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Studies on Constituents of Medicinal Plants. XXII.[1] Constituents of *Schizandra nigra* Max. (4)[2]

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Androsin, β-sitosteryl glucoside and a new lignan glycoside named schizandriside were isolated from the woody part of *Schizandra nigra* Max., in addition to schizandronic acid, schizandrolic acid, schizandronol, oplodiol and (+)-catechin-7-β-D-glucopyranoside. Schizandriside (I) was shown to be (+)-isolariciresinol-2α-β-D-xylopyranoside on the basis of spectral and chemical data.

Keywords——lignan glycoside; (+)-isolariciresinol xyloside; ^{13}C-NMR; *Schizandra nigra* Max.; Magnoliaceae; schizandriside

The authors have already reported the structures of schizandronic acid,[4] schizandrolic acid,[5] schizandronol,[5] oplodiol[5,6] and (+)-catechin-7-β-D-glucopyranoside[7] isolated from the woody part of *Schizandra nigra* Max. This paper deals with the structure elucidation of a new lignan glycoside, named schizandriside, also isolated from the woody part. The ethyl acetate-soluble fraction of the methanolic extract afforded β-sitosteryl glucoside, androsin[7] and schizandriside (I), $C_{25}H_{32}O_{10}$, colorless needles of mp 225—226°, $[\alpha]^{26°}=+30.1°$ (c=0.97, EtOH). On acetylation with acetic anhydride and pyridine, I afforded a haxa-acetate (II), $C_{37}H_{44}O_{16}$, as an amorphous powder. Schizandriside (I), on hydrolysis with β-glycosidase (emulsin, Sigma) or 10% H_2SO_4, afforded D-xylose and (+)-isolariciresinol (III),[8] mp 156°, $[\alpha]^{29°}=+69.5$ (c=2.02, acetone). The value of the molecular rotation of I ($148.1°\times10^2$) minus that of (+)-isolariciresinol ($250.2°\times10^2$) is $-102.1°\times10^2$, which is nearly equal to the value ($-108°\times10^2$) of the molecular rotation of methyl β-D-xyloside, but is not equal to the calculated value ($+108°\times10^2$) of methyl β-L-xyloside, obtained by using Hudson's A (181°) and B (72°) values.[9] These findings suggest[9,10] that schizandriside (I) is (+)-isolariciresinol β-D-xyloside. In order to determine the position of attachment and the ring size of the D-xylose moiety, the ^{13}C nuclear magnetic resonance (NMR) spectrum of II was studied. It could be interpreted as shown in Table I, taking the ^{13}C NMR of (+)-isolariciresinol tetra-acetate[11] into consideration. Schizandriside hexa-acetate (II) exhibits ^{13}C NMR signals assignable to the carbons of the aglycone at the δ values shown in Table I; except for the $C_{2\alpha}$ carbon, each signal is essentially the same as the signal of the corresponding carbon of (+)-isolariciresinol tetra-acetate,[11] suggesting that

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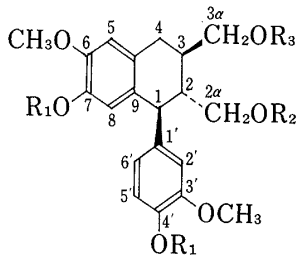
the D-xylose moiety is attached at the $C_{2a}H_2OH$ group as β-D-xyloside. Compound II also exhibits ^{13}C NMR signals of the β-D-xyloside moiety at the δ values shown in Table I, each of which is equal to that of the corresponding carbon of methyl tri-O-acetyl-β-D-xylopyranoside,[12] suggesting that the D-xylose moiety is present on the aglycone part of II as β-D-xylopyranoside. The glucosidation shift[13] of tetra-O-acetyl glucopyranoside on the α-carbon of the R–CH_2OH group was reported to be +6—+7 ppm and the acetylation shift[14] on the α-carbon of the R–CH_2OH group was reported to be +1.6 ppm. On the assumption that the glucosidation shift is similar to the xylosidation shift, the calculated δ values of the ^{13}C chemical shifts of the C_{2a} and C_{3a} carbons of (+)-isolariciresinol-2α-β-D-xylopyranoside haxa-

