Methyl Haloacetone Ketals as Steroidal Alcohol Derivatives in Gas–Liquid Chromatography and Combined GLC–Mass Spectrometry

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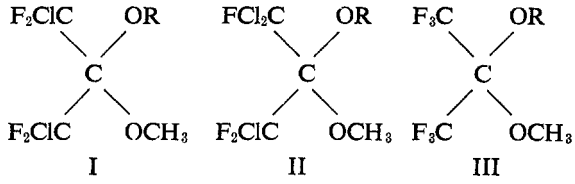
GAS-LIQUID CHROMATOGRAPHY of high-molecular weight polar compounds such as steroid alcohols is difficult because of the tendency of such compounds with free hydroxyl groups to become irreversibly absorbed and thermally dehydrated. Recourse to derivatives is common. Acetates are often utilized because they are easy to prepare and well-defined chemically; halogenated analogs, perfluoroacetates and perfluorobutyrates also have useful electron-capturing properties (*1–4*). Trimethylsilyl derivatives are popular because they are easy to prepare and result in exceptionally nonpolar derivatives.

Unfortunately, TMS derivatives are hydrolytically unstable, and the acyl derivatives have undesirable thermal and mass spectral properties. Finally, side reactions due to enolization may occur during the preparation of some of these derivatives particularly when pyridine is used to absorb the relatively strong acids liberated in the reaction. Because of this, alternative alcohol derivatives were sought which would be easy to prepare and which would combine the best features of the above adducts.

sym-Dichlorotetrafluoroacetone (4FK) and 1,1,3-trichlorotrifluoroacetone (3FK) are reported to form hemiacetals readily with lower alcohols (*5, 6*). The hemiacetal hydroxyl group generated is reported to undergo methylation with dimethyl sulfate and potassium carbonate as well as with methyl iodide and silver oxide (*7*).

We have now found that C_{18}, C_{19}, and C_{21} steroidal alcohols will react with these reagents and with gaseous hexafluoroacetone as well. The hemiketals obtained on evaporation of excess reagent were well-crystallized compounds, easily recrystallized from nonhydroxylic solvents and stable to thin-layer chromatography (TLC) on silica gel. Although they may be recommended as general derivatives of high molecular weight alcohols, they were quantitatively degraded to the parent alcohol on gas chromatography.

To stabilize the hemiketals, advantage was taken of the high acidity of the remaining hydroxyl group due to the electron withdrawing effects of the halogen and alkoxy substituents. Thus, rapid and quantitative conversion to the corresponding methyl ethers was affected by brief treatment with ethereal diazomethane. The *O*-methyldichlorotetrafluoroketals [2,4-MCFK, (I)], *O*-methyltrichlorotrifluoroketals [3,3-MCFK, (II)], and *O*-methylhexafluoroketals [6-MFK, (III)], formed in this manner



crystallized easily, were thermally and chemically stable, and especially suitable for GLC and combined GLC–mass spectrometry.

EXPERIMENTAL

The steroid hemiketals of 3FK and 4FK were made by dissolving the appropriate steroid in an excess of the reagent in benzene (20–60% v/v) and allowing the solution to stand at room temperature overnight. Reagents were then evaporated on a water bath at 60 °C with a stream of N_2. In the case of hexafluoroacetone, the steroid was dissolved in chloroform, cooled to −30 °C, and sufficient hexafluoroacetone condensed to give a visible increase in volume.

When the hemiketal itself was the desired product, it was recrystallized from petroleum ether. Thus the dehydroepiandrosterone hemiketal of 4FK melted at 138–139 °C compared with the parent steroid [Lit. dimorphic needles, mp 140–141°, plates mp 152–153° (*8*)]. In most cases the intermediate hemiketal, though crystalline, was immediately converted to its *O*-methyl ether by dissolving it in ethereal diazomethane at −10 °C. Immediate evolution of nitrogen ensued but the mixture was generally allowed to stand at −10 °C for 6–8 hr. The solvents were then evaporated and the usually crystalline product was subjected directly to combustion analysis with satisfactory results for several of the adducts listed in Table I. When partial conversion (85–97% by combustion analysis) occurred, recrystallization from aqueous ethanol achieved the necessary purification and satisfactory analyses were obtained for all compounds listed. The properties of the compounds are listed in Table I.

Under the conditions employed above, hydroxyl groups at the C-20 position are too sterically hindered to react with the reagent, but the less hindered C-17 position of testosterone is successfully derivatized. The *O*-methyl ethers are remarkably stable to hydrolysis either by aqueous *N* alkali or *N* hydrochloric acid.

