Two New Tetranor-Cycloartane Glycosides from Cimicifuga Rhizome

Makiko NISHIDA,[a] Hitoshi YOSHIMITSU,*,[a] 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 June 4, 2003; accepted July 11, 2003

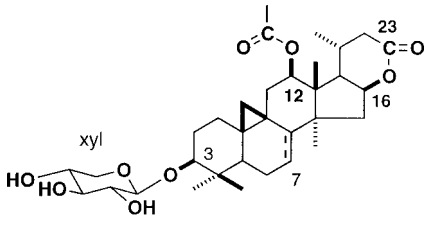
Two new tetranor-cycloartane glycosides (1, 2) were isolated from Cimicifuga Rhizome. Their structures were determined by spectroscopic analysis. These compounds suggested the existence of a biogenetic pathway into C-23 lactone-type cycloartane glycosides.

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 tetranor-cycloartane glycosides (**1**, **2**), together with cimicifugoside (0.085%)[1] and actein (0.008%)[1] from Cimicifuga Rhizome. This paper describes the structural elucidation of the tetranor-cycloartanes based on two dimensional (2D) NMR spectroscopic analysis and hydrolysis, and the biogenetic pathway into C-23 lactone-type cycloartane glycosides from cimicifugoside and actein. The $CHCl_3$ 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** (0.0004%) and **2** (0.0002%).

Compound (**1**) was obtained as a white powder, $[\alpha]_D$ −75.0° (MeOH). The molecular formula of **1** was determined as $C_{33}H_{50}O_9$ by high resolution (HR)-FAB-MS showing a $[C_{33}H_{50}O_9Na]^+$ ion peak at *m/z* 613.3348. One cyclopropane methylene at δ 0.21 (d, J=4.3 Hz) and 0.58 (d, J=4.3 Hz), four quaternary methyls at δ 0.86, 1.02, 1.25 and 1.33, a secondary methyl at δ 0.98 (J=6.1 Hz), an acetyl methyl at δ 2.14 and an anomeric proton at δ 4.86 (d, J=7.3 Hz) on the ^{1}H-NMR spectrum of **1** were very similar to those of actein except for the side chain. A comparative study of the ^{13}C-NMR spectrum of **1** with that of actein indicated that **1** was a tetranor-cycloartan 3-*O*-β-D-xyloside with an acetoxyl group at C-12, resulting from a loss of four carbons, C-24, C-25, C-26 and C-27 of actein. On acid hydrolysis (refluxed with 2 N hydrochloric acid for 1 h), **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. 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 connectivity (HMBC) experiments. The ^{1}H–^{1}H COSY and HMBC led us to the plane structure of **1** as an 12-acetoxy-tetranor-cycloartan 3-*O*-xyloside. The long-range correlation cross-peaks between an acetyl methyl proton (δ 2.14) and an acetyl carbon (δ 170.5); H-12 (δ 5.08) and an acetyl carbon (δ 170.5); H-16 (δ 4.81) and C-23 (δ 173.5) resulted in the acetoxyl group at C-12 and the six-membered lactone ring between C-23 and C-16. The nuclear Overhauser effect (NOE) correlations, H-29/H-3 and H-5, H-30/H-19, H-19/H-8 and H-18, H-18/H-8, H-15β and H-20, H-17/H-12 and H-16, H-21/H-17 and H-22α, H-28/H-12, H-15α and H-17 and H-15α/H-16 in the NOESY and NOEDS spectrum, suggested 3*S*, 12*R* and 16*S* configurations. From the above evidence, the structure of **1** was determined to be 12β-acetoxy-3β-hydroxy-24,25,26,27-tetranor-cycloartan-23,16β-olide 3-*O*-β-D-xylopyranoside.

Compound (**2**) was obtained as a white powder, $[\alpha]_D$ −134.9° (MeOH). The molecular formula of **2** was determined as $C_{33}H_{48}O_9$ by HR-FAB-MS showing a $[C_{33}H_{48}O_9Na]^+$ ion peak at *m/z* 611.3188. The ^{1}H-NMR spectrum of **2** and **1** were almost identical, with the appearance of an olefinic proton signal at δ 5.12. In the ^{13}C-NMR data of **2**, signals due to the aglycon moiety, except for the signals of the A, B and C rings, and the sugar moiety were in good agreement with those of **1**. The above evidence indicated that **2** was a 7-en analogous of **1**. Furthermore, in the HMBC, the methyl proton signal at δ 1.06 (H-28) showed long-range correlation with δ 147.2 (C-8). The coupling patterns and constants of the H-3 (dd, J=4.0, 11.6 Hz), the H-12 (dd, J=3.8, 9.2 Hz) and the H-16 (ddd, J=3.9, 8.1, 8.5 Hz) suggested 3*S*, 12*R*



