FLUORINE-19 NUCLEAR MAGNETIC RESONANCE SPECTROSCOPY OF STEROL AND BILE ACID TRIFLUOROACETATES

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Received December 8, 1969

ABSTRACT

A new analytical method for hydroxysteroids by ^{19}F-NMR-spectroscopy of the trifluoroacetyl derivatives is described.

INTRODUCTION

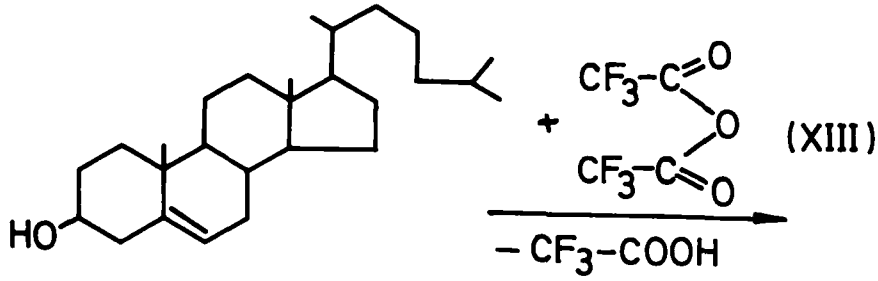
^{19}F-NMR spectroscopy has been applied to several classes of natural products in this laboratory. In the peptide and carbohydrate field it has been demonstrated that the ^{19}F-NMR spectra can be used with advantage for fast analysis and conformational assignments of trifluoroacetyl (TFA) derivatives of amino acids, peptides (1) and carbohydrates (2,3). Amino and hydroxyl groups of the so far investigated natural products react with trifluoroacetic anhydride to the corresponding trifluoroacetyl derivative. Every attached trifluoroacetyl group in the molecule causes one characteristic singlet peak in the fluorine spectrum. The purpose of this investigation is to demonstrate in what manner the ^{19}F-NMR signal of the trifluoroacetyl group at C_3 of several sterols and cholic acids is influenced even by far removed changes in the steroid molecule.

EXPERIMENTAL

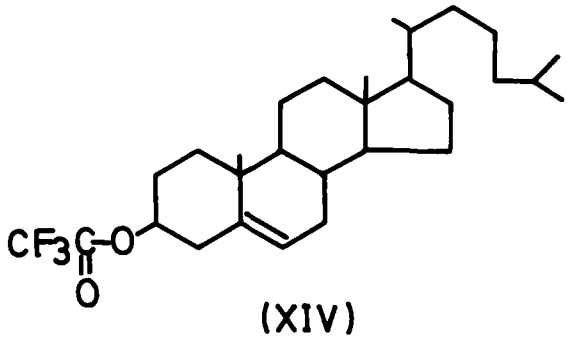
^{19}F-NMR spectra were measured on a Varian HA-60 (56.4MHz) instrument. All measurements were made at 25° C in CCl_4 containing 2 % methyl trifluoroacetate as internal standard. The steroid concentration was 0.2 molar. If the solutions prepared for measurements

are free of trifluoroacetic acid the δ values of the fluorine signals are not influenced by the steroid concentration. With the recently developed instrument Varian XL-100 (94.1 MHz for ^{19}F) we could get well re - solved fluorine spectra with samples of 1 mg of steroid derivative.

The trifluoroacetates were prepared as follows: The steroid (e. g. II) was dissolved in the least possible amount of trifluoroacetic anhydride (XIII). After a reaction time of 15 minutes at room temperature the solution was evaporated to dryness. Dissolving the residue in CCl_4 and evaporating again was done twice thereafter. The O-TFA-steroid (e. g. XIV) was then dried at 0.1 mm Hg over P_2O_5 and KOH for 10 hours.



(II)



(XIV)

RESULTS

The measured -values of trifluoroacetylated sterols and bile acids are presented in the following table:

TABLE

Chemical shifts (δ-values) of O-TFA sterols and bile acids

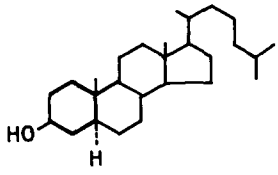
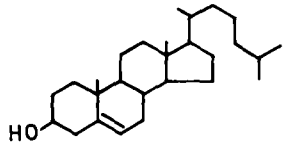
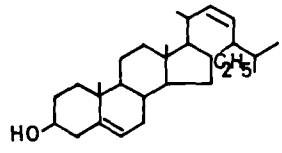
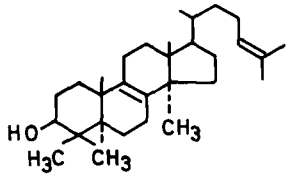
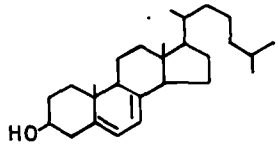
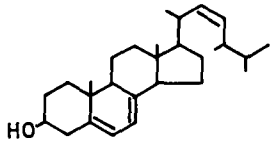
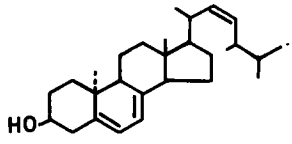
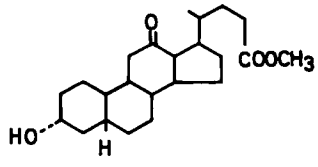
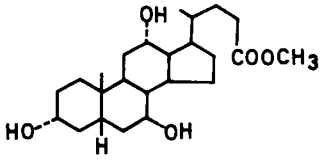
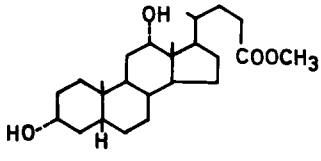
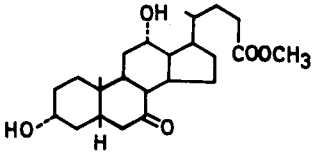
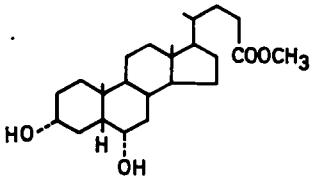
O-TFA-derivative	δ-value (ppm) C_3	C_6	C_{12}
 cholestanol (I)	0,395		
 cholesterol (II)	0,355		
 stigmasterol (III)	0,362		
 lanosterol (IV)	0,375		
 7-dehydrocholesterol (V)	0,35		
 ergosterol (VI)	0,33		

TABLE (continued)

	O-TFA-derivative	δ-value (ppm) C_3	C_6	C_{12}
	lumisterol (VII)	0,13		
	3 α-hydroxy-12-ketocholanic acid (methyl ester)(VIII)	0,385		
	cholic acid (methyl ester)(IX)	0,79	0,86 1,03	
	deoxycholic acid (methyl ester)(X)	0,39		0,475
	3 α, 12 α-dihydroxy-7-ketocholanic acid (methyl ester)(XI)	0,39		0,435
	hyodeoxycholic acid (methyl ester)(XII)	0,26	0,145	

DISCUSSION

The table and figure show the results of our fluorine resonance measurements: Compared to ^{1}H-NMR the ^{19}F-NMR spectra look rather simple. No confusion of overlapping multiplets is possible as no spin-spin coupling does occur.