Table I. ^{13}C NMR Data for II (25.15 MHz, $CDCl_3$, ppm)

C	II	(+)-Isolariciresinol tetra-acetate[11]	C	II	Methyl tri-O-acetyl-β-D-xylopyranoside[12]
1	46.8(d)	47.2	1″	101.1(d)	101.0
2	44.1(d)	43.5	2″	71.1(d)	70.2
3	34.6(d)	35.2	3″	71.5(d)	71.0
4	33.1(t)	33.1	4″	68.8(d)	68.3
5	111.9(d)	111.7	5″	62.0(t)	61.3
6	149.3(s)	149.2	$OCOCH_3$	20.7(q)	—
7	137.9(s)	137.9	$OCOCH_3$	168.9(s)	—
8	123.5(d)	123.6		169.7(s)	
9	131.5(s)	131.0		169.9(s)	
10	134.3(s)	134.0		171.1(s)	
1′	138.6(s)	138.4			
2′	113.1(d)	113.1			
3′	151.3(s)	151.0			
4′	143.0(s)	142.7			
5′	122.7(d)	122.7			
6′	121.7(d)	121.5			
2α	67.1(t)	63.0			
3α	65.9(t)	66.2			
OCH_3	56.1(q)	55.9			

Letters in parentheses designate the signal multiplicity with off-resonance decoupling.
Abbreviations: s, singlet; d, doublet; t, triplet; q, quartet



I : $R_1=R_3=H$, $R_2=\beta$-D-xylopyranosyl
II : $R_1=R_3=Ac$, R_2=tri-O-acetyl-β-D-xylopyranosyl
III: $R_1=R_2=R_3=H$

Chart 1

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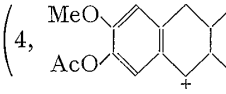
14) The ^{13}C NMR chemical shifts of Ph–CH_2OH and Ph–CH_2OAc were reported to be δ 64.5 and δ 66.1 ppm. L.F. Johnson and W.C. Jankowski, "Carbon-13-NMR Spectra," WileyInterscience, New York, N.Y., Spectrum Nos. 246 and 345.

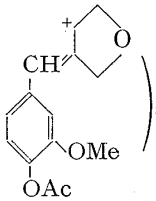
acetate could be δ 67.4—68.4 ppm (63.0−1.6+6 (or 7)) and δ 66.2 ppm, respectively, and the calculated ^{13}C chemical shifts of the $C_{2\alpha}$ and $C_{3\alpha}$ carbons of (+)-isolariciresinol-3α-β-D-xylopyranoside haxa-acetate could be δ 63.0 ppm and δ 70.6—71.6 ppm (66.2−1.6+6 (or 7)), respectively. Compound II exhibits ^{13}C NMR signals of the carbons of the $-CH_2OR$ groups at δ 67.1 ppm and at δ 65.9 ppm. Schizandriside (I), after permethylation by the Hakomori method, followed by hydrolysis with 10% H_2SO_4, afforded colorless needles of mp 146°, having a ^1H NMR spectrum almost identical with that[15] of 3α,7,4′-tri-O-methyl-(+)-isolariciresinol. Thus, the spectral and chemical data suggest schizandriside (I) to be (+)-isolariciresinol-2α-β-D-xylopyranoside, as shown in Chart 1. Two β-D-glucopyranosides and one α-L-arabinofuranoside have recently been reported as glycosides of (+)-isolariciresinol.[15]

