CHROM. 5084

COMPARATIVE GAS CHROMATOGRAPHIC STUDIES OT CORTICOSTEROID BORONATES

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(Received October 13th, 1970)

SUMMARY

Cyclic esters formed by reaction of corticosteroids with methyl-, *n*-butyl-, *tert.*-butyl-, cyclohexyl- and phenylboronic acid have been studied, and found to be satisfactory derivatives for analytical gas chromatography. The composition of the esters has been confirmed by mass spectrometry: molecular ions were observed for all but one of the 75 boronates investigated. The problem of achieving stoichiometric reactions for analytical use has been examined.

INTRODUCTION

Previous work[1,2] has shown that corticosteroids can be effectively stabilised for gas chromatography in the form of cyclic esters formed with phenylboronic acid or *n*-butylboronic acid. Analogous derivatives have now been prepared with methylboronic, *tert.*-butylboronic and cyclohexylboronic acid. Methylboronates are notable for the small increment in molecular weight attending their formation: they accordingly have short retention times, and give easily measurable mass spectra. It was envisaged that the bulky *tert.*-butyl group might confer greater stability towards hydrolysis, but such an effect has not been observed: however, the retention times of *tert.*-butylboronates are conveniently short because of the steric properties of the *tert.*-butyl group.

In this paper, gas chromatographic data are surveyed for cyclic boronates of a variety of corticosteroids. The potential applications of boronic acids as reagents for corticosteroids are discussed.

The use of methylboronic and *tert.*-butylboronic acid has been briefly reported in a preliminary note[3].

EXPERIMENTAL

Materials

Steroids were obtained from commercial suppliers. Methylboronic acid was prepared (by Mr. D. S. STEVENSON) by the method described by MCCUSKER *et al.*[4]; *tert.*-butylboronic acid was prepared by a variation of the method of SNYDER *et al.*[5],

in which a fractionating column was used[4] to reduce losses during the isolation of the product. This acid was sensitive to air and was best handled under nitrogen, but it could be stored either dry or in solution in ethyl acetate at 0° without appreciable decomposition. All evaporations were carried out under vacuum or in a stream of nitrogen.

Chromatographic methods

Gas–liquid chromatography (GLC) was carried out with a Perkin-Elmer F-11 chromatograph using 6 ft. glass columns, 4 mm I.D., packed with 1 % OV-17 on Gas-Chrom Q, 100–120 mesh. Gas chromatography–mass spectrometry (GC–MS) was conducted with an LKB 9000 instrument using 3 or 6 ft. glass columns, 4 mm I.D., packed with 1 % OV-17 on Gas-Chrom Q, 100–120 mesh. The ionising voltage was 70 eV, accelerating voltage 3.5 kV, and electron multiplier voltage 3.1 kV. Tabulated mass spectra are to be submitted to the Mass Spectrometry Data Centre (A.W.R.E., Aldermaston, Great Britain). Thin-layer chromatography (TLC) of corticosteroid boronates was carried out using "ChromaR sheet 500" (Mallinckrodt) with chloroform as mobile phase.

Preparation of boronate esters

In the standard procedure, the steroid (10 μmole) and the appropriate boronic acid (10 μmole) were dissolved in ethyl acetate (1 ml) and the mixture was kept at room temperature for 5 min. Under these conditions, 17α,20-diols, 20,21-diols and 17α,20,21-triols were fully converted to boronates as indicated by TLC, and the reaction mixtures gave single peaks when examined by GLC. The products from 17,21-dihydroxy-20-ketones were mainly cyclic boronates as judged by GLC, but small peaks due to loss of the side-chain were present: these were considerably reduced by the addition of a slight excess (10 %) of the boronic acid. A larger excess of reagent could be tolerated where other hydroxyl groups were absent. Yields from the 20,21-ketols were much lower, but could be improved by the use of up to 3 molar equivalents of boronic acid.

Further transformations of cyclic boronates

Trimethylsilyl ethers of 3α,17α,20-trihydroxysteroid 17α,20-boronates and 3α,11β, 20,21-tetrahydroxysteroid 20,21-boronates. The cyclic boronate isolated by evaporation of the solution prepared as above was dissolved in dry pyridine (0.1 ml). Hexamethyldisilazane (HMDS; 0.1 ml) and trimethylchlorosilane (TMCS; trace) were added and the mixture was kept at room temperature for 5 min. The pyridine and reagents were removed by evaporation, and the residue was extracted with cyclohexane (1 ml). Samples (1 μl) of the solution were examined by GLC and GC–MS. The mass spectra of the products confirmed the presence of the trimethylsilyl ether group and the boronate ring. Strong peaks at *m/e* 73 and 75 have been disregarded in assigning base peak which for the purpose of the present paper are defined as the most intense peaks above *m/e* 80.

The following base peaks, molecular ions (intensities as % of base peak, in parentheses), and retention indices* were observed:

* Retention indices cited in the experimental section were determined on columns with OV-17 stationary phase, by programmed temperature gas chromatography, from 230° at 2°/min.

