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CHARACTERIZATION OF PROSTAGLANDINS BY COMBINED GAS–LIQUID CHROMATOGRAPHY AND CHEMICAL IONIZATION MASS SPECTROMETRY*

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SUMMARY

This report describes the advantages of chemical ionization mass spectrometry coupled with gas–liquid chromatography for the analysis of prostaglandins and similar biologically active metabolites. By the use of this technique, a more thorough knowledge of the chemical structure of numerous widely used derivatives of various prostaglandins is possible. As indicated in the discussion, care must be exercised in the choice of specific types of derivative for the purpose of structural identification of the intact prostaglandins and their intact metabolites.

INTRODUCTION

The use of mass spectrometry for the elucidation of the structure of prostaglandins is almost as numerous as the rapidly evolving bibliography concerning prostaglandins. In most instances after derivatization the prostaglandin analogues have been analyzed by gas–liquid chromatography (GLC) coupled with an electron impact (EI) mass spectrometer[1–7]. In numerous instances EI mass spectra of prostaglandins and their derivatives exhibit a very low or non-existent abundance of ion fragments in the molecular ion region[2]. In order to enhance the abundance of ions in EI spectra, one usually reduces the electron voltage to 10–20 eV. On the contrary, with the use of a decreased electron voltage there also is an increased requirement in sample size.

Chemical ionization (CI) mass spectrometry is a type of high-pressure mass spectrometry in which the compound of interest interacts with the reactant or carrier gas ions. The reactant ions are formed by a combination of EI and ion–molecule recombination. The primary, secondary, and tertiary reactant ions then combine with the desired sample and in the process transfer massive entities including protons (H^+),

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hydride ions (H^-), and alkyl carbonium ions (RCH_2^+) to the desired sample molecule. The amount of energy involved in the processes of CI mass spectrometry is relatively low, depending upon the reactant gas used. Because of the low level of energy transferred in the CI analyses, there is a general enhancement in the abundance of ions formed in the molecular region. For a more detailed discussion on CI mass spectrometry, the reader is referred to articles by Munson and Field[8], Munson[9], and Fales *et al.*[10].

By the use of CI mass spectrometry coupled with GLC, one is able to obtain the representative mass spectrum including the molecular ion and other pertinent mass fragments which unequivocally identify the particular compound under consideration even in low nanogram quantities[11,12]. Furthermore, by the complementary characterization of crudely purified environmental agents and their metabolites by GLC combined with CI and EI mass spectrometry, one can identify the chemical structure of numerous polyfunctional metabolites and reaction products of complex environmental agents[11–14].

The purpose of the present investigations was to develop a more descriptive analytical tool for the chemist and biologist in order to identify conclusively the chemical structure of nanogram quantities of biologically active prostaglandins and their metabolites.

EXPERIMENTAL

Materials

Prostaglandins (PG) E_2, $F_{1\alpha}$, and $F_{2\alpha}$ were provided through the courtesy of Dr. John Pike, the UpJohn Company (Kalamazoo, Mich., U.S.A.). Trifluoroacetic anhydride, trimethylsilylimidazole (TSIM), and bis(trimethylsilyl)trifluoroacetamide (BSTFA) were purchased from Pierce (Rockford, Ill., U.S.A.). Diazomethane was prepared[15] fresh daily from N-methyl-N-nitroso-*p*-toluenesulfonamide (Aldrich, Milwaukee, Wisc., U.S.A.). All organic solvents were Baker Analyzed quality and/or freshly distilled prior to use. PGB_2 was prepared from PGE_2 by treatment with 0.5 *M* KOH at room temperature for 1 h followed by acidification to pH 3.5 with 1 *N* HCl and extraction with diethyl ether. The organic phase after being washed with water was evaporated to dryness.

Methods

Derivatization. Methyl esters of the specific prostaglandin were prepared with a fresh solution of diazomethane as reported earlier[15]. The acetate derivatives were synthesized with a mixture of pyridine–acetic anhydride (1:1)[15]. The trimethylsilyl ethers were prepared at room temperature using a mixture of TSIM–BSTFA (2:1)[15]. The trifluoroacetate of $PGF_{2\alpha}$ was prepared as described by Thompson *et al.*[3]. All freshly prepared derivatives of the prostaglandins were analyzed by GLC coupled with CI and EI mass spectrometry.

GLC/EI mass spectrometry. Unless otherwise stated EI mass spectra were obtained using a Finnigan (Sunnyvale, Calif., U.S.A.) Model 1015C Quadrupole mass spectrometer[14] interfaced with (1) a Varian Aerograph (Palo Alto, Calif., U.S.A.) Model 1400 gas chromatograph or more recently with (2) a Finnigan Model 9500 gas chromatograph and all-glass transfer interface line with single-stage glass jet

separator. The manifold temperature was maintained at 175°. Routinely spectra were collected at 70 eV, 20 eV and occasionally at 10 eV with 3000 V applied from the high-voltage power supply[14]. With either of the above interfaced gas chromatographs, a 152 × 0.2 cm I.D. glass column packed with 3% OV-1 on Gas-Chrom Q (80–100 mesh) with a measure helium flow-rate of 35–40 ml/min was used with an overall pressure of 5×10^{-5} to 10^{-6} Torr. Unless otherwise stated for the analysis of all derivatives except the trifluoroacetate, the gas chromatograph column temperature was 220° for 5 min and then programmed at 10°/min to 240°. The injector temperature was maintained at 250°. The ionizer was initiated 5 min after injection of the sample. For the trifluoroacetate, the column temperature was maintained at 190° for 5 min and then programmed at 10°/min to 240°.

GLC–CI mass spectrometry. All CI mass spectra were obtained with a Finnigan Model 1015C Quadrupole with (1) a Varian Aerograph Model 1400 gas chromatograph or more recently with (2) a Finnigan Model 9500 gas chromatograph. The manifold temperature was maintained at 175° with an interface temperature of 225°. The ionizer heater was kept off in order to minimize ion fragmentation due to thermal effects. Under the above conditions, the ionizer temperature because of manifold heat transfer is only 50–75° maximally. For methane or isobutane, the ion source pressure is maintained at 600–1000 μ with an overall pressure of about 10^{-5} Torr. The ion source conditions were as reported earlier[11,12].

