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On the Constituents of *Linaria japonica* Miq. I. The Structure of Linarioside, a New Chlorinated Iridoid Glucoside and Identification of Two Related Glucosides

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The iridoid glucoside composition of *Linaria japonica* Miq., a Japanese folk medicine known as a laxative and diuretic, has been clarified to comprise antirride (Ia), antirrhinoside (IIa) and linarioside (IIIa). Linarioside is a new iridoid glucoside possessing a chlorohydrin moiety and the structure has been established as IIIa on the basis of physicochemical evidences and conversion into the corresponding epoxide antirrhinoside (IIa). Antirrhinoside is the major component of the plant and has been revealed to possess a weak and slow laxative property.

In addition, the detailed proton magnetic reronance examinations on these glucosides and their derivatives have been presented.

Linaria japonica Miq., a perennial scrophulariaceous herb, grows on the sandy place along the seashore in Japan. It has been claimed[2] to have been used as a laxative and diuretic in Japanese folk medicine and flavonoid glycosides[3] are the only constituents hitherto elucidated from the plant. On the other hand, some Europian *Linaria* species, also having medical qualities,[4] have been investigated and flavonoid pigments[5] and an alkaloid[6] have been isolated. Recently, Esposito, *et al.*[7] elucidated an iridoid diglucoside 10-O-glucosyl-aucubin from *Linaria vulgaris* Mill. From the same plant, Sticher[8] reported the isolation of antirrhinoside (IIa), an iridoid glucoside initially obtained[9] from two species of *Antirrhinum* (Scrophulariaceae), which is a closely related genus to *Linaria*.

During the continuative phytochemical investigations[10] on the scrophulariaceous plants, a new naturally occurring chlorinated iridoid glucoside named linarioside (IIIa)[11] has been elucidated from *Linaria japonica* Miq., collected in Kanazawa, together with two known iridoid glucosides: antirride (Ia)[12] and antirrhinoside (IIa), the former being previously isolated from *Antirrhinum* species. In the present paper, we describe the details of the studies on these iridoid glucosides.

The glycoside portion obtained from the aerial part through the usual work up was chromatographed using charcoal-Celite and the polar fraction was treated with AcOEt–EtOH

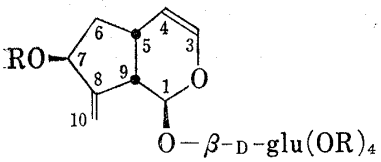
1) Location: *Toneyama, Toyonaka, Osaka.*
2) S. Omura (ed.), "Sogo Yakuyo Shokubutsu," Nankodo, Tokyo, 1944, p. 122.
3) T. Nakaoki, N. Morita, H. Mototsune, A. Hiraki, and T. Takeuchi, *Yakugaku Zasshi*, **75**, 172 (1955).
4) *a*) O. Gessner, "Die Gift- und Arzneipflanzen von Mitteleuropa," Carl Winter Universitätsverlag, Heidelberg, 1953, p. 294; *b*) H.A. Hoppe, "Drogenkunde," Cram de Gruyter & Co., Hamburg, 1958, p. 525.
5) *a*) B. Valdés, *Phytochemistry*, **9**, 1253 (1970); *b*) L.P. Kuptsova and A.I. Ban'kovskii, *Khim. Prir. Soedin.*, **6**, 128 (1970) [*C.A.*, **73**, 106300 (1970)].
6) *a*) D. Gröger and S. Johne, *Planta Medica*, **13**,182 (1965); *b*) S. Johne and D. Gröger, *Pharmazie*, **23**, 35 (1965).
7) P. Esposito and M.L. Scarpati, *Gazz, Chim. Ital.*, **100**, 836 (1970).
8) O. Sticher, *Phytochemistry*, **10**, 1974 (1971).
9) M.L. Scarpati, M. Guiso, and P. Esposito, *Gazz. Chim. Ital.*, **98**, 177 (1968).
10) I. Kitagawa, K. Hino, T. Nishimura, E. Iwata, and I. Yosioka, *Chem. Pharm. Bull.* (Tokyo), **19**, 2534 (1971), and the works on the other scrophulariaceous plants cited therein.
11) Preliminary report on the structure of linarioside: I. Kitagawa, T. Tani, K. Akita, and I. Yosioka, *Tetrahedron Letters*, **1972**, 419.
12) M.L. Scarpati and M. Guiso, *Gazz. Chim. Ital.*, **99**, 807 (1969).

Linaria japonica Miq. (aerial part)
(air-dried 190 g)

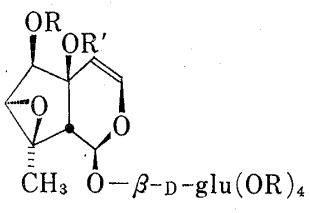
1) extd. with MeOH at reflux
2) evapd.
3) partitioned into *n*-BuOH–water mixture

- aq. layer → evapd. → residue (16 g) → charcoal-Celite column chromatog. with MeOH → polar fraction → extd. with AcOEt–EtOH (10: 3)
  - ppt. → D-mannitol (380 mg)
  - filtrate → evapd. → residue → silica gel column chromatog. with AcOEt–EtOH
    - glucoside mixture → silica gel column chromatog. with $CHCl_3$–MeOH
      - L-side I (270 mg)
      - L-side III (1.04 g)
    - L-side II (2.30 g)
- ppt. → extd. with AcOEt
  - filtrate
  - ppt. (43.5 g) → charcoal-Celite column chromatog. with MeOH → polar fraction → extd. with AcOEt–EtOH (10: 3)
    - ppt. → D-mannitol (2.69 g)
    - filtrate → evapd. → residue → silica gel column chromatog. with AcOEt–EtOH
      - glucoside mixture → silica gel column chromatog. with $CHCl_3$–MeOH
        - L-side I (620 mg)
        - L-side III (1.97 g)
      - L-side II (3.10 g)
- *n*-BuOH layer

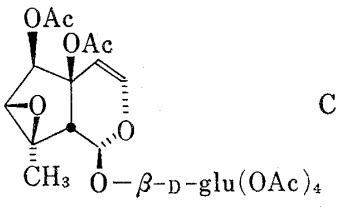
Chart 1. Isolation Procedure of L-Side I, II, and III



