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Further Study on the Constituents of *Rhododendron brachycarpum*

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Abstract □ From the leaves of *Rhododendron brachycarpum*, rhododendrin, grayanotoxin I and guaijaverin were isolated and characterized by spectral data.

Key words □ *Rhododendron brachycarpum*, Ericaceae, rhododendrin, grayanotoxin I, guaijaverin, ^{13}C-NMR

In the previous paper[1], the isolation of quercetin, avicularin, quercitrin and hyperin from the leaves of *Rhododendron brachycarpum* (Ericaceae) was reported. In a course of continuous work on this plant part, additional three compounds were isolated. The ethylacetate soluble fraction from the MeOH extract was chromatographed repeatedly on silica gel and Sephadex columns to give compounds **1**, **2**, and **3**.

Compound **1**, mp 188-189°, $[\alpha]_D^{20}$-40°, showed the presence of a hydroxyl group (3350 cm^{-1}), aromatic ring (1620, 1610, 1520 cm^{-1}) and glycoside bond (1100-1000 cm^{-1}) in its IR spectrum. Its NMR spectra showed the presence of a 1,4-di-substituted benzene ring (two *ortho*-coupled doublets each of two protons, at δ 6.96 and 6.68 ppm, J = 8.1Hz, two doublets at 114.86 and 129.05 ppm), two methylenes (30.02 and 38.8 ppm), one oxygen-bearing methine (72.60 ppm), one methyl (19.56 ppm) and D-glucose (100.72, 73.41, 76.61, 70.16, 76.83 and 61.17 ppm), indicating that it was identical with rhododendrin which was previously isolated from this plant. In fact, our compound gave comparable physical constants[2-4]. The ^{1}H-NMR, ^{13}C-NMR and MS of **1** have been elucidated for the first time.

Compound **2**, mp 247-8°, $[\alpha]_D^{20}$-5° gave brown coloration in Liebermann-Burchard reaction and showed strong absorption bands at 3520 and 3350 cm^{-1} (hydroxyl) and 1720 and 1235 cm^{-1} (ester) in its IR spectrum. The mass spectrum did not show a molecular ion peak but the ion of highest mass appeared at *m/z* 394 (M^+-H_2O) and other prominent ion peaks at *m/z* 376 (M^+-$2H_2O$), 358 (M^+-$3H_2O$), 343 [M^+-($3H_2O$ + CH_3)], 316 [M^+-($2H_2O$ + HAc)] and 298 [M^+-($3H_2O$ + HAc)]. The ^{13}C-NMR spectrum showed absorption for twenty two carbons, six of which appeared in the region characteristic of oxygen-bearing tetrahedral carbons (73-84 ppm) and two of which were signals for acetyl group (21.4 and 170.4 ppm). Therefore, **2** was suggested to be a tetracyclic diterpenoid having five hydroxyl groups and one acetoxyl group. The ^{1}H-NMR (400 MHz, pyridine-d_5, TMS) exhibited two tertiary methyls at δ 1.26 and 1.65 ppm, two tertiary methyls on hydroxyl-carrying carbons at 1.47 and 1.84 ppm, one tertiary proton at 3.25 ppm (1H, t, J = 8.2Hz), two tertiary protons on hydroxyl-carrying carbons at 3.90 ppm (1H, brs) and 4.16 ppm (1H, dd-like) and one tertiary proton on an acetoxyl-carrying carbon at 6.19 ppm (1H, s). Assuming that **2** had an A-nor-B-homo-*ent*-kaurane skeleton, its structure was most likely grayanotoxin I which is widespread in the *Rhododendron* species[5]. The identity was confirmed by comparison of its physical properties and spectral data with those reported in the literature[6-8].

Compound **3**, mp 236-8°, $[\alpha]_D^{20}$-50° showed positive Mg + HCl, Zn + HCl and Molisch test. Its IR spectrum showed a broad hydroxyl and α,β-unsaturated carbonyl absorptions at 3350 and 1660 cm^{-1}, respectively and C-O stretching bands at 1070 and 1030 cm^{-1}, indicating its glycosidic nature. The UV spectrum exhibiting band I peak at 360 nm was very similar to those reported for a number of 3-hy-

droxyl substituted flavonols[9]. The ^{1}H-NMR showed two *meta*-coupled doublets of one proton with a J value of 2.0Hz at 6.2 ppm (H-6) and 6.4 ppm (H-8), and an *ortho*-coupled doublet of one proton at 6.84 ppm (J = 8.5Hz, H-5′), a *meta*-coupled doublet of one proton at 7.51 ppm (J = 2.0Hz, H-2′) and a double doublet of one proton at 7.65 ppm (J = 2.0 and 8.5Hz, H-6′) and sugar proton signals at 3.0-5.5 ppm, but no signal for H-3, indicating that this compound was a quercetin glycoside.

