Journal of Chromatography, 142 (1977) 533–547

CHROM. 10,282

GAS–LIQUID CHROMATOGRAPHY–MASS SPECTROMETRY OF THROMBOXANE B_2 AND ITS DETECTION IN SEMEN AND HUMAN AORTA BY SELECTED ION MONITORING

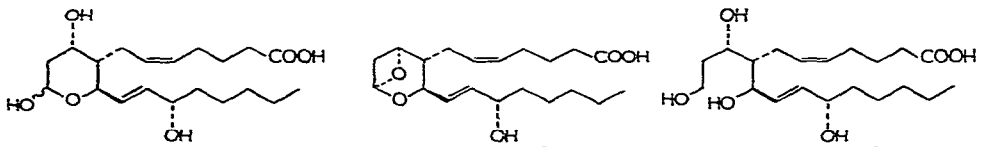
ANDREW G. SMITH*
Department of Chemistry, University of Glasgow, Glasgow G12 8QQ (Great Britain)
W. ARTHUR HARLAND
Department of Forensic Medicine, University of Glasgow, Glasgow G12 8QQ (Great Britain)
and
CHARLES J. W. BROOKS
Department of Chemistry, University of Glasgow, Glasgow G12 8QQ (Great Britain)

SUMMARY

The gas–liquid chromatographic and mass spectrometric properties of some derivatives of thromboxane B_2 methyl ester have been examined. Mass spectra were usually more informative at lower electron energies. The O-methyl- and O-*n*-propyloxime tri-trimethylsilyl ethers, and the tri-*tert.*-butyldimethylsilyl ether were used for selected ion monitoring (SIM). Thromboxane B_2 was detected by SIM in bovine semen and in human aorta.

INTRODUCTION

Thromboxane B_2 (the hemiacetal derivative of (8*S*,9*R*,12*S*)-[(1*S*)-1-hydroxy-3-oxopropyl]-9,12-dihydroxy-*Z*-5,*E*-10-heptadecadienoic acid)** (structure 1) was first identified by Hamberg and Samuelsson[1,2] as a major metabolite of prostaglandin G_2 and of arachidonic acid in suspensions of human platelets. It was later shown to be formed from the unstable active compound thromboxane A_2[3] (structure 2) which is highly potent in causing platelet aggregation and largely corresponds to the "rabbit aorta-contracting substance" of Piper and Vane[4]. The original identification of thromboxane B_2 was mainly based on gas–liquid chromatography–mass spectrometry



* Present address: Biochemical Pharmacology Section, MRC Toxicology Unit, Medical Research Council Laboratories, Woodmansterne Road, Carshalton, Surrey SM5 4EF, Great Britain.

** In this paper, the thromboxane B skeleton is numbered by analogy with prostaglandins, *i.e.* the oxopropyl side-chain is numbered C-9–C-11.

(GLC–MS) and the *in vitro* formation from arachidonic acid has since been demonstrated by GLC–MS in a number of tissues, either by obtaining complete mass spectra or by selected ion monitoring (SIM). The tissues that have been studied include platelets, guinea pig lung[5,6], spleen, kidney, liver, heart, stomach, whole blood and brain[7], rat brain[7] and carrageenin-induced granuloma[8],and human umbilical artery[9,10]. The significance of thromboxanes in relation to our studies of human atherosclerosis led us to compare the GLC and MS[1] properties of various thromboxane B_2 derivatives with the object of establishing analytical conditions and reference data, especially from the point of view of SIM. Further MS data have been published[8] while our work was in progress. We were also interested in discovering whether thromboxane B_2 could be detected in tissues without previously incubating them with arachidonic acid.

EXPERIMENTAL

Materials

Synthetic thromboxane B_2 was kindly supplied by Dr. U. F. Axen of Upjohn (Kalamazoo, Mich., U.S.A.)[11–13]. Methoxyamine hydrochloride was purchased from Kodak Ltd. (Kirkby, Great Britain). [$^2H_3$]Methoxyamine hydrochloride (Regis, Morton Grove, Ill., U.S.A.) was a gift from Dr. W. J. A. VandenHeuvel (Merck Sharp & Dohme, Rahway, N.J., U.S.A.), and *n*-propoxyamine hydrochloride was a gift from Dr. A. Chimiak (Technical University of Gdansk, Gdansk, Poland). Methaneboronic acid was purchased from Alfa Inorganics (Beverly, Mass., U.S.A.) and *n*-butaneboronic acid from Callery Chemical (Callery, Pa., U.S.A.). N,O-Bis(trimethylsilyl)trifluoroacetamide was obtained from Pierce and Warriner (Chester, Great Britain) and bis([$^2H_9$]trimethylsilyl)acetamide (99 atom% ^2H) from British Oxygen (London, Great Britain). A reagent mixture (TBDMS reagent) comprising *tert.*-butyldimethylsilylchlorosilane–imidazole–dimethylformamide (1:1:6, w/w/w) was supplied by Applied Science Labs. (State College, Pa., U.S.A.). Sephadex LH-20 (Pharmacia, Uppsala, Sweden) was exhaustively extracted in a Soxhlet apparatus with chloroform–hexane–methanol (70:30:5, v/v/v) before use.

Derivatization

Thromboxane B_2 was methylated before further derivatization, using a solution of diazomethane in diethyl ether. The ether was then removed under a stream of nitrogen.

Trimethylsilyl (TMS) ether. Thromboxane B_2 methyl ester (50 μg) in dry pyridine (25 μl) was heated for 30 min at 85° with bis(trimethylsilyl)trifluoroacetamide (10 μl).

Methane- and n-butaneboronate trimethylsilyl ether. The methyl ester (50 μg) was heated at 85° for 30 min with alkaneboronic acid (molar proportion 5:1) in pyridine (25 μl). The TMS ether was then formed as above.

O-Methyloxime tri-trimethylsilyl (MO–tri-TMS) ether. Thromboxane B_2 methyl ester (50 μg) was heated at 85° for 3 h in pyridine (50 μl) with methoxyamine hydrochloride (molar proportion 5:1). This was followed by trimethylsilylation as above. The *n*-propyloxime analogue was formed in a similar manner.

Tri-tert.-butyldimethylsilyl (tri-TBDMS) ether. Thromboxane B_2 methyl ester (50 μg) was heated overnight at 85° under nitrogen with TBDMS reagent (25 μl).

