

Analytical chemical studies on steroids

Part XIV. Determination of C-13-epimeric 17-oxosteroids by gas chromatography*

In a preceding paper the authors reported the gas chromatographic separation of several pairs of C-14-epimeric 5 α -androstanes¹. In extension of the previous work, an interest in the C/D-*cis*-fused ring system prompted us to investigate the stereochemistry of ring D in the 13 α -series. As to the formation of the 13 α -steroids, photoepimerization of the usual 17-oxosteroids is well known since the first success by BUTENANDT and his co-workers². However, a quantitative examination of these photochemical reactions has not yet been made. In this paper we wish to report a gas chromatographic method for the separation and determination of C-13-epimeric 17-oxosteroids and its application to the analysis of the crude product obtained by irradiation with ultraviolet light.

Experimental

Materials

13 α -Dehydroisoandrosterone** was prepared from dehydroisoandrosterone by U.V. light irradiation, followed by Girard's separation according to the procedure of BOTS³ (m.p. 186–187°). 13 α -Isoandrosterone was obtained from 13 α -dehydroisoandrosterone by usual hydrogenation over 5% palladium-on-charcoal (m.p. 129–131°; reported m.p. 124–126°⁴). The samples not mentioned above were commercially available.

Gas chromatography

The apparatus used for this work was a Shimadzu Model GC-1C gas chromatograph equipped with a hydrogen flame ionization detector and a U-shaped stainless steel column (3 m \times 3 mm I. D.). The column was packed with 1.5% SE-30 on a support of Chromosorb W (60–80 mesh). The detector and flash heater were kept at 250°, while the column temperature was 240°. Nitrogen was used as carrier gas at a flow rate of 90 ml/min.

Photochemical reaction

A solution of dehydroisoandrosterone (1 g) dissolved in 95% ethanol (200 ml) was placed in an irradiation vessel and irradiated with light of $\lambda > 280 m\mu$ using a 450 W mercury lamp UM-452 in an atmosphere of nitrogen under stirring. After a definite reaction time had elapsed, a 1 ml aliquot of the resulting solution was taken up and evaporated *in vacuo*. To the residue thus obtained an acetone solution (1 ml) containing a known amount of androsta-1,4-diene-3,17-dione (*ca.* 2 mg) was added, and 1–2 μ l of this solution was injected into the sample chamber. The reversed photo-reaction of 13 α -dehydroisoandrosterone to the usual 13 β -epimer and subsequent gas chromatographic analysis of the irradiation product were carried out in the same manner as described above.

* The systematic designations for the trivial names used in this report are: dehydroisoandrosterone, 3 β -hydroxyandrost-5-en-17-one; isoandrosterone, 3 β -hydroxy-5 α -androst-17-one.

** The authentic sample was kindly donated by Dr. L. J. CHINN, G. D. Searle & Co., to whom the authors' thanks are due.

Results and discussion

It is generally accepted that one of the chief advantages of a nonselective phase such as methyl silicone polymer lies in its effectiveness for different molecular shapes⁵. As is shown in Table I, this generalization is evident from the successful separation of two pairs of C-13-epimeric 17-oxosteroids (I and II) with SE-30 as the stationary

TABLE I

RELATIVE RETENTION TIMES OF C-13-EPIMERIC 17-OXOSTEROIDS

Conditions: stainless steel column (3 m × 3 mm I.D.); packing 1.5 % SE-30 on Chromosorb W (60–80 mesh); N₂ flow rate 90 ml/min; column temp. 240°; flash heater temp. 250°.

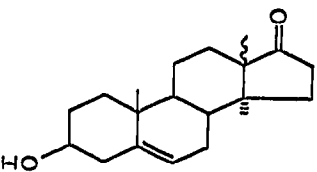
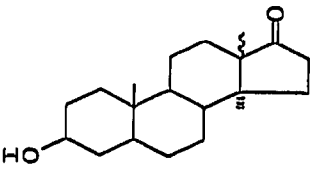
Compound structure	Configuration of C-13-CH ₃	Relative retention time
 (I)	β	0.40
	α	0.34
 (II)	β	0.42
	α	0.35
Cholestane		1.00 (26.5 min)