This assumption was supported by ^{13}C-NMR data, from which the sugar was suggested to be L-arabinose. In fact, acid hydrolysis of **3** afforded quercetin and L-arabinose.

A bathochromic shift in the UV spectrum induced by the addition of shift reagents (see Experimental) suggested that the sugar was attached to 3-hydroxyl group. This suggestion was supported by permethylation of **3** according to the Brimacombe's method[10] followed by methanolysis, which afforded 5,7,3′,4′-tetra-O-methyl quercetin. The configuration and conformation of sugar moiety was determined not only by the J value of the anomeric proton signal (Experimental) but also by application of the method of comparing molecular rotation described by Kovalev and Litvinenko[11]. Therefore, compound **3** was identified as quercetin 3-O-α-L-arabinopyranoside (guaijaverin) which was previously isolated from *Psidium guaijava*[12].

Compound **2** and **3** have not been isolated from this plant by any of the earlier authors.

EXPERIMENTAL METHODS

The mps were taken on a Thomas Hoover 6406-H apparatus and are uncorrected. The IR spectra were determined in KBr tablets on a Shimadzu spectrophotometer and the UV spectra were runned with CE 599 Universal automatic scanning spectrophotometer. The ^{1}H-NMR was recorded with a Varian FT-80A (80 MHz), Jeol-GX 270 (270 MHz) and Bruker WH-400 instrument (400 MHz) in DMSO-d_6 or pyridine-d_5 (free) and $CDCl_3$ (acetate) containing TMS as an internal standard and chemical shifts are given as δ (ppm). ^{13}C-NMR was recorded with a Varian FT-80A (20 MHz), Jeol-FX 900 (22.53 MHz) and Bruker WH-400 instrument (100 MHz) in the same solvent using the same internal standard and chemical shifts are given as δ (ppm). Mass spectra were taken a Hewlett-Packard 5985B GC/MS spectrometer operating at 70 eV. Optical rotation was measured on a Mitamura-Ricken polarimeter.

Extraction and Fractionation

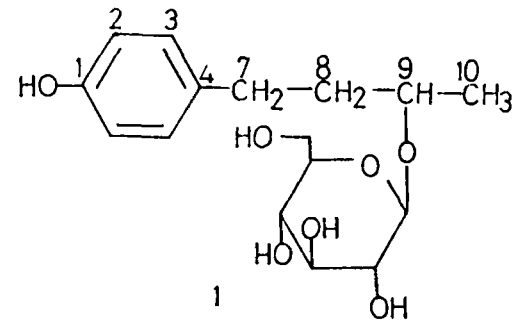
This was carried out as described previously[1].

Isolation

The ethylacetate extract was subjected to chromatography using SiO_2 ($CHCl_3$: MeOH: 7% HAc = 25:9:5, lower phase) and Sephadex LH-20 columns to yield grayanotoxin I(**2**) from early eluting fraction and rhododendrin(**1**) which was easily recrystallized from the mother liquor after separation of avicularin. The mother liquor which is nearly single spot in several solvent systems after separation of quercitrin was shown two spots in HCOOH:EtOAc:HAc:H_2O(11:100:11:27). This was subjected to chromatography using SiO_2($CHCl_3$:MeOH:H_2O = 80:20:1) column to yield upper major spot (quercitrin) and lower minor spot (guaijaverin,**3**) in the order of elution.

Compound 1 (rhododendrin)

