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Studies on the Constituents of *Enkianthus nudipes*. V.[1] A New Lignan Xyloside from the Stems

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Nudiposide (I), a new lignan xyloside isolated from the ericaceous plant *Enkianthus nudipes*, has been shown to be 2(*S*)-hydroxymethyl-3(*S*)-(β-D-xylosyloxymethyl)-6-hydroxy-5,7-dimethoxy-4(*R*)-(4′-hydroxy-3′,5′-dimethoxyphenyl)-tetralin.

A previous paper[1] in this series, reported the isolation of a new flavone glucoside "eudiposide" from the stems of *Enkianthus nudipes* (Honda) Ohwi (Ericaceae). The present paper describes the structure elucidation of a new lignan xyloside "nudiposide" from the same plant, and also reports the following compounds to be present: sucrose (II), ursolic acid (III), β-amyrin (IV), chrysin-7-D-glucoside (V), β-sitosterol-D-glucoside (VI), 2,6-dimethoxyquinone (VII), hyperoside (VIII), glucosyringic acid (IX) and quercetin-7-D-glucoside (X).

A methanolic extract of dried stems of the plant was treated as described in the experimental section to yield compounds (I) to (X). Nudiposide (I), obtained as colourless needles, mp 175—178°, $C_{27}H_{36}O_{12}$, $[\alpha]_D^{29.5}$ −67.1°, gave a brown colour with ferric chloride, and a blue colour with Gibbs reagent. The compound showed infrared (IR) absorption bands at 3420, 1612, 1514 and 1500 cm^{-1}, and showed a ultraviolet (UV) maximum at 280 nm. This information indicated the presence of a lignan structure of phenyltetralin type.

Hydrolysis of (I) with 5% HCl afforded compound (XI) and D-xylose. The latter was identified by paper chromatography (PC) and by comparing its specific rotation with that of authentic sample. Compound (XI), $C_{22}H_{28}O_8$, mp 171—172°, $[\alpha]_D^{31}$ −53.2°, showed IR absorption bands at 3420, 1610, 1515, 1500, 1455 cm^{-1} and a UV absorption at 279 nm. These data are identical with those of lyoniresinol (XVII), the structure and absolute configuration of which were elucidated by Yasue, *et al.*[3] The specific rotation of (XI), however, did not conform to that of lyoniresinol. Lyoniresinol obtained by Yasue, *et al.*[3] from *Lyonia ovalifolia* var. *elliptica*, and by Freudenberg, *et al.*[4] from *Alnus glutinosa*, both preparations showed positive values of specific rotation, $[\alpha]_D^{22}$ +68.4° and $[\alpha]_D^{25}$ +52° respectively. The negative value displayed by compound (XI) indicated it to be an enantiomer of (XVII). This was confirmed when the circular dichroism (CD) curves (Figure 1) of the dimethyl ether derivatives (XIV, XVIII) of (XI) and (XVII) were found to be symmetrically opposite. Finally, the position of the sugar linkage to compound (XI) (hereby named (−)-lyoniresinol) and the absolute configuration of the D-xylose moiety were determined as follows.

The hexa-O-methylether derivative (XV), $C_{33}H_{48}O_{12}$, of nudiposide, obtained by methylating (I) according to the Hakomori method, was hydrolyzed with 5% H_2SO_4 in water, to give alcohol (XII) and methanolyzed with 2N HCl in methanol, to give methyl 2,3,4-tri-O-methyl-D-xylopyranoside detected by GLC. Oxidation of (XII) with Jones reagent afforded carboxylic acid (XIII). The IR and proton magnetic resonance (PMR) spectra of both (XII) and (XIII) were found to be identical with those of authentic samples derived from lyoniside (XVI)

1) Part IV: M. Ogawa and Y. Ogihara, *Yakugaku Zasshi*, **95**, 655 (1975).
2) Location: *Tanabe-dori, Mizuhoku, Nagoya.*
3) M. Yasue and Y. Kato, *Yakugaku Zasshi*, **80**, 1013 (1960); *idem, ibid.*, **81**, 526 (1961); Y. Kato, *Chem. Pharm. Bull.* (Tokyo), **11**, 823 (1963).
4) K. Freudenberg and K. Weinges, *Tetrahedron Letters*, **1959**, 19; K. Weinges, *Chem. Ber.*, **94**, 2522 (1961).

