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Tannins from *Rubus coreanum*

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Abstract—Tannins were isolated from the stems of *Rubus coreanum* and identified as (–)-epicatechin , (+)-catechin , procyanidin B-4 and sanguiin H-4 by spectral analysis.

Keywords—*Rubus coreanum* · Rosaceae · tannin · flavan-3-ol · proanthocyanidin · Ellagitannin

Rubus coreanum (Rosaceae), which has been used in folk medicine as a tonic for aged people,[1),2)] is a fruit drug growing throughout southern Korea.[3),4)] From Rubus coreanum only the triterpenoids such as niga-ichigoside F_1, F_2, suavissimoside R_1, coreanoside F_1 are known.[5)-7)] As our part of research to find the biologically active components from plant polyphenols, we first isolated two flavan-3-ols, (–)-epicatechin(1) and (+)-catechin(2), a proanthocyanidin, procyanidin B-4(3) and an ellagitannin, sanguiin H-4(4) from the stems of this plant. This paper deals with the isolation and structural elucidation of these compounds.

Experimental

General Experimental Procedures - The ^{1}H- and ^{13}C-NMR spectra were recorded at 200,500 MHz (^{1}H-NMR) and 50,100MHz (^{13}C-NMR) with a Bruker AM-200 and Bruker AMX 500 spectrometer. Chemical shifts are given in δ (ppm) scale with TMS as internal standard. EIMS spectra were obtained with GC-MS/MS-DS, TSQ 700 (U.S.A) spectrometer. Negative FAB-MS were measured at 1.5 kv (accelerating voltage) with glycerol matrix. Chromatography was carried out on Amberlite XAD-2 (16-45mesh, Fluka), Sephadex LH-20 (25-100 μm, Pharmacia), MCI-gel CHP-20P (75-150 μm, Mitsubishi).

TLC was carried out on silicagel 60 F_{254} (Merck) and precoated cellulose F_{254}(Merck) in the solvent system benzene—ethyl formate—formic acid (1:7:1 v/v%) and 2% HOAc. Chromatogram was detected by illumination with U.V light and by use of four spray reagent : (i) $FeCl_3$, (ii) 10% - H_2SO_4, (iii) anisaldehyde - sulphuric acid, (iv) $NaNO_2$-HOAc.

Plant Material - The stems of *Rubus coreanum* were collected from the herbary of Chung Ang University, on september 1993. A voucher specimen is deposited in the herbarium of college of pharmacy, Chung Ang University.

Extraction and Isolation - The fresh stems (4 kg) were extracted with 80% aq. Me_2CO at room temperature (3 times). The acetone was removed *in vacuo* and the aqueous layer was defatted with ether. The extract were chromatographed on Amberlite XAD-2 (150x520 mm) with increasing concentration of methanol in water to give 6 fractions. Repeated CC of fraction 5 on Sephedex LH-20 (78x300 mm) with 80% EtOH followed by MCI-gel CHP-20P with H_2O-MeOH gradient system gave (–)-epicatechin(850 mg), (+)-catechin(50 mg), procyanidin B-4(1.5 g) and sanguiin H-4(60 mg).

Compound 1 - White needles, EIMS $[M]^+$ *m/z* 290, $[\alpha]_D^{20}$ -58.0° (c = 0.1, acetone), ^{1}H-NMR(Me_2CO-d_6 + D_2O) δ: 7.18 (1H, d, *J*=2Hz, H-2'), 6.85 (1H, dd, *J*=2, 8Hz, H-6'), 6.78 (1H, d, *J*=8Hz, H-5'), 6.09 1H, d, *J*=2.3Hz, H-8), 5.92 (1H, d, *J*=2.3Hz, H-6), 4.8 9 (1H, s, H-2), 4.21 (1H, m, H-3), 2.83 (1H, dd, *J*=4, 16Hz, H-4), 2.49 (1H, dd, *J*=5, 16Hz, H-4). ^{13}C-NMR (Me_2CO-d_6 + D_2O) : δ 79.3 (C-2), 66.8 (C-3), 28.9 (C-4), 157.5 (C-5), 95.6 (C-6), 157.5 (C-7), 96.1 (C-8), 157.0 (C-9), 99.7 (C-10), 132.2 (C-1'), 115.2 (C-2'), 145.2 (C-3'), 145.3 (C-4'), 115.5 (C-5'), 119.3 (C-6')