5β-pregnane-3α,17α,20α-triol 17,20-methylboronate 3-trimethylsilyl ether, base peak *m/e* 215, M^+ 432 (15 %); $I = 2840$;

5β-pregnane-3α,17α,20β-triol 17,20-methylboronate 3-trimethylsilyl ether, base peak *m/e* 215, M^+ 432 (11 %), $I = 2805$;

5β-pregnane-3α,17α,20β-triol 17,20-*tert.*-butylboronate 3-trimethylsilyl ether, base peak *m/e* 215, M^+ 474 (14 %); $I = 2845$;

5β-pregnane-3α,11β,20α,21-tetrol 20,21-*tert.*-butylboronate 3-trimethylsilyl ether*, base peak *m/e* 382, M^+ 490 (1.5 %); $I = 3465$;

5β-pregnane-3α,11β,20β,21-tetrol 20,21-*tert.*-butylboronate 3-trimethylsilyl ether*, base peak *m/e* 382, M^+ 490 (1.5 %); $I = 3400$.

Acetates of diol and triol boronates. The hydroxysteroid cyclic boronate (10 μmole) was dissolved in dry pyridine (0.5 ml) and a large excess (0.1 ml) of acetic anhydride was added. The mixture was left overnight at room temperature, and the reagents were removed by evaporation. The product was taken up in ethyl acetate (1 ml) and samples (1 μl) were examined by GLC. The presence of the acetate and the boronate group was confirmed by mass spectrometry. The following base peaks, molecular ions and retention indices were observed:

5β-pregnane-3α,17α,20α-triol 17,20-methylboronate 3-acetate, base peak, *m/e* 342, M^+ 402 (5 %); $I = 3085$;

17α,20β,21-trihydroxypregn-4-en-3-one *n*-butylboronate monoacetate[2], base peak *m/e* 43, M^+ 456 (20 %) $I = 3925$.

20-O-Methyloxime 17,21-n-butylboronate of 3α,17α,21-trihydroxy-5β-pregnan-20-one ("Tetrahydro S"). The formation of 3α,17α,21-trihydroxy-5β-pregnan-20-one 17α,21-*n*-butylboronate 20-O-methyloxime from 3α,17α,21-trihydroxy-5β-pregnan-20-one 17α,21-*n*-butylboronate has been described previously[2]. This compound has now been prepared from 3α,17α,21-trihydroxy-5β-pregnan-20-one 20-O-methyloxime. A mixture of 3α,17α,21-trihydroxy-5β-pregnan-20-one (3.4 mg) and O-methylhydroxylamine hydrochloride (5 mg) in dry pyridine (1.0 ml) was kept at 60° overnight, cooled and diluted with water. The product was extracted from the mixture with ethyl acetate, washed with water and dried with 5 Å molecular sieve. The solvents were evaporated, and the residue was dissolved in ethyl acetate (1.0 ml) together with *n*-butylboronic acid. The product was characterised by GC–MS: base peak *m/e* 43, M^+ 445 (34 %); $I = 3455$.

3α,17α,21-Trihydroxy-5β-pregnan-20-one 17,21-n-butylboronate 20-O-methyloxime 3-trimethylsilyl ether. The preparation of 3α,17α,21-trihydroxy-5β-pregnan-20-one 17α,21-*n*-butylboronate 20-O-methyloxime 3α-trimethylsilyl ether by the action of N,O-bistrimethylsilylacetamide (BSA) on the *n*-butylboronate O-methyloxime has been described previously[2]. The ether has also been prepared using N-trimethylsilyl-diethylamine in place of BSA. This reaction sequence gave similar results when an excess (3 molar proportions altogether) of *n*-butylboronic acid was used in the first step. Base peak *m/e* 397, M^+ 517 (25 %); $I = 3300$.

Relative stability of various cyclic boronates towards solvolysis

Examples have been given of the stability of *n*-butylboronates derived from

* Mass spectral data for these compounds were recorded with an LKB 9000 instrument in the Institute for Lipid Research, Baylor College of Medicine, Houston, Texas, U.S.A. We thank Dr. M. G. HORNING for providing this facility.

17,20-diols[1], 20,21-diols and 17,20,21-triols[2], in the presence of reagents for acetylation or trimethylsilylation. Selective removal of the boronate grouping by solvolysis with propane-1,3-diol has also been achieved. We have compared the effect of propane-1,3-diol on a series of boronates.

Propane-1,3-diol (20 μg) in ethyl acetate (20 μl) was added to the cyclic boronate in ethyl acetate (20 μl of solution prepared by the standard procedure). Samples (2 μl) were examined at intervals by GLC, with results cited in Table I.

TABLE I

EFFECT OF PROPANE 1,3-DIOL ON CORTICOSTEROID BORONATES IN ETHYL ACETATE SOLUTION

Degree of hydrolysis: (A) Hydrolysis complete in samples taken after 1 min; (B) hydrolysis complete in samples taken after 12 min; (C) partial hydrolysis after 1 day; (D) little or no hydrolysis after 1 day.

