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# Studies on the Constituents of the Aerial Parts of *Dioscorea tenuipes* Franch. *et* Savat. IV.[1] 2β,3α,4β-Trihydroxy-5β-pregn-16-en-20-one and Its 2- and 4-Monoacetates

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Three kinds of pregnane derivatives $G_5$, $G_6$, and $G_7$ were isolated from the aerial parts of *Dioscorea tenuipes* Franch. *et* Savat. They were characterized as 2β,3α,4β-trihydroxy-5β-pregn-16-en-20-one (IV) and its 2-(VII) and 4-monoacetates (VIII), respectively, which are corresponding to coexisting diotigenin (I) and its monoacetates (II and III).

**Keywords**——pregnane derivatives; cooccurrence with corresponding spirostane derivatives; *Dioscorea tenuipes*; silica gel chromatog.; NMR

In the preceding papers[1,3] of this series it was reported that the methanol extracts of the fresh aerial parts of *Dioscorea tenuipes* Franch. *et* Savat. contained diotigenin(2β,3α,4β-trihydroxy-5β-25L-spirostane) (I) and its 2- and 4-monoacetates (II and III, respectively) along with other related compounds.

This paper concerns isolation and characterization of three additional minor constituents, tentatively named $G_5$, $G_6$ and $G_7$.

The procedure of isolation is shown in Chart 1.

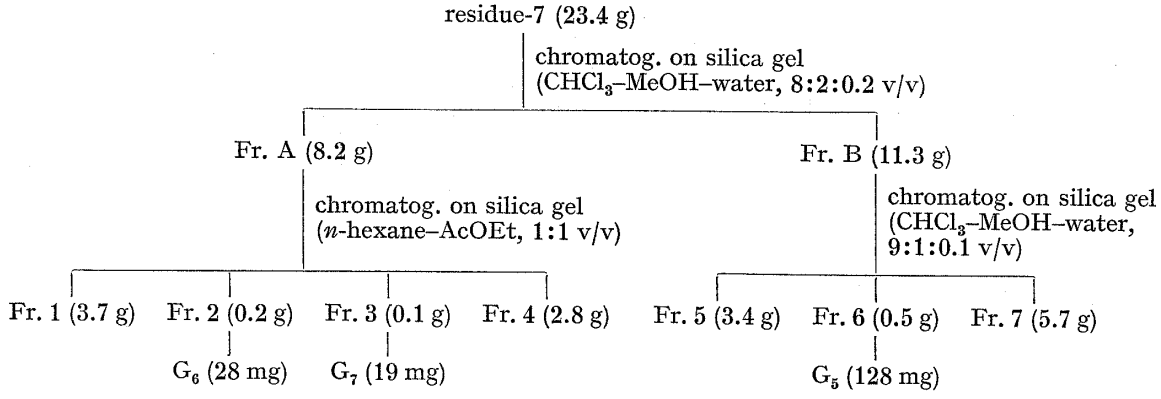


Chart 1

$G_5$ (IV), mp 255—257°, showed on its infrared (IR) spectrum the absorptions of an enone system and on the nuclear magnetic resonance (NMR) spectrum the signals due to one vinyl proton, two tertiary methyl and one acetyl groups. Usual acetylation of IV provided the triacetate (V), mp 185—187°. The NMR spectrum of V exhibits the signals of three protons adjacent to acetoxyl groups, and their chemical shifts and coupling patterns are quite similar to those of I triacetate (VI).[3,4] The above data suggest that IV is most likely to be a trihy-

1) Part III: S. Kiyosawa and T. Kawasaki, *Yakugaku Zasshi*, **95**, 424 (1975).
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3) *a*) S. Kiyosawa and T. Kawasaki, *Yakugaku Zasshi*, **95**, 94 (1975); *b*) *Idem, ibid.*, **95**, 102 (1975).
4) *a*) K. Takeda, T. Okanishi, A. Akahori, and F. Yasuda, *Chem. Pharm. Bull.* (Tokyo), **16**, 421 (1968); *b*) A. Akahori, F. Yasuda, and T. Okanishi, *ibid.*, **16**, 498 (1968).

droxypregn-16-en-20-one corresponding to coexisting I.[5] The structure was colloborated by direct comparison of IV with the authentic sample derived from VI according to the modified Marker method.[6]

Therefore IV is 2$\beta$,3$\alpha$,4$\beta$-trihydroxy-5$\beta$-pregn-16-en-20-one.

