556.
10. Djerassi, C., v. Mutzenbecher, G., Fajkos, J., Williams, D. H. and Budzikiewicz, H. (1965) *J. Am. Chem. Soc.* **87**, 817.
11. Kirson, I., Glotter, E., Abraham, A. and Lavie, D. (1970) *Tetrahedron* **26**, 2209.
12. Budzikiewicz, H. and Djerassi, C. (1962) *J. Am. Chem. Soc.* **84**, 1430.
13. Giral, F. and Rivera. C. (1975) *Phytochemistry* **14**, 793.
14. Labarrère, J. A., Chipault, J. R. and Lundberg, W. O. (1958) *Analyt. Chem.* **30**, 1466.

Phytochemistry, Vol. 27, No. 6, pp. 1895 1896, 1988.
Printed in Great Britain.

0031-9422/88 $3.00+0.00

AN ACYLATED SITOSTEROL GLUCOSIDE FROM *ALISMA PLANTAGO-AQUATICA*

GENG PEI-WU,* YOSHIYASU FUKUYAMA,† WANG REI,* BAO JINXIAN* and KAZUYUKI NAKAGAWA

Tokushima Research Institute, Otsuka Pharmaceutical Co. Ltd, 463-10 Kagasuno, Kawauchi-cho, Tokushima 771-01, Japan

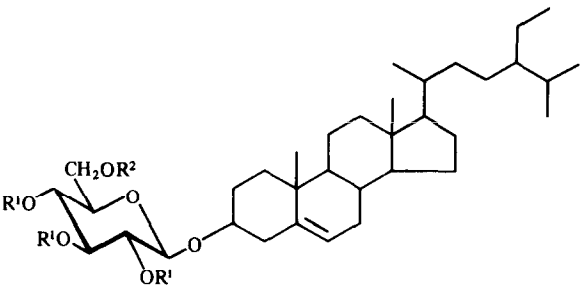
(*Received* 26 *October* 1987)

Key Word Index—*Alisma Plantago-aquatica*; Alismataceae; sitosterol-3-*O*-6-stearoyl-β-D-glucopyranoside; sitosterol; methyl stearate.

Abstract—A phytosterol glucoside acylated with stearic acid has been isolated from the methanol extract of the rhizome of *Alisma Plantago-aquatica*, and its structure has been determined as sitosterol-3-*O*-6-stearoyl-β-D-glucopyranoside by spectroscopic data and chemical conversions.

In a previous paper [1] we reported the structures of protostane-type triterpenoids, 16β-methoxy and 16β-hydroxyalisol B monoacetates isolated from the rhizome of *Alisma Plantago-aquatica* L. var. *orientale* Samuels (Alismataceae) [2]. A further study on the constituents of this medicinal plant has now resulted in the isolation of a new sitosterol glucoside acylated with stearic acid.

The IR, ^{1}H and ^{13}C NMR spectra of compound **1** displayed the presence of hydroxy (3400 cm^{-1}) and ester groups, and a sugar moiety, in addition to a saturated fatty acid residue. Methanolysis of **1** afforded two products. The less polar one was identified as methyl stearate by the ^{1}H NMR and mass spectra, whereas the polar product was identified as sitosterol-3-*O*-β-D-glucopyranoside by comparison with spectral data of the authentic sample after a conventional acetylation. This chemical evidence suggested that the stearic acid in **1** should be bonded to a hydroxy group of the glucose moiety in **3**. The ^{1}H NMR spectrum of **1** revealed the two double doublet signals (δ4.25, *dd*, $J=12.2$, 2.5 Hz and 4.52, *dd*, $J=12.2$, 4.0 Hz) which corresponded to the H-6 methylene group in the glucose moiety. Since these signals were not greatly shifted (δ 4.12 and 4.24) on acetyl-



1 R^1 = H, R^2 = $C(=O)(CH_2)_{16}Me$
2 R^1 = Ac, R^2 = $C(=O)(CH_2)_{16}Me$
3 $R^1 = R^2$ = H
4 $R^1 = R^2$ = Ac

*Present address: Beijing Institute of Pharmaceutical Industries, 12 Dog Qiao Road, Chao Yang Men Wei, Beijing, China.
†Author to whom correspondence should be addressed.

ation. This result clearly supported the bond of stearic acid via an ester linkage to the hydroxyl at C-6 in the glucose moiety. Thus, the structure of **1** was determined to be sitosterol-3-*O*-6-stearoyl-β-D-glucopyranoside.

This is the first isolation of a phytosterol acyl glycoside from the title plant although *A. Plantago-aquatica* elaborates a number of protostane-type triterpenoids [1, 2] and sesquiterpenes [3].

EXPERIMENTAL

^1H (200 MHz) and ^{13}C NMR (50.3 MHz); $CDCl_3$: TMS as int. standard; CC: silica gel (Wakogel C-300); TLC: precoated silica gel plates F_{254} (Merck, 0.25 mm). Spots were visualized by 40% $CeSO_4$–H_2SO_4.

Plant material. Alisma Plantago-aquatica was identified by Wang Rei. A voucher specimen has been deposited at Beijing Institute of Pharmaceutical Industries, China.