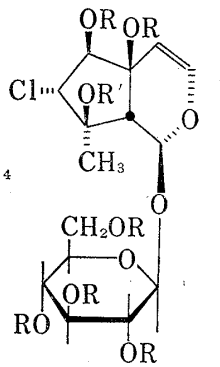
Ia : R=H antirride (=L-side I)
Ib : R=Ac



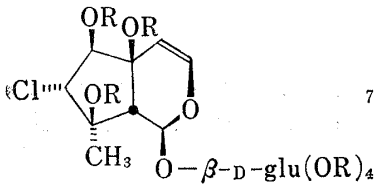
IIa : R=R'=H antirrhinoside (L-side II)
IIb : R=Ac, R'=H
IId : R=Me, R'=H
IIe : R=R'=Me
IIf : R=R'=$(CH_3)_3Si$



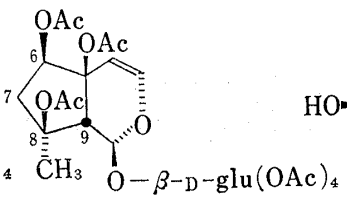
IIc



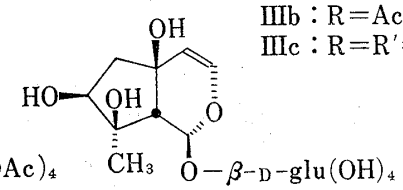
IIIa : R=R'=H linarioside (=L-side III)
IIIb : R=Ac, R'=H
IIIc : R=R'=Ac



IIId : R=$(CH_3)_3Si$



IV



V

Chart 2

(10: 3) mixture. The insoluble mass furnished D-mannitol, which was identified with an authentic sample by comparisons of melting point, infrared (IR) spectra and thin-layer chromatography (TLC) on cellulose powder.[13] Repeated column chromatography of the AcOEt–EtOH soluble portion using silica gel (*cf.* Chart 1) gave three glucosides (designated as L-side I, II and III) in about 0.6, 3.3 and 1.9% yields from the air-dried plant material. The similar treatment of subterranean part of the plant afforded two glucosides (L-side II and III) in 0.6 and 0.1% yields respectively.

L-Side I (Ia), mp 85.0—87.0°, colors pale yellow-brown with Trim and Hill reagent[14] and shows an IR absorption band at 1655 cm^{-1} characteristic of an enol-ether double bond of an iridoid glucoside.[15] Acetic anhydride in pyridine converted L-side I into a pentaacetate (Ib), mp 154.0—155.0°. The physical properties of L-side I and the pentaacetate resemble those[12] reported for antirride (Ia) and identity of L-side I with authentic antirride was established by the direct comparisons of both pentaacetates (melting point, TLC and IR).

Since some signals in the proton magnetic resonance (PMR) spectrum of antirride are unassigned in the literature,[12] we have carried out the decoupling experiment to achieve the complete assignment of Ib as described below (Table I). Upon irradiation of an olefinic region at δ 6.11, a broad doublet (J=6 Hz) at δ 4.66 collapsed to a broad singlet and a signal at δ 2.77 was deformed. When a vinylic proton at δ 4.66 was irradiated, a signal at δ 6.11 (d.d., J=6 and 2.5 Hz) was transformed into a sharp doublet (J=2.5 Hz) and the signals at δ 2.77 and 3.02 were deformed. Therefore the signals at δ 6.11, 4.66 and 2.77 are assigned to the protons at C_3, C_4 and C_5, respectively. Furthermore, irradiation of the C_9 proton signal at δ 3.02 sharpened the C_4 proton signal and also collapsed the C_1 proton doublet (δ 5.37, J=3 Hz) to a singlet. As given in Table I, further decoupling experiment has revealed a multiplet at δ 5.59 being an X part in an ABX system assignable to a proton at C_7. The assignments for antirride (Ia) shown in Table II are also based in part upon the decoupling experiment.

Table I. The Decoupling Experiment of Penta-O-acetyl-antirride (Ib)
(δ values at 100 MHz in $CDCl_3$, J and $W_{h/2}$ values in Hz)

Decoupled proton	Irradiated at						
	1.81 C_6-αH	2.14 C_6-βH	2.77 C_5-H	3.02 C_9-H	4.66 C_4-H	5.59 C_7-H	6.11 C_3-H
C_1-H (d, J=3)				singlet			
C_3-H (d.d., J=6, 2.5)			doublet (J=6)		doublet (J=2.5)		
C_4-H (br. d, J=6)			deformed	double doublet (J=6, 2)			broad singlet ($W_{h/2}$=9)
C_5-H (m)				*a*)	deformed		deformed
C_6-βH (m)			*a*)			*a*)	
C_6-αH (m)			deformed			deformed	
C_7-H (m)	deformed	deformed					
C_9-H (m)			*a*)		deformed		

abbreviations in Table I—V: br=broad, d=doublet, d.d=double doublet, m=multiplet, s=singlet, t=triplet, t like=triplet like
a) Signal patterns are unclear due to the side band overlapping.

L-Side II (IIa), the major glucoside, is a hygroscopic amorphous substance. It colors yellow-brown with Trim and Hill reagent and exhibits a distinctive band of enol-ether at

13) M. Tomoda, *Yakugaku Zasshi*, **87**, 207 (1967).
14) A.R. Trim and R. Hill, *Biochem. J.*, **50**, 310 (1952).
15) 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.

