Two New 15,16-Seco-cycloartane Glycosides from Cimicifuga Rhizome

Makiko NISHIDA,[a] Hitoshi YOSHIMITSU,*,[a] Masafumi OKAWA,[b] Tuyoshi IKEDA,[b] and Toshihiro NOHARA[b]

[a] *Faculty of Engineering, Kyushu Kyoritsu University; 1–8 Jiyugaoka Yahata-nishi-ku, Kitakyushu 807–8585, Japan: and* [b] *Faculty of Pharmaceutical Sciences, Kumamoto University; 5–1 Oe-honmachi, Kumamoto 862–0973, Japan.*

Received May 30, 2003; accepted August 7, 2003

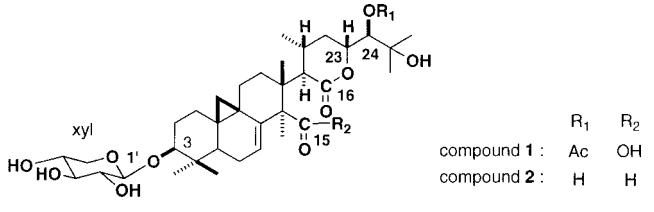
Two new 15,16-seco-cycloartane glycosides (1, 2) were isolated from Cimicifuga Rhizome. Their structures were determined by spectroscopic analysis.

Key words Cimicifuga Rhizome; cycloartane glycoside; *Cimicifuga* sp.; Ranunculaceae

Our study of the chemical constituents in Ranunculaceous plants resulted in the isolation of two new 15,16-seco-cycloartane glycosides (**1**, **2**) from Cimicifuga Rhizome. Cimicifuga Rhizome, originated from a rhizome of the genus *Cimicifuga* plants, has been used as anti-inflammatory, analgesic and antipyretic remedies in Chinese traditional medicine. This paper describes the structural elucidation of the new cycloartanes based on 2D NMR spectroscopic analysis and hydrolysis. The H_2O fraction of the MeOH extract was separated by MCI gel CHP20P, Sephadex-LH20 and silica gel column chromatographies and finally HPLC to give two compounds **1** and **2**.

Compound (**1**) was obtained as a white needle, $[\alpha]_D$ −31.2° (MeOH). The molecular formula of **1** was determined as $C_{37}H_{56}O_{12}$ by high resolution (HR)-FAB-MS showing a $[C_{37}H_{56}O_{12}Na]^+$ ion at *m/z* 715.3678. One cyclopropane methylene at δ 0.57 (d, J=3.7 Hz) and 1.18 (d, J=3.7 Hz), six quaternary methyls at δ 1.06, 1.33, 1.61, 1.67, 1.96 and 1.98, a secondary methyl at δ 1.05 (J=6.8 Hz), an acetyl methyl at δ 2.10, an olefinic proton at δ 5.86 (br d, J=6.8 Hz) and an anomeric proton at δ 4.88 (d, J=7.6 Hz) on the ^{1}H-NMR spectrum suggested **1** to be a cycloartane glycoside. On acid hydrolysis, **1** afforded D-xylose, the structure of which was confirmed by the ^{1}H-NMR coupling pattern and optical rotation using chiral detection in the HPLC analysis, together with several unidentified artificial sapogenols. Thirty carbon signals due to the aglycon part observed along with a xylose and an acetyl unit in the ^{13}C-NMR spectrum. The structural assignment was achieved by ^{1}H–^{1}H correlation spectroscopy (COSY), ^{1}H-detected heteronuclear multiple quantum coherence (HMQC) and heteronuclear multiple bond connection (HMBC) experiments. The ^{1}H–^{1}H COSY and HMBC led us to the plane structure of **1** as an 24-acetoxy-15,16-seco-cycloart-7-en 3-*O*-xyloside (Fig. 1). The long-range correlations between an acetyl methyl proton (δ 2.10) and an acetyl carbon (δ 170.9); H-24 (δ 5.38) and C-25 (δ 71.6) and an acetyl carbon (δ 170.9); two singlet methyl protons (δ 1.61, 1.67) and C-25 (δ 71.6) and C-24 (δ 79.7) indicated the terminal structure on the side chain. Furthermore, the long-range correlation cross-peaks between H-28 (δ 1.96) and C-13 (δ 43.9), C-14 (δ 57.1), C-8 (δ 144.3) and C-15 (δ 177.8); H-18 (δ 1.98) and C-12 (δ 33.2), C-13 (δ 43.9), C-17 (δ 56.7) and C-14 (δ 57.1); H-17 (δ 2.88) and C-18 (δ 23.0), C-20 (δ 27.6), C-13 (δ 43.9) and C-16 (δ 173.2); H-23 (δ 5.35) and C-16 (δ 173.2) resulted in the C–C bond cleavage between C-15 and C-16, and the six-membered lactone ring between C-16 and C-23. The nuclear Overhauser effect (NOE) correlations, H-29/H-3 and H-5, H-30/H-19, H-19/H-18, H-18/H-22β, H-20/H-22β and H-23, H-21/H-22α and H-17, H-28/H-17, H-23/H-27 and H-24/H-26 in the NOESY and NOEDS spectrum, suggested 3*S*, 13*R*, 14*R*, 17*R*, 20*R* and 23*R* configurations. The anomeric center of the xylose moiety was determined to be β-configuration from the large $^3J_{H1-H2}$ value. The $^4C_1$-conformation of xylose was shown by comparison of the carbon resonances for monosaccharide. From the above evidence, the structure of **1** was elucidated except for the stereo configuration at C-24.