After injection of the desired sample in the specific solvent, the ionizer is maintained off for a specified duration of time. The GLC column conditions were as indicated for EI.

Data acquisition. This dual Finnigan 1015C EI and CI mass spectrometer system[14] operated from a common electronic console is controlled by a System 150 data collection system (System Industries, Sunnyvale, Calif., U.S.A.) composed of a PDP-8E, magnetic tape drive, disk, plotter, teletype, and interfacing hard- and software. This laboratory is in a unique position of having both the EI and CI system from the same company so that they can be interchangeably interfaced to one computer system for collecting and processing of data. This enables one to make more confidently comparison statements regarding the advantages and disadvantages of one system over the other with a given series of compounds and other variables being the same.

RESULTS AND DISCUSSION

The consideration of prostaglandins as potentially hazardous environmental agents arises from at least two lines of thought. Because of the extreme potency and diverse effects produced by minute quantities of numerous prostaglandins on biological systems, the evaluation as to whether exogenous prostaglandins either relative to ingestion or to topical exposure should be considered environmental factors must be clarified. Secondly, one must determine whether endogenous prostaglandins play a significant role in the response of man and biological test systems to common environmental pollutants.

With respect to the biological activity of prostaglandins[16], those of the E type are potent bronchial dilators while prostaglandins of the F class are potent constrictors. Both classes of prostaglandins have been implicated in inflammatory responses.

The present investigations were undertaken to make available a more descriptive means of determination of chemical structure in order to study the biosynthesis and metabolism of prostaglandins in the lung. Correlations will be made relating the biological effects of common environmental pollutants on the systems responsible for the synthesis and metabolism of prostaglandins in the lung.

Care must be exercised in the choice of methods for administration of the prostaglandin sample into the mass spectrometer. Furthermore, as will be illustrated later, the type of derivative for a specific prostaglandin used in the identification and characterization by mass spectrometry will limit the degree of completeness of elucidation of chemical structure of these biologically active lipids.

As shown in Table I for PGE_2, the analytical conditions greatly alter the characteristics of the mass spectrum. In the EI spectra for both 10 eV and 70 eV, there are numerous indications of formation of ion fragments by the elimination of water ($M - x\ H_2O$) in sequence with fragmentation of the alkyl side chain and/or elimination of the carboxyl side chain. Very unexpected is the fragment ($M + 2\ H$) in the EI spectra which is present in an abundance of about 6% in the 10-eV spectrum and to a lesser extent (0.4%) in the 70-eV spectrum. In the isobutane spectrum of PGE_2 (Table I), three major ion fragments are formed by elimination of hydroxyl functions *m/e* 335, 317, and 299 —with a low abundance (0.8%) of $M + 1$ fragment and almost non-existent recombination fragments of the quasi molecular ion to yield *m/e* 391 (0.3%).

With the higher level of energy transfer using methane, PGE_2 has a base peak of *m/e* 317 as indicative with isobutane [$(M + 1) - 2\ H_2O$]. The ions in the quasi-molecular region ($M \pm 1$) are not significant. Furthermore, the carbonium ion formed by the elimination of one molecule of water followed by a recombination with methane ($C_2H_5^+$) to form *m/e* 363 seems to be very stable. This degree of stability of the ion formed by elimination of two molecules of water from the quasi-molecular ions ($M + 1$) to yield *m/e* 317 which may recombine with methane to yield *m/e* 345 is also comparable. As exemplified for other types of polyfunctional compounds[14], through an elimination process of the quasi-molecular ions ($M \pm 1$) coupled with a recombination with an ion of the reagent gas, a quite stable and abundant ion is formed. The instability of ions in the quasi-molecular region ($M \pm 1$) for PGE_2 as studied with methane and isobutane by CI mass spectrometry may be caused by thermal effects produced in vaporization of the underivatized prostaglandin at a probe temperature of 225–250°. On the contrary, the instability and low abundance of the quasi-molecular ($M \pm 1$) ions may be caused by a net overall interaction of the numerous functional groups of the molecule to yield a chemically and/or thermally labile component.

An earlier report[17] indicated some of the characteristics of PGA, and similar compounds using direct probe CI mass spectrometry with methane. Ions were of very low abundance in the molecular region[17]. Elimination of excess number of water molecules further complicated the complete understanding of these spectra[17].

Because of the high degree of polyfunctionality, the low abundance of ions in the molecular region, the elimination–recombination processes that take place with prostaglandins in the underivatized forms, and because of the needs for very elaborate means of purification prior to analysis by direct probe mass spectrometry, the most useful means of analysis would include derivatization after minimum purification followed by GLC coupled with CI and EI mass spectrometry.

TABLE I

PORTIONS OF MASS SPECTRA FOR UNDERIVATIZED PGE_2 (*m/e* 352)

Sample vaporized on direct probe at 225–250°.

*EI-70 eV**		*EI-10 eV****		*CI-Methane*§		*CI-Isobutane*§	
Fragment m/e	%	*Fragment m/e*	%	*Fragment m/e*		*Fragment m/e*	%
						391§§	0.3
				375§§	2.6		
						373	0.8
				363	13.4		
				355	0	355	1.3
354**	0.4	354**	5.3				
				345§§	9.4	345	0
				335§§	42.2	335§§	37.8
		334	2.6				
				317	100.0	317	100.0
316	0.4	316	4.4	316	5.7	316	3.0
				299	55.0	299	12.0
		298	3.5				
296	0.4	296	5.3				
				281	6.6	281	0.5
278	0.4	278	2.6				
				273	6.0	273	0.2
263	1.2	263	14.9	263	22.5	263	1.1
245	1.5	245	19.3	245	10.3	245	0.6
				233	5.1	233	0
227	1.1	227	12.3				
208	3.6	208	45.6	208	7.4	208	0.9
190	4.1	190	43.0	190	7.4	190	3.4
164	7.2	164	86.8				
				151	5.1	151	0.3
				119	2.8	119	0.8
109	13.2	109	78.9				
				107	3.4	107	0.8
105	14.6	105	36.8	105	1.7	105	0.3
				99	9.1	99	1.2
				95	3.4	95	0.9
94	17.0	94	100.0	94	2.2	94	0.5

* Base peak *m/e* 43.
** (M+2H).
*** Base peak *m/e* 94.
§ Base peak *m/e* 317.
§§ Elimination–recombination fragments.