TABLE II. The PMR Data given in δ Values (J and $W_{h/2}$ values in Hz)

	C_1-H	C_3-H	C_4-H	C_5-H	C_6-H	C_7-H	C_9-H	C_{10}-H(2—3)	C_1'-H
Antirride (Ia)[a]	5.48(d) (J=3)	6.37(d.d) (J=7, 2)	5.01(br.d) (J=7)	2.8—3.3	1.8 —2.3 (2H)	3.4—4.2	2.8—3.3	5.21—5.38 (2H)	4.91(d) (J=7.8)
Ib[b]	5.37(d) (J=3)	6.11(d.d.) (J=6, 2.5)	4.66(br.d) (J=6)	2.77(m)	2.14(m) 1.81(m)	5.59(t-like)	3.02(m)	4.80—5.34 (2H)	4.80—5.34
Antirrhinoside (IIa)[c]	5.47(d) (J=6.0)	6.46(d) (J=6.0)	4.97(br.d) (J=6.0)	—	4.06(d) (J=2.0)	3.57(d) (J=2.0)	2.48(br.d) (J=6.0)	1.47(s) (3H)	4.77(d) (J=7.5)
IIb[d]	5.17(d) (J=7.8)	6.35(d) (J=6.0)	4.87—5.34	—	4.87—5.34	3.57(br.s) ($W_{h/2}$=3.5)	2.50(d) (J=7.8)	1.53(s) (3H)	4.87—5.34
IIc[d]	5.47—5.60	6.39(d) (J=6.0)	5.47—5.60	—	4.88—5.18	3.57(d) (J=2.0)	2.77(br.s) ($W_{h/2}$=4.5)	1.41(s) (3H)	4.88—5.18
IId[d]	5.03(d) (J=8.5)	6.31(d) (J=6.0)	4.91(d) (J=6.0)	—	2.7 —3.7	2.7—3.7	2.38(br.d) (J=8.5)	1.48(s) (3H)	4.56(d)[e] (J=7.0)
IIe[d]	5.21(d) (J=5.5)	6.41(d) (J=6.5)	4.89(d) (J=6.5)	—	2.7 —3.7	2.7—3.7	2.64(br.d) (J=5.5)	1.43(s) (3H)	4.60(d)[e] (J=6.5)
IIf[f]	4.85(d) (J=10.0)	6.15(d) (J=6.0)	4.86(d) (J=6.0)	—	2.7 —4.0	2.7—4.0	2.32(br.d) (J=10.0)	[i]	4.61(d) (J=5.5)
Linarioside (IIIa)[g]	5.70(br.s) ($W_{h/2}$=2)	6.36(d) (J=6.5)	5.23(d.d.) (J=6.5, 1.5)	—	3.4 —4.1	3.4—4.1	2.51(br.s) ($W_{h/2}$=4)	1.21(s) (3H)	4.58(d) (J=7)
IIIb[d]	5.54(br.s) ($W_{h/2}$=2)	6.26(d) (J=6.5)	5.65(d.d.) (J=6.5, 1.5)	—	4.6 —5.3	4.6—5.3	3.41(br.s) ($W_{h/2}$=4)	1.33(s) (3H)	4.6—5.3
IIIc[b]	6.12(br.s) ($W_{h/2}$=2.5)	6.27(d) (J=7)	5.64(d.d.) (J=7, 2)	—	4.7 —5.4	4.7—5.4	3.65(br.s) ($W_{h/2}$=4)	1.53(s) (3H)	4.7—5.4
IIId[h]	4.99(d) (J=10.5)	6.36(d) (J=6)	5.05(d) (J=6)	—	3.0 —3.9	4.46(d) (J=11)	2.60(d) (J=10.5)	1.42(s) (3H)	4.74(d) (J=6.5)
Hepta-O-acetyl-harpagide (IV)[d),27]	6.03(br.s)	6.38(d) (J=6.5)	5.52(d)[e] (J=6.5)	—	4.8 —5.5	1.8—2.7 (2H)	3.19(br.s)	1.52(s) (3H)	4.8—5.5

a) 100 MHz in D_2O with TMS as an external standard
b) 100 MHz in $CDCl_3$
c) 100 MHz in D_2O
d) 60 MHz in $CDCl_3$
e) doublet with fine structure
f) 60 MHz in CCl_4
g) 60 MHz in D_2O
h) 100 MHz in $CDCl_3$ with $CHCl_3$ as an internal standard
i) Chemical shift is unclear due to the overlapping by the methyl signals of trimethylsilyl groups.

1658 cm^{-1} as well as a broad hydroxyl absorption band at 3487 cm^{-1} in its IR spectrum (KBr). In the PMR spectrum it shows the presence of two adjacent vinylic protons at δ 6.46 (d, J=6 Hz) and 4.97 (br. d, J=6 Hz), which are usually observed in the C_9-iridoid glucosides.[16] The presence of an epoxide, indicated by a positive epoxide test with sodium thiosulfate,[17] is further supported by a narrow doublet at δ 3.57 (J=2.0 Hz) ascribable to an epoxide proton (*cf.* catalpol: δ 3.71[10] and unedoside: δ 3.6[18]).

Acetylation of L-side II with acetic anhydride and pyridine afforded a pentaacetate (IIb), mp 140.0—141.0° and a hexaacetate (IIc), mp 178.0—179.0°, the latter of which possesses no hydroxyl function as shown by its IR spectrum (Nujol). All the chemical and physical properties of L-side II and its acetates have led to an assumption that L-side II is identical with antirrhinoside (IIa).[8,9] Since the direct comparison with the authentic sample was impossible and some assignments of the PMR data were lacking in the literatures, we have performed the decoupling experiment to afford the assignment of IIa and IIb as given in Table II. The double resonance experiment of IIa (Table III) has revealed that a characteristic broad doublet at δ 4.97 is assigned to the C_4 proton which couples with the C_3 proton (J=6 Hz) and the C_9 proton (J<1 Hz). This type of long range coupling between the protons at C_4 and C_9 of IIa has already been demonstrated in hexa-O-acetyl-harpagide (IV).[19]

Table III. The Decoupling Experiment of Antirrhinoside (IIa)
(δ values at 100 MHz in D_2O, J and $W_{h/2}$ values in Hz)

Decoupled proton	Irradiated at					
	2.48 C_9-H	3.37 C_2'-H	4.06 C_6-H	4.77 C_1'-H	5.47 C_1-H	6.46 C_3-H
C_1-H (d, J=6.0)	singlet like ($W_{h/2}$=2.0)					
C_4-H (br. d, J=6.0)	sharp doublet (J=6.0)					broad singlet ($W_{h/2}$=1.5)
C_7-H (d, J=2.0)			singlet			
C_9-H (br. d, J=6.0)					broad singlet ($W_{h/2}$=2.0)	
C_1'-H (d, J=7.5)		singlet				
C_2'-H (δ 3.3—4.1)				deformed		

