Communications to the Editor

Chem. Pharm. Bull.
33(10)4637—4639(1985)

OCCURRENCE OF PIEROSIDE C, A GRAYANOTOX-9-ENE DERIVATIVE, IN *PIERIS JAPONICA*

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A new diterpene glycoside, pieroside C, has been isolated together with grayanotoxin-VII and -XVIII from *Pieris japonica* D. DON(Ericaceae). Its structure was determined by chemical and spectroscopic means to be 3-O-(β-D-glucopyranosyl)-3,5,6,16-tetrahydroxygrayanotox-9-ene. This is the first report of a grayanotox-9-ene derivative occurring in nature.

KEYWORDS——*Pieris japonica*; Ericaceae; diterpene glucoside; grayanotox-9-ene; pieroside C; grayanotoxin-VII; grayanotoxin-XVIII

In previous papers we reported the isolation and characterization of two diterpene glycosides, pieroside A and B, from the leaves of *Pieris japonica* D. DON(Ericaceae).1,2) This paper describes the structure of another glycoside isolated from this plant, pieorside C, along with two known diterpenes.

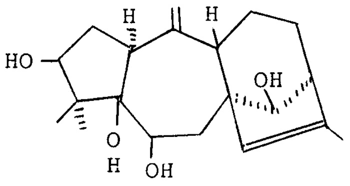
Grayanotoxin-VII(**1**),3) mp 182.5-183.5°C, and grayanotoxin-XVIII(**2**),4) mp 167-169°C, were isolated from the chloroform-soluble fraction of the methanol extract, and identified by spectroscopic analysis and/or comparison with the authentic sample.

Pieroside C(**3**)5) was obtained from the n-butanol-soluble fraction of the methanol extract in the course of the isolation of pieroside A and B after repeated chromatography.2) Its ^{13}C-NMR spectrum indicated the presence of twenty-six carbons, six of which were the signals characteristic of a glucose moiety[δ 104.9(d, C-1′), 77.8 and 77.7(each d, C-3′ and C-5′), 75.1(d, C-2′), 71.7(d,C-4′), and 62.7(t, C-6′)].4) Compound **3** gave a pentaacetate(**3b**)6) on acetylation. Enzymatic hydrolysis of **3** by naringinase gave a genuine aglycone(**3a**),7) $C_{20}H_{32}O_4$, whose ^{1}H-NMR and ^{13}C-NMR spectra showed the presence of the following groups: four tertiary methyls, one of which is on a double bond; six methylenes, four methines, two of which are adjacent to an oxygen; four quaternary carbons, two of which are adjacent to an oxygen; and two quaternary olefinic carbons. From the above data and biogenetic considerations, **3a** should have a grayanotoxane(A-nor-B-homo-ent-kaurane) skeleton, and no hydroxyl groups on the C-14 because of the absence of any singlets around δ 4.50 in the ^{1}H-NMR spectrum.8) Comparing the ^{1}H-NMR spectra of **2** and **3a**, the H-1 signal of **2**[δ 3.13(t, J=9 Hz)] shifted downfield to δ 3.50(t, J=10 Hz) in the spectrum of **3a**. From this the structure of **3a** was deduced to be 3,5,6,16-tetrahydroxygrayanotox-9-ene. The anomeric proton of **3** was observed at δ 4.93(d, J=7 Hz), so it must be a β-D-glucoside. The glucosidation position was determined by the ^{13}C-NMR spectrum.

Table I. ^{13}C-NMR Chemical Shifts (δ) of Grayanotoxins (C_5D_5N)

	5[11)]	4	6[11)]	2	3a	3
C- 1	51.7	51.5	45.9	44.5	44.7	44.7
- 2	35.8	35.8	39.4	39.4	36.8	35.7
- 3	82.6	82.5	81.2	81.2	80.4	87.8
- 4	51.8	51.8	50.6	50.6	50.4	50.4
- 5	84.6	84.7	83.9	83.5	85.0	83.8
- 6	74.2	74.6	69.5	70.6	68.1	69.2
- 7	44.4	50.4	42.2	46.7	46.6	46.5
- 8	52.6	46.5	50.6	44.7	48.9	49.2
- 9	55.2	53.2	50.8	52.3	139.1	139.6
-10	78.1	78.2	153.0	153.0	121.2	120.9
-11*	22.6	23.3	24.6	24.1	28.8	28.7
-12*	27.1	26.6	24.8	25.8	26.4	26.2
-13	56.4	50.8	54.3	47.9	47.4	47.4
-14	79.4	36.2	81.0	36.5	48.9	48.9
-15	60.4	61.3	60.7	62.5	57.0	56.7
-16	79.7	78.2	81.2	79.5	81.1	81.4
-17	23.9	24.5	24.6	25.4	24.5	25.9
-18	23.3	23.2	23.8	24.1	24.3	24.4
-19	19.8	19.7	18.7	19.2	20.2	19.4
-20	28.3	28.3	111.9	112.2	17.8	18.5

* The assignments may be reversed in each column.



