A NOVEL STEROIDAL SAPOGENIN, POGOSTEROL FROM *VERNONIA POGOSPERMA*

Joseph MUNGARULIRE,[a] Raphael M. MUNABU,[b] Chikako MURASAKI,[c] Tadashi EGUCHI,[c] Yoshinori FUJIMOTO,*[,c] Katsumi KAKINUMA,[c] Kimiko KOBAYASHI,[d] Masakazu URAMOTO,[d] and Nobuo IKEKAWA[e]

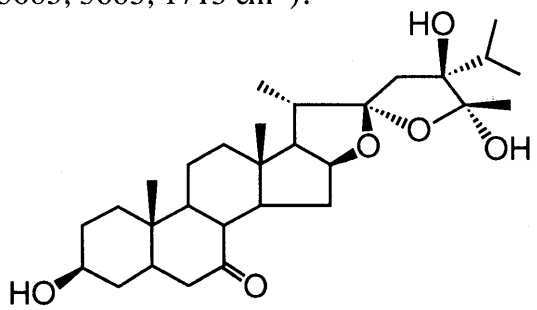
Institut de Recherche Scientifique et Technologique (I.R.S.T.), CURPHAMETRA,[a] B.P. 227 Butare, Rwanda, Chemistry Department, University of Nairobi,[b] Box 30197 Nairobi, Kenya, Department of Chemistry, Tokyo Institute of Technology,[c] Meguro-ku, Tokyo 152, Japan, The Institute of Physical and Chemical Research, (RIKEN),[d] Wako-shi, Saitama 351-01, Japan, College of Science and Engineering, Iwaki Meisei University,[e] Iwaki, Fukushima 970, Japan

The structure and stereochemistry of a novel steroidal sapogenin, pogosterol (**1**), isolated from the leaves of *Vernonia pogosperma* has been established from spectral and single crystal X-ray analysis.

KEYWORDS *Vernonia pogosperma*; Asteraceae; pogosterol; steroidal sapogenin

Vernonia pogosperma (Asteraceae), known as Umbimbafuro in Kinyarwanda, is a shrub growing in the intertropical Africa region,[1] and has been used in Rwanda for the treatment of hepatotoxicity and stomach disorders. In the course of our screening of biologically active substances of tropical plants, chloroform extract of the leaves of this plant was found to exhibit a cytotoxic activity against L-1210 cells. Chromatographic (silica gel and LH-20) separation of the extract and crystallization from methanol furnished the titled steroidal sapogenin (**1**), which we named pogosterol, mp 154-157°C, $[\alpha]_D^{23}$ -91.6° ($CHCl_3$, c=0.43).

The molecular formula of pogosterol (**1**) was assigned as $C_{29}H_{46}O_6$ on the basis of the FAB-MS (*m/z* 491 M+H) and ^{13}C-NMR spectral data, although the sample was hygroscopic and elemental analysis gave varying results depending on drying conditions of the sample. The IR spectrum indicated the presence of hydroxyl and carbonyl groups (3685, 3665, 1715 cm^{-1}).



pogosterol (**1**)

The ^{1}H-NMR spectrum of (**1**) exhibited signals for three tertiary (δ 0.73, 1.09 and1.36) and three secondary (δ 0.95, 0.97 and 1.00) methyl groups, and two oxymethine protons (δ 3.59 and 4.63).[2] The ^{13}C-NMR spectrum showed signals of 29 carbons including one carbonyl carbon (δ 210.98), two acetal carbons (δ 108.15 and 115.27), and three oxygenated carbons (δ 70.55, 81.70 and 81.29).[3] These data were reminiscent of a C_{29} steroidal sapogenin for (**1**). Analysis of the NMR data with the known steroids[4] suggested that pogosterol has a 7-oxo-3,16-dioxy-steroidal nucleus, which was subsequently supported by long-range C-H COSY NMR experiments. These experiments also indicated two and three bond connectivity which allowed us to figure out the side chain structure (Fig. 1). The mass fragment ions,[5] characteristic of steroidal sapogenins,[6] *e.g*, ions at *m/z* 316 (C-20/C-22 and C-16/C16-O cleavage), 287 (C-17/C-20 and C-16/C16-O cleavage-H) corroborated with the structure. Unexpectedly the signal (δ 2.06) ascribable to the C-23 methylene protons was observed as a sharp singlet in $CDCl_3$. By changing the NMR solvent to benzene-d_6, however, the signal was altered to an expected AB type [δ 2.0 and 2.4 (*J*=10 Hz)].

