Journal of Steroid Biochemistry, 1972, Vol. 3, pp. 741–747. Pergamon Press. Printed in Great Britain

TMS-ENOL-TMS: A NEW TYPE OF DERIVATIVE FOR THE GAS PHASE STUDY OF DIHYDROXYACETONE SIDE CHAIN SATURATED CORTICOSTEROID METABOLITES

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(*Received 2 September* 1971)

SUMMARY

Using base-catalysed silylating conditions, it is possible to prepare in a quantitative fashion a new type of derivative from hydroxyketosteroids. The gas phase behavior of these derivatives is excellent. This type of reaction is an effective means of stabilizing the dihydroxyacetone side chain of corticosteroid metabolites, and mass spectrometric data indicate a 17α, 21 di TMS-20 enol TMS structure. This new type of derivative should facilitate the gas phase study of corticosteroid hormones.

THE GAS phase analysis of the main corticosteroid metabolites possessing the dihydroxyacetone side chain raises a crucial problem since this structure is thermally unstable and easily lost during gas liquid chromatography [1]. Successful analysis of this type of structure can only be achieved by some kind of modification of the free compounds; several approaches have been investigated in the past and recently reviewed [2]. Apart from the chemical removal of the side chain prior to g.l.c., a number of derivatives have been introduced but they usually suffer from non-quantitative formation, although they may be useful for structural characterization [2]. At the present time, for quantitative studies, the 0-methyl-oxime-trimethylsilylethers [3] are the most satisfactory although care should be taken when they are used on a routine basis for the analysis of biological mixtures, since contaminents such as acid traces may lead to non reproducible silylating conditions.

A study of the trimethylsilyl (TMS) ethers of steroids of biological interest has shown that enol-TMS can be obtained from keto groups [4, 5] under acid-catalysed conditions and that these derivatives have good g.l.c. behavior.

This paper describes the use of base-catalysed silylating conditions to obtain a new type of derivatives from the corticosteroid dihydroxyacetone side chain. This derivative is quantitatively formed and exhibits excellent g.l.c. properties; its structure was assessed on the basis of mass spectrometric data.

EXPERIMENTAL

Typical corticosteroid metabolites from commercial sources (Mann Research Lab., New York) were taken as model compounds: 3α,21-dihydroxy-5β-pregnan-

11,20-dione (THA); 3α,11β,21-trihydroxy-5β-pregnan-20-one (THB); 3α,17α,21-trihydroxy-5β-pregnan-20-one (THS); 3α,17α,21-trihydroxy-5β-pregnan-11,20-dione (THE); 3α,11β,17α,21-tetrahydroxy-5β-pregnan-20-one (THF) and its 5α isomer (allo-THF); 5β-pregnane-3α, 11β, 17α, 20α, 21-pentol (Cortol).

The silylating agents used in this work (BSA = N,O-bis trimethylsilylacetamide; BSTFA = N,O-bis trimethylsilyltrifluoracetamide; TSIM = trimethylsilylimidazole) were purchased from Pierce Chemicals Co., Rockford, Illinois (U.S.A.). All reactions were carried out in tightly capped small test tubes.

Non catalysed silylation was carried out on 50 μg of steroid with 200 μl of BSA or BSTFA overnight at room temperature[4].

For the base catalysed reaction, 0·2 ml of a methanolic solution of sodium (or potassium) acetate (50 mg/ml) were first evaporated to dryness under a nitrogen stream in the reaction tube; 50 μg of the steroid was then added in ethanol (1 mg/ml). After complete evaporation of the solvent, 200 μl of BSTFA was added and the mixture left to react overnight at room temperature; 2–5 μl were directly injected onto the gas chromatograph or the gas-chromatograph-mass spectrometer.

For the quantitative study of the reaction, cholesterol butyrate (50 μg) was added as an internal standard prior to the silylating agent.

