PAPER ELECTROPHORESIS OF STEROIDS IN BORATE BUFFERS

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INTRODUCTION

Paper electrophoresis in borate buffers has been used extensively for the separation and identification of polyhydroxylated carbohydrates[1–3]. Carbohydrates, in which the hydroxyl groups are vicinal and *cis*-oriented, form negatively charged complexes with borate that migrate toward the anode. Complex formation between borate and steroidal *cis*-dihydroxyl groups is also known to occur. Paper chromatography in the presence of borate has been used to indicate the configuration of a number of vicinal hydroxyl groups of steroids[4,5]. Borate derivatives of two 16α,17α-dihydroxy steroids have been prepared and characterized by LEESON *et al.*[6]. BULASCHENKO *et al.*[7], in a study of steroid metabolism, employed paper electrophoresis in borate buffer for the recognition of steroids containing hydroxyl groups of C-20 and C-21. The application of paper electrophoresis to C-16,17-dioxygenated steroids, as well as certain other polyhydroxylated steroids, is described in this paper.

EXPERIMENTAL

Borate buffers were prepared by adjusting a 0.3 *M* boric acid solution to the desired pH with 10 *N* sodium hydroxide. The solution was then diluted with an equal volume of reagent-grade methanol.

Electrophoresis was performed on Whatman 3 MM paper that had been saturated with the electrolyte, blotted, and placed in a horizontal electrophoresis migration chamber*. The ends of the paper were dipped into the borate solution contained in the electrode vessels. The paper and the electrolyte were allowed to equilibrate for a period of 30–60 min prior to application of the steroids (10–50 μg) in methanolic solution. In most instances electrophoresis was carried out at room temperature for 5 h at 500 V (10.9 V/cm).

9α-Fluorocortisol (XVI)**, which does not form a charged complex with borate, was included in each experiment to serve as an indicator of electro-osmotic flow. The position of each steroid was measured relative to that of XVI, and the anodic migration (M_s) was expressed as a fraction of the distance travelled by the borate-complexing steroid, 9α-fluoro-16α-hydroxycortisol (XVII).

* Model E8co-2B, Research Specialities Co., Richmond, Calif.
** Refer to tables for identy of steroids.

The steroids were detected by methods commonly used in paper chromatographic analysis[8].

RESULTS AND DISCUSSION

Effect of pH

The rate of anodic migration of steroids in borate buffers was dependent on pH (Fig. 1). The mobility of a 16α,17α-dihydroxy-20-ketosteroid (XVII) increased sharply from pH 6.1 to about pH 8.5, and then decreased slightly. The migration rate of other steroids (I, IV, XXV) was less susceptible to changes in the pH 6–8 range, but increased rapidly under more alkaline conditions. All complexing steroids streaked at pH values below 7.0.

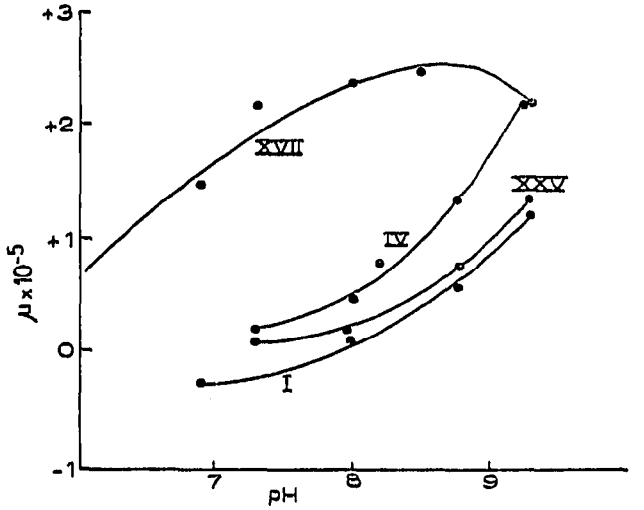

Fig. 1. Effect of pH upon electrophoretic migration. μ = Electrophoretic migration = $\frac{\text{velocity in cm/sec}}{\text{voltage/cm}}$. Negative value denotes cathodic migration. I = 16α-hydroxyandrost-4-ene-3,17-dione; IV = 16α,17α-dihydroxy-17β-methylestr-4-en-3-one; XVII = 9α-fluoro-11β,16α,17α,21-tetrahydroxypregn-4-ene-3,20-dione; XXV = 17α,20β,21-trihydroxypregn-4-en-3-one.