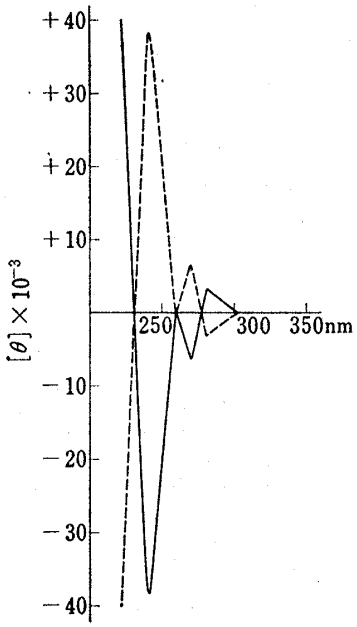


Fig. 1. CD Curves of XIV and XVIII

——: (−)-lyoniresinol dimethyl ether (XIV)
– – –: (+)-lyoniresinol dimethyl ether (XVIII)

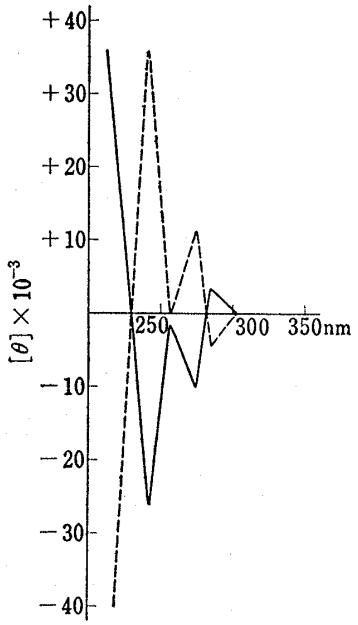
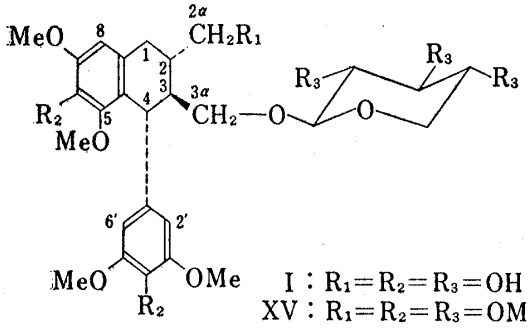
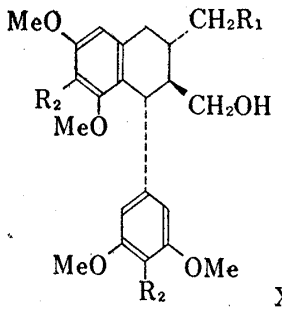


Fig. 2. CD Curves of I and XVI

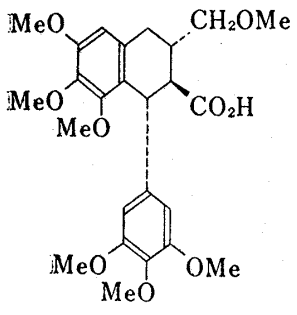
——: nudiposide (I)
– – –: lyoniside (XVI)



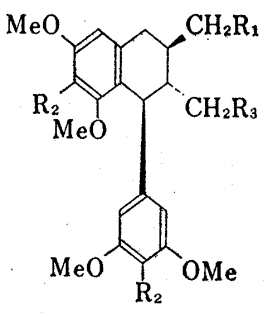
I : $R_1=R_2=R_3=OH$
XV : $R_1=R_2=R_3=OMe$



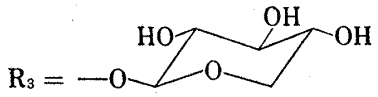
XI : $R_1=R_2=OH$
XII : $R_1=R_2=OMe$
XIV : $R_1=OH$, $R_2=OMe$