A distinctive spectral feature of IIc as compared with IIa and IIb (Table II) is a broad singlet at δ 2.77 ($W_{h/2}$=4.5 Hz) assignable to the C_9 proton. In case of IIa and IIb, the C_9 proton is observed as a doublet (J=6 Hz for IIa and 7.8 Hz for IIb respectively) due to the coupling with the C_1 proton. On the basis of these PMR evidences along with the Dreiding model inspection, the C_1 proton is concluded to be α-oriented and quasi-axial in IIa and IIb and quasi-equatorial in IIc, since the configuration of the glucosyloxy moiety at C_1 has previously been proved to be β.[9] As pointed out by Le Quesne,[20] the variety of splitting pattern of the C_9 proton among IIa, IIb and IIc is ascribed to the conformational difference of dihydropyrane ring which is significant in IIc as depicted in Chart 2. Since deacetylation of IIc with Amberlite IRA-400[17b] regenerated IIa, IIc is assured to retain the original carbon framework of IIa.

Furthermore, the β-linkage of glucose in IIa has been demonstrated by a doublet (J=7.5 Hz) at δ 4.77[21] which is assigned to the anomeric proton of IIa. The similar coupling

16) Reference 15), pp. 105—109.
17) *a*) W.C.J. Ross, *J. Chem. Soc.*, **1950**, 2257; *b*) J.M. Bobbitt, D.W. Siggles, S. Mahboob, H. Schmid, and W. von Philipsborn, *J. Org. Chem.*, **31**, 500 (1966).
18) T.A. Geissman, W.F. Knaack, Jr., and J.O. Knight, *Tetrahedron Letters*, **1966**, 1245.
19) I. Kitagawa, T. Nishimura, T. Takei, and I. Yosioka, *Chem. Pharm. Bull.* (Tokyo), **15**, 1254 (1967).
20) P.W. Le Quesne, *J. Chem. Soc.* (*C*), **1968**, 1661.
21) L.M. Jackman, *Fortschr. Chem. Org. Naturstoffe*, **23**, 315 (1965).

patterns are also observed in the PMR spectra (Table II) of methyl ethers (IId and IIe) and trimethylsilyl (TMS) ether (IIf). The somewhat deformed doublets[22] due to the anomeric protons are found at δ 4.56 (J=7 Hz) in the pentamethyl ether (IId) and at δ 4.60 (J=6.5 Hz) in the hexamethyl ether (IIe), which were prepared from IIa with CH_3I and Ag_2O.[23] The anomeric proton of TMS-ether (IIf), obtained by treatment of IIa with trimethylchlorosilane and hexamethyldisilazane in pyridine, is observed as a doublet (J=5.5 Hz) centered at δ 4.61. In good accord with a report by Kamerling, *et al.*,[24] the PMR study with a TMS-ether of glycoside is favorable in order to clarify the splitting mode of the anomeric proton. L-Side II is now concluded to be identical with antirrhinoside (IIa).[25]

L-Side III (IIIa, named linarioside), the secondary abundant glucoside, possesses a quite unstable and hygroscopic property. It gives a yellowish brown to dark greenish brown color with Trim and Hill reagent and a positive Beilstein test. The IR spectrum (KBr) of linarioside shows the absorption bands at 3450 cm^{-1} (broad) (hydroxyl) and at 1663 cm^{-1} (enol-ether). The enol-ether group in the glucoside is further characterized by the signals at δ 6.36 (d, J= 6.5 Hz) and 5.23 (d.d., J=6.5 & 1.5 Hz) in the PMR spectrum of linarioside. From the acid hydrolysis product, glucose was detected by paper partition chromatography (PPC) and TLC on cellulose powder. Therefore, linarioside has been presumed to be a halogenated C_9-iridoid glucoside.

On acetylation with acetic anhydride and pyridine, linarioside afforded a hexaacetate (IIIb) and a heptaacetate (IIIc). The hexaacetate (IIIb), $C_{27}H_{35}O_{16}Cl$, mp 174.0—176.5°, still shows a hydroxyl absorption band at 3450 cm^{-1} in its IR spectrum (Nujol) and a D_2O-exchangeable hydroxyl proton signal at δ 2.30 in its PMR spectrum. However, the heptaacetate (IIIc), $C_{29}H_{37}O_{17}Cl$, mp 148.5—150.0°, lacks the hydroxyl band in its IR spectrum (Nujol). It follows therefore that linarioside possesses a chlorine atom and totally seven hydroxyl functions.

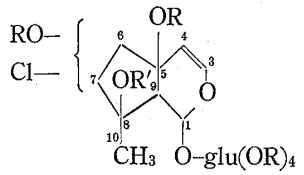
Examination of PMR spectrum of IIIc (Table II), which was confirmed by the decoupling experiment as given in Table IV, reveals the resemblance of IIIc to hepta-O-acetyl-harpagide (IV)[19,27] which possesses two tertiary acetoxyl functions at C_5 and C_8. There exist however two significant differences: IIIc lacks two methylene protons (δ 1.8—2.7 in IV) and shows a downfield shift of the C_9 proton (δ 3.65 in IIIc and δ 3.19 in IV). The absence of signals due to any methylene protons suggests that linarioside possesses some substituents at C_6 and C_7. When IIIb is further acetylated to IIIc, the methyl signal (C_{10}) is shifted lower (from δ 1.33 to 1.53), which indicates the newly acetylated hydroxyl being geminal to the methyl. The presence of free hydroxyl group in IIIb at C_8 bearing the methyl group rather than at C_5 is possibly supported by the fact that upon the reacetylation only a signal due to the nearby C_9 proton shows a downfield shift (from δ 3.41 in IIIb to δ 3.65 in IIIc) while the signal due to the C_4 proton is unaffected. As given in Table II, the acetylation of hydroxyl function at C_5 results in the downfield shift of C_4 and C_9 protons of IIIa(from δ 5.23 and 2.51 in IIIa to δ 5.65 and 3.41 in IIIb respectively). Hereupon, a partial structure [A] is advanced for linarioside and its derivatives.

