TRANSFORMED STEROIDS.

118.* THE GEOMETRIC ISOMERISM OF 20-ETHOXYCARBONYLHYDRAZONES OF 3β-HYDROXY-16,17α-CYCLOPROPANOPREGN-5-EN-20-ONE

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In the reaction of the 3β-acetate of 3β-hydroxy-3'H-cyclopropano[16,17α]pregn-5-en-20-one (I) with ethoxycarbonylhydrazine in AcOH at 20°C (exposure for up to 6 days), a chromatographically separable equilibrium mixture of the 20Z-ethoxycarbonylhydrazone of 3β-acetoxy-3'H-cyclopropano[16,17α]pregn-5-en-20-one (II), with mp 190-195°C, and the 20E-ethoxycarbonylhydrazone of 3β-acetoxy-3'H-cyclopropano[16,17α]pregn-5-en-20-one (III) with mp 186-193°C (ratio 2:1) is obtained. The geometric isomers obtained are distinguished by IR, PMR, and CD spectroscopy. It has been shown that the observed geometric isomerism takes place via an intermediate enehydrazine form.

While the tendency of hydrazones to take part in geometric isomerism of the Z,E type is known, it has not been discovered among the 20-hydrazones of steroids of the pregnane series, in spite of wide-ranging and systematic investigations of this class of compounds with the aim of using them for the stereospecific directed synthesis of 16,17α-substituted biologically active pregnanes. The only exception is the formation of the E- isomer of a 20-acylhydrazone detected on the reaction of an acylhydrazine with the acetate of 3β-hydroxypregna-5,16-dien-20-one, which on further treatment with acetic acid was converted into the Z- isomer [2]. (The Z- isomer is characterized by the syn arrangement of the 31-CH_3 group and the ethoxycarbonylamino grouping, and the E- isomer corresponds to their anti arrangement.) Back-isomerization was not detected to any appreciable degree whatever under the conditions used (AcOH, 20°C, 24 h). We have detected geometric isomerization of the Z,E type in the series of 20-ethoxycarbonylhydrazones of 3β-hydroxy-3'H-cyclopropano[16,17α]pregn-5-en-20-one. Thus, on prolonged exposure of the 3β-acetate of 3β-hydroxy-3'H-cyclopropano 16,17α pregn-5-en-20-one (I) to ethoxycarbonylhydrazine (ECH) in AcOH (up to 6 days), two chromatographically separable iosmers were formed which, according to their chemical and physicochemical characteristics can be characterized as the Z- and E- isomers (III and IV, respectively). The saponification of (III) and (IV) with sodium ethanolate or potassium bicarbonate in methanol gave their 3-hydroxy analogs (V) and (VI) having similar melting points and not differing chromatographically or mass-spectrometrically and not isomerizing into one another in neutral and alkaline media (monitored by PMR spectroscopy). The spectral differences of the isomers (II) and (IV) are given in Table 1. While their UV, mass, and IR spectra are completely identical in solution there are appreciable differences in the PMR and IR spectra of their crystalline forms. It is known that in the PMR spectra of unsymmetrical ketones α-protons present in the syn position to the amine nitrogen absorb in a stronger field than in the corresponding anti isomers [3]. A comparison from this point of view of the protons of the 21-CH_3 groups from the spectra of (II) and (III) (1.73 and 1.96 ppm) and their hydroxy analogs (V) and (VI) (1.73 and 1.94 ppm) permitted the hydrazones (III) and (IV) to be assigned to the Z- form and (V) and (VI) to the E- form. The chemical shifts of the protons of the 18-angular methyl group and of the amine proton differed considerably. The downfield shift of the signal of the NH proton in the E- form of (III) cannot be explained by a greater degree of conjugation in this isomer, since their UV spectra are identical and it is more probably caused by the descreening effect of the spatially adjacent substituents. It must be mentioned that in the circular dichroism (CD) spectra Cotton effects of the same sign appear in the region of the azomethine chromophores of compounds (III) and (IV). This difference may serve

*For Communication 117, see [1].

