Studies on the Constituents of the Aerial Parts of *Dioscorea tenuiipes* 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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(Received May 6, 1970)

Three kinds of pregnane derivatives G₅, G₆, and G₇ were isolated from the aerial parts of *Dioscorea tenuiipes* 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 tenuiipes*; 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 tenuiipes* Franch. et Savat. contained diotigenin(2β,3α,4β-trihydroxy-5β-255-spirostone) (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 G₅, G₆, and G₇.

The procedure of isolation is shown in Chart 1.

<table>
<thead>
<tr>
<th>Fr. A (8.2 g)</th>
<th>Fr. B (11.3 g)</th>
</tr>
</thead>
<tbody>
<tr>
<td>chromatog. on silica gel (CHCl₃-MeOH—water, 8:2:0.2 v/v)</td>
<td>chromatog. on silica gel (CHCl₃-MeOH—water, 9:1:0.1 v/v)</td>
</tr>
<tr>
<td>Fr. 1 (3.7 g)</td>
<td>Fr. 2 (0.2 g) Fr. 3 (0.1 g) Fr. 4 (2.8 g) Fr. 5 (3.4 g) Fr. 6 (0.5 g) Fr. 7 (5.7 g)</td>
</tr>
<tr>
<td>G₅ (28 mg) G₆ (128 mg)</td>
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</tr>
</tbody>
</table>

Chart 1

G₆ (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 acetoxy 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-

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droxyprogren-16-en-20-one corresponding to coexisting I.\textsuperscript{5) The structure was collaborated by direct comparison of IV with the authentic sample derived from VI according to the modified Marker method.\textsuperscript{6)  

Therefore IV is 2β,3α,4β-trihydroxy-5β-pregn-16-en-20-one.

\( \text{G}_6 \) (VII), mp 217—219\degree (decomp.), was thought to be a monoacetate of IV on the basis of its molecular formula \( \text{C}_{23}\text{H}_{34}\text{O}_5 \) (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\textsuperscript{8a), III\textsuperscript{8b) and diotigenin 2,4-diacetate,\textsuperscript{9a} 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.

\( \text{G}_7 \) (VIII), mp 178—181\degree (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 \textit{Paris polyphylla} Sm. of a triglycoside of 3β-hydroxypregna-5,16-dien-20-one together with the corresponding spirostanol glycoside, dioscin, has previously been reported.\textsuperscript{7) The present finding of cooccurrence of IV, VII and VIII with I, II and III seems also to be worth of note.

\begin{center}
\begin{tabular}{ll}
I & \( R_1=R_2=R_3=H \) \\
II & \( R_1=\text{Ac}, R_2=R_3=H \) \\
III & \( R_1=R_2=H, R_3=\text{Ac} \) \\
VI & \( R_1=R_2=R_3=\text{Ac} \) \\
IV & \( R_1=R_2=R_3=H \) \\
V & \( R_1=R_3=R_4=\text{Ac} \) \\
VII & \( R_1=\text{Ac}, R_2=R_3=H \) \\
VIII & \( R_1=R_2=H, R_3=\text{Ac} \)
\end{tabular}
\end{center}

Formulae 1

\section*{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

\textsuperscript{5) 2β,3α,4β-Triacetoxy-5β-pregn-16-en-20-one (V), colorless needles (from n-hexane), mp 191—193\degree, [\( \alpha \)]\textsubscript{D} +74.2\degree (MeOH), was prepared from VI by Takeda, \textit{et al.}•\textsuperscript{8a)  
Model A-60A (60 MHz) spectrometer and chemical shifts are given in δ (ppm) scale with tetramethylsilane as internal standard (s, singlet; t, triplet; d, doublet; m, multiplet; br, broad). Mass spectra were recorded on a RMU 6E (Hitachi) mass spectrometer (ionizing electron, 70 eV; ionizing current, 50 μA; source temperature, 120—130°C). Column chromatography was carried out with Kiesel Gel 60 (70—230 mesh ASTM) (Merck).

Isolation of G₄, G₅, and G₆—As described previously (refer to Charts 1, 1-A and 1-B in Part 1(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 extracts of the aerial parts of D. tenuepis. The remaining compounds were combined (tentatively named extract 7, 23.4 g from 400 g of the MeOH extracts) and fractionated in a column of Sephadex G-25.  

G₄ (2β,3α,4β-Trihydroxy-5β-pregnen-16-en-20-one) (IV) — Colorless plates (from AcOEt), mp 255—257; [α]D -58.1° (c=0.86). 1R ν max cm⁻¹: 3380 (OH), 1672 and 1584 (enone), no spiroketal absorptions. NMR: 0.88 (3H, s, 18-CH₃), 1.05 (3H, s, 19-CH₃), 2.25 (3H, s, 21-CH₃), 4.76 (3H, s, OH×3), 6.68 (1H, d, d, J=2, 3 Hz, vinyl proton). Mass Spectrum m/e: 348 (M⁺), 335 (M⁺-CH₂), 305 (M⁺-CH₃CO).  Anal. Calcd. for C₂₇H₄₆O₅: 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 Ac₂O (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°, [α]D +63.3° (c=0.50). 1R ν max cm⁻¹: 1745 (CH₂COO), 1667 and 1588 (enone). NMR: 0.88 (3H, s, 18-CH₃), 1.07 (3H, s, 19-CH₃), 1.09 (3H, s, CH₃COO×2), 2.03 (3H, s, CH₃COO), 2.25 (3H, s, 21-CH₃), 4.8—5.6 (3H, CH₂COOCH<×3), 6.68 (1H, d, d, J=2, 3 Hz, vinyl proton) (VI: 0.76 (3H, s, 18-CH₃), 1.09 (3H, s, 19-CH₃), 2.02 (6H, s, CH₃COO×2), 2.04 (3H, s, CH₃COO), 4.8—5.6 (3H, CH₂COOCH<×3)). Mass Spectrum m/e: 474 (M⁺), 459 (M⁺-CH₃), 431 (M⁺-CH₃CO), 414 (M⁺-CH₃COOH), 371 (414-CH₃CO), 354 (414-CH₂COO), 294 (534-CH₃COOH), 251 (294-CH₂COO).