compound **1** : 7,8-2H
compound **2** : Δ^5

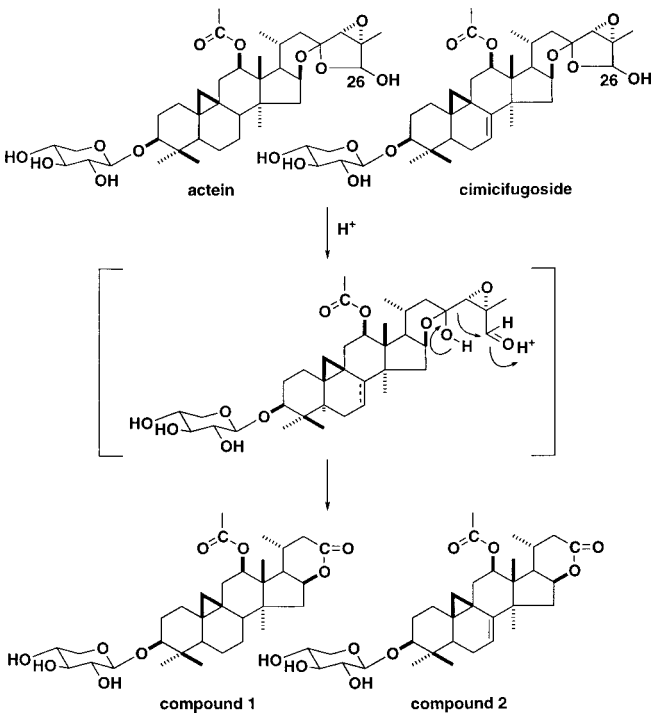


Chart 1

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

and 16*S* configurations, respectively. Consequently, the structure of **2** was determined to be 12β-acetoxy-3β-hydroxy-24,25,26,27-tetranor-cycloart-7-en-23,16β-olide 3-*O*-β-D-xylopyranoside.

Actein and cimicifugoside were stable in MeOH at 50 °C for 1 h, which had nothing to form artifacts. Firstly, MCI gel CHP20P column chromatography with MeOH–H_2O of the $CHCl_3$ fraction furnished tetranor-cycloartan-type glycosides (**1**, **2**) and 24,26-oxygenated cycloartan-type glycosides (actein and cimicifugoside) in the separate fractions. Compounds **1** and **2** were obtained on treatment of actein and cimicifugoside with 1% hydrochloric acid, together with several unidentified artificial sapogenols, respectively. Accordingly, compounds **1** and **2** might be biosynthetically derived from genuine glycosides of the 24,26-oxygenated cycloartan-type such as actein and cimicifugoside through Chart 1. A similar biogenetic pathway from 23,26-oxygenated spirostane-type glycosides to C-22 lactone-type glycosides has been proposed by Nafady *et al.*[4)]

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., In Y., Ishida T., Miyase T., Kusano G., *Chem. Pharm. Bull.*, **46**, 467—472 (1998).

2) ^{1}H-NMR spectra of **1** (in pyridine-d_5) δ: 1.14, 1.53 (each 1H, H-1), 1.91, 2.28 (each 1H, H-2), 3.47 (1H, dd, *J*=4.3, 11.6 Hz, 3-H), 1.27 (1H, H-5), 0.72, 1.49 (each 1H, H-6), 0.95, 1.25 (each 1H, H-7), 1.62 (1H, dd, *J*=5.2, 11.6 Hz, H-8), 1.17 (1H, dd, *J*=3.8, 16.2 Hz, H-11), 2.72 (1H, dd, *J*=8.9, 16.2 Hz, H-11), 5.08 (1H, dd, *J*=3.8, 8.9 Hz, H-12), 1.83 (1H, dd, *J*=5.2, 13.5 Hz, H-15), 2.00 (1H, dd, *J*=8.2, 13.5 Hz, H-15), 4.81 (1H, ddd, *J*=5.2, 8.2, 8.3 Hz, H-16), 2.14 (1H, dd, *J*=8.3, 11.0 Hz, H-17), 1.25 (3H, s, H-18), 0.21, 0.58 (each 1H, d, *J*=4.3 Hz, H-19), 2.01 (1H, m, H-20), 0.98 (3H, d, *J*=6.1 Hz, H-21), 2.27 (1H, dd, *J*=13.2, 14.6 Hz, H-22), 2.48 (1H, dd, *J*=3.2, 14.6 Hz, H-22), 0.86 (3H, s, H-28), 1.33 (3H, s, H-29), 1.02 (3H, s, H-30), 2.14 (3H, s, Ac), 4.86 (1H, d, *J*=7.3 Hz, xyl H-1), 4.04 (1H, dd, *J*=7.3, 8.7 Hz, xyl H-2), 4.17 (1H, dd, *J*=8.7, 8.7 Hz, xyl H-3), 4.25 (1H, m, xyl H-4), 3.75 (1H, dd, *J*=10.3, 11.1 Hz, xyl H-5), 4.37 (1H, dd, *J*=5.0, 11.1 Hz, xyl H-5). ^{13}C-NMR spectra of **1** (in pyridine-d_5) δ: 32.0 (C-1), 30.0 (C-2), 88.1 (C-3), 41.3 (C-4), 47.1 (C-5), 20.5 (C-6), 25.7 (C-7), 46.0 (C-8), 20.3 (C-9), 27.0 (C-10), 36.5 (C-11), 76.7 (C-12), 48.7 (C-13), 48.4 (C-14), 43.9 (C-15), 80.4 (C-16), 53.7 (C-17), 13.4 (C-18), 29.7 (C-19), 26.9 (C-20), 22.1 (C-21), 38.7 (C-22), 173.5 (C-23), 19.6 (C-28), 25.8 (C-29), 15.4 (C-30), 21.5 (Ac), 170.5 (Ac), 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).