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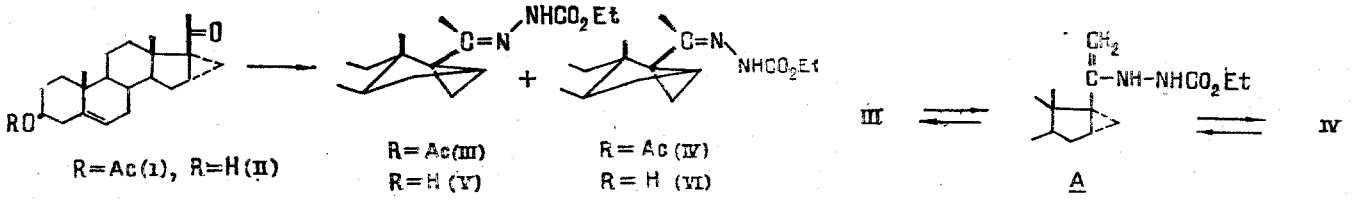
TABLE 1

Compound	PMR spectrum, δ, ppm, $CHCl_3$			IR spectrum (ν_{max}^{KBr}, cm^{-1})	CD spectrum (C_2H_5OH)	
	18-CH_3	21-CH_3	NH		λ_{max}	$\Delta\varepsilon$
III	0,78	1,74	7,43	1042, 1230, 1260, 1380, 1440, 1520, 1712, 1736, 3300	240	−2,52
IV	0,85	1,96	8,26	1042, 1212, 1250, 1380, 1495, 1730, 1760, 3390	233	+6,75
V	0 78	1,73	7,38	1058, 1242, 1380, 1440, 1545, 1710, 3030, 3230, 3420		
VI	0,86	1,94	8,28	1065, 1335, 1375, 1430, 1470, 1725, 3030, 3065, 3395, 3440		

as a diagnostic method in the assignment of steroid 20-hydrazones to the Z- and E- forms in those numerous cases when only one geometric isomer has been isolated.



A chromatographic examination of the course of the reaction of (I) with ECH in acetic acid showed that the Z- isomer (III) is formed first and this isomerizes with an increase in the reaction time (from 1 to 6 days) into the E- isomer (IV), the ratio of the isomers becoming constant after only 72 h and reaching an equilibrium in which the Z- form (III) always predominates (∿2:1). In inert solvents, and in an alkaline medium, the two isomers are extremely stable, but in glacial acetic acid and, particularly, in a mixture with chloroform (apparently containing catalytic amounts of HCl) the interconversion of these forms takes place, and the stable Z- form predominates. On being heated in AcOH with acetylacetone, the two forms are converted into the initial cyclopropane derivative (I).

To elucidate the mechanism of this reaction we have performed the interconversion of the Z- and E- isomers in perdeuterated acetic acid. It was found that the protons of the 21-CH_3 group are replaced by deuterium, which follows both from mass-spectrometric results and from the PMR spectra of the isomers (III) and (IV) isolated from the equilibrium mixture obtained by isomerizing one of the two geometric isomers (disappearance of the three-proton signal of the 21-CH_3 group). It is interesting to note that the proton of the hydrazone fragment does not participate in deuterium exchange, in view of which it is highly logical to assume the initial isomerization of the hydrazone form into the tautomeric enehydrazine form A as an intermediate in the observed geometric isomerization. Apparently, not the last role in the stabilization of the enehydrazine form is played by the cyclopropane ring in a conformation of the molecule in which there are only possibilities for its conjugation not with the azomethine but with the methylene double bond. To this we may add the fact that the UV spectra of the hydrazones (III) and (IV), which are identical (λ_{max} 226 nm, ε 13,600, and λ_{max} 228 nm, ε 13,650, respectively) scarcely differ from that of the 16,17-unsubstituted analog — the 3-acetate of the 20-ethoxycarbonylhydrazone of 3β-hydroxypregn-5-en-20-one (λ_{max} 227 nm, ε 13,200). This means that the hydrazone group in (III) and (IV) is not conjugated with the cyclopropane ring. All this, in combination with the results of a consideration of steric factors, permits the assumption for the hydrazones (III) and (IV), in contrast to the ketone (I) [3, 4], of that geometry of the C-17—C-20 bond in which the cyclopropyl and hydrazone groups are cis-oriented with respect to one another. The absence of the enehydrazine form from neutral solutions [5] naturally excludes the possibility of geometric isomerism. In an acid medium, however, especially in AcOH, the capacity of the hydrazones for enolization and tautomeric transformation into the enehydrazine form is known [5, 6]. Alkaline catalysis iodine, or the irradiation of (II) with UV light do not cause its isomerization.

