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Characterization of cholestanols and cholestanones by thin-layer and gas chromatography

Steroid boranes. II.

The thermal isomerization of organoboranes derived from steroid olefins, followed by oxidation with alkaline hydrogen peroxide yields a complex mixture of 5α- and 5β-cholestanols, differing only in the position and stereochemistry of the hydroxyl groups[1].

In this paper we report the retention times and R_F values of a series of isomeric cholestanols and cholestanones, as well as those of some of their derivatives, which were used to identify the components of a mixture of stanols resulting from the isomerization and hydroboration of cholest-5-ene.

Experimental

Steroids. The steroids used in the investigation were prepared by well-known published methods[2].

Thin-layer chromatography. All chromatograms were run on Silica Gel G (E. Merck). The plates were stored in a desiccator after activation at 110°. The plates were prepared on a Shandon plate spreader and coated to a thickness of 0.25 mm.

The solvent systems used were: cholestanoles: two developments with benzene (Matheson, Coleman and Bell, Spectrograde); cholestanones: one development with benzene.

Detection: vanillin–sulphuric acid–ethanol spray; the plate is heated to 110° for 5 min after spraying. Cholestanols give blue-violet spots, whereas the cholestanones give colours from yellow to violet[3].

Vapour phase chromatography. A Pye Panchromatograph with argon ionization detector and 7 ft. × 1/4 in. glass columns was used. The solid support was Gas Chrom Q 100/120 mesh (Applied Science Laboratories, Inc.). The liquid phase used was 3 % HI-EFF 8BP (cyclohexane–dimethanol succinate, Applied Science Laboratories, Inc.). The carrier gas was argon (Agamex). The conditions are given in Table I.

Trimethylsilyl ethers of the alcohols were prepared with BSA reagent (Pierce Chemical, Inc.) according to instructions supplied by the manufacturer.

TABLE I

CONDITIONS FOR VAPOUR PHASE CHROMATOGRAPHY

	Temperature (°C)		*Flow (ml argon/ min)*
	Column	*Detector*	
Free alcohols	225	235	68
Trimethylsilyl ethers Trifluoroacetates	200	225	91
Ketones O-Methyloximes	225	235	68

Trifluoroacetates were prepared by allowing the alcohols to react with trifluoroacetic anhydride (Distillation Products, Inc.) at room temperature and evaporating to dryness with a stream of dry nitrogen. The residues were dissolved in chloroform for injection into the column.

O-Methyloximes were prepared by treating the ketone with O-methoxyamine (Applied Science Laboratories, Inc.)[4].

1-Microlitre injections of 5 mg/ml chloroform solutions of the steroids and their derivatives were made.

Results

The retention times (5α-cholestane = 1.0) and R_F factors are listed in Table II.

TABLE II

RETENTION TIMES AND R_F VALUES OF STEROID ALCOHOLS AND STEROID KETONES

Steroid alcohols	*Retention time (5α-cholestane = 1.0)*			*R_F value of free alcohol*
	Free alcohol	*TMS ether*	*Trifluoro-acetate*	
5α-Cholestan-1α-ol	—	0.77	—	0.80
5α-Cholestan-1β-ol	—	1.18	—	0.74
5α-Cholestan-2α-ol	6.81	1.81	1.68	0.32
5α-Cholestan-2β-ol	—	1.50	—	0.50
5α-Cholestan-3α-ol	5.75	1.38	1.49	0.43
5α-Cholestan-3β-ol	6.52	2.24	2.08	0.25
5β-Cholestan-3α-ol	7.12	1.83	1.69	0.39
5α-Cholestan-4α-ol	3.69	1.96	1.62	0.39
5β-Cholestan-4β-ol	4.56	1.41	1.21	0.48
5α-Cholestan-6α-ol	5.45	1.40	1.38	0.53
5β-Cholestan-6β-ol	—	1.03	—	0.64
5α-Cholestan-7α-ol	—	1.41	1.45	0.83
5α-Cholestan-7β-ol	—	0.698	0.71	0.69

Steroid ketones	*Retention time*		*R_F value of free ketone*
	Free ketone	*O-Methyl oxime*	
5α-Cholestan-2-one	5.47		0.25
5α-Cholestan-3-one	6.48	4.82	0.20
5β-Cholestan-3-one	5.64	4.08	0.23
5α-Cholestan-4-one	4.8	3.29	0.27
5β-Cholestan-4-one	3.8	2.35	0.41
5α-Cholestan-6-one	4.88	2.61	0.31
5β-Cholestan-6-one	4.04	2.56	0.24
5α-Cholestan-7-one	4.2	2.59	0.53

Discussion

Under the conditions of vapour phase chromatography used, the retention times of the free stanols were rather long and the peaks broad, resulting in considerable overlapping and poor resolution. The sharpest peaks and best resolution were obtained with the TMS ethers of the cholestanols. In cases where mixtures could not

be resolved using TMS ethers, trifluoroacetates were tried, as well as conversion of the stanols to ketones. The comparison of the R_F values of the cholestanols and cholestanones helped resolve the last doubts on the identity of the components of almost any mixture of the above-tabulated cholestanols.

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Gas chromatographic separation of 4'-nitroazobenzene-4-carboxylic acid esters of alcohols

The basic reagent 4'-nitroazobenzene-4-carboxylic acid chloride was first prepared by HECKER[1] in 1955, and later on used as a reagent for hydroxyl compounds as reported by BUTENANDT[2,3] during investigations on 'bombicol'. The separation of amines as 4'-nitroazobenzene-4-carboxamides has been described earlier[4]. In the same way the gas chromatographic separation of esters can be helpful in the identification of saturated and unsaturated aliphatic and aromatic alcohols as well as phenols.

Experimental

Apparatus. For chromatography, 1-m and 2-m stainless steel tubes (4-mm I.D.) packed with 2.5 % w/w silicone grease (E. Merck AG., Darmstadt, Germany) on 60 to 80 mesh Chromosorb G, acid washed and DMCS treated, were used in fractometer type F6 (Perkin Elmer Bodenseewerk, Überlingen, Germany). The fractometer was equipped with a flame-ionization detector and a 2.5-mV recorder (Siemens-Kompensograph L 288 × 288) with a paper feed of 0.5 cm/min.

Procedure. The 4'-nitroazobenzene-4-carboxylic acid esters were prepared according to HECKER[1] and purified to the melting points listed there. Previously unrecorded derivatives were crystallized to a constant melting point. Samples of 1 to 2 μg in about 5 μl of benzene were injected into the chromatograph.