Journal of Chromatography, 76 (1973) 502–504

CHROM. 6388

Note

Analysis of steroids

XXIII. Microdetermination of Δ^5-steroids after thin-layer chromatographic separation

The steroids that contain a double-bond in the Δ^5 position are very important among natural steroids and intermediates for the manufacture of steroid drugs. Δ^5-3-Hydroxy steroids of plant or animal origin are the most important starting materials for the preparation of hormonal steroids. Consequently, the determination of this bonding system is very important. The usual methods for its determination are the colorimetric method by LIEBERMANN AND BURCHARD and the titration of the double-bond with bromine. The disadvantage of the LIEBERMANN–BURCHARD method is that its applicability depends on the nature of the side-chain at the 17-position and other groups attached to the steroid skeleton; *e.g.*, this method may be used succesfully for the determination of cholesterol, while the sapogenins, such as diosgenin, and hormonal steroids, such as dehydroepiandrosterone, do not give the reaction[1]. The direct titration is performed with a 0.1 *N* solution of bromine in glacial acetic acid, containing sodium acetate and mercury(II) chloride as catalysts, using visual or potentiometric end-point detection[2]. Neither of these procedures can be applied directly in the determination of mixtures of structurally very similar steroids, which usually occur in the starting materials for steroid syntheses.

In our work, the diosgenin and cholesterol contents of crude extracts were determined. In both instances the direct titration involves a large positive error because of the presence of other Δ^5-steroids and unsaturated non-steroidal materials. For this reason the determination was performed after preliminary TLC separation. Under such conditions, the amount of the material available for the analysis is restricted to hundreds of micrograms. The colorimetric methods are generally preferred for the determination of such small amounts. However, for the above reasons, instead of the LIEBERMANN–BURCHARD method, the generally applicable bromometric titration was adapted to the micro-scale. For this purpose, 0.01 *N* titrant was used and the volume required was about 0.2 ml.

Experimental and results

The TLC separation was carried out on a previously purified Kieselgel G layer. In order to remove the oxidisable impurities, the silica gel was refluxed with three portions of methanol prior to use. After drying the gel at room temperature, 20 × 20 cm chromatoplates were prepared with layers 0.25 mm thick. The plates were heated for 1 h at 105° and divided into lanes of 1.5-cm width. About 200-μg amounts of steroid were applied as streaks to each lane, leaving two lanes empty. The development was performed in an unsaturated chamber containing a mixture of 97 parts of dichloromethane and 3 parts of methanol. After development, both the centre and the side lanes were sprayed with concentrated sulphuric acid, the other lanes having

been covered with glass plates. This enables the spots to be made sufficiently visible at room temperature. In this way heating the layer to higher temperatures, which would influence the elution and the consistency of the layers, can be avoided. The silica gel from each of two lanes located on the basis of the above spraying was collected and used for a single determination.

The elution was performed with 2 ml of a mixture of equal volumes of chloroform and methanol. Quantitative elution could be achieved, which was checked by TLC and GLC investigations. The silica gel was also removed from areas of the two empty lanes, equal to the area of the spot of diosgenin. The empty gel was eluted under the same conditions, and its bromine consumption was determined. A silica gel was used

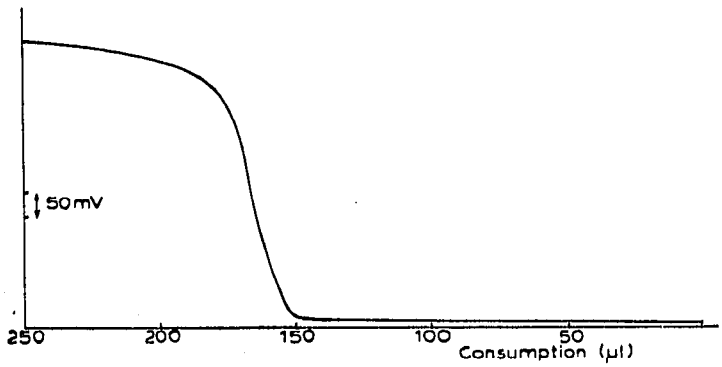

Fig. 1. Titration curve of diosgenin eluted from the silica gel.

such that the blank consumption was less than 10% of the expected consumption of the steroid. After the elution, the solution obtained was evaporated to dryness, the residue was dissolved in 1 ml of glacial acetic acid containing 0.5 *M* sodium acetate and 0.08 *M* mercury(II) chloride. The titration was carried out with a 0.01 *N* solution of bromine in acetic acid, using a Radiometer autotitrator (TTT-2; SBR-2) with a Pt–calomel electrode pair and a 0.25-ml autoburette (ABU-11). The volume required was approximately 0.2 ml. The blank consumption of the empty layer has been taken into account in each instance. The potential change was about 500 mV at the vicinity

TABLE I

TITRATION OF DIOSGENIN WITH BROMINE (0.01 *N* SOLUTION IN ACETIC ACID)

Diosgenin taken (µg)	*Volume of bromine solution (µl)*	*Diosgenin found (µg)*	*Recovery (%)*
115	57.0; 58.5	118; 121	102.3; 105.0
160	79.0; 74.0	163; 153	102.0; 95.5
245	118; 122	244; 252	99.5; 102.9
300	142; 150	291; 310	97.1; 103.3
350	168; 172	347; 355	99.1; 101.5
440	210; 215	434; 444	98.6; 100.9
480	230; 235	475; 485	99.0; 101.1

of the end point (Fig. 1). It can be seen in Table I that the bromine consumption is a linear function of the amount of diosgenin, and the recoveries varied in the range 97–105%. A volume of bromine solution of 1 μl is equivalent to 2.06 μg of diosgenin. For 400 μg of diosgenin, the relative standard deviation of the method is $\pm 3.5\%$. This method can also be successfully applied in the determination of cholesterol in crude extracts.

The authors thank Miss J. SÜTÖ for technical assistance.

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Received September 14th, 1972