Gas–liquid chromatography was carried out with a Model 400 F & M instrument (F & M Scientific Co., Avondale, Pa.) equipped with a tritium foil electron capture detector employing a pulsed DC voltage and a Glowall Model 320 instrument (Willow Grove, Pa.) equipped with a hydrogen flame detector. Column packings were 1% silicone gum nitrile (XE-60) on 80–100 mesh Diataport S (F & M Scientific

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Table I. Properties of *O*-Methylhaloacetone Ketals

Compound	MP	Formula	R_t:OV-17[a]	R_t:XE-60[b]	R_f:TLC[c]
3-Methylestradiol-17β-2,4-MCFK	109–110°	$C_{23}H_{28}F_4O_3Cl_2$	2.40	2.08	
Androsterone-3α-2,4-MCFK	120–1°	$C_{23}H_{32}F_4O_3Cl_2$	0.87	1.50	0.78
Androsterone-3α-6-MFK	147–9°	$C_{23}H_{32}F_6O_3$	0.42	0.89	
Etiocholanolone-3α-2,4-MCFK	110–11°	$C_{23}H_{32}F_4O_3Cl_2$	1.52	1.73	0.71
Dehydroepiandrosterone-3β-2,4-MCFK	132–5°	$C_{23}H_{30}F_4O_3Cl_2$	1.93	1.78	0.71
Testosterone-17β-2,4-MCFK	140–1°	$C_{23}H_{30}F_4O_3Cl_2$	3.00	4.82	0.45
Testosterone-17β-6-MFK	142–7°	$C_{23}H_{30}F_6O_3$	0.84	2.47	
Pregnanediol-3α-2,4-MCFK	161–2°	$C_{25}H_{38}F_4O_3Cl_2$	3.00	2.33	0.41
Pregnanediol-3α-3,3-MCFK	147–8°	$C_{25}H_{38}F_3O_3Cl_2$	4.14	4.57	0.06
Pregnenolone-3β-2,4-MCFK	166–7°	$C_{25}H_{34}F_4O_3Cl_2$	2.85	3.02	0.77
Pregnenolone-3β-6-MFK	159–163°	$C_{25}H_{34}F_6O_3$	0.78	1.73	
Allopregnanolone-3β-2,4-MCFK	140–3°	$C_{25}H_{30}F_4O_3Cl_2$	2.93	3.19	0.77

[a] Cholestane = 1.00; 8.2 min on 3% OV-17 at 250 °C, 20 psi inlet.
[b] Cholestane = 1.00; 7.0 min on 2% XE-60 at 200 °C, 20 psi inlet.
[c] Silica Gel G, benzene–ethyl acetate, 9/1 (v/v).

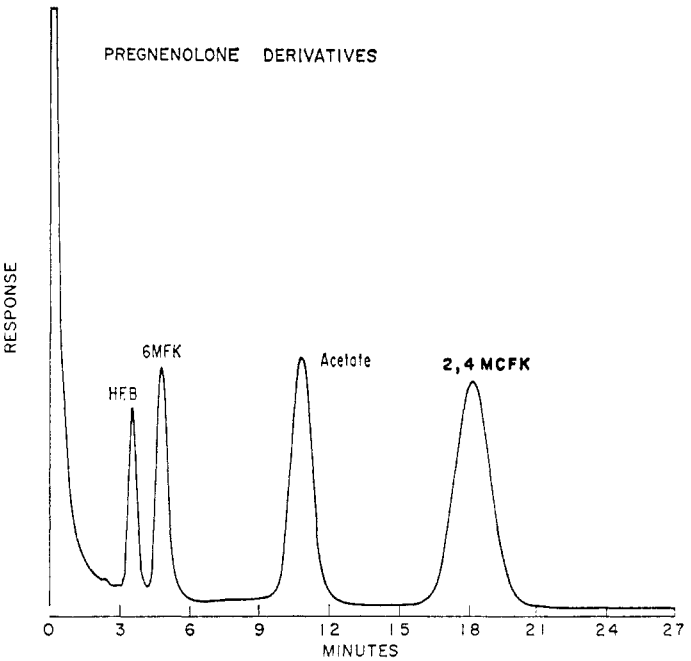

Figure 1. Gas-liquid chromatogram of *O*-methyl ketals of pregnenolone on 3% OV-17 at 250 °C

Co.) or 3% phenylsiloxane (OV-17) on Gas Chrom Q (Applied Science Co., State College, Pa.). Electron capture detector conditions for optimum signal/noise ratio were: carrier gas, 5% methane in argon without purge; pulse interval, 150 μsec; range and attenuation setting resulting in 6.2×10^{-10} A at full scale chart deflection. The hydrogen flame detector was optimized at 3×10^{-11} A at full scale chart deflection.

