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Studies on the Constituents of the Leaves of *Pieris japonica* D. Don[1]

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Four new diterpenoids, pieristoxins H(I), I(II), J(III) and K(IV), were isolated from the leaves of *Pieris japonica* D. Don, together with the known diterpenoids, asebotoxin IV(XI) and VII(XII). The chemical structures of I and II were established to be 7-O-hydroxy and 14-O-lactoyl grayanotoxin III, respectively. III and IV were shown to be esters of pieristoxin G(VI), one of the most highly oxygenated grayanoids.

Keywords——*Pieris japonica*; Ericaceae; asebi; diterpenoids; pieristoxin H; pieristoxin I; pieristoxin J; pieristoxin K; grayanoid; toxin

Pieris japonica D. Don (Japanese name, asebi) is a well-known toxic plant. In previous work,[3] we isolated several new diterpenoids from the leaves of asebi and described the stereochemistry of pieristoxin G (P G). In the course of detailed studies on the diterpenoids, we isolated P H (I), P I (II), P J (III), P K (IV), asebotoxin IV (A IV) and A VII as minor diterpenoids from the leaves of asebi, among which four diterpenoids (P H—K) were new. The present paper describes the isolation of these diterpenoids and the elucidation of their structures.

In P H (I, $C_{20}H_{32}O_7$, mp 242—245°), the band at 855 cm^{-1} in the infrared (IR) spectrum of I was assumed to be due to epoxide. The nuclear magnetic resonance (NMR) spectrum indicated the presence of epoxide, having peaks (3.20, 4.10 ppm, each 1H, d, $J=3.0$ Hz) characteristic of epoxide-containing analogs, such as P B (V) and P G (VI), and of the -CH(OH)-CH(OH)- moiety (4.08, 4.23 ppm, 2H, ABq, $J=7.0$ Hz). I was reduced with lithium aluminium hydride ($LiAlH_4$) in dry tetrahydrofuran for 48 hr to give deacyl pieristoxin C (VII). These results suggested that P H was $2\beta,3\beta$-epoxy-7α-hydroxy grayanotoxin III.

P I (II, $C_{23}H_{38}O_8$, amorphous) was found to have an ester linkage by IR spectroscopy (1710 cm^{-1}) and was hydrolyzed with ion exchange resin IRA 400 in MeOH to give grayanotoxin III (G III, VIII). The reaction of II with CH_3CHO–$HClO_4$ gave an acetal (1.31, 4.96 ppm, $\begin{matrix}-\mathrm{O}\\-\mathrm{O}\end{matrix}\!\!>\!\mathrm{CHCH_3}$), which was hydrolyzed and subsequently ozonolyzed[4] to give 6-O-acetyl G III). Oxidation of II with chromium trioxide in pyridine gave the diketone (1735, 1724 and 1705 cm^{-1}), which was hydrolyzed with ion exchange resin IRA 400 in MeOH to afford 3,6-diketo G III (IX). These results suggested that the acyl group in II was located at the C-14 position. The NMR spectra of diketo P I and 5,6-O-ethylidene P I showed the presence of a lactoyl moiety (1.22 ppm, 3H, d, $J=7.0$ Hz and 1.22 ppm, 3H, d, $J=9.0$ Hz). In addition, after the hydrolysis of II the acid portion was methylated with CH_2N_2 ether solution. The retention time of the methyl ester in gas chromatography (GLC) was in good agreement with that of methyl lactate. Based on these results, the structure of P I was elucidated as 14-O-lactoyl G III.

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1) Location: *Omiya 5-16-1, Asahi-ku, Osaka 535, Japan.*

2) This work was presented at the 16th Annual Meeting of the Kyushu Branch of the Pharmaceutical Society of Japan, Fukuokashi, July 1979.

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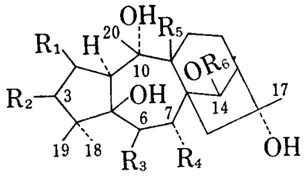

	R$_1$	R$_2$	R$_3$	R$_4$	R$_5$	R$_6$
I:	⁄O∖		OH	OH	H	H
II:	H	OH	OH	H	H	$\mathrm{CO\overset{OH}{C}HCH_3}$
III:	⁄O∖		OCOCH$_3$	OH	OH	H
IV:	⁄O∖		OCOCH$_3$ OCOC$_2$H$_5$	OCOC$_2$H$_5$ OCOCH$_3$	OH	H
V:	H	OH	OH	H	H	$\mathrm{CO\overset{OH}{C}HCH_3}$
VI:	⁄O∖		OH	OH	OH	H
VII:	H	OH	OH	OH	H	H
VIII:	H	OH	OH	H	H	H
IX:	H	=O	=O	H	H	H
X:	⁄O∖		OH	OCOCH$_3$	OH	H
XI:	H	OH	OCOC$_2$H$_5$	OH	H	H
XII:	⁄O∖		OCOCH$_3$	OH	OH	COC$_2$H$_5$