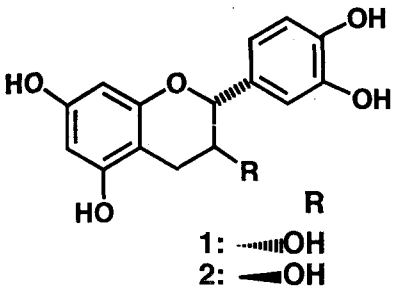
Compound 2 - Off-white needles, EIMS $[M]^+$ *m/z* 290, $[\alpha]_D^{20}$ + 12.0° (c=0.1 , acetone), ^{1}H-NMR (Me_2CO-d_6 + D_2O) δ: 6.89 (1H, m, H-2'), 6.78 (1H, m, H-5'), 6.76 (1H, m, H-6'), 6.02(1H, d, *J*=2.3Hz, H-8), 5.87 (1H, d, *J*=2.3Hz, H-6), 4.55 (1H, d, *J*=7.3Hz, H-2), 3.99(1H, m, H-3), 2.52-2.89 (2H in total, m, H-4). ^{13}C-NMR (Me_2CO-d_6 + D_2O) : δ 82.9 (C-2), 68.6 (C-3), 29.1 (C-4), 157.5 (C-5), 96.6 (C-6), 158.0 (C-7), 95.7 (C-8), 156.0 (C-9), 99.7 (C-10), 132.3 (C-1'), 115.7 (C-2'), 146.0 (C-3'), 145.9 (C-4'), 116.1 (C-5'), 120.3 (C-6')

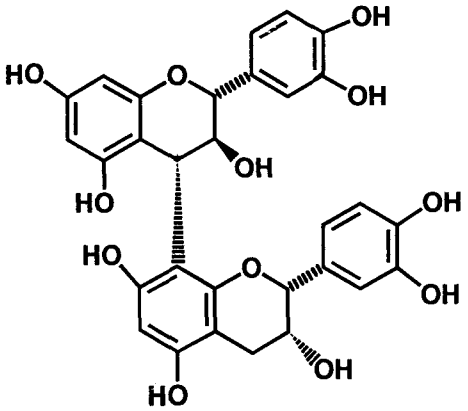
Compound 3 - Brown amorphous powder, Negative FAB-MS $[M-H]^-$ *m/z* 577, $[\alpha]_D^{20}$-177.0° (c=0.1, acetone), ^{1}H-NMR (Me_2CO-d_6 + D_2O) δ: 7.27[1H, d, *J*=1.9Hz, H-2' (u or t)], 7.03 (1H, d, *J*=1.9Hz, H-2' (u or t)], 6.90[1H, dd, *J*=1.9, 8.0Hz, H-6' (u or t)], 6.88[1H, dd, *J*=1.9, 7.9Hz, H-6' (u or t)], 6.84 [1H, d, *J*=7.9Hz, H-5' (u or t)], 6.82 [1H, d, *J*=8.0Hz, H-5' (u or t)], 6.80[d, J = 1.9 Hz, H-2' (conformer)], 6.76 [(d, *J*=1.9Hz, H-2' (conformer)], 6.73[d, *J*=8.1Hz, H-5' (conformer)], 6.69[d, *J*=8.1Hz, H-5' (conformer)], 6.54 [(dd, *J*=1.9, 8.1Hz, H-6' (conformer)], 6.41 [(dd, *J*=1.9, 8.1Hz, H-6' (conformer)], 6.21[s, H-6 (conformer, t)], 6.08 [1H, s, H-6(t)], 5.97 [d, J = 2.4 Hz, H-8(conformer u)], 5.96[d, *J*=2.4Hz, H-6(conformer u)], 5.89[1H, d, *J*=2.4Hz, H-8 (u)], 5.87[1H, dd, *J*=2.4Hz, H-6(u)], 4.99[1H, s, H-2(t)], 4.88[0.5H, s, H-2 (conformer u or t)], 4.70[1H, d, *J*=7.7Hz, H-2(u)], 4.60[1H, dd, *J*=7.7, 9.7Hz, H-3(u)], 4.51[m, H-2(conformer u or t)], 4.47[1H, d, *J*=9.7 Hz, H-4(u)], 4.39 [m, H-3 and H-4(conformer)], 4.28[1H, m, H-3(t)], 4.13[m, H-3(conformer)] ^{13}C-NMR(Me_2CO-d_6 + D_2O) δ: 79.8 (C-2t), 83.3 (C-2u), 73.1 (C-3u), 66.8 (C-3t), 38.1 (C-4u), 29.0 (C-4t), 154.7-158.2 (C-5u, 5t, 7u, 7t, 9u, 9t), 97.2 (C-6u), 96.4 (C-6t), 96.8 (C-8u), 107.7 (C-8t), 106.4 (C-10u), 101.3 (C-10t), 131.2-132.1 (C-1'u and t), 114.3-120.6 (C-5'u and t, C-6'u and t), 144.8-145.7(C-4'u and t, C-3'u and t) u:upper unit t: terminal unit