Steroid	*Degree of hydrolysis of boronates*		
	Methyl	*tert.-Butyl*	*n-Butyl*
17α,20α-Diol	C	D	C
17α,20β-Diol	D	D	D
20,21-Diol	C	C	C
17α,20α,21-Triol	D	D	D
17α,21-Diol-20-one (3 examples)	A	B	B
20,21-Ketol	A	A	A

Formation of boronates in the presence of excess reagent

Effect of solvent

Methylboronic acid (0.6 mg, 10 μmole) in ethyl acetate (0.1 ml) was added to a solution of the steroid (10 μmole) in ethyl acetate (1 ml). After 5 min a sample of this solution (1 μl) was examined by GLC. Additional methylboronic acid (1.0 mg) was added to the solution, and further samples (1 μl) were examined by GLC. This was repeated with *n*-butylboronic acid and *tert.*-butylboronic acid in each of the following solvents: pyridine, ether, hexane, cyclohexane, dimethylformamide, dioxan and acetone. With steroids containing free hydroxyl groups at positions 3, 11 or 20, marked reduction in peak height and increased tailing were invariably observed in the presence of an excess of the boronic acid.

Derivative formation

TMS ethers. The cyclic boronate, prepared in the presence of an excess of the boronic acid in pyridine, was treated with HMDS and TMCS as described above. The product was extracted with cyclohexane and samples (1 μl) were examined by GLC. Satisfactory peaks corresponding to boronate trimethylsilyl ethers were obtained from 17,20-diols, 20,21-diols and 17,20,21-triols, but 17,21-dihydroxy-20-ketones and 20,21-ketols gave mixtures of products with loss of the boronate grouping. Treatment with BSA instead of HMDS and TMCS gave similar results.

Acetates. Preparation of acetates was successful for boronates of side-chain diols and triols, but again hydrolysis of the boronate ester occurred with the 17,21-dihydroxy-20-oxosteroid boronates and 20,21-ketols.

Attempted displacement of acyclic boronates by reagents forming cyclic boronates

2-Hydroxycyclohexanone or ephedrine[2] was added in excess to a solution of

3α,17α,21-trihydroxy-5β-pregnan-20-one 17α,21-*n*-butylboronate prepared in the presence of an excess of *n*-butylboronic acid, and samples (1 μl) of the mixture were examined by GLC. Again partial hydrolysis of the 17,21-dihydroxy-20-oxosteroid cyclic boronate occurred. Similar results were obtained with 3β, 21-dihydroxypregn-5-en-20-one *n*-butylboronate.

RESULTS AND DISCUSSION

The five boronic acids studied were generally similar in their reactions with the corticosteroids. However, there were distinct differences in the reactivity of the several types of corticosteroid, and in the properties of the derived esters, as already observed with *n*-butylboronates[1]. 17,20-Diols, 20,21-diols, and especially 17,20,21-triols yielded stable esters which resisted hydrolysis and could be submitted to thin-layer chromatography. 17,21-Dihydroxy-20-ketones gave esters which were easily solvolysed by propane-1,3-diol: in this respect *tert.*-butylboronates were only marginally more stable than methylboronates. The reaction times for ester formation were generally short; up to 5 min at room temperature was sufficient for the formation of all the boronates studied, and most reactions appeared to be complete within 1 min. Under these conditions, single peaks were produced by the boronates of the 17,20-diols, 20,21-diols, and 17,20,21-triols, on admixture of equimolar proportions of the steroid and boronic acid. A slight excess of the boronic acid was needed to produce a single peak with the 17α,21-dihydroxy-20-ketones; with equimolar proportions, a small peak due to the 17-oxosteroid (produced by thermal decomposition of the unreacted steroid)[6] was always present (Fig. 1). The formation of cyclic boronates of 20,21-ketols was incomplete even when several molar proportions of reagent were present: moreover, the use of an excess of boronic acid was impracticable for corticosteroids containing hydroxyl groups additional to those in the side-chain.

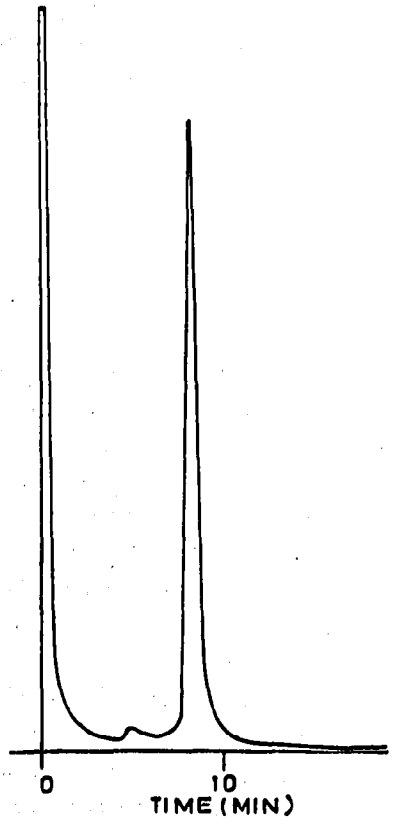
Gas chromatographic properties

The derivatives reported here gave satisfactory gas chromatographic peaks, with stabilisation of the corticosteroid side-chains, except for the ketol boronates, which showed evidence of partial decomposition. Retention indices are summarised in Table II. The comparatively short retention times of methyl- and *tert.*-butylboronates are evident. They are further illustrated in Fig. 2, which shows the separation of various boronates of cortisone, and in Fig. 3, which depicts the separation of methylboronates of a range of corticosteroids.

The cyclic boronates of 17β-side-chain diols and triols were sufficiently stable for the formation of derivatives of unreacted hydroxyl and carbonyl groups to be achieved. Solvolysis of the boronate ring was, however, observed with many of the cyclic boronates of the 17α,21-dihydroxy-20-ketones and 20,21-ketols.

