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Note

Lipophilic gel chromatography of steroids

Structure–standard elution volume relationships

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Lipophilic gel chromatography on chemically modified dextrans has found widespread application in the analysis of naturally occurring and synthetic steroids and their metabolites in biological fluids[1–5]. The separation of unconjugated steroids by lipophilic gels appears to depend predominantly on the number of hydroxyl groups in the naturally occurring steroids[1,6]. Recently work has shown that a similar relationship appears to hold for the chromatography of synthetic steroidal spirolactones on Lipidex 5000 gel with toluene as eluent[7]. The present paper describes a more detailed study of the relationship between hydroxyl group number and standard elution volume (SEV) for two classes of synthetic steroids.

MATERIALS AND METHODS

AnalaR-quality solvents were redistilled before use. All other reagents were supplied by Hopkin and Williams (Chadwell Heath, Great Britain) unless otherwise stated.

Reference compounds Ia, VIIa, VIIb, VIIc, VIIe, IXa and Xb were supplied by G. D. Searle & Co. (Morpeth, Great Britain); IIa and IVa by the Analytical Development Laboratory (G. D. Searle & Co. Ltd., High Wycombe, Great Britain), and Ib, Ic, IVb, IVc and VIIIa–d by Dr. A. Karim (Department of Drug Metabolism and Radiochemistry, Searle Labs., Skokie, Ill., U.S.A.).

Compounds IIa, IIb and VI were synthesised as previously described[7], and Va and Vb were prepared by borohydride reduction of IVb and IVc, respectively, using the same method as that for IIIb[7]. The structures of the reference compounds are shown in Fig. 1.

Lipophilic gel chromatography

Columns of Lipidex 5000 (Packard Instruments Ltd., Caversham, Great Britain) with a bed volume of 12.8 to 14.2 ml were prepared and used essentially as described previously[7], except that the 17α-ethinyl steroids were eluted with toluene at a flow-rate of 0.14 ml/min. Fractions (1 ml) were collected on a LKB Ultrorac frac-

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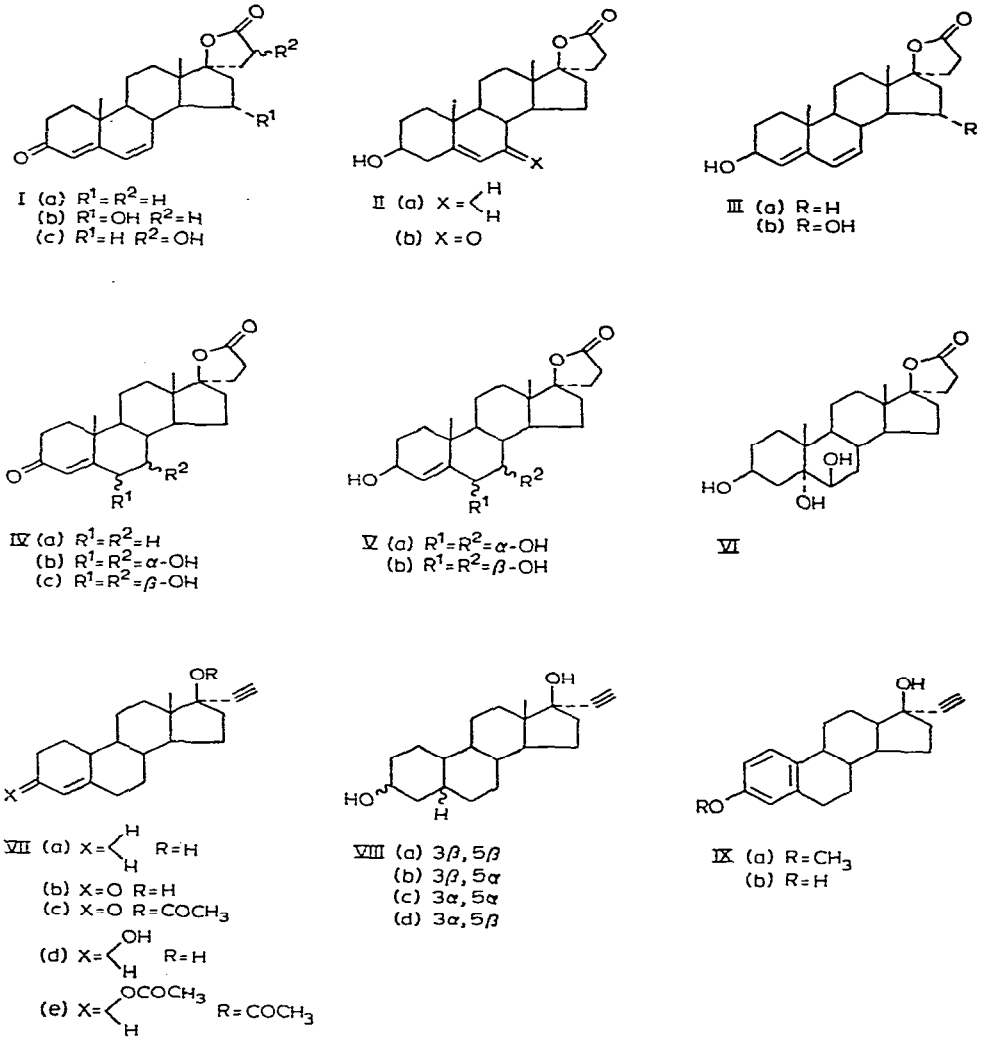