Compound 4 - Brown amorphous powder, Negative FAB-MS $[M-H]^-$ *m/z* 633, $[\alpha]_D^{20}$+99.0° (c=0.1, acetone), ^{1}H-NMR (Me_2CO-d_6 + D_2O) δ: 7.26 (2H, s, galloyl-H), 6.81 (1H, s, HHDP-H), 6.60 (1H, d, *J*=4Hz, glc-1), 6.53 (1H, s, HHDP-H), 5.53 (1H, t, *J*=9Hz, glc-3), 5.27 (1H, dd, *J*=4, 9Hz, glc-2). ^{13}C-NMR (Me_2CO-d_6 + D_2O) : δ 119.9 (galloyl C-1), 110.4 (2C, galloyl C-2, 6), 145.9 (2C, galloyl C-3, 5), 139.8 (galloyl C-4), 165.9 (galloyl-COO-), 114.3, 114.5 (HHDP C-1, C-1'), 125.9, 126.4 (HHDP C-2, C-2'), 107.2, 107.5 (HHDP C-3, C-3'), 144.3, 144.4 (HHDP C-4, C-4'), 136.1, 136.4 (HHDP C-5, C-5'), 145.1, 145.2 (HHDPC-6, C-6'), 169.3, 170.2 (HHDP-C=0-), 91.0 (glc-1), 73.7 (glc-2), 76.0 (glc-3), 66.8 (glc-4), 77.9 (glc-5), 60.8 (glc-6).

Results and Discussion

Compound 1 - White needles, $[\alpha]_D^{20}$-58.0· (c=0.1, acetone), gave a blue color with ferric chloride reagent and an orange-red color with anisaldehyde-sulphuric acid reagent which supposed to have a flavan-3-ol unit. The ^{1}H-NMR spectrum of 1 exhibited a ABX type of B-ring at δ 6.78 (1H, d, *J*=8Hz, H-5'), 6.85 (1H, dd, *J*=2, 8Hz, H-6') and 7.18 (1H, d, *J*=2Hz, H-2') and two meta coupled doublet signals (*J*=2.3Hz) at δ 5.92 (H-6) and 6.09 (H-8) suggesting 5,7-dihy-





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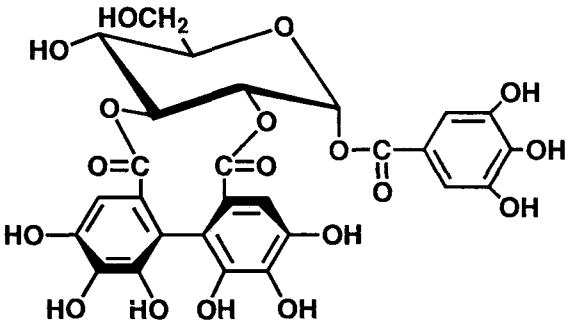