Chart 1

The IR spectrum of P J (III, $C_{22}H_{32}O_8$, mp 258—260°) exhibited bands at 1726 and 1250 cm^{-1} (ester), and its NMR spectrum showed signals due to an acetyl group at 2.12 ppm (3H, s) and the -CH(OR)CH(OH)- moiety at 4.25 and 5.82 ppm (2H, ABq, J=9.0 Hz). Hydrolysis of III with IRA 400 in MeOH gave PG (VI). These data are very similar to those for 7-O-acetyl P G (X) derived from P G by acetylation with AcOH–AcOK. In the NMR spectrum of III, the 18-methyl signal and a proton (–CHOR) in the environment –CH(OR)CH(OH)– appeared at 1.09 and 5.92 ppm, respectively, but in X they appeared at 1.52 and 5.82 ppm. Though X consumed the reagent on periodate oxidation, III did not. Thus, P J was considered to be 6-O-acetyl P G.

The IR spectrum of P K (IV, $C_{25}H_{38}O_{10}$, mp 278—281°) showed the presence of ester (1730, 1700 and 1225 cm^{-1}) and its NMR spectrum showed the presence of a propionyl group (1.12 ppm, 3H, t, J=7.0 Hz and 2.50 ppm, 2H, q, J=7.0 Hz), an acetyl group (2.14 ppm, 3H, s), and a –CH(OR)CH(OR)– moiety (5.47, 5.60 ppm, 2H, ABq, J=9.0 Hz). Alkaline hydrolysis of IV gave P G (VI). On the basis of the above evidence, P K was determined to be 6-O-acetyl-7-O-propionyl P G or 7-O-acetyl -6-O-propionyl P G.

Crystals of mp 216—218° and an amorphous material obtained from the $CHCl_3$ extract were identical with asebotoxin IV (XI) and VII (XII), respectively, on the basis of their spectral data and their general properties.

Experimental

All melting points are uncorrected. The IR absorption spectra were determined on a Shimadzu IR-400 spectrometer. The NMR spectra were measured with Hitachi R-22 and Varian EM-360 machines and chemical shifts are given in the δ(ppm) scale with tetramethylsilane as an internal standard. Mass spectra were recorded on a Japan Electron Optics Co. JMS-01SG mass spectrometer.

Isolation of Diterpenoids——The leaves of asebi collected on Mt. Rokko, Hyogo-ken, were extracted with MeOH. The subsequent extraction procedures were as described in the preceding paper.[3] Each extract was chromatographed on a column of silica gel, eluting successively with n-hexane, benzene and EtOAc containing increasing amounts of MeOH (up to 10%). The $CHCl_3$ extract contained A IV, A VII, I, II, and IV, the EtOAc extract contained A VII and II, and the BuOH extract contained III.

Acebotoxin IV(XI)——From the $CHCl_3$ extract, colorless needles, mp 216—218°, were obtained, yield 0.83×10^{-4}%. IR $\nu_{\max}^{\mathrm{KBr}}$ cm^{-1}: 3550, 3450 (OH), 1735 (ester). NMR (C_5D_5N): 1.01, 1.52, 1.55, 1.79 (each

3H, s), 1.20 (3H, t, $J=7.0$ Hz, $CH_2C\underline{H}_3$), 2.50 (2H, q, $J=7.0$ Hz, $CO\underline{C}\underline{H}_2CH_3$), 3.28 (1H, d, $J=3.0$ Hz), 3.84 (1H, d, $J=3.0$ Hz), 4.16, 5.87 (2H, ABq, $J=9.0$ Hz). The IR and NMR data were identical with those for A IV.

Asebotoxin VII(XII)——From the $CHCl_3$ and EtOAc extract, a mixture of XII and II was obtained as a white powder. The mixture was chromatographed on a column of alumina and subjected to prep-TLC, eluting with *n*-hexane–benzene, to give finally XII as a crystalline powder, yield 2.1×10^{-3}%. IR ν_{max}^{KBr} cm^{-1}: 3370 (OH), 1715 (ester). NMR ($CDCl_3$): 1.16, 1.23, 1.28, 1.57 (each 3H, s), 1.16 (3H, t, $J=7.0$ Hz, $OCH_2C\underline{H}_3$) 2.40 (2H, q, $J=7.0$ Hz), 3.20, 3.74 (2H, d, $J=3.0$ Hz), 5.27, 3.56 (2H, ABq, $J=10.0$ Hz), 5.96 (1H, s).

Pieristoxin H(I)——From the $CHCl_3$ extract, colorless needles were obtained, mp 242—245°, yield 0.5×10^{-4}%. *Anal.* Calcd for $C_{20}H_{32}O_7$: C, 62.48; H, 8.39. Found: C, 62.18; H, 8.32. High resolution mass spectrum Calcd for $C_{20}H_{28}O_5$ (M^+-2H_2O): 348.19366. Found: 348.19672. IR ν_{max}^{KBr} cm^{-1}: 3520, 3480, 3080 (OH), 855 (epoxide). NMR (C_5D_5N): 1.22, 1.50, 1.56, 1.80 (each 3H, s), 2.83 (1H, s), 3.20, 4.10 (2H, d, $J=3.0$ Hz, epoxide), 4.28, 4.52 (2H, ABq, $J=9.0$ Hz, –CH(OH)CH(OH)–), 4.65 (1H, s, $C\underline{H}OH$). MS: *m/e* 366 (M^+-18), 348 ($M^+-18\times2$), 330 ($M^+-18\times3$), 312 ($M^+-18\times4$). I (5 mg) was reduced with $LiAlH_4$ in dry tetrahydrofuran for 48 hr to give colorless needles, mp 255—257°. The IR and TLC of this material were identical with those of deacyl P C(VII).