Fig. 1. Chemical structures.

tion collector (LKB, South Croydon, Great Britain). The elution volumes of the reference compounds were determined by gas chromatography (for the spirolactones) or thin-layer chromatography (for the 17α-ethinyl compounds) analysis of the fractions, as described by Boreham *et al.*[7], and SEV values were calculated by the method of Brooks and Keates[6].

RESULTS AND DISCUSSION

The SEV data show the group resolution of the steroidal spirolactones (Table I) and the 17α-ethinyl compounds (Table II). This fractionation appears to depend predominantly on the number of hydroxyl groups in the steroid, in agreement with previous observations for naturally occurring steroids[1,6,8]. The SEV ranges for compa-

TABLE I

ELUTION DATA (SEV VALUES) FOR REFERENCE STEROIDAL SPIROLACTONES ON LIPIDEX 5000 IN TOLUENE

Compound	*Average SEV*	*Average log_{10} SEV*
3-Keto fraction		
Canrenone (Ia)	67	1.83
6,7-Dihydrocanrenone (IVa)	66	1.82
Monohydroxy fraction		
3β-Hydroxy-3-deoxocanrenone (IIIa)	107	2.03
Andrenolactone (IIa)	113	2.05
Keto-hydroxy fraction		
15α-Hydroxycanrenone (Ib)	201	2.30
21ξ-Hydroxycanrenone (Ic)	184	2.26
7-Ketoandrenolactone (IIb)	61	1.79**
Dihydroxy fraction		
3β,15α-Dihydroxy-3-deoxocanrenone (IIIb)	419	2.62
Keto-dihydroxy fraction		
6α,7α-Dihydroxy-6,7-dihydrocanrenone (IVb)	>800*	
6β,7β-Dihydroxy-6,7-dihydrocanrenone (IVc)	460	2.66
Trihydroxy fraction		
3β,6α,7α-Trihydroxy-3-deoxo-6,7-dihydrocanrenone (Va)	>800*	
3β,6β,7β-Trihydroxy-3-deoxo-6,7-dihydrocanrenone (Vb)	>800*	
5α,6β-Dihydroxy-5,6-dihydroandrenolactone (VI)	>800*	

Linear regression analysis of log_{10} SEV *vs.* number of hydroxyl groups gave $y = 0.4075x + 1.7887$ (corr. 0.951)

* SEV could not be determined, as compounds were not eluted by toluene.

** Anomalous result for a keto-hydroxy compound.

TABLE II

ELUTION DATA (SEV VALUES) FOR REFERENCE 17α-ETHINYL STEROIDS ON LIPIDEX 5000 IN TOLUENE

The polar compounds (SEV > 600) were eluted with toluene–isopropanol (3:1, v/v).

Compound	*Average SEV*	*log_{10} SEV*
Non-polar fraction		
Ethynodiol diacetate (VIIe)	58	1.76
3-Keto fraction		
Norethisterone acetate (VIIc)	62	1.79
Monohydroxy fraction		
Lynestrenol (VIIa)	106	2.03
Mestranol (IXa)	106	2.03
Keto-hydroxy fraction		
Norethisterone (VIIb)	112	2.05
Ethisterone	111	2.05
11β-Methylnorethisterone	109	2.04
Dihydroxy fraction		
3β,5β-Diol (VIIIa)	179	2.25
3β,5α-Diol (VIIIb)	192	2.28
3α,5α-Diol (VIIIc)	181	2.26
3α,5β-Diol (VIIId)	202	2.31
Ethinyloestradiol (IXb)	>600*	

Linear regression analysis of log_{10} SEV *vs.* number of hydroxyl groups gave $y = 0.2476x + 1.7847$ (corr. 0.995).

