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DIENOL HEPTAFLUOROBUTYRATES AS DERIVATIVES FOR GAS LIQUID CHROMATOGRAPHY OF STEROIDAL Δ^4-3-KETONES

DETERMINATION OF THE STRUCTURE OF THE ISOMERIC DIENOL ESTERS

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Abstract—The reaction of testosterone-17β-acetate (3) with heptafluorobutyric anhydride in high purity and anhydrous acetone at room temperature leads to the preferential formation of 1 with less than 1% of the isomeric 2,4-diene. The reaction is pseudo-unimolecular and after 30 min, 99% of the starting material is esterified. The two isomers have been fully characterized (UV, IR, NMR, CD, MS) and their sensitivities to electron capture detection are compared.

INTRODUCTION

Since the introduction by Exley *et al.*[1] and Dehennin *et al.*[2] of dienol heptafluorobutyrates (HFB) as suitable derivatives for the ultramicro determination of 4-ene-3-one steroid hormones by GLC with electron capture detection (ECD), some controversy has risen in the literature about the reaction conditions which lead to quantitative formation of dienol esters.

Devaux and Horning[3] recommend the preferential formation of 2,4-androstadiene-3,17β-diol-diheptafluorobutyrate as a testosterone derivative for ECD, without isolation and unequivoqual characterization of the compound.

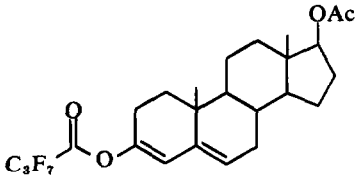
This investigation deals with the reaction of heptafluorobutyric anhydride (HFBAnh) with 3-oxo-4-androstenyl-17β-acetate (3) in acetone, but the results can be generalized to other 4-ene-3-one steroids and to other perfluoroanhydrides.

RESULTS AND DISCUSSION

Structure determination of dienol heptafluorobutyrates

Acid catalysis. Malhotra and Ringold[4] have shown that the enolization of steroidal α,β-unsaturated ketones under strong acidic conditions leads to preferential but not exclusive formation of the thermodynamically more stable heteroannular $\Delta^{3,5}$-diene by loss of the 6β-axial hydrogen under stereoelectronic control[5] of the reaction.

HFBAnh is the anhydride of a strong acid ($K_a = 0{\cdot}68$)[6] which is liberated during the reaction. The reaction of 4-ene-3-one steroids with HFBAnh in acetone or benzene leads to the preferential formation of the 3,5-dienol-3-HFB. The heteroannular diene structure of this compound is supported by various spectral data. The UV absorption maximum at 227 nm shows a slight hypsochromic shift of approximately 10 nm when compared to the calculated value according to the Woodward and Fieser's rules.[7] This can be explained by the inductive effect of the HFB group.



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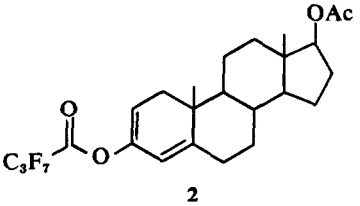

2

The presence of a double bond between C5 and C6 is clearly evidenced by the methanolysis of 1 in MeOH containing 0·01 mM Et_3N.

Fig 1 shows that after 30 min almost all of the compound has been transformed into the corresponding 5-ene-3-one (dotted line). Upon addition of a drop of conc HCl, the immediate isomerization into the 4-ene-3-one is observed (bar line; $\lambda_{max} = 240$ nm). The facile methanolysis of the dienol HFB might be due to the strong -I effect of the HFB group which increases the electron deficiency of the carbonyl C atom of the ester group. The subsequent protonation occurs at the vinylic C4 and thus indicates the intermediate formation of the 3,5-dienolate ion.[4]

