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# The Chemical Studies on the Oriental Plant Drugs. XIX.[1] Some New Constituents of Licorice Root. (1). The Structure of Licoricidin

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A new isoflavan derivative, licoricidin, $C_{25}H_{32}O_5$, mp 161—162.5°, $[\alpha]_D^{22.5°}$ +20° (MeOH) was isolated from licorice root. According to the results mostly deduced from nuclear magnetic resonance and mass spectral analyses, licoricidin would be represented as being 3′,6-diisopentenyl-2′,4′,5-trihydroxy-7-methoxyisoflavan (IXa).

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

In the course of study to find the active principle of licorice root which would be effective for stomach ulcer, some new phenolic constituents were isolated from the commercial licorice root, *Glycyrrhiza glabra* sp. (Leguminosae).

One of the new constituents, the first isoflavan isolated from plants, has been named licoricidin, first Licoricidin, $C_{26}H_{32}O_5$, colourless needles, mp 161—162.5° (from $CH_2Cl_2$), $[\alpha]_D^{22.5°}$ +20° (MeOH), was isolated using the silica gel column chromatography from benzene-soluble and sodium carbonate-insoluble portion of methanolic extracts of licorice.

Although it shows no positive ferric and diazo reactions, it possesses phenolic hydroxyls forming triacetate, $C_{32}H_{38}O_8$, mp 137—138.5° ($\nu_{max}$ 1770 $cm^{-1}$ (phenolic acetate C=O)). On catalytic hydrogenation, licoricidin afforded a tetrahydro-derivative which gave triacetate, $C_{32}H_{42}O_8$, mp 132—133°.

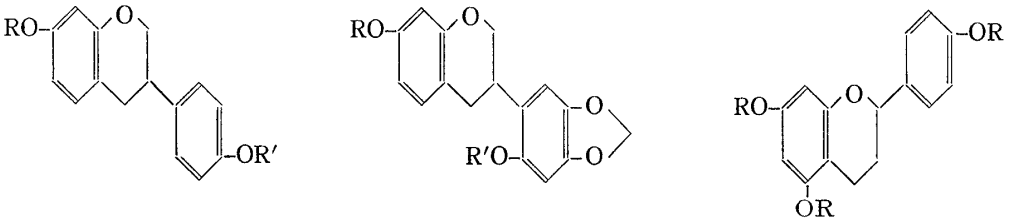
The nuclear magnetic resonance (NMR) spectrum of licoricidin (in *d*-DMSO) revealed the presence of two almost equivalent isopentenyl groups ($\delta$ 1.62 (6H, broad singlet) 1.70 (6H, broad singlet), 5.15 (2H, multiplet) and 3.22 (4H, multiplet)), aromatic methoxyl group (3.61 (3H, singlet)), aromatic protons (6.09 (1H, singlet), 6.31 (1H, doublet, $J$=8.6 cps), 6.71 (1H, doublet, $J$=8.6 cps)) and hydroxyls (8.07 (1H, singlet), 8.94 (1H, singlet) and 9.08 (1H, singlet); disappeared by the addition of $D_2O$).

The ultraviolet (UV) spectrum of licoricidin triacetate resembled those of equol[4] diacetate (Ib), *dl*-dihydromaackiain diacetate[5] (IIb) and methyl ether of a flavan[6] (IIIb) isolated by Birch from *Xanthorrhoea preisii* (Liliaceae) (Table I).

1) Part XVIII: N. Aimi, H. Fujimoto and S. Shibata, *Chem. Pharm. Bull.* (Tokyo), **16**, 641 (1968).
2) Location: *Bunkyo-ku, Hongo, Tokyo.*
3) Glycyrrhizin and its aglycone, glycyrrhetic acid (L. Ruzicka, O. Jeger and W. Ingold, *Helv. Chim. Acta*, **26**, 2278 (1943); J.M. Beaton and F.S. Spring, *J. Chem. Soc.*, **1955**, 3126), glabric acid (J.M. Beaton and F.S. Spring, *J. Chem. Soc.*, **1956**, 2417) and liquoric acid (M.H.A. Elgama, M.B.E. Fayez and G. Snatzke, *Tetrahedron*, **21**, 2109 (1965)) were known as the triterpenoid constituents of licorice root. Glycyrrhizin and glycyrrhetic acid were shown to have adrenal steroid-like activities. Liquiritin, its aglycone, liquiritigenin (J. Shinoda and S. Ueeda, *Ber.*, **67**, 434 (1934)), isoliquiritin and its aglycone, isoliquiritigenin (B. Puri and T.R. Seshadri, *J. Sci. Ind. Res.*, **13**, 475 (1954)), were known as the phenolic constituents. Isoliquiritigenin shows a remarkable spasmolytic action. Neoliquiritin, neoisoliquiritin and licurazid were reported (V.I. Litvinenko, *Farmatsevt. Zh.* (Kiev), **18**(5), 20 (1963)[*C.A.*, **60**, 6700*g* (1964)]] as the glycosides of liquiritigenin and isoliquiritigenin, respectively.
4) G.F. Marrian and D. Beall, *Biochem. J.*, **29**, 1586 (1935).
5) S. Shibata and Y. Nishikawa, *Chem. Pharm. Bull.* (Tokyo), **11**, 167 (1963).
6) A.J. Birch and M. Salahu-din, *Tetrahedron Letters*, **1964**, 2211.