Compound (**2**) was obtained as a white needle, $[\alpha]_D$ −39.8° (MeOH). The molecular formula of **2** was determined as $C_{35}H_{54}O_{10}$ by HR-FAB-MS showing a $[C_{35}H_{54}O_{10}Na]^+$ ion at *m/z* 657.3622. The ^{1}H-NMR spectrum of **2** and **1** were almost identical, with the remarkable difference being the H-24 (δ 3.76) signal which was 1.62 ppm upfield, the appearance of the aldehyde (δ 9.85) signal and the disappearance of the acetyl signal. Meanwhile, in the ^{13}C-NMR spectrum of **2**, the signals due to the A-ring of the aglycon moiety and the sugar moiety were in good agreement with those of **1**. On acid hydrolysis, **2** afforded D-xylose together with several unidentified artificial sapogenols. Furthermore, the HMBC showed long-range correlations between H-24 (δ 3.76) and C-25 (δ 72.5); two singlet methyl protons (δ 1.69, 1.74) and C-25 (δ 72.5) and C-24 (δ 80.0); H-28 (δ 1.62) and C-13 (δ 43.2), C-14 (δ 59.6), C-8 (δ 140.4) and C-15 (δ 200.4); H-18 (δ 1.56) and C-12 (δ 31.3), C-13 (δ 43.2), C-17 (δ 55.6) and C-14 (δ 59.6); H-17 (δ 2.74) and C-18 (δ 22.2), C-20 (δ 28.2), C-13 (δ 43.2) and C-16 (δ 173.8); H-23 (δ 5.15) and C-16 (δ 173.8). The above data suggested that **2** was accompanied with the disappearance of an acetyl group at C-24 and the presence of an alde-



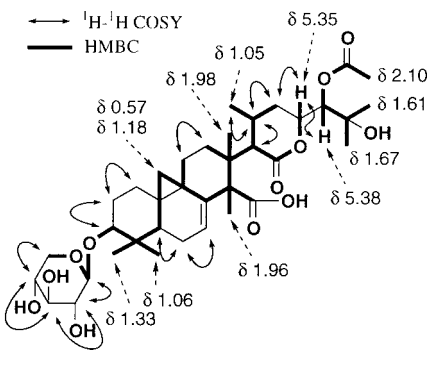


Fig. 1. ^{1}H–^{1}H COSY and HMBC Correlations of **1**

* To whom correspondence should be addressed. e-mail: yoshimit@kyukyo-u.ac.jp

hyde group at C-15 of **1**. The NOE correlations, H-29/H-3 and H-5, H-30/H-19, H-19/H-18, H-18/H-15 and H-22β, H-20/H-22β and H-23, H-21/H-22α and H-17, H-28/H-17, H-23/H-27 and H-24/H-26, indicated that C-3, C-13, C-14, C-17, C-20 and C-23 of **2** had the same configurations as **1** had, respectively.