The dimethylformamide was then removed and the derivative purified by elution through a column (5 × 0.5 cm) of Sephadex LH-20 eluted with 2 ml of chloroform–hexane–methanol (10:10:1, v/v/v).

Reduction of thromboxane B_2 methyl ester. Methyl ester (50 μg) dissolved in methanol–2-propanol (1:1, v/v) was reduced with excess sodium borohydride. After extraction with ethyl acetate, the sample was divided into two portions and derivatized as the methaneboronate di-TMS ether and as the tetra-TMS ether (see above).

All samples were dissolved in ethyl acetate (25 μl) prior to GLC and GLC–MS.

Extractions

A human aorta (from a male, aged 63 years, who had suffered an abdominal aortic aneurysm) was obtained 4 h *post mortem* and the grossly normal media tissue (28.5 g) from the thoracic region was thoroughly washed and extracted as described previously[14], to yield an organic acidic fraction. This extract was then methylated and treated with TBDMS reagent (50 μl) as above. After the removal of reagents by passage through Sephadex LH-20, the fraction was subjected to thin layer chromatography (TLC) on silica gel G with hexane–diethyl ether (75:25, v/v) as the mobile phase. An area corresponding to thromboxane B_2 methyl ester tri-TBDMS ether was removed for examination by GLC–MS.

Substandard bull semen (130 ml) was obtained from the Scottish Milk Marketing Board, Southbar Cattle Breeding Centre (Inchinnan, Great Britain) and kept at −70° until required. The semen was acidified to pH 3 with hydrochloric acid and extracted with 500 ml of chloroform–methanol (4:1, v/v) by a Folch extraction[15] overnight under nitrogen at −5°. The chloroform layer was then filtered and evaporated to dryness under vacuum at 40°. The residue was methylated with an ethereal solution of diazomethane, filtered and the diethyl ether removed under nitrogen. The residue was dissolved in benzene (10 ml). A portion (0.5 ml) was methylated following removal of the benzene, and then heated with excess methoxyamine hydrochloride in pyridine. The extract was subjected to TLC on silica gel, using chloroform–methanol (9:1, v/v) as mobile phase, and an area corresponding in R_F value to authentic thromboxane B_2 methyloxime methyl ester was removed and treated with bis(trimethylsilyl)trifluoroacetamide before analysis. Another portion of the lipid (0.5 ml) was methylated and treated in the same reaction and purification sequence as described for the aortal extract, to give a TBDMS derivatized fraction.

GLC and GLC–MS

A Pye 104 gas chromatograph with a flame-ionization detector (FID) was used for GLC. Glass columns were used of either 3 m × 4 mm I.D. packed with 1% OV-1 on Gas-Chrom Q (100–120 mesh) operating at 245°, or 1.5 m × 4 mm I.D. packed with 5% SP-2340 on Chromosorb W AW (100–120 mesh) at 225°. In both cases nitrogen (50 ml/min) was used as the carrier gas. Kováts retention indices were calculated from comparative measurements on *n*-alkanes and are recorded to the nearest 5 units.

Electron impact mass spectra (20 and 70 eV) were obtained using an LKB 9000 mass spectrometer equipped with a 1% OV-1 column (3 m × 3 mm I.D.) at 250–285°. The temperature of the separator and the source was 270°, the flash heater was at 270°, the trap current was 60 μA and helium was used as the carrier gas (flow-rate,

30 ml/min). SIM was accomplished with the accelerating voltage alternator system of the LKB 9000. MS sensitivity was increased, and resolution was reduced to approximately 350, by opening the electron multiplier slits. The chemical ionization (isobutane) mass spectrum was obtained using a DuPont 21-490F instrument as previously described[16].

RESULTS AND DISCUSSION

Thromboxane B_2 must be converted to suitable derivatives[1,8] before it can be examined by GLC and GLC–MS. The formation of the methyl ester as a first step is convenient, especially if any other type of chromatography is involved before a GLC stage. The use of the methyl ester also leads to only a small increase in the molecular weight. The GLC retention indices of the derivatives formed in this study, on both non-polar (OV-1) and polar (SP-2340) type phases, are shown in Table I. Electron impact mass spectra were usually more informative at 20 eV than at 70 eV, the higher electron energy inducing too much fragmentation, yielding ions of low *m/e* values. Even at 20 eV, for most derivatives, molecular ions and related high-mass ions were of low intensity. The GLC and MS properties of the various derivatives are discussed separately below.

TABLE I

GLC DATA (KOVÁTS RETENTION INDICES) FOR DERIVATIVES OF THROMBOXANE B_2 METHYL ESTER

For conditions see Experimental.

Derivative	$I^{245°}_{OV\text{-}1}$	$I^{225°}_{SP\text{-}2340}$
Tri-TMS ether	2760	3495
Cyclic 9,11-methaneboronate TMS ether	2670	
Cyclic 9,11-butaneboronate TMS ether	2900	
MO–tri-TMS ether	2725, 2795*	3485, 3525*
Propyloxime tri-TMS ether	2920	3660
Tri-TBDMS ether	3405	4065
Reduced form tetra-TMS ether	2820	3650
Reduced form methaneboronate di-TMS ether	2770	3485

* Major peak.

Tri-trimethylsilyl ether

The tri-TMS ether of thromboxane B_2 possessed good chromatographic properties. The mass spectrum at 70 eV showed a base peak at *m/e* 73 ($[(CH_3)_3Si]^+$). No molecular ion was observed, while the ions of *m/e* 510 ($[M - TMSOH]^+$) and *m/e* 256 ($[(CH_3)_3SiO–CH=CH–CH_2–CH=CH–(CH_2)_3–COOCH_3]^+$)[1] were of 0.3% and 26% relative abundance, respectively. The main features of the mass spectrum were similar to those reported by Ho *et al.*[17] and Chang *et al.*[8]. The 20-eV spectrum is shown in Fig. 1 with an indication of the major fragment ions. In this case *m/e* 256 was the base peak (as in reported mass spectra at undefined electron energies[1,5]) and though a molecular ion at *m/e* 600 was observed, it was only of 0.5% abundance. The high relative abundance of the ion of *m/e* 256 in the 20-eV spectrum reflects its established[2,6,9,10,18] suitability for use in SIM of thromboxane B_2.