Table I. UV Spectra of Flavan and Isoflavans

| | $\lambda_{max}^{EtOH}$ mμ (log ε) |
|---|---|
| Licoricidin triacetate | 274(3.43), 281(3.44) |
| Equol diacetate (Ib) | 276(3.48), 282(3.46) |
| *dl*-Dihydromaackiain diacetate (IIb) | 284(3.81) |
| Flavan of *Xanthorrhoea preisii* (IIIb) | 235(4.42, shoulder), 274(3.36), 280(3.26, shoulder) |




Ia : R=R'=H
Ib : R=R'=Ac

IIa : R=R'=H
IIb : R=R'=Ac

IIIa : R=H
IIIb : R=Me

This would suggest that licoricidin is a flavan or isoflavan. The latter would be the case, since the NMR spectrum of licoricidin gave no signal of ($C_2$) proton of flavan nucleus which should appear at about $\delta$ 5.0, while the NMR spectral pattern of the hetero-ring system of licoricidin triacetate (Fig. 1) showed a close resemblance with that of equol (Ia) (Fig. 2).

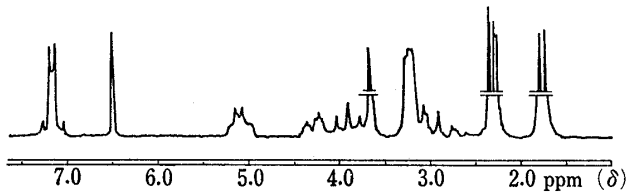



Fig. 1. NMR Spectrum of Licoricidin Triacetate (100 Mc/sec in $CDCl_3$)

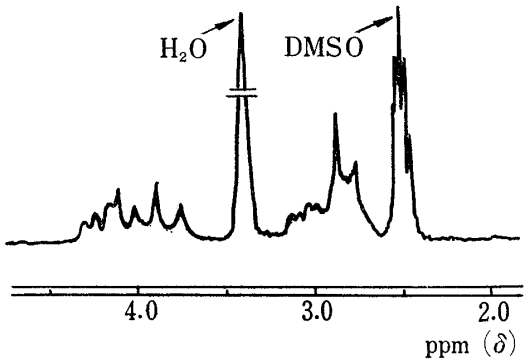
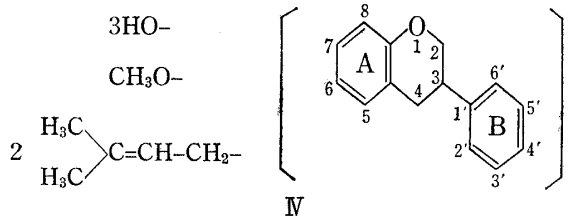



Fig. 2. NMR Spectrum of Equol (100 Mc/sec in *d*-DMSO)

Thus licoricidin would possibly be an isoflavan as being formulated by the following partial structure (IV).

The mass spectrum of licoricidin gave fragments at *m/e* 233 (V) and 220 (VI), which would be derived from the ring A. The peaks at *m/e* 204 and 148 would correspond to the fragments, VII and VIII, which are arisen from the ring B.[7]

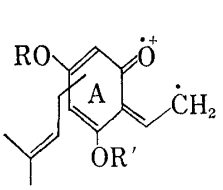



IV

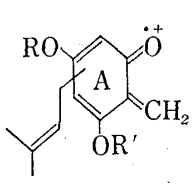
The aromatic proton signals of licoricidin at $\delta$ 6.31 and 6.71 showing an *ortho* coupling ($J$=8.6 cps), and the unvarying UV spectrum by the addition of aluminum chloride revealed the location of substituents in the ring B as formulated (VII).

