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Note

A new derivative for the gas–liquid chromatography with electron capture detection of steroidal secondary alcohols

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Heptafluorobutanoic (HFB) esters of various steroids have been used in quantitative determinations by gas–liquid chromatography (GLC) with electron capture detection (ECD). Unsaturation of the parent steroid is nevertheless a prerequisite for high sensitivities and thus saturated steroidal HFB esters are endowed with poor affinities for thermal electrons[1].

In this paper, we describe a Friedel–Crafts acylation that leads to the introduction of an exocyclic conjugated heptafluorobutanoyl group.

EXPERIMENTAL

Steroids with a secondary hydroxyl group at the 3α, 3β, 17α or 17β position ($2 \cdot 10^{-3}$ *M*) are dissolved in 0.1 ml of dry acetone plus 0.1 ml of trimethylchlorosilane (TMCS). Heptafluorobutanoic anhydride (0.05 ml) is then added and the mixture is allowed to stand at room temperature for 1 h. Subsequent evaporation under nitrogen, dissolution in *n*-hexane and analysis by GLC (1% SE-30 or 1% OV-7 on Gas-Chrom Q, 100–120 mesh; 150 × 0.3 cm column; 200°) and gas chromatography–mass spectrometry (GC–MS)[1] demonstrates the formation of the compounds listed in Table I.

The structure of the new derivative was elucidated by UV (Cary 15), IR (Perkin-Elmer 237) and NMR spectrometry (Jeol C60 HL).

TABLE I

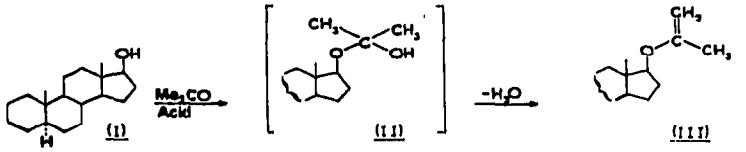
YIELDS OF REACTION PRODUCTS

Parent steroid	C_3F_7CO–O– *ester (%)*	*New derivative,* C_3F_7CO–$CH(CH_3)$–C–O– *(%)*
5α-Androstan-3α-ol	7	93
5α-Androstan-3β-ol	35	65
5α-Androstan-17β-ol	5	95

DISCUSSION

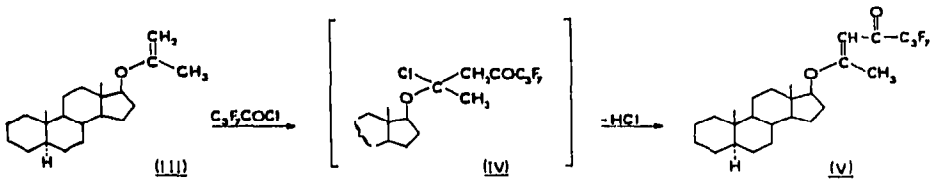
The normal reaction between a steroidal secondary alcohol and HFB anhydride in acetone solution leads to the formation of an HFB ester[2]. This reaction is strongly inhibited by an increasing proton concentration[3] and by compounds that have Lewis acid properties (TMCS).

Adlercreutz *et al.*[4] used TMCS as an acidic catalyst for the synthesis of acetonides and we have evidence that 5α-androstan-17β-ol (I) reacts with an acetone–TMCS (1:1) solution to form the 17β-isopropenyl ether (III) via the intermediate hemiketal (II). Small amounts ($\leqslant 5\%$) of 17β-trimethylsilyl ether can also be detected.



Compound III (5α-androstan-17β-yl-2′-propenyl ether) is an enol ether that is too labile to be purified by partition or adsorption chromatography. It is sufficiently stable in dry *n*-hexane containing a trace amount of pyridine and can therefore be analyzed by GC–MS: $I_{200}^{SE\text{-}30} = 2235$; $I_{200}^{OV\text{-}7} = 2410$; $M = 316$ ($C_{22}H_{36}O$); $[M^+] = 5$; $[M^+ - 15] = 2$; *m/e* [259] = 52, [258] = 33, [163] = 48, [135] = 36, [81] = 100, [67] = 100.

It is thus established that III is an identified intermediate in the reaction course that leads to the final compound 5α-androstan-17β-yl-(1′-heptafluorobutanoyl)-2′-propenyl ether (V) through the following reaction scheme:

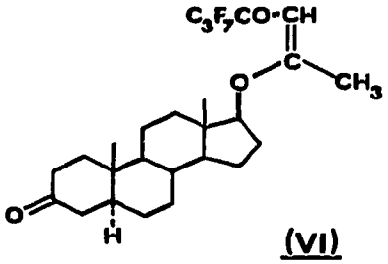


The role of TMCS in these reactions is three-fold: through its acidic properties, it inhibits the formation of the classical HFB ester; it favours hemiketal formation and through reaction with $(C_3F_7CO)_2O$ it forms C_3F_7COCl wich is the active acylating species here.

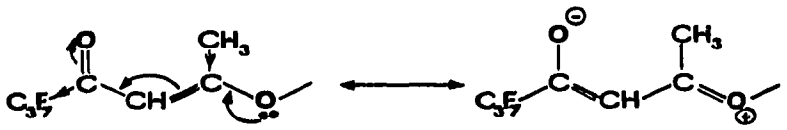
Compound V has excellent GLC properties ($I_{200}^{SE\text{-}30} = 2598$; $I_{200}^{OV\text{-}7} = 2651$) and its relative molar electron-absorbing activity [relative to testosterone bis(heptafluorobutanoylate)] is considerably higher than that of the corresponding HFB ester (Table II).

TABLE II

RELATIVE SENSITIVITIES FOR ECD

Compound	*Relative molar electron-absorbing activity*
5α-Androstan-17β-yl HFB	0.8
V	27
 (VI)	74

This can be explained by the mesomerism of the electrophore:



where the separation of the opposite charges favours the capture of a thermal electron. This emphasizes the important influence of conjugated unsaturation on the electron affinity.

The high electron affinity of VI, which is interesting for the quantitative determination of dihydrotestosterone by GLC with ECD, is due to a long-range interaction between the 3-keto function and the 17β–O–C$=$CH–COC_3F_7 derivative. This corroborates the previous statement, made by Lovelock *et al.*[5], on the marked electron absorption of steroids that possess the structure $-CO-CH=CR_1R_2$, provided that there is in the molecule an opportunity for further electronic interaction.

CONCLUSION

The new derivative, $C_3F_7CO-CH=C(CH_3)-O-$, has some selectivity, as steroids with phenolic or tertiary hydroxyl groups do not form the intermediate hemiketals.

There is a severe competition between the formation of the HFB ester (35%) and the 1-heptafluorobutanoyl-2-propenyl ether (65%) in the case of steroids with equatorial hydroxyl groups (Table I). This is due to the four-fold higher reactivity of equatorial alcohol groups towards $(C_3F_7CO)_2O$, compared with their axial isomers[3]. It is therefore advisable to use the 1-heptafluorobutanoyl-2-propenyl ether derivative

only with axial alcohol groups and to make a prior check on the relative rates of the two competing reactions.

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