Boronates of 17,20-diols, 20,21-diols and 17,20,21-triols were stable towards silylating reagents, and where the steroid boronate still contained free hydroxyl groups, the fully derivatised compound could be obtained. Consequent improvement in GLC peak shape, compared with that of the hydroxysteroid boronate, was frequently observed. The mass spectra of these compounds confirmed the presence of both the boronate ring and the trimethylsilyl groups.

Acetates of hydroxysteroid boronates could also be prepared by the use of



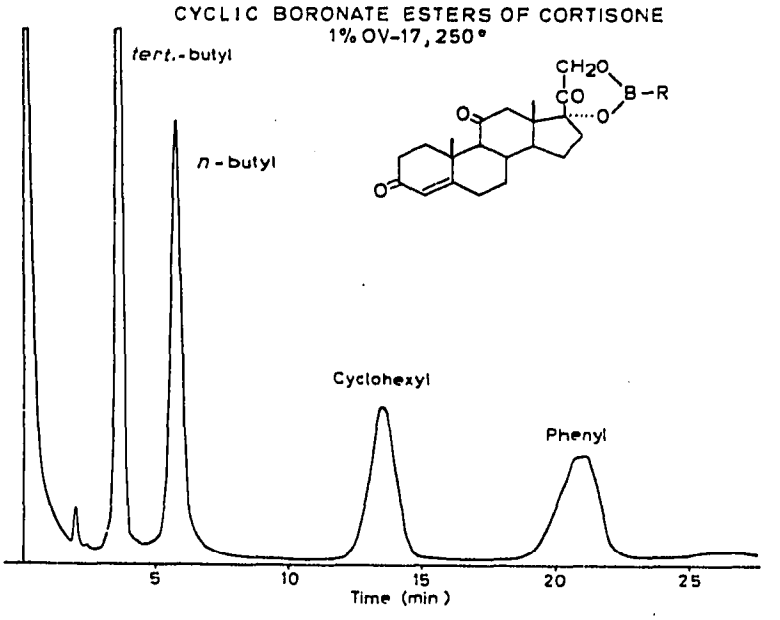


Fig. 1. Isothermal (250°) trace of 3α,17α,21-trihydroxy-5β-pregnan-20-one 17,21-methylboronate prepared by admixture of equimolar proportions of the steroid and methylboronic acid. The sample injected (in 1 μl of ethyl acetate) represents 0.01 μmole of steroid. The small peak preceding the main peak is due to 3α-hydroxy-5β-androstan-17-one.

Fig. 2. Isothermal (250°) separation of the *tert.*-butyl-, *n*-butyl-, cyclohexyl-, and phenylboronates of cortisone on 1% OV-17 (6 ft.). The sample injected (in 4 μl of ethyl acetate) represents 0.01 μmole of each steroid. The methylboronate has a retention index very similar to that of the *tert.*-butylboronate and is not separated under these conditions.

acetic anhydride in pyridine, but neither trimethylsilyl ethers nor acetates of the 17α,21-dihydroxy-20-ketone or 20,21-ketol boronates could be obtained without considerable solvolysis of the boronate rings.

The carbonyl group of cyclic boronates derived from 17,21-dihydroxy-20-oxo-steroids has been shown[2] to form an O-methyloxime derivative without destruction of the boronate ester. We have now shown that the resulting methyloxime boronate can react further with silylating reagents, with retention of the boronate ring, in contrast to the compounds where the 20-oxo group is not protected. Somewhat improved yields were noted when the methyloxime group was introduced before the boronate. Of the reagents investigated for the silylation of these derivatives, BSA and N-trimethylsilyl-diethylamine appeared to be the most satisfactory.

Mass spectrometric properties

The most valuable feature of the mass spectra of corticosteroid boronates is the general prominence of molecular ions (Table II), or of the ions of *m/e* (M-18) where free hydroxyl groups were present. In most cases, fragment ions containing

TABLE II

GAS CHROMATOGRAPHIC AND MASS SPECTROMETRIC DATA FOR CORTICOSTEROID BORONATES

Abbreviated nomenclature: P = pregnane; ^{4}P = pregn-4-ene; ^{5}P = pregn-5-ene.