Gas chromatographic analyses were performed on twelve foot silanized glass columns filled with 100–120 mesh gas chrom P coated with 1% (w/w) stationary phase (OV-1 or OV-101 and OV-17) according to Horning *et al.*[7]. Retention parameters were expressed as methylene units (M.U.) as previously described[7].

Mass spectra were obtained thanks to the courtesy of Dr. C. J. W. Brooks (Glasgow) on an LKB 9000 gas chromatograph-mass spectrometer; the separator temperature was kept at 250°C. In all cases the spectra were recorded at 70 eV ionization energy as well as 12 eV energy in order to obtain a better definition in the high masses range.

RESULTS AND DISCUSSION

When a typical dihydroxyacetone side chain metabolite (e.g. THS, THE, THF, 50–100 μg) is submitted to g.l.c. after overnight treatment at room temperature with a silylating agent (e.g. BSTFA, 100–200 μl), a complex mixture of products is obtained (Fig. 1).

When the silylation reaction is carried out in the presence of a nucleophilic agent such as potassium (or sodium) acetate in solid form (10 mg) a single product is obtained with excellent gas chromatographic properties (Fig. 2).

The quantitative aspect of this reaction can only be estimated by reference to a stable internal standard. Cholesterol butyrate was used for this purpose. A reproducible response coefficient (close to 1) was regularly found for all model compounds studied. This demonstrates a complete and reproducible conversion to a stable derivative; no trace of by-product was seen whatsoever (Fig. 2).

Table 1 gives the retention parameters obtained on two different phases for the different model compounds after reaction under acetate catalysis. These values show that rather poor separation is obtained between these compounds on a non selective phase (OV-1) whereas a better resolution is obtained on OV-17.

Cortol was treated in the same conditions in order to check if the base catalysis was effective on the silylation of hindered hydroxyl group. The result (Table 1) shows that under the base catalysed silylating conditions used here a cortol tri

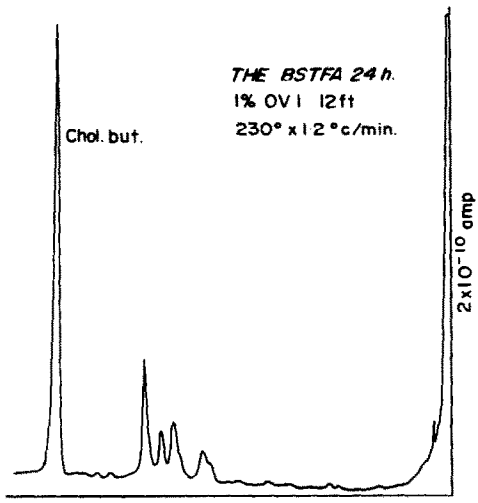

Fig. 1. G.l.c. analysis (temperature programming) of THE (50 μg) after 24 h standing in a silylating agent (BSTFA, 200 μl). An equal amount of cholesterol butyrate (Chol. But.) was added before the reaction.

Table 1. TMS-enol-TMS: methylene unit values; BSTFA (K. acetate) 25°C 20 h

		1% OV-1	1% OV-17
Tetrahydrodehydrocorticosterone	THA	31·29	32·36
Tetrahydrocorticosterone	THB	31·56	32·78
Tetrahydrodeoxycortisol	THS	29·81	29·92
Tetrahydrocortisone	THE	30·98	31·58
Tetrahydrocortisol	THF	31·24	32·02
Allo-THF		31·54	32·15
Cortol*		32·66	33·91

*The retention times indicate that a cortol tri TMS (3,20,21 tri-trimethyl silyloxy) is obtained under the conditions used [7].

TMS is formed [7]; this indicates that the silylation of the hydroxyl groups is not catalysed under these conditions since the hindered 11β- and 17α-tert hydroxyl groups remain free. Thus, this catalysis appears to influence preferentially the enolization process, by contrast to the usual acid catalysed reaction [4, 5].

