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LINARIOSIDE, A NEW CHLORINE CONTAINING IRIDOID GLUCOSIDE, FROM LINARIA JAPONICA MIQ.

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In our continuing investigations on the crude drugs originated from Scrophulariaceous plants, we have examined the glycosidic components of *Linaria japonica* Miq.(a former Japanese folk medicine as a diuretic, *Scrophulariaceae*) collected on the seashore to elucidate the biologically active principle of the plant, since the flavonoids have been the only constituents previously clarified[1]. The present communication deals with the chemical evidences of a new iridoid glucoside named linarioside leading to the structure(I), which is the first example of a chlorine containing iridoid glucoside found in nature.

Repeated chromatography (using active charcoal-celite and silica gel) of the glycosidic portion obtained through the ordinary procedure from the methanolic extract of aerial parts furnished two iridoid glucosides in ca. 5% yield (based on the air-dried plant material) along with D-mannitol. The physical properties including the PMR data of the main glucoside (yield: 3.3%) and its penta- and hexa-acetates are superimposable with those reported for antirrhinoside (IV), which was initially revealed from *Antirrhinum* species (*Scrophulariaceae*) by Scarpati et al.[2] and has recently been isolated from *Linaria vulgaris* Mill.[3], although the direct comparison was not undertaken.

Linarioside(I), the second major glucoside component, is a quite unstable and hygroscopic amorphous substance. It is colored dark purple by conc. HCl and yellow-brown to dark green-brown by Trim and Hill reagent[4], and shows a positive Beilstein test. It exhibits the characteristic enol ether absorption band[5] at 1663 cm^{-1} and a broad hydroxyl band at 3460 cm^{-1} in its IR spectrum(KBr). On acid hydrolysis it gave glucose as detected by PPC and TLC(cellulose powder).

Acetylation of I with acetic anhydride and pyridine afforded a hexaacetate(II), $C_{27}H_{35}O_{16}Cl$,[6]

mp. 174.0-176.5°(colorless needles from methanol); $[\alpha]_D^{11°}$ -105°(dioxane); IR(Nujol, cm^{-1}): 3450(hydroxyl), 1760, 1725, 1250, 1215(acetate), 1655(enol ether); PMR(60 MHz, $CDCl_3$, τ): 7.7(1H, broad signal, exchangeable with D_2O), and a heptaacetate(III), $C_{29}H_{37}O_{17}Cl$, mp. 148.5-150.0°(colorless needles from methanol); $[\alpha]_D^{11°}$ -107°(dioxane); IR(Nujol): no hydroxyl band, thus indicating linarioside(I) to possess totally seven hydroxyl functions.

Assuming an iridoid glucoside framework for linarioside as for accompanied antirrhinoside, the PMR data of heptaacetate(III) is favorably comparable with those of hepta-O-acetyl-harpagide(VIII)[7] except that the signals for methylene protons at C_7(7.3-8.2 τ in the latter) are not observed in the former, whereas the significant downfield shift of C_9 proton signal is noticed in the former (Table I). The unacetylated hydroxyl group in the hexaacetate(II) is presumed to be at C_8 by the fact that the signals due to the protons at C_1, C_9, and C_{10} of II are observed at the diamagnetically shifted position as compared with those of the hepta-acetate(III). Consequently, the partial structure(A) has become plausible for linarioside(I) and its acetates(II and III).

Table I. The PMR Data of II, III, and VIII
(τ values in $CDCl_3$, J values in Hz)

	II (60 MHz)	III (100 MHz)	VIII (60 MHz)[7]
C_1-H	4.46 (br.s. $W\frac{h}{2}$ =2)	3.88 (br.s. $W\frac{h}{2}$ =2.5)	3.97 (br.s.)
C_3-H	3.74 (d. J=6.5)	3.73 (d. J=7)	3.62 (d. J=6.5)
C_4-H	4.35 (d.d. J=6.5, 1.5)	4.36 (d.d. J=7, 2)	4.48 (d.-like, J=6.5)
C_6-H	4.7-5.4	4.6-5.3	4.5-5.2
C_7-H(2H)	4.7-5.4 (1H)	4.6-5.3 (1H)	7.3-8.2 (2H)
C_9-H	6.59 (br.s. $W\frac{h}{2}$ =4)	6.35 (br.s. $W\frac{h}{2}$ =4)	6.81 (br.s.)
C_{10}-3H	8.67 (s.)	8.47 (s.)	8.48 (s.)

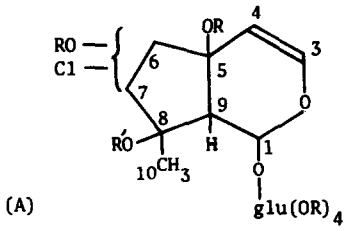
(br.: broad, s.: singlet, d.: doublet, d.d.: double doublet, d.-like: doublet like)

Furthermore, the partial structure(A) has been supported by the double resonance experiment performed on the protons at C_1, C_3, C_4, and C_9 of the heptaacetate(III), which is in good accord with our previous decoupling data for hexa-O-acetyl-harpagide(VII)[8] where the long range coupling between two protons at C_4 and C_9 has been demonstrated(Table II).

