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N-Butylboronate derivatives of the F prostaglandins

Resolution of prostaglandins of the E and F series by gas–liquid chromatography

Several reports have appeared on the separation of prostaglandins according to the degree of unsaturation by gas–liquid chromatography (GLC)[1]. The F compounds have been successfully analyzed as their TMSi derivatives[2,3]. The E prostaglandins have been converted into the B derivatives by alkali treatment and analyzed as the methyl esters or acetylated methyl esters[4,5]. The E prostaglandins have been found suitable for GLC as their methoxime trimethylsilyl derivatives (MO-TMSi)[6]. Recently, resolution of the E and F prostaglandins in a mixture was achieved as the MO-TMSi and TMSi derivatives, respectively, on columns of OV-17 and OV-22 but not SE-30 (ref. 7). All the methods mentioned above except the last fail to resolve a mixture of E and F prostaglandins and therefore must be preceded by a chromatographic class separation (thin-layer or column chromatography) prior to analysis by GLC. It was shown recently that β-dihydroxy groups in steroids could be reacted with *n*-butylboronic acid in a variety of solvents quickly and quantitatively to produce cyclic *n*-butylboronic (NBB) esters[8]. We have found this reagent suitable for derivatization of the F_α prostaglandins. The selective, quick and quantitative formation of the cyclic NBB-TMSi derivatives of the F_α prostaglandins together with their GLC properties relative to the MO-TMSi derivatives of the E prostaglandins is presented in this report. This method can be used for the direct GLC analysis of four parent prostaglandins, *viz.* E_1, E_2, $F_{1\alpha}$, $F_{2\alpha}$ in a mixture without prior class separation.

Methods

Materials. Prostaglandins E_1, E_2, $F_{1\alpha}$, $F_{2\alpha}$ were generously supplied by Dr. J. E. PIKE, The Upjohn Company, Kalamazoo. Gas chromatography was performed on an F & M Model 402 gas chromatograph fitted with a 4-ft. glass column packed with 3 % SE-30 ultraphase on Chromosorb W (Applied Science Laboratories) maintained at 220° and equipped with a flame ionization detector. Mass spectra were recorded at 70 eV on an LKB 9000 gas chromatograph–mass spectrometer fitted with the same column support (260°) as the gas chromatographic analyses. Tri-Sil Z and methoxime (MOX) reagents were purchased from Pierce Chemical Company. Dimethoxypropane was purchased from Aldrich Chemical Co., Milwaukee, and *n*-butylboronic acid was obtained from Applied Sciences Laboratories.

Derivatives for gas chromatography. A mixture of 30 μg each of prostaglandins E_1, E_2, $F_{1\alpha}$, and $F_{2\alpha}$ was prepared in a microtube (total volume 100 μl) fitted with a rubber septum. MOX reagent (20 μl) was added and the solution was left at room temperature overnight. This converted the E prostaglandins to the 9-methoxime derivatives without altering the F prostaglandins. The solvent was removed by a fine stream of nitrogen and 30 μl of a solution of *n*-butylboronic acid (2.5 mg) in dimethoxypropane (1 ml) was added. This converted the F prostaglandins to the 9,11-*n*-butylboronate derivatives without altering the methoxime derivatives of the E prostaglandins. After 2 min at 60° the solvent was evaporated and 20 μl of Tri-Sil Z

was added to convert the remaining functional groups of the prostaglandins, *viz.* the 15-hydroxyl and the carboxyl at C-1 to the trimethylsilyl derivatives. After 5 min at 60° the samples were placed in a desiccator until analysis could be performed by gas chromatography. Several control experiments were carried out with individual prostaglandins to test whether the F prostaglandins were degraded in the presence of MOX reagent or the E prostaglandins as MO derivatives were degraded in the presence of the NBB reagent.

Solvolysis of $PGF_{1\alpha}$-NBB-TMSi. Experiments were carried out to check the stability of $PGF_{1\alpha}$-NBB-TMSi. $PGF_{1\alpha}$ (30 μg) was converted to the NBB-TMSi derivative as described above. In one experiment 35 μg of *n*-butylboronic acid was used, in another 70 μg was used. The samples were placed in a desiccator at room temperature and aliquots at various time intervals were injected into the gas chromatograph and the areas under the peaks due to $PGF_{1\alpha}$-NBB-TMSi and $PGF_{1\alpha}$-TMSi calculated (see Table II).

Results and discussion

The resolution of prostaglandins E_1 and E_2 as methoxime trimethylsilyl derivatives and prostaglandins $F_{1\alpha}$ and $F_{2\alpha}$ as *n*-butylboronate trimethylsilyl derivatives is shown in Fig. 1. A very good separation between the E and F classes was obtained. Good resolution within each class according to degree of unsaturation was also observed under the operating conditions used. As has already been reported[6,7], the methoxime derivatives of the E prostaglandins produce two peaks with each compound, a minor and a major peak due to *syn–anti* isomerism about the methoxime group.

