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Behaviour of dehydroisoandrosterone, testosterone and their conjugates on DEAE-Sephadex

Recent publications have shown the value of DEAE-Sephadex chromatography in separating several types of phenolic steroid conjugates[1–4]. The present report concerns the behaviour of two neutral C_{19} steroids and their conjugates in simple NaCl concentration gradients on DEAE-Sephadex columns.

Experimental

Reagents. DEAE-Sephadex (A-25) of medium porosity was purchased from Pharmacia (Canada) Ltd., Montreal, Quebec, and packed to yield columns of 58 × 1 cm as described elsewhere[4].

Steroids and conjugates. [7-^{3}H]Dehydroisoandrosterone (DHA) of specific activity (S.A.) 1.6 Ci/mmole was purchased from New England Nuclear Corp., Boston, Mass., U.S.A. and [4-^{14}C]testosterone (T) of S.A. 29.2 Ci/mole was purchased from Radiochemical Centre, Amersham, Bucks., Great Britain (now Amersham-Searle). The conjugates were purchased from New England Nuclear Corp. These were [7-^{3}H]dehydroisoandrosteron-3-yl-β-D-glucopyranosiduronate (DHAG) of S.A. 10 Ci/mmole, the NH_4^+ salt of [7-^{3}H]dehydroisoandrosteron-3-yl-sulphate (DHAS) of S.A. 10 Ci/mmole, [1,2-^{3}H]testosteron-17-yl-β-D-glucopyranosiduronate (TG) of S.A. 50 Ci/mmole, and the NH_4^+ salt of [7-^{3}H]testosteron-17-yl-sulphate (TS) of S.A. 8 Ci/mmole.

Methods. Aqueous solutions of various mixtures of the above labelled compounds, containing a drop of methanol to facilitate solution of the free steroids, were applied to DEAE-Sephadex columns which were developed with linear concentration gradients of NaCl in H_2O as described elsewhere[2,4]. The mixing vessel in each case contained 400 ml H_2O and the donor vessel contained 400 ml of either 0.2, 0.3, 0.4 or 0.8 *M* NaCl. 5-ml fractions of eluate were collected and radioactivity was determined by liquid scintillation spectrometry[5].

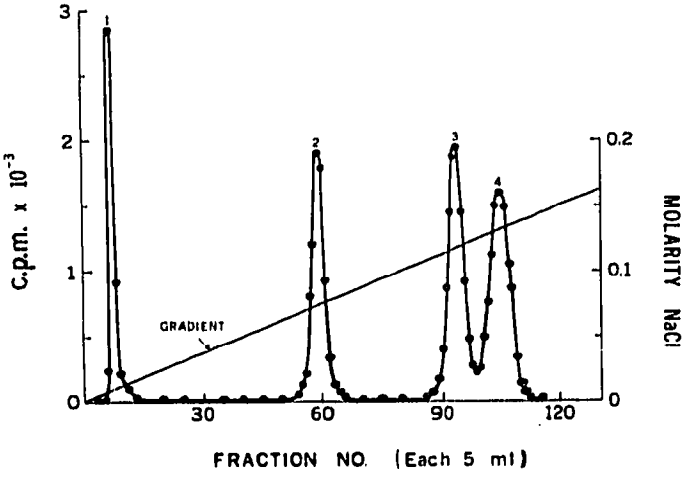

Fig. 1. DEAE-Sephadex chromatography in a linear gradient (0–0.2 *M* NaCl) of: peak 1, DHA or T; peak 2, DHAG or TG; peak 3, TS; peak 4, DHAS.

Results and discussion

In none of the gradients could DHA be separated from T nor could DHAG be separated from TG. However, the free steroids were easily separable from the glucosiduronates in all gradients. The sulphates, in turn, were well separated from the free steroids and glucosiduronates and, furthermore, DHAS and TS were separable from each other to varying extents in the different gradients. The greatest degree of separation between the two sulphates occurred in the 0–0.2 *M* NaCl gradient (Fig. 1) and in the 0–0.3 *M* gradient. The chromatographic mobilities of DHA and T were independent of the gradient employed but, as expected, behaviour of the conjugates was dependent on salt concentration. The sulphates of DHA and T were eluted in positions similar to certain oestrogen monoglucosiduronates[2,4] and considerably before oestrogen monosulphates[2].

It is of interest to note that when, in one experiment, employing the 0–0.2 *M* NaCl gradient, labelled oestrone and 17β-oestradiol were chromatographed along with ^{3}H-DHA, the oestrogens were eluted between fractions 12 and 20 while DHA appeared between fractions 6 and 11. It would appear from this and from the behaviour of the conjugates that the presence of a phenolic group allows a greater retention by the DEAE-Sephadex (see ref. 4).

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