Methaneboronate trimethylsilyl ether

It appeared to us that the particular hemiacetal form of thromboxane B_2 possessing the *cis* 9,11-diol system might be conveniently stabilised by formation of a cyclic alkaneboronate. (A similar process is effective for hemiacetals of 18,21-dihydroxy-20-oxosteroids[19].) The GLC of prostaglandins of the F_α series as *n*-butaneboronates was first demonstrated by Pace-Asciak and Wolfe[20], and we have previously examined the electron impact and chemical ionization mass spectra of a range of 9,11-cyclic alkaneboronates of prostaglandins $F_{1\alpha}$, $F_{2\alpha}$ and $F_{3\alpha}$ as the methyl ester 15-TMS ethers[16]. These derivatives of prostaglandins F_α possessed good GLC properties. However, thromboxane B_2 methyl ester 9,11-methaneboronate 15-TMS ether gave a low response on both polar and non-polar phases during GLC, with a strongly tailing "solvent front" on the chromatogram. The FID response was less than 25% of that for the tri-TMS ether derivative. Only a small proportion of the tri-TMS ether was present as a contaminant, indicating that the poor response was not due merely to incomplete formation of the methaneboronate. *n*-Butaneboronic acid gave a poorer response, and no chromatographic peak at all was observed with cyclohexane- and benzeneboronic acids. The 70-eV mass spectrum of thromboxane B_2 methyl ester methaneboronate 15-TMS ether is shown in Fig. 2. The ion of *m/e* 409 ($[M - 71]^+$) arising from cleavage of the C-15:C-16 bond adjacent to the 15-trimethylsilyloxy group was of only moderate intensity, whereas an equivalent ion is usually the base peak at 70 eV in the spectrum of prostaglandin F_α analogues[16]. The presence of the cyclic methaneboronate group was shown by the ion at *m/e* 97 (base peak) corresponding to $[CH_3BO_2C_3H_3]^+$. The molecular ion was of low abundance (*m/e* 480; 1%). The *n*-butaneboronate analogue gave a similar fragmentation pattern with a moderately intense $[M - 71]^+$ ion (14%) at *m/e* 451. The ion of *m/e* 139 (80%) could be attributed to $[CH_3(CH_2)_3BO_2C_3H_3]^+$ arising by cleavage of the pyran ring, and the base peak of the spectrum was *m/e* 73 ($[(CH_3)_3Si]^+$). The ion of *m/e* 199 ($[C_{11}H_{23}OSi]^+$, *i.e.* C-13–C-20 side-chain) was much higher in these alkaneboronate spectra than in those of other thromboxane B_2 derivatives. The chemical ionization (isobutane) mass spectrum of the methaneboronate contained a strong molecular ion (*m/e* 480; 35%) and accompanying $[M - 1]^+$ and $[M + 1]^+$ ions of lesser intensity. The base peak was at *m/e* 391 ($[M - 89]^+$), corresponding to the elimination of the trimethylsilyloxy group as previously observed in the spectra of prostaglandin F_α alkaneboronates[16].

O-Methyloxime tri-trimethylsilyl ether

In thromboxane B_2 the hemiacetal form is in equilibrium with the hydroxy-aldehyde and is thus susceptible to reaction with an alkoxyamine hydrochloride to form the O-alkyloxime. Hamberg and Samuelsson[1] used the methyl ester MO–tri-TMS ether in their original identification of thromboxane B_2, Chang *et al.*[8] employed the same derivative and also the TMS ester MO–tri-TMS ether when demonstrating the transformation of arachidonic acid to thromboxane B_2 by granulation tissue. Similarly Falardeau *et al.*[21] converted thromboxane B_1 to its MO–TMS ether derivative and Dawson *et al.*[22] used the methyloxime derivative in their identification of 15-oxothromboxane B_1 in the perfusate of immunologically challenged guinea pig lung. In our examination of the GLC properties of the O-methyloxime of thromboxane B_2 methyl ester tri-TMS ether, the derivative was found to chromatograph well and gave a FID response approximately equal to the methyl ester tri-TMS ether derivative.

