1537, 1485. $\delta_{ppm}^{d_6\text{-acetone}}$: 1.41 (6H, s, CH_3 of chromene), 5.67 (1H, d, 10, Hβ of chromene), 6.36 (1H, d, 8.5, H-5′), 6.80 (1H, d, 10, Hα of chromene), 7.0—7.20 (3H, H-6, H-8, and H-6′), 8.16 (1H, d, 8.5, H-5), 8.33 (1H, s, H-2), 9.70 (2H, broad s, OH-2′ and OH-7, exchangable with D_2O). *m/e*: 336 (M^+). *Anal.* Calcd. for $C_{20}H_{16}O_5$: C, 71.42; H, 4.80. Found C, 71.22; H, 4.80.

Glabrene (IV)——Glabrene was recrystallised from benzene–acetone (95: 5) to form slightly brown plates, mp 198—202° (decomp.). λ_{max}^{EtOH} nm (log ε): 248 (4.25), 281 (4.26), 297 (4.28), 324 (4.32). ν_{max}^{KBr} cm^{-1}: 3290, 2960, 1639, 1612, 1598, 1498, 1442. $\delta_{ppm}^{d_6\text{-acetone}}$: 1.44 (6H, s, CH_3 of chromene), 4.97 (2H, s, H-2), 5.63 (1H, d, 10, Hβ of chromene), 6.3—6.4 (4H, m, H-4, H-6, H-8, and H-5′), 6.67 (1H, d, 10, Hα of chromene), 6.90 (1H, d, 8, H-6′ or H-5), 7.02 (1H, d, 8, H-5 or H-6′), 8.39 and 8.62 (1H each, s, 2′ and 7-OH, disappeared with D_2O). *m/e*: 322 (M^+). *Anal.* Calcd. for $C_{20}H_{18}O_4$: C, 74.52; H, 5.63. Found: C, 74.37; H, 5.70.

Glabrone Diacetate (II)——A mixture of glabrone (25 mg), pyridine and acetic anhydride was left overnight at room temperature, and then poured into ice water. Insoluble material was collected and recrystallised from ethanol to give a diacetate as colorless prisms (25 mg), mp 218—220°. λ_{max}^{EtOH} nm (log ε): 233 (4.55 sh.), 247 (4.58) and 302 (3.77). $\nu_{max}^{CHCl_3}$ cm^{-1}: 2900, 1768, 1651, 1618, 1483. $\delta_{ppm}^{CDCl_3}$: 1.44 (6H, s, CH_3), 2.13 (3H, s, OAc), 2.36 (3H, s, OAc), 5.66 (1H, d, 10, Hβ of chromene), 6.27 (1H, d, 10, Hα of chromene), 6.74 (1H, d, 8, H-5′), 7.05 (1H, d, 8, H-6′), 7.22 (1H, q, 8 and 2, H-6), 7.28 (1H, d, 2, H-8), 7.84 (1H, s, H-2), 8.28 (1H, d, 8, H-5). *m/e*: 420 (M^+). *Anal.* Calcd. for $C_{24}H_{20}O_7$: C, 68.56; H, 4.80. Found: C, 68.34; H, 4.80.

Glabrone Dimethyl Ether (III)——With diazomethane glabrone (26.5 mg) was methylated in the usual manner to give a dimethyl ether as colorless plates (16 mg) recrystallised from dil. EtOH. mp 124—125.5°. λ_{max}^{EtOH} nm: 236 (inf.), 248, 300 (sh.). $\nu_{max}^{CHCl_3}$: 3000, 1630, 1613, 1570, 1503, 1482. $\delta_{ppm}^{CDCl_3}$: 1.44 (6H, s, $2\times CH_3$), 3.57 (3H, s, OMe), 3.90 (3H, s, OMe), 5.60 (1H, d, 10, Hβ of chromene), 6.57 (1H, d, 10, Hα of chromene), 6.59 (1H, d, 8, H-5′), 6.82 (1H, d, 2, H-8), 6.94 (1H, q, 8 and 2, H-6), 7.10 (1H, d, 8, H-6′), 7.92 (1H, s, H-2), 8.16 (1H, d, 8, H-5). *m/e*: 364 (M^+, 40%), 349 (M–15, base peak), 333 (M–31, 11%). *Anal.* Calcd. for $C_{22}H_{20}O_5$: C, 72.51; H, 5.53. Found: C, 72.44; H, 5.61.

Glabrene Diacetate (V)——Glabrene was acetylated with pyridine and acetic anhydride in the usual manner. The product was crystallised from EtOH to give a diacetate as colorless needles, mp 148—150°.

6) A.J. Brink, G.J.H. Rall, and J.P. Engelbrecht, *Tetrahedron*, **30**, 311 (1974).
7) R.S. Burden, J.A. Bailey, and G.W. Dawson, *Tetrahedron Letters*, **1972**, 4175.
8) D.R. Perrin, *Tetrahedron Letters*, **1964**, 29.
9) B.S. Joshi and V.N. Kamat, *J. Chem. Soc. Perkin I*, **1973**, 907.

λ_{max}^{EtOH} nm (log ε): 242 (4.35), 275 (4.21), 283 (4.21), 325 (4.16). $\nu_{max}^{CHCl_3}$ cm^{-1}: 2970, 1760, 1608, 1495. $\delta_{ppm}^{CDCl_3}$: 1.41 (6H, s, 2×CH_3), 2.26 (3H, s, OAc), 2.30 (3H, s, OAc), 5.07 (2H, s, H-2), 5.64 (1H, d, 10, Hβ of chromene), 6.32 (1H, d, 10, Hα of chromene), 6.6—6.7 (4H), 7.0—7.20 (2H). *m/e*: 406 (M^+). *Anal.* Calcd. for $C_{24}H_{22}O_6$: C, 70.92; H, 5.46. Found: C, 70.63; H, 5.55.

Tetrahydroglabrene (VI)——A solution of 50 mg of glabrene in ethanol (18 ml) was hydrogenated in the presence of prereduced platinum oxide (30 mg). Removal of the solvent and recrystallisation of the residue from dil. methanol gave tetrahydroglabrene (43 mg), mp 187—189°. λ_{max}^{EtOH} nm (log ε): 282 (3.74 inf.), 284 (3.76), 288 (3.59 inf.). ν_{max}^{KBr} cm^{-1}: 3360, 2940, 1601, 1502, 1468. $\delta_{ppm}^{d_6\text{-acetone}}$: 1.32 (6H, s, 2×$CH_3$), 1.81 (2H, t, 6.5, H-3 of chroman), 2.68 (2H, t, 6.5, H-4 of chroman), 2.98 (2H, d, 8, H-4*ax* and H-4*eq*), 3.3 (1H, m, H-3*ax*), 3.91 (1H, t, 10, H-2*ax*), 4.21 (1H, d.q, 10, 3 and 1, H-2*eq*), 6.2—6.4 (3H), 6.77 (1H, d, 8, H-5 or H-6′), 6.85 (1H, d, 8, H-6′ or H-5), 8.08 (2H, broad s, OH-2′ and OH-7, exchanged with D_2O). *m/e*: 326 (M^+, 76%), 204 (base peak), 191 (97), 148 (58), 135 (48), 123 (42). *Anal.* Calcd. for $C_{20}H_{22}O_4$: C, 73.60; H, 6.79. Found: C, 73.57; H, 6.83.

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