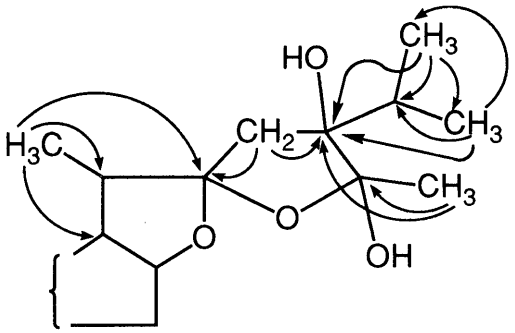


Fig. 1. Two and Three Bond Connectivity Revealed by Long-Range C-H COSY NMR Experiments

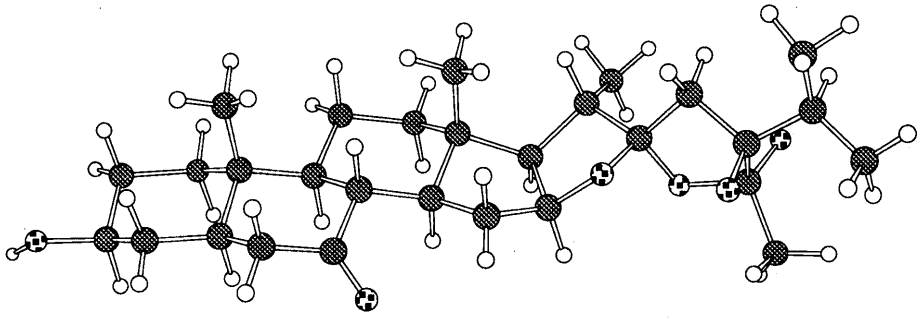


Fig. 2. Crystal Structure of Pogosterol (**1**)

At this stage, it seemed appropriate not only to propose a plain structure for (**1**), but to establish stereochemical configurations of the chiral centers on the side chain, and we undertook an X-ray diffraction analysis on a crystal obtained from methanol.[7] The molecular structure and relative stereochemistry of (**1**) was unequivocally established and is shown in Fig. 2. 22*S*, 24*S* and 28*R*-stereochemistry was inferred by assuming the absolute configuration of natural steroids.

Spirostane and furanospirostane are well-known steroidal sapogenin skeletons. To our knowledge pogosterol (**1**) is the first example that bears a new sapogenin skeleton, *i.e.*, C16-C22 and C22-C28 ether

linkage. Pogosterol exhibited a weak cytotoxic activity (IC_{50}: 1.7 μg/ml) against L-1210 cells *in vitro*. GC-MS analysis of a sterol fraction separated from the chloroform extract indicated the occurrence of less common Δ^7-stigmasterol and Δ^7-sitosterol in addition to stigmasterol and sitosterol. This seems quite suggestive of the biogenetic relationships between Δ^7-sterols and 7-oxo-steroid such as (**1**).

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REFERENCES AND NOTES

1) G.Troupin, "Flore du Rwanda (Spermatophytes)", Vol. 3, AGECOOP/INRS, Rwanda, 1985.

2) ^{1}H-NMR (270 MHz, $CDCl_3$) δ: 0.73 (s, 18-H_3), 0.95 (d, *J*=6.7 Hz, 26-H_3), 0.97 (d, *J*=7.0 Hz, 27-H_3), 1.00 (d, *J*=6.7 Hz, 21-H_3), 1.09 (s, 19-H_3), 1.36 (s, 29-H_3), 2.06 (s, 23-H_2), 2.37 (t, *J*=13.1 Hz, 6β-axial-H), 2.49 (t , *J*=11.3 Hz, 8β-axial-H), 2.59 (m, 15-H), 3.59 (m, 3α-axial-H), 4.00 (brs, 28-OH), 4.59 (brs, 24-OH), 4.63 (q, *J*=7.4 Hz, 16α-H).

3) ^{13}C-NMR (67.5 MHz, $CDCl_3$) δ: 11.90 (C-19), 14.64 (C-27), 15.83 (C-26), 16.30 (C-18), 18.52 (C-21), 21.61 (C-11), 23.41 (C-29), 31.03 (C-2), 31.26 (C-25), 32.25 (C-15), 36.08 (C-1), 36.08 (C-10), 37.88 (C-4), 38.58 (C-12), 38.58 (C-20), 40.68 (C-13), 43.51 (C-23), 45.88 (C-6), 46.58 (C-5), 48.50 (C-14), 49.29 (C-8), 55.04 (C-9), 60.23 (C-17), 70.55 (C-3), 81.29 (C-24), 81.70 (C-16), 108.15 (C-28), 115.27 (C-22), 210.98 (C-7).

4) H. Eggert and C. Djerassi, *J. Org. Chem.*, **38**, 3788 (1973); J. W. Blunt and J. B. Stothers, *Org. Mag. Res.*, **9**, 439 (1977).

5) EI-MS of (**1**) *m/z*: 454 (M-2H_2O), 436 (M-3H_2O), 430, 412, 385, 287 and 43.

6) C. Djerassi and W. H. Faul, *Org. Mass Spectrometry*, **3**, 1187 (1970).

7) Crystal data: $C_{29}H_{46}O_6$•CH_3OH, monoclinic, space group $P2_1$, *a*=17.280 (2), *b*=6.3414 (8), *c*=14.096 (1) Å, *β*=110.10(1)°, *V*=1451 (5) Å^3, Z=2, Dc=1.197 gcm^{-3}, λ(Cu Kα)=1.54184, μ=6.4 cm^{-1}, F(000)=572, R=0.059 for 2324 unique reflections with $|F_0|>3\sigma(F_0)$. The structure was solved by the direct method using MULTAN78. The positions for the protons of hydroxyls at C-24 and C-28 were not determined by the X-ray analysis.

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