TABLE II

ANALYTICAL RESULTS OF SYNTHETIC MIXTURES OF C-13-EPIMERIC DEHYDROISOANDROSTERONES*

Sample No.	Calculated (mg)		Found (mg)	
	13α	13β	13α	13β
1	0.200	0.800	0.205 (102.5)	0.809 (101.1)
2	0.400	0.600	0.397 (99.3)	0.594 (99.0)
3	0.599	0.400	0.594 (99.2)	0.400 (100.0)
4	0.799	0.200	0.793 (99.2)	0.200 (100.0)

* Figures in parentheses indicate the recovery in percentage.

phase. In both cases 13α-steroids are eluted before the corresponding *trans*-fused isomers. It is of particular interest that the difference in C/D-ring juncture is not reflected in the retention times of the C-14-epimers as previously reported, but distinctly in those of the C-13-epimers.

Examinations were then made on the separation and determination of C-13-epimeric dehydroisoandrosterones. First, several compounds were tested for use as internal standard and in consequence androsta-1,4-diene-3,17-dione was chosen as the most suitable one. Calibration curves were constructed by plotting the ratio of

peak area of each sample to the standard against the weight ratio of these two respectively, whereby good linearity was observed. The synthetic mixtures of a pair of epimers were prepared and subjected to the analysis employing the established method. As can be seen in Table II the present method proved to be quite satisfactory as regards recovery.

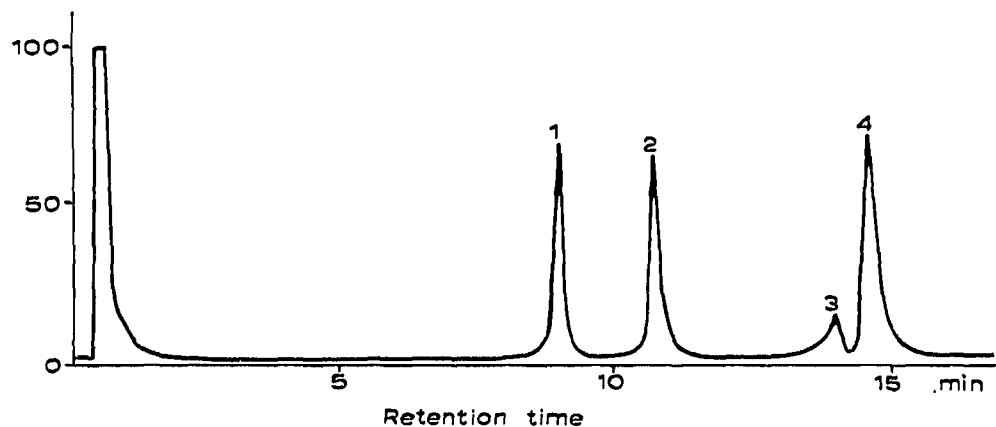


Fig. 1. Gas chromatogram of the irradiation product derived from dehydroisoandrosterone. 1 = 13α -dehydroisoandrosterone; 2 = dehydroisoandrosterone; 3 = unknown by-product; 4 = androsta-1,4-diene-3,17-dione (internal standard).