The 14*R* configuration of **1** was supported by the following ^{1}H-NMR data. The H-18 and H-20 signals, which were observed at δ 1.56 and 2.18 in **2**, were shifted to lower field to appear at δ 1.98 and 2.43, respectively, in **1** (Fig. 2). Meanwhile, the 24*R* configuration of **1** determined that the signal due to H-22α (δ 1.56) was shifted upfield by 0.37 ppm and the signal due to H-23 (δ 5.35) shifted downfield by 0.15 ppm in the latter compound, in a comparative study of the ^{1}H-NMR spectrum of **1** with that of **2**. These shifts were caused by the carbonyl group of an acetyl group at C-24 in **1** (Fig. 2). A similar shift pattern was observed in the 24-*epi*-24-*O*-acetyl-7,8-didehydrohydro-shengmanol 3-*O*-β-D-xylopyranoside (24*R*-type), and not in the C-24 epimer (24*S*-type).[1)] Furthermore, the 24*R* configuration of **2** was determined by the following evidence. **2** had the same NOE correlations of H-23/H-27 and H-24/H-26 and the coupling constants of H-24 (br s) as **1** had. The signal due to H-27 (δ 1.74) in **2** was shifted to lower field by 0.13 ppm as compared with that in **1**, which must be caused by the C-24 hydroxyl group.

These new cycloartane glycosides have structural peculiarities, namely, C–C bond cleavage between C-15 and C-16. Meanwhile, the 24-*O*-acetylhydroshengmanol xyloside showed slow chemical equilibration between the hemiketal form and its keto-alcohol form in solution.[2)] On treatment with heating at 50 °C for 1 h in MeOH, 24*R*- and 24*S*-24-*O*-acetyl-7,8-didehydrohydroshengmanol xylosides yielded several unidentified artificial glycosides, respectively. But the HPLC and TLC analyses didn't show the presence of **1** and **2** in these artificial glycosides. Accordingly, the 24-*O*-acetylhydroshengmanol analogous having a six-membered hemiketal ring between C-16 and C-23, a hydroxyl group at C-15 and an acetyl group at C-24[1)] might biosynthetically cause C–C bond cleavage between C-15 and C-16.

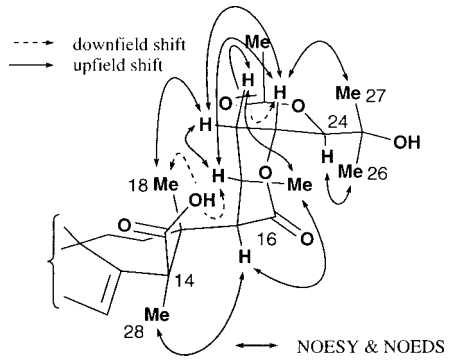


Fig. 2. NOE Correlations of **1** in Pyridine-d_5

Acknowledgements We are grateful to Prof. H. Okabe and Dr. J. Kinjo in Department of Pharmaceutical Sciences, Fukuoka University, for measurements of HR FAB-MS.