Experimental

The following instruments were used for determining physical data. Melting point: Yanagimoto micro-melting point apparatus (a hot plate type); ultraviolet (UV) spectra (in EtOH): Hitachi 323 recording spectrometer; infrared (IR) spectra (in KBr) (cm^{-1}): Nippon Bunko IR-G spectrometer; ^{13}C NMR spectra (δ value, ppm): JNM-PS-100 high resolution instrument at 25.15 MHz; ^1H NMR: JNM-PS-100 instrument at 100 MHz and JNM-PMX 60 NMR spectrometer at 60 MHz, with $(CH_3)_4Si$ as an internal reference; mass (MS) spectra: JMS-01SG spectrometer (direct inlet, 75 eV); gas–liquid chromatography (GLC): Shimadzu 4BPF gas chromatograph; optical rotation (at 589 nm): Nippon Bunko DIP-SL automatic polarimeter. thin–layer chromatography (TLC) was carried out on glass plates coated with silica gel G (Merck), unless otherwise stated.

Isolation——The ethyl acetate-soluble fraction[4] (60 g) of the methanolic extract of the woody part of the plant was chromatographed on silica gel (900 g) with $CHCl_3$–MeOH (10: 1), and three fractions *Rf* 0.44 (1.3 g), *Rf* 0.22 (183 mg), *Rf* 0.15 (630 mg) (TLC, $CHCl_3$–MeOH=10: 1) were separated. The fraction of *Rf* 0.44 afforded β-sitosteryl glucoside, mp 295—298°, after crystallization from MeOH (mixed mp and IR). The fraction of *Rf* 0.22 was rechromatographed on silica gel (50 g) with $CHCl_3$–MeOH (10: 1) to afford colorless needles (IV) of mp 225—227°, after crystallization from MeOH. Yield: 50 mg. UV λ_{max} (nm, log ε): 225 (4.22), 268.5 (4.07), 303.5 (3.81). ^1H NMR (60 MHz, CD_3OD): 2.60 (s, 3H, $COCH_3$), 3.80 (s, 3H, OMe), 7.17 (dull s, 1H, aromatic proton), 7.56 (m, 2H, aromatic protons), 3.2—3.5 (m, 6H, sugar protons). *Anal.* Calcd. for $C_{15}H_{20}O_8$: C, 54.87; H, 6.14. Found: C, 54.73; H, 6.04. Compound IV was hydrolyzed with 10% H_2SO_4 on a water bath for 2 hr to afford D-glucose and colorless plates of mp 116—117°, after crystallization from benzene; the latter was found to be identical with acetovanillone[7] by mixed mp and IR. The UV and IR spectra of IV and androsin[7] were identical, indicating that IV is androsin. The fraction of *Rf* 0.15 was chromatographed on silica gel with $CHCl_3$–MeOH (10: 1) to afford schizandriside (I), $C_{25}H_{32}O_{10}$, as colorless needles of mp 225—226°, after crystallization from MeOH. Yield: 77 mg. UV λ_{max} (nm, log ε): 233 (shoulder, 4.18), 285.5 (3.89). IR ν_{max}^{KBr} (cm^{-1}): 3400, 2850—2700, 1605, 1515, 1450, 1445, 1435, 1370, 1275, 1090, 890—865. *Anal.* Calcd. for $C_{25}H_{32}O_{10}\cdot H_2O$: C, 58.81; H, 6.71. Found: C, 58.66; H, 6.52.

Acetylation of I——A mixture of I (80 mg) in acetic anhydride (1 ml) and pyridine (1 ml) was allowed to stand at room temperature for 20 hr and the reaction mixture was poured into ice-water to afford a powder, which was chromatographed on silica gel (25 g) with benzene–ethyl acetate (5: 1). The fraction of *Rf* 0.17 (TLC, benzene–ethyl acetate=5: 1) was crystallized from MeOH–H_2O to afford the hexa-acetate (II) of I as an amorphous powder of mp 88—89°. IR ν_{max}^{KBr} (cm^{-1}): 2900, 1760, 1740, 1730, 1605, 1510, 1465, 1450, 1420, 1370, 1245—1195, 1035, 900, 830. MS *m/e* (relative intensity): 744 (2, M^+), 486 (2, M^+−triacetylxylosyl+H), 485 (4, M^+−triacetylxylosyl), 469 (8, M^+−triacetylxyloxyl), 426 (40, *m/e* 486−CH_3COOH), 425 (63, *m/e* 426−H), 409 (12, *m/e* 426−OH), 408 (10, *m/e* 426−H_2O), 384 (22, *m/e* 426−C_2H_2O), 383 (37, *m/e* 384−H), 367 (20, *m/e* 409−C_2H_2O), 365 (49, *m/e* 409−CH_2–CH_2O), 261 (4, ),[16]