Extraction and isolation. Air-dried and powdered rhizome (5 kg) of *A. Plantago-aquatica* collected in China was extracted with MeOH (30 l) at room temp. for 29 days. The MeOH extract was evapd *in vacuo* to give a crude extract (400 g), 170 g of which was separated by CC on silica gel using a CH_2Cl_2–MeOH gradient into 11 fractions: fr.1 (CH_2Cl_2, 100%), frs 2–4 (CH_2Cl_2–MeOH, 19:1), frs 5–7 (CH_2Cl_2–MeOH, 9:1), frs 8, 9 (CH_2Cl_2–MeOH, 4:1), fr. 10 (CH_2Cl_2–MeOH, 2:1), fr. 11 (CH_2Cl_2–MeOH, 1:1). Fr 7 (5.6 g) was further chromatographed on Sephadex LH-20 (MeOH) followed by CC on silica gel ($CHCl_3$–MeOH, 10:1) to afford sitosterol-3-*O*-6-stearoyl-β-stearoyl-β-D-glucopyranoside **1** (40 mg) as a colourless material; $[\alpha]_D^{21} - 53.3°$ (*c* 1.5; $CHCl_3$); IR $\nu_{max}^{CHCl_3}$ cm^{-1}: 3400 (OH), 1720 (C=O); ^1H NMR: δ 0.68 (3H, *s*), 0.85–0.97 (15 H, 5 × Me), 1.01 (3H, *s*), 1.25 (*br s*), 4.25 (1H, *dd*, *J* = 12.2, 2.5 Hz, H-6′), 4.39 (1 H, *d*, J = 7.7 Hz, H−1′), 4.52 (1 H, *dd*, *J* = 12.2, 4.0 Hz, H−6′), 5.37 (1 H, *m*, H-6); ^{13}C NMR: δ 11.92 (*q*), 12.02 (*q*), 14.16 (*q*), 18.86 (*q*), 19.10 (*q*), 19.44 (*q*), 19.86 (*q*), 22.75 (*t*), 23.15 (*t*), 25.09 (*t*), 29.25 (*d*), 29.25–30.02 (*t*), 32.00 (*t*), 32.14 (*d*), 32.04 (*t*), 36.75 (*d*), 36.76 (*s*), 39.05 (*t*), 39.68 (*t*), 42.41 (*s*), 45.90 (*d*), 50.25 (*d*), 56.31 (*d*), 56.67 (*d*), 63.77 (*t*), 70.59 (*d*), 73.42 (*d*), 73.76 (*d*), 76.44 (*d*), 79.86 (*d*), 101.40 (*d*), 122.08 (*d*), 140.48 (*s*), 174.15 (*s*).

Acetylation of **1**. A mixture of **1** (4 mg), Ac_2O (2 drops) and pyridine (0.5 ml) was stood at room temp. for 12 hr. Usual work-up afforded **2** (4.1 mg) as a colourless powder; ^1H NMR: δ 0.67 (3 H, *s*), 0.98 (3 H, *s*), 0.79–0.95 (15 H, 5 × Me), 1.26 (*br s*), and 2.01, 2.02 and 2.05 (each 3 H, *s*, OAc), 2.32 (2 H, *t*, *J* = 7.6 Hz), 3.50 (1 H, *m*, H-3), 3.67 (1 H, *ddd*, *J* = 9.5, 5.5, 2.9 Hz, H-5′), 4.12 (1 H, *dd*, *J* = 12.1, 2.9 Hz, H-6′), 4.24 (1 H, *dd*, *J* = 12.1, 5.5 Hz, H-6′), 4.58 (1 H, *d*, *J* = 8.0 Hz, H-1′), 4.95 (1 H, *dd*, *J* = 9.5, 8.0 Hz, H-2′), 5.05 (1 H, *dd*, *J* = 9.5, 9.5 Hz, H-4′), 5.21 (1 H, *dd*, *J* = 9.5, 9.5 Hz, H-3′), 5.36 (1 H, *m*, H-6).

Methanolysis of **1**. To a soln of **1** (11 mg) in dry MeOH (1 ml) was added NaOMe (0.5 mg) and the reaction mixture was stirred at room temp. under an argon atmosphere for 2 hr. H_2O (2 ml) was added and then extracted with EtOAc. The combined organic layers were washed with brine, dried over $MgSO_4$ and the evapn of solvent left a residue (11 mg), which was purified by CC on silica gel eluting with $CHCl_3$–MeOH (15:1) to give **3**, and methyl stearate; MS *m/z*: 298 [M^+]. The compound **3** was subjected to an acetylation (Ac_2O–pyridine) and usual work-up afforded **4**, which was identical with sitosterol-2,3,4,6-tetraacetyl-β-D-glucopyranoside in the spectral data (^1H NMR, IR and mass spectra).

REFERENCES

1. Geng, P. W., Fukuyama, Y., Yamada, T., Wang, R., Bao, J. and Nakagawa, K. (1988) *Phytochemistry* (in press).
2. Murata, T., Imai, Y., Hirata, K. and Miyashita, M. (1970) *Chem. Pharm. Bull.* (Tokyo)**18**, 1347.
3. Oshima, H., Iwakawa, T. and Hikino, H. (1983) *Phytochemistry* **22**, 183.