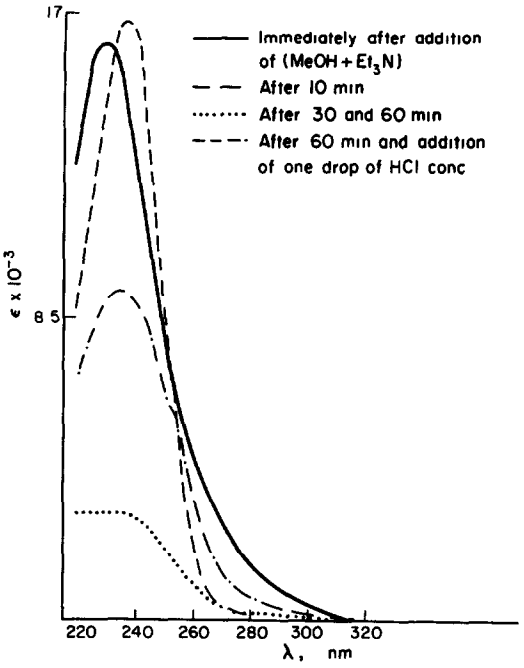

Fig 1. UV absorption spectra of the 3,5-dienol-3-HFB (1).

The IR spectrum has two characteristic absorption peaks of medium intensity in the region of C=C absorption. One peak at 1668 cm^{-1} is assigned to the Δ^3 double bond and the other at 1640 cm^{-1} to the Δ^5 double bond. An absorption peak at 898 cm^{-1} in the fingerprint region allows easy recognition of this isomer.

Both vinylic protons of **1** (5·88 at C4 and 5·53 at C6) have chemical shifts similar to the corresponding trichloroacetates[8] (5·91 and 5·53), but they are deshielded when compared with the respective protons of the 3,5-dienol-3-acetate (5·61 and 5·31).[9] This is explained by the joint effects of CO mesomerism and -I effect of the C_3F_7 atom group which render delocalization of the lone pair electrons of the ester C—O—C oxygen over the Δ^3 double bond impossible.

Mass spectrometric fragmentation of **1** has been discussed recently.[10]

The heteroannular transoid diene structure of **1** is clearly demonstrated by the high negative Cotton effect ($[\theta]_{226} = -31{,}250$). This means that when the Δ^3 double bond and the central single bond define a horizontal plane, the Δ^5 double bond is pointed downwards.[11]

Base catalysis. Weak acid and strong base favour kinetic control[12] of the enolization with preferential formation of the homoannular $\Delta^{2,4}$ diene by loss of the most acidic 2β-axial proton.[4]

The reaction of 4-ene-3-one steroids with HFB-Anh in benzene containing up to 15% pyridine leads to the preferential formation of 2,4-dienol-3-HFB. Compound **2** has a higher methanolysis rate than **1** because **2** is the less stable kinetic isomer. Protonation at the vinylic C2 of the intermediate 2,4-dienolate ion leads to the corresponding 4-ene-3-one (Fig 2).

In the region of C=C stretching vibration, the absorption peak at 1668 cm^{-1} is assigned to the Δ^2 double bond and the peak at 1608 cm^{-1} to the Δ^4 double bond.

The vinylic proton at C2 has a chemical shift (5·35) which is lower than the corresponding shift of the 2-enol-3-trichloroacetate[8] (5·45). This is due to the lower -I effect of the C_3F_7 versus the CCl_3 atom group. The chemical shift (5·50) of the vinylic proton at C4 is similar to the respective shift of the 2,4-dienol-3-acetate (5·45),[13] but there is important shielding when compared to the analogous proton in the 3,5-diene (5·88), where the Δ^3 double bond is highly polarized.

The mass spectrum of **2** is very similar to **1** and does not allow differentiation of the two isomers. There is some extra stabilization of the molecular ion of the $\Delta^{2,4}$ diene, which is the base peak of the spectrum.

The high positive Cotton effect $[\theta]_{267} = 31{,}200$ indicates the presence of a homoannular cisoid diene chromophore in **2**. According to the "cisoid diene" rule this diene is skewed in the sense of a right handed helix.

