Phytochemistry, 1980, Vol. 19, pp. 2479-2481. © Pergamon Press Ltd. Printed in England. 0031-9422/80/1101-2479 $02.00/0

A DITERPENE GLYCOSIDE AND LIGNANS FROM SEED OF *THUJOPSIS DOLABRATA*

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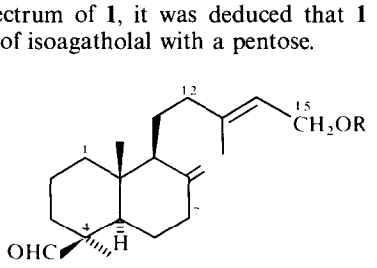
(*Received* 14 *February* 1980)

Key Word Index—*Thujopsis dolabrata* var. *hondae*; Cupressaceae; seed; new diterpene glycoside; isoagatholal-15-*O*-β-D-xylopyranoside; desoxypodophyllotoxin; β-peltatin.

The chemical investigation of the ether extract of seeds of *Thujopsis dolabrata* Sieb. et Zucc. var. *hondae* Makino led to an isolation of a new diterpene glycoside and two lignans. This paper deals with the isolation and structure of the new compound and the identification of the lignans.

Column chromatography of the ether on Si gel afforded an amorphous compound, **1**, and two crystalline compounds, **2** and **3**. Compound **1**, $C_{25}H_{40}O_6$, mp 132–140° (dec.) $[\alpha]_D^{24}$ − 35°, showed the presence of an aldehyde group (2725, 1715 cm^{-1}), an exocyclic methylene (3090, 1640, 895 cm^{-1}) and hydroxyl groups (3380, 1040 cm^{-1}) in the IR spectrum. The ^1H NMR and ^{13}C NMR spectra of **1** (Tables 1 and 2) were very similar to those of isoagatholal (**4**), $[\alpha_D]$ + 22.5°, isolated from the *n*-hexane extract, except for (a) additional signals in the ^1H NMR spectrum of **1** at δ 3.07–4.17, ascribed to protons attached to carbons bearing oxygen atoms and (b) the absence of five signals in the ^{13}C NMR spectrum of **1** in the region of δ 65.2–101.9, ascribed to carbons attached to oxygen atoms. Judging from the above facts and the *J* value of the signal due to H-1′ (*d*, *J* = 8 Hz), observed in the ^1H NMR spectrum of **1**, it was deduced that **1** was a β-glycoside of isoagatholal with a pentose.



1 R = D-xylose
4 R = H

Hydrolysis of **1** with trifluoroacetic acid gave D-xylose as a sugar, which was identified by GLC of its TMS derivative. However, the aglycone, isoagatholal, was not isolated but instead a complex mixture was obtained. Enzymatic hydrolysis of **1** with a glycosidase mixture, prepared from *Charonia lampas*, afforded isoagatholal, identified by TLC. Comparison of the ^{13}C NMR chemical shift data for the sugar moiety of **1** with those [1–3] for four stereoisomers of methyl xylosides (Table 3) established that **1** was the β-D-xylopyranoside of isoagatholal. Although the absolute configuration of the aglycone remained to be confirmed, the structure of the compound was most probably represented by formula **1**, from a consideration of the co-occurrence of **1** with (+)-isoagatholal, which will be reported in a forthcoming paper.

Compound **2**, mp 167–168° and compound **3**, mp 240–246° (dec.), were identified with desoxypodophyllotoxin and β-peltatin, respectively.

EXPERIMENTAL

Mps are uncorr. ^1H NMR and ^{13}C NMR spectra were recorded with TMS as an internal standard.

Extraction and isolation. Seed (920 g), collected in Aomori Prefecture in autumn 1977, was homogenized in *n*-hexane and extracted with *n*-hexane followed by Et_2O. The Et_2O extract (57 g) was chromatographed on charcoal (70 g) eluting with MeOH and $CHCl_3$ successively. The $CHCl_3$ eluate (25 g) was chromatographed on Si gel (Wakogel C-200, 130 g) eluting with $CHCl_3$, Et_2O and MeOH successively.

