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SYSTEM FOR THE IDENTIFICATION OF NOVEL PROSTAGLANDINS

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SUMMARY

A system for structural analysis based on thin-layer and gas chromatographic and mass spectrometric data is presented. It permits the rapid and unequivocal identification of structurally closely related prostaglandins and their products containing microbiologically introduced hydroxyl groups at positions C-16, -17, -18, -19 and -20. Novel criteria are described by which distinctions can be made between stereoisomers.

INTRODUCTION

The system introduced here was designed for the routine identification of prostaglandins. It appeared to be useful in the identification of unknown prostaglandins and in particular permits closely related isomeric compounds to be distinguished. An example of the compounds studied is given in Fig. 1.

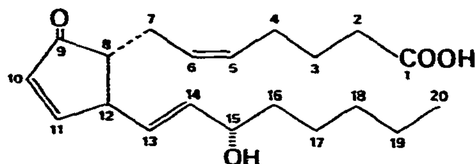

Fig. 1. Structure of PGA_2.

There are several series of prostaglandins, which differ from each other in the type of the five-membered ring (see Fig. 2). Numerical subscripts in the abbreviated

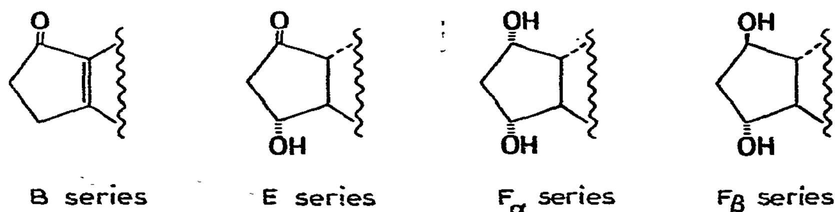

Fig. 2. Prostaglandin series.

nomenclature denote the number of specifically located double bonds in the side-chains, *e.g.*, *cis*-5 and *trans*-13 in PGA_2. Compounds such as $PGF_{1\alpha}$ contain only one, namely the *trans*-13 double bond.

The identification system was developed in order to elucidate the structure of novel prostaglandins obtained by conversion of natural prostaglandins and synthetic 11-deoxyprostaglandins with the aid of microorganisms[1,2], the main aim being hydroxylation. An analogous research programme using microbial conversions was carried out earlier with steroids[3].

Microbial conversions of prostaglandins have been reported, although they mostly refer to reductions of the 9- or 15-keto group and the Δ^{10} or the Δ^{13} double bond[4,5]. One instance of hydroxylation, however, has been described, *viz.*, the hydroxylation of PGA_2 at the C-18 position, combined with a reduction of the Δ^{10} bond[6].

In our laboratories a large number of novel hydroxyprostaglandins were prepared and isolated and identification data for about 70 of them are presented in this paper. The chromatographic and spectral data obtained from some series of these related compounds are suitable for establishing by comparison the common features of, *e.g.*, hydroxylation in a given position in a prostaglandin molecule. It appears that all modifications in a prostaglandin molecule can be detected on the basis of accompanying changes in the mass spectrum, the gas chromatographic (GC) retention time and the R_F value in thin-layer chromatography (TLC). The identification system started from the methods used by Bergström *et al.*[7], Gréen[8] and Granström and Samuelsson[9]. During our investigations, new rules and criteria were established so that the system could be extended to a versatile and very useful method for the identification of different types of natural and unnatural prostaglandins.

EXPERIMENTAL

As the system is designed for the routine analysis of prostaglandins of widely divergent types, the need arose for a standard procedure meeting all requirements and this procedure is outlined below.

Materials

The (±)-11-deoxyprostaglandins as well as some other prostaglandins that were used as starting compounds were provided by Syntex Research, Palo Alto, Calif., U.S.A. $PGF_{1\alpha}$, $PGF_{1\beta}$ and $PGF_{2\beta}$ were prepared by reduction of PGE_1 and PGE_2. The starting compound 11-deoxy-PGE_2 was obtained by microbial conversion of PGA_2.