The mass spectral analysis of licoricidin mentioned above has been confirmed by the comparison with that of equol, which gave the corresponding fragments, *m/e* 135 (V'), 122 (VI') and 120 (VII').

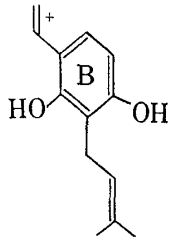
7) H. Audier, *Bull. Soc. Chim. France*, **1966**, 2892.



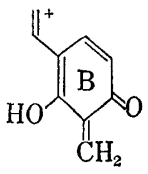
$R=CH_3$ or H
$R'=H$ or $CH_3$
*m/e* 233 V



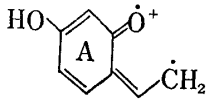
$R=CH_3$ or H
$R'=H$ or $CH_3$
*m/e* 220 VI



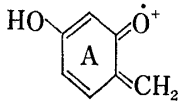
*m/e* 204 VII



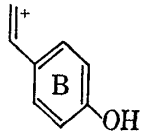
*m/e* 148 VIII



*m/e* 135 V′



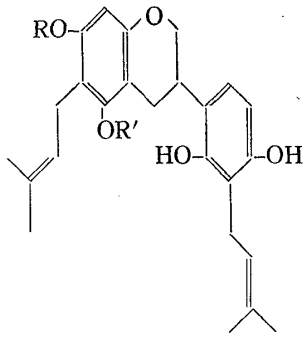
*m/e* 122 VI′



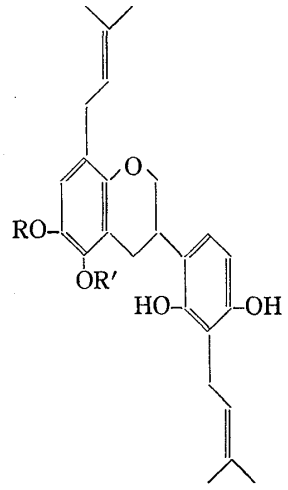
*m/e* 120 VII′

The above results led to the following four possible formulae (IXa, b) (Xa, b) for licoricidin.

On refluxing a solution of licoricidin in methanolic hydrochloric acid, the isopentenyl side chains were cyclized to form two 2,2–dimethylchroman rings whose geminal methyls (12H) gave a broad singlet at $\delta$ 1.27 in the NMR spectrum. This ruled out the formula (Xa) which can give only one 2,2–dimethylchroman system.

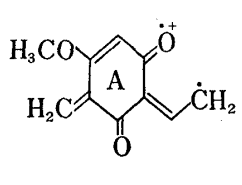


IXa : $R=CH_3$, $R'=H$
IXb : $R=H$, $R'=CH_3$

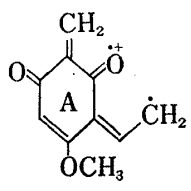


Xa : $R=CH_3$, $R'=H$
Xb : $R=H$, $R'=CH_3$

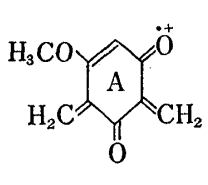
In the mass spectrum of licoricidin, the peaks at *m/e* 177 and 164 which would correspond to 233 (V–56) and 220 (VI–56) revealed the splitting of isopentenyl group[8] as being formulated XI or XII and XIII or XIV, respectively.



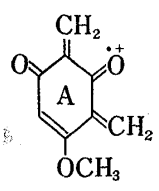
XI



XII



XIII



XIV

8) E. Ritchie, W.C. Taylor and J.S. Shannon, *Tetrahedron Letters*, **1964**, 1437; J.A. Diment, E. Ritchie and W.C. Taylor, *Aust. J. Chem.*, **20**, 565 (1967).

This result could rule out the possibility of formula (IXb) for licoricidin.

It has generally been observed a lower shift (about 0.4 ppm) of NMR signal of aromatic proton at *para* position of phenolic hydroxyl by acetylation.[9]

The NMR signal of aromatic proton of the ring A of licoricidin ($\delta$ 6.20) and tetrahydrolicoricidin ($\delta$ 6.15) were shifted to $\delta$ 6.50 ($\Delta$ −0.30 ppm) and $\delta$ 6.42 ($\Delta$ −0.27 ppm), respectively, on acetylation. It has also been observed that methylation of a hydroxyl group produces a downfield shift (0.2 ppm )of the signal of aromatic proton in the *ortho* position, whereas a somewhat smaller effect (about 0.1 ppm )and little or no effect are revealed on that of *p*- and *m*-proton,[10] respectively.