The mass spectra obtained for the derivatives of THS, THE and THF are shown in Fig. 3. In all cases a good molecular ion is seen from which a typical trimethylsilanol moiety is lost yielding a M-90 ion. The prominent ion at m/e 331 in the three spectra can be assigned to the favored ring D cleavage as indicated; a simultaneous hydrogen transfer will yield a stable m/e 331 bearing 3 TMS ether groups. These features can be understood on the basis of the structures proposed in Fig. 3.

These results suggest that the TMS formation from the 17αOH occurs before the 20-keto enol TMS reaction is completed. Otherwise the steric hindrance introduced by the bulky 20 enol TMS grouping would limit the reaction at the 17α

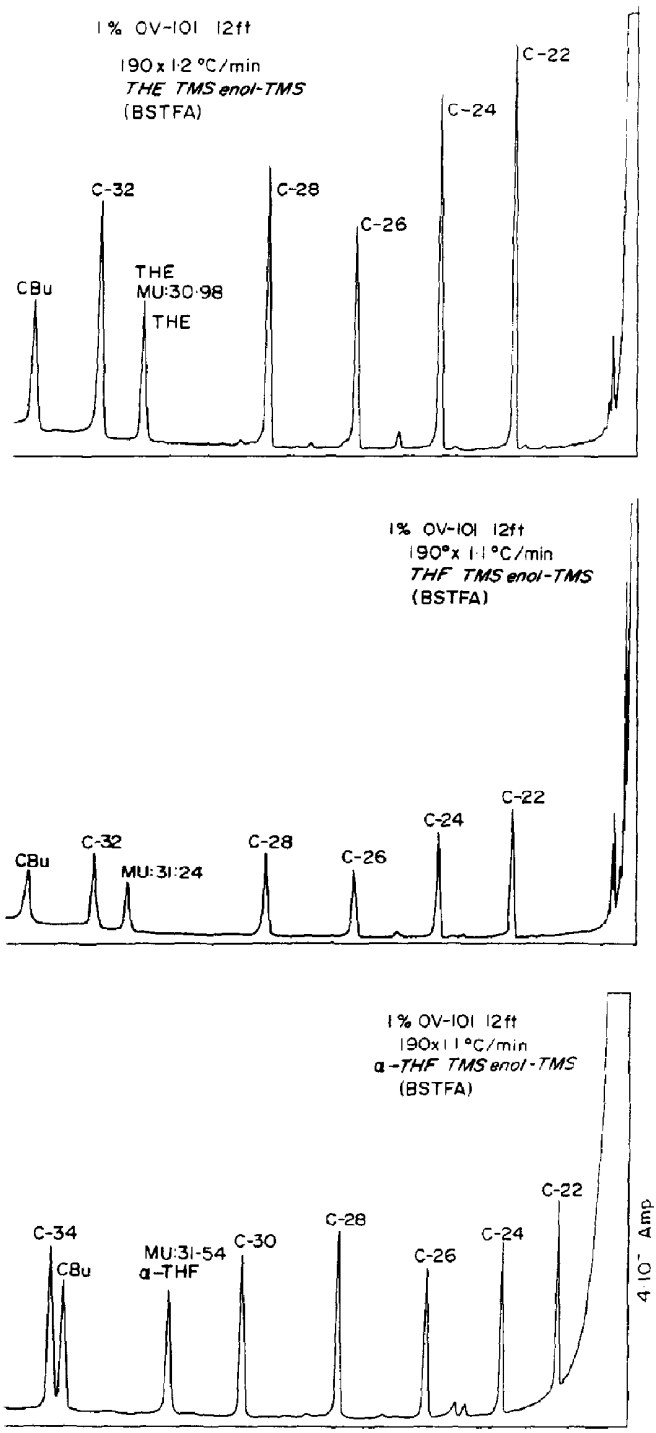

Fig. 2. G.l.c. analysis (temperature programming) of THE, THF and allo-THF (50 μg) after overnight treatment with BSTFA (200 μl) in the presence of potassium acetate (10 mg). Cholesterol Butyrate (50 μg) was added before starting the reaction. Even carbon number straight chain hydrocarbons (C22–C32) were used for the calculation of methylene unit values (M.U.).