Table II compares the EI and CI spectra of the underivatized $PGF_{1\alpha}$ by direct probe at 225–250°. In comparison to PGE_2 upon EI conditions, $PGF_{1\alpha}$ has a tendency to give up two protons in the molecular region with characteristics similar to hydrocarbons[8]. With methane there are not any detectable ions in the quasi-molecular region (M ± 1). With isobutane, $PGF_{1\alpha}$ forms a very weak ion (M − 1) at *m/e* 355.

As indicated earlier for PGE_2, $PGF_{1\alpha}$ using methane seems to form a much more stable ion by elimination of water with recombination with the methane fragment ($C_2H_5^+$) to produce the ion *m/e* 349 (5.1 %) than by direct recombination of the

TABLE II

PORTIONS OF MASS SPECTRA FOR UNDERIVATIZED $PGF_{1\alpha}$ (*m/e* 356)
Sample vaporized on direct probe at 225–250°

*EI-70 eV**		*CI-Methane****		*CI-Isobutane*§§	
Fragment m/e	*%*	*Fragment m/e*	*%*	*Fragment m/e*	*%*
				355§§§	1.7
354**	1.0				
		349§	5.1		
		339	25.4	339	100.0
		337	6.8	337	6.3
		335	3.4	335	7.4
		321§	100.0	321	73.7
		317	5.0	317	8.0
		303	93.2	303	30.9
296	1.0				
		295	83.1	295	71.4
		285	8.5	285	2.2
		277	37.3	277	6.3
266	1.7	266	32.2	266	17.7
		259	10.2	259	1.7
		257	5.1	257	23.0
		249	10.2	249	1.0
231	1.4	231	3.4	231	0
209	1.4	209	0	209	0
		207	10.2	207	1.1
		205	37.3	205	4.6
				195	2.3
191	1.4				
		189	10.2		
168	2.8				
165	2.4				
		163	33.9	163	12.6
159	1.7				
149	3.5				
123	5.6				
		109	16.9	109	2.3
107	10.5				
99	17.5	99	57.7	99	5.7
95	21.7				
81	49.0				

* Base peak *m/e* 43.
** (M−2H).
*** Base peak *m/e* 321.
§ Elimination–recombination fragments.
§§ Base peak *m/e* 339.
§§§ (M−1).

quasi-molecular ion with the ($C_2H_5^+$) ion. Numerous undesirable properties exist with the direct probe analysis of the prostaglandin in the underivatized forms by EI and CI mass spectrometry.

Because of the possibility of mislabeling of the above $PGF_{1\alpha}$ as exemplified by characteristics in the EI spectra and including the reactivity of the double bonds in this form, the $PGF_{1\alpha}$ and $PGF_{2\alpha}$ were first converted to the respective methyl esters

and then the free hydroxyl groups were silylated. Table III illustrates the usefulness of this derivative of the F type prostaglandin as characterized by CI mass spectrometry. Using methane as carrier gas, it is clear that one can very easily differentiate $PGF_{1\alpha}$ from $PGF_{2\alpha}$. In these spectra (Table III), the quasi-molecular ion (M — 1) is abundant (≈7%) with the recombination fragments $(M + C_2H_5)^+$, and $(M + C_3H_5)^+$ very noticeable. The base peak of $PGF_{1\alpha}$ and $PGF_{2\alpha}$ is the molecular ion less two tri-

TABLE III

METHANE CI MASS SPECTRA OF THE METHYL ESTER TRIMETHYLSILYL ETHER DERIVATIVES OF $PGF_{1\alpha}$ (*m/e* 586) AND $PGF_{2\alpha}$ (*m/e* 584)

$PGF_{1\alpha}$		$PGF_{2\alpha}$	
Fragment m/e	%	*Fragment m/e*	%
		625***	0.7
615*	4.3	613*	10.3
585**	6.9	583**	7.2
571	52.6	569	82.2
515	15.5	513	11.9
497	83.6	495	48.9
481	11.2	479	25.8
407	100.0	405	100.0
381	87.9	379	20.1
317	86.2	315	58.7
310	12.1	289	10.1
291	13.8	173	13.9
173	13.8	73	31.4
73	27.6		

* Recombination fragments $(M+C_2H_5)^+$.
** M−1.
*** Recombination fragment $(M+C_3H_5)^+$.

methylsilanol (TMSOH) groups, 407 and 405 respectively, with abundant $(M - CH_3)^+$ and $(M - TMSOH)^+$ fragments. The CI spectra of the two F prostaglandins (Table III) are complex with respect to the presence of ions other than those of the quasi-molecular region and their recombinations. In the area of these spectra, as we have seen for other polyfunctional compounds, the low-molecular-weight ions generated in the CI processes are very descriptive of the molecule. In addition, characteristic data of EI spectra greatly complement the CI data and conversely the CI data complement the EI data to the extent that in some cases one type of information alone would not solve conclusively the structural determination. The ion *m/e* 173, being generated by cleavage of the bond adjacent to the C_{15}-silylated hydroxyl group, is very informative of the chemical structures of the methyl side chain from C_{15} to C_{20}.

Fig. 1 illustrates the advantages of CI mass spectrometry over EI mass spectrometry. As seen in the EI spectrum, the highest mass fragment present is *m/e* 513, coinciding with the elimination of the five-carbon side chain. As discussed earlier, EI and CI spectra complement each other in that fragments which may be low or nonexistent in one analytical condition are abundant and very necessary for structural determination.