NMR spectra were determined on a Varian A-60 spectrometer using tetramethylsilane as internal reference. Mass spectra were determined on an LKB-9000 combined GLC–mass spectrometer using a 3% OV-17 on 100 mesh Gas Chrom Q (Applied Science Co.) column at 220 °C. The separator was 250° C and source was 290° C. Silica gel G or GF (Merck) was used for thin-layer chromatography.

RESULTS AND DISCUSSION

Chromatographic and Chemical Properties. Table I lists the derivatives with the mp's, relative retention times on XE-60 and OV-17 liquid phases, and R_f values on a typical TLC system. Symmetrical peak shapes were observed for these derivatives even when chromatographed in 1-mμg amounts, indicating their lack of interaction with adsorptive sites.

Figure 1 compares the 6-MFK and 2,4-MCFK derivatives of pregnenolone with its acetate and heptafluorobutyrate on an OV-17 column. The hexafluoroacetone adduct elutes near the heptafluorobutyrate and would be especially advantageous for higher molecular weight or polyhydroxylated compounds. The trichlorotrifluoroacetone adducts [(3,3-MCFK (II)] are retained longest of the series (Table I) because of their additional molecular weight due to the chlorine atoms.

Figure 2 demonstrates the stability of these compounds to refluxing alkali. The heptafluorobutyrate as well as the acetate has been hydrolyzed to a mixture of pregnenolone and its 17-epimer while the haloacetone adducts have undergone epimerization only. The *O*-methyl group of the 2,4-MCFK derivatives is observed as a rather broad band in the NMR spectrum at δ 3.56, and its intensity may be useful in determining the number of alcohol groups derivatized. The 3,3-MCFK derivatives show an analogous band at δ 3.30.

GLC–Mass Spectral Properties. The mass spectra of the

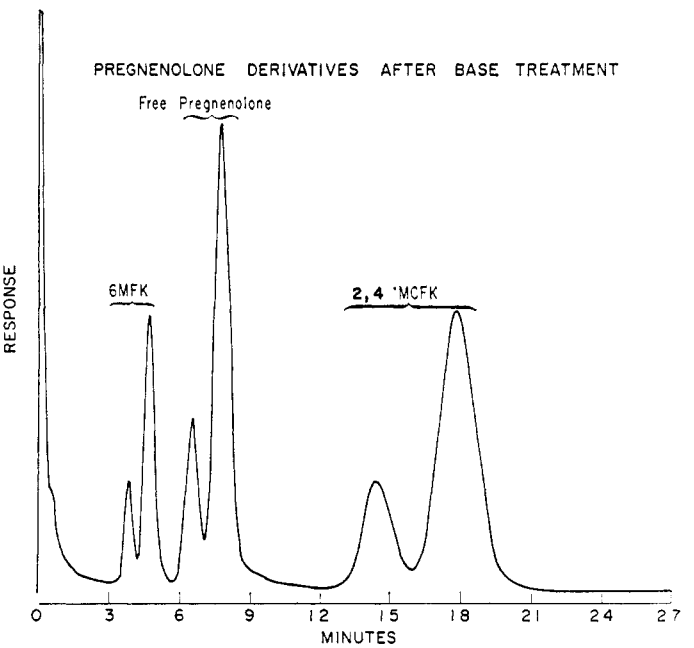

Figure 2. Gas-liquid chromatogram of same mixture as in Figure 1 after treatment with base