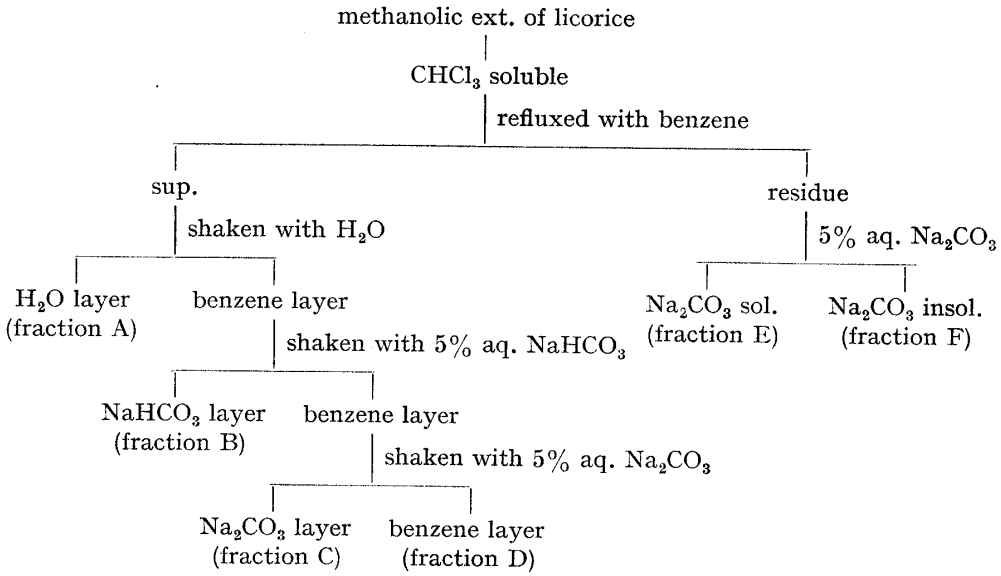
The signal of aromatic proton of the ring A of tetrahydrolicoricidin ($\delta$ 6.15) was shifted to $\delta$ 6.25 ($\Delta$ −0.10 ppm )on methylation.

This would indicate the *para* position of phenolic hydroxyl of ring A of licoricidin must be free as being formulated IXa.

## Experimental

All the NMR spectra were measured with tetramethylsilane as the internal reference. The chemical shifts are shown in $\delta$ value (ppm).

**Separation of Methanolic Extracts of Licorice**——A chloroform-soluble part of methanolic extracts of licorice was refluxed with benzene, and divided into benzene-soluble and benzene-insoluble portions. Each portion was separated into sodium carbonate-soluble and insoluble parts.




**Isolation of Licoricidin**——The fraction D was eluated on the silica gel column chromatography using methylene chloride. The middle part of fraction gave a crystalline compound, licoricidin, which was recrystallized from methylene chloride and chloroform to form colourless needles, mp 161—162°, $[\alpha]_D^{22.5°}$ +20° ($c$=1, MeOH). (The yield from the methanolic extracts was 0.002%.) It gave no colouration with $FeCl_3$ and diazo reagent, and yellow colour with conc. $H_2SO_4$. *Anal.* Calcd. for $C_{26}H_{32}O_5$: C, 73.56; H, 7.60. Found: C, 73.38; H, 7.78. UV $\lambda_{max}^{EtOH}$ m$\mu$ (log $\varepsilon$): 281 (3.69 sh), 284 (3.71). IR $\nu_{max}^{KBr}$ cm$^{-1}$: 3520, 3395, 2940, 1616, 1606, 1502. $\nu_{max}^{CHCl_3}$ cm$^{-1}$: 3595, 3410, 2920, 1617, 1594, 1480. NMR ($d$-DMSO): 1.62 (6H, s), 1.70 (6H, s), *ca.* 2.5—3.5 (7H, m. overlapped with the signal of $H_2O$ and DMSO) 3.61 (3H, s), 3.86 (1H, tr), 4.11 (1H, br. d), 5.15 (2H, m), 6.09 (1H, s), 6.31 (1H, d), 6.71 (1H, d), 8.07 (1H, s), 8.94 (1H, s), 9.08 (1H, s)

**Licoricidin Triacetate**——A mixture of licoricidin (100 mg), acetic anhydride (1 ml) and pyridine (1.5 ml) was kept at room temperature overnight, and then poured into ice water. The solid was collected and recrystallized from methanol to give licoricidin triacetate (75 mg) as colourless needles, mp 131—132.5°, $[\alpha]_D^{15°}$

9) W.E. Hillis and D.H.S. Horn, *Aust. J. Chem.*, **18**, 531 (1965); **19**, 705 (1966); K.G.R. Pachler and D.G. Roux, *J. Chem. Soc.* (C), **1967**, 604.