* SEV could not be determined, as compound was not eluted by toluene.

rable groups of compounds show some differences between the spirolactone and 17α-ethinyl steroid groups; these differences may reflect the presence of the lactone ring, which provides an additional polar substituent in the spirolactones.

There was a linear relationship between the $\log_{10}$ SEV and the number of hydroxy groups in the steroid, for the spirolactones and 17α-ethinyl steroids, as shown by regression analysis (Tables I and II, respectively). However, certain compounds have SEV values that deviate from this relationship. Thus, 7-ketoandrenolactone (IIb) has an SEV of 61, in contrast to the value of 180 to 200 expected for a keto-hydroxy steroid. This may reflect an effect of the position of the keto substituent on the chromatography of a 3β-hydroxy compound, as the other keto-hydroxy compounds retain a 3-keto group. Substitution at C-7 has been found to give anomalous SEV values in other classes of steroids, *e.g.*, 7β- and 7α-hydroxycholesterol, which have SEV values much smaller than those expected for dihydroxy steroids[1]. In contrast to IIb, IVb (a keto-dihydroxy spirolactone) has an SEV >800, much larger than that of its stereoisomer IVc (Table I); and ethinyloestradiol (IXb) has a larger SEV (>600) than the other dihydroxy 17α-ethinyl steroids (Table II).

Adsorption on lipophilic gels is considered to result from hydrogen bonding between ether linkages of the gel network and hydrogen donors in the solute[8,9]. It is possible that the stereochemistry of the hydroxy groups in IVb results in increased hydrogen bonding and thus greater adsorption of the compound by the gel. Ethinyl-oestradiol (IXb) is a phenolic steroid, which would be expected to be more extensively retained than other dihydroxy compounds because of the ionisable phenolic hydroxy group.

TABLE III

ELUTION DATA (SEV VALUES) FOR SOME NATURALLY OCCURRING ANDROSTANE DERIVATIVES*

Compound	*SEV*	*$\log_{10}$ SEV*
Non-polar fraction		
4-Androsten-3,17-dione	60	1.78
Monohydroxy fraction		
3α-Hydroxy-5α-androstan-17-one	90	1.95
3β-Hydroxy-5-androsten-17-one	90	1.95
3α-Hydroxy-5β-androstan-17-one	90	1.95
17β-Hydroxy-4-androsten-3-one	110	2.04
Dihydroxy fraction		
5-Androstene-3β,17β-diol	210	2.32
3β,15α-Dihydroxy-5-androsten-17-one	185	2.28
3β,15α-Dihydroxy-5α-androstan-17-one	190	2.28
3β,16α-Dihydroxy-5-androsten-17-one	175	2.24
3β,17β-Dihydroxy-5-androsten-7-one	220	2.34
Trihydroxy fraction		
5-Androstene-3β,16β,17β-triol	450	2.65
5-Androstene-3β,7α,17β-triol	400	2.60
5-Androstene-3β,7β,17β-triol	400	2.60
3β,16β,17β-Trihydroxy-5-androsten-7-one	600	2.78

Linear regression analysis of $\log_{10}$ SEV *vs.* number of hydroxyl groups gave $y = 0.3172x + 1.6787$ (corr. 0.979).

* SEV data taken with permission from Anderson *et al.*[1].

The linear relationship between $\log_{10}$ SEV and the number of hydroxyl groups confirms the importance of the hydroxy substituents in the lipophilic gel chromatography of steroids. In addition, this relationship may allow the prediction of SEV values for other closely related steroids or the estimation of hydroxyl group numbers from SEV data. The latter is of particular value in studies of steroid-drug metabolism.

The relationship between structure and SEV observed for the two classes of synthetic steroids in this study appears to apply to naturally occurring steroids. Thus, the data in Table III show a linear relationship between $\log_{10}$ SEV and hydroxyl number for some 14 steroids of the androstane series studied by Anderson *et al.*[1] The over-all results of the present investigation confirm the value of lipophilic gel chromatography for the group separation of synthetic steroids and provide additional information about the relationship between structure and SEV for various classes of steroids.

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