Fermentation

Two milligrams of a starting prostaglandin dissolved in 50% aqueous ethanol were added to 10 ml of a culture of a microorganism. The wide variety of microorganisms and the methods used are summarized in refs. 1 and 2. The mixture was shaken, with rotation, at 26° for 24 h, acidified to pH 3 with 10% aqueous citric acid solution and extracted 3–6 times with ethyl acetate.

Thin-layer chromatography

The crude extracts were purified by preparative TLC on plates pre-coated

with silica gel (0.25 mm Merck Fertigplatten F_{254}). Eluent I, which was used in most instances, consisted of a mixture of ethyl acetate, *n*-heptane, acetic acid, methanol and water (45:40:4:2:1). Eluent II, which was employed for more polar compounds, was a mixture of the same components in the ratio 40:20:4:6:3. The prostaglandins were extracted with 3 ml of methanol with a recovery better than 50% for the more polar compounds.

Five microlitres of the extract were applied to a thin-layer plate and chromatographed once more under the conditions described above but with the difference that 3 μg of PGE_2 and $PGF_{2\alpha}$ were applied as well, so that the system could be standardized and quantified. After drying the plate, the spots were detected by spraying with a solution of copper(II) acetate in 15% aqueous orthophosphoric acid, followed by heating at 230° for 1–2 min. The R_F values were normalized on the basis of the determined mean values for the standards.

Derivatization

For further analysis, it was necessary to confer good thermal properties to the prostaglandins by derivatization. Good GC properties and an informative mass spectrometric (MS) fragmentation pattern were obtained with methyl ester, methoxime trimethylsilyl ether derivatives. An aliquot of the above extract, sufficient to contain about 200 μg of the compounds to be analysed, was taken. The sample was esterified with diazomethane in diethyl ether for 15 min, the solution was evaporated to dryness, the residue was dissolved in 0.2 ml of dry pyridine and 5 mg of methoxylamine hydrochloride were added. The reaction mixture was allowed to stand overnight at room temperature, and a diethyl ether–water extraction was carried out. The organic layer was evaporated to dryness under a stream of nitrogen and the residue silylated with 15 μl of a mixture of bistrimethylsilyltrifluoroacetamide (BSTFA), trimethylchlorosilane (TMCS) and pyridine (10:1:5). These conditions ensured complete silylation without dehydration even when tertiary hydroxyl groups were present. In special instances, silylation was achieved by a reaction with 15 μl of bistrimethyl-d_9-silylacetamide (BSA_{9d}).

Gas chromatography

The silylation mixture was injected directly into the gas chromatograph [Pye Unicam Model 104, equipped with a 5 ft. × 2.3 mm I.D. silanized glass column, containing 3% OV-17 on Gas-Chrom Q (100–120 mesh); oven temperature, 235°; injector temperature, 260°; carrier gas (nitrogen) flow-rate, 35 ml/min]. GC retention times were transformed into *C* values[7]. For this procedure, a mixture of normal fatty acid methyl esters was used as a standard. The *C* value was derived from a plot of the retention time (logarithmic scale) *versus* the number of carbon atoms in the acids (linear scale).

Mass spectrometry

For the GC–MS experiments, the gas chromatograph was coupled to an AEI MS 902 mass spectrometer (electron energy, 70 eV; trap current, 500 μA; source temperature, 250°) via a Watson–Biemann separator. The GC conditions were as described above, except that the column length was 3 ft. and the carrier gas was helium at a flow-rate of 15 ml/min.

Spectra file

For identification purposes, it was necessary to have a set of easily accessible reference spectra. A mass spectrum of each new compound was drawn on a linear mass scale in such a way that the smallest peaks needed for significant identification were just visible (height about 1 mm). As a consequence, a few peaks were too high to be depicted full size; their relative intensities (%) are labelled on the top of the spectra (Figs. 3–12).