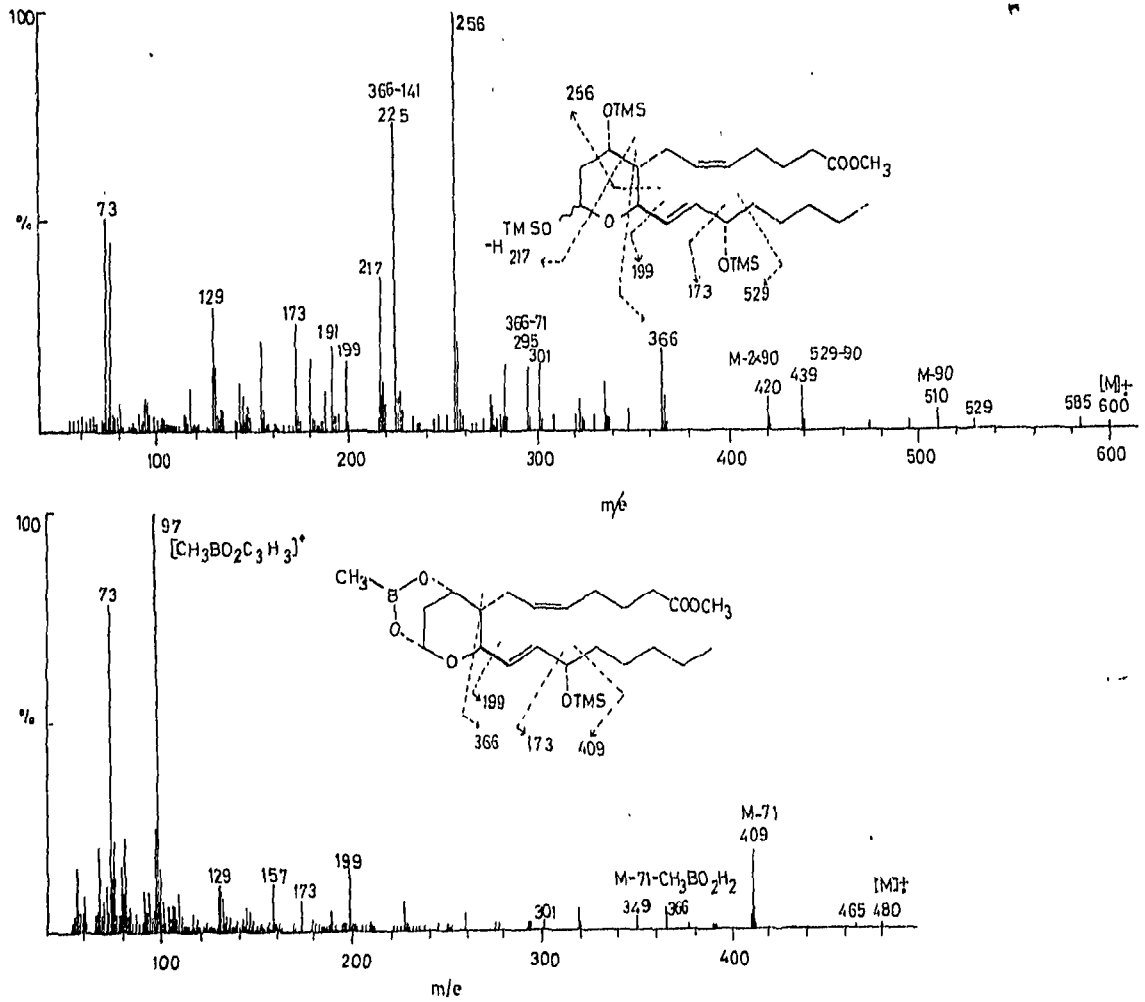


Fig. 1. Mass spectrum (20 eV) of the methyl ester tri-TMS ether of thromboxane B_2. The ions of *m/e* 73, 129, 173, 199, 217, 225, 256, 295, 301, 323, 366 and 600 were shifted to *m/e* 82, 138, 191, 208, 235, 234, 265, 304, 319, 332, 375 and 627, respectively, in the spectrum of the [$^2H_9$]TMS ether analogue.

Fig. 2. Mass spectrum (70 eV) of the cyclic methaneboronate methyl ester trimethylsilyl ether of thromboxane B_2. The ions *m/e* 73, 173, 199, 349, 409 and 480 were shifted to *m/e* 82, 182, 208, 358, 418 and 489, respectively, in the mass spectrum of the [$^2H_9$]TMS ether analogue.

Two peaks were observed (Table I; Fig. 3), a major and a minor which varied in relative proportions. These were ascribed to the *syn* and *anti* isomers. Formation of the methyloxime under conditions involving a large excess of methoxyamine hydrochloride with longer reaction times, gave only the second eluting isomer. The methyl ester O-*n*-propyloxime tri-TMS ether gave a single broad peak.

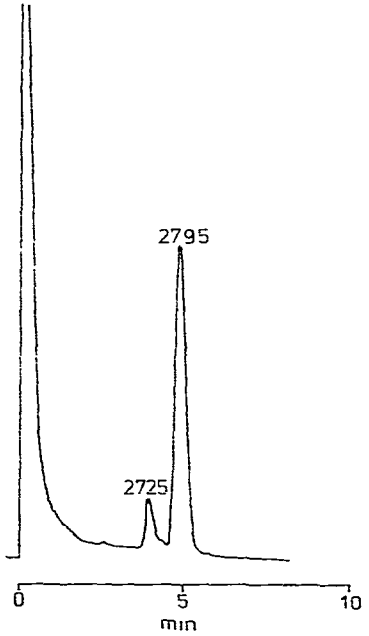


Fig. 3. GLC of the methyl ester O-methyloxime tri-TMS ether of thromboxane B_2 (200 ng). Column 3 m × 4 mm I.D., 1% OV-1 at 245° with nitrogen carrier gas (flow-rate 50 ml/min).

The mass spectra of the methyloxime and *n*-propyloxime derivatives were determined at both 20 eV and 70 eV. Even at 20 eV the intensities of ions of high *m/e* value were extremely low and many were not observed at all at 70 eV. The major fragmentation patterns are shown in Fig. 4 and Table II. In agreement with previous work[1,8] the major ions of the methyloxime spectra were *m/e* 301 arising by cleavage across the middle of the molecule (between the C-8 and C-12 bond) which was the base peak of spectra at 20 eV, *m/e* 211 (loss of TMSOH from *m/e* 301), *m/e* 191, *m/e* 174 (cleavage of the C-8:C-9 bond), *m/e* 173 (cleavage of the C-14:C-15 bond), *m/e* 142 and *m/e* 73 ($[TMS]^+$). Appropriate mass increments were observed for corresponding ions in the spectra of the $[^2H_3]$methyloxime, $[^2H_9]$TMS, and *n*-propyloxime analogues (Table II). The minor GLC peak of the methyloxime possessed similar 20-eV and 70-eV spectra to the major peak, except that the ions of *m/e* 173, 174 and 142 were of much lower intensity.

Reduction of thromboxane B_2

Reduction of thromboxane B_2 methyl ester with sodium borohydride yielded the known acyclic compound (methyl ester of structure 3), the tetra-TMS derivative

