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THE USE OF DIFFERENT SILYLATING AGENTS FOR STRUCTURE ANALYSES OF STEROIDS

HELMUT GLEISPACH
University Childrens Hospital, A-8010 Graz (Austria)

SUMMARY

A number of different silylating agents and their mixtures are available with different activities and, if combined, mutual stimulation or even suppression of their behaviour may occur. We were therefore interested in determining the optimal reaction conditions and also the possibility of using them for structure analyses. Different steroids have been treated with silylating agents under various conditions. The silyl ethers were dissolved in carbon tetrachloride and analysed by gas–liquid chromatography–mass spectrometry. It could be proved that every hydroxyl group in a steroid could be transformed into its silyl ether depending on the silylating mixture used. Oxo groups in the α-position to a hydroxyl group can react with formation of the enediol trimethylsilyl ether, whereas free oxo groups react under strong conditions with formation of the enol trimethylsilyl derivative.

INTRODUCTION

Since the first description of trimethylsilyl (TMS) ether derivatives[1] and their application to steroids[2] for analyses by gas–liquid chromatography (GLC), the determination of steroids by GLC has become more and more important for the detection of the whole steroid pattern. For the analysis of the structure of an unknown steroid, combined GLC–mass spectrometry (MS) is the most widely used technique. But even when GLC–MS is used it is necessary to form different derivatives so as to be certain about the location of the reactive groups. Many silylating agents with different and specific activities are available today. N,O-Bis(trimethylsilyl)acetamide (BSA), for example, has been used by Horning *et al.*[3] and also in this laboratory[4], whereas the high reactivity of bis(trimethylsilyl)trifluoroacetamide (BSTFA) has been applied by Ros[5] for the detection of urinary steroid profiles by GLC. Chambaz[6] used BSA combined with potassium acetate for the formation of the 20,21-enediol TMS ethers of corticosteroids.

EXPERIMENTAL

Method and material

An RMU-6-M Hitachi Perkin-Elmer mass spectrometer coupled with an all-

glass interface to a Perkin-Elmer 990 gas chromatograph was used for the analyses.

GLC conditions. A 2-m glass column filled with 3% OV-1 on Gas-Chrom Q was used with helium as carrier gas at a flow-rate of 30 ml/min. The column temperature was 220–270° and the injection and manifold temperatures were 270°.

MS conditions. The mass range was 1000, the temperature of the substance-heater was 140–180° and that of the chamber was 190°. The acceleration potential was 70 eV.

Reagents

Pure steroids from E. Merck (Darmstadt, G.F.R.) or Ikapharm (Ramat-ban, Israel) were used as test substances. The silylating agents were obtained from Merck or from Macherey, Nagel & Co. (Düren, G.F.R.).

Procedure

In order to be certain about the results, every derivative was examined by the direct inlet system and also by GLC–MS. Sometimes in the GLC–MS analysis, more than one peak was obtained after silylation of the steroid (results of a semi-quantitative reaction) and it could be demonstrated that the different peaks were mono-, di-, tri- or even penta-TMS derivatives of the same steroid.

Systematic names, abbreviations and structures of the silylating agents used are shown in Table I.

The reactions of the steroids with the silylating agents were carried out in a solution of the silylating agent in pyridine (1:1) or in tetrahydrofuran (1:1) or by using the pure silylating agent without solvent. It could be proved that it is not necessary to use a solvent when employing the silylating agents mentioned above, as they develop a sufficiently high solvent action for the steroids to react.

Reaction–temperature and reaction–time studies

The reaction of the various silylating agents with the different reactive groups of the steroids was carried out by incubating 100 μg of the steroid with an excess of the silylating agent over a period of 24 h at room temperature or for 1/2, 1 or 5 h at 60°. Immediately after the reaction, the excess of silylating agent was removed by blowing a stream of dry nitrogen on to the surface of the reaction mixture at room temperature. The residue was dissolved in 0.1 ml of carbon tetrachloride and 2 μl were injected into the chromatograph or 10 μg of each sample were introduced into the mass spectrometer using the direct inlet system. The most reproducible results and the lowest formation of decomposition products were observed using incubation at room temperature over 24 h. No differences in the reaction products were observed if the treatment at room temperature was prolonged for more than 24 h. At 60°, it was found that after short incubation times the reaction was not complete, and after longer incubation times decomposition of steroids often occurred. For this reason, the results discussed were obtained after incubation of 100 μg of the steroid with 0.2 ml of the pure reagent or of mixtures of the reagents. The only exception was in the case of hexamethyldisilazane (HMDS), of which 0.5 ml was used.