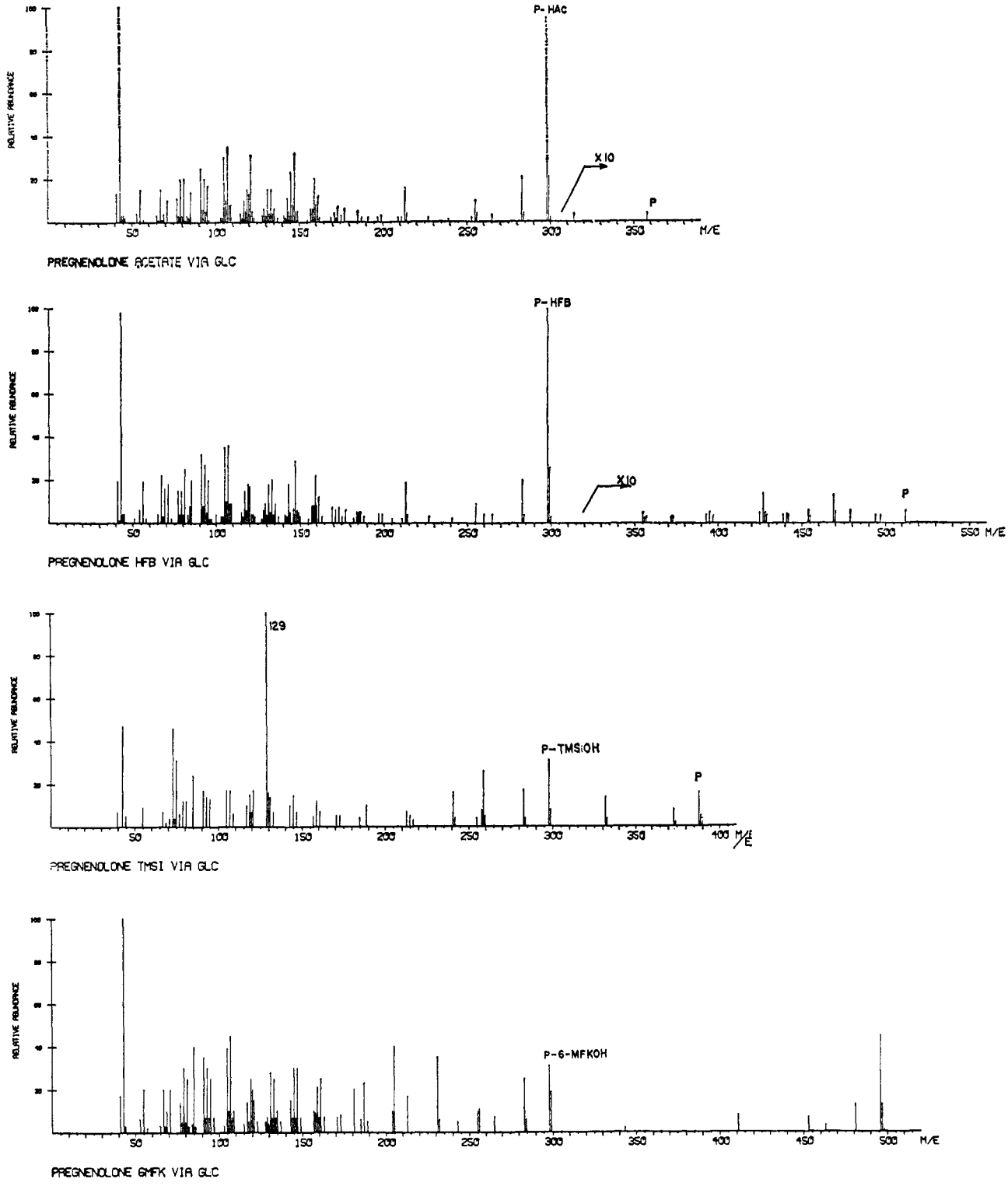

Figure 3. Mass spectra of pregnenolone derivatives *via* GLC–MS interface

acetate, heptafluorobutyrate, trimethylsilyl ether, and 6-MFK-derivatives of pregnenolone are compared in Figure 3. All were run *via* the GLC inlet with a separator temperature of 250 °C and the ion-chamber at 290 °C. Identical spectra were obtained *via* the direct insertion probe and the extensive deacylation of the acyl derivatives is therefore due to the relatively high ion-chamber temperature rather than the separator. Unfortunately, the source temperature must be maintained at this high value in the GLC–MS of high molecular weight compounds in order to ensure rapid scavenging and pump-out of samples so that adjacent peaks are not cross-contaminated.

The trimethylsilyl derivative is more thermally stable and shows a satisfactory molecular ion but the most abundant ion at *m/e* 129 is due to $[(CH_3)_3SiO{=}CHCH{=}CH_2]^+$ involving C_1, C_2, and C_3 in one of the unusual rearrangements to which silyl derivatives are prone (*9*).

