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Chemical Studies on the Oriental Plant Drugs. XXXVI.[1] Structure of Licoricone, a New Isoflavone from Licorice Root

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A new isoflavone, licoricone, $C_{22}H_{22}O_6$, mp 250—251°, was isolated from the root of *Glycyrrhiza uralensis* Fisch. et DC. The chemical structure of licoricone was studied spectroscopically to lead a partial formula (VII). Finally an X-ray crystallographic analysis of its monobromoacetate (VIII) was undertaken to formulate licoricone as I.

Several constituents of licorice root have been investigated chemically under the correlation with their pharmacological activities.[3]

In the course of study of the anti-gastric ulcer principles of licorice root, some phenolic constituents, licoricidin, glycyrol, 5-O-methylglycyrol and isoglycyrol were isolated. The structures of these compounds were elucidated on the basis of chemical and spectral data.[3,4]

Recently we obtained a new isoflavone named licoricone from licorice, the root of *Glycyrrhiza uralensis* Fischer. et DC. Separation of the sodium carbonate-soluble fraction of the methanolic extracts of the root by silica gel column chromatography gave colourless crystals of licoricone (I), $C_{22}H_{22}O_6$, mp 250—251°. The ultraviolet (UV) spectrum of licoricone shows isoflavone-type absorptions and the NMR spectrum gave a sharp singlet at δ 8.01, assignable to $H_{(2)}$ of isoflavone skeleton. Although licoricone gave no ferric reaction, the presence of two phenolic hydroxyls was revealed by the formation of a diacetate (II), $C_{26}H_{26}O_8$, mp 172.5—173.5° and a dimethyl ether (III), $C_{24}H_{26}O_6$, mp 179.5—181°. Furthermore, the nuclear magnetic resonance (NMR) spectrum showed the presence of one γ,γ-dimethylallyl group, two aromatic methoxyl groups, four aromatic protons and two phenolic hydroxyls. One of the aromatic proton signals at δ 7.92 (d, J=9 Hz) is coupled with that at δ 6.96 (q, J=9, 2.5 Hz) which is coupled again with that at δ 6.89 (d, J=2.5 Hz). The lowest signal (δ 7.92) was assigned to the proton at $C_{(5)}$ which must be deshielded by a carbonyl at the 4-position. Accordingly, the signals at δ 6.96 and 6.89 were assigned to the protons at $C_{(6)}$ and $C_{(8)}$, respectively.

The UV absorption maximum at 284 nm underwent bathochromic shift to 303 nm in the presence of NaOAc, suggesting that one of the hydroxyls must be located at $C_{(7)}$ position.[5]

On treatment of licoricone (I) with methanolic HCl, two products, tentatively named product A (IV) and B (V), were obtained respectively by the addition of methanol and water to the double bond of a γ,γ-dimethylallyl group without formation of a chroman ring, suggesting that the γ,γ-dimethylallyl group is not located at the position adjacent to a hydroxyl group.

In the mass spectrum of licoricone a peak at m/e 351 (44%) was observed, which corresponded to the fragment VI derived from licoricone with a loss of methoxy group. As already

1) Part XXXV: M. Takai, H. Yamaguchi, T. Saitoh, and S. Shibata, *Chem. Pharm. Bull.* (Tokyo), **20**, 2488 (1972).
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3) S. Shibata and T. Saitoh, *Chem. Pharm. Bull.* (Tokyo), **16**, 1932 (1968), and the references cited therein.
4) T. Saitoh and S. Shibata, *Chem. Pharm. Bull.* (Tokyo), **17**, 729 (1969).
5) L. Jurd, "The Chemistry of Flavonoid Compound," ed. by T.A. Geissman, Pergamon Press, 1962, p. 107.

reported,[6] this fragmentation reveals the presence of a methoxyl group at $C_{(2')}$ position of B ring of isoflavone.

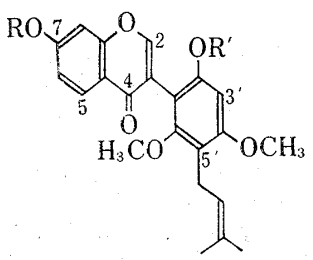
From these results licoricone could be formulated by the partial structure VII, but no conclusive evidence could be provided for the final structure only by the chemical and spectral experiments.

Therefore, an X-ray structure anaylsis of a heavy-atom derivative of licoricone was undertaken.