10) T.J. Batterham and R.J. Highet, *Aust. J. Chem.*, **17**, 428 (1964).

+48.8° ($c$=0.5, $CHCl_3$). UV $\lambda_{max}^{EtOH}$ m$\mu$ (log $\varepsilon$): 274 (3.43 sh), 281 (3.44). IR $\nu_{max}^{KBr}$ cm$^{-1}$: 2930, 1760, 1613, 1590, 1480, 1205; $\nu_{max}^{CCl_4}$ cm$^{-1}$: 2920, 1770, 1614, 1590, 1480, 1200. *Anal.* Calcd. for $C_{32}H_{38}O_8$: C, 69.80; H, 6.96. Found: C, 70.10; H, 6.83. NMR ($CDCl_3$): 1.70 (6H, s), 1.76 (6H, s), 2.30 (3H, s), 2.33 (3H, s), 2.37 (3H, s), *ca.* 2.6—3.3 (7H, m), 3.74 (3H, s), 3.96 (1H, t), 4.30 (2H, m), 5.13 (2H, m), 6.50 (1H, s), 7.13 (2H, q).

**Tetrahydrolicoricidin**——A solution of licoricidin (100 mg) in absolute ethanol (35 ml) was hydrogenated for 38 min at room temperature using $PtO_2$ (50 mg) as a catalyst. The product which was failed to crystallize gave the same *Rf* value as licoricidin on the thin-layer chromatogram. In the NMR spectrum (in $CDCl_3$), however, the signals, $\delta$ 0.89 (6H, broad singlet) and 0.98 (6H, broad singlet), appeared and the signals of olefinic proton disappeared.

**Tetrahydrolicoricidin Triacetate**——Tetrahydrolicoricidin (oily, 65 mg) was dissolved in a mixture of acetic anhydride (0.5 ml) and pyridine (1 ml). On standing overnight at room temperature, the mixture was poured into ice water. The precipitate was filtered and recrystallized from methanol to givet tetrahydrolicoricidin triacetate as colourless needles (55 mg), mp 132—133. UV $\lambda_{max}^{EtOH}$ m$\mu$ (log $\varepsilon$): 276 (3.39, shoulder), 282 (3.42). IR $\nu_{max}^{KBr}$ cm$^{-1}$: 2955, 1755, 1613, 1586, 1480, 1470, 1200; $\nu_{max}^{CCl_4}$ cm$^{-1}$: 2970, 1772, 1616, 1593, 1481, 1193. *Anal.* Calcd. for $C_{32}H_{42}O_8$: C, 69.29; H, 7.63. Found: C, 69.59; H, 7.64.

**Acid-catalized Cyclization of Licoricidin**——(Formation of Isolicoricidin A and B): A mixture of licoricidin (40 mg), conc. hydrochloric acid (2.8 ml) and methanol (10 ml) was refluxed for 2 hr. Water (15 ml) was added and then methanol was removed under diminished pressure. The product was failed to be crystallized. In the NMR spectrum (in $CCl_4$) of the product, the signals of olefinic proton and methyl group on double bond disappeared, while a new broad singlet (6H) appeared at $\delta$ 1.26. Almost equivalent doublet signals ($J$=9.0 cps, *ortho* coupling) displayed at $\delta$ 6.66 and 6.74 in aromatic proton region, besides at $\delta$ 5.99 (1H, singlet) and 6.25 (1H, doublet, $J$=9.0 cps). It shows that C-3′ isopentenyl group on B ring was cyclized with C-2′ or C-4′ hydroxyl groups to form chroman ring. The product formed by the former ring closure was named isolicoricidin A, and by the latter case isolicoricidin B, though two products could not be separated in a pure state.

**Acknowledgement** We wish to thank Mrs. S. Mihashi (née Shimamura) for her assistance in experimental work. Thanks are also due to Nihon Kayaku Co. for providing materials.

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