

CHROM. 8881

## Note

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### Analysis of steroids

#### XXVI. Transformation of 3-methoxy-2,5(10)-diene steroids during their gas chromatographic analysis

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A great number of steroids undergo decomposition or transformation when chromatographed at elevated temperatures (200–250°) which is usually necessary for the evaporation of these less volatile materials. The best known examples are the cleavage of the 17-side chain of corticosteroids<sup>1,2</sup>, formation of D-homo derivatives from 17-hydroxy-20-keto pregnane derivatives<sup>3</sup>, dehydration of 4-en-3-hydroxy steroids<sup>4</sup>, formation of cyclo derivatives from 3-sulphonyloxy-5-ene steroids<sup>5</sup>, the fission of the alkoximes of ketosteroids to nitriles<sup>6</sup> and the decomposition of cholesterol heptafluorobutyrate to the corresponding 3,5-diene<sup>10</sup>. A common feature of these and some other reactions of this type is that they are either not unidirectional and quantitative under chromatographic conditions or it is very difficult to use them as the basis of reliable analytical procedures.

The present paper aims to extend the number of the above reactions by describing our observations on the re-arrangement of 3-methoxy-2,5(10)-dienes to 3-methoxy-3,5-dienes which under suitable conditions have been found to meet the requirements of quantitative analytical methods.

### EXPERIMENTAL

A Hewlett-Packard 7620 gas chromatograph was used equipped with a flame ionization detector (FID).

Methanolic solutions of the samples were injected directly onto the column (6 ft. × 4 mm I.D., packed with 80–100 mesh Gas-Chrom Q coated with 1% QF-1, 3% OV-210, 3% SE-30, 3% JXR or, 3% OV-225). Temperatures: column oven 230°, vaporizer zone and detector 250°. The flow-rate of the carrier gas (nitrogen) was 45 ml/min.

The same instrument was used for trapping the reaction products. The FID was replaced by a water-cooled U-tube and all solid materials eluted from the column after the repeated injections of the methanolic solution were collected.

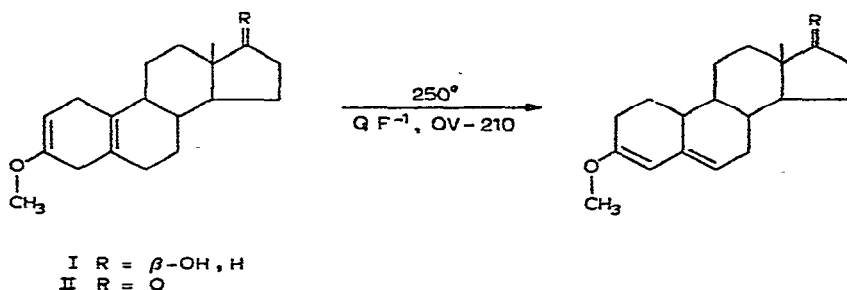
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The ultraviolet (UV) and infrared (IR) spectra of the trapped materials were recorded by means of Pye-Unicam SP-1800 and Perkin-Elmer 257 instruments, respectively.

## RESULTS AND DISCUSSION

During our analytical studies aimed at developing a quantitative method for the simultaneous determination of 3-methoxy-2,5(10)-oestradien-17 $\beta$ -ol (I) and 3-methoxy-2,5(10)-oestradien-17-one (II) it was found that their separation was excellent when the ketone-selective silicone QF-1 was used as the stationary phase (retention time for I 8.0 min and for II 9.8 min). The symmetry and reproducibility of the peaks enabled us to determine 1% of I in the presence of II.



These good results seemed to be surprising to us as 3-alkoxy-2,5(10)-dienes are well known to be unstable, aromatization and formation of  $\Delta^4$ -3-ketones being the main reaction routes of their transformation. Taking this into consideration it seemed to be unlikely that I and II were chromatographed intact. To clear up this problem the materials eluted from the column were trapped and subjected to spectroscopic investigation. The UV spectra of the materials obtained (white needles) in methanol show an intense maximum at 242 nm which is not affected by treatment with sodium borohydride<sup>7-9</sup>. This indicates the presence of the conjugated 3-methoxy-3,5-diene grouping (the parent non-conjugated 3-methoxy-2,5(10)-diene system is spectrophotometrically inactive). Further evidence supporting this structure was furnished by the IR spectra: the  $\nu_{\text{C}=\text{C}}$  vibrations appearing with medium intensity at 1627 and 1651  $\text{cm}^{-1}$  are also characteristic of the 3-methoxy-3,5-diene system.

The trapped materials were dissolved in methanol and re-chromatographed on the same column. The excellent agreement of the retention times and the acceptable agreement of the peak areas indicated that more than 85% of I and II were instantaneously transformed to the corresponding 3,5-dienes (see reaction equation) so that neither side reactions nor remarkable losses need be considered during the chromatographic process.

We repeated these investigations with all stationary phases listed under Experimental. It is interesting to note that similar results were only obtained using OV-210. All of the other columns resulted in chromatograms of poor quality and the spectra of the trapped materials showed that they were mixtures of various deriv-

atives. As both QF-1 and OV-210 contain fluoroalkyl groups it seems to be likely that the reason for the unidirectional re-arrangement in the case of these stationary phases is the directing effect of this grouping on the transformation of 3-methoxy-2,5(10)-dienes.

#### ACKNOWLEDGMENT

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