Short Communications

Use of an asphalt fraction for the gas-liquid chromatography of steroids

The rather high temperatures which have to be employed for the gas–liquid chromatography (GLC) of steroids have until recently restricted the choice of stationary phases to a small number of thermostable substances. Non-polar phases resistant to high temperatures are not difficult to find, and successful applications of the method, using Apiezon and the silicones, have been made for the separation of sterols differing in the number of carbon atoms or in configuration.

For the separation of more closely related sterols, for example pairs differing only by one double bond, a polar stationary phase is necessary, and such phases are notoriously thermolabile. BEERTHUIS AND RECOURT[1] separated cholesterol and cholestanol by converting them into the acetates and chromatographing on a polyester. The disadvantages of this method for routine use are the necessity for a preliminary acetylation of the mixture and the fact that polyesters are markedly unstable at temperatures above 190°, so that one is forced to accept relatively long retention times.

Recently VANDENHEUVEL, HAAHTI AND HORNING have described[2] a new polar stationary phase, QF-1, which is a fluorinated alkyl silicone and as such is both polar and stable at high temperatures. They noted a separation of cholesterol and cholestanol using this material, and obtained a separation factor of 1.09. We had meanwhile discovered that the already known phases[3] Apiezon L and SE-30, which when used alone are incapable of separating these two sterols, may be successfully modified to do so by the addition of a fraction obtained from air-blown asphalt. This fraction has the necessary separating properties because of its content of high-molecular-weight condensed aromatic rings.

The fraction is obtained by repeatedly extracting the asphalt, first with *n*-hexane and subsequently with benzene–absolute ethanol (1:1, v/v). The residue is readily soluble in benzene and in chlorinated hydrocarbons, but virtually insoluble in petroleum ether, ether, and ethyl acetate. It is not suitable for use as such for GLC since its melting point is too high and the consequent high viscosity leads to "tailing". At the temperatures we employ (about 240°) the asphalt fraction is apparently held in solution by Apiezon or SE-30; the viscosity is reduced and the tailing avoided.

The column filling found thus far to be the most suitable for our purpose, and which is resistant to temperatures up to 260° for long periods, consists of Chromosorb P (Johns-Manville) which has first been made hydrophobic with dimethyl-dichloro-

silane and subsequently impregnated with 1–4 % (by weight) Apiezon L or SE-30 and 0.5–1 % of the asphalt fraction described. Appropriate amounts of both substances are dissolved in benzene, and the mixed solution is applied to the support in the usual manner. Columns containing Apiezon and the asphalt fraction gave longer retention times for the steroids than those employing SE-30 as diluent. Some separations of steroid mixtures are shown in Figs. 1 and 2. The apparatus consisted of a "Pye" Argon Chromatograph. The samples (10–20 μg) were introduced as follows: a glass rod provided with a small metal spring was dipped into the steroid solution. After evaporation of the solvent this rod was brought into the column.

The use of Chromosorb P as supporting medium led to the remarkable observation that sterols have the same retention times as their acetates or benzoates,

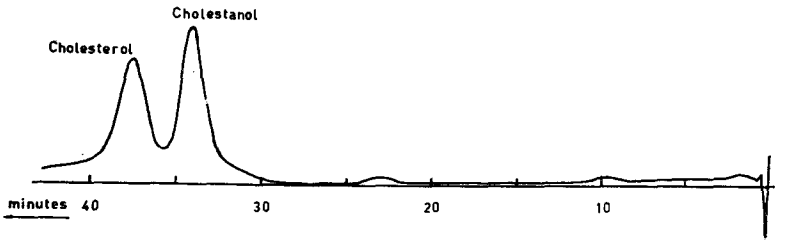

Fig. 1. Separation of cholesterol and cholestanol by gas–liquid chromatography, using Apiezon L and an asphalt fraction (see text) as stationary phase. Column 140 cm; Chromosorb P, 1.5 % Apiezon L, 0.8 % asphalt fraction; 250°; 2 atm.

