

Isolation and Structure Elucidation of Triterpenoidal Constituents from the Leaves of *Rhododendron brachycarpum*

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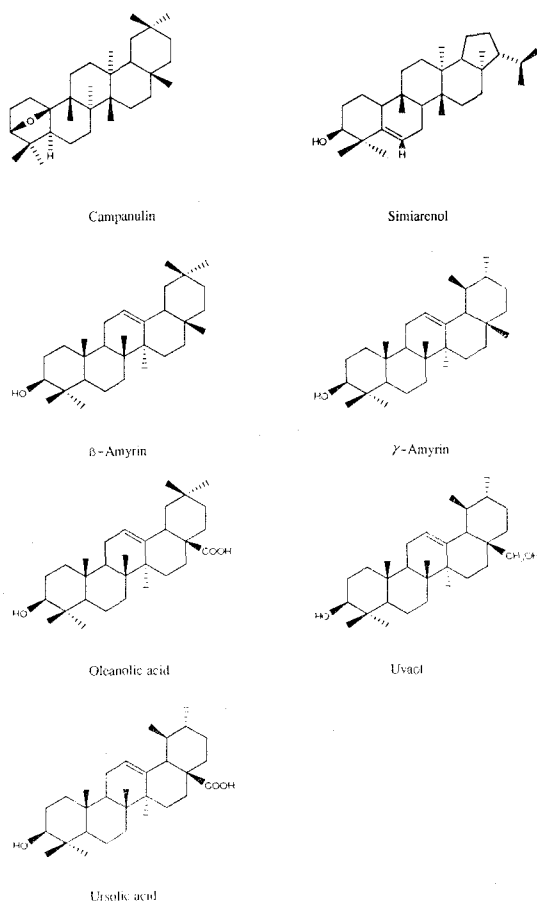
Abstract—Campanulin, α -amyrin, β -amyrin, uvaol, simiarenol, ursolic acid and oleanolic acid were isolated from the ethyl acetate fraction of the leaves of *Rhododendron brachycarpum* and their structures were identified by spectroscopic and chemical methods.

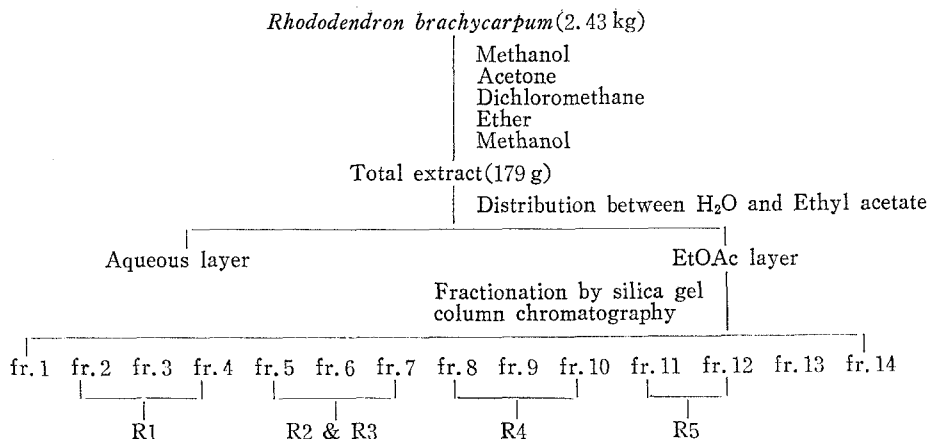
Keywords—*Rhododendron brachycarpum* • campanulin • α -amyrin • β -amyrin • uvaol • simiarenol • ursolic acid • oleanolic acid

As a part of our continuing search for biologically active compounds from natural sources, we have investigated metabolites produced by *Rhododendron brachycarpum* which is widely distributed throughout far east countries such as Japan, China and Korea. Although it has been used traditionally as antipyretics and antirheumatic agents, studies on chemical components of this poisonous shrub are scarce¹⁾. Now we report the isolation and structure elucidation of seven triterpenoids from the title plant.

Results and Discussion

Column chromatography of the ethyl acetate fraction of *Rhododendron brachycarpum*, followed by further purification through either crystallization or reverse phase HPLC, afforded seven triterpenes as summarized in Fig. 1. As shown in scheme 1, seven components were isolated from the ethylacetate fraction and all of them exhibited positive reactions in Lieber-