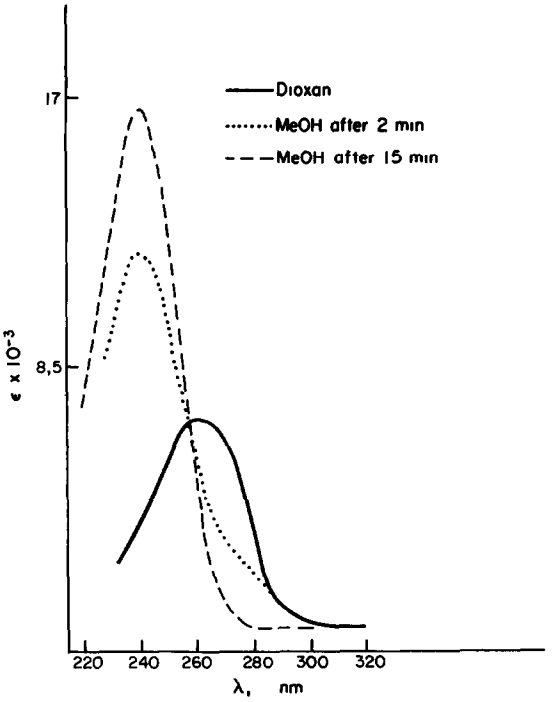

Fig 2. UV absorption spectra of the 2,4-dienol-3-HFB (2).

Reaction kinetics. It is generally accepted that the formation of the enol is the slow step of acid catalysed reactions, leading either to the halogenation, deuteration, racemization or esterification of carbonyl compounds.[15] In the case of base catalysis, the reaction step which limits the overall reaction rate is considered to be the formation of the enolate.

In presence of a large excess (100 fold) of reagent, the dienol esterification becomes a pseudo-first order reaction, as shown in Fig 3. The experimental rate constant k_{exp} is slightly lower for the dienol esterification ($k_{exp} = 0{\cdot}154$ min^{-1}) when compared to the esterification of a phenolic OH group ($k_{exp} = 0{\cdot}187\ min^{-1}$) under the same conditions. Approximately 99% of the 4-ene-3-one steroid is transformed into the 3,5-dienol-3-HFB after 30 min in acetone containing 10% HFBAnh at room temperature. These are in our hands the most suitable conditions for the micro preparation of these esters prior to analysis by GLC.

The reaction in benzene-pyridine (85–15) containing 10% HFBAnh at 60°C is also pseudo-unimolecular. The reaction rate is much slower and does not increase by increasing base concentration or base strength (triethylamine). The yield of the 2,4-dienol-3-HFB never exceeds 85% because the equilibrium mixture contains nearly 15% of the 3,5-diene. The inability to evaporate the reaction mixture entirely (micro scale reaction), is also a drawback for subsequent injection into a GLC column, fitted with a ECD. The remaining

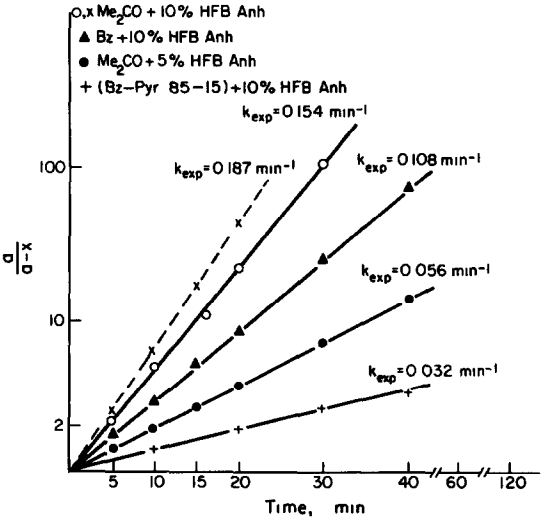

Fig 3. Reaction rates for the conversion of a 4-ene-3-one to a 2,4-dienol-3-HFB (+——+), of a 4-ene-3-one to a 3,5-dienol-3-HFB (●——●; △——△; ○——○) and of 1,3,5(10)-estratrien-3-ol to 1,3,5(10)-estratriene-3-HFB (×-------×).

pyridinium-HFB has considerable tailing in GLC and TLC.