XIII



XVI : $R_1=R_2=OH$



XVII : $R_1=R_2=R_3=OH$
XVIII : $R_1=R_3=OH$, $R_2=OMe$

Chart 1

by Yasue, *et al.*,[5] except optical rotations. In addition, the CD curves of lyoniside and (I) (Figure 2) were found to be symmetrically opposite. This evidence confirmed that the sugar moiety was linked to (I) in the 3α-position. The PMR spectrum of the hexamethyl ether derivative (XV) of nudiposide, showed a doublet signal of one proton at δ 4.24 (J=7.0 Hz). This signal was attributed to the anomeric proton of D-xylose, which, on the basis of the coupling constant, indicated the β-configuration.[6]

The data outlined above established the structure of nudiposide as: 2(*S*)-hydroxymethyl-3(*S*)-(β-D-xylosyloxymethyl)-6-hydroxy-5,7-dimethoxy-4(*R*)-(4′-hydroxy-3′,5′-dimethoxyphenyl)-tetralin. Compounds (II) to (X) were identified by comparison with respective authentic samples.

Experimental

Melting points were determined with a Yanagimoto Micro Melting Point apparatus and uncorrected. IR spectra were obtained with a Jasco IR-S and a Jasco IRA-2. Optical rotations were measured with a Yanagimoto model OR-10 polarimeter, and CD data were obtained with a J-20 (Jasco). UV spectra were determined with a Hitachi Eps-3T, and PMR spectra with a JNM-MH-60 and a JNM-4H-100 spectrometers. Thin-layer chromatography (TLC) was carried out on plates coated with Kieselgel G (Merck), in order to check the purity of all products. Column chromatography was carried out on Silicic acid (Mallinckrodt: 100 mesh). Gas chromatograph used was a JEOL JGC-1100 with hydrogen flame injection detector.

Extraction and Isolation Procedure——Crushed stems of *Enkianthus nudipes* (6 kg) were air dried (20°), and extracted with MeOH (25 liters) under reflux. The filtered extract was concentrated to *ca.* 3 liters and allowed to stand at room temperature to yield sucrose (II) (18 g). Water (5 liters) was added to the filtrate and the mixture was extracted, firstly with ether and secondly with *n*-butanol. The aqueous phase remaining was concentrated to give a residue (540 g), which was subsequently extracted with MeOH under reflux. Evaporation of the ether extract yielded a residue which was dissolved in $CHCl_3$ and upon standing at room temperature gave ursolic acid (III), (180 mg) mp 275—280° (from EtOH). The remaining filtrate was concentrated to a minimum volume and chromatographed on silica gel (100 g) employing (a) cyclohexane, (b) cyclohexane: $CHCl_3$ (9: 1, 8: 2, 1: 1, 3: 7, v/v) and (c) $CHCl_3$ as eluent solvent. The $CHCl_3$ eluate gave β-amyrin (IV) (120 mg), mp 195—197° (from MeOH).

Evaporation of the *n*-butanol extract yielded a residue which was extracted three times with ethyl acetate under reflux. The combined extracts were concentrated and chromatographed on silica gel (600 g) with $CHCl_3$: MeOH (9: 1, v/v). The eluate afforded chrysin-7-D-glucoside (V) (320 mg), mp 208—210° (from EtOH). The eluate solutions from ethyl acetate extraction systems were combined, evapolated to a small volume and chromatographed on silica gel with (a) $CHCl_3$ and (b) $CHCl_3$: MeOH (95: 5 v/v). The following compounds were isolated successively from the fractions with system (b): β-sitosterol-D-glucoside (VI) (61 mg), mp 277—282° (from $CHCl_3$–MeOH); 2,6-dimethoxyquinone (VII) (20 mg), mp 250° (from acetone); hyperoside (VIII) (60 mg), mp 234—235° (from MeOH); and nudiposide (I) (800 mg), as colourless needles, mp 175—178° (from acetone–C_6H_6). *Anal.* Calcd. for $C_{27}H_{36}O_{12}$: C, 58.69; H, 6.57. Found: C, 58.41; H, 6.80. $[\alpha]_D^{29.5}$ −67.1° (c=1.43, EtOH). UV λ_{max}^{EtOH} nm (log ε): 280, (3.57), UV $\lambda_{max}^{EtOH+NaOH}$ nm: 256, 297. IR ν_{max}^{KBr} cm^{-1}: 3420, 2940, 1612, 1514, 1500.