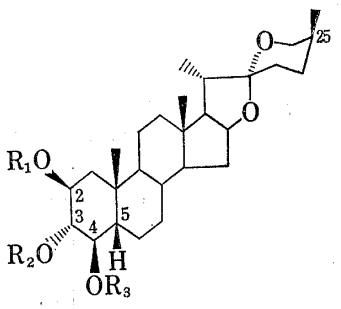
$G_6$ (VII), mp 217—219° (decomp.), was thought to be a monoacetate of IV on the basis of its molecular formula $C_{23}H_{34}O_5$ ($M^+$, *m/e* 390), IR and NMR spectra and the fact that VII was hydrolyzed with 2% KOH in dil. methanol to give IV. On the NMR spectrum of VII the proton adjacent to an acetoxyl group appears as a multiplet at 4.78 ppm, while the protons of two methine groups bearing hydroxyl functions are observed at 3.38 (triplet, $J$=9 Hz) and 3.85 ppm (double doublets, $J$=10, 9 Hz). By comparisons with the spectra of II,[3a] III[3b] and diotigenin 2,4-diacetate,[3a] they are assigned, respectively, to the protons at $C_2$, $C_3$ and $C_4$.

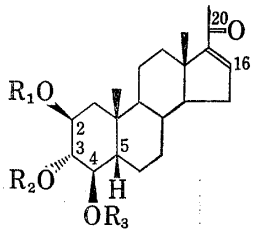
Accordingly VII is considered to be 2-monoacetate of IV.

$G_7$ (VIII), mp 178—181° (decomp.), was hydrolyzed, alike VII, to yield IV, and the molecular formula and the IR and NMR spectra indicate it to be another monoacetate of IV. VIII shows on its NMR spectrum a one-proton double doublets ($J$=10, 9 Hz) at 4.68 ppm, another one-proton triplet ($J$=9 Hz) at 3.42 and a one-proton multiplet at 3.87. In the same way as in the case of VII, they are attributed, respectively, to the proton adjacent to acetoxyl group at $C_4$ and the methine protons at $C_3$ and $C_2$ bearing hydroxyl groups.

Consequently VIII is characterized as 4-monoacetate of IV.

Isolation from *Paris polyphylla* Sm. of a triglycoside of 3$\beta$-hydroxypregna-5,16-dien-20-one together with the corresponding spirostanol glycoside, dioscin, has previously been reported.[7] The present finding of cooccurrence of IV, VII and VIII with I, II and III seems also to be worth of note.




I : $R_1$=$R_2$=$R_3$=H

II : $R_1$=Ac, $R_2$=$R_3$=H

III : $R_1$=$R_2$=H, $R_3$=Ac

VI : $R_1$=$R_2$=$R_3$=Ac

IV : $R_1$=$R_2$=$R_3$=H

V : $R_1$=$R_2$=$R_3$=Ac

VII : $R_1$=Ac, $R_2$=$R_3$=H

VIII : $R_1$=$R_2$=H, $R_3$=Ac

Formulae 1

## Experimental

Melting points were determined on a micro melting point apparatus (Yanagimoto) and are uncorrected. Optical rotations were measured in $CHCl_3$ solution on a NEP-2 (Rex) polarimeter and IR spectra were obtained in a KBr disk with a Shimadzu IR 27C spectrometer. NMR spectra were taken in $CDCl_3$ solution on a Varian

5) 2$\beta$,3$\alpha$,4$\beta$-Triacetoxy-5$\beta$-pregn-16-en-20-one (V), colorless needles (from *n*-hexane), mp 191—193°, $[\alpha]_D$ +74.2° (MeOH), was prepared from VI by Takeda, *et al.*[4a]

6) D.H. Gould, H. Staeudle, and E.B. Hershberg, *J. Am. Chem. Soc.*, **74**, 3685 (1952).

7) T. Nohara, H. Yabuta, M. Suenobu, R. Hida, K. Miyahara, and T. Kawasaki, *Chem. Pharm. Bull.* (Tokyo), **21**, 1240 (1973).