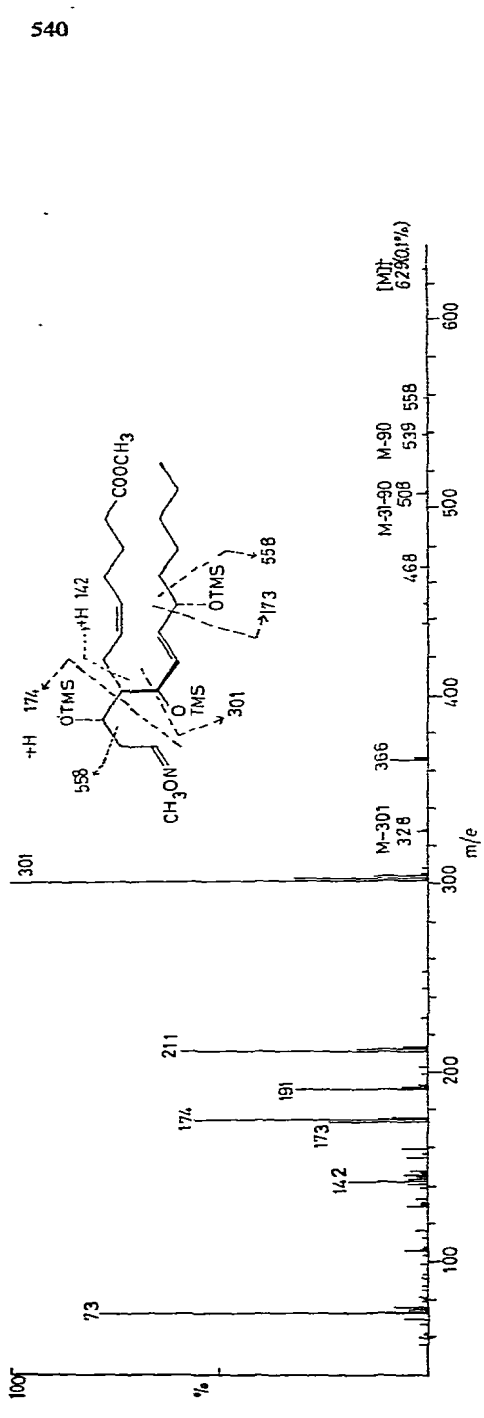


Fig. 4. Mass spectrum (20 eV) of the major peak of thromboxane B_2 methyl ester MO-tri-TMS ether ($I^{245°}_{GV-1}$ 2795).

Fig. 5. Mass spectrum (70 eV) of the cyclic methaneboronate methyl ester di-TMS ether of the product of borohydride

BLE II

; DATA FOR METHYL- AND *n*-PROPYLOXIME TRI-TMS ETHERS OF THROMBOXANE B^2 ETHYL ESTER

rivative	*m/e values of some characteristic fragment ions (relative abundances in parentheses)*												
	eV	*M*⁻	*Base peak*	*Others***									
)–tri-TMS ether*	20	629 (0.1)	301	558 (1)	539 (1)	508 (3)	468 (2)	366 (9)	328 (3)	211 (59)	199 (1)	191 (31)	174 (55)
										173 (25)	155 (5)	142 (19)	73 (78)
	70	629 (–)	73	526 (1)	366 (2)	328 (1)	301 (22)	211 (17)	199 (2)	191 (5)	174 (7)	173 (8)	155 (4)
												147 (11)	142 (9)
$_3$]MO–tri-TMS ether*	70	632 (0.1)	73	508 (1)	366 (1)	301 (27)	211 (19)	199 (1)	191 (8)	177 (11)	173 (7)	147 (8)	142 (10)
)–tri-[$^2H_9$]TMS ether*	70	656 (–)	82	319 (26)	220 (18)	209 (1)	208 (1)	183 (8)	182 (8)	164 (5)	151 (10)		
)pyloxime tri-TMS ether	20	657 (0.1)	301	598 (0.5)	567 (0.1)	508 (1)	211 (23)	202 (33)	173 (17)	142 (19)	73 (18)		
	70	657 (–)	73	301 (6)	211 (10)	202 (2)	191 (2)	173 (3)	147 (3)	142 (3)			

* Spectrum of the major peak (second eluting) of the *syn* and *anti* isomers; spectra of the first eluting peak re similar except that the ions *m/e* 142, 173 and 174 were of much lower abundance.

** *m/e* 558 ([*M* – C-16–C-20]⁺), 539 ([*M* – TMSOH]⁺), 508 ([*M* – (TMSOH + RO)]⁺), 468 ([558 – ISOH]⁺), 328 ([*M* – 301]⁺), 301 ([TMSO=CH–CH=CH–CH(OTMS)–$(CH_2)_4CH_3$]⁺), 211 ([301 – ISOH]⁺), 199 ([C-13–C-20 side-chain]⁺), 191 ([TMSO=CH–OTMS]⁺), 174 ([TMSO=CH–CH_2–CH= $)CH_3$]⁺), 173 ([TMSO=CH–$(CH_2)_4CH_3$]⁺), 73 ([TMS]⁺). See also Fig. 4.

of which showed the expected GLC and MS properties[1]. No molecular ion was observed (at 70 eV) though ions at *m/e* 494 ([*M* – (2 × TMSOH)]⁺) and *m/e* 411 ([*M* – (TMSOH + 173)]⁺) were consonant with the presence of four hydroxyl groups in the parent compound. The presence of a C-15-trimethylsilyloxy group was illustrated by the presence of intense ions at *m/e* 301 and *m/e* 211 (see methyloxime spectra). Other strong ions were observed at *m/e* 219, 173, 147, 129, 103, 81, 73 (base peak) and 69. The arrangement of the hydroxyl groups of this compound made it seem likely that it would form at least one type of cyclic boronate. Treatment of the reduced thromboxane methyl ester with methaneboronic acid, followed by trimethylsilylation, yielded a single product with excellent GLC properties. The 70-eV mass spectrum (Fig. 5) showed a molecular ion at *m/e* 554. The derivative was shown to be a cyclic 9,12-methaneboronate by the presence of ions of *m/e* 199 and *m/e* 103 and by the absence of significant ions at *m/e* 301 and *m/e* 219.

Tri-tert.-butyldimethylsilyl ether

Although TMS ethers give diagnostically useful ions and have been widely used, molecular ions or [*M* – TMSOH]⁺ ions, etc. may be of extremely low intensity when two, three or more hydroxyl groups are present in the parent molecule. The use of TBDMS ethers as an alternative has been explored with polyhydroxylated compounds such as steroids[23,24] and prostaglandins[25]. The advantage of these deriva-