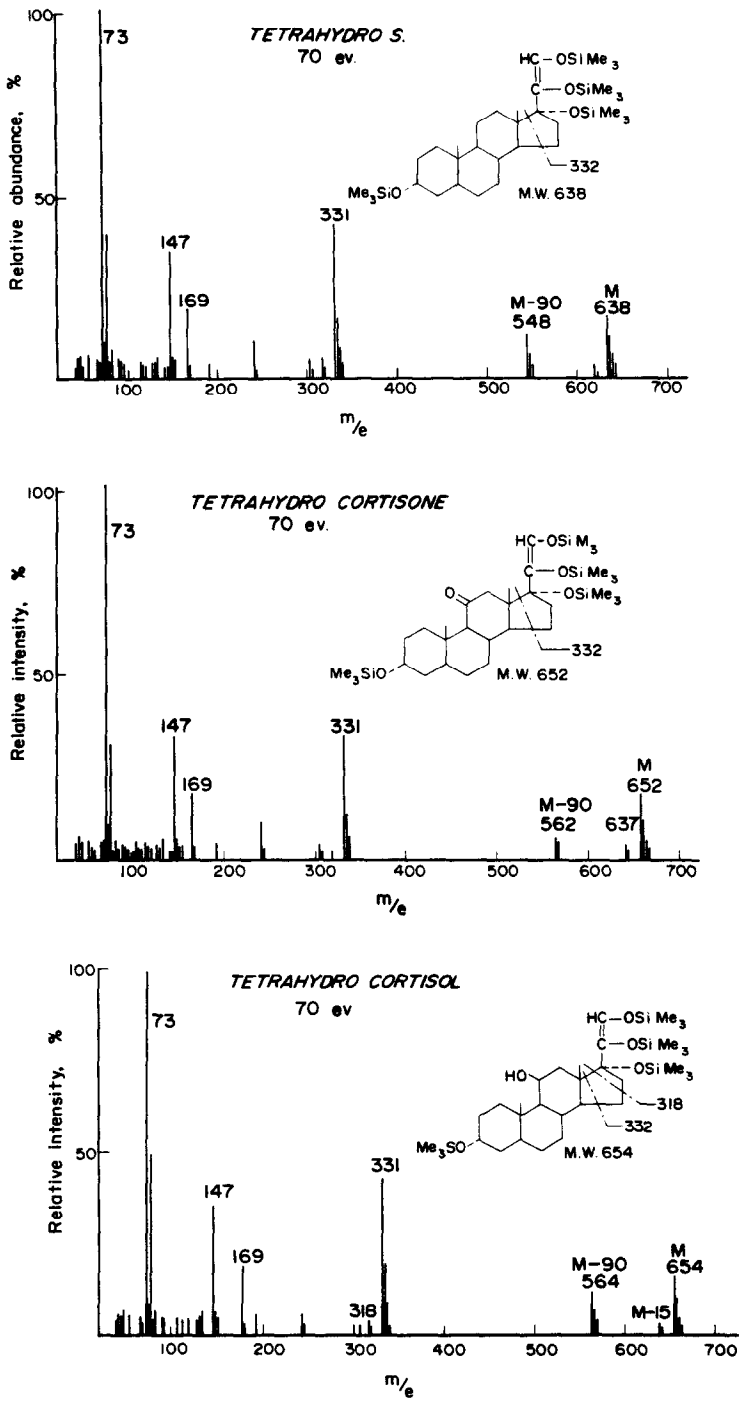

Fig. 3. Mass spectra of the THS, THE, THF derivatives. (For comment see text.)

position, as in the case of cortol under the same conditions [5]. Further investigation of the 17α,21-di TMS, 20 enol TMS structure postulated here for the derivatized side chain is in progress. Another possibility for the structure of the derivative

would be a base-catalysed D-Homo rearrangement followed by the enol-TMS formation from the D ring ketogroup, although the mass spectrometric data do not support this possibility.