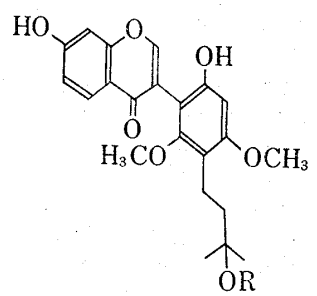
Treatment of licoricone with bromoacetyl bromide and a few drops of pyridine in $CHCl_3$ afforded a dibromoacetate as a major product which was shown by the thin-layer chromatography (TLC) of the reaction mixture, but during the purification by silica gel column chromatography partial debromoacetylation took place to give a monobromoacetate.

Licoricone monobromoacetate (VIII), mp 205—208°, $C_{24}H_{23}O_7Br$, formed colourless triclinic prisms elongated along the c axis when it was recrystallized from MeOH. The lattice constants (a=11.70Å, b=13.11Å, c=8.16Å, α=103.8°, β=91.6°, γ=105.4°) and space group (P_1) were determined from precession photographs taken with CuKα radiation considering that this compound is optically inactive. The density measured by the floatation method using the mixture of carbon tetrachloride and hexane was 1.426 g. cm^{-3} which agrees well with the calculated value 1.438 g. cm^{-3} assuming that two structure units are contained in the unit cell. Three dimensional diffraction data were collected on a Rigakudenki four-circle single-crystal diffractometer automatically controlled by a HITAC 10 computer with MoK α radiation.

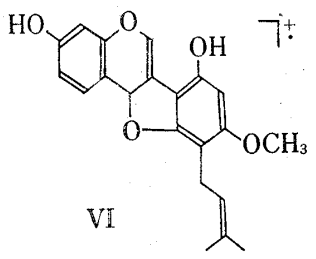
The total of 2664 independent observed structure factors were thus obtained. The structure was solved by the heavy atom method with the use of several repeated cycles of Fourier and difference Fourier syntheses coupled with the structure factor calculations. Refinement of the structural parameters was made by the block-matrix least-squares calculations to an R value of 0.17, in which anisotropic thermal vibrations of the bromine atom were allowed for. Further refinement was carried out by the least-squares method including all the thirty-two atoms with the individual anisotropic thermal vibrations and two cycles of calculations gave the R value of 0.11 for 2664 non-zero observed reflexions. The molecular structure of the bromo compound determined by the present X-ray analysis is formulated as VIII.



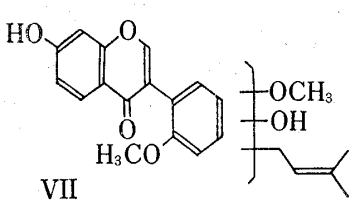
I : R=R'=H
II : R=R'=$COCH_3$
III : R=R'=CH_3
VIII : R=H, R' =$COCH_2Br$



IV : R=CH_3
V : R=H



VI



VII

Consequently, the structure of licoricone has now been unequivocally formulated as 2',7-dihydroxy-4',6'-dimethoxy-5'-γ,γ-dimethylallylisoflavone (I), which is the first naturally occurring flavonoid compound having phloroglucinol-type O-functions in the B ring.

The full details of the present X-ray investigation of licoricone monobromoacetate will be reported elsewhere.

6) R.V.M. Campbell, S. H.Harper, and A.D. Kemp, *J. Chem. Soc.* (*C*), **1969**, 1787.

Experimental

Isolation of Licoricone——The sodium carbonate-soluble part of methanolic extracts of licorice root was chromatographed over silica gel using a mixture of benzene and acetone (10: 1) as the solvent. Concentration of the middle part of the fractions gave colourless needles of licoricone (I), mp 250—251° (The yield from the methanolic extract was 0.004%). Both $FeCl_3$ and Mg-HCl reactions are negative.

UV λ_{max}^{EtOH} nm (ε): 238 (20640), 248 (19720), 284 (10240), 302 (7800); $\lambda_{max}^{EtOH\text{-}NaOAc}$ nm: 250, 253 (inf.), 303 (sh.), 330. IR $\nu_{max}^{CHCl_3}$ cm^{-1}: 3500, 3160, 1631, 1612, 1585, 1570; ν_{max}^{KBr} cm^{-1}: 3140, 1628, 1611, 1584. NMR (60 Mc, d_6-DMSO): δ 1.63 (3H, br. s), 1.69 (3H, br. s, $-CH_2-CH=C(C\underline{H}_3)_2$), 3.20 (2H, br. d, $J=7$ Hz, $-C\underline{H}_2-CH=C(CH_3)_2$), 3.40 (3H, s), 3.77 (3H, s) (OCH_3), 5.14 (1H, br. t, $J=7$ Hz, $-CH_2=C\underline{H}-C(CH_3)_2$), 6.35 (1H, s, H-3′), 6.89 (1H, d, $J=2.5$ Hz, H-8), 6.96 (1H, d. d, $J=9$, 2.5 Hz, H-6), 7.92 (1H, d, $J=9$ Hz, H-5), 8.01 (1H, s, H-2), 9.2 (1H, br.), 10.6 (1H, br.) (-OH, disappeared after the addition of D_2O). Mass Spectrum *m/e*: 382 (M^+, base peak), 367 (M-CH_3, 57%), 351 (M-OCH_3, 44), 327 (M-$CH=C(CH_3)_2$, 17), 314 (M-$CHCH=C(CH_3)_2$, 19), 257 (25), 137 (37). *Anal.* Calcd. for $C_{22}H_{22}O_6$: C, 69.10; H, 5.80. Found: C, 68.95; H, 5.87.