3) ^{1}H-NMR spectra of **2** (in pyridine-d_5) δ: 1.14, 1.58 (each 1H, H-1), 1.89, 2.27 (each 1H, H-2), 3.45 (1H, dd, *J*=4.0, 11.6 Hz, 3-H), 1.20 (1H, dd, *J*=5.5, 12.6 Hz, H-5), 1.57, 1.87 (each 1H, H-6), 5.12 (1H, br d, *J*=6.8 Hz, H-7), 1.26 (1H, dd, *J*=3.8, 16.0 Hz, H-11), 2.92 (1H, dd, *J*=9.2, 16.0 Hz, H-11), 5.20 (1H, dd, *J*=3.8, 9.2 Hz, H-12), 2.15 (1H, dd, *J*=3.9, 12.9 Hz, H-15), 2.25 (1H, dd, *J*=8.5, 12.9 Hz, H-15), 4.91 (1H, ddd, *J*=3.9, 8.1, 8.5 Hz, H-16), 2.14 (1H, dd, *J*=8.1, 11.2 Hz, H-17), 1.28 (3H, s, H-18), 0.52, 1.04 (each 1H, d, *J*=4.3 Hz, H-19), 2.02 (1H, m, H-20), 0.98 (3H, d, *J*=6.4 Hz, H-21), 2.29 (1H, dd, *J*=13.3, 14.6 Hz, H-22), 2.48 (1H, dd, *J*=3.2, 14.6 Hz, H-22), 1.06 (3H, s, H-28), 1.34 (3H, s, H-29), 1.03 (3H, s, H-30), 2.14 (3H, s, Ac), 4.85 (1H, d, *J*=7.6 Hz, xyl H-1), 4.05 (1H, dd, *J*=7.6, 8.7 Hz, xyl H-2), 4.17 (1H, dd, *J*=8.7, 8.7 Hz, xyl H-3), 4.25 (1H, m, xyl H-4), 3.75 (1H, dd, *J*=10.2, 11.2 Hz, xyl H-5), 4.36 (1H, dd, *J*=5.2, 11.2 Hz, xyl H-5). ^{13}C-NMR spectra of **2** (in pyridine-d_5) δ: 30.3 (C-1), 29.6 (C-2), 87.8 (C-3), 40.5 (C-4), 42.4 (C-5), 21.9 (C-6), 114.5 (C-7), 147.2 (C-8), 21.4 (C-9), 28.5 (C-10), 36.4 (C-11), 76.3 (C-12), 47.9 (C-13), 50.9 (C-14), 42.8 (C-15), 80.5 (C-16), 54.1 (C-17), 14.8 (C-18), 29.1 (C-19), 26.9 (C-20), 22.0 (C-21), 38.6 (C-22), 173.4 (C-23), 26.8 (C-28), 25.8 (C-29), 14.3 (C-30), 21.5 (Ac), 170.6 (Ac), 107.5 (xyl C-1), 75.7 (xyl C-2), 78.7 (xyl C-3), 71.3 (xyl C-4), 67.2 (xyl C-5).

4) Nafady A. M., El-Shanawany M. A., Mohamed M. H., Hassanean H. A.-H., Zhu X.-H., Yoshihira T., Okawa M., Ikeda T., Nohara T., *Tetrahedron Lett.*, **44**, 3509—3511 (2003).