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droxylation pattern of A-ring. The ^{1}H-NMR spectrum of **1** further showed a singlet signal at 4.89 induced by H-2, a multiplet signal at δ 4.21 by H-3 and two double-doublet signals at δ 2.83 (J=4, 16Hz) and 2.49 (J=5, 16Hz) by H-4 indicated (-)cis catechin type.[8),9)] The EIMS spectrum showed a molecular ion peak at *m/z* 290. From these results, **1** was characterized as (-)-epicatechin.

Compound 2 - off white needles, $[\alpha]_D^{20}$+ 12.0° (c = 0.1, acetone), gave positive results to $FeCl_3$(dark green color) and anisaldehyde-sulphuric acid(pink) and showed almost same R*f* value as (-)-epicatechin on TLC. The ^{1}H-^{13}C-NMR spectra were similar to those of **1** except one proton doublet signal at δ 4.55 (J=7.3Hz, H-2) indicating trans catechin.[10)] The EIMS spectrum of **2** also showed its molecular ion peak at *m/z* 290. Thus **2** was elucidated as (+)-catechin.

Compound 3 - $[\alpha]_D^{20}$-177.0° (c=0.1, acetone), was obtained as brown amorphous powder. This compound gave a blue color with ferric chloride reagent and an orange-red color with anisaldehyde-sulphuric acid reagent and showed the low R*f* value comparing with **1** or **2** on TLC which is typical of proanthocyanidin polymer. The negative FAB-MS spectrum of **3** exhibited $[M-H]^-$ peak at *m/z* 577, which suggested that this compound possesses a sturctural skeleton of a B-type proanthocyanidin dimer.[11)] The ^{1}H-NMR spectrum of **3** displayed complex patterns due to the existence of conformers generated by the restricted interflavan rotation.[12),13),14)] Its distinctive H-2 terminal singlet signal at δ 4.99, a doublet signal at δ 4.70[J=7.8Hz, H-2 (upper)], a double doublet signal at δ 4.60[J=7.8, 9.7Hz, H-3 (upper)], and a doublet signal[J=9.7Hz, H-4(u)] at δ 4.47 revealed the presence of [4→8]-linked upper catechin and terminal epicatechin.[15)] And a quite similar C-2 upper signal (little effect on the C-2 resonance) at δ 83.3 compared with catechin (δ 82.9) also indicated C-4 as a trans orientation in the ^{13}C-NMR spectrum of **3**.[15)]

Therefore **3** was characterized as procyanidin B-4.[14),15)] The chemical shifts of NMR data from the reference were coincident with **3**.[16)] Direct comparison with authentic standard supported this conclusion(CO-TLC).[17)]

Compound 4 - $[\alpha]_D^{20}$+99.0° (c=0.1, acetone), was characterized as an ellagitannin by its blue colouration with ferric chloride and reddish-brown colouration with sodium nitrate acetic acid.[18)] The ^{1}H-NMR spectrum of **4** showed a galloyl group at δ 7.26 (2H, s) and HHDP-H(each 1H, s) at δ 6.81 and δ 6.53. ^{1}H-NMR spectrum of **4** showed an anomeric proton doublet at δ 6.60 and its coupling constant (J=4Hz) suggested to be α-configuration.[19)] The ^{13}C-NMR spectrum of **4** also showed one glucose moiety at δ 91.0, 77.9, 76.0, 73.7, 66.8, 60.8. The ^{1}H-NMR spectrum of 4 also showed 2, 3-HHDP glucose moiety from the low field shifted a double-doublet signal (J=4, 9Hz, glc-2) at δ 5.27 and a triplet signal(5=9Hz, glc-3) at δ 5.53.[20),21)] The negative FAB-MS spectrum showed a molecular ion peak at m/z 633. From these results, **4** was identified as sanguiin H-4 unambiguously.[22)]

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