Another widely used derivative of the F series of prostaglandins is the methyl

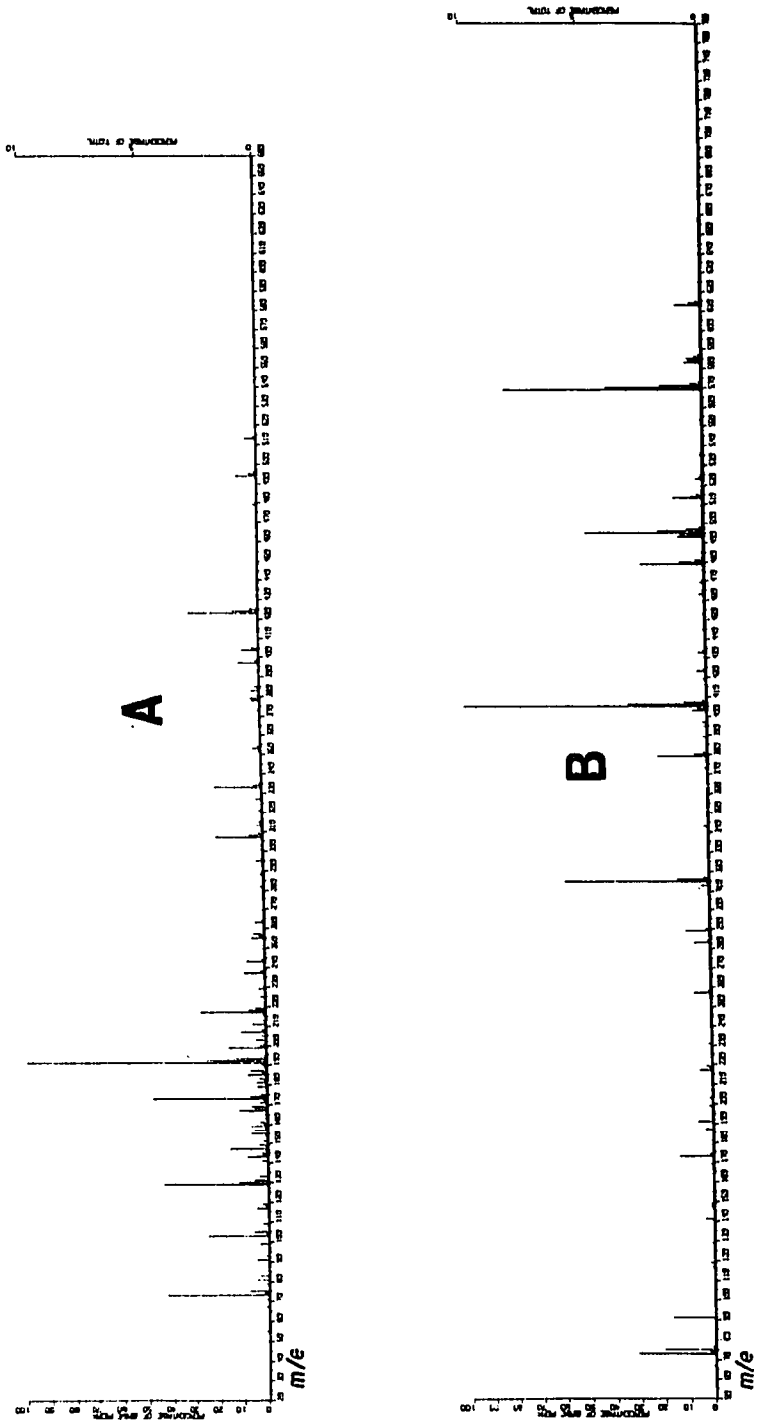


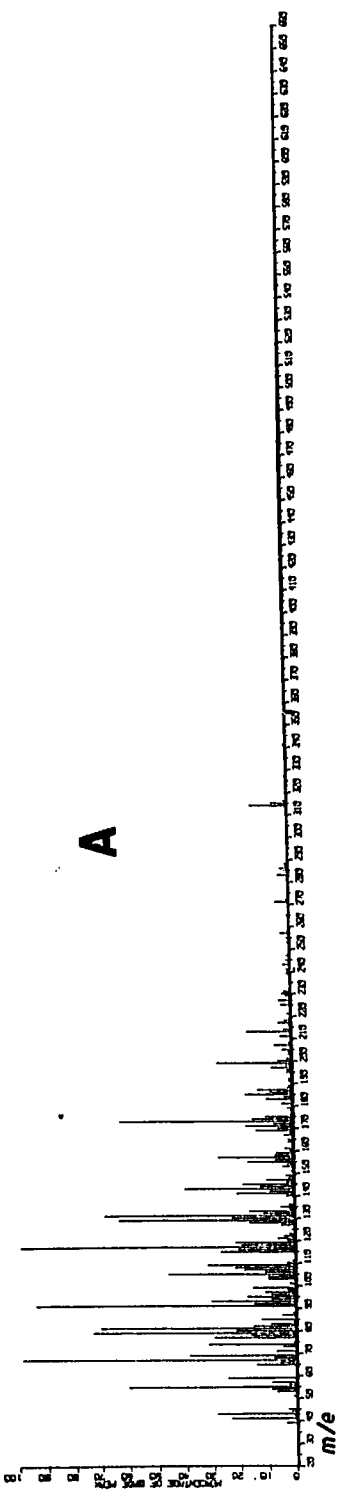
Fig. 1. 20-eV EI and methane CI mass spectra of the methyl ester trimethylsilyl ether derivative of $PGF_{2\alpha}$. (A) 20-eV EI spectrum. (B) Methane CI spectrum.