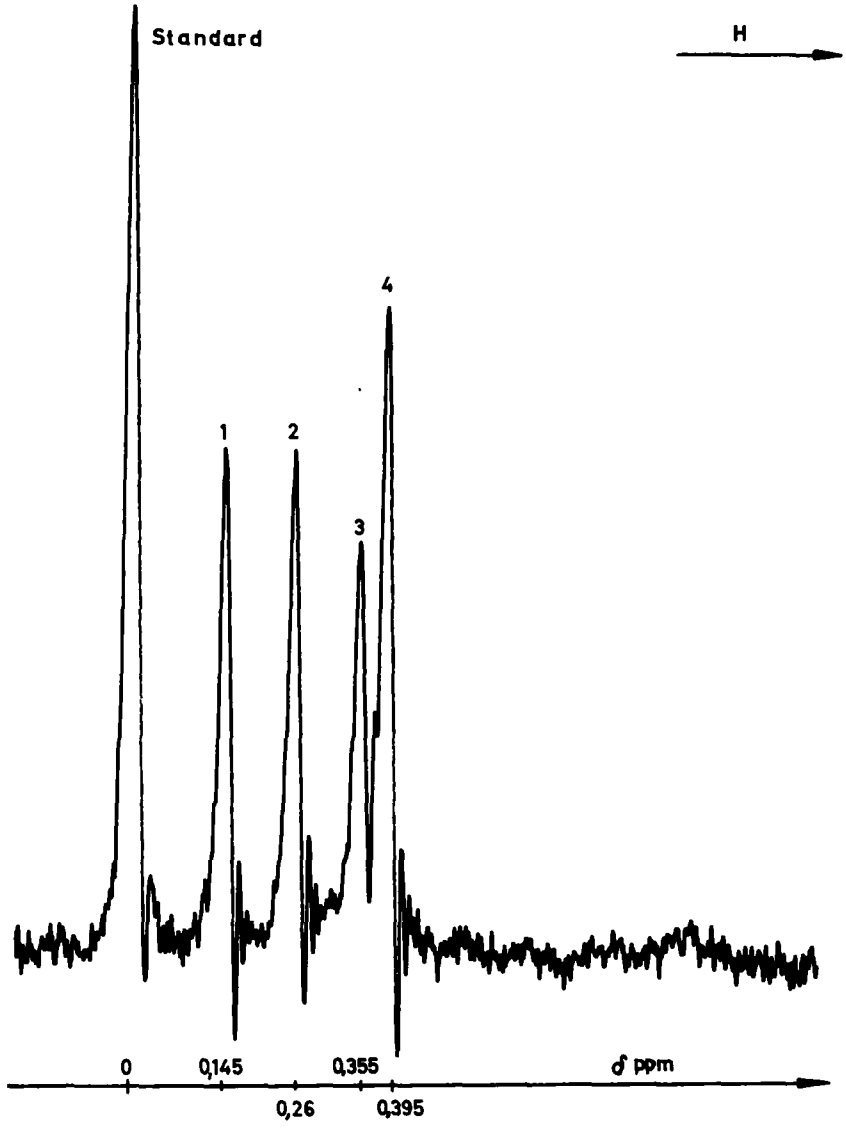


Figure: ^{19}F-NMR-spectrum of a mixture of O-trifluoroacetyl-
hyodeoxycholic acid (peaks 1 and 2)
cholesterol (peak 3)
cholestanol (peak 4)
(internal standard: methyl trifluoroacetate)

The above listed sterols have only one single hydroxyl group (always at position three) attached to the steroid skeleton; we expect in the fluorine spectra of their O-TFA-derivatives only one single peak. This series of compounds illustrates the influences of the chemical environment on the δ -value of the CF_3 signal due to the trifluoroacetyl group at C_3. The CF_3 signals of O-trifluoroacetyl-cholestanol(I), cholesterol (II) and stigmasterol (III) show that π bonds close to C_3 cause downfield shifts. The α- or ß-linkage of the methyl group attached to C_{10} in ergosterol (VI) and lumisterol (VII) characteristically influences the chemical shift of the CF_3 signal due to the C_3 O-trifluoroacetyl group. Nearby O-trifluoroacetyl groups cause a downfield shift of the CF_3 signal at C_3 as seen by comparing the ^{19}F-NMR-data of deoxycholic (X), 3 α, 12 α-dihydroxy-7-ketocholanic (XI) and hyodeoxycholic acid (XII) trifluoroacetates. From the seven sterols listed there are only two pairs whose signals could not be resolved by the Varian HA-60 if measured as a mixture: Cholesterol (II) and stigmasterol (III) and cholesterol (II) and 7-dehydrocholesterol (V). However, there is a slight but significant difference in the δ-values of the fluorine signals of the O-TFA-derivatives of cholesterol (II) and stigmasterol (III). A change in the far removed side chain still shifts the signal of the trifluoroacetyl group at C_3. The same effect is observed by comparing 7-dehydrocholesterol (V) with ergosterol (VI), however in this case the fluorine signals can be resolved well. The advantages of ^{19}F-NMR-spectroscopy for analytical purposes are obvious:

1) As the fluorine signals are very sensitive to the chemical environment characteristic parameters even for chemically very similar steroids are obtained.

2) The fluorine signals mostly appear as well separated singlets and they do not depend on concentration in the absence of trifluoroacetic acid.

3) Using the reaction conditions described above amino- and hydroxyl-groups will react specifically with trifluoroacetic anhydride. The method provides a suitable tool for identification and quantitative analysis of hydroxysteroids in steroid mixtures.

4) Additionally, the same derivatives can be used for analysis by mass spectrometry and gas liquid chromatography. Precious steroids can be gained back by hydrolysis of their trifluoroacetates.

ACKNOWLEDGEMENTS

We wish to thank E. Merck (Germany) and F. Hoffmann-La Roche (Switzerland) for steroid samples. We are indebted to the Deutsche Forschungsgemeinschaft for financial assistance. W. Voelter wishes to thank Deutsche Forschungsgemeinschaft for a Habilitationsstipendium.

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3. Jung, G., Voelter, W., Breitmaier, E. and Bayer, E., Liebigs Annalen, in press