Pieristoxin I(II)——A mixture of II and XII was obtained as a white powder from the $CHCl_3$ extract. As mentioned above, II was finally obtained as a crystalline powder, yield 2.0×10^{-3}%. *Anal.* Calcd for $C_{23}H_{38}O_8$: C, 62.42; H, 8.66. Found: C, 62.08; H, 8.82. IR ν_{max}^{KBr} cm^{-1}: 3320 (OH), 1710 (C=O). II (10 mg) was treated with ion exchange resin IRA 400 (OH form) in MeOH to give colorless needles. This material was identical with G III(VIII) on TLC, IR and NMR. II (30 mg) was treated with $CH_3CHO-HClO_4$ in refrigerator overnight and the usual work-up provided a crystalline powder (20 mg). IR ν_{max}^{KBr} cm^{-1}: 3360 (OH), 1730 (C=O). NMR ($CDCl_3$): 0.78, 1.04, 1.34, 1.42 (each 3H, s), 1.22 (3H, d, $J=9.0$ Hz, $-CH(OH)C\underline{H}_3$), 1.31 (3H, d, $J=4.8$ Hz, $CHC\underline{H}_3$), 4.96 (1H, q, $J=4.8$ Hz, $\begin{smallmatrix}-O\\-O\end{smallmatrix}\!>C\underline{H}CH_3$), 5.76 (1H, s, $C\underline{H}OR$). Hydrolysis and subsequent ozonolysis of the acetal gave 6-O-acetyl G III). IR ν_{max}^{KBr} cm^{-1}: 3420 (OH), 1730, 1250 (ester). NMR (C_5D_5N): 1.33, 1.45, 1.63, 1.80 (each 3H, s), 2.08 (3H, s, $COCH_3$). II (5 mg) was oxidized with chromium trioxide in pyridine to give diketo P I, which was hydrolyzed with IRA 400 in MeOH to give colorless needles, mp 288—290°. This material was identical with 3,6-diketo G III(IX) derived from G I (14-O-acetyl G III). IR ν_{max}^{KBr} cm^{-1}: 3500, 3280, 2980 (OH), 1720, 1700 (C=O). After removal of G III from the hydrolyzed mixture of II, the residue was methylated with CH_2N_2 ether solution. The products were examined by GLC (column, 25% PEG 20M on Shimalite NAM; 3 mm × 2 m; column temp., 180°; carrier gas, N_2; t_R (min), 4.3).

Pieristoxin J(III)——The BuOH extract was chromatographed on a column of active carbon–celite (1:1), eluting with 30—40% MeOH. The resulting material was further chromatographed on silica gel, eluting with EtOAc. Fractions 6—7 provided colorless needles, mp 258—260°, yield 1.2×10^{-4}%. *Anal.* Calcd for $C_{22}H_{34}O_9$: C, 59.71; H, 7.75. Found: C, 59.83; H, 7.74. High resolution mass spectrum Calcd for $C_{22}H_{32}O_8$ (M^+-H_2O): 424.2097. Found: 424.206 ± 0.005. IR ν_{max}^{KBr} cm^{-1}: 3500 (OH), 1726, 1250 (ester). NMR (C_5D_5N): 1.09, 1.54, 1.56, 2.10 (each 3H, s), 2.15 (3H, s, $COCH_3$), 3.30, 4.08 (2H, d, $J=3.0$ Hz, epoxide), 4.52, 5.82 (2H, ABq, $J=9.0$ Hz, –CH(OR)CH(OH)–). III was hydrolyzed with IRA 400 in MeOH to give P G(VI), which was identical with an authentic sample as judged by IR and NMR spectroscopy and TLC.

Pieristoxin K(IV)——The $CHCl_3$ extract yielded colorless needles, mp 278—281°, yield 0.5×10^{-4}%. *Anal.* Calcd for $C_{25}H_{38}O_{10}$: C, 60.22; H, 7.68. Found: C, 60.06; H, 7.76. IR ν_{max}^{KBr} cm^{-1}: 3440 (OH), 1730, 1700, 1220 (ester), 860 (epoxide). NMR (C_5D_5N): 1.10, 1.48, 1.48, 2.03 (each 3H, s), 2.14 (3H, s, $COCH_3$), 1.12 (3H, t, $J=7.0$ Hz, $CH_2C\underline{H}_3$), 3.20, 4.12 (2H, d, $J=3.0$ Hz, epoxide), 5.47, 5.63 (2H, ABq, $J=9.0$ Hz, –CH(OR)CH(OR)–). Hydrolysis of IV with 5% methanolic KOH gave P G(VI), which was identical with an authentic sample (mixed fusion, TLC and IR spectra).