Scheme 1

mann Burchard test. Campanulin (R1) was the major triterpene and was easily crystallized from the methylene chloride-acetone mixed solvent as a colorless needle. The spectroscopic data including X-ray crystallographic data of R1 were exactly same as those of authentic sample.²⁻⁵⁾ R2 from fraction 5, 6 was crystallized in ether solution as a white needle and the structure was determined as simiarenol by the comparison of the spectral data with those of authentic simiarenol. Its acetate shows a lowfield triplet (4.72) for a proton on carbon bearing an acetate, the coupling pattern is indicative of an equatorial C₃ proton having eq/ax and eq/eq coupling. The base peak *m/z* 274 corresponds formally to a retro-diels-alder cleavage of ring B. The loss of allylic C₂₆ methyl group gives rise to the other major peak *m/z* 259. A weak peak (M-43) and a strong peak (*m/z* 231) due to the loss of C₃H₇ from the molecular ion *m/z* 274, reflect the presence of isopropyl group. R4 from fraction 8, 9, 10 was uvaol and was also confirmed with authentic specimen.^{4, 7, 13)} The crude R5 obtained after evaporation of fraction 11, 12 was isolated by acid-base treatment. The semicrystalline R3 and R5 were proved to be mixtures of two components by analytical HPLC and R5 was esterified with diazomethane and acetylated for the conven-

ience of structure determination. In the case of α , β -amyryns and oleanolic and ursolic acids, these two mixtures could not be separated by ordinary column chromatography, but by reverse phase prep-HPLC. Therefore the structures of the above four triterpenes were only confirmed by co-IR and co-mp with authentic samples⁸⁻¹²⁾ after separation by reverse phase prep-HPLC with RI detector. The ratios of above two mixture were calculated as follows; α -amyryn : β -amyryn = 87 : 13, ursolic acid : oleanolic acid = 95 : 5.

Experimental

General procedures—All melting points were taken on Buchi 510 and uncorrected. IR spectra were recorded in KBr disc on Fts-80 spectrometer and NMR spectra were run at GSX 270 spectrometer with TMS as an internal standard and all carbon multiplicities were determined by DEPT method. Mass data was taken on JMS-DX 300 spectrometer operating at 70 eV.

Plant materials—Leaves of *Rhododendron brachycarpum* were collected at the Odae Mountain, Kangwon Province in Korea at March 1990.

Extraction and isolation—The leaves of

R. brachycarpum were percolated successively with MeOH, acetone, CH_2Cl_2 , EtOAc, MeOH at room temperature. All fractions were combined, evaporated and distributed between H_2O and EtOAc. The EtOAc fraction was chromatographed on a silica column with following solvent system gradiently: n-hexane, n-hexane: EtOAc(15:1—1:1), benzene: acetone(10:1 →5:1), CH_2Cl_2 : MeOH(10:1).

Separation of α , β -amyriins—The mixture was separated by using H_2O : MeOH(97:3) as an eluent in preparative HPLC system with C_{18} column.

Acetylation—Triterpene alcohol was mixed with acetic anhydride and pyridine. After 24 hrs at room temperature, the reaction was stopped by addition of cold water.

Methylation of acid—Triterpenoidal acids were converted into its methyl ester by treatment with ethereal diazomethane at room temperature.

Separation of acid mixture—The acid fraction was basified with 0.1 N KOH and extracted with CH_2Cl_2 , acidified with 0.1 N HCl and extracted with CH_2Cl_2 . The crude acid mixture was separated by preparative HPLC using H_2O : MeOH: EtOAc (90:10:0.1) as a solvent system with C_{18} column.

Campanulin(R1)

Mp: 196~198°; ^1H - NMR (CDCl_3 , ppm): 3.75(1H, d,d); ^{13}C NMR (CDCl_3 , ppm): 31.9 (C-1), 24.7(2), 84.3(3), 43.3(4), 53.1(5), 19.8(6), 20.2(7), 41.9(8), 36.7(9), 93.6(10), 30.6(11), 30(12), 39.2(13), 39.3(13), 31.8 (15), 35.9(16), 30.1(17), 43.6(18), 35.0(19), 28.3(20), 33.4(21), 38.7(22), 24.4(23), 23.0 (24), 20.5(25), 19.3(26), 18.5(27), 31.9(28), 32.8(29), 34.1(30); Mass, m/z (relative intensity %): 426(M^+ , 100), 343(30), 205(39), 137 (80)

Simiarenol(R2)

Mp: 190~192°; ^1H - NMR (CDCl_3 , ppm):

3.48(1H, m), 5.63(1H, m), 0.82(3H, d, C_{29}), 0.89(3H, d, C_{30}); ^{13}C - NMR (CDCl_3 , ppm): 122.30, 143.00; Mass, m/z (relative intensity, %): 426(M^+ , 30), 274(100), 159(85), 231(50)

Simiarenol acetate

^1H - NMR (CDCl_3 , ppm): 5.53(1H, m), 4.69 (1H, m), 2.01(3H, s, OCOCH_3)

β -Amyrin(R3)

Mp: 196~197°; ^1H - NMR (CDCl_3 , ppm): 1.074(3H, s), 0.803(3H, s), 0.795(3H, s), 1.013(3H, s), 1.00(3H, s), 0.975(3H, s); ^{13}C - NMR (CDCl_3 , ppm): 38.1(1), 27.8(2), 78.9 (3), 39.5(4), 55.7(5), 19.3(6), 33.2(7), 40.2 (8), 48.3(9), 37.3(10), 23.3(11), 121.6(12), 144.8(13), 42.1(14), 26.6(15), 27.8(16), 33.1 (17), 48.8(18), 46.6(19), 32.3(20), 34.3(21), 38.1(22), 29.3(23), 16.1(24), 16.0(25), 16.9 (26), 26.7(27), 28.1(28), 33.3(29), 23.8(30); Mass, m/z (relative intensity): 426(M^+), 218, 203