The concentrated methanolic extract from the residue of the original aqueous phase was chromatographed on silica gel (700 g) with $CHCl_3$: MeOH (9: 1, 8: 2, 7: 3, 1: 1, v/v). The $CHCl_3$: MeOH (9: 1, v/v) eluate yielded glucosyringic acid (IX) (100 mg), mp 210—212° (from MeOH). The (8: 2) eluate was rechromatographed on silica gel with $CHCl_3$: MeOH (7: 3, v/v) to give quercetin-7-D-glucoside (X) (30 mg), mp 246—248° (from aqueous MeOH).

Acidic Hydrolysis of I——A suspension (I) (200 mg) in 5% HCl (25 ml) was refluxed under nitrogen for 2.5 hr. An ethyl acetate extract of the resultant mixture was concentrated to a minimum volume and chromatographed on silica gel with $CHCl_3$: MeOH (9: 1, v/v) to yield (−)-lyoniresinol (XI) (120 mg) as colourless needles, mp 171—172° (from H_2O). *Anal.* Calcd. for $C_{22}H_{28}O_8$: C, 62.84; H, 6.71. Found: C, 62.53; H, 7.03. $[\alpha]_D^{31}$ −53.2° (c=2.01, acetone). UV λ_{max}^{EtOH} nm (log ε): 279 (3.57). Paper chromatography of the aqueous layer showed the existence of D-xylose, which was purified by DCC[7] (solvent system, $CHCl_3$–MeOH–H_2O 35:65:40) with the ascending method having 1200 theoretical plates, (23.5 mg), $[\alpha]_D^{24}$ +25° (c=1.84. H_2O).

(−)-Lyoniresinol Dimethyl Ether (XIV)——A solution of (XI) (73 mg) in dry acetone (16 ml) with K_2CO_3 (470 mg) was treated with Me_2SO_4 (0.81 ml) and refluxed for 87 hr. The filtered solution was concentrated to give a residue which was dissolved in ethyl acetate. After being shaken with 2% NaOH, washed with wa-

5) J. Sakakibara, H. Ina, and M. Yasue, *Yakugaku Zasshi*, **94**, 1377 (1974).
6) B. Casu, M. Reggiani, G.G. Gallo, and A.V. Gervani, *Tetrahedron Letters*, **1964**, 2839; **1965**, 2253.
7) T. Tanimura, J.J. Pisano, Y. Ito, and R.L. Bowman, *Science*, **169**, 54 (1970).

ter, and dried over Na_2SO_4, the ethyl acetate layer was concentrated to a minimum volume and purified on silica gel with $CHCl_3$: MeOH (4: 1) to give (XIV) (40 mg) as colourless needles, mp 158—160° (from aqueous MeOH). *Anal.* Calcd. for $C_{24}H_{32}O_8$: C, 64.27; H, 7.19. Found: C, 64.44; H, 7.16. $[\alpha]_D^{28}$ −20.7° (c=0.58, EtOH), UV λ_{max}^{EtOH} nm (log ε): 274 (3.26). IR ν_{max}^{KBr} cm^{-1}: 3425, 2940, 2900, 2839, 1587, 1494, 1455. PMR ($CDCl_3$) δ: 1.68—1.94 (2H, m, C-2, C-3), 2.60—2.74 (2H, broad d, C-1), 3.23 (3H, s, C-5 OCH_3), 3.79 (9H, s, 3×OCH_3), 3.80 (3H, s, OCH_3), 3.86 (3H, s, OCH_3), 3.97 (1H, d, J=7.0 Hz, C-4), 6.32 (2H, s, C-2′, C-6′), 6.44 (1H, s, C-8).