A definitive proof of the structure IIIa for linarioside has been obtained by correlation with concurrent antirrhinoside as below. On treatment with hot methanolic KOH followed

22) The anomeric proton signal of methyl 2,3,4,6-tetra-O-methyl-β-D-glucopyranoside is also observed as a deformed doublet: D. Gagnaire and L. Odier, *Carbohydrate Research*, **11**, 33 (1969).
23) R. Kuhn, H. Trischmann, and I. Low, *Angew. Chem.*, **67**, 32 (1955).
24) J.P. Kamerling, M.L.A. de Bie, and J.F.G. Vligenthart, *Tetrahedron*, **28**, 3037 (1972).
25) According to the pharmacological assay (K. Yamauchi, S. Imura, R. Amano, and S. Kuwano, *Shoyakugaku Zasshi*, **22**, 147 (1968)) kindly undertaken by Dr. S. Kuwano of Kotaro Kanposeiyaku Co., Ltd., antirrhinoside shows a weak and slow laxative effect: ED_{50} 1.5—1.6 g/kg mouse (Litchfield-Wilcoxon method) (*cf.* catalpol: ED_{50} 344 mg/kg[26]; water maceration extract of rhubarb: ED_{50} 300 mg/kg).
26) I. Kitagawa, T. Nishimura, A. Furubayashi, and I. Yosioka, *Yakugaku Zasshi*, **91**, 593 (1971).
27) H. Lichti and A. von Wartburg, *Helv. Chim. Acta*, **49**, 1552 (1966).

Table IV. The Decoupling Experiment of Hepta-O-acetyl-linarioside (IIIc)
(δ values at 100 MHz in $CDCl_3$, J and $W_{h/2}$ values in Hz)

Decoupled proton	Irradiated at		
	3.65 C_9-H	3.78 C_5'-H	5.64 C_4-H
C_1-H (br. s, $W_{h/2}=2.5$)	singlet		
C_3-H (d, $J=7$)			singlet
C_4-H (d.d., $J=7$ and 2)	doublet ($J=7$)		
C_9-H (br. s, $W_{h/2}=4$)			singlet
C_6'-2H (AB part in ABX) $J_{AB}=12$, $J_{AX}=5$, $J_{BX}=2.5$		AB quartet ($J=12$)	



[A] IIIa : R=R'=H
IIIb : R=Ac, R'=H
IIIc : R=R'=Ac

Chart 3

by acetylation, linarioside afforded in a good yield an epoxy-acetate, which was identical with penta-O-acetyl-antirrhinoside (IIb) in all respects (melting point, TLC behavior, specific rotation and IR spectrum). Based on due mechanistic consideration on the formation of 7β,8β-epoxide from a precursory chlorohydrin, linarioside must possess an α-oriented chlorine atom at C_7, since the presence of a hydroxyl at C_8 has been already clarified. Consequently the structure of linarioside is now formulated as IIIa. Formation of an alternative 6β,7β-epoxide was not observed.

The location of chlorine atom at C_7 has been reinforced by the examination of PMR spectrum of IIIc in comparison with IV, the former shows a paramagnetically shifted signal due to the proton at C_9. The observation is in accordance with the work by Lack, *et al.*[28] who have found in the PMR studies of various halogenated compounds that a significant downfield shift is observed for a proton locating at a γ position to the halogen atom. Furthermore, the PMR spectrum[29] of TMS-ether (IIId) was examined. A doublet at δ 4.46 ($J=11$ Hz) is assignable to the proton at C_7 bearing the chlorine atom and the assignment has been substantiated by the decoupling experiment (Table V), in which irradiation at δ 3.40 (C_6-H) collapsed a doublet at δ 4.46 (C_7-H) to a singlet. It should be pointed out here that the proof of a proton on the carbon possessing a chlorine atom rules out a 7β-hydroxy-8α-chloro-structure for linarioside. In addition, a fairly large coupling constant ($J=11$ Hz) between C_6-αH and C_7-βH has been clarified although both protons are *trans* and standing on a five-membered ring and a smaller J value is expected (*cf.* dihydrogaliridoside (V)[30]: C_7 proton at δ 4.68 (d.d., $J=8.6$ & 11.0 Hz)). β-Linkage of the glucose moiety in linarioside (IIIa) has been demonstrated by an anomeric proton signal appearing as a doublet at δ 4.58 ($J=7$ Hz) in IIIa and at δ 4.74 ($J=6.5$ Hz) in TMS-ether (IIId). On the basis of the foregoing findings, the structure of linarioside is established as IIIa possessing a 7α-chloro-8β-hydroxyl moiety.[31]

Linarioside (IIIa) is the first example of a chlorine containing iridoid glucoside in nature. Detailed examinations of the fresh plant material by TLC have confirmed that linarioside is indeed a naturally occurring substance and is not formed as an artefact during the extraction and/or separation procedure.

28) *a*) R.E. Lack and A.B. Ridley, *J. Chem. Soc.* (*B*), **1968**, 721; *b*) R.E. Lack, J. Nemorin, and A.B. Ridley, *J. Chem. Soc.* (*B*), **1971**, 629.
29) The spectrum was measured with chloroform as an internal standard. The chemical shifts were given on the basis of chloroform proton signal at δ 7.29.
30) O. Sticher, *Helv. Chim. Acta*, **53**, 2010 (1970).
31) The biological examination of linarioside will be undertaken in near future.

TABLE V. The Decoupling Experiment of Hepta-O-trimethylsilyl-linarioside (IIId)
(δ values at 100 MHz in $CDCl_3$, J and $W_{h/2}$ values in Hz)

Decoupled proton	Irradiated at 2.60 C_9-H	3.40 C_6-H	4.46 C_7-H	4.74 C_1'-H	6.36 C_3-H
C_1-H (d, J=10.5)	singlet				
C_4-H (d, J=6)					singlet
C_6-H (δ 3.0—3.9)			deformed		
C_7-H (d, J=11)		singlet			
C_1'-H (d, J=6.5)		broad singlet[a] ($W_{h/2}$=3.5)			
C_2'-H (δ 3.3—3.5)				deformed	

a) Due to the simultaneous irradiation on C_2,-H.

