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DITERPENE GLYCOSIDES FROM *PIERIS JAPONICA*

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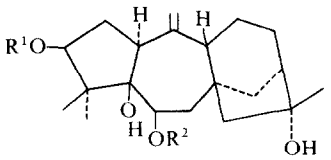
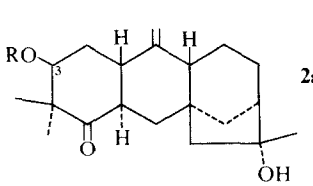
Pieris japonica (Ericaceae), a poisonous shrub widely distributed in Japan, contains a number of toxic diterpenoids [1]. In a previous paper we reported the isolation and structure determination of a new diterpene, asebotoxin-X, and a new diterpene glycoside, pieroside A [2]. In this paper, we wish to present the isolation and identification of grayanoside B(**1a**) and a new glycoside, pieroside B(**2a**) from the *n*-butanol-soluble fraction of a methanol extract of *P. japonica*.

The identity of grayanoside B(**1a**) was established as follows. Enzymatic hydrolysis of **1a** afforded a genuine aglycone, which was identified as grayanotoxin-XVIII (**3**), previously isolated by us from *Leucothoe grayana* [3]. Acetylation of **1a** gave an acetate (**1b**), which was identical with pentaacetylgrayanoside B in all respects [3].

Enzymatic hydrolysis of pieroside B(**2a**) by naringinase afforded a genuine aglycone (**4a**), $C_{20}H_{30}O_3$, whose IR spectrum showed a carbonyl absorption at 1705 cm^{-1}. Acetylation of **4a** gave a monoacetate (**4b**), $C_{22}H_{32}O_4$, whose ^{13}C NMR and ^1H NMR spectra indicated the presence of the following groups: three tertiary methyls, one secondary acetoxyl group, six methylenes, four methines, two quaternary carbons, one tertiary hydroxyl group, one exo methylene, and one carbonyl carbon. From these data, **4a** was presumed to be leucothol A and was identical with the specimen synthesized from the aglycone of grayanoside C [4]. Acetylation of **2a** gave a tetraacetate (**2b**), $C_{34}H_{48}O_{12}$, whose ^{13}C NMR spectrum (d_5-pyridine) suggested the presence of a glucose moiety [δ 62.6 (*t*), 69.4, 72.2, 72.2, 73.3, 102.2 (*d*)] [5]. Comparison





4a R = H
4b R = Ac

of the ^{13}C NMR spectrum of **4b** with that of **2b** indicated that only the signal of C-3 [δ 79.4 (*d*)] shifted downfield to δ 87.8. In the ^1H NMR spectrum the anomeric proton of **2a** and **2b** was observed at δ 4.85, *d*, $J = 7$ Hz and 4.55, *d*, $J = 7$ Hz, respectively. Consequently the structure of **2a** was elucidated as 3-*O*-(β-D-glucopyranosyl)-leucothol A. This is the first example of a leucothane glycoside found in nature and is of great biogenetic interest.

EXPERIMENTAL

Mps were uncorr. ^1H NMR spectra were measured at 100 MHz. ^{13}C NMR spectra were measured at 25 MHz. The δ values are expressed in ppm downfield from TMS as an internal standard. MS (20 eV) were taken with a direct inlet. Plants were collected at Gifu-Prefecture, Japan, in March.

Extraction and isolation of grayanoside B(**1a**) *and pieroside B*(**1b**). Dried leaves (18.2 kg) were extracted with hot MeOH. The MeOH extracts were diluted with H_2O and extracted with $CHCl_3$, EtOAc and *n*-BuOH, successively. Part of the *n*-BuOH extract was chromatographed on a Si gel column. The $CHCl_3$–MeOH (9:1) eluate was applied to prep. TLC. Repeated chromatography by Si gel and silanized Si gel [eluants: $CHCl_3$–MeOH (17:3) and MeOH–H_2O (1:1), respectively] gave pure **1a** and **2a**. Enzymatic hydrolysis and acetylation of the glycosides were carried out as described before [2–4].