ester trifluoroacetates[3]. We have examined the possible use of this specific derivative for metabolite identification purposes. Thompson *et al.*[3] reported the characteristics of the 27.5-eV EI spectrum of the trifluoroacetate of $PGF_{2\alpha}$. Under the reported conditions[3], the highest mass fragment detectable was *m/e* 542, which was considered to be $(M - 114)^3$. Fig. 2 illustrates the characteristics of the 70-eV and 20-eV EI spectra for this derivative of $PGF_{2\alpha}$. The ion region from *m/e* 542 to 314 is very sparsely populated. On the contrary, this same sample when analyzed by CI mass spectrometry with methane and isobutane yields very abundant *m/e* 543, 429, and 315 fragments. The methane spectrum (Fig. 3) has *m/e* 571 and 583 fragments indicative of the respective recombination ions $(M + C_2H_5)^+$ and $(M + C_3H_5)^+$. The isobutane spectrum (Fig. 3) has a base peak of 543 $(M + 1)$ with less abundant 429, and 315 fragments with a *m/e* 581 recombination fragment $(M + C_3H_3)^+$. Similar recombination fragments $(M + C_3H_3)^+$ have been characteristic in isobutane spectra of other prostaglandin derivatives. Fig. 4A is the reconstructed gas chromatogram of the CI analysis of the trifluoroacetate derivative of $PGF_{2\alpha}$. Fig. 4B is the limited mass search for *m/e* 542–544 of this analysis. Under the presently reported analytical conditions, we have not been able to detect any ions or recombination ions of the methyl ester "tri" trifluoroacetate of $PGF_{2\alpha}$ *m/e* 656 using limited mass computer analysis or by varying analytical parameters. Furthermore, as exemplified by the recombination fragments in the methane and isobutane spectra, it was concluded that the major chromatographable product was the methyl ester "di" trifluoroacetate with a molecular weight of 542. As will be seen later for the acetate of $PGF_{2\alpha}$, in the presence of traces of acid either trifluoroacetic acid or acetic acid, $PGF_{2\alpha}$ tends to eliminate probably the C_{15} hydroxyl group and/or the respective acetate or trifluoroacetate to yield the diene. Chemical structures and structures of the possible component ions of the trifluoroacetate anhydride reaction are given in Fig. 5.

The general instability of natural prostaglandins in basic and acidic media has resulted in numerous challenges to the synthetic chemist in this area[6]. The general instability of the various types of hydroxyl groups of the E prostaglandin is well documented[6]. On the contrary, very little consideration has been given to this instability for the polyfunctional F type prostaglandins. Earlier investigators noted that $PGF_{2\alpha}$ was stable at room temperature at pH 5–11 for months[6] while substantial loss of biological activity occurred at pH 1–4.

As shown in the present report, the prostaglandin $PGF_{2\alpha}$ seems to have a great tendency to eliminate and/or dehydrate in the presence of acid. Because of this tendency, the trifluoroacetate and the later described acetate derivatives are not the most desirable derivatives for the structural characterization of the intact prostaglandins of the F type.

Derivatization of methyl ester of $PGF_{2\alpha}$ with acetic anhydride–pyridine resulted in the formation of a single chromatographable component similar in chemical structure to that of the trifluoroacetate. The isobutane spectrum of this acetate derivative has characteristic fragments at *m/e* 435, 375, 315, 283, 217, 187, and 61, with a base peak of *m/e* 315. The methane spectrum has mass fragments at *m/e* 435, 375, 315, 283, 187 and 61. These data indicate that only two acetate groups were present in the molecule. Elimination and/or dehydration seems to take precedence in this derivatization similar to that described for the trifluoroacetate. There was no detectable "tri" acetate derivative of $PGF_{2\alpha}$ present.



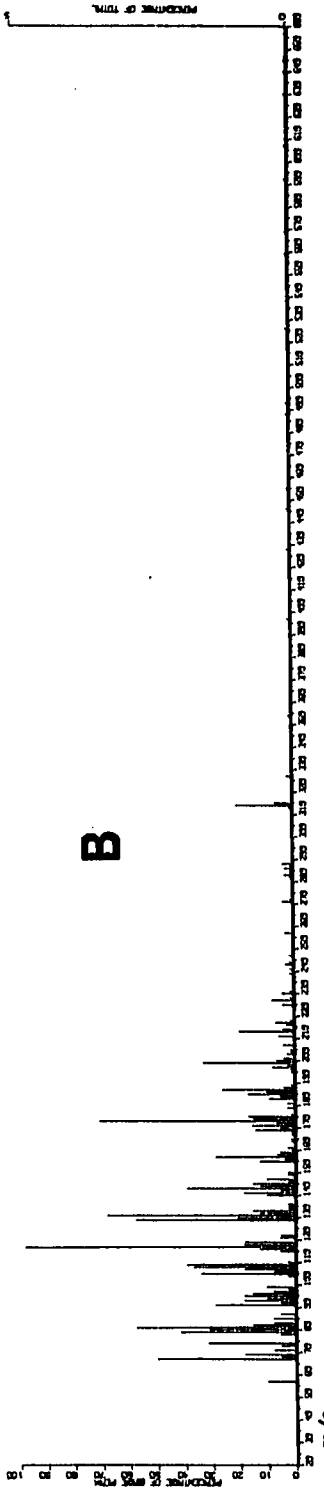


Fig. 2. EI mass spectra of the methyl ester trifluoroacetate derivative of $PGF_{2\alpha}$. (A) 70 eV. (B) 20 eV.