The geometric isomerism found is an exceptionally rare phenomenon in the chemistry of the steroid 20-hydrazones which is observed only in the case of the 20-hydrozones of 3β-hydroxypregna-5,16-dien-20-one where the successive nature of the formation of the Z, E isomers, with the greater stability of the Z- isomer, is directly opposite to the situation reported elsewhere [2].

EXPERIMENTAL

Melting points were determined on a Kofler block. PMR spectra were taken on a Tesla BS-497 spectrometer (100 MHz) with HMDS as internal standard. IR spectra were taken on a UR-10 instrument and mass spectra on a Varian MAT CH-6 instrument with the direct introduction of the sample into the ion source, and CD spectra on a Jobin-Yvon III instrument in ethanol. The analysis of the compounds corresponded to the calculated figures.

20Z-Ethoxycarbonylhydrazone of 3-Acetoxy-3'H-cyclopropano[16,17]pregn-5-en-20-one (III). A solution of 1.5 g of (I) and 3 g of H_2N-$NHCO_2C_2H_5$ in 75 ml of HOAc and 6 ml of $CHCl_3$ was kept at 20°C for 3 days. The solvent was partially evaporated in vacuum, the residue was diluted with water, and the precipitate that deposited was filtered off, washed with water, dried, and recrystallized from ether. This gave 0.95 g of (III), $C_{27}H_{40}O_4N_2$, mp 190-195°C. PMR spectrum (δ, ppm): 0.78 s (18-CH), 0.96 s (19-CH_3), 1.22 t (CH_3 group of O-C_2H_5), 1.74 s (21-CH_3), 1.94 s (3-OAc), 4.16 q (CH_2 group of O-C_2H_5), 7.43 (NH). Mass spectrum (m/e): 456 (M^+), 441 ($M^+ - CH_3$), *426.5 (456→441), 396 (M^+ −HOAc), 381 ($M^+ - CH_3$−HOAc), 368 ($M^+ - CH_3 - CO_2C_2H_5$), 323 (M^+ −HOAc−$CO_2C_2H_5$), 308 (M^+ −HOAc−$CO_2C_2H_5$−CH_3).

Isomerization of the 20Z-Ethoxycarbonylhydrazone (III) in AcOH. A solution of 0.05 g of (II) in 0.5 ml of AcOH and 1 ml of $CHCl_3$ was kept at 20°C for 4 days, after which it was boiled for 7 h. The chloroform was evaporated off, the residue was diluted with water, and the precipitate was filtered off. This gave 0.045 g of a substance the separation of which by TLC [SiO_2, benzene−ether (2:1)] yielded, in addition to 0.027 g of (II), 0.016 g of (IV), $C_{27}H_{40}O_4N_2$, mp 186-193°C (from ether). Mass spectrum (m/e): 456 (M^+), 441 ($M^+ - CH_3$), *426.5 (456→441), 396 (M^+ −AcOH), 381, 368, 323, 308.

Isomerization of the 20E-Ethoxycarbonylhydrazone (IV) in AcOH. A solution of 0.025 g of (IV) in 1.25 ml of AcOH was kept at 20°C for 4 days. After the working up procedure described above, 0.025 g was obtained of a mixture the TLC of which [benzene−ether (7:1)] gave 0.014 g of (II) and 0.07 g of (IV).

20Z-Ethoxycarbonylhydrazone of 3β-Hydroxy-3'H-cyclopropano[16,17α]pregn-5-en-20-one (V). A solution of 0.3 g of (III) in 30 ml of CH_3OH was treated with 0.15 g of $KHCO_3$ in 1 ml of H_2O. The reaction mixture was boiled for 2 h, after which 2/3 of the solvent was evaporated off, the residue was diluted with water, and the resulting precipitate was filtered off. This gave 0.29 g of a product the crystallization of which from CH_3OH yielded 0.25 g of (V) with mp 182-186°C. Mass spectrum (m/e): 414 (M^+), 399 ($M^+ - CH_3$), 326 ($M^+ - CH_3 - CO_2C_2H_5$).