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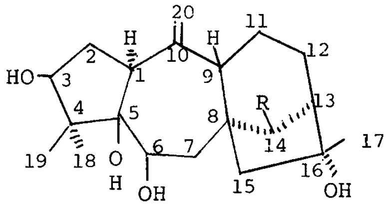
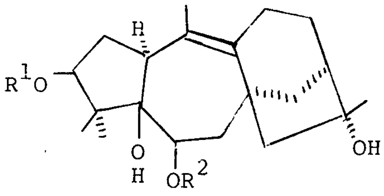
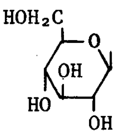
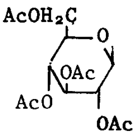
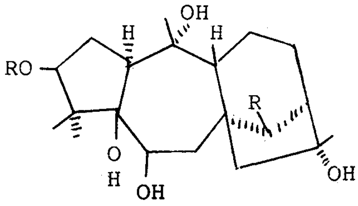


	R
2	H
6	OH



	R^1	R^2
3		H
3a	H	H
3b		Ac



	R
4	H
5	OH

The assignments of the ^{13}C-NMR spectra of grayanotoxins have been reported by several investigators.[9,10,11] However, no complete assignments of grayanotoxins having no hydroxyl groups on C-14 have appeared. So we tried to assign the spectra of 14-deoxygrayanotoxin-III(**4**)[12] and 14-deoxygrayanotoxin-II(**2**)(grayanotoxin-XVIII) by analogy with those of grayanotoxin-III(**5**) and grayanotoxin-II(**6**) (Table I). Due to the lack of the 14-hydroxyl group, the C-8 and C-13 of **4** and **2** shifted upfield. The assignments of the spectra of **3** and **3a** were achieved by comparing them with the spectrum of **2**. The downfield shift of more than 12 ppm on the C-14 of **3** and **3a** would be caused by the presence of a double bond between the C-9 and -10. The C-3 signal of aglycone **3a** appeared at δ 80.4, while that of glucoside **3** was observed at δ 87.8. Other carbinyl carbon signals scarcely shifted on glucosidation. Hence ß-D-glucoside bond must be attached at the C-3 of the aglycone. From the above argument, it was concluded that pieroside C(**3**) is 3-O-(ß-D-glucopyranosyl)-3,5,6,16-tetrahydroxy-grayanotox-9-ene. The grayanotox-9-ene derivative was the first one found in nature.

ACKNOWLEDGEMENT The authors are grateful to Mr. T. Ohki (The Bio-dynamics Research Institute) and Mr. J. Nakagami (Taisho Pharmaceutical Co. & Ltd.) for the measurement of high resolution MS and ^{13}C-NMR spectra.

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4)J. Sakakibara, N. Shirai, T. Kaiya, and H. Nakata, Phytochemistry, **18**, 135(1979). ^{1}H-NMR of **2**(C_5D_5N) δ:1.12(3H,s), 1.55(6H,s), 3.13(1H,t,J=9 Hz), 3.93(1H,dd,J=2 and 6 Hz), 4.21(1H,dd,J=2 and 9 Hz), 5.16(2H,s, H-20).
5)Viscous syrup, $[\alpha]_D^{21.5}$ -14.46°(c=2.45, MeOH), ^{1}H-NMR(C_5D_5N) δ:1.08, 1.45, 1.59, 1.74(each 3H,s), 4.00-4.52(many protons), 4.93(1H,d,J=7 Hz).
6)Colorless powder, mp 262°C(dec.), ^{1}H-NMR($CDCl_3$) δ:0.90, 0.95, 1.39, 1.69(each 3H,s), 1.98, 2.00, 2.01, 2.07, 2.09(each 3H,s), 4.22(2H,m), 4.52(1H,d,J=7 Hz), 5.15(4H,m). MS m/z:648(M^+-AcOH), 630, 612.
7)^{1}H-NMR(C_5D_5N) δ:0.91, 1.30, 1.58, 1.84(each 3H,s), 3.50(1H,t,J=10 Hz), 3.83(1H, t, J=5 Hz), 4.22(1H, m). MS m/z:336.2311(M^+, calcd for $C_{20}H_{32}O_4$, 336.2300).
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(Received August 30, 1985)