RESULTS

The system of identification was developed starting from a series of 11-deoxy-prostaglandins. This proved to be a good starting point, especially for the MS analysis. In general, the derivatives used give rise to a well defined fragmentation. Highly intense MS peaks are obtained upon cleavage in the C-12 side-chain, and the same is true for the products formed from these starting prostaglandins by hydroxylation in the C-16/20 part of the aliphatic chain. There is no question of pattern complication in the mass spectrum caused by the 11α-hydroxyl group, but this effect is noticed when the system is extended to the analysis of prostaglandins of the E and F series. The results of the MS analysis are illustrated below and some typical spectra are presented in Figs. 3–12. The conclusions in the Discussion, however, are based on the spectra of all compounds listed in Table I.

The GC *C* values of the derivatives of the starting prostaglandins and a large number of conversion products are given in Table I. The *C* values are the means of 2–49 measurements. In a few instances where single measurements are tabulated, the *C* value is given with only one decimal figure. Each measurement is based on a sample extracted from a culture and purified, derivatized, chromatographed and finally identified by MS. The experiments were carried out randomly over a period of 4 years, involving occasional changes in experimentors and GC columns. The *C* values have standard deviations ranging from 0.04 to 0.11 (mean 0.07 *C* unit).

In the gas chromatograms of the derivatives of the prostaglandins of the A, E and 11-deoxy-E series, two peaks occur which may be attributed to the *syn*- and *anti*-isomers of the methoxime[8,10,11]. The distance between the two peaks is about 0.5 *C* unit. It appears that the isomers are not formed in equal amounts. Among the prostaglandins of the A series, the area of the first eluting GC peak is 30–50% larger, while in the E series the second GC peak exceeds the first by a factor of 4–6. With the B prostaglandins one peak is detected and probably only one of the isomers can be formed here[10].

R_F values which are of importance for the identification system are listed in Table II. Some of the values are based on single measurements. Mean R_F values from 2–26 observations had standard deviations between 0.008 and 0.016 R_F unit (mean 0.012).

The starting prostaglandins are usually converted by the selected[1,2] microorganisms into 20-hydroxyprostaglandins or mixtures of 18- and 19-hydroxy compounds. In some instances, 16- or 17-hydroxy compounds are found as by-products in 18- and 19-hydroxylation. Chain degradation to 2,3-dinor- and 2,3,4,5-tetranor-prostaglandins as well as combinations of chain degradation and hydroxylation also