A solution of 0.015 g of (II), 0.02 g of ECH, and 0.5 ml of AcOH was kept at 20°C for 18 h. After the working-up procedure described above and crystallization from ether, 0.012 g of (V) was obtained with mp 182-186°C, identical with that described above.

20E-Ethoxycarbonylhydrazone of 3β-Hydroxy-3'H-cyclopropano[16,17α]pregn-5-en-20-one (VI). A solution of 0.05 g of (IV) in 5 ml of CH_3OH containing 0.025 g of $KHCO_3$ in 0.5 ml of H_2O was boiled for 1 h, after which it was diluted with water and the precipitate that deposited was filtered off. This gave 0.045 g of a product the crystallization of which from ether yielded 0.03 g of (IV) with mp 187-190°C. Mass spectrum (m/e): 414 (M^+), 399 ($M^+ - CH_3$), 326 ($M^+ - CH_3 - CO_2C_2H_5$).

SUMMARY

The reaction of 16,17α-cyclopropano-20-ketosteroids with ethoxycarbonylhydrazine forms interconverting geometric Z- and E- isomers with respect to the azomethine bond. An enehydrazine structure has been put forward as an isomerization intermediate.

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TOMATOSIDE A FROM THE SEEDS OF *Lycopersicum esculentum*

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Results are given which confirm the structure of the furostanol glycoside from tomato seeds forming wastes of the preserving industry. From a butanolic extract of the seeds of *Lycopersicum esculentum* Mill. we have isolated the furostanol glycoside tomatoside A (I) the structure of which has been established as 25(S)-5α-furostan-3β,22α,26-triol 26-O-β-D-glucopyranoside 3-O-[O-β-D-glucopyranosyl-(1→2)-O-β-D-glucopyranosyl-(1→4)-β-D-galactopyranoside]. At the same time, by enzymatic and chemical transformations three new spirostanol glycosides of neotigogenin have been obtained: tomatoside B (III), which is 25(S)-5α-spirostan-3β-ol 3-O-[O-β-D-glucopyranosyl-(1→2)-β-D-galactopyranoside], 25(S)-5α-spirostan-3β-ol 3-O-[O-β-D-glucopyranosyl-(1→4)-β-D-galactopyranoside] (V), and 25(S)-5α-spirostan-3β-ol 3-O-β-D-galactopyranoside (IV).

We have reported previously [1] that the seeds of *Lycopersicum esculentum* Mill. (tomato) contain about 0.3% of neotigogenin (II). Continuing a study of tomato seeds, from the total extractive substances we have isolated two compounds with similar R_f values (Ia/Ib). A positive reaction with the Ehrlich reagent [2], the presence in the IR spectrum of a broad band of low intensity at 900 cm^{-1} [3] with the absence of bands indicating that the compounds belong to the spirostan series [4, 5] and a characteristic behavior on two-dimensional TLC [6] permit these substances to be assigned to the furostanol glycosides.

It is known that 22-OH furostanols, on being heated with methanol, form the 22-O-methyl ethers which readily undergo the reverse transition [7, 8]. When the combined glycosides (Ia/Ib) were heated in water and methanol, the individual compounds (Ia) and (b) were isolated. The PMR spectrum of glycoside (Ib) has a three-proton singlet at 3.12 ppm [3]. In the PMR spectrum of compound (Ia) there are no resonance signals of the protons of methoxy groups. Consequently, substance (Ia) is a 22-OH furostanol glycoside. This is a native compound and we have called it tomatoside A. Compound (Ib) is its 22-O-methyl ether. Because of the ease of interconversion of glycosides (Ia) and (Ib), the subsequent operations to prove the structure of tomatoside A (Ia) were performed with the product (Ia/Ib).

In 1973, Japanese workers [9], who were interested in the bitter principal of tomato seeds, described the structure of a new tetraside of the furostan series which they called TFI. The marked difference in the melting points of tomatoside A (Ia) and TFI (247-250°C and 217-220°C, respectively) caused some doubts as to the complete identity of the structures of these glycosides. The aim of the present work was to establish the structure of tomatoside A (Ia) and to obtain a number of model glycosides of neotigogenin.

The complete acid hydrolysis and methanolysis of product (Ia/Ib) led to neotigogenin [1, 2] and a mixture of carbohydrates. Analysis of the hydrolysates by TLC and GLC [11] showed the presence of D-glucose and D-galactose in a ratio of 3:1.

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