CHROM. 4702

Thin-layer chromatography of the chromogens from the Allen reaction

As part of an investigation of the mechanism of the ALLEN[1] reaction, separation of the components of the reaction mixtures has been effected by thin-layer chromatography. The chromogens were prepared by a modification[2] of the ALLEN reaction. The steroids used were 3β-hydroxyandrost-5-en-17-one (dehydroepiandrosterone, DHEA), 17β-hydroxyandrost-4-en-3-one (testosterone, TT) and androst-4-ene-3,17-dione (androstenedione, AD). The steroids were dissolved (100 mg per ml) in 66% H_2SO_4 (Analar grade, B.D.H.), and from this solution two sets of derivatives were prepared.

Firstly, the acidic solutions were poured into a tenfold excess of water, and the resultant precipitate was filtered off under reduced pressure provided by a water-pump, washed with water and acetone, and dried. In the case of TT and AD, the derivatives were crystallised from methanol. In the second experiment, the acidic solutions were heated in a boiling water-bath for 4 min (DHEA) or 30 min (TT and AD), the resulting blue-green solutions were poured into a tenfold excess of water and the precipitates recovered as previously. The two derivatives each from DHEA, TT and AD were dissolved in 1,2-dichloroethane (B.D.H.), spotted onto pre-coated aluminium-backed alumina plates (Merck) with fluorescence indicator and developed using the Eastman chromatographic apparatus with ethyl acetate–benzene (Analar grade, B.D.H.) as solvent. Optimal concentrations were ethyl acetate–benzene in a ratio of 1:10 for DHEA chromogens and 1:2 for TT and AD chromogens.