259 (32, triacetylxylosyl), 247 (3, ),[16] 199 (29, *m/e* 259−CH_3COOH), 157 (63, *m/e* 199−C_2H_2O), 139 (53, *m/e* 199−CH_3COOH), 97 (60, *m/e* 139−C_2H_2O).

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^{1}H NMR ($CDCl_3$): 1.5—2.4 (m, 2H), 2.04 (s, 6H, 2×OAc), 2.07 (s, 6H, 2×OAc), 2.21 (s, 3H, OAc), 2.32 (s, 3H, OAc), 2.86 (d, 2H, J=7), 3.0—3.4 (m, 2H), 3.75 (s, 3H, OMe), 3.78 (s, 3H, OMe), 3.9—4.4 (m, 6H), 4.7—5.0 (m, 2H), 5.08 (d, 1H, J=8), 6.31 (s, 1H, C_8–H), 6.56—6.74 (m, 3H), 6.94 (d, 1H, J=8, $C_{5'}$–H). *Anal.* Calcd. for $C_{37}H_{44}O_{16}$: C, 59.67; H, 5.96. Found: C, 59.79; H, 5.99.

Hydrolysis of I——a) I (120 mg) in 1 ml of 10% H_2SO_4 was warmed on a water bath for 12 hr and the reaction mixture was extracted with ethyl acetate. The ethyl acetate-soluble fraction was chromatographed on silica gel (40 g) with $CHCl_3$–MeOH (7: 1). The fraction of *Rf* 0.44 (TLC, $CHCl_3$–MeOH=7: 1) (80 mg) was rechromatographed on silica gel (30 g) with ethyl acetate to afford (+)-isolariciresinol (III),[8] *Rf* 0.32 (TLC, ethyl acetate) as colorless needles of mp 157° (27 mg), after crystallization from $CHCl_3$–acetone. UV λ_{max}^{MeOH} (nm, log ε): 232.5 (shoulder, 4.18), 285 (3.89). *Anal.* Calcd. for $C_{20}H_{24}O_6$: C, 66.65; H, 6.71. Found: C, 66.37; H, 6.74.

This compound (III) was methylated with diazomethane to afford colorless needles of mp 174°. $[\alpha]^{29°}$=+13.8° (c=1.30, $CHCl_3$). M^+=388. The methylate was identical with an authentic sample of (+)-isolariciresinol dimethyl ether[8] of mp 175—177° on the basis of mixed mp and IR. Compound III was acetylated with acetic anhydride and pyridine to afford the tetra-acetate[8] as colorless needles of mp 169°, after crystallization from MeOH. *Rf* 0.1 (TLC, benzene–ethyl acetate=20: 1). *Anal.* Calcd. for $C_{28}H_{32}O_{10}$: C, 63.62; H, 6.10. Found: C, 63.18; H, 6.10. The aqueous layer, obtained from the acidic hydrolysate, was passed through a column of ion-exchange resin (Amberlite IRA-400) and concentrated. The resulting product showed a spot at *Rf* 0.46 on TLC (Silica Rider 5B, impregnated with 0.02 M CH_3COONa, $CHCl_3$–MeOH=3: 2). Under the same conditions, D-xylose showed a spot at *Rf* 0.46 and L-arabinose gave one at *Rf* 0.40.

