생 약 학 회 지
Kor. J. Pharmacogn.
17(3): 195~198(1986)

Isolation of β-Peltoboykinolic Acid from *Rodgersia podophylla*

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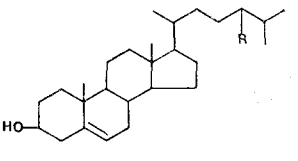
Abstract—From the rhizomes of *R. podophylla* (Saxifragaceae) β-peltoboykinolic acid, mp 249~51°, was isolated and characterized by spectral data. β-Sitosterol and campesterol were also identified.

Keywords—*Rodgersia podophylla* · Saxifragaceae · triterpene · β-peltoboykinolic acid · sterol · β-sitosterol · campesterol

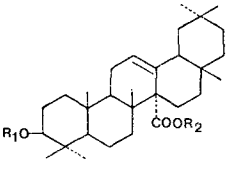
The rhizome of *Rodgersia podophylla* A. Gray (Saxifragaceae) is used as an antipyretic analgesic to headache and boneache, and an antiseptic to malignant sores, enteritis and bacillary dysentery[1]. The phytochemical studies on the *Rodgersia* plants have afforded mono- and diglycosides of kaempferol and quercetin[2]. We report here the results of our investigation of *R. podophylla*.

Column chromatographic separation of the chloroform soluble fraction of the methanol extract gave compounds **1** and **2** in the order of elution. Compound **1**, mp 136~8°, showed positive Liebermann-Burchard reaction and shows hydroxy band at 3,420cm^{-1} and double bond at 1,640, 842 and 796cm^{-1} in its ir spectrum, indicating characteristic of sterols. GC/MS analysis of this compound showed that it was a mixture of two sterols: a major sterol, **1a** (RRt 1.59), accounting for 97% of the mixture, and a minor sterol, **1b** (RRt 1.29), accounting for only 3% of the mixture. The mass spectrum of the major component (**1a**) gave a molecular ion peak at *m/z* 414 and abundant fragments at *m/z* 396, 381, 273, 255, 231, 213 and 81. This fragmentation pattern was consistent with that of authentic β-sitosterol, and was also in agreement with the literature[3,4]. The mass spectrum of the minor component (**1b**) indicated that the fragmentation pattern was similar to that of **1a**: *m/z* 400(M^+), 385, 382, 367, 273, 255, 231, 213 and 81, indicating the presence of 24-CH_3 sterol rather than 24-C_2H_5 one[4]. This result suggested that the minor component (**1b**) seems to be campesterol. The nmr spectrum of this mixture showed two angular methyl singlets at δ0.67 (H-18) and 1.00 (H-19) and three secondary methyl doublets at δ0.91 (H-21), 0.81 and 0.83 (H-26/27) ppm. This spectrum was found to be identical to that of authentic β-sitosterol[3]. Compound **1** was therefore identified as a mixture of β-sitosterol and campesterol in a ratio of 97% and 3%.

Compound **2**, mp 249~51°, showed positive Liebermann-Burchard test (violet) and absorption



1 R = C_2H_5 (**1a**)
R = CH_3 (**1b**)



2 R_1 = R_2 = H
3 R_1 = Ac, R_2 = H
4 R_1 = H, R_2 = CH

bands at 3,470cm^{-1} (OH), 1,685cm^{-1} (COOH) and 1,629, 840, 829 and 814cm^{-1} due to trisubstituted double bond in its ir spectrum. It gave a monoacetate (3), mp 242°, on acetylation with Ac_2O/pyridine and a monomethyl ester (4), mp 111~9° and 164~5° (double mp) by methylation with CH_2N_2. The nmr spectrum of 2 showed seven tertiary methyl singlets at δ0.77 ~1.03ppm, a double doublet at δ3.21 (J=9.7 and 5.8Hz) assignable to a C-3 methine proton and a triplet at δ5.68 (J=2.3 Hz) due to an olefinic proton of the trisubstituted double bond. The mass spectrum of 2 revealed a molecular ion peak at m/z 456 with a base peak at m/z 190 and abundant fragments at m/z 248 (81.4%) and 203 (52.1%) due to characteristic retro Diels-Alder fragmentation[5]. These results suggested that 2 is an oleanen group with one hydroxyl at C-3 and one carboxyl in D/E rings. The carboxyl group in 2 seems to be located in β, γ-position to the double bond because 2 readly loses carbon dioxide on melting[6~8]. This result further supported by the fact that the chemical shift of an olefinic proton was downfield shifted to δ5.68ppm[8,9] and the resonances of the olefinic carbons (C-12; 126.29ppm and C-13; 137.60ppm) were consistent with the reported for the olean-12-en-27-oic acid series[9]. Therefore the compound 2 would be 3β-hydroxyolean-12-en-27-oic acid (β-peltoboykinolic acid[10]) and direct comparison with an authentic sample established its identity (mmp*, tlc, ms and nmr). This is the first report of the isolation of β-peltoboykinolic acid from the genus *Rodgersia*. From the chemotaxonomic point of view, it is of interest to note that triterpenoids possessing olean-12-en-27-oic acid series were forund only in the genera *Cinchona*[7], *Peltoboykinia*[10], *Astilbe*[8], *Cornula-ca*[11] and *Cordia*[9]. The presence of β-peltoboykinolic acid (2) has hitherto been found in Saxifragaceous plants[8,10].

