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Isolation of (+)-Catechin from the Roots of *Rosa rugosa*

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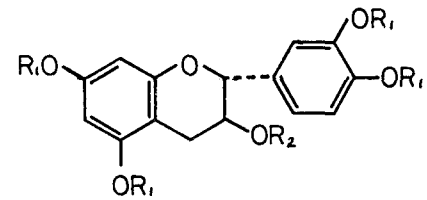
Abstract—From the roots of *Rosa rugosa*(Rosaceae), (+)-catechin, and a mixture of β-sitosterol and campesterol glucosides were isolated and characterized by the physicochemical and spectral data.

Keywords—*Rosa rugosa* · Rosaceae · (+)-catechin · sterol-glucosides · ^{13}C-NMR

The roots of *Rosa rugosa* Thunb.(Rosaceae) have been used as a folkloric medicine for treating diabetics. On the other hand, in a preliminary screening test for the hypolipemic action of several crude drugs, this plant showed significant activity.[1] The phytochemical studies on this plant have afforded β-sitosterol, campesterol and quercetin.[2,3]

We now report the results of our investigation of *R. rugosa*. Compound **1**, mp 276~9°, gave a positive reaction in the Liebermann-Burchard and Molisch tests and showed hydroxyl (3400 cm^{-1}), double bond (1640 and 840—790 cm^{-1}), and glycoside bond (1100—1000 cm^{-1}) absorptions in its IR spectrum. Judging from its ^{13}C-NMR specrtra, it was found that **1** contained one mole of sugar (δ100.8ppm). Acid hydrolysis of **1** gave a genin, mp 128~31°, which was identified as a mixture of β-sitosterol (65%) and campesterol (35%) (MS and GLC) and D-glucose(co-TLC).

The β-configuration for the glucoside linkage was determined by the *J* value of the anomeric proton signal (*J*=7Hz). Therefore, compound **1** was identified as a mixture of β-sitosterol and compesterol glucosides. Compound **2**, mp 174~8°, $[\alpha]_D^{17}$+30°, showed positive $FeCl_3$ test (green) and hydroxyl (3300 cm^{-1}), aromatic ring (1630 and 1525 cm^{-1}) absorptions in its IR spectrum, and a single absorption peak characteristic of a catechin at 281.5 nm in its UV spectrum.[4] It gave a pentaacetate(**2a**), mp 130~2°, on acetylation with Ac_2O/pyridine and a tetramethyl ether(**2b**), mp 143~4°, by methylation with CH_2N_2. The MS spectrum showed a molecular ion at *m/z* 290(21.1%) and other fragment peaks at *m/z* 152(RDA fragment with B ring, 43.4), 139(RDA fragment with A ring +H, 100), 123(152-CHO, 69.7) and 109(123-CO, 8.0). The ^{1}H-NMR spectrum showed the two signals at 5.89(1H, d, *J*=2Hz) and 5.69 (1H, d, *J*=2Hz) ascribable to H-8 and H-6 on



2	R_1 = H	R_2 = H
2a	R_1 = OAc	R_2 = OAc
2b	R_1 = Me	R_2 = H

the A-ring of a flavan skeleton and the signals at 6.72(1H, d, J=2Hz), 6.69(1H, d, J=8Hz) and 6.60(1H, dd, J=8 and 2Hz) assignable to the protons of a 1,3,4-trisubstituted benzene ring. The signals at 4.48(1H, d, J=7Hz, H-2), 3.88~3.75(1H, m, H-3), 2.51(2H, ABX type, J=16.0, 8.0 and 5.5Hz, H_2-4) suggested the presence of a catechin moiety. These spectral data were in agreement with those for the structure of (+)-catechin. It was further identified by direct comparisons with an authentic sample (mmp, co-TLC and ^{1}H-NMR).

Experimental

Apparatus

Melting points were determined on a Thomas Hoover 6404-H apparatus and were uncorrected. The UV spectra were runned on a Shimadzu MPS-50L recording spectrophotometer and the IR spectra were recorded on a Shimadzu spectrophotometer. The ^{1}H- and ^{13}C-NMR spectra were recorded on a Jeol GX-270 and Jeol FX-90Q spectrometer with TMS as an internal standard and chemical shifts are given as δ (ppm). Mass spectra were taken on a Jeol 01-SG-2 spectrometer and optical rotations were obtained on a Mitamura-Ricken polarimeter. The GLC was carried out with a Hitachi GL 163 gas chromatography equipped with a FID. The chromatographic column was 1 m×3 mm tube contained Chromosorb W(80~100 mesh) coated with 2% OV-17(column temp; 290°, injector temp; 300°, N_2 gas; 45 ml/min)

Plant material

The *R. rugosa* used was purchased from the Chinese herb medicine shop at the Pyongwha market, Pusan and its properties were assurately tested with the collected roots from the herbal garden of College of Pharmacy, Pusan National University.