	Steroid	*Boronate type*	*Retention index*	*Mass spectrometric data*			
				M^+		$(M-18)^+$	
				m/e	*% of base peak*	*m/e*	*% of base peak*
1	5β-P-3α,17α,20α-triol	Methyl	3010[a]	360	13	342	100
		tert.-Butyl	3050[a]	402	18	384	100
		n-Butyl	3265	402	8	384	53
		Cyclohexyl	3590[b]	428	11	410	100
		Phenyl	3775[b]	422	26	404	100
2	5β-P-3α,17α,20β-triol	Methyl	2970[a]	360	10	342	100
		tert.-Butyl	3010[a]	402	12	384	100
		n-Butyl	3265	402	5	384	56
		Phenyl	3775[b]	(not recorded)			
3	5β-P-3α,11β,17α,20β-tetrol	Methyl	3255[b]	376	1	358	48
		tert.-Butyl	3270[b]	418	1	400	46
		n-Butyl	3480	418	2	400	48
4	^{4}P-20β,21-diol-3-one	Methyl	3380[b]	356	18	338	1
		tert.-Butyl	3520[b]	398	12	380	1
		n-Butyl	3680	398	6	380	1
		Cyclohexyl	4030[b]	424	56	406	4
		Phenyl	4330[b]	418	10	400	1
5	5α-P-3α,11β,20α,21-tetrol	Methyl	3460[b]	376	4	358	70
		tert.-Butyl	3600[b]	418	5	400	72
6	5α-P-3α,11β,20β,21-tetrol	Methyl	3470[b]	376	2	358	59
		tert.-Butyl	3565[a]	418	1	400	63
7	^{4}P-17α,20α,21-triol-3-one	Methyl	3595[b]	372	30	354	11
		tert.-Butyl	3650[b]	414	23	396	10
		n-Butyl		414	35	396	13
		Cyclohexyl	4205[b]	(not recorded)			
8	^{4}P-17α,20β,21-triol-3-one	Methyl	3620[b]	372	26	354	8
		tert.-Butyl	3650[b]	414	25	396	7
		n-Butyl	3835[b]	414	100	396	23
		Cyclohexyl	4080[b]	440	48	422	9
		Phenyl	4345[b]	434	40	416	5
9	5α-P-3β,11β,17α,20β,21-pentol	Methyl	3650[b]	392	1	374	12
		tert.-Butyl	3715[b]	(434)	0	416	16
10	5β-P-3α,17α,20α,21-tetrol-11-one	Methyl	3490[a]	390	25	372	56
		tert.-Butyl	3570[a]	432	30	414	80
		n-Butyl	3800	432	95	414	100
11	^{4}P-17α,21-diol-3,20-dione	Methyl	3360[b]	370	73	352	2
		tert.-Butyl	3400[b]	412	71	394	2
		n-Butyl	3580	412	74	394	2
		Cyclohexyl	3965[b]	438	34	420	2
		Phenyl	4120[b]	432	41	414	1
12	5β-P-17α,21-diol-3,20-dione	Methyl	3175[a]	372	45	354	4
		tert.-Butyl	3215[a]	414	25	396	3
		n-Butyl	3400	414	46	396	2
		Cyclohexyl	3710[b]	440	16	422	2
		Phenyl	3885[b]	434	62	416	2

(*Continued on page 200*)

TABLE II (*continued*)

	Steroid	*Boronate type*	*Retention index*	*Mass spectrometric data*			
				M^+		$(M-18)^+$	
				m/e	*% of base peak*	*m/e*	*% of base peak*
13	5β-P-3α,17α,21-triol-20-one	Methyl	3180[b]	374	4	356	27
		tert.-Butyl	3220[b]	416	4	398	25
		n-Butyl	3345	416	8	398	39
14	[4]P-17α,21-diol-3,11,20-trione	Methyl	3450[b]	384	73	366	2
		tert.-Butyl	3490[b]	426	73	408	2
		n-Butyl	3660	426	47	(408)	0
		Cyclohexyl	4065[b]	452	50	434	2
		Phenyl	4230[b]	446	55	428	1
15	5β-P-17α,21-diol-3,11,20-trione	Methyl	3300[b]	386	35	368	1
		tert.-Butyl	3305[b]	428	18	410	1
		n-Butyl	3600	428	21	410	1
		Cyclohexyl	3825[b]	454	11	436	1
		Phenyl	3990[b]	448	36	430	1
16	5β-P-3α,17α,21-triol-11,20-dione	Methyl	3270[b]	388	10	370	48
		tert.-Butyl	3270[a]	430	11	412	49
		n-Butyl	3465	430	13	412	42
17	[4]P-11β,17α,21-triol-3,20-dione	Methyl	3630[b]	386	31	368	38
		tert.-Butyl	3660[b]	428	35	410	39
		n-Butyl	3890[b]	428	43	410	47
18	5β-P-3α,11β,17α,21-tetrol-20-one	Methyl	3360[a]	390	1	372	15
		tert.-Butyl	3400[a]	432	1	414	12
		n-Butyl	3605	432	5	414	10
19	[4]P-21-ol-3,20-dione	Methyl	3310[b]	354	19	(336)	0
		tert.-Butyl	3480[b]	396	37	(378)	0
20	5α-P-21-ol-3,20-dione	Methyl	3220[b]	356	14	(338)	0
		tert.-Butyl	3380[b]	398	24	(380)	0
21	[5]P-3β,21-diol-20-one	Methyl	3150[b]	356	25	338	8
		tert.-Butyl	3325	398	22	380	8
		n-Butyl	3470[b]	398	66	380	12
		Phenyl	3850[b]	418	10	400	12

[a] 240° [b] 250° } otherwise, measured by programmed temperature gas chromatography, 200–280°.

boron were produced which were partially characteristic of the corticosteroid side-chain involved. However, except for the boronates of 17,20-diols and 20,21-ketols, such ions were not usually among the most abundant. Fig. 4 shows typical mass spectra, recorded for the methylboronate and *tert.*-butylboronate of 5β-dihydro-S (17α,21-dihydroxy-5β-pregnane-3,20-dione). Both derivatives give the same base peak, resulting from scission of ring D. The *tert.*-butylboronate is notable for the strong peak arising through loss of the *tert.*-butyl group. Further details of the observed mass spectral fragmentations will be discussed in a separate communication.