* There is a discrepancy between the mp of our sample and that recorded in literature[10]. The mp of the authentic sample donated by Prof. Nagai showed 250~2° and no depression in mixed mp with our sample.

Experimental

General experimental procedures—Melting points were determined on a Mitamura-Riken apparatus and are uncorrected. Ir spectra were recorded on a Perkin-Elmer 283B spectrophotometer. ^{1}H and ^{13}C-nmr spectra were recorded on a Varian FT-80A spectrometer and are given in ppm (δ) downfield from an internal TMS standard. Mass spectra were determined on a Hewlett-Packard 5985B GC/MS instrument at 70eV using direct inlet system. The GC/MS system was used with a OV-101 on fused silica capillary column (12m, 280°). Cholesterol was used as an internal standard. Optical rotations were obtained on a Rudolph Autopol III autometic polarimeter in $CHCl_3$ solution.

Plant material—The *R. podophylla* used was from a collection made in the spring of 1985 at Mt. Kae Bang, Kangwon province. A voucher specimen (CMK-103) is available for inspection at the herbarium of the Department of Pharmacy, Kangwon National University, Korea.

Extraction and isolation—Air-dried rhizomes (3kg) was exhaustively extracted with MeOH. The MeOH extract was partitioned between $CHCl_3$ and H_2O. The aqueous layer was extracted with BuOH. The $CHCl_3$ soluble fraction was chromatographed on silica gel and eluted with benzene-ether (5:1). Fraction 5 was concentrated and crystallized from MeOH to give compound 1 as needles. Fractions 6 and 7 were combined, concentrated and recrystallized from acetone to afford compound 2 as needles.

Compoun 1—mp 136~8°, $[\alpha]_D^{18}$ −29.6° (c, 0.3)

ir ν_{max}^{KBr} cm^{-1} 3420, 1057 (OH), 1640, 842, 796 (double bond). ^{1}H-nmr ($CDCl_3$, TMS) δ 0.67

(3H, s, H-18), 0.81 (3H, d, J=6.67Hz, H-26 or H-27), 0.83 (3H, d, J=6.75Hz, H-26 or H-27), 0.91 (3H, d, J=6.20Hz, H-21), 1.00 (3H, s, H-19), 3.47 (1H, bs, 3-OH), 3.50 (1H, m, H-3), 5.34 (1H, bd. J=4.2Hz, H-6).

GC/MS, m/z (rel. int.) **1a**: 414 (M^+, 48.1), 399 (M^+-CH_3, 16.8), 396 (M^+-H_2O, 37.4), 381 (399-H_2O, 20.6), 329 (M^+-C_6H_{13}, 9.9), 303 (M^+-$C_7H_{11}O$, 9.2), 273 (M^+-sc, 10.7), 255 (M^+-sc-H_2O, 28.2), 231 (M^+-sc-C_3H_6, 18.3), 229 (M^+-sc-C_3H_8, 15.3), 213 (231-H_2O, 40.5), 81 (100), (sc=side chain). **1b**: 400 (M^+, 72.2), 385 (M^+-CH_3, 17.5), 382 (M^+-H_2O, 44.0), 367 (385-H_2O, 26.5), 315 (M^+-C_6H_{13}, 13.2), 289 (M^+-$C_7H_{11}O$, 13.4), 273 (M^+-sc, 14.5), 255 (M^+-sc-H_2O, 35.0), 231 (M^+-sc-C_3H_6, 19.6), 229 (M^+-sc-C_3H_8, 15.3), 213 (231-H_2O, 44.4), 81 (100).

Compound 2—mp 249~51°, $[\alpha]_D^{22}$+119° (c, 0.1) (Lit.[10]), mp 220~222°, $[\alpha]_D^{25}$+114°)

ir ν_{max}^{KBr} cm^{-1} 3470 (OH), 1685(COOH), 1629, 840, 829, 814 (double bond). ^{1}H-nmr ($CDCl_3$, TMS) δ 0.77 (3H, s, CH_3), 0.84 (9H, s, 3×CH_3), 0.96(6H, s, 2×CH_3), 1.03 (3H, s, CH_3), 3.21(1H, dd, J=5.8 and 9.7Hz, H-3), 5.68 (1H, t, J=2.3Hz, H-12).

ms, m/z (rel. int.) 456 (M^+, 6.4), 438 (M^+-H_2O, 9.3), 412 (M^+-CO_2, 6.4), 397(412-CH_3, 10.0), 248(D/E ring, 81.4), 234(11.4), 233 (248-CH_3, 8.6), 208 (40.0), 207 (A/B ring, 43.6), 203 (248-COOH, 52.1), 190(100), 189(22.1), 179(21.3), 175(42.1), 135(60.0), 133 (28.6).