Model A-60A (60 MHz) spectrometer and chemical shifts are given in $\delta$ (ppm) scale with tetramethylsilane as internal standard (s, singlet; t, triplet; d.d., double doublets; m, multiplet; br, broad). Mass spectra were recorded on a RMU 6E (Hitachi) mass spectrometer (ionizing potential, 70 eV; ionizing current, 50 $\mu$A; source temperature, 120—130°). Column chromatography was carried out with Kiesel Gel 60 (70—230 mesh ASTM) (Merck).

**Isolation of $G_5$, $G_6$, and $G_7$**——As described previously (refer to Charts 1, 1-A and 1-B in Part I[3a]) of this series) thirteen homogeneous crystalline compounds and an oil (Fr. 10 in Chart 1) were isolated from the residues 4, 5, and 6 obtained from the MeOH extractives of the aerial parts of *D. tenuipes*. All the remainders of isolation of the above compounds were combined (tentatively named residue 7, 23.4 g from 400 g of the MeOH extractives) and fractionated as shown in Chart 1.

**$G_5$ (2$\beta$,3$\alpha$,4$\beta$-Trihydroxy-5$\beta$-pregn-16-en-20-one) (IV)**——Colorless plates (from AcOEt), mp 255—257°, $[\alpha]_D^{19}$ +58.1° ($c$=0.86). IR $\nu_{max}$ cm$^{-1}$: 3380 (OH), 1672 and 1584 (enone), no spiroketal absorptions. NMR: 0.88 (3H, s, 18-$CH_3$), 1.05 (3H, s, 19-$CH_3$), 2.25 (3H, s, 21-$CH_3$), 4.76 (3H, s, OH×3), 6.68 (1H, d.d., $J$=2, 3 Hz, vinyl proton). Mass Spectrum $m/e$: 348 ($M^+$), 333 ($M^+-CH_3$), 305 ($M^+-CH_3CO$). *Anal.* Calcd. for $C_{21}H_{32}O_4$: C, 72.38; H, 9.26. Found: C, 72.23; H, 9.21.

**Triacetate (V) of IV**——IV (37 mg) was acetylated with pyridine (1 ml) and $Ac_2O$ (1 ml) at room temperature overnight. The product (32 mg) was crystallized from $n$-hexane to give V as colorless needles (18 mg), mp 185—187°, $[\alpha]_D^{16}$ +63.3° ($c$=0.50). IR $\nu_{max}$ cm$^{-1}$: 1745 ($CH_3COO$), 1667 and 1588 (enone). NMR: 0.88 (3H, s, 18-$CH_3$), 1.07 (3H, s, 19-$CH_3$), 1.99 (6H, s, $CH_3COO$×2), 2.03 (3H, s, $CH_3COO$), 2.25 (3H, s, 21-$CH_3$), 4.8—5.6 (3H, $CH_3COOC\underline{H}<$×3), 6.68 (1H, d.d., $J$=2, 3 Hz, vinyl proton) (VI: 0.76 (3H, s, 18-$CH_3$), 1.09 (3H, s, 19-$CH_3$), 2.02 (6H, s, $CH_3COO$×2), 2.04 (3H, s, $CH_3COO$), 4.8—5.6 (3H, $CH_3COOC\underline{H}<$×3)). Mass Spectrum $m/e$: 474 ($M^+$), 459 ($M^+-CH_3$), 431 ($M^+-CH_3CO$), 414 ($M^+-CH_3COOH$), 371 (414−$CH_3CO$), 354 (414−$CH_3COOH$), 294 (354−$CH_3COOH$), 251 (294−$CH_3CO$).

**Modified Marker's Degradation of VI**——VI (320 mg) in $Ac_2O$ (5 ml) was refluxed with $AlCl_3$ (70 mg) for 3 hr. The reaction mixture was cooled, and AcONa (188 mg), AcOH (11 ml), and then $CrO_3$ (560 mg) in AcOH-water (2: 1 v/v) (2 ml) were added under stirring at 10—14°. After further stirring for 20 min at room temperature, $NaHSO_3$ (100 mg) was added and the mixture was concentrated *in vacuo* upto 10 ml, diluted with saline (3 ml), benzene (10 ml) and 13% $K_2CO_3$ solution in water (5 ml), and stirred for 4 hr at room temperature. The benzene layer was separated, washed with water and evaporated. The residue (130 mg) was chromatographed over silica gel (eluent, $n$-hexane–AcOEt 1: 2 v/v) and a homogeneous (thin-layer chromatography) fraction was crystallized from AcOEt to give colorless plates (85 mg), mp 254—257°. They were identified with IV on mixed fusion and by comparisons of IR and NMR spectra.