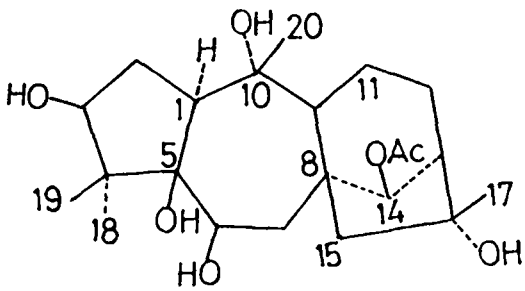
Colorless needles from MeOH, mp 188-9°, $[\alpha]_D^{20}$-40°(c 0.5, MeOH), $FeCl_3$ and Molisch tests; positive, IR ν_{max}^{KBr} Cm^{-1}; 3350(OH), 1620,1610,1520 (aromatic), 1100-1000(glycoside), UV λ_{max}^{KBr} nm (MeOH) (logε); 225 (3.94), 280 (3.24), MS (*m/z*,%); 328 (M^+, 0.6), 166 ($M^+ - C_6H_{10}O_5$, 9.6), 148 ($M^+ - C_6H_{12}O_6$, 45), 121 (HO-  -CH_2-CH_2^+, 20.5), 107 (HO-  -CH_2^+, 100), ^{1}H-NMR (DMSO-d_6 + D_2O, 270 MHz)δ; 6.96 (2H,d,J = 8.1Hz, H-3 and H-5), 6.68 (2H,d,J = 8.1 Hz, H-2 and H-6), 4.17 (1H,d,J = 7.8Hz, anomeric), 3.78-3.65 (1H,m,H-9), 2.95 (2H,t,J = 4Hz, H-7), 1.81-1.54 (2H,m,H-8), 1.10 (3H,d,J = 6.1Hz, -CH_3), ^{13}C-NMR (DMSO-d_6, 22.53 MHz)δ; 155.00 (s, C-1), 132.19 (s,C-4), 129.05 (d,C-3 and C-5), 114.86 (d, C-2 and C-6), 100.72 (d, C-1″), 76.83 (d, C-5″), 76.61 (d, C-3″), 73.41 (d, C-2″), 72.60 (d, C-9), 70.16 (d, C-4″), 61.17 (t, C-6″), 38.8 (t, C-7), 30.02 (t, C-8), 19.56 (q,C-10).



Compound 2 (grayanotoxin I)

Colorless needles from MeOH, mp 247-8°, $[\alpha]_D^{20}$

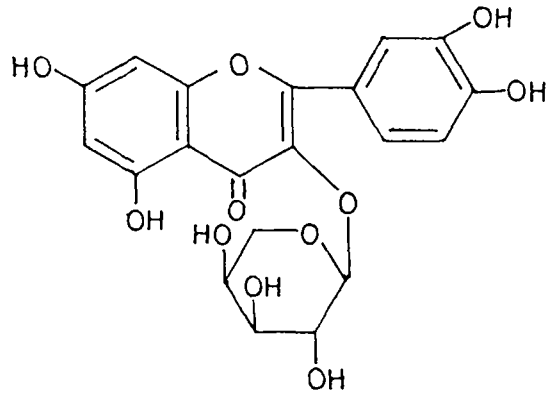
-5°(c 0.5,MeOH), IR ν_{max}^{KBr} Cm^{-1}; see text, ^{1}H-NMR (pyridine-d_5,400 MHz) δ;6.19 (1H, brs,H-14), 4.16 (1H,dd-like,H-6), 3.90 (1H,brs, H-3), 3.25 (1H,t,J = 8.2Hz,H-1), 2.00 (3H,s, -OAc), 1.84 (3H,s,-CH_3), 1.65 (3H,s,-CH_3), 1.47 (3H,s,-CH_3), 1.26 (3H, s,-CH_3),MS (*m/z*,%);394 (M^+-H_2O,0.7), 376 (M^+-$2H_2O$, 8.5),358 (M^+-$3H_2O$,19.9), 343 (M^+-$3H_2O$-CH_3,2.7),316(M^+-$2H_2O$-HAc, 8.5), 298 (M^+-$3H_2O$-HAc,10.7), 283 (M^+-$3H_2O$-HAc-CH_3,3.8), 265 (M^+-$3H_2O$-HAc-$2CH_3$,16.8), ^{13}C-NMR (pyridine-d_5,100 MHz)δ;170.4 (acetyl carbonyl), 84.6 (C-5), 83.0 (C-14),82.9 (C-3), 78.8 (C-16), 78.1 (C-10), 73.8 (C-6), 61.4 (C-15), 55.8 (C-9), 55.2 (C-13), 51.9 (C-4), 51.5 (C-1), 51.2 (C-8), 44.2 (C-7), 36.0 (C-2), 28.5 (C-20), 27.5 (C-12), 24.1 (C-17), 23.6 (C-18), 22.6 (C-11), 21.4 (acetyl methyl), 19.9 (C-19).