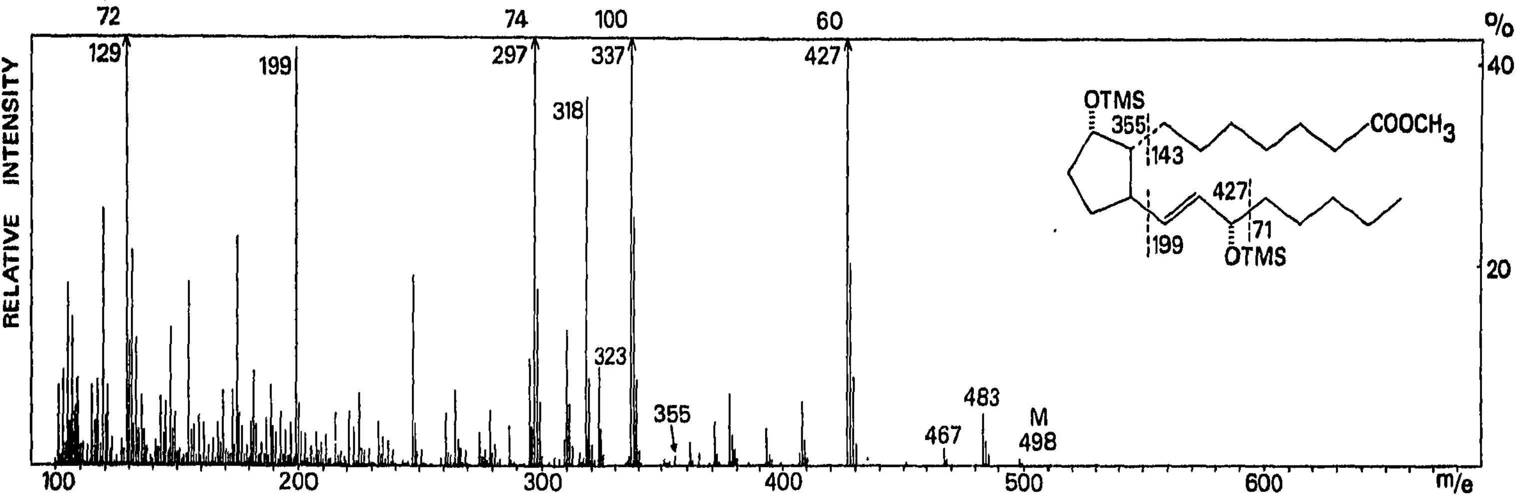

Fig. 3. Mass spectrum of the ME-TMS derivative of 11-deoxy-PGF$_{1\alpha}$.

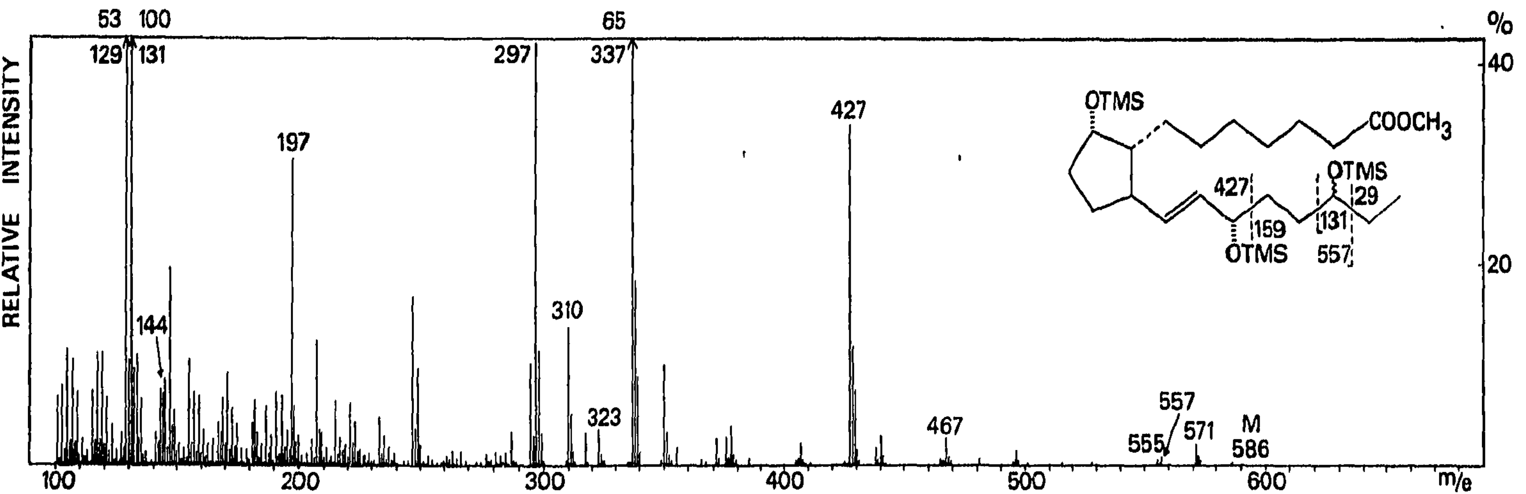

Fig. 4. Mass spectrum of the ME-TMS derivative of 18-hydroxy-11-deoxy-PGF$_{1\alpha}$.

Fig. 5. Mass spectrum of the ME-TMS derivative of 18-hydroxy-11-deoxy-$PGF_{1\beta}$.

Fig. 6. Mass spectrum of the first-eluting isomer of the ME-MO-TMS derivative of 19-hydroxy-11-deoxy-PGE_1.

Fig. 7. Mass spectrum of the second-eluting isomer of the ME-MO-TMS derivative of 19-hydroxy-11-deoxy-PGE_1.

Fig. 8. Mass spectrum of the second-eluting isomer of the ME-MO-TMS_{9d} derivative of 19-hydroxy-11-deoxy-PGE_1.

