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A SPIROSTANOL GLYCOSIDE FROM AERIAL PARTS OF *DIOSCOREA TENUIPES**

SHIU KIYOSAWA, KATSUMI GOTO, KENJI SAKAMOTO and TOSHIO KAWASAKI†‡

Kyoto College of Pharmacy, Nakauchi-cho Misasagi Yamashina-ku, 607 Kyoto, Japan; †Faculty of Pharmaceutical Sciences, Kyushu University, Maedashi 3-1-1, Higashi-ku, 812 Fukuoka, Japan

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Key Word Index—*Dioscorea tenuipes*; Dioscoreaceae; structure determination; ^{1}H NMR; spirostan-tetrol glycoside; 1β-hydroxydiotigenin 1-*O*-arabinoside.

Abstract—A new spirostanol glycoside isolated from aerial parts of *Dioscorea tenuipes* was characterized as 5β,25-L-spirostan-1β,2β,3α,4β-tetrol (1β-hydroxydiotigenin or 4β-hydroxyneotokorogenin) 1-*O*-α-L-arabinopyranoside on the basis of chemical and ^{1}H NMR spectral data.

INTRODUCTION

From the fresh aerial parts of *Dioscorea tenuipes* Franch et Sav. collected during September and October at the Ozeki-path in the suburbs of Otsu City, 17 new steroid compounds have so far been isolated together with several known substances such as diotigenin (**1**), neotokorogenin (**2**), tenuipegenin§ taraxerol and phytosterols[2]. Sixteen of the new compounds were characterized as, for example, a glycoside of **2**, acylates of **1**, and the corresponding furostanol-, 20,22-secofurostanol- and pregnane-glycosides[2–10]. This paper describes the structure determination of the seventeenth compound, compound S_2 (**3**)[2].

RESULTS AND DISCUSSION

The IR spectrum of compound S_2 (**3**) showed the characteristic absorptions of the 25-L-spirostane nucleus[11–13] whilst the ^{1}H NMR spectrum of peracetate (**4**) exhibited six acetoxy signals and a one-proton doublet assignable to an anomeric sugar proton. Compound **3** was hydrolysed with acid to give L-arabinose and an aglycone $C_{27}H_{44}O_6$ (**5**). Therefore, **3** was a monoarabinoside of **5** which was different from its congener tenuipegenin, a 25-L-spirostan-tetrol[1, 2], in mp and $[\alpha]_D$, and was regarded as its isomer.

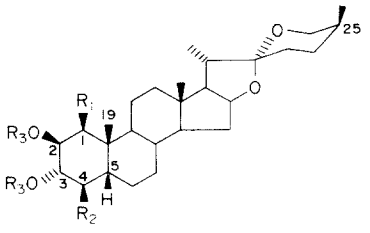
Permethylation of **3** followed by methanolysis of the resulting hexamethylate (**6**) provided methyl 2,3,4-tri - *O* - methyl - L - arabinopyranoside and a trimethyl ether (**7**) of **5**. Compound **7** gave a monoacetate (**8**) and a monoketone (**9**) on acetylation and oxidation, respectively. The ^{1}H NMR spectral data of **7–9** were compared with those[14] of the trimethyl ether (**10**) and 2,3-dimethyl ether (**11**) of tokorogenin (**12**), the 1-acetate (**13**) and 1-dehydro compound (**14**) of **11**, and the trimethyl ether (**15**) of **1** (Table 1). A three-proton singlet ascribable to H_3-19 of **7** was shifted upfield by δ 0.15 from that of **10** and showed the same chemical shift as that of **11**. Furthermore, the δ-value of H_3-19 of **8** was practically identical with those of **13** and **15**. These data indicated the presence of a 1β(axial)-hydroxy group and *cis* juncture of the A/B rings in the spirostane nucleus of **7**[15]. The proton geminal to the 1β-hydroxy and 1β-acetoxy groups, respectively, in **7** and **8** appeared as a doublet (J = 2 Hz) as in the cases of **11** and **13**, and the one-proton doublet (J = 10 Hz) at δ 3.99 in **9** had the same δ and J values as the H-2 of **14**. Therefore, **9** had an α(axial)-H, which was coupled with the 3β(axial)-H, and hence the β(equatorial)-methoxy group at C-2. Two double doublets, both showing J = 9 and 10 Hz, at δ 3.14 and 3.76 and a multiplet at δ 1.39 in the spectrum of **9** were assigned to the protons geminal to the methoxy groups at C-3 and 4 and the angular proton at C-5. By means of the double resonance technique, they were proved, respectively, to be due to 3β(axial)-, 4α(axial)- and 5β(axial)-protons.