Finally, the conclusive evidence of the structure(I) for linarioside has been achieved as follows. Treatment of III with methanolic KOH followed by acetylation furnished in a

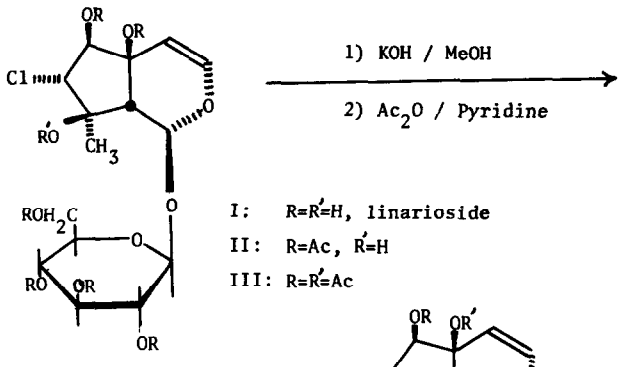
Table II. The Decoupling Experiment of III
(100 MHz, $CDCl_3$)

irradiated at / decoupled protons	365 Hz (C_9-H)	564 Hz (C_4-H)
C_1-H (3.88 τ, br.s. $W_{\frac{1}{2}}$=2.5)	sharp singlet	
C_3-H (3.73 τ, d. J=7)		singlet
C_4-H (4.36 τ, d.d. J=7, 2)	sharp doublet (J=7)	

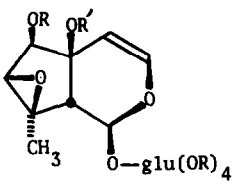


(A)

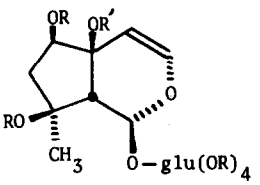
R=R'=H for I
R=Ac, R'=H for II
R=R'=Ac for III



I: R=R'=H, linarioside
II: R=Ac, R'=H
III: R=R'=Ac



IV: R=R'=H, antirrhinoside
V: R=Ac, R'=H



VI: R=R'=H, harpagide
VII: R=Ac, R'=H
VIII: R=R'=Ac

good yield(ca. 70% or even more) an epoxide, which was found identical in all respects(mixed mp, TLC, $[\alpha]_D$, and IR) with the pentaacetate(V) of antirrhinoside. Based on the mechanistic consideration on ready formation of antirrhinoside(IV) possessing a β-epoxy function, the functional group pattern including stereochemistry of linarioside(I) has become unambiguous. Although two alternative β-epoxides(6,7- and 7,8-) are possibly derivable, the 7,8-epoxide is formed almost preferentially. The true reason is a subject of further study.

The additional support for the location of chlorine atom at C_7 is given in the PMR data of heptaacetate(III). In the PMR studies on various halogenated compounds, Lack et al.[9] have pointed out that the signal of proton at γ position to the halogen atom is observed at

downfield due to an anisotropy and/or a field effect of the halogen atom. Referring to their observations, the downfield appearance of C_9 proton signal in III as compared with that of VIII could presumably be ascribed to its γ location from the chlorine atom, i.e. at C_7. Incidentally, all the PMR data of linarioside derivatives(II and III) are now assigned as given in Table I.

The co-occurence of a chlorohydrin(I) and its related epoxide(IV) in the same plant is of much interest from the biogenetic view-point and is reminiscent of the finding by Kupchan and his co-workers[10], who have isolated eupatorin and its related chlorohydrin eupachlorin from *Eupatorium* species (*Compositae*).

The biological activities of these glucosides are under investigation.

References and Footnotes

1) a) T.Nakaoki, N.Morita, H.Mototsune, A.Hiraki and T.Takeuchi, Yakugaku Zasshi, 75, 172 (1955);

Europian *Linaria* species have also been used as a folk medicine[1b] and shown to contain alkaloid[1c] and flavonoid pigments[1d].

b) O.Geßner, "Die Gift- und Arzneipflanzen von Mitteleuropa," Carl Winter Universitätsverlag, Heidelberg, 1953, p.294;

c) D.Gröger and S.Johne, Planta Medica, 13, 182 (1965);

d) B.Valdés, Phytochemistry, 9, 1253 (1970).

2) M.L.Scarpati, M.Guiso and P.Esposito, Gazz. Chim. Ital., 98, 177 (1968).

3) O.Sticher, Phytochemistry, 10, 1974 (1971).

4) A.R.Trim and R.Hill, Biochem. J., 50, 310 (1952).

5) J.M.Bobbitt and K.P.Segebarth, "Cyclopentanoid Terpene Derivatives," ed. by W.I.Taylor and A.R.Battersby, Marcel Dekker, Inc., New York, 1969, pp.101-104.

6) All the compounds given with chemical formulae gave satisfactory analytical values.

7) H.Lichti and A.von Wartburg, Helv. Chim. Acta, 49, 1552 (1966).

8) I.Kitagawa, T.Nishimura, M.Takei and I.Yosioka, Chem. Pharm. Bull.(Tokyo), 15, 1254 (1967).

9) R.E.Lack, J.Nemorin and A.B.Ridley, J. Chem. Soc.(B), 629 (1971).

10) S.M.Kupchan, J.E.Kelsey, M.Maruyama, J.M.Cassady, J.C.Hemingway and J.R.Knox, J. Org. Chem., 34, 3876 (1969).