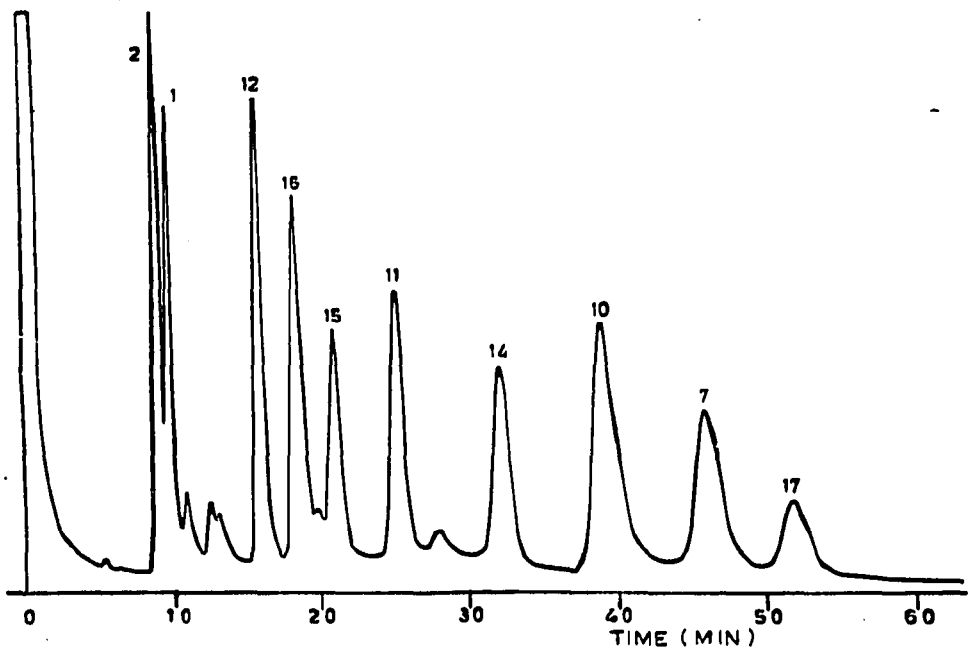


Fig. 3. Isothermal (230°) separation of the methylboronates of ten corticosteroids (approx. 1 μg of each) on 1% OV-17 (12 ft.). The numbers of the peaks refer to the structures given in Table II. The three small peaks between peaks 1 and 12 are due to 17-oxosteroids produced by thermal decomposition of unreacted 17α,21-dihydroxy-20-oxosteroids (see RESULTS AND DISCUSSION), and the peak between Nos. 11 and 14 was caused by an unidentified impurity present in No. 17.

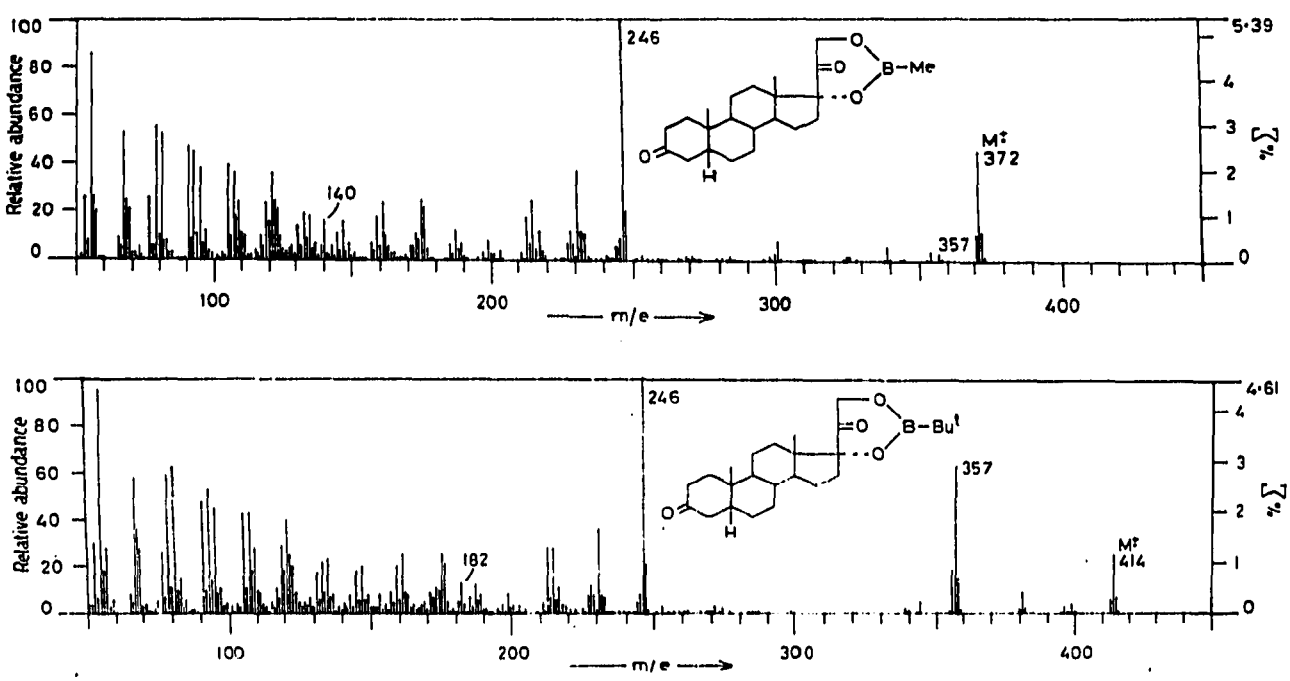


Fig. 4. Mass spectra of the methyl- and *tert.*-butylboronate of 5β-dihydro S (17α,21-dihydroxy-5β-pregnane-3,20-dione).