b) Emulsin (Sigma, 500 mg) was added to a solution of I (150 mg) in 80 ml of AcOH–AcONa–buffer (pH 4.8) and the mixture was incubated at 30—34° for 4 days with stirring, then extracted with ethyl acetate. The ethyl acetate-soluble fraction was chromatographed on silica gel with ethyl acetate–benzene (5: 1). The fraction of *Rf* 0.65 (TLC, ethyl acetate–benzene=5: 1) afforded III (15 mg), mp 155—156.5°, after crystallization from $CHCl_3$–acetone. The aqueous layer, obtained from the enzymatic hydrolysate of I, was evaporated *in vacuo* and the resulting product was methylated with 1% HCl–MeOH at 95°. The methylate thus obtained, after being passed through columns of ion-exchange resins (Amberlite 120B and IRA-400), was trimethylsilylated with hexamethyldisilazane and trimethylchlorosilane in pyridine. The product thus obtained showed three peaks[17] at t_R 15.1, 17 and 21 min on the gas–liquid chromatogram (1.5% SE 30 on a support of 60—80 mesh Chromosorb W, 2.5×4 mm, 155°, N_2 gas, 35 ml/min, FID). Under the same conditions, authentic methyl trimethylsilyl D-xyloside showed peaks at t_R 15.1, 17 and 21 min. The aqueous layer showed a spot of D-xylose at *Rf* 0.46 (Silica Rider 5B, impregnated with 0.02 M CH_3COONa, $CHCl_3$–MeOH=3: 2).

Permethylation[18] of I, followed by Hydrolysis——To a solution of I (200 mg) in dimethylsulfoxide (DMSO) (10 ml), a carbanion solution (2 ml), prepared from NaH (1 g) and DMSO (11 ml) under N_2 gas was added, and the mixture was stirred at room temperature for 1 hr under N_2 gas. After 1 hr, MeI (2 ml) was added and the mixture was stirred for 1 hr under N_2 gas. Water was then added to the reaction mixture, which was extracted with $CHCl_3$. The $CHCl_3$-soluble fraction was chromatographed on silica gel (60 g) with benzene–acetone (20: 1) and the fraction of *Rf* 0.12 (TLC, benzene–acetone=20: 1) was again chromatographed on silica gel (9 g) with ether–hexane (1: 1). The fraction of *Rf* 0.15 (TLC, ether–hexane=1: 1) was chromatographed on silica gel (16 g) with benzene–ether (2: 1). The fraction of *Rf* 0.32 (25 mg) (TLC, benzene–ether=2: 1) thus obtained was hydrolyzed in MeOH with 5 ml of 10% H_2SO_4 on a water bath for 7 hr and the hydrolysis product was extracted with $CHCl_3$. The $CHCl_3$–soluble fraction was chromatographed on silica gel (6 g) with benzene–ether (2: 1) then the fraction of *Rf* 0.15 (TLC, benzene–ether=2: 1) was chromatographed on silica gel (6 g) with benzene–AcOEt (1: 1). The fraction of *Rf* 0.35 (TLC, benzene–AcOEt=1: 1) thus obtained afforded colorless needles of mp 146°. ^{1}H NMR ($CDCl_3$): 1.44—1.86 (m, 1H, C_3–H), (the multiplet became td, J=10 and 2, when irradiated at δ 2.74), 2.02—2.32 (m, 1H, C_2–H), 2.74 (br. d, 2H, J=8, C_4–H_2), 3.26—4.00 (m, 4H, $C_{3\alpha}$– and $C_{2\alpha}$–H_2), 3.40 (s, 3H, $C_{3\alpha}$–OMe), 3.57, 3.80, 3.84, 3.88 (each, s 3H, OMe), 4.98 (br. d, 1H, J=11, C_1–H), 6.22 (s, 1H, C_8–H), 6.58 (s, 1H, C_5–H), 6.60 (d, J=3, 1H, $C_{2'}$–H), 6.74 (dd, J=3 and 8, 1H, $C_{6'}$–H), 6.81 (d, J=8, 1H, $C_{5'}$–H).

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