Thus, **9** was defined as 2β,3α,4β - trimethoxy - 5β,25 - L - spirostan - 1 - one, **7** as the corresponding 1β-hydroxy compound, and **6** was considered to have a 2,3,4 - tri - *O* - methyl - arabinopyranose moiety combined with the 1-hydroxy group of **7**. The J values of anomeric proton signals on the ^{1}H NMR spectra of **4** and **6** were in good agreement with those of tokoronin peracetate[14] and permethylate[14],

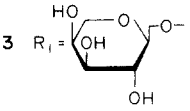
* Part 6 in the series "Constituents of Aerial Parts of *Dioscorea tenuipes*". For Part 5 see ref. [8].

‡ To whom correspondence should be addressed.

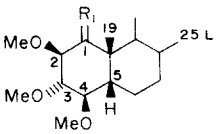
§This compound was isolated from the aerial parts of *D. tenuipes* complex in the Rokko population. It is believed to be a 25 - L - spirostan - tetrol, but the structure has not been established[1].

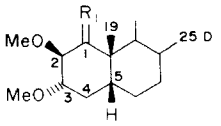


1 $R_1=R_3=H$, $R_2=OH$
2 $R_1=OH$, $R_2=R_3=H$
5 $R_1=R_2=OH$, $R_3=H$



3 $R_1=$ $R_2=OH$, $R_3=H$





7 $R_1=$ OH
8 $R_1=$ OAc
9 $R_1=O$
15 $R_1=H_2$

10 $R_1=$ OMe
11 $R_1=$ OH
13 $R_1=$ OAc
14 $R_1=O$

respectively, indicating α-linkage of the L-arabinopyranose.

Consequently, **3** is 5β,25-L-spirostan-1β,2β,3α,4β-tetrol 1-*O*-α-L-arabinopyranoside.

Compound **3** is a new natural product and the aglycone **5** might be called 4β-hydroxyneotokorogenin or 1β-hydroxydiotigenin, an isomer of compound T_1 (24α-hydroxyisodiotigenin)[10] and also probably of tenuipegenin[1]. It is noted that **1** occurred in the free state and as its acylates but not glycoside, while **2** and **5**, both bearing a 1β-hydroxy group, were present as 1-*O*-arabinosides and not acylates.

EXPERIMENTAL

All mps were uncorr. Optical rotations: 20–28° using a 1 dm cell; ^{1}H NMR: 60 and 100 MHz, $CDCl_3$, TMS as int. standard.

Compound S_2 (**3**). Collected by repeating the procedure shown in Chart 1-B of Part 1[2] of this series. Colourless needles (MeOH), mp 248–249° (dec.), $[\alpha]_D$ − 12.7° (MeOH; *c* 0.75). IR ν_{max}^{KBr} cm^{-1}: 3360 (OH), 987, 915 > 895, 852 (25-L-spirostane). (Found: C, 62.24; H, 9.08. $C_{32}H_{52}O_{10}\cdot H_2O$ requires: C, 62.52; H, 8.85%.)

Hexa-acetate (**4**) *of* **3**. Prepared by acetylation of **3** (110 mg) with Ac_2O–pyridine at room temp. overnight. Colourless plates (129 mg) (MeOH), mp 146–148°, $[\alpha]_D$ − 1.3° ($CHCl_3$; *c* 0.4). IR ν_{max}^{KBr} cm^{-1}: no OH, 1740 (OAc), 987, 916 > 896, 852 (25-L-spirostane); ^{1}H NMR: δ 0.74 (3H, *s*, H-18), 0.97 (3H, *d*, *J* = 6 Hz, H-27), 1.18 (3H, *s*, H-19), 1.20 (3H, *d*, *J* = 6 Hz, H-21), 1.95–2.18 (18H, OAc ×6), 4.57 (1H, *d*, *J* = 6 Hz, H-1 of ara). (Found: C, 62.21; H, 7.60. $C_{44}H_{64}O_{16}$ requires: C, 62.24; H, 7.60%.)

Hydrolysis of **3** *with acid*. Compound **3** (350 mg) in 2 N HCl–50% MeOH (15 ml) was refluxed for 3 hr. The reaction mixture was extracted with *n*-BuOH. The organic layer was evaporated, the residue (120 mg) was passed through a Si gel column (eluent, $CHCl_3$–MeOH–H_2O, 9:1:0.1) and crystallized from MeOH to yield an aglycone (**5**) as colourless needles (87 mg), mp 308–310°, $[\alpha]_D$ − 28.0° (MeOH; *c* 0.5). IR ν_{max}^{KBr} cm^{-1}: 3380 (OH), 985, 923 > 897, 850 (25-L-spirostane). (Found: C, 69.57; H, 9.84. $C_{29}H_{44}O_6$ requires: C, 69.80; H, 9.55%.) The aq. layer was evaporated *in vacuo* to give a syrup, $[\alpha]_D$ + 106.5° (H_2O; *c* 1.2), R_f on Si gel TLC (solvent, $CHCl_3$–MeOH–H_2O, 8:2:0.2): 0.13 ($[\alpha]_D$ and R_f identical with those of L-arabinose).