Structure of cyclic boronates formed from 17,20,21-triols

The boronates formed by 17,20,21-triols have been tentatively regarded as six-membered esters involving the 17- and 21-hydroxyl groups[2]. Such a structure is similar to that established for the two boronate rings in galactitol bis-phenylboronate[7]. On the other hand, a five-membered structure has been proposed[7–9] for glycerol phenylboronate. We have not yet been able to assign firmly the structures of the boronates of 17,20,21-triols. The dioxaborinane form is consistent with the smooth oxidation of the *tert.*-butylboronates of both 17α,20α,21- and 17α,20β,21-trihydroxypregn-4-en-3-one with dimethylsulphoxide–acetic anhydride[10,11] to the corresponding ester of 17α,21-dihydroxypregn-4-ene-3,20-dione; this was identified by its retention index and mass spectrum. (Attempts to effect the reverse reaction, by reduction of the steroidal dihydroxyacetone boronate with sodium borohydride, were unsuccessful, because of solvolysis of the boronate ester.) The stability of the triol boronates is also most easily understood in terms of a six-membered formulation in which the 20-oxygen atom co-ordinates with the boron atom[7]. Certain of the mass spectrometric data appear to indicate a five-membered ester structure, but the possibility that this arises during fragmentation, perhaps via the co-ordinated structure proposed, is considered reasonable. We are continuing investigations of this structural problem.

Structure of cyclic boronates derived from 20,21-ketols

These are presumed to involve the enol form of the 20-oxo group, as shown by a marked reduction of the carbonyl absorption in the infrared: the remaining carbonyl peak was produced by unreacted ketol. The proton magnetic resonance spectrum of a solution prepared from 21-hydroxy-5α-pregnane-3,20-dione and 3 molar equivalents of methylboronic acid showed no signal at the position expected for an olefinic proton, indicating a Δ^{17} rather than Δ^{20} structure for these derivatives.

Analytical formation of boronate esters in the presence of excess of reagent

In order to apply boronic acids for the gas-phase characterisation of natural steroids, it would be convenient, even if not essential, to use the reagents in excess. Unreacted boronic acids are readily eluted during gas chromatography, in the form of their trimeric anhydrides (boroxines), so that the excess of reagent presents no direct problem. The reagent may, however, interact with isolated hydroxyl groups, yielding esters of low volatility which impair the gas chromatographic analysis. We have examined two approaches to this problem.

Improvement in the selectivity of reaction. It was envisaged that a suitable choice of reaction medium, and selection of the most effective boronic acid, might permit satisfactory formation of the cyclic ester without concomitant reaction of isolated hydroxyl groups. Variation of the solvent, however, produced little or no observable effect on the reaction as far as the formation of interfering acyclic boronates was concerned. Ethyl acetate, cyclohexane, hexane, ether, dioxane, N,N-dimethylformamide, acetone and pyridine were investigated, but drastic reductions in peak heights were observed in all cases as soon as the boronic acid was added in excess. Moreover, this effect was observed for all the boronic acids studied.

Use of a second reagent. The aim here was to remove the relatively unstable acyclic boronate groups without affecting the cyclic esters. Various silylating and

acylating reagents were found to displace the interfering acyclic boronate groups, restoring well-shaped GLC peaks for cyclic boronates formed from 17,20-diols, 20,21-diols and 17,20,21-triols. This is illustrated in Fig. 5. Fig. 5a is a gas chromatographic trace of 5β-pregnane-3α,17α,20β-diol 17,20-*n*-butylboronate, prepared by mixing the reagents in equimolar proportions. An aliquot taken after addition of more *n*-butylboronic acid shows a sharp decrease in peak height, with substantial tailing (Fig. 5b). Addition of HMDS–TMCS leads to trimethylsilylation of the 3α-hydroxyl group with displacement of the interfering boronic acid, and restoration of a satisfactory peak (Fig. 5c), more symmetrical than that of the boronate of the free triol (Fig. 5a).