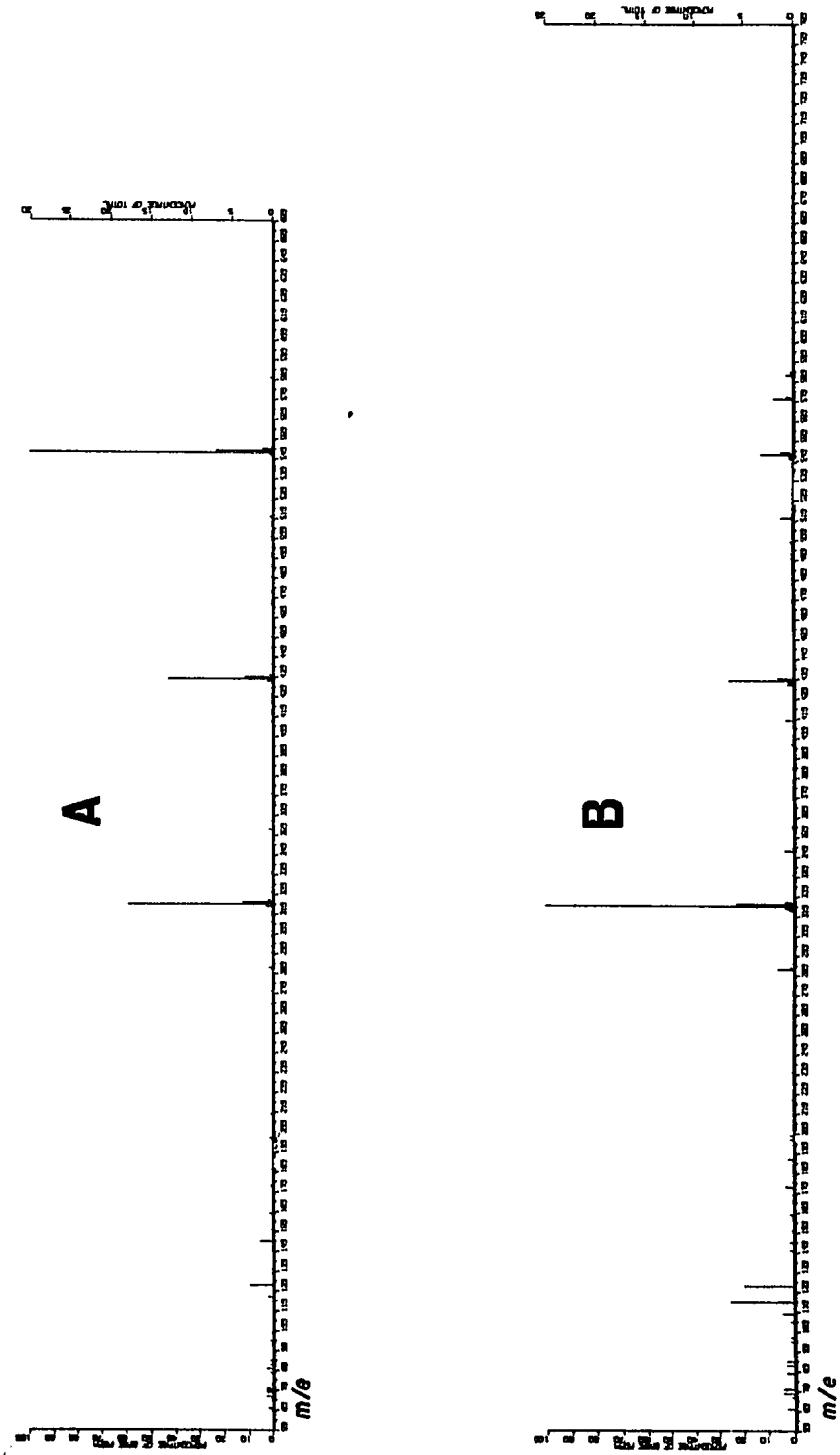


Fig. 3. CI mass spectra of the methyl ester trifluoroacetate derivative of $PGF_{2\alpha}$. (A) Isobutane. (B)Methane.

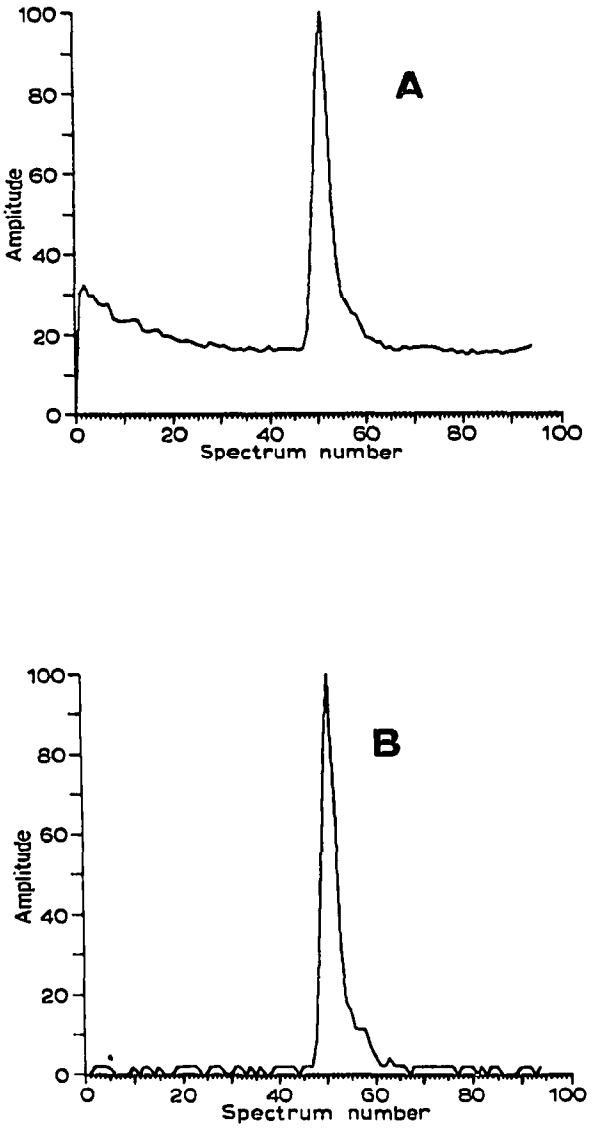


Fig. 4. Isobutane CI mass chromatograms of the methyl ester trifluoroacetate derivative of $PGF_{2\alpha}$. (A) Reconstructed gas chromatogram. (B) Limited mass search *m/e* 542–544.

An earlier report[15] indicated the undesirability of the formation of the acetate derivatives of the E prostaglandins with respect to the separation of PGE_1 and PGE_2. In addition, results from the present investigations indicate that the component analyzed by GLC–CI mass spectrometry after derivatization of PGE_2 with acetic anhydride–pyridine is not the intact prostaglandin but is an elimination product very similar to those described for the acetate and trifluoroacetate derivatives of $PGF_{2\alpha}$. The CI methane spectrum of this PGE_2 acetate contains characteristic ion fragments at *m/e* 432, 419, 405, 391, 371, 359, 331, 299, 243, and 190 with a base peak of 61. The