RESULTS

The results obtained after incubation for 24 h at room temperature are shown

TABLE I

SILYLATING AGENTS USED IN THIS INVESTIGATION

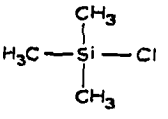
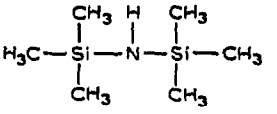
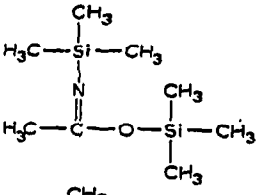
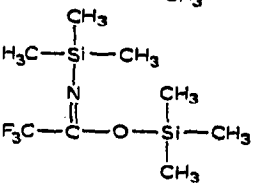
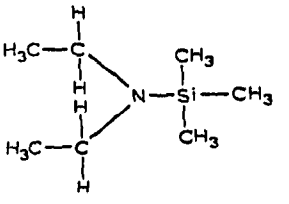
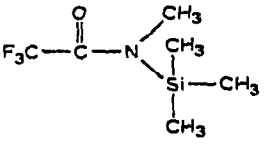
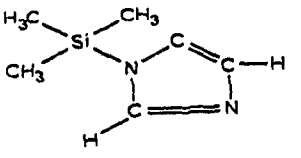
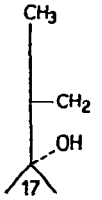
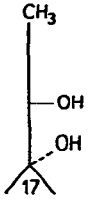
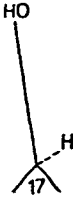
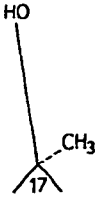
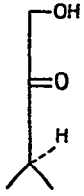
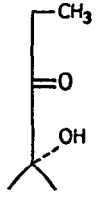
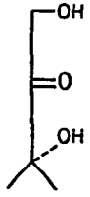
Systematic name	*Abbreviation*	*Formula*
Trimethylchlorosilane	TMCS	
Hexamethyldisilazane	HMDS	
N,O-Bis(trimethylsilyl)acetamide	BSA	
N,O-Bis(trimethylsilyl)trifluoroacetamide	BSTFA	
Trimethylsilyldiethylamine	TMSDEA	
N-Methyl-N-trimethylsilyltrifluoroacetamide	MSTFA	
Trimethylsilylimidazole	TMSI	

TABLE II

GROUPS SILYLATED BY THE DIFFERENT REAGENTS AFTER 24 h AT ROOM TEMPERATURE

Positive reactions are indicated by +, semi-quantitative reactions by ± and negative reactions by −.

Silylating agent	*Group*															
	3α-OH; 3β-OH	*HO-Ph*	*11β-OH*	*16α-OH*					*20α-OH; 20β-OH*	*21-OH*				*3-Oxo*	*17-Oxo*	*20-Oxo*
HMDS	±	+	−	−	−	−	−	−	−	+	−	−	−	−	−	−
TMCS	±	+	−	−	−	−	−	−	+	±	−	−	−	−	−	−
HMDS + TMCS	+	+	−	+	−	−	+	−	+	+	−	−	−	−	−	−
BSA	+	+	−	+	−	−	+	−	+	+	−	−	−	−	−	−
BSA + TMCS	+	+	+	+	−	−	+	−	+	+	−	−	−	−	−	−
TMSDEA	+	+	−	+	−	−	+	−	+	+	−	−	−	−	−	−
TMSDEA + TMCS	+	+	+	+	−	−	+	−	+	+	−	−	−	−	−	−
BSTFA	+	+	−	+	−	−	+	−	+	+	−	−	−	−	−	−
BSTFA + TMCS	+	+	+	+	−	−	+	−	+	+	−	−	−	−	−	−
MSTFA	+	+	−	+	−	−	+	±	+	+	±	−	±	−	±	±
MSTFA + TMCS	+	+	+	+	−	−	+	−	+	+	−	−	−	−	−	−
MSTFA + potassium acetate	+	+	−	+	−	−	+	±	+	+	+	+	+	±	+	+
TMSI	+	+	−	+	+	+	+	−	+	+	−	−	−	−	−	−
TMSI + MSTFA	+	+	−	+	+	+	+	−	+	+	−	−	−	−	−	−

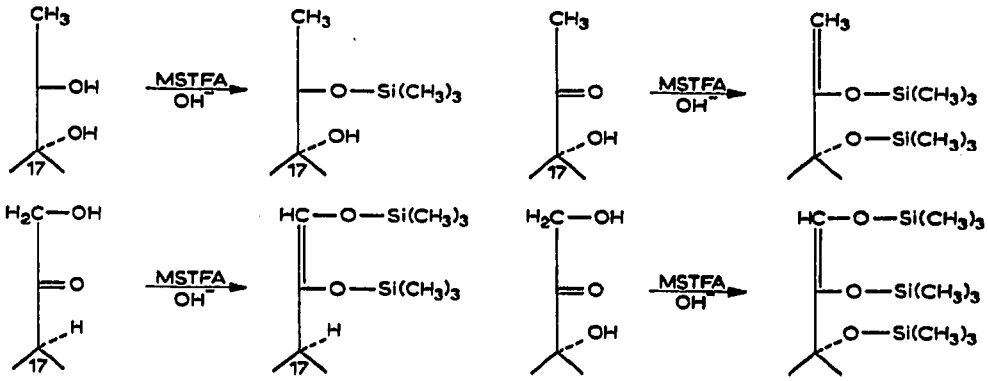


Fig. 1. Reaction of the side-chain with MSTFA and potassium acetate.