Modified Marker's Degradation of VI — VI (320 mg) in Ac₂O (5 ml) was refluxed with AlCl₃ (70 mg) for 3 hr. The reaction mixture was cooled, and AcOAc (188 mg), AcOH (11 ml), and then CrO₃ (560 mg) in AcOH-water (2:1 v/v) (2 ml) were added under stirring at 10—14°C. After further stirring for 20 min at room temperature, NaHSO₄ (100 mg) was added and the mixture was concentrated in vacuo until 10 ml, diluted with saline (3 ml), benzene (10 ml) and 13% K₂CO₃ 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.

G₅ (2-Monoacetate of IV) (VII) — Colorless needles (from AcOEt), mp 217—219° (decomp.), [α]D +3.8° (c=0.53). 1R ν max cm⁻¹: 3380 (OH), 1730 (CH₂COO), 1648 and 1583 (enone), no spiroketal absorptions. NMR: 0.88 (3H, s, 18-CH₃), 1.05 (3H, s, 19-CH₃), 2.08 (3H, s, CH₃COO), 2.22 (3H, s, 21-CH₃), 2.85 (2H, br, OH×2), 3.38 (1H, t, J=9 Hz, HOCH<), 3.85 (1H, d, d, J=10, 9 Hz, HOCH<), 4.78 (1H, m, CH₂COOCH<), 6.88 (1H, d, d, J=3, 2 Hz, vinyl proton) (II: 0.76 (3H, s, 18-CH₃), 1.02 (3H, s, 19-CH₃), 2.08 (3H, s, CH₃COO), 3.41 (1H, t, J=9 Hz, HOCH<), 3.83 (1H, d, d, J=10.5, 9 Hz, HOCH<), 4.84 (1H, m, CH₂COOCH<). III (in pyridine-d₅): 0.83 (3H, s, 18-CH₃), 1.02 (3H, s, 19-CH₃), 2.05 (3H, s, CH₃COO), 4.12 (1H, t, J=9 Hz, HOCH<), 4.51 (1H, m, HOCH<), 4.75 (2H, br, OH×2), 5.47 (1H, d, d, J=10, 9 Hz, CH₂COOCH<). Mass Spectrum m/e: 390 (M⁺), 375 (M⁺-CH₃), 347 (M⁺-CH₃CO), 287 (M⁺-CH₂COO), 287 (330—CH₃CO).  Anal. Calcd. for C₂₇H₄₅O₆: C, 70.51; H, 8.78. Found: C, 70.51; H, 8.82. VII (12 mg) was hydrolyzed with 2% KOH in MeOH-water (2:1 v/v) (5 ml), and the hydrolysate was crystallized from AcOEt to give colorless plates (4 mg), mp 254—256°, identical with IV on mixed fusion.

G₆ (4-Monoacetate of IV) (VIII) — White powder (from AcOEt) (mp 178—181° (decomp.)), [α]D +34.3° (c=0.35). 1R ν max cm⁻¹: 3380 (OH), 1737 (CH₂COO), 1647 and 1583 (enone). NMR: 0.88 (3H, s, 18-CH₃), 1.05 (3H, s, 19-CH₃), 2.08 (3H, s, CH₃COO), 2.25 (3H, s, 21-CH₃), 3.42 (1H, t, J=9 Hz, HOCH<), 2.85 (2H, br, OH×2), 3.87 (1H, m, HOCH<), 4.68 (1H, d, d, J=10, 9 Hz, CH₂COOCH<), 6.68 (1H, d, d, J=3, 2 Hz, vinyl proton). Mass Spectrum m/e: 390 (M⁺), 375 (M⁺-CH₃), 347 (M⁺-CH₂COO), 330 (M⁺-CH₃CO), 287 (330—CH₃CO).  Anal. Calcd. for C₂₇H₄₅O₈: C, 70.74; H, 8.78. Found: C, 70.47; H, 8.57. VIII (8 mg) was hydrolyzed in the same way as VII and the product was crystallized from AcOEt to give colorless plates (3 mg), mp 254—256°, identical with IV.

Acknowledgements — The authors thank the former president Prof. M. Tomita, Prof. T. Sawada and the late Dr. M. Hutoh of Kyoto College of Pharmacy for their interests and encouragements, and Drs. T. Komori, K. Miyahara, and T. Nohara of Kyushu University for their valuable discussions. Thanks are also due to Mr. Y. Fujimura (NMR), Dr. K. Hashimoto, Miss M. Fujioka (mass spectra) and Dr. K. Umemoto (elemental analysis) of Kyoto College of Pharmacy and to the members (elemental analysis) of central analysis room of Kyoto University.