CHROM. 4159

DIMETHYLDIACETOXY SILANE AS A SILYLATING REAGENT

A NEW TECHNIQUE FOR FORMING SILICONIDES OF CORTICOSTEROIDS

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SUMMARY

Dimethyldiacetoxysilane is a convenient reagent for thermal stabilisation of suitable corticosteroids to give siliconides with good gas chromatographic properties. The conversion to the derivative is high and the simplicity and speed of the procedure allows rapid analysis of corticosteroid mixtures.

The technique is applicable to steroids with the 17α,21-dihydroxy-20-oxo side chain, but not to such steroids as corticosterone where siliconide formation is not possible. Steroids with unhindered hydroxyl groups form thermally stable silyl derivatives with this reagent.

INTRODUCTION

One of the major problems in the gas chromatography of steroids has been that of chromatographing such compounds as 11β,17α,21-trihydroxypregn-4-ene-3,20-dione (cortisol) at high temperatures. The formation of simple derivatives such as trimethylsilyl ethers of the hydroxy groups does not stabilise the side chain sufficiently for chromatography. Until GARDINER AND HORNING[1] introduced the methyl oxime trimethylsilyl ether procedure in 1966 the only technique for measurement of these steroids was by chromatography of the 17-oxo steroids produced when the molecule was degraded, either oxidatively before introduction to the chromatograph, or thermally on the chromatographic column. The technique of forming the methyl oxime of the 20-ketone and trimethylsilyl ethers of either the 21-hydroxyl or both the 17α- and 21-hydroxyls allowed the gas phase chromatography of corticosteroids without loss of structural identity by degradation. Although this technique has been reported to be quantitative[2], it is lengthy and requires a certain experience from the operator.

It has recently been shown in these laboratories[3] that reaction of the dihydroxy acetone side chain, of compounds such as cortisone, with a difunctional silane gives a cyclic silyl derivative which incorporates the side chain in the ring. This derivative is thermally stable; the minimal increase in molecular weight coupled with the formation of silicon–oxygen bonds with their relatively poor electron donating properties,

results in short retention times and symmetrical peaks, during gas chromatography.

The difficulty in forming these siliconides has been due to the difunctional silane reacting with other hydroxyl groups of the steroid, to give thermally unstable derivatives. It is now reported that dimethyldiacetoxysilane (DMDAS) reacts with corticosteroids having the 20-oxo-17α,21-dihydroxy side chain to form siliconides and that t also reacts with unhindered hydroxyl groups to give thermally stable silyl derivaiives. DMDAS does not react with hindered hydroxyl groups (such as 11β) because of the steric bulk of the reagent (Fig. 1).

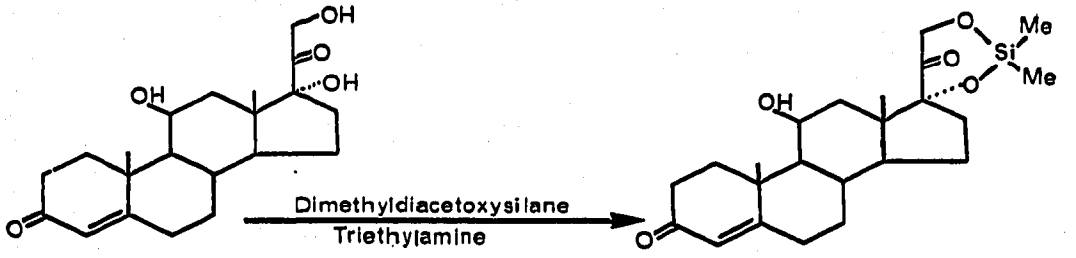

Fig. 1.

EXPERIMENTAL

Gas chromatography was carried out using a Hewlett Packard F. & M. 402 gas chromatograph with flame ionisation detector. Column temperature 245°, column packing 1 % OV-1 on 100–120 mesh acid washed, silanised, Chromosorb G and carrier gas nitrogen flowing at 30 ml/min.