Licoricone Diacetate (II)——Licoricone (90 mg), Ac_2O (1.5 ml) and pyridine (2 ml) were mixed under ice cooling and the mixture was allowed to stand overnight. After working up as usual, the product was recrystallized from acetone to yield colourless needles of licoricone diacetate (II) (73 mg), mp 172—173.5°. UV λ_{max}^{EtOH} nm (ε): 257 (39200), 298 (11500). IR $\nu_{max}^{CHCl_3}$ cm^{-1}: 1770 (OAc), 1648 (C=O), 1620, 1575, 1515. NMR (60 Mc, CCl_4): δ 1.63, 1.71 (1Me each, s, $-CH_2-CH=C(C\underline{H}_3)_2$), 1.97, 2.26 (1Me each, OAc), 3.33 (2H, br. d, $J=7$ Hz, $-C\underline{H}_2-CH=C(CH_3)_2$, 3.47, 3.81 (1Me each, OCH_3), 5.16 (1H, br. t, $J=7$ Hz, $-CH_2-C\underline{H}=C(CH_3)_2$, 6.52 (1H, s, H-3′), 7.13 (1H, d. d, $J=8.5$, 2.5 Hz, H-6), 7.27 (1H, d, $J=2.5$ Hz, H-8), 7.84 (1H, s, H-2), 8.24 (1H, d, $J=8.5$ Hz, H-5). *Anal.* Calcd. for $C_{26}H_{26}O_8$: C, 66.95; H, 5.58. Found: C, 67.38; H, 5.62%.

Licoricone Dimethyl Ether (III)——Licoricone (I) (129 mg) was methylated with Me_2SO_4 (1 ml) and K_2CO_3 (1.5 g) in acetone (20 ml), and the crude product was recrystallized from acetone and then from MeOH to yield colouless needles of licoricone dimethyl ether (73 mg), mp 179.5—181°. UV λ_{max}^{EtOH} nm (ε): 247 (24010), 284 (12450), 304 (10100). IR $\nu_{max}^{CHCl_3}$ cm^{-1}: 1643, 1630, 1613, 1585, 1573. NMR (60 Mc, $CDCl_3$): δ 1.65, 1.74 (1 Me each, $-CH_2CH=C(C\underline{H}_3)_2$), 3.32 (2H, br. d, $J=7$ Hz, $-C\underline{H}_2CH=C(CH_3)_2$), 3.49, 3.75, 3.87, 3.91 (1 Me each, OCH_3), 5.20 (1H, br. t, $J=7$ Hz, $-CH_2C\underline{H}=C(CH_3)_2$), 6.37 (1H, s, H-3′), 6.90 (1H, d, $J=2.5$ Hz, H-8). 6.97 (1H, d. d, $J=9$, 2.5 Hz, H-6), 7.79 (1H, s, H-2), 8.19 (1H, d, $J=9$ Hz, H-5). *Anal.* Calcd. for $C_{24}H_{26}O_5$: C, 70.23; H, 6.39. Found: C, 69.97; H, 6.39%.