tives lies in the often increased simplicity of the spectra relative to the respective TMS ethers, and in the occurrence of intense ions ($[M - 57]^+$) corresponding to loss of the *tert*.-butyl group (molecular ions are usually of very low abundance). The $[M - 57]^+$ ion is often the base peak of a spectrum. The low intensity of ions of higher *m/e* value in the spectrum of thromboxane B_2 methyl ester tri-TMS ether indicated that this compound might give a more informative spectrum as the methyl ester tri-TBDMS ether. GLC indicated that under the conditions described in Experimental, a single product was formed with good chromatographic properties (Table I; Fig. 6). The mass spectrum (20 eV) showed a number of characteristic ions, of moderate or high intensity, in the high-mass region (Fig. 7). The $[M - 57]^+$ ion, *m/e* 669, was the base peak and no molecular or $[M - CH_3]^+$ ions were observed. A prominent ion at *m/e* 298 was probably formed by cleavage of the C-8:C-12 and C-9:C-10 bonds (equivalent to the ion of *m/e* 256 in the spectrum of the tri-TMS ether). Other intense ions were observed at *m/e* 537 ($[M - (57 + TBDMSOH)]^+$), 371 ($[M - (298 - 57)]^+$), 301, 351, 267, 261, 241 (equivalent to 199 in TMS ether), 239, 215 (equivalent to 173 in TMS ether), 171, 129, 75 and 73. At 70 eV only a low intensity $[M - 57]^+$ ion was present, most of the ionization current being represented in the low-mass ions at *m/e* 69, 73 and 75. Although no decomposition was observed during GLC at 305° (1% OV-1) with an injector temperature of 350°, some decomposition of the tri-TBDMS ether was observed at high injection temperatures on the LKB 9000 during GLC–MS. A lower intensity $[M - 57]^+$ ion was observed when spectra were obtained on a VG MM16F mass spectrometer and it is worth noting that Kelly and Taylor[25] reported marked variations in TBDMS ether spectra recorded on different mass spectrometers.

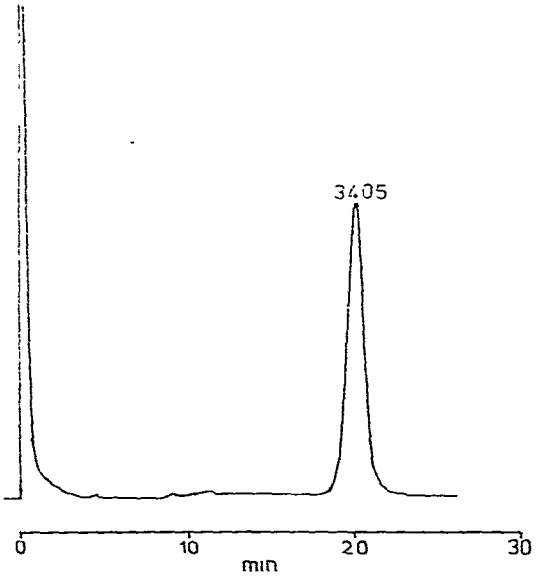


Fig. 6. GLC of thromboxane B_2 (2 μg) methyl ester tri-TBDMS ether. Conditions as for Fig. 3.

During preliminary investigations of TBDMS ether formation, the reagent used was diluted with pyridine redistilled from potassium hydroxide. In this case, only partial derivatization occurred even after prolonged periods. GLC (1% OV-1) showed a major product with a shorter retention time ($I = 3305$) than the methyl ester tri-TBDMS ether (see Table I). MS showed it to be the di-TBDMS ether ($[M - 57]^+$ ion at *m/e* 555) and trimethylsilylation gave a new compound ($I = 3200$) with the expected $[M - 57]^+$ ion at *m/e* 627 and an ion at *m/e* 537 corresponding to a further loss of trimethylsilanol. The absence of an ion at *m/e* 298 and the presence of moderately intense ions at *m/e* 241, 215, 371 and 156 suggested that the original underivatized hydroxyl group had been that at C-9. Other intense ions were observed at *m/e* 129, 219, 167, 351 and 365.

The attempted formation of the TBDMS ether thromboxane B_2 cyclic methaneboronate methyl ester proved unsuccessful.

Selected ion monitoring

The ion of *m/e* 256 in the mass spectrum of the tri-TMS ether of thromboxane B_2 methyl ester has been used by Hamberg and co-workers[2,6,9,10,18] for SIM. [$^2H_8$]-Thromboxane B_2 was employed as an internal standard. We decided to investigate other derivatives as candidates for SIM based on ions of higher mass value than *m/e* 256 and therefore potentially more specific for thromboxane B_2. The ion of *m/e* 301 in the spectrum of the O-methyloxime tri-TMS ether was found to give good results, although with the standard mixture two peaks were observed for the *syn* and *anti* isomers. With the instrumentation and conditions previously described, the limit of detection was approximately 25 pg of thromboxane B_2 at 22 eV. The presence of the ion of *m/e* 301 in the spectrum of the O-*n*-propyloxime tri-TMS ether of thromboxane B_2 methyl ester indicated the possibility of the use of this derivative as an internal standard and "carrier" at least after initial extraction stages. The detection of thromboxane B_2 in bull semen by SIM of the ion of *m/e* 301 is illustrated in Fig. 8. The results indicated a concentration of 5.4 ng/ml of semen. However, this may well be an underestimate as no deuterated thromboxane B_2 was available for use as an internal standard. Only the major methyloxime isomer was observed, presumably because of the large excess of methoxyamine hydrochloride used.

The $[M - 57]^+$ ion of thromboxane B_2 methyl ester tri-TBDMS ether was also examined for its potential in SIM. The much higher *m/e* value of this ion (*m/e* 669) relative to *m/e* 301 or *m/e* 256 (coupled with the characteristic retention index of the derivative) affords improved analytical specificity. The differences in the mass spectra of this derivative at 20 and 70 eV led us to investigate the changes in the signal amplitude at *m/e* 669 at different electron voltages (Fig. 9). Sensitivity was very low at 70 eV and optimum signal was achieved at 25 eV (the limit of detection being 100 pg of thromboxane B_2). By monitoring *m/e* 669, thromboxane B_2 was detected in an extract of bull semen (approximately 6.5 ng/ml) and in an extract of human aortal media (1.5 ng/g wet tissue). The SIM chromatograms are shown in Fig. 10. The use of chemical ionization MS for prostaglandins is sometimes advantageous over electron impact MS when undertaking SIM[16], and its potential for thromboxanes should therefore be explored.