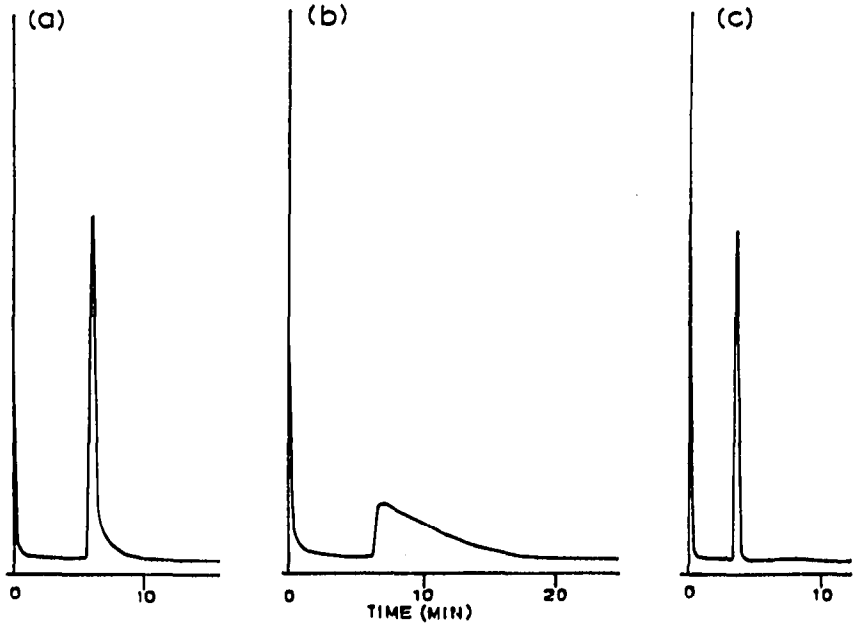


Fig. 5. (a) GLC trace of 5β-pregnane-3α,17α,20β-triol 17,20-*n*-butylboronate (0.01 μmole) obtained on 1% OV-17 at 230°. (b) The effect of adding an excess of *n*-butylboronic acid to (a). (c) The effect of trimethylsilylation of the 3α-hydroxyl group: this displaces the acyclic boronate group present in (b) without affecting the cyclic ester (17, 20). The aliquot used represents 0.005 μmole of steroid.

Use of these reagents with dihydroxyacetone or ketol boronates apparently caused solvolysis of the boronate ring, unless, in the case of the dihydroxyacetone, the 20-oxo fraction was first protected (*e.g.* as its O-methyloxime). Such a three-stage reaction is clearly inconvenient for analytical use.

We have also examined the effect of adding an excess of a reagent of low molecular weight, designed to form a cyclic boronate less stable than the desired steroidal boronate, but more stable than the interfering acyclic boronate. 2-Hydroxycyclohexanone and ephedrine[2] were investigated as possible reagents, but were ineffective. Their use also resulted in cleavage of the 17α,21-dihydroxy-20-ketosteroid and 20,21-ketol boronates.

CONCLUSIONS

Although boronic acids show promise as reagents for the gas-phase characterisation of corticosteroids, their use in quantities in excess of the amount necessary

for complete formation of the cyclic ester still presents a problem where isolated hydroxyl groups are present. This problem may be satisfactorily overcome for cyclic boronates of 17,20-diols, 20,21-diols and 17,20,21-triols by the additional formation of trimethylsilyl or acetyl derivatives of these groups, resulting in compounds showing excellent GLC properties. Boronates of 17α,21-dihydroxy-20-oxosteroids and of 20,21-ketols are too unstable to be treated in this way. Accordingly, boronates are convenient for characterisation of 17α,21-dihydroxy-20-oxosteroids only where the latter have been isolated in almost pure form and in approximately known amount. The low molecular weight increment accompanying the formation of methylboronates makes these esters particularly suitable for the characterisation of polyhydroxysteroids and other compounds of high molecular weight containing suitably-disposed hydroxyl groups.

ACKNOWLEDGEMENTS

We are indebted to the Medical Research Council for a research grant. The LKB 9000 gas chromatograph–mass spectrometer was provided by a grant (No. B/SR/2398) from the Science Research Council. We thank Professor R. A. RAPHAEL, F.R.S. for his encouragement, Dr. I. SANGSTER for providing some of the data in Table II, and Mrs. M. KIRKLAND for experimental assistance.

REFERENCES

1 C. J. W. BROOKS AND J. WATSON, in C. L. A. HARBOURN (Editor), *Gas Chromatography 1968, Proc. 7th Intern. Symp., Copenhagen*, Institute of Petroleum, London, 1969, p. 129.
2 G. M. ANTHONY, C. J. W. BROOKS, I. MACLEAN AND I. SANGSTER, *J. Chromatog. Sci.*, 7 (1969) 623.
3 C. J. W. BROOKS AND D. J. HARVEY, *Biochem. J.*, 114 (1969) 15P.
4 P. A. MCCUSKER, E. C. ASHBY AND H. S. MAKOWSKI, *J. Amer. Chem. Soc.*, 79 (1957) 5179.
5 H. R. SNYDER, J. A. KUCK AND J. R. JOHNSON, *J. Amer. Chem. Soc.*, 60 (1938) 105.
6 W. J. A. VANDENHEUVEL AND E. C. HORNING, *Biochem. Biophys. Res. Comm.*, 3 (1960) 356.
7 E. J. BOURNE, E. M. LEES AND H. WEIGEL, *J. Chem. Soc.*, (1965) 3798.
8 R. A. BOWIE AND O. C. MUSGRAVE, *J. Chem. Soc.*, (1963) 3945.
9 B. SERAFIN AND G. MILKOWSKA, *Roczniki Chem.*, 38 (1964) 897.
10 B. LINDBERG AND K. N. SLESSOR, *Acta Chem. Scand.*, 21 (1967) 910.
11 J. S. BRIMACOMBE, A. HUSAIN, F. HUNEDY AND M. STACEY, *Advan. Chem. Ser.*, No. 74 (1968) 56.