Derivatives were formed by evaporating the steroid sample at the bottom of a 2 × 75 mm glass tube, adding 20 μl of a solution of dimethyldiacetoxysilane (2 %) and triethylamine (2%) in dry hexane, and sealing the top of the tube in a flame. The sealed tubes were kept at 40° for 2 h when the tubes were broken open and the mixture injected directly into the chromatograph. The derivatives in presence of the reagent in the sealed tube remain stable for at least 48 h. The percentage conversion to siliconide was estimated by comparing the peak area of the siliconide in question with that of a known amount of preformed crystalline siliconide (3β-acetoxy-16α,17α-dihydroxypregn-5-en-20-one siliconide, ref. 4) introduced as an internal standard.

Dimethyldiacetoxysilane was prepared by refluxing 1 mole of dimethyldichlorosilane with 2 moles of acetic anhydride for 4 h then slowly distilling off the acetyl chloride produced, through a Dufton column. The temperature was raised and unreacted acetic anhydride was distilled off. The temperature was further raised and dimethyldiacetoxysilane was distilled off between 164 and 168°. The product was redistilled and the fraction boiling at 164–166° collected, literature[5] b.p. 44–45° at 3 mm.

RESULTS AND DISCUSSION

Dimethyldiacetoxysilane reacts with *cis*-diols and steroids with the 17α,21-dihydroxy-20-oxo side chain to form siliconide derivatives which are analogous to acetonides. A similar reaction with 16α,17α-hydroxy groups has recently been reported[6]. These siliconides may be formed under mild conditions in the presence of triethylamine which acts as a scavenger towards the acetic acid formed in the reaction and as

such, pushes the equilibrium over in favour of the steroid derivatives. The similarity between acetonides and siliconides does not extend to the method of formation; in acetonide formation no reaction occurs at isolated hydroxyl groups; however, when difunctional silanes are used to form siliconides, provision must be made for such reaction. In a previous approach to forming siliconides of cortisol[3], tetramethyldisilazane was added to the reagent to react with hydroxyl groups which did not participate in siliconide formation, but this brought associated problems of low yields because of the competitive nature of the reaction.

When reacting with an isolated hydroxyl DMDAS gives a derivative of the type shown in Fig. 2 and as this reagent is a mixed anhydride with acetic acid it is noteworthy that in the presence of a mild base, negligible amounts of acetate are formed.

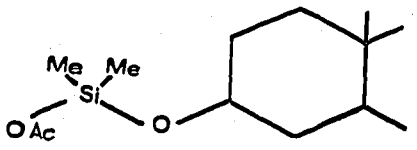

Fig. 2.

This was shown by simple time controlled experiments with the reagent and 3β-hydroxyandrost-5-en-17-one. The products were examined by gas–liquid chromatography and at no time was there more than a trace of acetate present. Since a mixed derivative is formed there should be the possibility of further reaction with a free hydroxyl to give intermolecular silyl bridges; this undesirable side reaction is minimised by using a very dilute solution of reagent and base in hexane. A second advantage of using a dilute solution is that the likelihood of two molecules of DMDAS reacting with both 17α-hydroxy and the 21-hydroxy groups of corticosteroids is minimised. Such a combination would lead to thermally unstable derivatives. A disadvantage of using a dilute reagent is that there is little in reserve to react with traces of moisture as well as the compound to be silanised. This means that the sample must be scrupulously dried before the reagent mixture is added.

DMDAS reacts to stabilise all compounds where the dihydroxy acetone side chain is present, it also reacts with hydroxyl compounds in the same way as trimethylsilylating reagents[7] to give thermally stable non-polar derivatives. It does not, however, stabilise compounds such as corticosterone (11β,21-dihydroxypregn-4-en-3,20-dione) where the possibility of siliconide formation does not exist. The reagent would stabilise 20,21-diols by forming the siliconide in a similar way to acetonide formation[8].