It is of interest from the biogenetic view-point that a chlorohydrin (IIIa) and a corresponding epoxide (IIa) occur together in the same plant, in which the chlorohydrin is presumed to be derived from the epoxide. The analogous co-existance of chlorohydrin and epoxide in the sesquiterpene group has already been shown by Kupchan, *et al.*[32]: euparotin (epoxide) and a related chlorohydrin eupachlorin from *Eupatorium rotundifolium* (Compositae). In addition, some chlorohydrin type sesquiterpene[33] and diterpene[34] lactones have recently been reported.

Experimental[35]

Isolation of Glucosides——a) Air-dried aerial part (cut, 190 g, collected at Uchinada in Kanazawa in September 1967) was extracted with MeOH under reflux and treated as given in Chart 1. Combined extracts were partitioned into *n*-BuOH–water mixture as usual. The residue (16 g) obtained from an aqueous layer was chromatographed on 1: 1 mixture of active charcoal (120 g, Seisei-Shirasagi, Takeda Chem. Ind.) and Celite 535 (120 g, Wako Pure Chem. Ind.) developing with MeOH (totally 8 liter). The earlier eluate gave resinous substance which was treated with AcOEt–EtOH (10: 3) mixture. The soluble portion was then purified by passing through a silica gel column with AcOEt–EtOH (10: 3) mixture (totally 16.5 liter) to afford antirrhinoside (IIa, L-side II, 2.30 g) and a glucoside mixture, which was further purified by a silica gel column with $CHCl_3$ and $CHCl_3$–MeOH mixture of increasing polarity to give antirride (Ia, L-side I, 270 mg) and linarioside (IIIa, L-side III, 1.04 g). The AcOEt–EtOH (10: 3) mixture insoluble portion was repeatedly recrystallized from EtOH to give a compound (380 mg) melting at 165.5—167.0°, which was identified with authentic D-mannitol (mp 166.0—168.0°) by mixed mp (165.5—167.0°), IR (Nujol) and TLC (cellulose powder MN-300, Macherey, Nagel & Co.) with acetone–*n*-BuOH–H_2O (7: 2: 1) and AcOEt–pyridine–H_2O (40: 11: 6).

The precipitates formed during the *n*-BuOH–water partition were collected by filtration and treated with AcOEt and the insoluble portion (43.5 g) was treated similarly as given in Chart 1 to give the additional amounts of D-mannitol (2.69 g), antirride (620 mg), antirrhinoside (3.10 g) and linarioside (1.97 g).

Antirride (Ia), 0.6% yield from the air-dried plant material, was crystallized from acetone to give colorless needles of mp 85.0—87.0°, $[\alpha]_D^{23}$ −116.0° (c=0.42). *Anal.* Calcd. for $C_{15}H_{22}O_8 \cdot 3/2H_2O$: C, 53.36; H,

32) S.M. Kupchan, J.E. Kelsey, M. Maruyama, J.M. Cassady, J.C. Hemingway, and J.R. Knox, *J. Org. Chem.*, **34**, 3876 (1969).
33) A.G. González, J. Bermejo, J.L. Bretón, and J. Triana, *Tetrahedron Letters*, **1972**, 2017.
34) W.B.T. Cruse and M.H.G. James, *Chem. Commun.*, **1971**, 1278.
35) Melting points were taken on the Yanagimoto Micro-meltingpoint Apparatus (a hot-stage type) and are uncorrected. Specific rotations were measured at room temperature in dioxane with the Rex Photoelectric Polarimeter NEP-2 (1=1 cm), the IR spectra were taken with the Hitachi EPI-S2 and EPI-G3 Spectrometers, and the PMR spectra were taken with the Hitachi R-20A and Varian A-60 and HA-100 Spectrometers (tetramethylsilane (TMS) as the internal standard in $CDCl_3$ and sodium-2,2-dimethyl-2-silapentane-5-sulfonate (DSS) as the internal standard in D_2O unless otherwise indicated). Camag D-5 silica gel was employed for TLC and Merck silica gel (70—230 mesh) was used for column chromatography.

7.12. Found: C, 53.37; H, 7.29. IR ν_{max}^{KBr} cm^{-1}: 3350 (br), 2898, 2873, 1655, 1050, 996, 750. PMR data are as given in Table II.

Antirrhinoside (IIa), 3.3% yield, is a hygroscopic amorphous substance, $[\alpha]_D^{28}$ −76.3° (c=0.51). IR ν_{max}^{KBr} cm^{-1}: 3487 (br), 2900 (br), 1658, 1230. PMR data are as given in Table II and the decoupling experiment as shown in Table III.

Linarioside (IIIa), 1.9% yield, is a quite unstable and hygroscopic substance, $[\alpha]_D^{11}$ −51.0° (c=0.98). IR ν_{max}^{KBr} cm^{-1}: 3450 (br), 2960, 1663, 1238. PMR data are as given in Table II.

b) Air-dried subterranean part (cut, 980 g, collected at Uchinada in September 1970) was extracted with hot MeOH 4 times and filtered while hot. The brown resinous residue (317 g) obtained by evaporation of the solvent was treated with n-BuOH and the solution was evaporated and chromatographed on active charcoal–Celite 535 (1: 1) mixture with aid of MeOH. A glycoside fraction thus obtained was rechromatographed on silica gel with $CHCl_3$ and $CHCl_3$–MeOH mixture to afford antirrhinoside (IIa), linarioside (IIIa) and a mixture of two glucosides. The mixture was further purified by column chromatography on silica gel using n-BuOH saturated with water as the eluant to give IIa and IIIa in totally 0.6 and 0.1% yields from the air-dried plant material.