Stability in the reaction medium. The dienol HFB are stable in their respective reaction media. This has been checked for a reaction duration of 24 hours. For the isomerization reaction:

$$\text{2,4-dienol-3-HFB} \rightleftharpoons \text{3,5-dienol-3-HFB}$$

the free energy differences between the isomers can be calculated, using the equation $\Delta F = -RT\ln K$. For the reaction in acetone containing 10% HFBAnh at room temperature, the extra stabilization of the 3,5-diene structure is 3·0 kcal/mole. The extra stability of the 2,4-diene structure for the reaction in benzene–pyridine (85–15) containing 10% HFBAnh at 60° is much lower, only 1·1 kcal/mole.

The 3,5-dienol-3-HFB is also perfectly stable in the reaction medium used for the preparation of the corresponding 2,4-diene. When the 2,4-dienol-3-HFB is dissolved in the reaction mixture used for the preparation of the 3,5-diene, a rapid isomerization:

$$\text{2,4-dienol-3-HFB} \longrightarrow \text{3,5-dienol-3-HFB}$$

is observed (Table 1).

The conversion of the 2,4-dienol-3-HFB into the 3,5-dienol-3-HFB must proceed through protonation at C2 with intermediate formation of the 4-ene-3-one. The slow reaction step seems to be the protonation which depends on the initial $[H^+]$ of the reaction medium.

Gas chromatographic behaviour and response to electron capture. Base line separations of isomers 1 and 2 can be achieved with 2000 theoretical plates on columns packed with polar (1% OV-7) or non polar (1% OV-101) stationary phases.

The retention indices[17] of 2 ($I_{210}^{OV-101} = 2525$; $I_{210}^{OV-7} = 2616$) are slightly lower than those of 1 ($I_{210}^{OV-101} = 2566$; $I_{210}^{OV-7} = 2654$). This can be explained by the homoannular cisoid diene structure of 2, where ring A with its HFB group attached at C3, is more planar than in the case of the heteroannular transoid diene (1).

Relative molar electron absorbing activities can be calculated from the measurement of the response to ECD. The following experimental

Table 1

Reaction time in a mixture (Me_2CO + 10% HFBAnh) at room temperature	Composition of the mixture as the ratio [2,4-diene/3,5-diene] in %
0	98·4
5 min	34·2
30 min	13·0
1 hr	10·5
24 hr	0·5

relationship was found; molar electron absorbing activity (2) = 1·9 × [molar electron absorbing activity (1)].

This supports our previous statement[10] on the important contribution of molecular structure and polarization effects to the overall electron affinity of perfluorinated steroid derivatives. The increased sensitivity to ECD of 2 is probably due to the conformation of the cisoid diene structure, which is particularly suitable for direct interaction via the intramolecular space between the ester carbonyl oxygen and the Δ^2 double bond. This enhances the δ^+ charge at C2 and favours electron capture at this place of the molecule. The polarization of the second double bond (Δ^4) in α of the HFB group is negligible, so that no contribution to electron absorbing activity can be expected here.

EXPERIMENTAL

M.ps were taken in glass capillaries and are uncorrected. The UV spectra were recorded on a Cary 15 spectrophotometer. The IR spectra were determined on a Perkin Elmer 237 grating spectrophotometer. The NMR spectra were recorded on a Varian A 60 spectrometer with solutions made in $CDCl_3$ and peak positions are indicated in ppm downfield from TMS, serving as an internal standard. CD was measured on the Roussel-Jouan dichrograph. GLC with FID or ECD and combined GC-MS were performed as described previously.[10]

Solvents and reagent. Me_2CO, C_6H_6 (Mallinckrodt, Nanograde) and pyridine (E. Merck) were made anhydrous and distilled. Special care must be taken for the Me_2CO which must be free of any base. Heptafluorobutyric anhydride (Pierce Chemical Cy) was prepared by refluxing the corresponding acid on P_2O_5 and fractional distillation (b.p. 106–7° at 760 mm Hg). Reaction conditions: The concentration of the steroid was $4 \cdot 10^{-3}$ M (a). Reactions in Me_2CO were run at room temp and reactions in C_6H_6 or in C_6H_6-pyridine (85–15) were performed at 60°. The concentrations of HFBAnh were either 10% (0·4 M) or 5%. At regular time intervals a sample of the mixture was analysed by GLC and the concentrations of dienol ester (x) were determined on a standard curve. This curve was obtained by plotting peak heights versus known concentrations of pure crystallized reference compound. The ratio a/a–x was subsequently calculated.