The formation of DMDAS derivatives allows good separation by gas–liquid chromatography of 17α,21-dihydroxypregn-4-ene-3,11,20-trione (cortisone), cortisol, 3α,11β,17α,21-tetrahydroxy-5β-pregnan-20-one (tetrahydrocortisol) and synthetic corticosteroids such as 9α-fluoro-16β-methyl-11β,17α,21-trihydroxy pregna-1,4-diene-3,20-dione (betamethasone). Fig. 3 shows the separation of an artificial mixture of corticosteroids. The tetrahydro derivatives such as tetrahydrocortisol have similar retention times to the parent compounds on non-polar (OV-1) columns but they may be separated on the same columns by temperature programming.

The percentage conversion to the derivatives, estimated as described under EXPERIMENTAL was 75–95% depending on the steroid. For some compounds (*e.g.* cor-

tisol) there was evidence of a small secondary peak (see Fig. 4) which is possibly due to reaction of the reagent at the 11 position.

Dimethyldiacetoxy silane has wide potential uses as a silylating reagent; it will certainly be useful for identification of corticosteroids by gas phase techniques and could prove useful in the quantitative analysis of corticosteroids from biological sources.

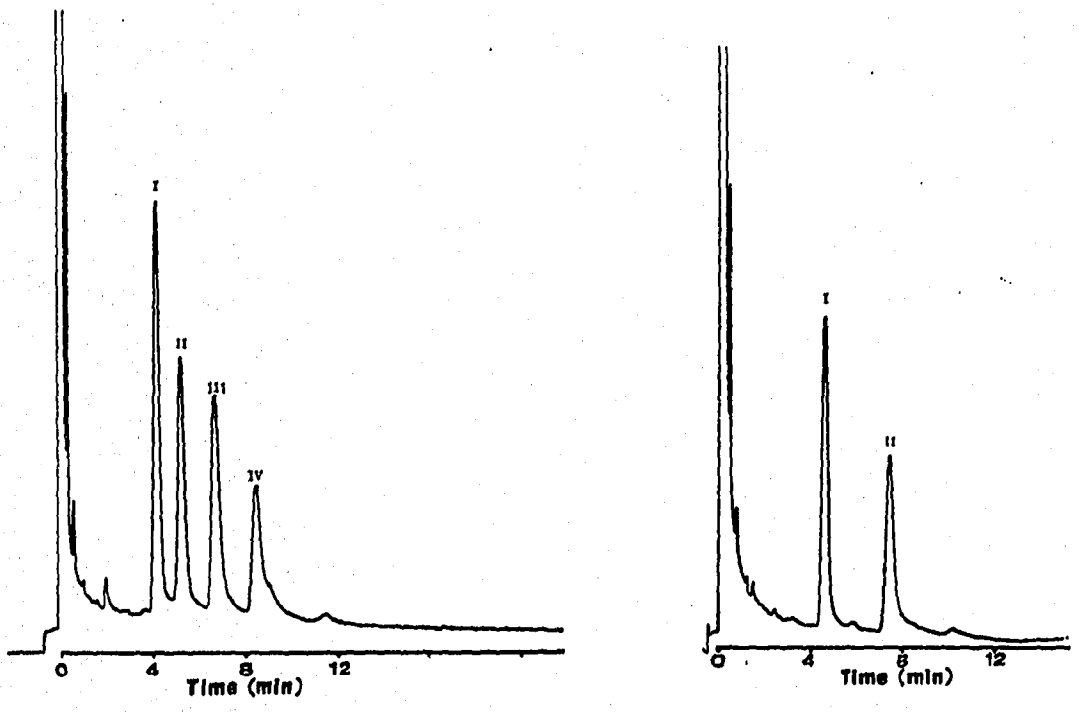

Fig. 3. Gas chromatograph trace of a synthetic mixture of cortisone (II), cortisol (III), and betamethasone (IV), as siliconide derivatives. The derivatives were formed from 1 μg of each compound, An internal standard (I) of the preformed siliconide of 3β-acetoxy-16α,17α-dihydroxypregn-5-en-20-one (1 μg) was used.

Fig. 4. 1 μg of cortisol (II) as siliconide, internal standard (I) as in Fig. 3.

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