Penta-O-acetyl-antirride (Ib)——A solution of Ia (190 mg) in pyridine (4 ml)-acetic anhydride (4 ml) mixture was kept at 31° for 14 hr, treated with ice–water and extracted with ether. The ether extract (254 mg) obtained after usual work up was crystallized from MeOH to give colorless needles (211 mg), which were recrystallized from EtOH to give a pentaacetate (Ib) of mp 154.0—155.0°, $[\alpha]_D^{13}$ −158.1° (c=1.05). *Anal.* Calcd. for $C_{25}H_{32}O_{13}$: C, 55.40; H, 6.08. Found: C, 55.07; H, 5.94. IR ν_{max}^{Nujol} cm^{-1}: no hydroxyl band, 1758, 1738, 1650, 1240, 1219, 897. PMR ($CDCl_3$, 100 MHz) δ: 4.28 (1H, A part in ABX, J_{AX}=4, J_{AB}=12) (C_6'-H), 4.12 (1H, B part in ABX, J_{BX}=2, J_{AB}=12) (C_6'-H), 3.72 (1H, X part in ABX, m) (C_5'-H), 2.08, 2.06, 2.02 (3H each, s), 2.00 (6H, s) (–OCOC$\underline{H}_3$×5), and the other signals as given in Table II. Ib was used for the decoupling experiment as given in Table I. The identity of the pentaacetate (Ib) with authentic penta-O-acetyl-antirride was established by the direct comparisons (mp, mixed mp, IR ($CHCl_3$) and TLC (silica gel, benzene–AcOEt (1: 1)).

Penta-O-acetyl-antirrhinoside (IIb) and Hexa-O-acetyl-antirrhinoside (IIc)——IIa (540 mg) was acetylated with acetic anhydride (8 ml) in pyridine (8 ml) at 31° for 30 hr. After ordinary treatment, a reaction product was crystallized from absolute EtOH to give a pentaacetate (IIb) as colorless needles, mp 140.0—141.0°, $[\alpha]_D^{28}$ −85.5° (c=0.78). *Anal.* Calcd. for $C_{25}H_{32}O_{15}$: C, 52.44; H, 5.63. Found: C, 52.21; H, 5.64. IR ν_{max}^{Nujol} cm^{-1}: 3500, 1757, 1666, 1230. PMR ($CDCl_3$, 60 MHz) δ: 4.28 (2H, deformed doublet, J=3.5) (C_6'-2H), 3.39—3.65 (1H, m) (C_5'-H), 3.20 (1H, s, disappeared on D_2O treatment) (C_5-O$\underline{H}$), 2.19, 2.11, 2.02 (3H, each, s), 2.07 (6H, s) (–OCOC$\underline{H}_3$×5), and other signals as given in Table II.

The mother layer after removing IIb was evaporated and the residue was purified by preparative TLC (AcOEt–ether (30: 1)) to afford an additional amount of the pentaacetate (IIb) (totally 466 mg) and a hexaacetate (IIc) (20 mg) which was crystallized from absolute EtOH to give colorless plates, mp 178.0—179.0°, $[\alpha]_D^{19}$ −174.7° (c=0.78). *Anal.* Calcd. for $C_{27}H_{34}O_{16}$: C, 52.77; H, 5.58. Found: C, 53.04; H, 5.58. IR ν_{max}^{Nujol} cm^{-1}: no hydroxyl band, 1757 (br), 1653, 1226. PMR ($CDCl_3$, 60 MHz) δ: 2.09, 2.03, 1.97, 1.91 (3H, each, s), 2.00 (6H, s) (–OCOC$\underline{H}_3$×6), and other signals as given in Table II.

Hydrolysis of Hexa-O-acetyl-antirrhinoside (IIc) with Amberlite IRA-400——IIc (100 mg) was dissolved in MeOH (1 ml) and treated with Amberlite IRA-400 (OH form) (3 g) and water (1 ml). After occasional stirring for 30 min at 70—80°, the reaction mixture was filtered and the filtrate was purified by preparative TLC ($CHCl_3$–MeOH (3: 1)) to give an amorphous powder (32 mg), which was identified with antirrhinoside (IIa) by TLC (n-BuOH–MeOH–H_2O (14: 1: 4) and AcOEt–EtOH–H_2O (5: 2: 1)), IR (KBr) and PMR (in D_2O).

Penta-O-methyl-antirrhinoside (IId) and Hexa-O-methyl-antirrhinoside (IIe)——To a solution of IIa (300 mg) in DMF (6 ml) were added Ag_2O (1 g) and CH_3I (1 ml) and the mixture was stirred at room temperature for 48 hr, filtered and poured into water (100 ml). The total solution was extracted with $CHCl_3$ and the extract was washed, dried and evaporated. The residue was then separated by preparative TLC (ether) yielding a pentamethyl ether (IId) (81 mg) and a hexamethyl ether (IIe) (124 mg). IId was crystallized from n-hexane–MeOH to give colorless needles, mp 135.0—136.0°, $[\alpha]_D^{13}$ −78.8° (c=0.83). *Anal.* Calcd. for $C_{20}H_{32}O_{16}$: C, 55.51; H, 7.46. Found: C, 55.35; H, 7.23. IR $\nu_{max}^{CCl_4}$ cm^{-1}: 3575, 2950, 2870, 1659, 1125, 1106, 1080, 1060, 1018. PMR ($CDCl_3$, 60 MHz) δ: 3.57, 3.53, 3.34 (3H each, s), 3.48 (6H, s) (–OC$\underline{H}_3$×5), and the other signals as given in Table II. Crystallization of IIe, $[\alpha]_D^{13}$ −151.3° (c=0.46), was unsuccessful. IR $\nu_{max}^{CCl_4}$ cm^{-1}: no hydroxyl band, 2950, 2850, 1657, 1130, 1105, 1070, 1055, 1030. PMR ($CDCl_3$, 60 MHz) δ: 3.57, 3.52, 3.48, 3.46, 3.35, 3.18 (3H each, s) (–OC$\underline{H}_3$×6), and the other signals as given in Table II.