in Table II. It is obvious that HMDS or TMCS alone have only a very small reactivity, whereas all of the other reagents form silyl derivatives with the sterically non-hindered hydroxyl groups. An exception is TMSI, which reacts particularly well with the sterically hindered 17α-hydroxyl group but not so easily with non-hindered hydroxyl groups. The sterically hindered 11β-hydroxyl group can be silylated only by using BSA, MSTFA or TMSDEA combined with the acidic catalyst TMCS. Only MSTFA reacts with oxo groups without a catalyst, but even then the reaction is not quantitative. TMSDEA, BSA and MSTFA combined with potassium acetate as a basic catalyst react quantitatively (more than 90%) with the 20-oxo groups of the corticoids with formation of the 20,21-enediol or the 17α-hydroxy-20,21-enediol-TMS derivatives,

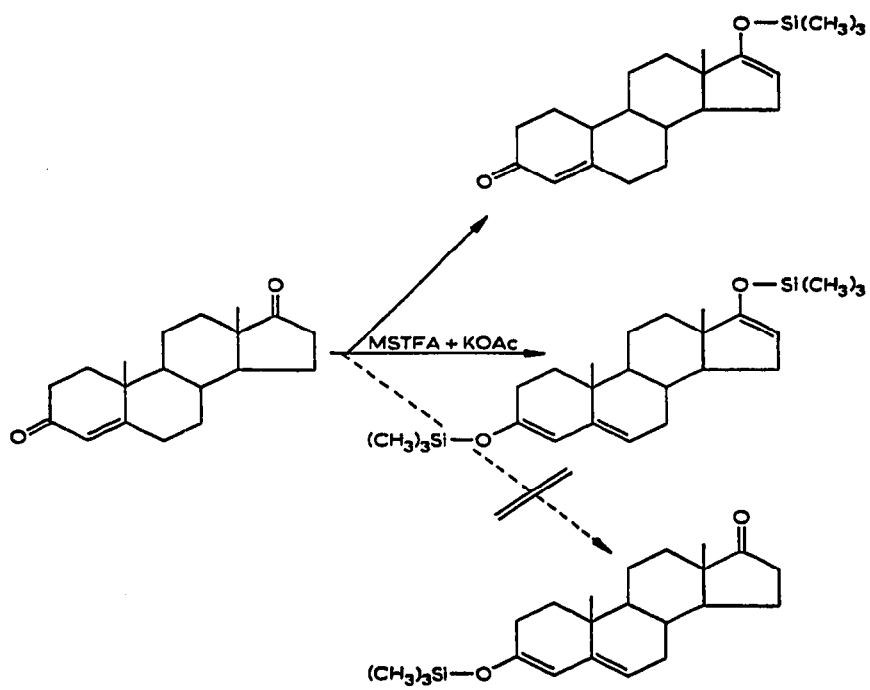


Fig. 2. Reaction of androstenedione with MSTFA and potassium acetate.

as shown in Fig. 1. Therefore, these reagents can be used for stabilisation of the 17α-hydroxy-20-oxo and the 20-oxo-21-hydroxy groups, as well as for the silylation of the dihydroxyacetone group of the side-chain with formation of the 20,21-ene-17,20,21-trihydroxy-TMS derivative. The fact that MSTFA with potassium acetate as catalyst reacts in the case of the dihydroxyacetone group even with the 17α-hydroxyl group is proved not only by the molecular weight of the silyl derivatives but also by the molecular weight of the fragments obtained by MS.

In comparison, it is interesting that MSTFA combined with potassium acetate also reacts with the 17α-hydroxy-20-oxo group with formation of the corresponding enediol-TMS derivatives, whereas it reacts only with the 3α- and the 20β-hydroxyl groups in the case of pregnane-3α,17α,20β-triol. MSTFA also reacts under the influence of a basic catalyst with the 3-oxo and 17-oxo groups but not with the sterically hindered 11-oxo group. In the case of androstenedione (Fig. 2), two products are obtained: depending on the reaction conditions, either the 3,17-disilyl derivative or the 17-enol-TMS ether is obtained, whereas we were unable to find a means of forming the mono-3-enol-TMS derivative.

DISCUSSION

HMDS or TMCS alone can be used for the partial silylation of 3α-, 3β-, 21- and 3-phenolic hydroxyl groups. Sterically non-hindered hydroxyl groups can be silylated with BSA, BSTFA, TMSDEA or MSTFA. Silylation of the sterically hindered 17α-hydroxyl group can be carried out by use of TMSI, whereas 11β-hydroxyl groups can be silylated with a mixture of TMSDEA, BSA or MSTFA with TMCS. The use of MSTFA with a basic catalyst such as potassium acetate ensures the formation of the 20,21-enediol-TMS or of the 20,21-ene-17,20,21-triol-TMS derivatives of the glucocorticoids and also the formation of the enol-TMS ethers of oxo steroids. Therefore, of the GLC analysis of the different silyl derivatives obtained from one steroid under varying conditions gives the possibility of more or less checking the structure of a steroid by comparison of the retention times. By using GLC–MS, the detection of the position of hydroxyl and oxo groups of an unknown steroid is possible after formation of particular TMS derivatives using different silylating agents.

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