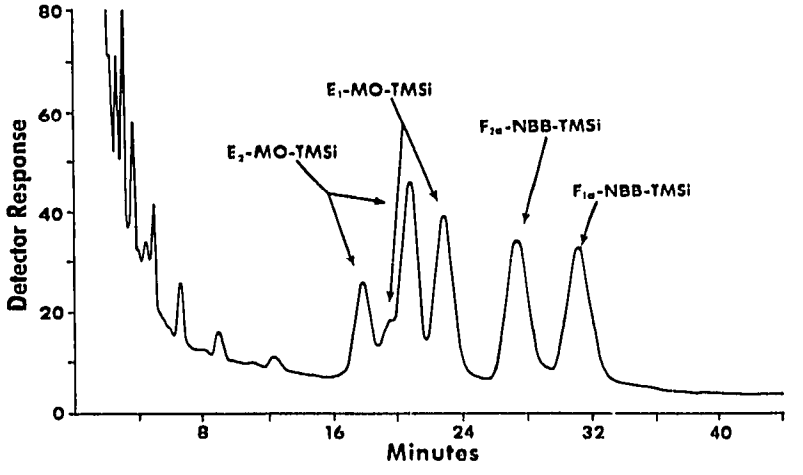

Fig. 1. Gas–liquid chromatogram of a mixture of four prostaglandins derivatized in the order MOX, NBB, TMSi (see *Methods* for details).

The NBB-TMSi derivatives were excellent derivatives for the F_α prostaglandins because of the rapidity and ease of formation at room temperature. One peak was observed for each compound. They possess much longer retention times relative to the MO-TMSi derivatives of the E prostaglandins (Table I) and can be useful on this basis to resolve a mixture of the two classes of prostaglandins directly without prior class-separating chromatographic operations. Furthermore, NBB derivatization is

TABLE I

RETENTION TIMES OF SOME PROSTAGLANDIN DERIVATIVES

Compound[a]	*C value*[b]
$PGF_{1\alpha}$-NBB-TMSi	26.90
$PGF_{2\alpha}$-NBB-TMSi	26.48
$PGF_{1\alpha}$-TMSi	25.48
$PGF_{1\beta}$-TMSi	24.88
$PGF_{2\alpha}$-TMSi	24.90
PGE_1-MO-TMSi	25.72 (25.24)[c]
PGE_2-MO-TMSi	25.48 (24.90)[c]
PGB_1-MO-TMSi	25.40

[a] NBB = *n*-butylboronate; TMSi = trimethylsilyl; MO = methoxime; PG = prostaglandin.
[b] Retention times are expressed as C values (see ref. 9).
[c] Minor isomer observed.

specific to the *cis* configuration of the 9,11-hydroxyl groups since the F_β prostaglandins do not react with this reagent.

A disadvantage of the NBB-TMSi is their ease of solvolysis. Table II shows the effect of storage on a sample of $PGF_{1\alpha}$-NBB-TMSi in a solution of Tri-Sil Z left at room temperature in a septum-covered vial in a desiccator. Solvolysis of $PGF_{1\alpha}$-NBB-TMSi with subsequent appearance of $PGF_{1\alpha}$-TMSi can lead to erroneous results in quantitation of a mixture of prostaglandins unless proper precautions are taken to minimize solvolysis, since the retention times of the PGF-TMSi compounds resulting from the solvolysis of the PGF-NBB-TMSi are very similar to the MO-TMSi derivatives of the E prostaglandins (Table I). As shown in Table II, appearance of $PGF_{1\alpha}$-TMSi is fairly slow and does not start before 3 h at room temperature. Therefore analysis should be carried out as quickly as possible to minimize these errors.

Since water is one of the products resulting from the formation of the NBB derivatives, it is important to remove this from the reaction mixture to avoid rapid hydrolysis of the NBB derivatives. Use is made here of dimethoxypropane as solvent for the derivatization. In the presence of water dimethoxypropane is hydrolysed to

TABLE II

STABILITY OF $PGF_{1\alpha}$-NBB-TMSi[a]

Time (h)[b]	*Composition (%)*[c]		*Composition (%)*[c]	
	$PGF_{1\alpha}$-NBB-TMSi[d]	*$PGF_{1\alpha}$-TMSi*	*$PGF_{1\alpha}$-NBB-TMSi*[e]	*$PGF_{1\alpha}$-TMSi*
0–2	100	0	100	0
5	95	5	96	4
24	33	67	64	36

[a] PG = prostaglandin; NBB = *n*-butylboronate; TMSi = trimethylsilyl.
[b] Refers to period after formation of derivative in a solution of Tri-Sil Z (see *Methods*).
[c] Composition is determined by GLC.
[d] Concentration of *n*-butylboronic acid 35 μg/30 μg $PGF_{1\alpha}$.
[e] Concentration of *n*-butylboronic acid 70 μg/30 μg $PGF_{1\alpha}$.

acetone and methanol which are removed with nitrogen when the reaction is terminated.

