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STUDIES OF ALDOSTERONE 20,21-CYCLIC BORONATES BY GAS–LIQUID CHROMATOGRAPHY AND MASS SPECTROMETRY

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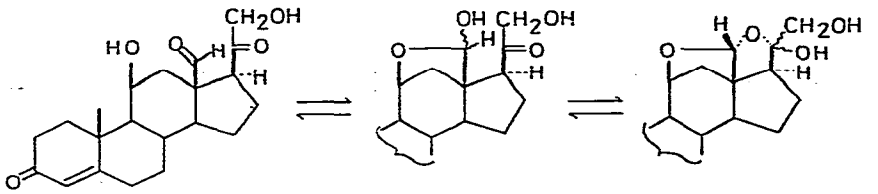
SUMMARY

Aldosterone is simply and rapidly converted at room temperature into cyclic 20,21-methane- and 1-butaneboronate derivatives. Analyses by gas–liquid chromatography–mass spectrometry (GLC–MS) in each instance afford a single chromatographic peak for which electron impact and chemical ionization mass spectrometric data are reported. Complete derivatization is indicated by a comparison of the field desorption mass spectra of aldosterone and aldosterone methaneboronate. The new derivatives provide a convenient means of characterization of aldosterone by GLC–MS.

INTRODUCTION

Aldosterone (11β,21-dihydroxy-3,20-dioxo-4-pregnen-18-al) can exist in several tautomeric forms. In the original reports on the structure and properties of aldosterone[1,2], the hydroxy-aldehyde form (1) and the 18:11-hemiacetal (2) were recognized. The occurrence of the 18:11,20-acetal 20:18-hemiketal form (3) in solution was indicated by infrared spectrometry[3]. The structure and stereochemistry shown in (4) were later established for a crystalline monohydrate of aldosterone by the X-ray crystallographic work of Duax and Hauptman[4]. These workers suggested that the stability of this tautomer might lead to its persistence as an important form in solution, and a study by nuclear magnetic resonance spectroscopy has confirmed the preponderance of (3) in $CDCl_3$ solution[5]. Protection of the 20,21-diol grouping in (3) by the formation of cyclic acetals has been reported, *e.g.*, the acetonide (5) is obtained by exchange with acetone ketals[6].

Cyclic alkaneboronates are readily obtained under mild conditions from a wide range of 1,2- and 1,3-diols[7], and the value of these derivatives for gas-phase characterization of various corticosteroids has been demonstrated in detail[8–11]. In particular, 18-hydroxy-11-deoxycorticosterone (18,21-dihydroxy-4-pregnene-3,20-dione) readily afforded a 20,21-methaneboronate useful for gas-phase characterization[12,13].



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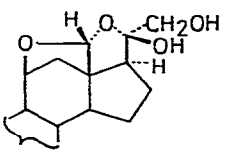

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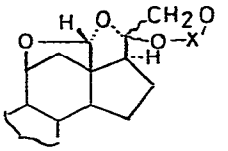

5 X = $C(CH_3)_2$

6 X = BCH_3

7 X = $B(CH_2)_3CH_3$

We describe here the preparation of analogous cyclic boronates derived from the tautomeric form (3) of aldosterone, and discuss their possible utility in relation to other characteristic derivatives of the hormone*.

EXPERIMENTAL

Materials

Aldosterone 21-acetate was a gift from Professor C. H. Robinson. Methaneboronic acid was obtained from Alfa Inorganics (Ventron-Hicol, Rotterdam, The Netherlands) and 1-butaneboronic acid from Callery (Callery, Pa., U.S.A.).

Methods

Aldosterone 21-acetate was hydrolysed with methanolic potassium hydrogen carbonate according to the procedure of Simpson *et al.*[1]. Micro thin-layer chromatography on silica gel using chloroform–ethyl acetate (1:1), indicated complete hydrolysis. Cyclic boronate derivatives were prepared by dissolving aldosterone in ethyl acetate or pyridine and addition of the appropriate boronic acid (1.1 molar proportions). Pyridine was removed under a stream of nitrogen and the product dissolved in ethyl acetate immediately prior to analysis. Gas–liquid chromatography (GLC) was performed using a Pye 104 instrument equipped with a glass column (2 m × 3.5 mm I.D.) packed with 1% OV-1 on Gas-Chrom Q (100–120 mesh) with nitrogen (40 ml/min) as carrier gas.

