PHYTOECDYSONES OF SERRATULA

II. VITICOSTERONE E FROM Serratula sogdiana AND ITS PARTIAL SYNTHESIS

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As we have reported previously [1], an ethanolic extract of the flowers of Serratula sogdiana Bge. family Compositae contains not less than five phytoecdysones according to thin-layer chromatography (TLC). The qualitative compositions of extracts of the flowers and of the leaves of the plant are different.

When a methanolic extract of the leaves of S. sogdiana was chromatographed on a column of alumina, in addition to ecdysterone (I) [1] we isolated the least polar compound (II) with mp 197-198°C, the inclusion of which in the class of phytoecdysones is confirmed by its UV and IR spectra. $\lambda_{max}^{C_2H_5OH}$ 245 nm (log ε 4.00); ν_{max}^{KBr} 3400 cm^{-1} (OH) 1660 cm^{-1} (C=C-C=O. The IR spectrum also shows the absorption band of an ester group at 1725 cm^{-1}.

The mass spectrum has peaks with m/e 444 (M −60−18), 426, 411, 408, 393, 375, 363, 345, 327, 310, 301, 300, 99, 81, which are characteristic for the fragmentation of ecdysterone [1, 2]. This shows that compound (II) and ecdysterone are based on the same hydrocarbon skeleton and functional groups.

The quantitative oxidation of (II) with sodium periodate showed that its molecule contains two free diol groupings.

The NMR spectrum of (II), like that of ecdysterone, contains the signals of angular methyl groups of a steroid nucleus and methyl groups of a side chain (Table 1), and also a three-proton singlet at 1.82 ppm corresponding to an acetyl group. The absence of signals of protons geminal to an acetylated hydroxyl shows the tertiary nature of the acetyl group. A downfield shift of the resonance signals from the C_{26} and C_{27} methyl groups as compared with those for ecdysterone confirms the presence of an acetyl group at C_{25}.

The spectral results presented and also the values of the physicochemical constants of the substance enable compound (II) to be identified as viticosterone E – a phytoecdysone isolated previously [3] from Vitex megapotamica (Spreng.) Moldenke, family Verbenaceae.

TABLE 1. Chemical Shifts of the Signals of the Protons of Compounds (I-VII), ppm

Compound	$C_2 + C_3$, m	C_7, br. s	C_{18}, s	C_{19}, s	C_{21}, s	$C_{26/27}$, s
I	3,90—4,15	6,07	1,08	0,95	1,45	1,26
II	3,90—4,18	6,11	1,09	0,94	1,48	1,30 1,38
III	4,05-4,30	5,74	0,74	0,92	1,12	1,20
IV	4,0 —4,30	5,75	0,74	0,94	1,10	1,27
V	3,65—4,0	5,75	0,74	0,91	1,10	1,27 1,35
VI	4,85—5,29	5,80	0,98	0,80	1,21	1,14 1,17
VII	4,86—5,24	5,80	0,97	0,81	1,21	1,37

Notes. The spectra of compounds (I) and (II) were taken in C_5D_5N and those of (III) and (IV) in $CDCl_3$; m – multiplet; br. s – broadened singlet; s – singlet.

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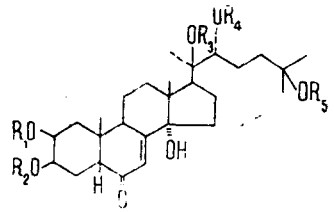
The structure of viticosterone E was established on the basis of spectral characteristics alone. To confirm the proposed structure, we have performed a partial synthesis of viticosterone E from ecdysterone.



	R_1	R_2	R_3	R_4	R_5
I	H	H	H	H	H
II	H	H	H	H	Ac
III	$>CMe_2$		$>CMe_2$		H
IV	$>CMe_2$		$>CMe_2$		Ac
V	H	H	$>CMe_2$		Ac
VI	Ac	Ac	H	Ac	H
VII	Ac	Ac	H	Ac	Ac

Ecdysterone was converted into the diacetonide (III) [1, 2], which was then acetylated at the free hydroxy group. In the acetate (IV) so obtained, the signals of the protons of the methyl groups at C_{26} and C_{27} were shifted downfield as compared with (III) (see Table 1), showing that it was in fact the hydroxy group at C_{25} that had been acetylated.