Fig. 9. Mass spectrum of the second-eluting isomer of the ME-MO-TMS derivative of 19-hydroxy-11-deoxy-15-epi-2,3,4,5-tetranor-PGE_1.

Fig. 10. Mass spectrum of the ME-TMS derivative of 19-hydroxy-11-deoxy-20-ethyl-15-methyl-$PGF_{1\alpha}$.

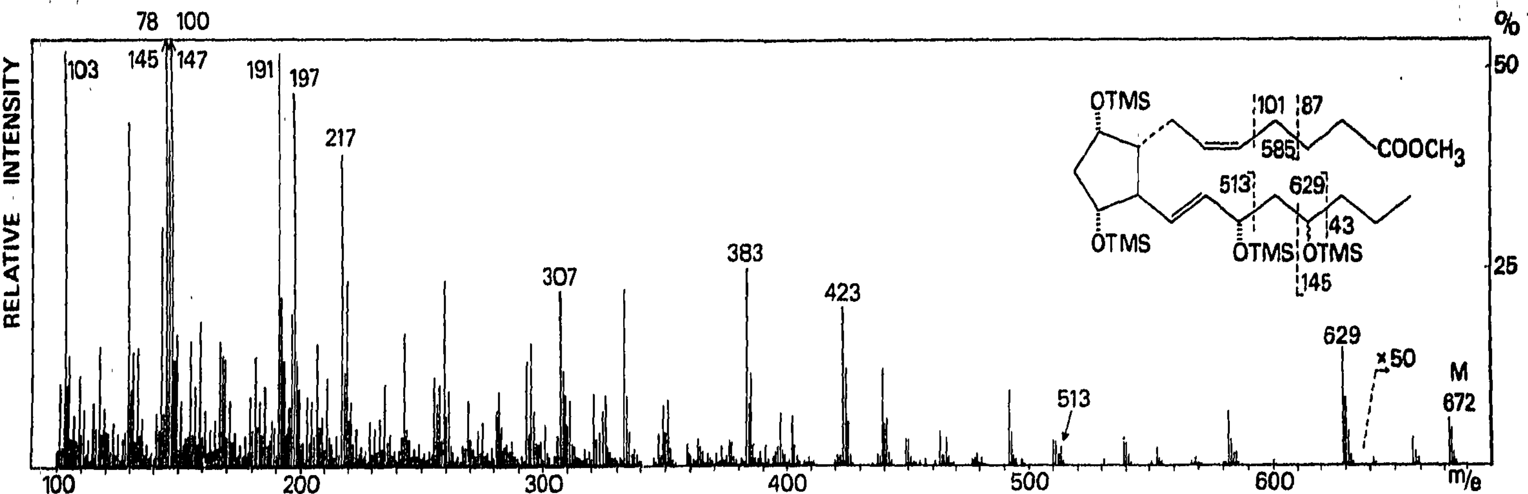

Fig. 11. Mass spectrum of the ME-TMS derivative of 17-hydroxy-$PGF_{2\alpha}$.