Nudiposide Hexamethyl Ether (XV)——Compound (I) (350 mg) in DMSO (5 ml) was treated with a solution of NaH (700 mg) in DMSO (30 ml) and CH_3I (1.05 ml) according to the Hakomori method. The crude methylether of (I), here obtained, was purified on silica gel with C_6H_6 to afford (XV) (350 mg) as colourless powder mp 38—40° (from C_6H_6–hexane), *Anal.* Calcd. for $C_{33}H_{48}O_{12}$: C, 62.25; H, 7.60. Found: C, 61.75; H, 7.68. UV λ_{max}^{EtOH} nm (log ε): 274 (3.30). PMR ($CDCl_3$) δ: 3.32, 3.36, 3.52, 3.58, 3.64 (3H each, s, OCH_3), 3.81 (9H, s, 3×OCH_3), 3.84, 3.89 (3H each, s, OCH_3), 4.24 (1H, d, J=7.0 Hz, anomeric proton), 4.40 (1H, d, J=6.0 Hz, C-4).

(−)-Lyoniresinol Trimethyl Ether (XII)——Compound (XV) (250 mg) was refluxed in EtOH (5 ml) and 5% H_2SO_4 (15 ml) for 30 hr, and the reaction mixture was diluted with H_2O (50 ml) and extracted with ethyl acetate. The ethyl acetate layer was treated as described for (XIV) and chromatographed on silica gel with ethyl acetate to yield colourless syrup (XII). *Anal.* Calcd. for $C_{25}H_{34}O_8$: C, 64.92; H, 7.41. Found: C, 64.67; H, 7.28. UV λ_{max}^{EtOH} nm (log ε): 274 (3.28). IR $\nu_{max}^{CHCl_3}$ cm^{-1}: 3500, 3000, 2940, 2840, 1590, 1490, 1465. PMR ($CDCl_3$) δ: 1.72—1.98 (2H, m, C-2, C-3). 2.66 (2H, broad d, C-1), 3.22 3.36 (3H each, s, OCH_3), 3.74 (9H, s, 3×OCH_3), 3.77, 3.82 (3H, each s, OCH_3), 3.44 (2H, d, J=5.0 Hz, C-2 α), 3.62 (2H, broad d, C-3 α), 4.12 (1H, d, J=8.0 Hz, C-4), 6.34 (2H, s, C-2′, C-6′), 6.45 (1H, s, C-8).

Oxidation of (XII) with CrO_3 to (XIII)——Compound (XII) (120 mg) in acetone (10 ml) was oxidised with Jones reagent (CrO_3 1 g, H_2SO_4 0.9 ml, H_2O 7.2 ml) for an hour, and the reaction mixture was diluted with H_2O, then extracted with ethyl acetate. The extract was washed with water and chromatographed on silica gel with C_6H_6: MeOH (9: 1) to afford a colourless powder (XIII) (48 mg) mp 154—157° (from MeOH), *Anal.* Calcd. for $C_{25}H_{32}O_9$: C, 63.01; H, 6.77. Found: C, 63.35; H, 6.57. UV λ_{max}^{EtOH} nm (log ε): 273.5 (3.32). IR $\nu_{max}^{CHCl_3}$ cm^{-1}: 3000, 2940, 2840, 2750, 2400, 1705, 1595, 1495, 1465. PMR ($CDCl_3$) δ: 2.38—2.10 (1H, m, C-2), 3.26 (6H, s, 2×OCH_3), 3.72, 3.74, 3.75, 3.78, 3.84 (3H each, s, OCH_3), 3.38 (2H, d, J=5.0 Hz, C-2 α), 4.52 (1H, d, J=9.0 Hz, C-4), 6.27 (2H, s, C-2′, C-6′), 6.45 (1H, s, C-8).

Detection of Methylated Sugar——The sugar portion of the methanolysate was examined by GLC comparing with authentic sample. Methyl 2,3,4-tri-O-methyl-D-xylopyranoside: t_R (min) 2.3 (α), 3.2 (β). The conditions (GLC) applied in this experiment are as follows. Column, 10% DEGS on chromosorb W, 3 mm×2 m; column temperature, 170°; carrier gas, N_2, 1.0 kg/cm^2.

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