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Note

Separation of some 16-androstenes on hydroxyalkoxypropyl-Sephadex (Lipidex™)

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Interest has been aroused recently in the odoriferous 16-androstenes in view of their effects as pheromones in pigs[1]. In our studies on the metabolism and estimation of this group of steroids, it has become increasingly necessary to achieve a separation of the various compounds from each other. Chromatography on columns of alumina has been used extensively in the past with reasonable success[2] but this method does not resolve 5α-androst-16-en-3α-ol from 4,16-androstadien-3-one. Following the reports[3–5] that oestradiol, cortisol and 17-hydroxyprogesterone can be separated on Sephadex LH-20, similar methods were applied to mixtures of 16-androstenes using non-polar solvents such as benzene and *n*-pentane, but no separations have so far been achieved. Jänne *et al.*[6] have improved on the Sephadex LH-20 separation by using a lipophilic Sephadex derivative, hydroxyalkoxypropyl-Sephadex[7] (Lipidex™), which has allowed relatively non-polar steroids such as testosterone and progesterone to be resolved. This report describes how Lipidex™ can be utilized to separate some of the very non-polar 16-androstenes, such as 5α-androst-16-en-3-one and 4,16-androstadien-3-one.

EXPERIMENTAL

[5α-^{3}H]5α-Androst-16-en-3-one (15.01 Ci/mmole) was generously supplied by Dr. W. Hafferl, Syntex Research, Palo Alto, Calif., U.S.A. [5α-^{3}H]5α-Androst-16-en-3α- and -3β-ols were prepared from [5α-^{3}H]5α-androst-16-en-3-one by reduction with KBH_4 followed by separation by thin-layer chromatography[8]. [7α-^{3}H]Androsta-4,16-dien-3-one (125 Ci/mole) was synthesized by the method of Wilkinson *et al.*[9].

Lipidex™ (obtained from Packard-Becker B. W. Chemical Operations, Groningen, The Netherlands) was allowed to equilibrate for at least 24 h in the same solvent system as was to be used for elution. A glass column (I.D. 9 mm), fitted with a plug of fat-free cotton wool, was then packed with 12.5 g of equilibrated Lipidex™ in *n*-pentane–cyclohexane (99.5:0.5) and the gel allowed to settle by gravitation. The resulting gel column was 500 mm long. The labelled 16-androstenes, first separately and then as mixtures, were applied to the top of the column bed in 0.1 ml of the eluting solvent mixture. Elution was carried out with *n*-pentane–cyclohexane (99.5:0.5) with a flow-rate of 60 ml/h. Fractions (1 ml) were collected, transferred to counting pots and scintillant was added before radioactivity was measured in a liquid scintillation spectrometer.

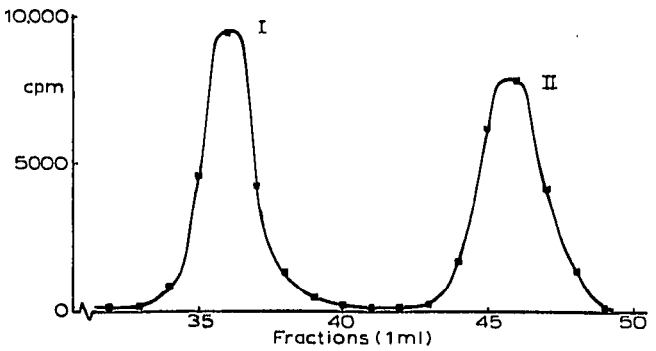

Fig. 1. Separation of [5α-^{3}H]5α-androst-16-en-3-one (I) from [7α-^{3}H]androsta-4,16-dien-3-one (II) on a column (length 500 mm) of Lipidex™. The eluting solvent used was *n*-pentane–cyclohexane (99.5:0.5).

RESULTS AND DISCUSSION

Fig. 1 illustrates a typical separation of two 16-androstenes, 5α-androst-16-en-3-one and 4,16-androstadien-3-one. Other 16-androstenes have been applied to Lipidex™ using the same conditions as described above, and Table I summarizes the elution peak volumes and the elution fractions containing each steroid.

The column has been useful in separating 5α-androst-16-en-3-one and 4,16-androstadien-3-one extracted from human peripheral blood plasma samples. Approximately 70% recovery was obtained for each steroid, as shown by the addition of tracer amounts of each labelled steroid to the plasma prior to extraction. The resolution of 4,16-androstadienone and 5α-androst-16-en-3α-ol, not possible with alumina chromatography[2], has also been achieved with Lipidex™. This separation would be a particularly useful step to include in a radioimmunoassay method for each 16-androstene.

TABLE I

SEPARATION OF SOME 16-ANDROSTENES ON LIPIDEX™ WITH *n*-PENTANE–CYCLOHEXANE (99.5:0.5) AS SOLVENT SYSTEM

Column height, 500 mm; flow-rate, 60 ml/h.

Steroid	*Approx. elution peak (ml)*	*Elution fraction (ml)*
5α-Androst-16-en-3-one	36	33– 40
4,16-Androstadien-3-one	46	43– 49
5α-Androst-16-en-3α-ol	99	87–111
5α-Androst-16-en-3β-ol	123	111–135

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REFERENCES

1 H. C. B. Reed, D. R. Melrose and R. L. S. Patterson, *Brit. Vet. J.*, 130 (1974) 61.
2 D. B. Gower, *J. Steroid Biochem.*, 3 (1972) 45.
3 A. R. Bourne, *J. Chromatogr.*, 92 (1974) 465.
4 B. R. Carr, G. Mikhail and G. L. Flickinger, *J. Clin. Endocrinol.*, 33 (1971) 358.
5 P. Eneroth and E. Nyström, *Biochim. Biophys. Acta*, 144 (1967) 149.
6 O. Jänne, D. Apter and R. Vihko, *J. Steroid Biochem.*, 5 (1974) 155.
7 J. Ellingboe, E. Nyström and J. Sjövall, *J. Lipid Res.*, 11 (1970) 266.
8 P. J. Brophy and D. B. Gower, *Biochem. J.*, 128 (1972) 945.
9 M. Wilkinson, M. M. Coombs and D. B. Gower, *J. Label. Compounds*, 6 (1970) 386.