Acetate of **1a**(**1b**). Recrystallization from *i*-PrOH gave colourless needles, mp 213–214°, identical in all respects to the authentic pentaacetylgrayanoside B.

Aglycone of **1a**(**3**) (= *grayanotoxin*-XVIII). Recrystallization from EtOAc gave colourless crystals, mp 166–167°, identical in mmp and IR with the authentic grayanotoxin-XVIII.

Pieroside B(**2a**). Amorphous powder $[\alpha]_D^{23.5}$ −11.8° (MeOH, $c = 2.0$). ^1H NMR (d_5-pyridine): δ 1.15 (3 H, *s*), 1.52 (6 H, *s*), 3.8–4.6 (many protons), 4.85 (1 H, *d*, $J = 7$ Hz), 4.81, 5.00 (each 1 H, *s*).

Acetate of **2a**(**2b**). Recrystallization from MeOH gave colourless needles, mp 267° (decomp.). (Found: C, 63.24; H, 7.43. Calc. for $C_{34}H_{48}O_{12}$: C, 62.95; H, 7.46%). IR ν_{max}^{KBr} cm^{-1}: 3485, 1750, 1702, 1640. ^1H NMR ($CDCl_3$): δ 1.06, 1.19, 1.39 (each 3 H, *s*), 1.99, 2.01, 2.04, 2.06 (each 3 H, *s*), 4.14 (2 H, *m*), 4.55 (1 H, *d*, $J = 7$ Hz), 4.85–5.11 (5 H, *m*). ^{13}C NMR (d_5-pyridine): δ 21.2, 24.4, 25.2 (*q*, –Me), 20.4 × 2, 20.6 × 2, (*q*, –COMe), 21.6, 24.9, 30.5, 36.2, 39.1, 55.5 (*t*, $-CH_2-$), 43.2, 48.3, 49.4, 49.9 (*d*, –CH–), 46.0, 49.6 (*s*, –C–), 78.6 (*s*, –C–OH), 87.8 (*d*, –CH–OH), 105.1 (*t*, $>C=CH_2$), 152.0 (*s*, $>C=CH_2$), 213.0 (*s*, $>C=O$), 62.6 (*t*, C′–6), 69.4 (*d*, C′–4), 72.2 × 2 (*d*, C′–2 and C′–5), 73.3 (*d*, C′–3), 102.2 (*d*, C′–1), 169.3, 169.6, 170.1, 170.3 (*s*, –COMe).

Aglycone of **2a**(**4a**) (= *leucothol A*). Recrystallization from EtOAc gave colourless plate crystals, mp 245°. (Found: C, 75.37; H, 9.74. Calc. for $C_{20}H_{30}O_3$: C, 75.43; H, 9.50%). IR ν_{max}^{KBr} cm^{-1}: 3500, 1705, 1642. Identical in all respects to the authentic leucothol A.

Acetate of **4a**(**4b**). ^1H NMR ($CDCl_3$): δ 1.03, 1.25, 1.38 (each 3 H, *s*), 1.99 (3 H, *s*), 4.88, 4.93 (each 1 H, *d*, $J = 1$ Hz), 5.16 (1 H, *m*). ^{13}C NMR (d_5-pyridine): δ 20.7, 21.0, 24.4, 24.8 (*q*, –Me), 21.6, 24.8, 29.1, 36.0, 38.9, 55.3 (*t*, $-CH_2-$), 43.4, 48.0, 49.3, 49.8 (*d*, –CH–), 45.8, 48.1 (*s*, –C–), 78.5 (*s*, –C–OH), 79.4 (*d*, –CH–OR), 105.9 (*t*, $>C=CH_2$), 150.8 (*s*, $>C=CH_2$), 169.8 (*s*, –COMe), 212.7 (*s*, $>C=O$). MS *m/e* 360 (M^+, $C_{22}H_{32}O_4$), 342 ($M^+ - 18$), 300 ($M^+ -$ HOAc).

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