Acid Treatment of Licoricone (I)——After addition of conc. HCl (5 ml) to the solution of licoricone (210 mg) in MeOH (20 ml), the mixture was refluxed for 2 hr on a water-bath. The reaction mixture was diluted with water and extracted with $CHCl_3$. The $CHCl_3$ layer was washed with water, dried over anhyd. $MgSO_4$ and concentrated to dryness. The crude products were then chromatographed on a silica gel column using a mixture of benzene and acetone (9: 1 and 7: 3) as the eluting solvent. Evaporation of the solvent of the early fraction gave a colourless solid which was recrystallized from acetone to yield colourless needles of product A (IV) (130 mg) mp 265.5—267° (decomp.). UV λ_{max}^{EtOH} nm (ε): 239 (21400), 248 (21440), 284 (11680), 303 (sh., 9770). IR ν_{max}^{KBr} cm^{-1}: 3485, 3160, 1630, 1615, 1587, 1575. NMR (100 Mc, d_6-DMSO): δ 1.14 (2 Me, s, $-CH_2CH_2C(OCH_3)(C\underline{H}_3)_2$), 1.57 (2H, m, $-CH_2C\underline{H}_2C(OCH_3)(CH_3)_2$), 3.15, 3.43, 3.76, (1 Me each, OCH_3), 3.32 (2H, br. s, OH, disappeared after deuterium exchange), 6.35 (1H, s, H-3′), 6.92 (1H, d, $J=2.5$ Hz, H-8), 6.97 (1H, d. d, $J=2.5$, 9 Hz, H-6), 7.99 (1H, d, $J=9$ Hz, H-6), 8.07 (1H, s, H-2). The signal of the benzyl protons was overlapped with that of DMSO. Mass Spectrum *m/e*: 414 (M^+, $C_{23}H_{26}O_7$), 382 (M-MeOH), 367 (M-MeOH-CH_3), 351 (M-MeOH-OCH_3), 327 (M-$CH_2C(OCH_3)(CH_3)_2$, base peak). *Anal.* Calcd. for $C_{23}H_{26}O_7$: C, 66.65; H, 6.32. Found: C, 66.37; H, 6.31.

Another product was obtained from the later fractions of the column chromatography and recrystallized from $CHCl_3$ to afford colourless needles of product B (V) (20 mg), mp 250—251°. UV λ_{max}^{EtOH} nm (ε): 239 (20640), 248 (20600), 284 (11420), 303 (sh, 9600). IR ν_{max}^{KBr} cm^{-1}: 3480, 3160, 1635 (sh.), 1625, 1615, 1586, 1575. Mass Spectrum *m/e*: 400 (M^+, $C_{22}H_{24}O_7$, 11%), 385 (M-CH_3, 4), 382 (M-H_2O, 7), 369 (M-OCH_3, 30), 327 (M-$CH_2C(OH)(CH_3)_2$, base peak), 313 (M-$CH_2CH_2C(OH)(CH_3)_2$, 20). *Anal.* Calcd. for $C_{22}H_{24}O_7$: C, 65.99; H, 6.04. Found: C, 66.29; H, 6.16.

Licoricone Monobromoacetate (VIII)——To the solution of licoricone (I) (100 mg) in $CHCl_3$ (5 ml) containing a few drops of pyridine, bromoacetyl bromide (1 ml) was added dropwise under ice-cooling. After a few minutes, ice water was added to the reaction mixture, which was then extracted with $CHCl_3$. The chloroform layer was washed with water, dried over anhyd. Na_2SO_4 and evaporated to give yellow film, which was chromatographed on a silica gel column (Wakogel, 100 g) using a mixture of benzene and acetone (15: 1) as the solvent. A band giving blue fluorescence under an UV lamp was collected and evaporated to yield colourless film, which was crystallized from MeOH to afford colourless prisms of licoricone monobromoacetate (VI) (59 mg), mp 205—208°. NMR (100 Mc, $(CD_3)_2CO$ and d_6-DMSO): δ 1.67, 1.77 (1 Me each, $-CH_2CH=C(C\underline{H}_3)_2$), 3.50, 3.87 (1 Me each, OCH_3), 4.00 (2H, s, $-OCOCH_2Br$), 5.20 (1H, br. t, $-CH_2C\underline{H}=C(CH_3)_2$), 6.70 (1H, s, H-3′), 6.89 (1H, d, $J=2.5$ Hz, H-8), 6.93 (1H, d. d, $J=2.5$, 9 Hz, H-6), 7.87 (1H, s, H-2), 7.98 (1H, d, $J=9$ Hz, H-5), 10.6 (1H, br, -OH). Mass Spectrum *m/e*: 504 (M^+, Br^{81}), 502 (M^+, Br^{79}), 487 (M-OH,

Br^{81}), 485 (M-OH, Br^{79}), 473 (M-OCH_3, Br^{81}), 471 (M-OCH_3, Br^{79}), 449 (M-C_4H_7, Br^{81}), 447 (M-C_4H_7, Br^{79}), 424 (M-Br+1), 382[7] (M-$COCH_2$ Br+1), 367 (382-CH_3), 365 (382-OH, base peak), 351 (382-OCH_3). *Anal.* Calcd. for $C_{24}H_{23}O_7Br$: C, 57.26; H, 4.57. Found: C, 56.93; H, 4.60.

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7) This ion corresponds to the M^+ ion of licoricone (I).