As was to be expected, the constants of the diacetonide acetate (IV) were identical with those of the diacetonide of viticosterone E [3].

The diacetonide acetate (IV) was hydrolyzed with dilute acetic acid to remove the protective isopropylidene groupings. A mixture of two substances with R_f 0.20 and 0.32 was formed. The reaction products were separated chromatographically. The less polar compound (V) had in the mass spectrum a peak with m/e 502 (M−60). The NMR spectrum of (V) contained signals at 1.39 and 1.41 ppm, corresponding to one isopropylidene grouping, and also a broad two-proton multiplet in the 3.65-4.0-ppm range (see Table 1) relating to the protons of a free diol grouping at C_2 and C_3 [2]. As in the acetonides (III) and (IV) the signal of the C_{21}-methyl group of (V) was shifted downfield as compared with that of viticosterone (II). Consequently, compound (V) must be assumed to be the 20,22-monoacetonide of viticosterone E.

As reported previously [2], the 20,22-monoacetonide grouping is resistant to acid hydrolysis.

The compound with R_f 0.20 was obtained in very small amount and was shown by chromatography to be identical with natural viticosterone E (II).

The hydrolysis of (IV) with dilute (6%) hydrochloric acid also gave a mixture of (V) and (II) in which the proportion of the latter was very small. The use of more concentrated solutions of acids may cause the splitting off of the labile 14α-hydroxy group [2].

We also obtained viticosterone E from ecdysterone (I) by another method. Substance (I) was acetylated with acetic anhydride in pyridine. The resulting mixture of the 2,3,22-triacetate (VI) and the 2,3,22,25-tetraacetate (VII) [1, 2] was separated on a column. When the tetraacetate (VII) was subjected to partial hydrolysis with an aqueous methanolic solution of potassium bicarbonate, the acetyl substituents of the secondary hydroxy groups were saponified. By separating the reaction mixture on a thin layer, viticosterone (II), identical with the natural material in its R_f values, melting point, and IR spectrum, was isolated. This method gave a better yield of viticosterone E than that passing through the diacetonide of ecdysterone (III).

EXPERIMENTAL

Chromatography was carried out with type-KSK silica gel and alumina (activity grade IV) prepared by the usual method. The phytoecdysterones were shown up by treatment with vanillin–sulfuric acid [2].

The IR spectra were obtained on a UR-20 spectrophotometer (KBr), the NMR spectra on a JNM-4H-100 MHz instrument with HMDS as internal standard (δ scale), and the mass spectra on an MKh-1303 instrument fitted with a system for the direct introduction of the substance into the ion source at an ionizing voltage of 40 eV and a temperature of 140-170°C.

Isolation of Viticosterone E (II). The air-dry leaves of S. sogdiana (23.5 kg) were extracted with 140 liters of methanol. The extract was concentrated, diluted with water, and treated with petroleum ether. Then the aqueous layer was exhaustively extracted with butanol. Distillation of the solvent yielded 850 g of a dry pulverulent extract. Part of this material (45 g) were chromatographed on a column of alumina (1.4 kg). On elution with a mixture of chloroform and methanol (30:1) 0.33 g (0.027%) of viticosterone E (II), $C_{29}H_{46}O_8$ was obtained with mp 197-198°C (acetone–hexane), $[\alpha]_D^{20}+60.0°$ (c 1.31; methanol), R_f 0.81 [chloroform–ethanol (4:1) system on silica gel]. Literature data [3] – mp 198-199°C.

2,3,20,22-Diacetonide of Ecdysterone (III) from (I). A suspension of 1.7 g of ecdysterone in 230 ml of anhydrous acetone was treated with 30 mg of phosphotungstic acid, and the reaction mixture was shaken at room temperature until the ecdysterone had dissolved completely. Then the solution was concentrated in vacuum to small volume, diluted with water, and neutralized with sodium bicarbonate solution. The neutral solution was extracted with ether and the extract was chromatographed on a column of silica gel (125 g). Chloroform elution gave 1.38 g of ecdysterone diacetonide (III), $C_{33}H_{52}O_7$, with mp 232-233.5°C (ether–hexane), $[\alpha]_D^{24}+40.5°$ (c 0.88; methanol) [1]. Literature data [2] – mp 234-236°C, $[\alpha]_D^{19}+37°$ (methanol).