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Table II. Comparative Electron Capture of Halogenated Steroid Derivatives

Compound	Electron capture (peak area/mass of derivative, testosterone-17β-HFB = 100)
Testosterone-17β-heptafluorobutyrate	100
Testosterone-17β-2,4-MCFK	33
Testosterone-17β-chloroacetate	22
Testosterone-17β-6-MFK	2
Androsterone-3α-2,4-MCFK	16
Androsterone-3α-chloroacetate	16
Androsterone-3α-heptafluorobutyrate	22
Pregnanediol-3α-2,4-MCFK	4
Pregnanediol-3α-3,3-MCFK	7
Pregnenolone-3β-2,4-MCFK	4
Allopregnanolone-3β-2,4-MCFK	6
3-Methylestradiol-17β-2,4-MCFK	6

The 6-MFK derivative (Figure 3) exhibits the most abundant molecular ion of the series and its molecular ion is the second most abundant ion in its spectrum, exceeded only by *m/e* 43 from the acyl side chain. In the case of androsterone 6-MFK and testosterone 6-MFK, the molecular ion is the largest peak in the spectrum.

The chlorine-containing adducts (3,3-MCFK and 2,4-MCFK) also show good molecular ions but their intensities are much more dependent on ion-chamber temperature than the 6-MFK derivatives.

Electron-Capture Properties. On a weight basis, the 2,4-MCFK derivative of testosterone was the most active of those tested (Table II). The derivatives appear to be comparable in activity to the chloroacetates, and less active than the heptafluorobutyrates.

A higher chlorine content as in the 3,3-MCFK derivative of pregnenolone almost doubles the activity compared to the 2,4-MCFK derivative. On the other hand, the chlorine-free 6-MFK derivatives are less active than the heptafluorobutyrates which contain a higher fluorine content. Thus, steric and electronic factors are clearly involved in the electron capturing process.

Linear response, using the 2,4-MCFK derivatives of testosterone, pregnanediol, and 3-methylestradiol, was obtained in the range of 1–5 mμg, confirming the thermal stability and lack of adsorption of these haloketal derivatives.

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Microanalytical Method for Determination of Perbromate Ion in the Presence of Macro Amounts of Other Bromine Anions

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THE NEED for a sensitive, rapid, and convenient analytical method for the determination of perbromate ion in its compounds and in the presence of much larger concentrations of bromate and/or bromide ion has been greatly stimulated by the recent successful preparation of perbromic acid and three of its alkali-metal salts (*1*, *2*), and by the discovery of BrO_4^- ion in radiolyzed, crystalline $KBrO_3$ and $CsBrO_3$ (*3*).

Previous analyses (*1*, *2*) for perbromate have employed either reduction to Br_3^- by 12*M* HBr followed by Br_3^- oxidation of I^- to I_3^- which was titrated with thiosulfate, or, reduction to Br^- by Mo(VI) catalyzed $SnCl_2$ in 6*M* HCl followed by chlorine oxidation of Br^- to BrCN in neutral cyanide solution, reduction of BrCN by acid iodide, and titration of I_3^- with thiosulfate. Both methods are accurate, but they are also long, tedious, and work best for relatively pure BrO_4^- ion samples. If appreciable amounts of other bromine oxyanions are present, *i.e.*, BrO_3^-, a preliminary step must be performed in *ca.* 1.5*M* HBr to reduce said species to Br_2 which then is sparged from solution with an inert gas.

The microanalytical procedure developed in this investigation is an adaptation of the crystal violet solvent extraction–spectrophotometric method for the determination of ClO_4^- ion (*4*). Concentrations of 1 to 10 × $10^{-6}M$ BrO_4^- ion can be determined in the presence of 1000 times larger amounts of bromate or bromide ion with good accuracy.

EXPERIMENTAL

High purity $KBrO_4$ was recrystallized from an aqueous solution initially *ca.* 0.2*M* in $KBrO_4$ and containing less than 1% F^- ion and/or lower bromine oxidation states. The recrystallized salt was vacuum dried at room temperature over $N_2(l)$ for >48 hours before analysis and preparation of a standard solution. Infrared analysis showed neither H_2O vibrational bands nor any absorptions by bromine oxyanions of lower oxidation state, *e.g.*, BrO^-, BrO_2^-, or BrO_3^-. A 5.226 × $10^{-3}M$ standard $KBrO_4$ solution was prepared and checked for F^- and Br^- ion contamination with specific ion electrodes. The standard solution was found to be $\leq 1 \times 10^{-5}M$ in F^- and $\leq 5 \times 10^{-7}M$ in Br^- ions, which were considered negligible. The other inorganic chemicals were reagent grade and were used without further purification.

A 1.00 × $10^{-3}M$ solution was prepared from National Aniline "Certified" crystal violet (91% total dye content as the chloride salt) and deionized water. This solution was filtered through a fine sintered glass filter to remove insoluble

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