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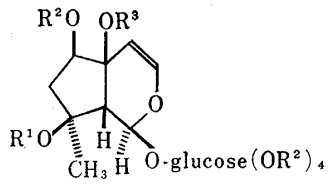
On the Iridoid Constituent isolated from the Roots of *Scrophularia buergeriana* Miq.

Although Xuanshen (Japanese name : Genjin, 玄参), the roots of *Scrophularia ningpoensis* Hemsl. (in China) or *S. buergeriana* Miq. (in Japan) (Japanese name : gomanohagusa) (*Scrophulariaceae*), has long been known as an important oriental medicament,[1] the study clarifying its chemical constituent especially in relation to its biological activity has never been provided. In order to find out the active principle, we have first attempted the chemical investigation on the glycosidic fraction obtained from Xuanshen, the dried roots of *S. buergeriana* Miq.*[1] In this communication, we wish to report our preliminary result on the iridoid (monoterpene glucoside) components, of which one of the major has now been revealed to consist of harpagoside (I)[2] (70~80%) and 8-(O-methyl-*p*-coumaroyl)-harpagide (II) (20~30%). These seem to be partly responsible for easy darkening of the plant material.



I : harpagoside
R^1=*trans*-cinnamoyl
$R^2=R^3=H$

II : R^1=O-methyl-*p*-coumaroyl
$R^2=R^3=H$

III : hexa-O-acetyl harpagide
$R^1=R^2=Ac$, $R^3=H$

IV : hepta-O-acetyl harpagide
$R^1=R^2=R^3=Ac$

Chart 1.

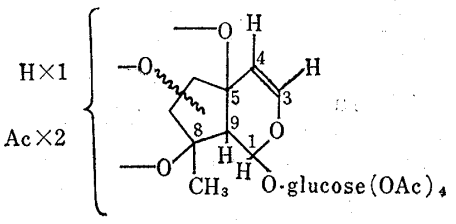
n-Butanol extraction of the methanol extracts prepared from commercial Xuanshen followed by passing through active carbon column in acetone solution, afforded an amorphous colorless glucoside (*ca.* 0.7% yield) named tentatively G-side I, which showed single spot on thin layer chromatogram (TLC) (silica gel G, Merck, Rf=0.65 using *n*-butanol-acetic acid-water; 4:1:5, upper layer; red-purple by Godin reagent[3]). G-side I is quite hygroscopic, alters to dark upon standing at room temperature for a long while and fairly unstable against either acid or alkali. For instance, in 5% methanolic hydrogen chloride at room temperature it exhibits initially red color, which changes to red-purple and finally to dark purple. In addition to the above described properties,[4] a characteristic infrared absorption band (in nujol) of G-side I at 1650 cm^{-1} attributed to an enol ether function[5] along with two bands at 3350 (hydroxyl), 1680 cm^{-1} (conjugated ester carbonyl) has led us to assume G-side I to be an iridoid analogue possessing additionally unsaturated ester linkage. The assumption has been substantiated by the following evidences

On barium hydroxide treatment G-side I yielded *trans*-cinnamic acid, O-methyl-*p*-coumaric acid and a neutral colorless product (named G-ol). The latter, although unsuccessful to get in a crystalline form, was found similar to G-side I in color reactions (with the acid and Godin reagent) and gave glucose (detected by Avicel TLC[6]) on acid

*1 Identified by Drs. S. Takahashi and T. Namba of this faculty.

1) "Chung Yao Chih" (中薬誌), Vol. I, pp. 122. Peking (北京), 1959.
2) H. Lichti, A. von Wartburg : Helv. Chim. Acta, **49**, 1552 (1966). Isolated from the roots of *Harpagophytum procumbens* DC. (*Pedaliaceae*).
3) P. Godin : Nature, **174**, 134 (1954); A. P. MacLennan, H. M. Randall, W. D. Smith : Anal. Chem., **31**, 2020 (1959).
4) H. Inouye, T. Arai : This Bulletin, **12**, 888 (1964).
5) A. J. Birch, J. Grimshaw, H. R. Juneja : J. Chem. Soc., **1961**, 5194, also literatures cited therein.
6) M. L. Wolfrom, D. L. Patin, R. M. de Lederkremer : J. Chromatog., **17**, 488 (1965).

hydrolysis. Acetylation of G-ol by acetic anhydride and pyridine afforded a hexaacetate (Ⅲ), $C_{27}H_{36}O_{16}$, m.p. 224~227°, $[\alpha]_D$ −131°(c, 1.0 in chloroform) : IR ($CHCl_3$) cm^{-1} : 3508 (hydroxyl), 1742, 1234 (acetate), 1645 (enol ether C=C) and a heptaacetate (Ⅳ), $C_{29}H_{38}O_{17}$, m.p. 185~190°, $[\alpha]_D$ −129°(c, 1.0 in chloroform) : IR (nujol) : no hydroxylic absorption band. The former yielded the latter by reacetylation. The fact that the heptaacetate lacks hydroxylic absorption band in its infrared spectrum indicates G-ol to have totally seven hydroxyl groups. Adopting an iridoid carbon skeleton to G-ol, the nuclear magnetic resonance spectrum (NMR*2) (Table I) of Ⅲ could be assigned with partial depiction (A). A decoupling experiment further clarified that a lowest field signal at τ 3.72 (doublet, J=6 c.p.s.) assignable to C_3-H was found to couple with a proton at C_4 (τ 5.01, d.-like,*3 J=6 c.p.s.). The double resonance experiment also disclosed a slightly coupling phenomenon between two protons at C_1 (τ 3.97, broad singlet) and C_9 (τ 6.99, broad singlet). A sharp singlet at τ 8.55 (three protons) could be ascribed to the methyl group attached to C_8 bearing an oxygen function. Furthermore, the absence of additional significant coupling of C_4-H to any adjacent proton except C_3-H suggests that C_5 possesses no hydrogen. These data could satisfactorily be interpreted on the basis of a partial formula (A).