C-16,17-Dioxygenated steroids (cf. Table I)

The anodic migration of 16α,17α-dihydroxysteroids could be prevented by acetylation of one of the *cis* hydroxyl groups, *e.g.* compounds VIII and IX. At high pH values however, some hydrolysis of the ester probably occurred with subsequent delayed migration of the free steroid.

16α-Hydroxylated steroids that contain a 17β-hydroxy group (II, III), or that lack any oxygen function at C-17 (VI, X) displayed a slight cathodic migration, for which we can offer no explanation. A 16β,17α-dihydroxysteroid (XIII), which would not be expected to complex with borate, exhibited two anodic migrating zones, perhaps caused by isomerization to compounds XII, XIX, and/or XX. Such isomerization has been shown to occur when 16β-acetoxy-17α,21-dihydroxypregn-4-ene-3,20-dione was subjected to basic conditions[9].

The presence of a C-20 ketone apparently contributes to the rate of anodic migration of 16α,17α-dihydroxysteroids, since blockage of the C-20 ketone (XV), or its absence (IV), resulted in a lowered migration rate. Moreover, compound XVIII,

TABLE I

ANODIC MIGRATION OF C-16- AND C-17-OXYGENATED STEROIDS

Steroid		M_s[a]	
		pH 8.5	*pH 9.3*
I	16α-Hydroxyandrost-4-ene-3,17-dione	0.28	0.50
II	16α,17β-Dihydroxyandrost-4-en-3-one		[b]
III	16α,17β-Dihydroxy-17α-methylestr-4-en-3-one		[b]
IV	16α,17α-Dihydroxy-17β-methylestr-4-en-3-one	0.55	0.91
V	16β-17β-Dihydroxy-16α-methylestr-4-en-3-one		0.74
VI	16α-Hydroxypregn-4-ene-3,20-dione		[b]
VII	17α-Hydroxypregn-4-ene-3,20-dione	0.00	
VIII	16α,17α-Dihydroxypregn-4-ene-3,20-dione	1.00	1.09
IX	16α-Acetoxy-17α-hydroxypregn-4-ene-3,20-dione	0.00	0.07
X	16α,21-Dihydroxypregn-4-ene-3,20-dione		[b]
XI	17α,21-Dihydroxypregn-4-ene-3,20-dione		0.00
XII	16α,17α,21-Trihydroxypregn-4-ene-3,20-dione	1.00	1.00
XIII	16β,17α,21-Trihydroxypregn-4-ene-3,20-dione		0.59 and 1.00
XIV	20-Ethylenedioxy-11β,17α,21-trihydroxypregn-4-en-3-one		0.00
XV	21-Acetoxy-20-ethylenedioxy-11β,16α,17α-trihydroxy-pregn-4-en-3-one	0.48	0.86
XVI	9α-Fluoro-11β,17α,21-trihydroxypregn-4-ene-3,20-dione	0.00	0.00
XVII	9α-Fluoro-11β,16α,17α,21-tetrahydroxypregn-4-ene-3,20-dione	1.00	1.00
XVIII	9α-Fluoro-11β,16α,17α,20β,21-pentahydroxypregn-4-en-3-one	0.79	1.00
XIX	16α,17α-Dihydroxy-17β-hydroxymethyl-D-homoandrost-4-ene-3,17a-dione	0.78[c]	
XX	16α,17aα-Dihydroxy-17aβ-hydroxymethyl-D-homoandrost-4-ene-3,17-dione	0.66[c]	
XXI	9α-Fluoro-11β,16α,17aα-trihydroxy-17aβ-hydroxymethyl-D-homoandrost-4-ene-3,17-dione	0.59[c]	0.74

[a] Anodic migration relative to that of 9α-fluoro-16α-hydroxycortisol (XVII).
[b] Slight migration towards the cathode.
[c] These migration rates were obtained at pH 8.0

which contains a 20β-hydroxyl group, migrated more slowly at lower pH values than the corresponding 20-ketosteroid (XVII).