The base catalysed reaction involved in this TMS enol TMS formation is being investigated in detail with a number of hydroxyketosteroids of biological interest. Preliminary results suggest that the various silylating agents available (e.g. BSA, BSTFA, TSIM) may behave differently in the reaction. The rate of formation of the enol TMS appears to be influenced by the stereochemical environment of the keto group involved. This effect was already noticed with acid catalysed silylations[8] and is of potential usefulness in characterization studies of unknown ketosteroids. Figure 4 shows the difference found in the rate of formation of the 20 enol TMS from three model corticosteroid metabolites with different substituents at the 11 position.

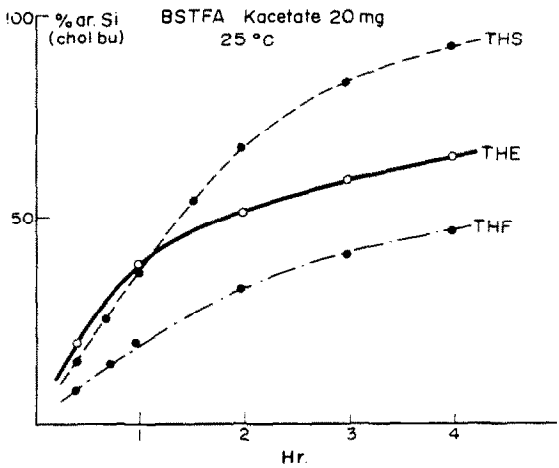

Fig. 4. Comparative rate of formation of the dihydroxyacetone side chain derivative for THS, THE and THF with BSTFA at 25°C (potassium acetate 20 mg). The yield (ordinate) is expressed as the percentage of product with respect to a fixed amount of internal standard (Si: Cholesterol Butyrate). With the three compounds, however, this yield was close to 100% after 20 h reaction.

The application of the TMS enol TMS derivatives to complex biological steroid mixtures (e.g. total neutral urinary steroid extract, including corticosteroid metabolites) for their gas chromatographic separation is of great potential value. However under the conditions used here, the 17-keto group yields an enol-TMS derivative but the reaction is not complete. On the other hand, the 3 keto groups studied were also converted to an enol-TMS in a non quantitative fashion and two isomeric products were found in the case of the 4-en,3-ketosteroid structure, as was already described with testosterone and acid catalysis[5, 6]. A systematic investigation is in progress in order to determine the experimental conditions needed to get a sole product for the main steroids of biological interest (i.e. either to avoid or to drive to completion the reaction for ketogroups other than at the 20 position). Preliminary results indicate that this might be achieved using different silylating agents and/or various nucleophilic catalysts. Other carboxylic salts as well as some amines or amides (e.g. piperidine, formamide) have also

been found to be effective catalysts of the enol TMS formation at the 20 position.

In conclusion, a TMS enol TMS derivative can be obtained under base-catalysed silylating conditions from hydroxyketosteroids. In the case of the saturated corticosteroid metabolites possessing the dihydroxyacetone side chain, this new derivative has excellent g.l.c. properties and a characteristic mass spectrometric fragmentation pattern. This new derivative is obtained in a very simple as well as quantitative and reproducible fashion.

ACKNOWLEDGEMENTS

This study was made possible thanks to the financial support from the Fondation pour la Recherche Médicale and the Institut National de la Santé et de la Recherche Médicale.

We are indebted to Dr. C. J. W. Brooks for very helpful discussions throughout this work.

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