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Glycosides of the Leaves of *Symplocos* spp. (Symplocaceae)

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A new dihydrochalcone-glucoside named confusoside (3) was isolated from the leaves of *Symplocos confusa*. This compound (3) was elucidated to be the 4′-*O*-β-D-glucoside of davidigenin (4) (2′,4,4′-trihydroxyldihydrochalcone) by ^{1}H and ^{13}C nuclear magnetic resonance spectroscopy. No dihydrochalcone-glycoside could be isolated from the leaves of the following plants of Symplocaceae: *S. glauca*, *S. lucida*, *S. myrtacea*, *S. liukiuensis*, *S. prunifolia*, *S. theophrastaefolia*, *S. cochinchinensis* and *S. chinensis* var. *leucocarpa*. However, verbenalin (6) was isolated from the leaves of *S. glauca*.

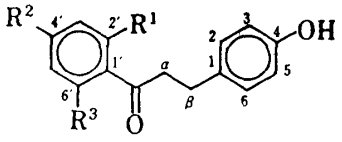
Keywords——*Symplocos* spp.; Symplocaceae; dihydrochalcone-glucoside; confusoside; davidigenin; ^{13}C NMR

As a part of our continuing studies on natural sweet glycosides, we reported the isolation of a sweet dihydrochalcone-glucoside, trilobatin (1), from the leaves of *Symplocos microcalyx* Hay. (Symplocaceae) and phloridin (2) from the leaves of *S. lancifolia* S. *et* Z. and *S. spicata* Roxb. Hort. Beng.[1] The present paper deals with a further investigation of the glycoside fraction of the leaves of plants of this family.

A crude glycoside fraction of the leaves of *S. confusa* Brand (syn.: *Corydyloblaste confusa* Ridley, Japanese name: Miyama-shirobai) was chromatographed on silica gel, affording a new glycoside (3) which was named confusoside. The presence of an infrared (IR) band ν_{max}^{Nujol} 1640 cm^{-1} (C=O), ultraviolet (UV) absorption maxima, λ_{max}^{EtOH} nm (ε): 227 (12300), 262 (13000) and 317 (5600) and ^{1}H nuclear magnetic resonance (NMR) (in CD_3OD) signals at δ 2.91 and 3.21 (2H, each, a pair of doublets, $J=6$ Hz, phenyl–CH_2–CH_2–$\overset{\downarrow}{C}$=O), as well as inspection of the ^{13}C NMR spectrum, indicated that 3 must be a dihydrochalcone derivative. On acid hydrolysis, 3 yielded glucose and an aglycone (4) which was identified as davidigenin, the aglycone of davidioside (5) (from *Vibrunum davidi*: Caprifoliaceae)[2] by comparison of the ^{13}C NMR signals with the reported data. The carbon signals due to the glycosyl moiety of 3 demonstrated the presence of one mol of a β-glucopyranosyl unit. Further, the carbon signals of 3 due to C-1—C-6 of the aglycone moiety appeared at almost the same positions as those of 4 and 5, excluding the presence of a glucosyl linkage at the 4-hydroxyl group. On the other hand, the resonance of 3 assignable to C-1′—C-6′ were found at quite different positions from those of 5; on going from 4 to 3, the signals due to C-4′ was displaced upfield by 0.9 ppm, while that of C-1′ was deshielded by 2.0 ppm. The negative optical rotation of 3 indicated that its β-glucosyl moiety belongs to the D-series. These results led us to formulate 3 as the 4′-*O*-β-D-glucopyranoside of 4. In contrast to 1 and 2, 3 does not taste sweet.

In a continuation of this study, a survey of glucoside fractions of the leaves of several other plants of this family was conducted. From the leaves of *S. glauca* (Thunb.) Koidz. (Japanese name: Mimizubai), Isoe reported the isolation of an iridoid glucoside, verbenalin (6),[3] which we also obtained from the same plant in a yield of 0.4%. From the leaves of *S. lucida* (Thunb.) S. *et* Z. (Japanese name: Kuroki), Inouye *et al.* isolated lignan-glucosides, (−)-pinoresinol β-D-glucoside and β-D-glucoside of (−)-pinoresinol monomethyl ether.[4] However, no dihydrochalcone-glucoside could be isolated from the leaves of either plant in the present work.

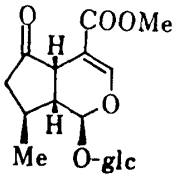
We also studied the leaf-glucoside fractions of *S. cochinchinensis* HAYATA (Japanese name: Aobanoki), *S. liukiuensis* MATSUM. (Japanese name Aobana-hainoki), *S. prunifolia* S. *et* Z. (Japanese name: Kurobai), *S. myrtacea* S. *et* Z. (Japanese name: Hainoki), *S. theophrastaefolia* S. *et* Z. (Japanese name: Kanzaburonoki) and *S. chinensis* DRUCE var. *leucocalpa* (NAKAI) OHWI (Japanese name: Sawafutagi). The yields of crude glycoside fractions (ether-insoluble and soluble in 1-butanol saturated with water) of methanolic extracts of the leaves of all of these plants were very low, and thin layer chromatography of the crude glycoside fraction of each plant did not show any significant spot of glycoside-like compound.