Electron impact (EI) and chemical ionization (CI) mass spectra were obtained during gas–liquid chromatography–mass spectrometry (GLC–MS) on a DuPont 21-490F instrument, fitted with a glass column similar to that used for GLC. The ion source temperature was 240° and the electron energy was 20 eV (EI) or 70 eV (CI).

* An oral presentation of part of this work was given at the Mass Spectrometry Group meeting, Cardiff, Great Britain, September 1977.

Isobutane and methane were used as CI reagent gases at pressures adjusted according to an empirical method described elsewhere[12].

Field desorption (FD) mass spectra were obtained using a Varian-MAT 731 instrument with a combined EI/FD source. Samples were applied to the FD emitter, in ethanol (aldosterone) or ethyl acetate (aldosterone methaneboronate), using a microlitre syringe. Spectra were recorded at an emitter current of approximately 15 mA. The ion source temperature was 200°.

RESULTS

GLC analysis of the products of methaneboronate and *n*-butaneboronate formation, after a reaction time of 5 min, indicated a single peak in each instance. No change in response was observed after longer reaction times, and the products are ascribed structures (6) and (7), respectively.

Retention data for methane- and butaneboronates are given in Table I. EI (20 eV) mass spectra, recorded during GLC–MS, showed extremely complex fragmentation patterns (Table I). Intense molecular ions were observed: for the methaneboronate, the exact mass was determined as *m/e* 384.21072 (calculated for $C_{22}H_{29}BO_5$: *m/e* 384.21079). Base peaks corresponded to the loss of a CH_2O_2 fragment [methaneboronate: *m/e* 338.20503 (calculated: *m/e* 338.20531)]. Isobutane CI mass spectra were very simple (Table I); similar results were obtained with methane as reagent gas. Protonated molecular ions, $[MH]^+$, were the base peaks; ions $[MH - H_2O]^+$ were also prominent.

TABLE I

GAS CHROMATOGRAPHIC AND MASS SPECTROMETRIC (ELECTRON IMPACT AND CHEMICAL IONIZATION) DATA FOR CYCLIC BORONATE ESTERS OF ALDOSTERONE

Derivative	*Retention index**	*Electron impact (20 eV) mass spectrum***						*Isobutane chemical ionisation mass spectrum***				
		M^+ ·	*Other ions****					MH^+	*Other ions*§			
Methaneboronate (6)	3170	384 (69)	338 (100)	111 (46)	162 (38)	339 (37)	131 (36)	385 (100)	384 (37)	386 (27)	367 (21)	383 (7)
			163 (35)	323 (32)	148 (32)	383 (30)	149 (30)					
1-Butaneboronate (7)	3450	426 (81)	380 (100)	153 (68)	356 (43)	397 (40)	191 (38)	427 (100)	428 (38)	409 (32)	426 (26)	408 (5)
			254 (37)	379 (34)	284 (33)	281 (32)	217 (32)					

* OV-1 liquid phase; 270°.

** Ions recorded as *m/e* (relative intensity).

*** 10 most abundant ions (above *m/e* 50) other than molecular ion.

§ All other ions (above *m/e* 70) of relative intensity >5%.

Evidence for complete conversion of aldosterone into the cyclic boronate derivative was obtained by comparison of the FD mass spectra of aldosterone and aldosterone methaneboronate (Table II). Aldosterone afforded a spectrum with a prominent molecular ion (*m/e* 360) and protonated molecular ion (*m/e* 361), together

TABLE II

FIELD DESORPTION MASS SPECTROMETRIC DATA FOR ALDOSTERONE AND ALDOSTERONE METHANEBORONATE

Ions recorded as *m/e* (relative intensity).