Hexa-O-trimethylsilyl-antirrhinoside (IIf)——To a solution of IIa (50 mg) in pyridine (1 ml) were added hexamethyldisilazane (1 ml) and trimethylchlorosilane (0.5 ml). The mixture was shaken vigorously for 30 sec and was allowed to stand for 30 min at room temperature, evaporated under reduced pressure and extracted with CCl_4. The residue (105 mg) obtained by evaporation of CCl_4 was purified by preparative TLC (benzene–ether (30: 1)) yielding an unstable hexatrimethylsilyl ether (IIf), $[\alpha]_D^{13}$ −8.0° (c=1.75). IR $\nu_{max}^{CCl_4}$ cm^{-1}: no hydroxyl band, 2970, 1654, 1248, 1136, 1115, 1082, 1060, 1020 (C–O–C, Si–O–C), 843, 750 (Si–CH_3). PMR data are as shown in Table II.

Acid Hydrolysis of Linarioside (IIIa) giving Glucose——A solution of IIIa (10 mg) in 2% H_2SO_4 (1 ml) and MeOH (1 ml) was refluxed for 30 min, neutralized with Dowex 44 (OH form) and filtered. The hydrolysate was examined by PPC (Toyo Roshi No. 50) developing three times with iso-PrOH–*n*-BuOH–H_2O (7: 1: 2) to confirm the presence of glucose as detected by aniline hydrogen phthalate. TLC on cellulose powder (MN-300) with acetone–*n*-BuOH–H_2O (7: 2: 1) also showed the hydrolysate to be glucose.

Hexa-O-acetyl-linarioside (IIIb) and Hepta-O-acetyl-linarioside (IIIc)——Acetylation of IIIa (130 mg) with acetic anhydride (4 ml) in pyridine (4 ml) at 31° for 19 hr followed by usual work up yielded an acetate mixture (203 mg) which was separated by preparative TLC ($CHCl_3$–AcOEt (4: 1)) to give a hexaacetate (IIIb) (118 mg) and a heptaacetate (IIIc) (61 mg). IIIb was recrystallized from MeOH to give colorless needles, mp 174.0—176.5°, $[\alpha]_D^{11}$ −105.0° (c=0.67). *Anal.* Calcd. for $C_{27}H_{35}O_{16}Cl$: C, 49.81; H, 5.42; Cl, 5.45. Found: C, 49.96; H, 5.35; Cl, 5.34. IR ν_{max}^{Nujol} cm^{-1}: 3450, 1760, 1725, 1655, 1250 (br), 1215; PMR ($CDCl_3$, 60 MHz) δ: 2.30 (1H, m, exchangeable with D_2O) (C_5-OH), 2.10, 2.08, 2.04, 2.01, 1.98, 1.95 (3H, each, s) (–OCOC$\underline{H}_3$×6), and the other signals as given in Table II. IIIc was recrystallized from MeOH to give colorless needles, mp 148.5—150.0°, $[\alpha]_D^{11}$ −107.0° (c=0.62). *Anal.* Calcd. for $C_{29}H_{37}O_{17}Cl$: C, 50.25; H, 5.38; Cl, 5.12. Found: C, 50.07; H, 5.18; Cl, 5.17. IR ν_{max}^{Nujol} cm^{-1}: no hydroxyl band, 1760, 1735, 1650, 1243, 1223; PMR ($CDCl_3$, 100 MHz) δ: 4.31 (1H, A part in ABX, J_{AX}=5, J_{AB}=12) (C_6'-H), 4.17 (1H, B part in ABX, J_{AB}=2.5, J_{AB}=12) (C_6'-H), 3.78 (1H, X part in ABX, m) (C_5'-H), 2.15, 2.11, 2.06, 2.02, 1.98 (3H each, s), 2.08 (6H ,s) (–OCOC$\underline{H}_3$×7), and the other signals as given in Table II. IIIc was used for decoupling experiment as given in Table IV.

Alkaline Treatment of Linarioside (IIIa) and Hepta-O-acetyl-linarioside (IIIc) followed by Acetylation giving Penta-O-acetyl-antirrhinoside (IIb)——To a solution of IIIa (55 mg) in MeOH (3 ml) was added an alkaline solution (3 ml) (composition: KOH (1 g), water (1 ml) and MeOH (50 ml)). The mixture was left standing at 40—50° for 20 min, neutralized with Amberlite IRC-50 and filtered. The filtrate was purified by preparative TLC ($CHCl_3$–MeOH (2: 1)) to give a white amorphous powder (33 mg), which was identified with antirrhinoside (IIa) by TLC (*n*-BuOH–MeOH–H_2O (14: 1: 4) and AcOEt–EtOH–H_2O (5: 2: 1)). Acetylation of the powder with acetic anhydride (2 ml) in pyridine (2 ml) at 31° for 24 hr followed by usual work up gave a crystalline product which was recrystallized from MeOH to yield a pentaacetate (mp 141.0—142.5°) as colorless needles being identical with penta-O-acetyl-antirrhinoside (IIb) (mp 140.0—141.0°) by mixed mp (141.0—142.0°), IR , specific rotation and TLC (benzene–ether (1: 1), $CHCl_3$–AcOEt (3: 1) and *n*-hexane–AcOEt (1: 1)).

To a solution of IIIc (51 mg) in MeOH (4 ml) was added an alkaline solution (3 ml) (composition: KOH (1 g), water (1 ml) and MeOH (19 ml)) and the mixture was treated as above to give antirrhinoside (IIa) (19 mg), which was also converted to penta-O-acetyl-antirrhinoside (IIb) (17 mg) and identified with an authentic sample in all respects (mixed mp, IR, $[\alpha]_D$ and TLC).

Hepta-O-trimethylsilyl-linarioside (IIId)——IIIa (90 mg) was trimethylsilylated with hexamethyldisilazane (1.5 ml), trimethylchlorosilane (0.7 ml) and pyridine (1.5 ml) under conditions identical with those employed in case of antirrhinoside (IIa). The product (145 mg) is a quite unstable oily substance, $[\alpha]_D^{13}$ −9.9° (c=2.31), IR $\nu_{max}^{CCl_4}$ cm^{-1}: no hydroxyl band, 2950, 2900, 1650, 1247, 1120, 1093, 1063, 1025, 843; PMR data are as given in Table II. IIId was used for the decoupling experiment as shown in Table V.

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