(A)

Table I. (τ values in $CDCl_3$)

	G-ol hexaacetate =hexa-O-acetylharpagide (Ⅲ)	G-ol heptaacetate[a] =hepta-O-acetylharpagide (Ⅳ)
C_1-H	3.97(br. s.)	3.97(br. s.)
C_3-H	3.72(d., J=6 c.p.s.)	3.63(d., J=6.6 c.p.s.)
C_4-H	5.01(d.-like, J=6 c.p.s.)	4.50(q., J=6.6 and *ca.* 1.2 c.p.s.)
C_9-H	6.99(br. s.)	6.80(br. s.)
C_8-CH_3	8.55(s.)	8.50(s.)

a) measured at 60 Mc.
br.=broad s.=singlet d.=doublet q.=quartet.

On searching the literature, the physical constants of Ⅲ and Ⅳ have been found resembling to those of hexa-O-acetyl- and hepta-O-acetyl-harpagides, whose structures have recently been established by Lichti and Wartburg.[2] In fact, the direct comparison (mixed melting point, IR, TLC) of Ⅲ and hexa-O-acetylharpagide confirmed their identity. Accordingly, it follows that G-ol should be harpagide itself and G-side I must be its aromatic acid ester.

On acetylation with acetic anhydride and pyridine at room temperature, G-side I yielded a crystalline pentaacetate m.p. 193~195° (single spot on TLC), whose NMR spectrum demonstrates that the pentaacetate is a mixture of O-methyl-*p*-coumaroyl- and *trans*-cinnamoyl-harpagide derivatives. In addition, the NMR spectrum of G-side I taken at 85°C in D_2O indicates that G-side I is composed of harpagoside (I) (70~80%) and 8-(O-methyl-*p*-coumaroyl)-harpagide (Ⅱ) (20~30%).*4 Moreover, the complete coincidence of all of the other signals between G-side I and harpagoside[2] suggests that both aromatic acid residues constituting G-side I are connected to each C_8-OH of two harpagide molecules, and a sharp singlet at τ 8.55 in the NMR spectrum of G-side I in particular corroborates the assumption.*5

*2 The NMR spectra were taken at 100 Mc. unless stated otherwise.
*3 On account of multiplet signals due to glucose protons of Ⅲ appearing nearby, it was rather difficult to recognize a quartet from this signal, however, as described later a quartet pattern (caused by a long range coupling) of C_4-H has clearly been distinguished in Ⅳ (Table I).

Lichti and Wartburg postulated[2] a possible long range coupling between C_1-H and C_4-H with J=1.2 c.p.s. (upon harpagoside, harpagide and their derivatives), which stimulated us to perform a double resonance study. According to our decoupling experiment of Ⅳ (at 60 Mc.), contrary to their presentation, a proton coupling to C_4-H with J=*ca.* 1.2 c.p.s. was found to be C_9-H instead of C_1-H (Table I). Thus, upon irradiation at τ 6.80 (C_9-H), a quartet at τ 4.50 (J=6.6 and *ca.* 1.2 c.p.s.) was found to change into a doublet (J=6.6 c.p.s.), together with the sharpening of a broad singlet at τ 3.97 (C_1-H) as expected. This can reasonably be understood in view of a long range coupling across four σ bond.[7]

The glycoside portion other than G-side I (mostly remained in the water soluble part) has now been under study in this laboratory.

The authors wish to express their sincere thanks to Res. Lab. of Takeda Chemical Industries for the measurement and the decoupling experiment of NMR spectra, to Dr. Wartburg of Sandoz AG., Basel for providing them with an authentic sample of hexa-O-acetylharpagide, to Drs. S. Takahashi and T. Namba of this faculty for the identification of the plant material and also to Dr. Y. Sasaki of this faculty for the valuable discussion on the long range coupling.

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*4 Calculated by means of the integrated peak area due to total aromatic protons and also to two high field aromatic protons adjacent to methoxyl function in O-methyl-*p*-coumaroyl moiety appearing at τ 3.15 (multiplet), which were easily discriminated from other aromatic protons.

*5 All the attempts (chromatography, fractional recrystallization etc.) to separate G-side I or its pentaacetate into the two components were failed.

7) S. Sternhell: Rev. Pure and Appl. Chem., **14**, 15 (1964).

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Isolation of a Plant Growth Inhibitory Substance from Garden Peas (*Pisum sativum* L.) and Its Identification with (+)-Abscisin-II

The investigation on plant growth regulators has become active recently and the several active substances have been isolated, especially from the higher plants.

We have been attempting to isolate plant growth regulators from garden peas (*Pisum sativum* L.), using the rice seedlings growth test for bioassay, and found the presence of a gibberellin-like substance and a growth inhibitor. In this communication, we wish to report the isolation of the inhibitor and its identification with (+)-abscisin-Ⅱ.

The fresh garden peas (1300 kg.) were broken into fragments and extracted twice with methanol overnight at room temperature.

The extract was concentrated and the concentrate was extracted with ethyl acetate. The ethyl acetate fraction was concentrated and then extracted with 5% sodium hydron carbonate. The extract was acidified with 2*N* hydrochloric acid and extracted with ethyl acetate.