The mass spectra of the *n*-butylboronate trimethylsilyl derivatives of $PGF_{1\alpha}$ and $PGF_{2\alpha}$ are shown in Fig. 2. Very simple fragmentation patterns are observed consisting mainly of fragments of high intensity involving loss of C_5H_{11} (M-71), $TMSiOCH(CH_2)_4CH_3$ (M-173) and loss of TMSiOH (90) from these fragments. The base peak above *m/e* 90 in the $PGF_{1\alpha}$ derivative (Fig. 2a) results from the loss of 71 from the molecular ion, whereas both M-71 and M-173 are very intense in the spectrum of the $PGF_{2\alpha}$ derivative (Fig. 2b). The intensity of the molecular ion in both spectra is quite significant. Loss of *n*-butylboronic acid (102) from the molecular ion and also from the M-90 fragment is observed in the mass spectrum of the $PGF_{2\alpha}$ derivative (Fig. 2b) but these fragments are hardly seen in the $PGF_{1\alpha}$ derivative (Fig. 2a).

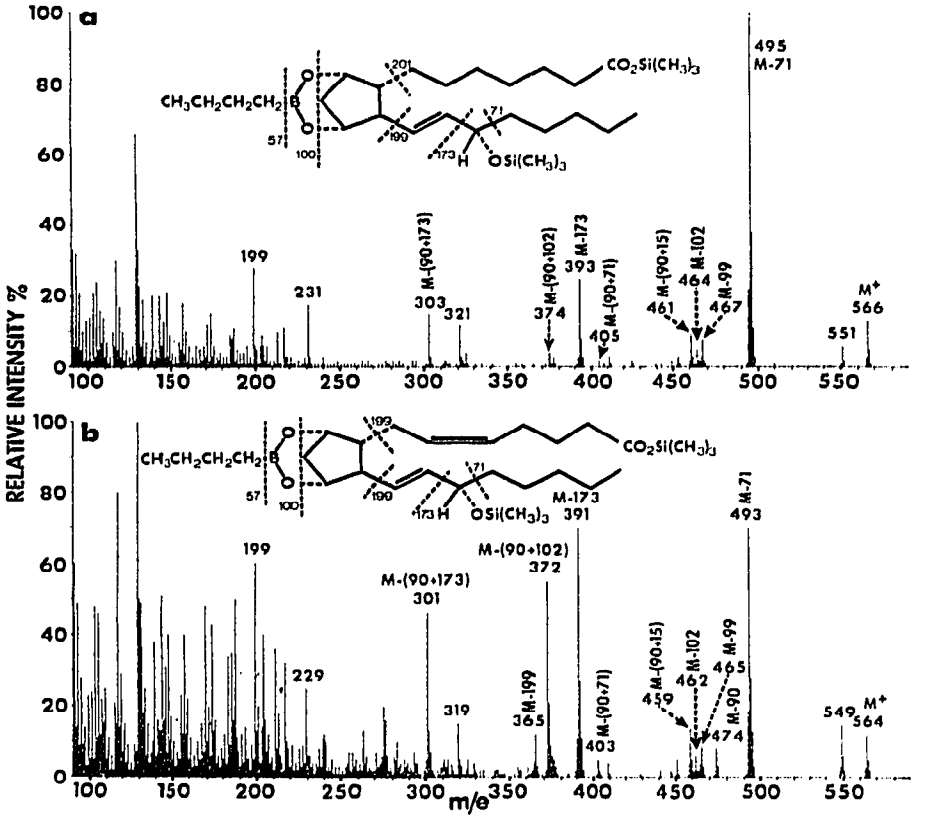

Fig. 2. Partial mass spectra of the *n*-butylboronate trimethylsilyl derivative of (a) $PGF_{1\alpha}$ and (b) $PGF_{2\alpha}$ recorded at 70 eV on an LKB-9000 GC–MS. A 3% SE-30 on Chromosorb W (HP) column was used at 260°. Relative abundance is expressed as percent of peak intensity above *m/e* 90.

It should be possible to obtain even better GLC separations of the F prostaglandins from the E prostaglandin with other longer-chain alkylboronate esters such as *n*-pentyl- or *n*-hexylboronates. Halogenated alkylboronate derivatives might prove useful in the detection of these compounds in the subnanogram level by electron capture gas chromatography.

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