References and Notes

1) Kusano A., Takahira M., Shibano M., Miyase T., Kusano G., *Chem. Pharm. Bull.*, **47**, 511—516 (1999).
2) Sakurai N., Kimura O., Inoue T., Nagai M., *Chem. Pharm. Bull.*, **29**, 955—960 (1981).
3) ^{1}H-NMR spectrum of **1** (in pyridine-d_5) δ: 1.36, 1.71 (each 1H, H-1), 2.00, 2.38 (each 1H, H-2), 3.53 (1H, dd, J=3.8, 11.3 Hz, 3-H), 1.36 (1H, H-5), 1.64, 1.95 (each 1H, H-6), 5.86 (1H, br d, J=6.8 Hz, H-7), 1.40, 2.10 (each 1H, H-11), 1.92 (2H, H-12), 2.88 (1H, d, J=3.0 Hz, H-17), 1.98 (3H, s, H-18), 0.57, 1.18 (each 1H, d, J=3.7 Hz, H-19), 2.43 (1H, m, H-20), 1.05 (3H, d, J=6.8 Hz, H-21), 1.56 (1H, dd, J=11.6, 13.8 Hz, H-22), 2.18 (1H, dd, 6.7, 13.8 Hz, H-22), 5.33 (1H, br d, J=11.6 Hz, H-23), 5.38 (1H, br s, H-24), 1.67 (3H, s, H-26), 1.61 (3H, s), 1.96 (3H, s, H-28), 1.33 (3H, s, H-29), 1.06 (3H, s, H-30), 2.10 (3H, s, Ac), 4.88 (1H, d, J=7.6 Hz, xyl H-1), 4.05 (1H, dd, J=7.6, 8.7 Hz, xyl H-2), 4.18 (1H, dd, J=8.7, 8.7 Hz, xyl H-3), 4.26 (1H, m, xyl H-4), 3.77 (1H, dd, J=10.3, 11.4 Hz, xyl H-5), 4.40 (1H, dd, J=5.0, 11.4 Hz, xyl H-5). ^{13}C-NMR spectrum of **1** (in pyridine-d_5) δ: 31.0 (C-1), 29.7 (C-2), 88.1 (C-3), 40.5 (C-4), 41.4 (C-5), 22.4 (C-6), 118.0 (C-7), 144.3 (C-8), 20.2 (C-9), 28.8 (C-10), 25.0 (C-11), 33.2 (C-12), 43.9 (C-13), 57.1 (C-14), 177.8 (C-15), 173.2 (C-16), 56.7 (C-17), 23.0 (C-18), 28.6 (C-19), 27.6 (C-20), 25.3 (C-21), 36.1 (C-22), 75.7 (C-23), 79.7 (C-24), 71.6 (C-25), 26.6 (C-26), 28.2 (C-27), 24.8 (C-28), 25.7 (C-29), 14.1 (C-30), 21.0 (Ac), 170.9 (Ac), 107.5 (xyl C-1), 75.6 (xyl C-2), 78.7 (xyl C-3), 71.3 (xyl C-4), 67.2 (xyl C-5).
4) ^{1}H-NMR spectrum of **2** (in pyridine-d_5) δ: 1.27, 1.69 (each 1H, H-1), 1.97, 2.33 (each 1H, H-2), 3.49 (1H, dd, J=3.9, 11.2 Hz, 3-H), 1.29 (1H, H-5), 1.54, 1.93 (each 1H, H-6), 5.28 (1H, br d, J=6.4 Hz, H-7), 1.29, 2.09 (each 1H, H-11), 1.84 (2H, H-12), 9.85 (1H, s, H-15), 2.74 (1H, d, J=4.4 Hz, H-17), 1.56 (3H, s, H-18), 0.51, 0.92 (each 1H, d, J=3.6 Hz, H-19), 2.18 (1H, m, H-20), 1.02 (3H, d, J=6.8 Hz, H-21), 1.93 (1H, dd, J=11.6, 13.0 Hz, H-22), 2.13 (1H, dd, 6.2, 13.0 Hz, H-22), 5.15 (1H, br d, J=11.6 Hz, H-23), 3.76 (1H, br s, H-24), 1.69 (3H, s, H-26), 1.74 (3H, s, H-27), 1.62 (3H, s, H-28), 1.31 (3H, s, H-29), 1.05 (3H, s, H-30), 4.87 (1H, d, J=7.3 Hz, xyl H-1), 4.06 (1H, dd, J=7.3, 8.7 Hz, xyl H-2), 4.18 (1H, dd, J=8.7, 8.7 Hz, xyl H-3), 4.26 (1H, m, xyl H-4), 3.77 (1H, dd, J=10.2, 11.0 Hz, xyl H-5), 4.40 (1H, dd, J=5.1, 11.0 Hz, xyl H-5). ^{13}C-NMR spectrum of **2** (in pyridine-d_5) δ: 31.1 (C-1), 29.6 (C-2), 88.0 (C-3), 40.5 (C-4), 40.7 (C-5), 22.6 (C-6), 122.2 (C-7), 140.4 (C-8), 19.1 (C-9), 28.7 (C-10), 25.0 (C-11), 31.3 (C-12), 43.2 (C-13), 59.6 (C-14), 200.4 (C-15), 173.8 (C-16), 55.6 (C-17), 22.2 (C-18), 28.8 (C-19), 28.2 (C-20), 25.0 (C-21), 36.5 (C-22), 78.3 (C-23), 80.0 (C-24), 72.5 (C-25), 26.1 (C-26), 29.4 (C-27), 18.9 (C-28), 25.6 (C-29), 14.0 (C-30), 107.6 (xyl C-1), 75.6 (xyl C-2), 78.7 (xyl C-3), 71.3 (xyl C-4), 67.2 (xyl C-5).