Semen has been shown to contain 19-hydroxyprostaglandins[26–30] and in view of the apparent presence of thromboxane B_2 in bovine semen it would be of interest

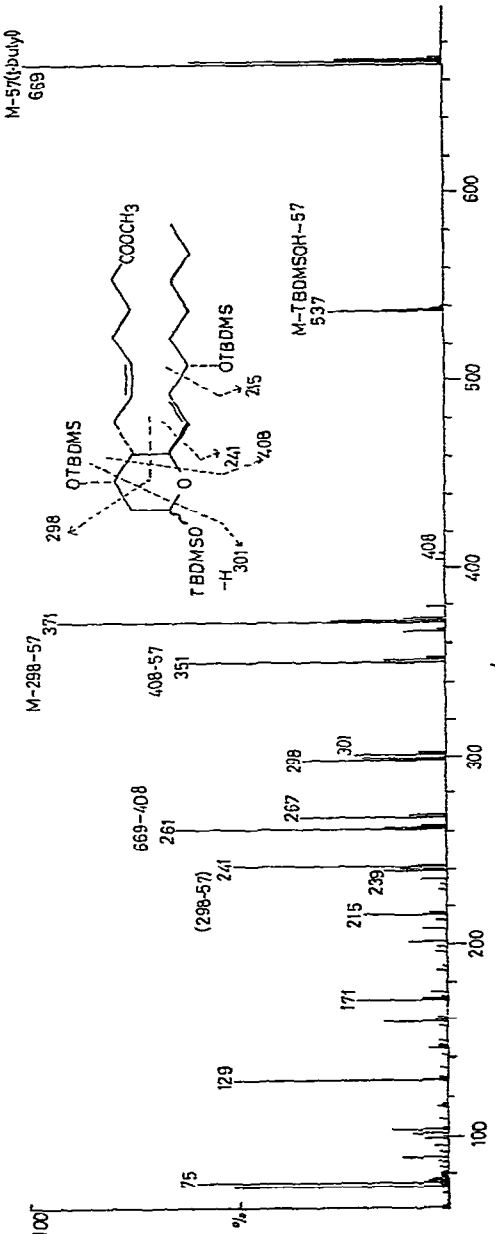


Fig. 7. Mass spectrum (20 eV) of thromboxane B_2 methyl ester tri-TBDMS ether.

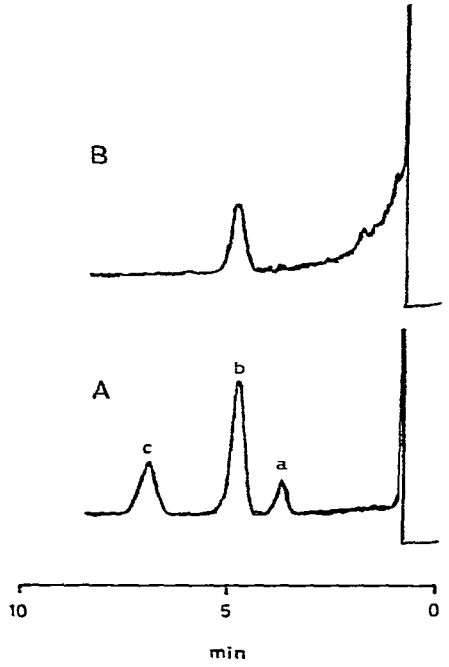


Fig. 8. Selected ion chromatograms (*m/e* 301) recorded during GLC–MS (22 eV) of (A) 1 ng of thromboxane B_2 as the MO–tri-TMS ether (peaks a and b) and 500 pg of the O-*n*-propyloxime analogue (c); (B) an extract of bovine semen derivatized with methoxyamine hydrochloride. Column, 3 m × 3 mm I.D., OV-1, temperature, 250°.

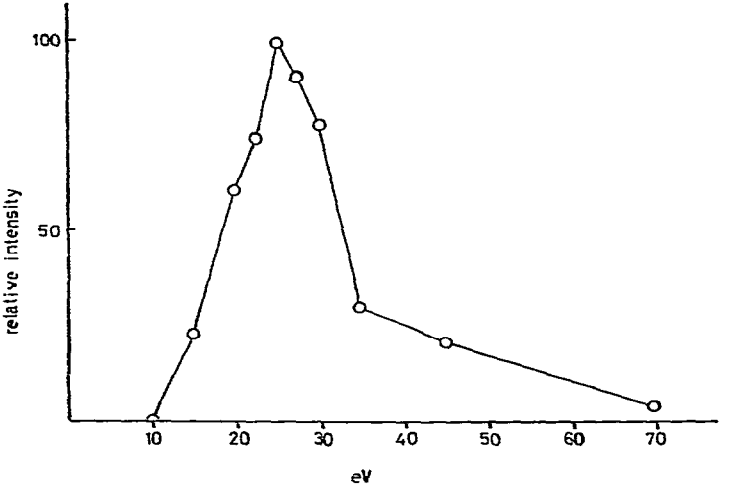


Fig. 9. Relative responses to the ion *m/e* 669 at different electron energies during selected ion monitoring GLC–MS of the methyl ester tri-TBDMS ether of thromboxane B_2 (5 ng). GLC conditions were as in Fig. 8 with a column temperature of 270°.

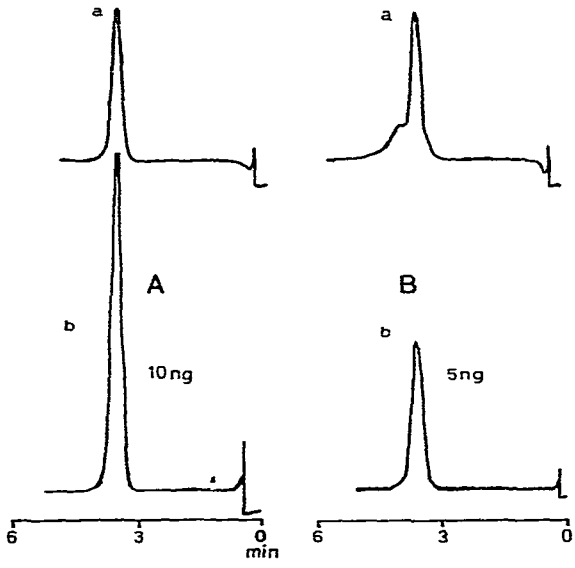


Fig. 10. Detection of thromboxane B_2 as the methyl ester tri-TBDMS ether derivative in extracts of (A) bovine semen and (B) human aortal media, by SIM of the ion *m/e* 669 ($[M-57]^+$) during GLC–MS at 25 eV; (a) extract, (b) reference compound. Column temperature 280°, 1% OV-1 column (3 m × 3 mm I.D.).

to determine whether the analogous 19-hydroxythromboxanes are also present. The relative and inter-linked roles of prostaglandins and thromboxanes in platelets and coronary arteries are now the subject of many investigations (see refs. 31–38 and works cited therein). We have previously identified prostaglandin $F_{2\alpha}$ in human aortal media[14] and the probable presence of thromboxane B_2 contrasts with the studies of Needleham *et al.*[37] who could not detect thromboxane synthetase in pig coronary artery microsomal fraction. Tuvemo *et al.*[9,10], however, have demonstrated the production of thromboxane B_2 from prostaglandin endoperoxides by human umbilical cord artery. The sensitivity of the thromboxane synthetase system to experimental conditions is well exemplified by the recent demonstration that thromboxanes are synthesized in microsomal preparations from ureter-obstructed rabbit kidneys, but not in those from normal kidneys[39].