2

Compound 3 (guaijaverin)

Yellowish needles from MeOH, mp 236-8°, $[\alpha]_D^{20}$-50°(c 0.5,MeOH), IR ν_{max}^{KBr} Cm^{-1};3350 (OH), 1660 (C = O), 1600, 1560, 1510 (C = C), 1350, 1290, 1190, 1070, 1030 (C-O), 970, 960, 900, 845, 800, 775, UV λ_{max} nm (MeOH) (logε);258 (4.32), 270 (sh,4.23), 298 (sh,3.93), 360 (4.24), λ_{max} nm (MeOH + NaOMe) 274 (4.37), 330 (sh,3.93), 410 (4.32), λ_{max} nm (MeOH + $AlCl_3$) (log ε) 276 (4.37), 306 (sh,3.83), 440 (4.34), λ_{max} nm (MeOH + $AlCl_3$ + HCl) (log ε) 270 (4.31), 304 (3.89), 365 (3.97), 406 (4.15), λ_{max}(MeOH + NaOAc) 274(4.32), 330 (4.05), 375 (4.16), λ_{max} nm (MeOH + NaOAc + H_3BO_3) (log ε) 262 (4.37), 298 (3.85), 382 (4.32), ^{1}H-NMR(DMSO-d_6,80 MHz) δ;12.63 (1H,brs,H-5, disappeared by addition of D_2O), 7.65 (1H,dd,J = 2.0 and 8.5 Hz,H-6'), 7.51 (1H,d,J = 2.0 Hz,H-2'), 6.84 (1H,d, J = 8.5Hz,H-5'), 6.40 (1H,d, J = 2.0 Hz,H-8), 6.20 (1H,d,J = 2.0 Hz,H-6), 5.37 (1H,d,J = 5.0 Hz,anomeric), ^{13}C-NMR (DMSO-d_6, 20MHz) δ;177.6 (C-4), 164.1 (C-7), 161.0 (C-5), 156.5 (C-2 and C-9), 148.5 (C-5'), 144.8 (C-3'), 133.9 (C-3), 122.0 (C-6'), 121.2 (C-1'), 116.1 (C-5'), 115.6 (C-2'), 104.1 (C-10), 101.8 (C-1''), 98.8 (C-6), 93.8 (C-8), 71.8 (C-2''), 70.9 (C-3''), 66.4 (C-4''), 64.8 (C-5'').



3

Acid hydrolysis of 3

Ten mg of **3** was refluxed with 5% H_2SO_4(30 m*l*) for 5 hr. After cooling, the reaction mixture was filtered. The alycone was crystallized from MeOH to afford quercetin as yellow needles, mp 315-6°. It was confirmed by direct comparison with an authentic sample (TLC, mmp and UV). The filtrate was neutralized with $BaCO_3$, filtered and concentrated. L-arabinose was identified by TLC (precoated cellulose, pyridine: EtOAc: HAc: H_2O = 36: 36: 7: 21, Rf 0.55).

Permethylation of 3 followed by acid hydrolysis

Fourty mg of **3** was permethylated using Brimacombe's method[10], and followed by the usual work up. Acid hydrolysis of the crude permethylether with 5% H_2SO_4 in 50% dioxane under reflux for 3 hr was followed by the usual work up. Crystallization of the aglycone from MeOH gave 5,7,3',4'-tetra-O-methyl quercetin, mp 193-5°, which was confirmed by direct comparison with an authentic sample (TLC, mmp and UV).

Acetylation of 3

A sample (30 mg) in pyridine and Ac_2O (1 m*l* each) was allowed to stand at room temperature overnight. The reaction mixture was poured into crushed ice and filtered. The precipitate was crystallized from $CHCl_3$-light petroleum (b.p. 100-120°) to give colorless needles (35 mg), mp 223-5°. IR λ_{max}^{KBr} Cm^{-1}; 1730,1250(acetate), ^{1}H-NMR($CDCl_3$,200 MHz) δ;8.08(1H,dd,J = 2.2 and 8.5Hz,H-6'), 7.96 (1H,d,J = 2.2Hz,H-2'), 7.35 (1H,d, J = 8.5 Hz,H-5'), 7.31(1H, d,J = 2.2 Hz,H-8), 6.84 (1H,

d,J = 2.2Hz,H-6), 5.56 (1H, d,J = 6.9 Hz,H-1″), 5.39 (1H,dd,J = 6.9Hz and 9.1Hz, H-2″), 5.20-5.18 (1H,m,H-4″), 5.12 (1H, dd,J = 3.5Hz and 9.1Hz, H-3″), 3.80 (1H, dd,J = 3.2Hz and 13.1Hz, H_a-5″), 3.51 (1H, d,J = 1.8Hz and 13.1Hz, H_e-5″), 2.45 (3H, s,-OAc), 2.35 (3H,s,-OAc), 2.34 (3H,s, -OAc), 2.32 (3H,s,-OAc), 2.13 (3H,s,-OAc), 2.09 (3H,s,-OAc), 2.05 (3H,s,-OAc).

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