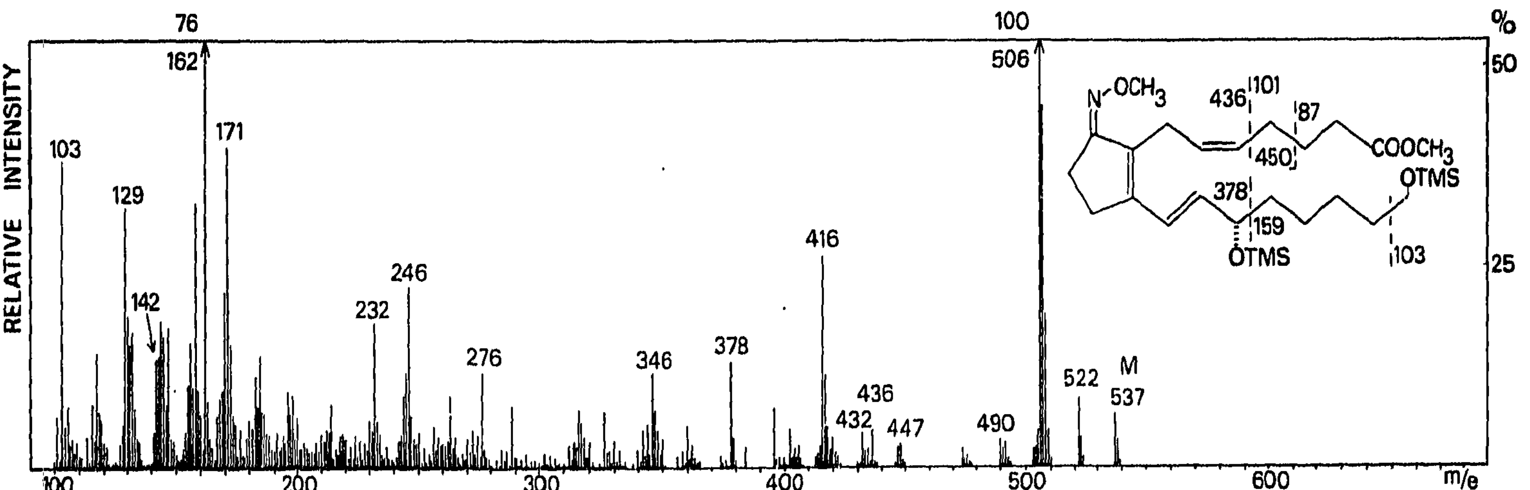

Fig. 12. Mass spectrum of the ME-MO-TMS derivative of 20-hydroxy-PGB_2.

TABLE I

C VALUES FOR PROSTAGLANDIN-METHYL ESTER-METHOXIME-TRIMETHYLSILYL ETHER DERIVATIVES ON OV-17

Mean *C* values from *n* measurements are tabulated. When two values are given, they refer to *syn*- and *anti*-isomers. Columns *i* give the increments to be added to the *C* values of the derivatives of the non-hydroxylated prostaglandins.

Starting prostaglandin (transformation products indented)	*Non-hydroxylated*		*Hydroxylated at C atom*														
			C-16			*C-17*			*C-18*			*C-19*			*C-20*		
	C	*n*	*C*	*n*	*i*	*C*	*n*	*i*	*C*	*n*	*i*	*C*	*n*	*i*	*C*	*n*	*i*
PGA_1	23.45								25.24		1.79	25.63		2.18			
	24.05	(2)							25.87	(8)	1.82	26.23	(3)	2.18			
PGA_2	23.30								25.17		1.87	25.53		2.23			
	23.85	(2)							25.70	(3)	1.85	26.10	(3)	2.25			
PGB_2	25.52	(4)							27.30	(11)	1.78	27.62	(37)	2.10	28.54	(9)	3.02
dinor-PGB_2	23.6	(1)															
tetranor-PGB_2	21.40	(2)															
13-*cis*-PGB_2												27.00	(7)		28.0	(1)	
11-Deoxy-PGE_1	23.57					—		—	25.44		1.87	25.73		2.16	26.7		3.1
	24.00	(3)				25.3	(1)	1.3	25.92	(9)	1.92	26.19	(33)	2.19	27.2	(1)	3.2
dinor-11-deoxy-PGE_1	21.6								—		—	—		—			
	22.1	(1)							23.97	(9)	1.9	24.20	(2)	2.1			
tetranor-11-deoxy-PGE_1	19.60														23.0		3.4
	20.10	(2)													23.4	(1)	3.3
13,14-dihydro-11-deoxy-PGE_1	23.80											—		—	27.12		3.32
	24.23	(3)										26.43	(10)	2.20	27.50	(4)	3.27
11-Deoxy-15-epi-PGE_1	23.60		—		—	—		—	25.53		1.93	25.82		2.22	—		—
	24.10	(2)	25.0	(1)	0.9	25.30	(2)	1.20	25.99	(10)	1.89	26.31	(16)	2.21	27.2	(1)	3.1
dinor-11-deoxy-15-epi-PGE_1	21.6								—		—						
	22.1	(1)							24.0	(1)	1.9						
tetranor-11-deoxy-15-epi-PGE_1	19.60								21.8		2.2	22.18		2.58			
	20.20	(2)							22.2	(1)	2.0	22.57	(3)	2.37			
11-Deoxy-PGE_2	23.49					—		—	25.30		1.81	25.66		2.17	26.59		3.10
	23.99	(8)				25.15	(2)	1.16	25.83	(21)	1.84	26.16	(28)	2.17	27.11	(15)	[illegible]