ACKNOWLEDGEMENTS

We thank Dr. U. F. Axen for the gift of thromboxane B_2, Dr. A. Chimiak for alkoxyamine reagents, Dr. W. J. A. VandenHeuvel for [$^2H_3$]methoxyamine hydrochloride, Mr. J. Isbister for arranging a supply of bull semen, Mr. C. G. Edmonds and Dr. S. J. Gaskell for assistance with GLC–MS, and the Medical Research Council for financial support (to C.J.W.B. and W.A.H.). The LKB9000 instrument was provided by S.R.C. grants Nos. B/SR/2398 and B/SR/8471.

REFERENCES

1 M. Hamberg and B. Samuelsson, *Proc. Nat. Acad. Sci. U.S.*, 71 (1974) 3400.
2 M. Hamberg and B. Samuelsson, *Proc. Nat. Acad. Sci. U.S.*, 71 (1974) 3824.
3 M. Hamberg, J. Svensson and B. Samuelsson, *Proc. Nat. Acad. Sci. U.S.*, 72 (1975) 2994.
4 P. J. Piper and J. R. Vane, *Nature (London)*, 223 (1969) 29.
5 M. Hamberg and B. Samuelsson, *Biochem. Biophys. Res. Commun.*, 61 (1974) 942.
6 M. Hamberg, *Biochim. Biophys. Acta*, 431 (1976) 651.
7 L. S. Wolfe, K. Rostworowski and J. Marion, *Biochem. Biophys. Res. Commun.*, 70 (1976) 907.
8 W.-C. Chang, S. Murota and S. Tsurufuji, *Prostaglandins*, 13 (1977) 17.
9 T. Tuvemo, K. Strandberg, M. Hamberg and B. Samuelsson, *Advan. Prost. Thromb. Res.*, 1 (1976) 425.
10 T. Tuvemo, K. Strandberg, M. Hamberg and B. Samuelsson, *Acta Physiol. Scand.*, 96 (1976) 145.
11 N. A. Nelson and R. W. Jackson, *Tetrahedron Lett.*, (1976) 3275.
12 R. C. Kelly, I. Schletter and S. J. Stein, *Tetrahedron Lett.*, (1976) 3279.
13 W. P. Schneider and R. A. Morge, *Tetrahedron Lett.*, (1976) 3283.
14 A. G. Smith, J. D. Gilbert, W. A. Harland and C. J. W. Brooks, *Biochem. Soc. Trans.*, 4 (1976) 108.
15 J. Folch, M. Lees and G. H. Sloane-Stanley, *J. Biol. Chem.*, 226 (1957) 497.
16 A. G. Smith and C. J. W. Brooks, *Biomed. Mass Spectrom.*, in press.
17 P. P. K. Ho, C. P. Walters and H. R. Sullivan, *Prostaglandins*, 12 (1976) 951.
18 K. Gréen, M. Hamberg and B. Samuelsson, *Advan. Prost. Thromb. Res.*, 1 (1976) 47.
19 S. J. Gaskell, C. G. Edmonds and C. J. W. Brooks, *Anal. Lett.*, 9 (1976) 325.
20 C. Pace-Asciak and L. S. Wolfe, *J. Chromatogr.*, 56 (1971) 129.
21 P. Falardeau, M. Hamberg and B. Samuelsson, *Biochim. Biophys. Acta*, 441 (1976) 193.
22 W. Dawson, J. R. Boot, A. F. Cockerill, D. N. B. Mallen and D. J. Osborne, *Nature (London)*, 262 (1976) 699.
23 G. Phillipou, D. A. Bigham and R. F. Seamark, *Steroids*, 26 (1975) 516.
24 S. J. Gaskell and C. J. W. Brooks, *Biochem. Soc. Trans.*, 4 (1976) 111.
25 R. W. Kelly and P. L. Taylor, *Anal. Chem.*, 48 (1976) 465.
26 P. L. Taylor and R. W. Kelly, *Nature (London)*, 250 (1974) 665.
27 P. L. Taylor and R. W. Kelly, *FEBS Lett.*, 57 (1975) 22.
28 I. Cooper and R. W. Kelly, *Prostaglandins*, 10 (1975) 507.
29 H. T. Jonsson, B. S. Middleditch and D. M. Desiderio, *Science*, 187 (1975) 1093.
30 H. T. Jonsson, B. S. Middleditch, M. A. Schexnayder and D. M. Desiderio, *J. Lipid Res.*, 17 (1976) 1.
31 S. Moncada, R. Gryglewski, S. Bunting and J. R. Vane, *Nature (London)*, 263 (1976) 663.
32 R. J. Gryglewski, S. Bunting, S. Moncada, R. J. Flower and J. R. Vane, *Prostaglandins*, 12 (1976) 685.
33 S. Moncada, R. J. Gryglewski, S. Bunting and J. R. Vane, *Prostaglandins*, 12 (1976) 715.
34 R. A. Johnson, D. R. Morton, J. H. Kinner, R. R. Gorman, J. C. McGuire, F. F. Sun, N. Whittaker, S. Bunting, J. Salmon, S. Moncada and J. R. Vane, *Prostaglandins*, 12 (1976) 915.
35 S. Kalsner, *Can. J. Physiol. Pharmacol.*, 53 (1975) 560.
36 F. Rioux and D. Regoli, *Can. J. Physiol. Pharmacol.*, 53 (1975) 673.
37 P. Needleham, P. S. Kulkarni and A. Raz, *Science*, 195 (1977) 409.
38 F. F. Sun, *Biochem. Biophys. Res. Commun.*, 74 (1977) 1432.
39 A. R. Morrison, K. Nishikawa and P. Needleman, *Nature (London)*, 267 (1977) 259.