α -Amyrin(R3)

Mp: 181~182°; ^1H - NMR (CDCl_3 , ppm): 5.13(1H, t), 3.23(1H, t); ^{13}C - NMR (CDCl_3 , ppm): 38.7(1), 27.3(2), 78.1(3), 38.1(4), 18.9(6), 33.1(7), 40.9(8), 47.0(9), 36.3(10), 23.0(11), 124.8(12), 140.1(13), 42.3(14), 28.1(15), 26.9(16), 34.3(17), 58.0(18), 38.9 (19), 38.8(20), 31.9(21), 41.6(22), 29.0(23), 15.3(24), 15.2(25), 16.2(26), 23.0(27), 28.0 (21), 17.8(29), 21.0(30); Mass, m/z (relative intensity, %): 426(M^+) 218, 203; IR $\nu_{\text{max}}^{\text{cm}^{-1}}$: 1630(C=C)

Uvaol(R4)

Mp: 228~230°; ^1H - NMR (CDCl_3 , ppm): 3.21(1H, m), 3.47(m), 5.15(m); ^{13}C - NMR (CDCl_3 , ppm): 138.8, 125.0, 79.0, 69.9, 55.2, 54.1, 47.7, 47.6, 42.4, 42.2, 40.1, 39.5, 39.4, 38.8, 38.0, 37.0, 35.4, 32.9, 31.0, 30.7, 28.1, 27.3, 26.1, 23.7, 23.6, 23.4, 21.3, 18.4, 17.4, 16.8, 15.7, 15.6; Mass, m/z (relative intensity %): 442(M^+) 234, 203; IR $\nu_{\text{max}}^{\text{cm}^{-1}}$: 1630(C=C), 3300(OH)

Diacetyl uvaol

Mp: 209~210°; ¹H-NMR (CDCl₃, ppm): 2.02(6H, s), 3.85(2H, d,d), 4.48(2H, d,d)

Ursolic acid(R5)

Mp: 292~293°; ¹³C-NMR (CDCl₃, ppm): 38.1(1), 23.2(2), 79.3(3), 38.7(4), 52.0(5), 18.2(6), 34.1(7), 38.3(8), 48.1(9), 36.3(10), 23.0(11), 125.2(12), 137.8(21), 42.3(14), 29.9(15), 22.5(16), 46.3(17), 54.9(18), 31.2(19), 30.8(20), 27.4(21), 37.7(22), 24.0(23), 17.4(24), 17.2(25), 15.5(26), 24.1(27), 176.3(28), 22.0(29), 23.3(30); Mass, *m/z*(relative intensity %): 456(M⁺), 247, 203, 189, 133; IR $\nu_{\max}^{\text{cm}^{-1}}$: 2600, 1680 (COOH)

Methyl ursolate

Mp: 168~170°; IR $\nu_{\max}^{\text{cm}^{-1}}$: 1710, 1165, 3300; Mass, *m/z*(relative intensity, %): 470, 262, 203, 189, 133; ¹H-NMR (CDCl₃, ppm): 3.20(1H, d,d), 3.58(3H, s), 5.20(1H, m); ¹³C-NMR (CDCl₃, ppm): 38.0(1), 28.1(2), 79.1(3), 39.4(4), 55.9(5), 19.1(6), 33.1(7), 40.3(8), 47.7(9), 37.7(10), 23.9(11), 125.1(12), 138.8(21), 42.3(14), 28.9(15), 24.7(16), 49.1(17), 51.9(18), 40.0(19), 39.8(20), 30.2(21), 36.1(22), 28.8(23), 15.1(24), 15.8(25), 17.1(26), 24.1(27), 178.2(28), 17.3(29), 21.0(30)

Methyl ursolate acetate

Mass, *m/z*(relative intensity %): 512(M⁺), 262, 203, 189, 133; ¹H-NMR (CDCl₃, ppm): 3.52(3H, s), 4.43(1H, m), 5.17(1H, m); ¹³C-NMR (CDCl₃, ppm): 179.9, 177.0, 169.9, 124.4, 137.2

Oleanolic acid(R5)

Mp: 298~300°; ¹H-NMR (CDCl₃, ppm): 3.20(1H, m), 2.83(1H, d,d), 5.28(1H, s);

¹³C-NMR (CDCl₃, ppm): 38.2(1), 27.0(2), 77.5(3), 39.0(4), 55.5(5), 18.3(6), 33.2(7), 39.0(8), 47.5(9), 36.9(10), 22.7(11), 123.0(12), 145.2(21), 42.1(14), 27.9(15), 23.0(16), 46.3(17), 41.5(18), 46.1(19), 30.8(20), 34.1(21), 33.6(22), 29.0(23), 16.3(24), 15.0(25), 17.4(26), 25.3(27), 180.7(28), 32.9(29), 23.6(30)

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