Ecdysterone Diacetonide Acetate (IV) from (III). The diacetonide (III) from the previous experiment (1.19 g) in 30 ml of pyridine was acetylated with 20 ml of acetic anhydride at 40°C for 45 h. The product was chromatographed on a column of silica gel (50 g). Chloroform elution yielded 323 mg of (IV), $C_{35}H_{54}O_8$, with mp 206-208°C (chloroform–ether), $[\alpha]_D^{24}+50.7°$ (c 0.78, chloroform) (literature data [3] – mp 202-204°C); further elution with chloroform gave 880 mg of the initial diacetonide (III).

Viticosterone E 20,22-Monoacetonide (V) and Viticosterone E (II) from (IV). The ecdysterone diacetonide acetate (IV) (132 mg) was hydrolyzed with a mixture of 11 ml of acetic acid, 4 ml of methanol, and 3 ml of water at room temperature for 3.5 h. Then the mixture was diluted with water, neutralized with potassium bicarbonate solution, and extracted with chloroform. The solvent was distilled off in vacuum. The dry residue consisted of a mixture of two substances with R_f 0.20 and 0.32 [silica gel; chloroform–methanol (19:1)].

This material (113 mg) was separated on a column of silica gel (45 g). Elution was performed with chloroform–methanol (30:1), giving 89 mg of the monoacetonide of viticosterone E (V), $C_{32}H_{50}O_8$, with mp 194-196°C (ether–hexane), $[\alpha]_D^{24}+90.0°$ (c 0.74; methanol), ν_{max}^{KBr} 3340 cm^{-1} (OH), 1660 (C=C–C=O), 1730 cm^{-1} (CH_3CO).

Further elution of the column with the same mixture gave 5 mg of a compound with R_f 0.20 identical in chromatographic behavior with natural viticosterone E (II). The yield (calculated on the ecdysterone) was 0.27%.

2,3,22-Triacetate (VI) and 2,3,22,25-Tetraacetate (VII) of Ecdysterone from (I). Ecdysterone (270 mg) in 10 ml of pyridine was acetylated with 7.5 ml of acetic anhydride at 40°C for 4 h. After the elimination of the solvent in vacuum, the dry residue was chromatographed on a column of silica gel (40 g). Elution with chloroform gave 75 mg of ecdysterone tetraacetate (VII), $C_{35}H_{52}O_{11}$, with mp 204-206°C (acetone–hexane) $[\alpha]_D^{24}+59.6°$ (c 0.89; methanol) [1]. Literature data [2] – mp 200.5-201°C, $[\alpha]_D^{19}+60°$ (methanol). Then elution was continued with chloroform–ethanol (19:1), giving 158 mg of ecdysterone triacetate (VI), $C_{33}H_{50}O_{10}$, with mp 196-198°C (ether) $[\alpha]_D^{24}+57.7°$ (c 0.75; methanol) [1]. Literature data [2] – mp 198.5-199°C, $[\alpha]_D^{19}+59°$ (methanol).

Viticosterone E (II) from the Tetraacetate (VII). A solution of 51 mg of the tetraacetate (VII) in 6 ml of methanol was treated with 65 mg of potassium bicarbonate in 5 ml of water and the mixture was left at 40°C for 6 h. Then the reaction mixture was diluted with water, neutralized with acetic acid, and extracted with ether. The ether was distilled off and the residue was chromatographed on a thin layer of silica gel in the chloroform–methanol (9:1) system. This gave 19.4 mg of viticosterone E (II) with mp 188-193°C (acetone–hexane). Yield (calculated on the ecdysterone) 6.6%. The R_f value in a thin layer and the IR spectrum were identical with the analogous indices of the natural compound.

SUMMARY

1. A phytoecdysone identified as viticosterone E has been isolated from a methanolic extract of the leaves of Serratula sogdiana Bge.

2. A partial synthesis of viticosterone E from ecdysterone has been effected by two methods – via the 2,3:20,22-diacetonide and via the 2,3,22,25-tetraacetate.

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