Compound I, a 16α-hydroxy-17-ketosteroid, exhibited an anodic migration at alkaline pH, perhaps due to enolization of the ketone. It may be noted, however (Fig. 1), that at acid pH a slight cathodic migration occurred, similar to that observed with compounds II, III, VI and X. If enolization is a factor in the anodic migration of compound I, it is difficult to explain the lack of migration of 20,21-α-ketolic steroids, which might also be expected to enolize at alkaline pH.

C-20-Hydroxysteroids (cf. Table II)

Steroids containing a glycol- or glycerol-type side chain migrated toward the anode more slowly than 16α,17α-dihydroxysteroids. A 17α,20β-dihydroxysteroid (XXIII) travelled at twice the rate of a 20β,21-dihydroxysteroid (XXIV). The 17α,20α-dihydroxysteroid (XXII), epimeric at C-20 to compound XXIII, behaved erratically and streaked slightly toward the anode at pH 9.3. 17α,20β,21-Trihydroxysteroids (XXV, XXVII) migrated faster than the steroids containing the glycol-type side chains.

TABLE II

ANODIC MIGRATION OF C-20-HYDROXYSTEROIDS

Steroid		M_s[a]	
		pH 8.5	*pH 9.3*
XXII	17α,20α-Dihydroxypregn-4-en-3-one		slight streak
XXIII	17α,20β-Dihydroxypregn-4-en-3-one		0.21
XXIV	11β,20β,21-Trihydroxypregn-4-en-3-one		0.11
XXV	17α,20β,21-Trihydroxypregn-4-en-3-one	0.31	0.72
XXVI	20β,21-Diacetoxy-17α-hydroxypregn-4-en-3-one		0.00
XXVII	9α-Fluoro-11β,17α,20β,21-tetrahydroxypregn-4-en-3-one	0.23	0.69

[a] Anodic migration relative to that of 9α-fluoro-16α-hydroxycortisol.

Steroids containing 1,3- or 1,3,5-cis-oriented hydroxyl groups

Neither 14α,17α,21-trihydroxypregn-4-ene-3,20-dione nor 15α,17α,21-trihydroxypregn-4-ene-3,20-dione migrated in borate buffers. These steroids contain 1,3-*cis*-oriented hydroxyl groups, the former in the 14α- and 17α-positions and the latter in the 15α- and 17α-positions. However, 7α,14α,17α,21-tetrahydroxypregn-4-ene-3,20-dione, which has 1,3,5-*cis*-oriented hydroxyl groups at the 7α-, 14α- and 17α-positions, did migrate in pH 9.3 buffer with an M_s value of 0.44.

Analogous observations have been reported by ANGYAL AND MCHUGH[10], who found that 1,3,5-*cis*-trihydroxy cyclitols, but not 1,3-*cis*-dihydroxy cyclitols, moved to the anode during paper electrophoresis in borate buffer.

Electrophoretic migration in other electrolytes

Anodic migration of the 16α,17α-dihydroxysteroids was also observed in alkaline phosphate, barbiturate, and tris-(hydroxymethyl)-aminomethane buffers. The mobility of the steroids in each of these buffers was erratic and much lower than that in borate solution. No satisfactory explanation for this phenomenon can be given at this time.

SUMMARY

Certain polyhydroxylated steroids form negatively charged complexes in borate buffer which migrate toward the anode during paper electrophoresis. The relative migration rates are tabulated.

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