Isoagatholal-15-O-β-D-xylopyranoside (**1**). The MeOH eluate (1.7 g) was recrystallized from EtOAc to afford an amorphous substance, mp 132–140° (dec.), $[\alpha]_D^{24}$ − 35° (*c* = 1.0, $CHCl_3$). (Found: C, 68.67; H, 9.22. $C_{25}H_{40}O_6$ requires: C, 68.77; H, 9.24%).

Desoxypodophyllotoxin (**2**). The first Et_2O eluate (2.3 g) deposited white solid, recrystallized from EtOH to yield colourless prisms, mp 167–168°, $[\alpha]_D^{28}$ − 120° (*c* = 1.0, $CHCl_3$). Mass measurement, Obs.: 398.1365; Calc. for $C_{22}H_{22}O_7$, 398.1365. UV λ_{max}^{EtOH} nm: 293 (log ε 3.69). IR ν_{max}^{KBr} cm^{-1}: 1775 (γ-lactone), 1590 (aromatic C=C). ^1H NMR ($CDCl_3$): δ 6.63 and 6.48 (each 1H, *s*, arom.), 6.33 (2H, *s*, arom.), 5.90 (2H, *s*, methylenedioxy), 4.63–4.37 (2H, *m*), 3.82 (3H, *s*, OMe), 3.76 (6H, *s*, OMe), 2.97–2.65 (5H). The physical and spectral data were in good agreement with those of desoxypodophyllotoxin [4].

β-Peltatin (**3**). The second Et_2O eluate (6.4 g) was rechromatographed on Si gel eluting with *n*-hexane–EtOAc (6:4) and then *n*-hexane–EtOAc (1:1). The latter eluate gave a semi-solid substance, recrystallized from EtOH to give colourless prisms, mp 240–246° (dec.), $[\alpha]_D^{28}$ − 130° (*c* = 1.0, $CHCl_3$). Mass measurement, Obs.: 414.1290, Calc. for $C_{22}H_{22}O_8$: 414.1314. UV λ_{max}^{EtOH} nm: 272 (log ε 3.25). IR ν_{max}^{KBr} cm^{-1}: 1770 (γ-lactone), 1630 and 1595 (arom. C=C). ^1H NMR ($CDCl_3$): δ 6.35 (3H, *s*, arom.), 6.21 (1H, *s*, arom.), 5.91 (2H, *s*, methylenedioxy), 5.59 (1H, *s*, phenolic OH), 4.40–4.63 (2H, *m*), 3.84 (3H, *s*, OMe), 3.79 (6H, *s*, OMe), 3.50–2.60 (5H). The physical and spectral data were in good agreement with those of β-peltatin [5].

Acid hydrolysis of **1**. Compound **1** (127 mg) was refluxed for 2 hr with aq. 2 N TFA (15 ml) containing MeOH (3 ml). The mixture was extracted with C_6H_6 (30 ml × 3). The C_6H_6 soln was washed

Table 1. ^{1}H NMR spectral data of the compounds **1** and **4** (δ ppm from internal TMS in $CDCl_3$)

Compound	H-9	H-14	H-15	H-16	H-17	H-18	H-19	H-20	H-1′	H-2′,3′,4′,5′$_{ax}$	H-5′$_{eq}$
1	2.44 *m*	5.29 *t* (J = 6.0)	4.24 *d* (J = 6.0)	1.65 *s*	4.86 *s* 4.53 *s*	1.02 *s*	9.70 *s*	0.57 *s*	4.17 *d* (J = 8.0)	3.68–3.07	3.90 *q* (J = 12.0 and 4.0)
4	2.44 *m*	5.35 *t* (J = 6.8)	4.11 *d* (J = 6.8)	1.65 *s*	4.86 *s* 4.53 *s*	1.02 *s*	9.70 *s*	0.57 *s*	—	—	—

Coupling constants in Hz.