Permethylation of **3**. Compound **3** (370 mg) was methylated by the Hakomori method[16] and the product was

Table 1. ^{1}H NMR data for compounds **7–11** and **13–15**

	H_3-19	H-1	H-2	H-3	H-4	H-5	OMe	OAc
7	1.14	3.88(*d*) (*J* = 2 Hz)	—	3.39(*dd*) (*J* = 10.5, 9 Hz)	—	—	3.45 3.54 3.61	—
8	0.96	5.43(*d*) (*J* = 2 Hz)	—	—	—	—	3.37 3.59 3.61	2.08
9	1.16	—	3.99(*d*) (*J* = 10 Hz)	3.14(*dd*) (*J* = 9, 10 Hz)	3.76(*dd*) (*J* = 9, 10 Hz)	1.39(*m*)	3.48 3.64 3.64	—
10	1.29	—	—	—	—	—	—	—
11	1.14	3.91(*d*) (*J* = 2 Hz)	3.22(*q*) (*J* = 2.5, 9 Hz)	—	—	—	3.44 3.44	—
13	0.95	5.45(*d*) (*J* = 1 Hz)	—	—	—	—	3.36 3.44	2.08
14	1.14	—	4.00(*d*) (*J* = 10 Hz)	—	—	—	3.43 3.47	—
15	0.97	—	3.10(*m*)	3.37(*t*) (*J* = 10 Hz)	—	—	3.42 3.58 3.63	—

chromatographed on a Si gel column (eluent, *n*-hexane–EtOAc, 1:1) to provide **6** as a glassy mass (403 mg). IR ν_{max}^{KBr} cm^{-1}: no OH, 986, 919 > 892, 850 (25-L-spirostane); ^{1}H NMR: δ 0.77 (3H, *s*, H-18), 0.99 (3H, *d*, J = 6 Hz, H-27), 1.14 (3H, *s*, H-19), 1.20 (3H, *d*, J = 6 Hz, H-21), 3.42–3.62 (18H, MeO ×6), 4.88 (1H, *d*, J = 2 Hz, H-1 of ara).

Methanolysis of **6**. Compound **6** (260 mg) was refluxed with 8% HCl in MeOH for 7 hr. The MeOH was removed *in vacuo*, H_2O added and the mixture was extracted with Et_2O. The extract was evaporated to dryness and the residue (235 mg) was chromatographed on Si gel (eluent, *n*-hexane–EtOAc, 1:1) to give a syrup, which was identified as methyl 2,3,4 - tri - *O* - methyl - α - L - arabinopyranoside (GC run in parallel with an authentic sample), and the methylated aglycone (**7**) as a white powder (MeOH) (140 mg). ^{1}H NMR: Table 1.

Acetate (**8**) *of* **7**. Compound **7** (90 mg) was acetylated and the product was crystallized from *n*-hexane to give **8** as colourless needles (61 mg), mp 192–194°, $[\alpha]_D$ − 20.9° ($CHCl_3$; *c* 0.79). IR ν_{max}^{KBr} cm^{-1}: 1748 (OAc), 987, 918 > 892, 850 (25-L-spirostane). ^{1}H NMR: Table 1. (Found: C, 70.34; H, 9.79. $C_{32}H_{52}O_7$ requires: C, 70.04; H, 9.55%.)

Dehydro-compound (**9**) *of* **7**. Compound **7** (150 mg) in Me_2CO (10 ml) was treated with Jones' reagent (1 ml) at room temp. for 5 min. The mixture was diluted with H_2O and extracted with Et_2O. The extract was evaporated to dryness and the residue was crystallized from MeOH to give **9** as colourless prisms (128 mg), mp 204–206°, $[\alpha]_D$ − 33.6° ($CHCl_3$; *c* 0.61). IR ν_{max}^{KBr} cm^{-1}: 1713 (carbonyl), 983, 923 > 900, 850 (25-L-spirostane). ^{1}H NMR: Table 1. (Found: C, 71.27; H, 9.63. $C_{30}H_{48}O_6$ requires: C, 71.39; H, 9.59%.)

Trimethyl ether (**15**) *of* **1**. Compound **1**[2] (112 mg) was methylated by the Hakomori method. Usual work-up and crystallization from MeOH of the product afforded **15** as colourless needles (75 mg), mp 183–185°, $[\alpha]_D$ − 17.6° ($CHCl_3$; *c* 1.28). ^{1}H NMR: Table 1.

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