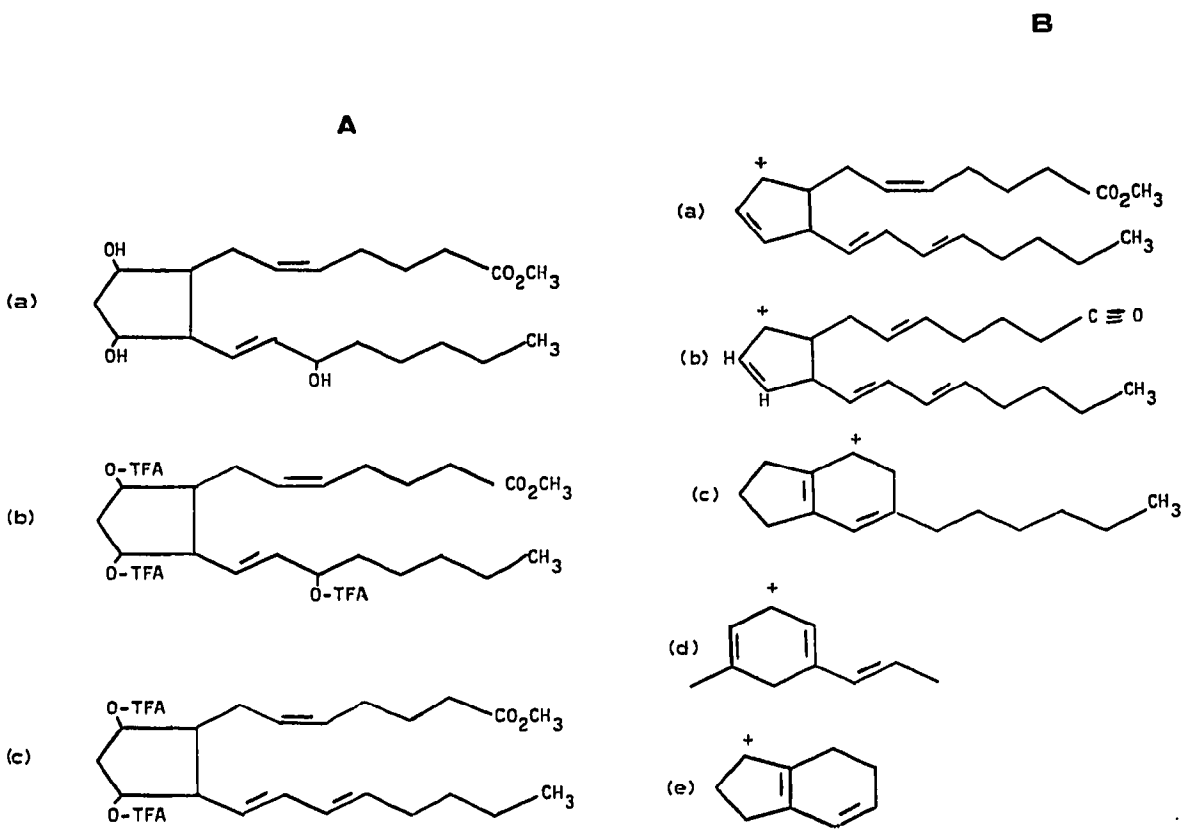


Fig. 5. Chemical structures and major ion fragments of the methyl ester trifluoroacetate derivative of $PGF_{2\alpha}$. (A) Chemical structures: (a) methyl ester of $PGF_{2\alpha}$; (b) "tri" trifluoroacetate of methyl $PGF_{2\alpha}$ (*m/e* 656); (c) major reaction product of trifluoroacetic anhydride plus methyl $PGF_{2\alpha}$ (*m/e* 542). (B) Major ion fragments: (a) *m/e* 314; (b) *m/e* 283; (c) *m/e* 199; (d) *m/e* 133; (e) *m/e* 117.

isobutane spectrum has mass fragments at *m/e* 433, 391, 371, 359, 331, 299, 190 and 61 with a base peak of 331.

Attempts to prepare the methyl ester trimethylsilyl ethers of the E prostaglandins were unsuccessful. As indicated earlier[15], it is not known whether the E prostaglandins failed to react or whether the derivatives did not elute from the chromatographic system. Present results using the GLC–CI mass spectrometer system suggest decomposition since there was continuous elution of non-differentiated components from the column.

The most desirable means of analysis of the E prostaglandins consists of conversion of these prostaglandins under alkaline conditions into the appropriate B prostaglandins followed by derivatization. Fig. 6 represents the CI mass spectra of the acetate derivative of PGB_2, which was prepared from PGE_2 as described above. The CI methane spectrum has characteristic ion fragments at *m/e* 431, 419, 391, 359, 331, 299, 85, 69, and 61 with a base peak of *m/e* 331. The very simple isobutane spectrum has mass fragments at *m/e* 429, 391, 331, and 61 with a base peak of 391. Unlike the CI mass spectra, in the 20-eV EI spectrum of PGB_2, the highest molecular weight

