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.

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[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.

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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

the purpose (1.5 cm in length and 2 mm in width) was cut from the center and bent towards it so that it was kept perpendicular to the plane of the paper.

Separation of the two compounds was accomplished by using two solvent systems, *viz.* (I) upper layer of solvent mixture from *n*-butanol–glacial acetic acid–water (40:10:50) and (II) upper layer of solvent mixture from *n*-butancl–dilute acetic acid (1.93 *N*)–water (40:10:50) for developing. Revelation was achieved by spraying the dried paper with 2 % alcoholic sodium hydroxide solution. The glucosides developed a yellow color with this solution and the free 1,8-dihydroxyanthraquinone derivatives a pink color. The yellow color of the sennosides changed gradually to brown after about a day. The average R_F values of sennosides A and B in solvent I are 0.79 and 0.68 respectively and in solvent II are 0.68 and 0.45 respectively.

By using the solvent system II which was found to be definitely better than solvent I for the separation of the glucosides, it was also possible to separate the free 1,8-dihydroxyanthraquinone derivatives, *viz.* chrysophanol, aloe-emodin, emodin and rhein from each of the two glucosides. It was observed that these compounds which also occur in senna, moved almost to the solvent front with R_F value of 0.93 when the solvent system II was employed.

The separation of the free anthraquinones from sennosides was confirmed by chromatography of 70 % alcoholic extract of senna (*Cassia angustifolia* Vahl) pods. In connection with this procedure used for confirmation, it might be mentioned that chromatography of this crude extract of senna pods revealed the presence of six bands other than those due to the two glucosides and free anthraquinones when solvent system II was used for development and alcoholic alkali was used for spraying. Further work on this aspect is in progress.

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[1] G. VALLETE AND M. L. HUREAU, *Therapie*, 12 (1957) 885.
[2] L. RUTTER, *Nature*, 161 (1948) 435.
[3] L. RUTTER, *Analyst*, 75 (1950) 37.

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