**$G_6$ (2-Monoacetate of IV) (VII)**——Colorless needles (from AcOEt), mp 217—219° (decomp.), $[\alpha]_D^{20}$ +3.8° ($c$=0.53). IR $\nu_{max}$ cm$^{-1}$: 3380 (OH), 1730 ($CH_3COO$), 1648 and 1583 (enone), no spiroketal absorptions. NMR: 0.88 (3H, s, 18-$CH_3$), 1.05 (3H, s, 19-$CH_3$), 2.08 (3H, s, $CH_3COO$), 2.22 (3H, s, 21-$CH_3$), 2.85 (2H, br, OH×2), 3.38 (1H, t, $J$=9 Hz, $HOC\underline{H}<$), 3.85 (1H, d.d., $J$=10, 9 Hz, $HOC\underline{H}<$), 4.78 (1H, m, $CH_3COOC\underline{H}<$), 6.68 (1H, d. d, $J$=3, 2 Hz, vinyl proton (II: 0.76 (3H, s, 18-$CH_3$), 1.02 (3H, s, 19-$CH_3$), 2.08 (3H, s, $CH_3COO$), 3.41 (1H, t, $J$=9 Hz, $HOC\underline{H}<$), 3.83 (1H, d.d., $J$=10.5, 9 Hz, $HOC\underline{H}<$), 4.84 (1H, m, $CH_3COOC\underline{H}<$). III (in pyridine-$d_5$): 0.83 (3H, s, 18-$CH_3$), 1.02 (3H, s, 19-$CH_3$), 2.05 (3H, s, $CH_3COO$), 4.12 (1H, t, $J$=9 Hz, $HOC\underline{H}<$), 4.51 (1H, m, $HOC\underline{H}<$), 4.75 (2H, br, OH×2), 5.47 (1H, d.d., $J$=10, 9 Hz, $CH_3COOC\underline{H}<$)). Mass Spectrum $m/e$: 390 ($M^+$), 375 ($M^+-CH_3$), 347 ($M^+-CH_3CO$), 330 ($M^+-CH_3COOH$), 287 (330−$CH_3CO$). *Anal.* Calcd. for $C_{23}H_{34}O_5$: C, 70.74; H, 8.78. Found: C, 70.51; H, 8.62. VII (12 mg) was hydrolyzed with 2% KOH in MeOH–water (2: 1 v/v) (3 ml), and the hydrolysate was crystallized from AcOEt to give colorless plates (4 mg), mp 254—256°, identical with IV on mixed fusion.

**$G_7$ (4-Monoacetate of IV) (VIII)**——White powder (from AcOEt) (mp 178—181° (decomp.)), $[\alpha]_D^{21}$ +34.3° ($c$=0.35). IR $\nu_{max}$ cm$^{-1}$: 3380 (OH), 1737 ($CH_3COO$), 1647 and 1583 (enone). NMR: 0.88 (3H, s, 18-$CH_3$) 1.05 (3H, s, 19-$CH_3$), 2.08 (3H, s, $CH_3COO$), 2.25 (3H, s, 21-$CH_3$), 3.42 (1H, t, $J$=9 Hz, $HOC\underline{H}<$), 2.85 (2H, br, OH×2), 3.87 (1H, m, $HOC\underline{H}<$), 4.68 (1H, d.d., $J$=10, 9 Hz, $CH_3COOC\underline{H}<$), 6.68 (1H, d.d., $J$=3, 2 Hz, vinyl proton). Mass Spectrum $m/e$: 390 ($M^+$), 375 ($M^+-CH_3$), 347 ($M^+-CH_3CO$), 330 ($M^+-CH_3COOH$), 287 (330−$CH_3CO$). *Anal.* Calcd. for $C_{23}H_{34}O_5$: C, 70.74; H, 8.78. Found: C, 70.47; H, 8.57. VIII (8 mg) was hydrolyzed in the same way as for VII and the product was crystallized from AcOEt to give colorless plates (3 mg), mp 254—256°, identical with IV.

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