Compound	MH^+	*Other ions**			
Aldosterone	361 (47)	329 (100)	343 (34)	330 (27)	360 (22)
		362 (13)	342 (11)		
Aldosterone methaneboronate (6)	385 (100)	386 (41)	384 (26)	285 (21)	387 (11)

* All other ions (above *m/e* 60) of relative intensity $\geqslant 10\%$.

with fragment ions of *m/e* 343 ($[MH - H_2O]^+$) and *m/e* 329 ($[M - CH_2OH]^+$). The FD mass spectrum of aldosterone methaneboronate included the protonated molecular ion, *m/e* 385, as base peak. No peaks attributable to free aldosterone were observed. A prominent ion of *m/e* 285 was present; the absence of a significant isotope peak at *m/e* 284 indicated that fragmentation involved loss of a boron-containing moiety. The ion is attributable to scission adjacent to the boronate ring with loss of a $CH_3BO_2CH_2CO$ fragment from the protonated molecular ion. An analogous fragmentation has been observed in the EI mass spectrum of 18-hydroxydeoxycorticosterone methaneboronate[12].

DISCUSSION

The first attempts at the analysis of aldosterone by GLC were based on its conversion into the 18,21-diacetate[14]. Unfortunately, this derivative readily undergoes transformation during GLC, with loss of one acetate group[15,16]. A more satisfactory derivative is the 18:11,21-acetal, obtainable by acid-catalysed dehydration[1]: this internally protected non-hydroxylic compound is stable to GLC[17], and has the advantages of low molecular weight and reconvertibility into aldosterone. The 3-enol heptafluorobutyrate of this internal acetal has been applied to the determination of aldosterone in plasma and urine[18–20]. Horning and Maume[21] observed the formation of multiple products from trimethylsilylation* of aldosterone: the 3,20-di-O-methyloxime 18,21-diheptafluorobutyrate and the corresponding di-O-methyloxime di-trimethylsilyl ether were more satisfactory, and the doublet peak (apparently from two isomers) of the latter derivative has been applied in the analysis of aldosterone[22]. Aldosterone 21-acetate can be conveniently characterized by GLC of the products obtained by methoximation followed by trimethylsilylation[21,23]. There appear to be no other reported aldosterone derivatives for GLC that retain the intact structure of the hormone. Many of the most practical methods for the determination of aldosterone are based upon its degradation to the 21-norlactone, first applied to GLC by Merits[24], and subsequently used for clinical determinations with electron-capture detection[25,26], or less sensitively with flame-ionization detection[27]: the 3-enol heptafluorobutyrate provides enhanced sensitivity in electron-capture GLC[28–30].

* A di-trimethylsilyl ether has been applied in analysis of urinary aldosterone by GLC–MS[31].

Table III shows the principal properties of the derivatives briefly surveyed above, together with data for the methaneboronate and 1-butaneboronate. The chief advantages of the latter types of derivative are the simplicity and rapidity of their formation under very mild conditions; their stability to GLC in which they afford single, well defined peaks; the abundance of their molecular ions and of the (M − 46) ions under electron impact; and the extreme simplicity of their isobutane CI mass spectra.

TABLE III

DERIVATIVES FOR GAS-PHASE CHARACTERIZATION OF ALDOSTERONE

*Derivative**	*Mol.wt.*	*Retention index*	*Column temp. (°C)*	*Mass spectrometric data (electron impact)*			*References*
				eV	*Base peak*	*M^+ (%)*	
18:11,21-Acetal	342	2975**	225	35	284	60	17, 18
18:11,21-Acetal 3-enol HFB	538			35	538	100	18
3,20-Di-O-methyloxime 18,21-di-TMS ether	562	3173, 3214**	180–200	70	459		21, 22
3,20-Di-O-methyloxime 18-TMS ether 21-acetate	532	3225**	225	70	459	<1	21, 23
Methaneboronate	384	3170***	270	20	338	69	This work
1-Butaneboronate	426	3450***	270	20	380	81	This work
21-Norlactone	328			30	284	60	27
21-Norlactone 3-enol HFB	524						30

* HFB = Heptafluorobutyrate; TMS = trimethylsilyl.
** SE-30.
*** OV-101.

These features make the cyclic alkaneboronates of aldosterone convenient for qualitative characterization of the hormone, although the derivatives share the limitations of other corticosteroid boronates in respect of quantitative applications[8–11]. For the determination of aldosterone and its congeners in biological fluids the elegant methods of Breuer and Siekmann[20] are at present the most satisfactory procedures that have been based on derivatives produced without degradation of the molecular structure of the substrates.

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