Extraction and Isolation

The dried roots(2.3 kg) were refluxed with MeOH. The MeOH extract was fractionated to yield $CHCl_3$(33 g), EtOAc(99 g), BuOH(38 g) and H_2O(67 g) soluble portions successively. The $CHCl_3$ soluble portion was chromatographed on Silica gel column with $CHCl_3$-MeOH(gradient) to give compound **1**. The EtOAc soluble portion was chromatographed on Silica gel column with $CHCl_3$-MeOH-7% HAc(25:8:5) to afford 10 fractions. Fraction 6 was concentrated and crystallized with H_2O to give compound **2**. The fractionation of other fractions are now in progress.

Compound 1 : Mp 276~9°, colorless needles from MeOH; IRν_{max}^{KBr}cm^{-1}: see text; ^{1}H-NMR (90MHz, DMSO-d_6) δ : 5.33(1H, m, vinylic), 4.86(1H, d, J=7Hz, anomeric), 1.22, 1.16, 0.95, 0.83, 0.77, 0.65(3H, each, 6× -CH_3); ^{13}C-NMR(22.5MHz, DMSO-d_6)δ: sugar; 100.8(C-1′), 76.7(C-3′ and 5′), 73.4(C-2′), 70.1(C-4′), 60.9(C-6′).

Compound 2 : Mp 174~8°, $[\alpha]_D^{17}$ +30° (c, 0.5, EtOH), colorless needles from H_2O; IR ν_{max}^{KBr} cm^{-1}: 330, 1630, 1525, 1470, 1290, 1150, 1080, 1030; UVλ_{max}^{MeOH}nm(logε): 281.5(3.50); ^{1}H-NMR (270MHz, DMSO-d_6)δ: see text; ^{13}C-NMR(22.5 MHz, DMSO-d_6)δ: 156.7(C-9), 156.1(C-5 and 7), 145.2(C-3′ and 4′), 131.6(C-1′), 119.8 (C-6′), 116.5(C-5′), 115.4(C-2′), 100.6(C-10), 96.6(C-8), 95.4(C-6), 81.6(C-2), 67.2 (C-3), 27.9(C-4); MS(m/z, rel. int.): see text.

Acid hydrolysis of 1 : 30 mg of **1** was refluxed with 5% H_2SO_4-MeOH(5 ml) for 4hrs. After cooling the reaction mixture was poured into water to give solids which were collected by filtration, when crystallized from MeOH, mp 128~31°; MS(m/z, rel. int.): 414[M_1]$^+$(100), 400[M_2]$^+$ (37.8), 329[M_1-C_5H_9O]$^+$ (100), 315 [M_2-C_5H_9O]$^+$(27.1), 303[M_1-$C_7H_{11}O$]$^+$(47.6), 289[M_2-$C_7H_{11}O$]$^+$(20.4), 275[M_1-$C_9H_{15}O$]$^+$ (22.7), 273[M-side chain]$^+$ (79.6), 261[M_2-$C_9H_{15}O$]$^+$ (11.1), 255[M-side chain-H_2O]$^+$

(76.9); GLC: t_R for β-sitosterol (3.0 min, 65%), campesterol (2.6 min, 35%).

The filtrate was concentrated *in vacuo* and refluxed with acid to hydrolyze methylglucoside. After neutralizing with $BaCO_3$, the aqueous solution was concentrated, in which only D-glucose was detected by TLC (precoated cellulose, pyridine:EtOAc:HAc:H_2O=5:5:1:3, Rf, 0.40).

Acetylation of 2: Compound **2** (50 mg) in pyridine (2 ml) and acetic anhydride (3 ml) was allowed to stand overnight at room temperature and the mixture was treated as usual to afford colorless needles from MeOH; mp 130~2°, $[\alpha]_D^{17}$ +25° (c, 1.0, $CHCl_3$); IRν_{max}^{KBr} cm^{-1}: 1750 and 1230 (acetate); ^{1}H-NMR (90MHz, $CDCl_3$) δ; 7.24—7.17 (3H, m, H-2′, 5′ and 6′), 6.66 (1H, d, J=2Hz, H-8), 6.60 (1H, d, J=2Hz, H-6), 5.26 (1H, dd, J=11.5 and 6Hz, H-3), 5.15 (1H, d, J=6Hz, H-2), 2.88 (1H, dd, J=16.5 and 5.0Hz, H_e-4), 2.66 (1H, dd, J=16.5 and 6.0 Hz, H_a-4), 2.88 (9H, s, 3 × -OAc), 2.10 (3H, s, -OAc), 2.00 (3H, s, -OAc).

Methylation of 2: A crude sample of **2** was methylated with etherial CH_2N_2 for 7 days, concentrated and chromatographed on Silica gel column. Elution with acetone:ether:N-hexane (2:5:5) gave tetramethylether and crystallized from MeOH as colorless needles, mp 143~4°; ^{1}H-NMR (270MHz, acetone-d_6) δ: 7.17—7.03 (3H, m, H-2′, 5′ and 6′), 6.28 (1H, d, J=2Hz, H-8), 6.19 (1H, d, J=2Hz, H-6), 4.78 (1H, d, J=8Hz, H-2), 4.21—4.15 (1H, m, H-3), 3.94 (9H, s, 3× -OMe), 3.87 (3H, s, -OMe), 3.10—2.59 (2H, m, H_2-4); MS (m/z, rel. int.): 346 (M^+, 8.6), 180 (RDA fragment with A ring +H, 100).

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