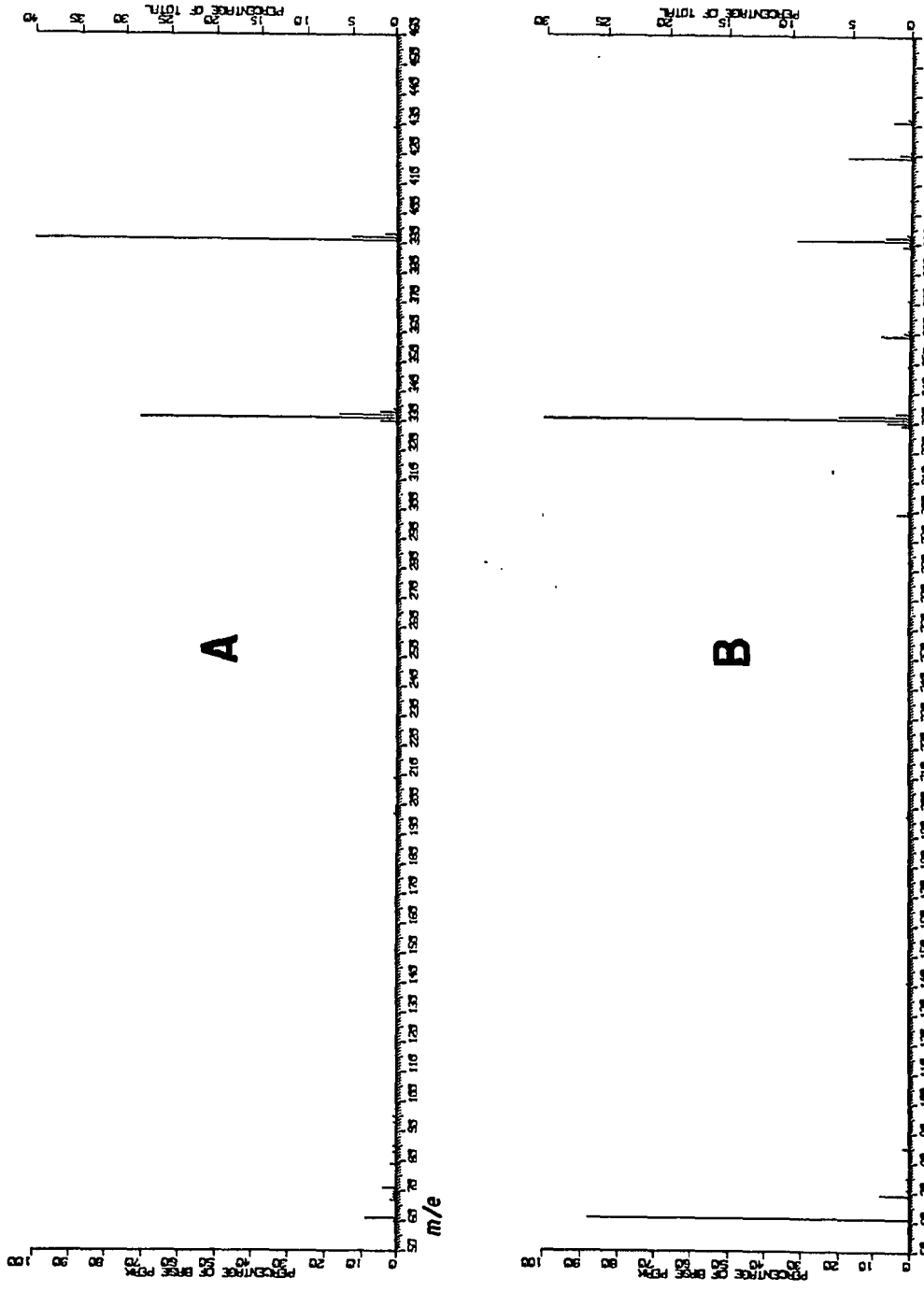


Fig. 6. CI mass spectra of the methyl ester acetate PGB_2. (A) Isobutane. (B) Methane.

fragment is *m/e* 330 (M − acetic acid) with abundant 247, 229, 217, 215, 203, 199, 187, 185, 175, 173, 171, 161, 159, 147, 133, 105 and a base peak of *m/e* 133. Fig. 7 summarizes the chemical structures of the most desirable derivatives of the E and F types of prostaglandin.

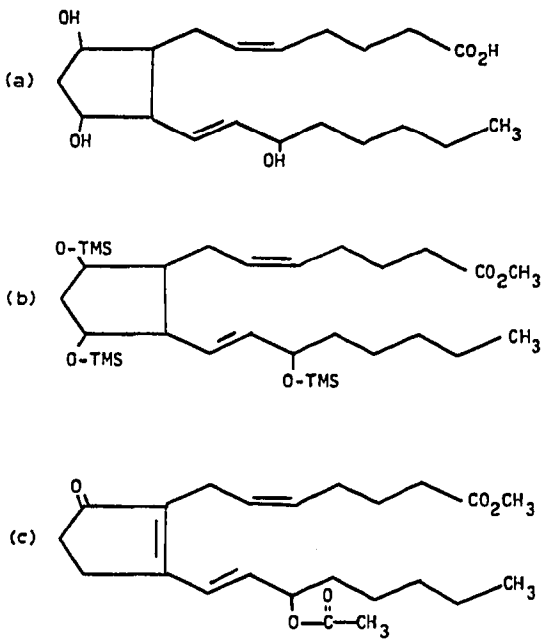


Fig. 7. Summary of chemical structures of the most desirable derivatives for the E and F types of prostaglandin. (a) $PGF_{2\alpha}$; (b) trimethylsilyl derivative of methyl $PGF_{2\alpha}$ (*m/e* 584); (c) acetate derivative of methyl PGB_2 (*m/e* 390).

As reported earlier for other polyfunctional compounds and their derivatives[11–14], because of the presence of a small number of ions in some CI mass spectra, the reporting laboratory recommends the complementary utilization of CI and EI mass spectrometry for the conclusive elucidation of the intact chemical structure of these lipids and other potentially hazardous environmental agents. As illustrated in this report, a combination of 10- or 20-eV and 70-eV EI data along with methane, isobutane and occasionally helium CI data facilitates the structure identification and characterization of unlimited types of organic compounds.

We have discussed only the qualitative uses of CI mass spectrometry for analysis of prostaglandins. Utilization of the CI mass spectrometry systems as a very sensitive means of quantitative analysis is very rewarding. The mass spectrometer is used as a specific ion detector in multiple ion detection or mass fragmentography. Instead of scanning a complete mass range, only specific ions are monitored. Quantitation in the low-picogram range is not uncommon by this technique. Enhancement of sensitivity by mass fragmentography has numerous advantages in the analysis and quantitation of drugs, environmental agents, their metabolites, and almost any compound that can be analyzed by GLC. A very recent report discusses the advantages of quantitative

mass fragmentography[18] in pharmacology and clinical medicine. By using GLC coupled with methane CI mass fragmentography, a sensitivity limit for the methyl ester trimethylsilyl ether derivative of the prostaglandin $PGF_{2\alpha}$ below 200 pg is obtained[18]. In the present report we have not stressed sample requirements, but using CI mass spectrometry with methane or isobutane we found 100–300 ng of sample to be very satisfactory for complete spectrum scanning. The level of detection for the discussed derivatives of the prostaglandins in the case of complete spectrum scanning or mass fragmentography will not be greatly limited by instrumentation but is limited mainly by the chemical lability of these polyfunctional lipids.

In concluding, we have described the advantages of utilization of GLC coupled with CI and EI mass spectrometry for the identification and characterization of prostaglandins and their analogues. The reporting laboratories have applied this powerful analytical tool to the investigation of the biosynthesis and metabolism of prostaglandins in the lung. Biological characterization and structure identification of prostaglandin metabolites will be discussed in a separate report.

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