3,5-*Androstadiene*-3,17β-*diol*-3-HFB-17-*acetate* (1). A soln of 0·5 g (3) in 5 ml dry Me_2CO containing 0·1 ml HFB acid was treated at room temp for 2 hr with 0·5 ml HFBAnh. The soln was evaporated under N_2 and the residue was crystallized from anhyd MeOH. The crystallized compound (0·44 g, 55%) contained less than 0·8% of 2, m.p. 121–2°. (Found: C, 56·48; H, 5·54; F, 25·18; $C_{25}H_{29}F_7O_4$ requires: C, 57·03; H, 5·51; F, 25·28%); UV (dioxan) λ_{max} 227 nm (ϵ 16,100); IR (KBr) 1775, 1740 cm^{-1} (ester C=O); 1668, 1640 cm^{-1} (C=C); 1140 cm^{-1} (ester C—O—C); NMR ($CDCl_3$) δ 0·84 (3H, s, 18-H_3); 1·04 (3H, s, 19-H_3); 2·05 (3H, s, 17-OAc); 4·66 (1H, t, J 7·5 Hz, 17-H); 5·53 (1H, t, J 2·5 Hz, 6-H); 5·88 (1H, d, J 1·5 Hz, 4-H) ppm; MS: M = 526·2; $[M^+]$ = 95; [M-15] = 9; [M-60] = 8; [M-(60 + 15)] = 16; *m/e* [369] = 5; [357] = 7; [355] = 13; [332] = 6; [319] = 6; [312] = 6; [297] = 6; [293] = 8; [253] = 12; [237] = 10; [197] = 5; [169] = 9; [146] = 100; [133] = 82; [119] = 28; [105] = 41; [91] = 39. CD $[\theta]_{226}$ −31,250.

2,4-*Androstadiene*-3,17β-*diol*-3-HFB-17-*acetate* (2). A soln of 1 g (3) in 10 ml dry C_6H_6 and 0·5 ml pyridine was treated for 24 hr at 60°C with 1 ml HFBAnh. The soln was evaporated under N_2 and the residue was crystallized from dry MeOH. The crude compound (0·84 g, 53%) contained 11% of 1. An aliquot (200 mg) was then chromatographed on a Florisil (100–120 mesh) column (30 × 1 cm) with C_6H_6-Et_2O (98–2) as an eluent (Flow 0·9 ml/min). The first 30 ml were discarded and the next 10 ml fraction contained 50 mg (2) with less than 1% (1), m.p. 112–3°. (Found: C, 56·69; H, 5·64; F, 25·13; $C_{25}H_{29}F_7O_4$ requires: C, 57·03; H, 5·51; F, 25·28%); UV (dioxan) λ_{max} 261 nm (ϵ 6,100). IR (KBr) 1775, 1730 cm^{-1} (ester C=O); 1668, 1608 cm^{-1} (C=C); 1140 cm^{-1} (ester C—O—C). NMR ($CDCl_3$) δ 0·84 (3H, s, 18-H_3); 1·08 (3H, s, 19-H_3); 2·05 (3H, s, 17-OAc); 4·66 (1H, t, J 7·5 Hz, 17-H); 5·35 (1H, t, J 2·0 Hz, 4-H); 5·50 (1H, t, 2-H). MS: M = 526·2; $[M^+]$ = 100; [M-15] = 2; [M-60] = 26; [M-(60 + 15)] = 33; *m/e* [369] = 4; [355] = 5; [330] = 9; [318] = 28; [317] = 32; [303] = 14; [269] = 8; [253] = 5; [169] = 5; [149] = 76; [147] = 92; [135] = 58; [119] = 20; [104] = 53; [91] = 33. CD $[\theta]_{267}$ +31,200.

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