	R^1	R^2	R^3
1:	OH	O-glc	OH
2:	O-glc	OH	OH
3:	OH	O-glc	H
4:	OH	OH	H
5:	O-glc	OH	H

glc; β-D-glucopyranosyl

Chart 1



6

TABLE I. ^{13}C NMR Signals in CD_3OD

	3	4	5[a)]
C-1	133.0	133.7	133.8
2, 6	130.3	130.4	130.4
3, 5	116.2	116.2	116.1
4	156.5	156.3	154.4
α	30.3	31.1	30.2
β	41.0	40.9	44.7
O=C	206.0	206.3	204.7
C-1′	115.9	113.9	121.2
2′	164.9	164.4	158.7
3′	105.1	103.7	104.0
4′	165.5	166.4	162.6
5′	109.3	109.2	111.0
6′	133.2	133.2	133.3
G-1	101.2		101.3
2	74.6		73.8
3	78.0		76.7
4	71.1		70.3
5	77.8		77.1
6	62.4		61.6

δ_C ppm from TMS.
a) In D_2O.[3)]

Experimental

General Procedures——NMR spectra were taken in CD_3OD on a JEOL PFT-100 spectrometer (internal standard: tetramethylsilane (TMS)), ^1H NMR at 100 MHz and ^{13}C NMR at 25.15 MHz. Melting points were taken on a micro hot stage and are uncorrected.

Collection of Plant Materials: *S. confusa*: in Amamiohshima in December, 1979. *S. glauca*: in Miyajima, Hiroshima in September, 1980. *S. cochinchinensis*: in Tokushima in December, 1979. *S. liukiuensis*: in Amamiohshima in December, 1979. *S. myrtacea*: in Miyajima, Hiroshima in July, 1979. *S. prunifolia*: in Miyajima, Hiroshima in April, 1980. *S. chinensis* var. *leucocalpa*: in Kiyosato, Yamanashi in July, 1979.

Confusoside (3) from the Leaves of *S. confusa*——Dried and powdered leaves (101 g) were extracted with hot MeOH. A suspension of the resulting MeOH extract in H_2O was washed with Et_2O and then extracted with 1-BuOH saturated with H_2O. The BuOH-layer was concentrated to dryness *in vacuo* to give a glycoside fraction (7.3 g), which was chromatographed on silica gel (solvent: EtOAc–EtOH–H_2O (100: 8: 1, homogeneous)) followed by centrifugal chromatography; on Hitachi CLC-5, disk: 30 cm, on silica gel: Funa Gel KT 2151 (150 Å), thickness: 3 mm, at 600 rpm, in EtOAc–EtOH–H_2O (100: 8: 1) and then EtOAc saturated with H_2O, affording **3**, pale yellow prisms, mp 117—118°C from EtOAc, $[\alpha]_D^{24}$ −58.3° (c=0.72, EtOH), yield: 0.5%. *Anal.* Calcd for $C_{21}H_{24}O_9$: C, 57.53; H, 5.98. Found: C, 57.35; H, 5.70.

A solution of a few mg of **3** in 10% aqueous H_2SO_4 (2 ml) was heated in a boiling water-bath for 50 min. After cooling, the reaction mixture was neutralized with $NaHCO_3$ and extracted with EtOAc. The EtOAc layer was concentrated to dryness and the residue was recrystallized from EtOAc–MeOH, affording **4**, colorless needles, mp 154—156°C, which was identified as davidigenin by comparison of its mp and the ^{13}C NMR spectrum with the reported data.[2)] In the aqueous layer, glucose was detected in the usual way by gas liquid chromatography as the trimethylsilyl ether.

Verbenalin (6) from Leaves of *S. glauca*——The glycoside fraction (5.8 g) obtained from the dried and powdered leaves (400 g) in the same way as above was chromatographed on silica gel (solvent: C_6H_6–acetone (7: 3)) to give **6**, colorless needles, mp 185—186°C from MeOH–acetone, $[\alpha]_D^9$ −177.1° (c=1.0, H_2O). UV

λ_{max}^{EtOH} nm (ε): 238 (9800). *Anal.* Calcd for $C_{17}H_{24}O_{10}$: C, 52.57; H, 6.23. Found: C, 52.31; H, 6.14. The identification of **6** was performed by comparison of its physical constants and ^1H and ^{13}C NMR data (in CD_3OD) with those reported for verbenalin.[5]

Survey of Glycoside Fractions of Other *Symplocos* spp.——MeOH extract obtained from dried leaves was suspended in H_2O and washed with Et_2O. Then the aqueous layer was extracted with 1-BuOH saturated with H_2O. The BuOH–layer was concentrated to dryness *in vacuo* to give a crude glycoside fraction. Thin layer chromatography was carried out on silica gel plates (Kieselgel $60F_{254}$ Merck Art. 5554) with EtOAc–EtOH–H_2O (8: 2: 1, homogeneous). Detection: H_2SO_4.

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References and Notes

1) T. Tanaka, K. Yamasaki, H. Kohda and O. Tanaka, *Planta Medica*, (Suppl.), 81 (1980).
2) S.R. Jensen, B.J. Nielsen and V. Norn, *Phytochemistry*, **16**, 2036 (1977).
3) Y. Isoe, Abstracts of Papers, 24th Annual Meeting of the Chemical Society of Japan, 1977 p. 1801.
4) H. Inouye, Y. Takeda and H. Nishimura, *Yakugaku Zasshi* **93**, 44 (1973).
5) F. Bailleul, P. Delaveau, A. Rabaron, M. Plat and M. Koch, *Phytochemistry*, **16**, 723 (1977).