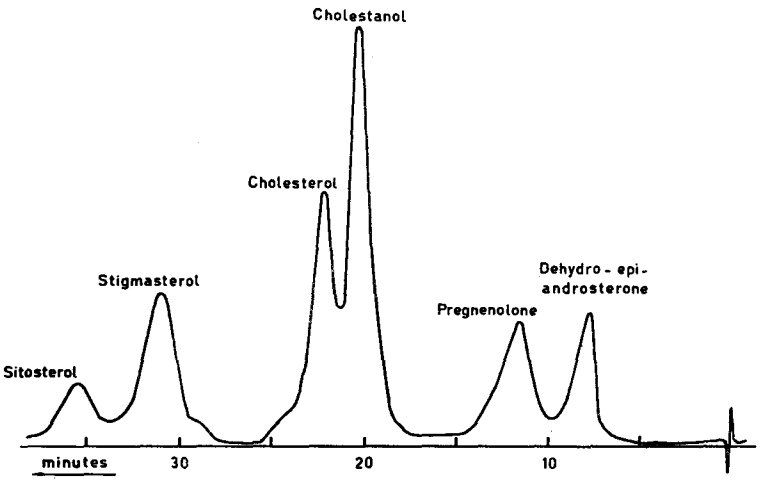

Fig. 2. Steroid GLC separations using SE-30 and the asphalt fraction as stationary phase. Note shorter retention times of cholestanol and cholesterol. Column 140 cm; Chromosorb P, 4 % SE-30, 0.4 % asphalt fraction; 240°; 2 atm.

irrespective of the stationary phase. By trapping the fractions leaving the column and subjecting them to "thin-layer" chromatography (on silica) and infra-red spectroscopy, it was possible to show that Chromosorb P strongly catalyses the removal of hydroxyl and acyl groups from sterols and sterol esters respectively to yield compounds with a new double bond, probably between C_2 and C_3. Thus cholestanol and

cholestanyl acetate both yield a cholestene, while cholesterol and cholesteryl acetate (or benzoate) yield a cholestadiene. The fact that this transformation takes place both quantitatively and virtually instantaneously at the top of the column was revealed by "thin-layer" chromatography and by the symmetrical shape of the peaks obtained from a mixture of cholestanol and cholesterol. It appears that the cholestene and cholestadiene are not further decomposed during their passage through the column, and that they are quickly and efficiently separated in the presence of the asphalt fraction.

The same dehydration and de-acylation on this support has been observed with sterols with shortened side-chain, for example pregnenolone, *epi*-dehydroandrosterone, and their acetates. The catalytic effect was also shown, though to only a small extent, when the Chromosorb P was replaced by acid- and ammonia-washed Celite 545. In the presence of the polyfluorethylene support "Haloport F" (F. and M. Scientific Corp., New Castle, Del., U.S.A.) all trace of the transformation had disappeared, but it was not possible to achieve satisfactory plate numbers with this material. The most successful combination remained SE-30, the asphalt fraction and Chromosorb P; because of the quantitative nature of the dehydration or de-acylation such columns are perfectly suitable for quantitative analysis.

This investigation was made with the support of the Netherlands Organisation for the Advancement of Pure Research (Z.W.O.).

Department of Physical Chemistry, University of Leiden (The Netherlands)

C. J. F. BÖTTCHER
J. W. A. MEIJER

[1] G. K. BEERTHUIS AND J. H. RECOURT, *Nature*, 186 (1960) 372.
[2] W. J. A. VANDENHEUVEL, E. O. A. HAAHTI AND E. C. HORNING, *J. Am. Chem. Soc.*, 83 (1961) 1513.
[3] E. C. HORNING, C. C. SWEELEY AND W. J. A. VANDENHEUVEL, *J. Am. Chem. Soc.*, 82 (1960) 3481.

Received August 15th, 1961

Paper chromatographic separation of sennoside A and sennoside B

Sennosides A and B, the active glucosides of senna, are known to differ in their biological activity and sennoside B is reported to be much more active than sennoside A[1]. The separation of the two stereoisomeric compounds is quite important for the evaluation of the activity of the crude drug and its extracts and the determination of the individual active glucosides.

By using a modified paper chromatographic technique of RUTTER[2,3], we have achieved the separation of the two sennosides. In the technique, a large filter (18 cm diameter) was employed, and as a developing "wick", a strip of paper just enough for