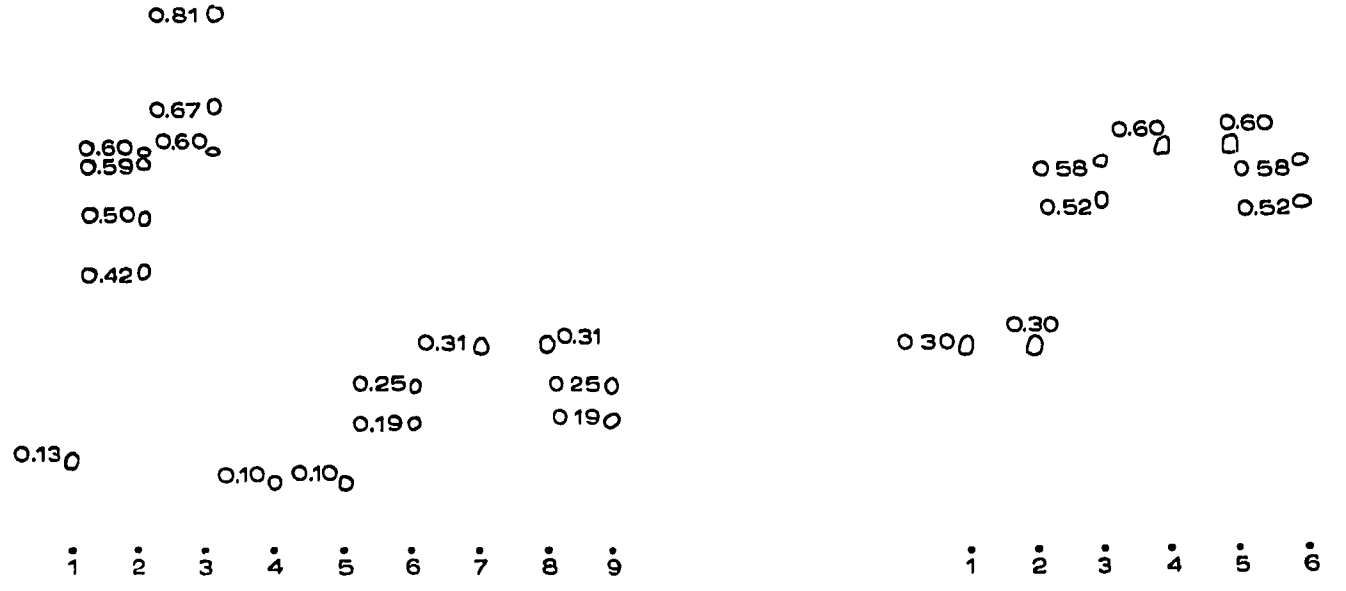

Fig. 1. Thin-layer chromatogram of chromogens from dehydroepiandrosterone (1–3), testosterone (4–6), and androstenedione (7–9), developed with ethyl acetate–benzene (1:10). 1, 4, 7 = parent steroid; 2, 5, 8 = derivative after solution in sulphuric acid without heating, 3, 6, 9 = derivative after solution in sulphuric acid and heating for 4 or 30 min.

Fig. 2. Thin-layer chromatogram of chromogens from testosterone (1–3) and androstenedione (4–6), developed with ethyl acetate–benzene (1:2). 1, 4 = parent steroid, 2, 5 = derivative after solution in sulphuric acid without heating; 3, 6 = derivative after solution in sulphuric acid and heating for 4 or 30 min.

The results are shown in Figs. 1 and 2; the R_F values for each component are indicated in these Figs. The chromogens were visualised by spraying with acetic anhydride–concentrated sulphuric acid (4:1)[3–5]. No further spots could be detected using phosphomolybdic acid[6,7], phosphotungstic acid or the ZIMMERMANN reagent[8] or by irradiation with UV light[9].

From these chromatograms it is concluded that: (1) DHEA is quantitatively converted into chromogens by solution in 66% H_2SO_4 without heating. Comparable treatment of TT and AD produces changes which are easily reversible when the acidic solutions are poured into water. It is most likely that the TT and AD are converted into their conjugate acids by protonation of the oxygen functions; these steroids were recovered in 61 and 93% yield, respectively. The identity of these compounds indicated by thin-layer chromatography was confirmed by IR spectroscopy of KBr pellets of the steroids and by melting point determinations. As protonation of the C-17 ketone group in androstenedione is easily reversible, it is concluded that changes which are observed in DHEA involve the 5-en-3β-ol group. The three spots obtained from DHEA are coloured yellow, red and orange, respectively, by the spraying reagent in passing from the starting point towards the solvent point. Visible absorption maxima in the sulphuric acid solution are obtained at 408 (yellow), 551 (purplish pink) and 486 nm (orange), which probably corresponds to the three chromogens in the chromatogram. (2) The reaction product obtained on heating with 66% H_2SO_4 is identical from TT and AD and different from the DHEA chromogen mixture—a hypothesis which has been advanced previously[2]. Thin-layer chromatography of TT and AD chromogen mixtures obtained after various heating intervals between 1 and 30 min confirmed this relationship (the difference is not due to differences in heating time) and indicated that TT reacts much more readily than AD. The IR spectra of KBr pellets of the TT and AD chromogen mixtures have identical vibration bands, which are different from those given by the DHEA chromogen mixture.

Supported by Grant No. MA-3208 of the Medical Research Council of Canada.

Department of Ophthalmology Research,
McIntyre Medical Sciences Centre, McGill University,
Montreal, Quebec (Canada)

H. A. JONES
E. PFENNINGER

1 W. M. ALLEN, S. J. HAYWARD AND A. PINTO, *J. Clin. Endocrinol*, 10 (1950) 54.
2 H. A. JONES, *Ph. D. Thesis*, University of Western Australia, 1968.
3 C. LIEBERMANN, *Ber.*, 18 (1885) 1803.
4 H. BURCHARD, *Chem. Zentr.*, 61 (1890) 25.
5 R. NEHER AND A. WETTSTEIN, *Helv. Chim. Acta*, 34 (1951) 2278.
6 D. KRITCHEVSKY AND M. R. KIRK, *Arch. Biochem. Biophys*, 35 (1952) 346.
7 L. L. SMITH AND T. FOELL, *J Chromatog*, 9 (1962) 339.
8 W. ZIMMERMANN, *Z. Physiol Chem.*, 233 (1935) 257.
9 R. TSCHESCHE, G. BIERNOTH AND G WULFF, *J. Chromatog.*, 12 (1963) 342.

Received March 10th, 1970