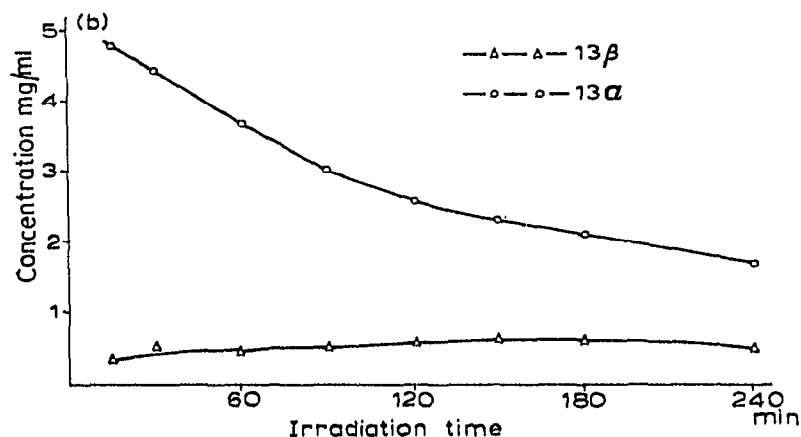
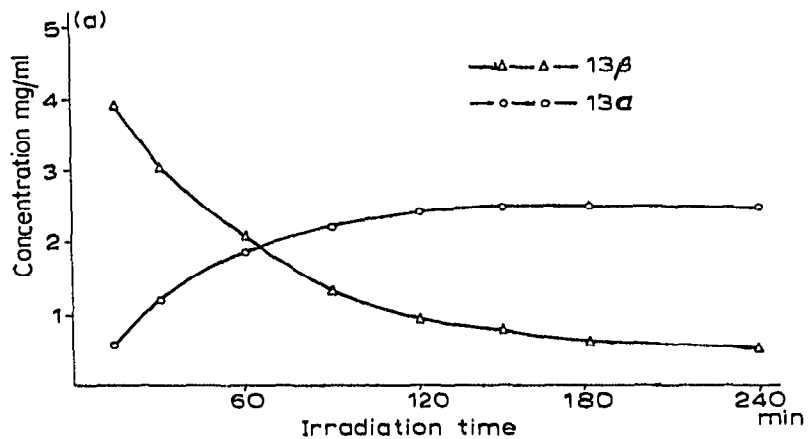


Fig. 2. (a) Change in composition of the irradiation product derived from dehydroisoandrosterone. (b) Change in composition of the irradiation product derived from 13α -dehydroisoandrosterone.

This procedure was now applied to the photochemical reaction of dehydroisoandrosterone. The amounts of the unchanged starting material and the resulting product were determined and plotted graphically against the irradiation time. As illustrated in Fig. 2a, transformation into the 13α -compound reached equilibrium in 150 min; on prolonged irradiation increasing amounts of an undesirable by-product appeared, whose structure was not characterized. Furthermore the reverse reaction⁶ was also explored in the same manner as described above. Upon irradiation with U.V. light 13α -dehydroisoandrosterone was transformed back again into its epimer, the amount of which finally reached a constant value almost equal to that in the reverse reaction (see Fig. 2b). The reversibility of the photochemical interconversion of 17α -oxo- 13β -steroid and its 13α -epimer has been clarified in a quantitative respect.

The present method for direct quantitation of the irradiation product itself is very useful for establishing the optimal conditions for the synthesis of 13α -steroid, because of its reliability and simplicity.

Further studies on the gas chromatographic separation of C- 13 -epimeric androstanes having various substituents in ring D are being conducted in this laboratory and will be reported in the near future.

Acknowledgement

This work was supported in part by a grant-in-aid from the Ministry of Education, Japan, which is gratefully acknowledged.

Pharmaceutical Institute, Tohoku University
School of Medicine, Sendai (Japan)

TOSHIO NAMBARA
TOSHIHIRO KUDO
HIROSHI HOSODA
SHUJIRO GOYA

- 1 T. NAMBARA AND R. IMAI, *J. Chromatog.*, 25 (1966) 248.
- 2 A. BUTENANDT, A. WOLFF AND P. KARLSON, *Ber.*, 74 (1941) 1308.
- 3 J. P. L. BOTS, *Rec. Trav. Chim.*, 77 (1958) 1010.
- 4 J. R. BILLEYER AND K. MIESCHER, *Helv. Chim. Acta*, 34 (1951) 2053.
- 5 E. C. HORNING, W. J. A. VANDENHEUVEL AND B. G. CREECH, in D. GLICK (Editor), *Methods of Biochemical Analysis*, Vol. XI, Interscience, New York, 1963, p. 69.
- 6 H. WEHRLI AND K. SCHAFFNER, *Helv. Chim. Acta*, 45 (1962) 385.

Received June 13th, 1967

J. Chromatog., 31 (1967) 210-213

Dry filling of capillary columns for liquid chromatography

We have previously described capillary teflon columns for adsorption and partition chromatography¹ and given funnel and pump filling liquid techniques for this type of column.

Working with the capillary columns we have found that a simpler dry filling technique offering many advantages in capillary column chromatography is just as effective for some column materials.

J. Chromatog., 31 (1967) 213-216