Table 2. ^{13}C NMR spectral data of the compounds **1** and **4** (δ ppm from internal TMS in $CDCl_3$)

Compound	C-1	C-2	C-3	C-4	C-5	C-6	C-7	C-8	C-9	C-10	C-11	C-12	C-13
1	38.5 *t*	19.4 *t*	38.5 *t*	48.7 *s*	55.0 *d*	22.1 *t*	34.5 *t*	147.3 *s*	56.1 *d*	40.1 *s*	24.1 *t*	38.5 *t*	142.0 *s*
4	38.4 *t*	19.3 *t*	38.5 *t*	48.6 *s*	55.0 *d*	22.1 *t*	34.5 *t*	147.3 *s*	56.1 *d*	40.1 *s*	24.1 *t*	38.5 *t*	139.7 *s*

	C-14	C-15	C-16	C-17	C-18	C-19	C-20	C-1′	C-2′	C-3′	C-4′	C-5′
1	119.6 *d*	65.7* *t*	16.5 *q*	107.3 *t*	24.4 *q*	205.7 *d*	13.7 *q*	101.9 *d*	72.9 *d*	75.8 *d*	69.7 *d*	65.2* *t*
4	123.5 *d*	59.2 *t*	16.3 *q*	107.3 *t*	24.4 *q*	205.5 *d*	13.6 *q*	—	—	—	—	—

* May be interchanged.

Table 3. Reported ^{13}C NMR spectral data of methyl xylosides [1] (δ ppm from internal TMS in D_2O)

	C-1	C-2	C-3	C-4	C-5	OMe
Methyl β-D-xylopyranoside	105.1	74.0	76.9	70.4	66.3	58.3
Methyl α-D-xylopyranoside	100.6	72.3	74.3	70.4	62.0	56.0
Methyl β-D-xylofuranoside	109.7	81.0	76.0	83.6	62.2	56.4
Methyl α-D-xylofuranoside	103.0	77.8	76.2	79.3	61.6	56.7

with H_2O, dried and concd *in vacuo* yielding an oil (87 mg) as an aglycone, which was found to be a complex mixture of products by means of TLC, IR and ^1H NMR. On the other hand, the combined aq. soln was evapd to dryness *in vacuo* furnishing a colourless syrupy liquid (48 mg), which was converted to the TMS derivative with TMS-PZ (Tokyo Kasei Kogyo Co.) and analysed by GLC (5% OV-17, 1.5 m × 3 mm, isothermal 130°, N_2 at 30 ml/min). Authentic lyxose, ribose, arabinose and xylose were subjected to silylation and GLC analyses in the same manner. The R_ts of the silylated sugar, derived from **1** were identical with that of silylated xylose.

Enzymatic hydrolysis of **1**. Compound **1** (150 mg) was suspended in a buffer soln (NaOAc–HOAc, pH 5.0, 15 ml) and mixed with a glycosidase mixture prepared from *Charonia lampas* (Seikagaku Kogyo Co., 100 mg). The mixture was kept at 36° for 10 days while being stirred. The reaction mixture was extracted with Et_2O. The Et_2O soln was worked up in the usual manner to give an oil (38 mg). TLC analysis developed with *n*-hexane–Et_2O (3:2) showed the presence of isoagatholal (R_f 0.23) together with several other products.

Acknowledgements—We wish to thank Mr. K. Kikutani, Aomori Regional Forest Office, for supplying us with the seed sample, and Dr. S. Sakurai, The Institute of Physical and Chemical Research, for the elementary analysis. We are also indebted to Miss N. Sawabe and Mrs. F. Hasegawa of this university for measurements of the ^1H NMR, ^{13}C NMR and mass spectra.

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