Acetylation of 2—To a crude sample of **2** Ac_2O/pyridine (1ml each) was added and the mixture was allowed to stand overnight at room temperature. The reaction mixture was poured onto crushed ice, filtered and subjected to silica gel column chromatography eluting with hexane-acetone (0 to 5%) to yield **3**. **3** was crystallized from MeOH to give needles (30mg).

mp 242°, $[\alpha]_D^{22}$+125.5° (c, 0.2) (Lit.[10]), mp 219~222°, $[\alpha]_D^{25}$+111°).

ir ν_{max}^{KBr} cm^{-1} 1719, 1282(OAc), 1696(COOH).

^{1}H-nmr ($CDCl_3$, TMS) δ 0.84 (15H, s, 5×CH_3), 0.98 (3H, s, CH_3), 1.03(3H, s, CH_3), 2.04 (3H, s, OAc), 4.49 (1H, dd, J=7.3 and 8.7Hz, H-3), 5.69 (1H, t, J=3Hz, H-12). ^{13}C-nmr ($CDCl_3$, TMS) δ 38.22 (C-1), 23.59 (C-2), 80.83 (C-3), 40.01 (C-4), 55.20 (C-5), 18.26 (C-6), 32.92 (C-7), 37.08 (C-8*), 49.28 (C-9), 37.68 (C-10*), 22.85 (C-11$^+$), 126.29 (C-12), 137.60 (C-13), 55.99 (C-14), 22.35 (C-15$^+$), 27.71 (C-16), 32.92 (C-17), 47.20 (C-18), 44.11 (C-19), 31.03 (C-20), 34.44 (C-21), 36.34 (C-22), 28.19 (C-23), 16.74 (C-24), 16.43 (C-25), 18.03 (C-26), 179.81 (C-27), 28.19 (C-28), 33.37 (C-29), 23.59 (C-30), 170.98 (CH_3**C**O), 21.15 (**C**H_3CO), *,+ interchangeable.

ms, m/z (rel. int.) 498 (M^+, 0.9), 483 (M^+-CH_3, 0.1), 480 (M^+-H_2O, 2.3), 465 (480-CH_3, 0.1), 454 (M^+-CO_2, 1.7), 438 (M^+-HOAc, 8.8), 423 (438-CH_3, 3.1), 249 (A/B ring, 18.9), 248 (D/E ring, 57.6), 203 (248-CCOH, 38.1), 190 (100), 189 (249-HOAc, 28.4), 175 (43.0), 147 (33.2), 135 (28.5), 133 (22.4).

Methylation of 2—A crude sample of **2** was methylated with etherial CH_2N_2, concentrated and chromatographed on silica gel. Elution with hexane-acetone (1 to 5%) gave **4** and crystallized from EtOH as fine needles (40mg).

mp 111-9° and 164~5° (double mp), $[\alpha]_D^{22}$+142.5° (c, 0.41) (Lit.[10]), mp 211~3°, $[\alpha]_D^{27}$+137°). ir ν_{max}^{KBr} cm^{-1} 3390 (OH), 1722 (ester). ^{1}H-nmr ($CDCl_3$, TMS) δ 0.76 (3H, s, CH_3), 0.83 (9H, s, 3×CH_3), 0.94 (6H, s, 2×CH_3), 1.00 (3H, s, CH_3), 3.20 (1H, dd, J=6.4 and 9.3Hz, H-3), 3.65 (3H, s, OCH_3), 5.60 (1H, t, J=3.0Hz, H-12). ms, m/z (rel. int.) 470 (M^+, 10.7), 455 (M^+-CH_3, 0.9), 452 (M^+-H_2O, 2.6), 411 (M^+-CH_3COO, 14.1), 410 (M^+-HOAc, 3.9), 262 (D/E ring, 92.2), 250 (45.1), 248 (14.5), 247 (C2-CH_3,

28.5), 221 (20.0), 208 (35.6), 207 (A/B ring, 58.4), 203 (262-$COOCH_3$, 65.0), 190 (100), 189 (207-H_2O, 34.5), 175 (47.5), 145 (25.5), 135 (38.0).

Acknowledgement—The authors thank Professor M. Nagai (Hoshi University, Japan) for the authentic sample of β-peltoboykinolic acid.

〈Received August 12, 1986: Accepted September 3, 1986〉

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