dinor-11-deoxy-PGE_2	21.7																
	22.1	(1)															
11-Deoxy-$PGF_{1\alpha}$	22.35	(2)				23.80	(2)	1.45	24.22	(5)	1.87	24.54	(5)	2.19			
tetranor-11-deoxy-$PGF_{1\alpha}$	18.80	(2)															
11-Deoxy-15-methyl-$PGF_{1\alpha}$	22.44	(5)							24.2	(1)	1.8	24.55	(2)	2.11			
11-Deoxy-15-epi-$PGF_{1\alpha}$	22.45	(2)	23.70	(2)	1.25	23.98	(4)	1.53	24.36	(37)	1.91	24.67	(49)	2.22	25.53	(7)	3.08
dinor-11-deoxy-15-epi-$PGF_{1\alpha}$	20.6	(5)															
13,14-dihydro-dinor-11-deoxy-15-epi-$PGF_{1\alpha}$	20.7	(1)															
11-Deoxy-$PGF_{1\beta}$	22.35	(3)				23.70	(2)	1.35	24.26	(10)	1.91	24.55	(11)	2.20	25.50	(4)	3.15
tetranor-11-deoxy-$PGF_{1\beta}$	18.77	(11)															
11-Deoxy-15-epi-$PGF_{1\beta}$	22.43	(6)				23.6	(1)	1.2	24.32	(20)	1.89	24.60	(25)	2.17	25.51	(19)	3.08
dinor-11-deoxy-15-epi-$PGF_{1\beta}$	20.4	(1)							22.4	(1)	2.0						
tetranor-11-deoxy-15-epi-$PGF_{1\beta}$	18.82	(14)															
11-Deoxy-15-methyl-20-ethyl-PGE_1	25.30											26.98		1.68	27.33		2.03
	25.80	(2)										27.46	(5)	1.66	27.77	(3)	1.97
11-Deoxy-15-epi-15-methyl-20-ethyl-PGE_1	25.40								—		—	26.92		1.52	27.41		2.01
	25.85	(2)							26.9	(1)	1.0	27.40	(4)	1.55	27.87	(7)	2.02
11-Deoxy-20-ethyl-$PGF_{1\alpha}$	24.20	(2)							25.55	(2)	1.35	25.90	(2)	1.70	26.27	(3)	2.07
11-Deoxy-15-methyl-20-ethyl-$PGF_{1\alpha}$	24.25	(2)							25.3	(1)	1.0	25.85	(2)	1.60	26.20	(3)	1.95
11-Deoxy-15-epi-15-methyl-20-ethyl-$PGF_{1\alpha}$	24.20	(2)							25.3	(1)	1.1	25.9	(1)	1.7	26.30	(2)	2.10
PGE_1	24.38					25.7		1.3	26.13		1.75	26.47		2.09			
	24.92	(5)				26.2	(1)	1.3	26.69	(11)	1.77	26.97	(9)	2.05			
dinor-PGE_1	22.40											24.5		2.1			
	22.90	(2)										25.0	(1)	2.1			
PGE_2	24.25								26.02		1.77	26.36		2.11			
	24.80	(2)							26.59	(12)	1.79	26.92	(13)	2.12			
$PGF_{1\alpha}$	23.58	(4)															
$PGF_{1\beta}$	23.00	(4)															
$PGF_{2\alpha}$	23.43	(3)				24.57	(3)	1.14	25.11	(12)	1.68	25.40	(11)	1.97			
$PGF_{2\beta}$	22.87	(2)															

TABLE II

R_F VALUES

The number of measurements is given in parentheses.

Starting prostaglandin	*Non-hydrox-ylated*	*Hydroxylated at C atom*				
		C-16	*C-17*	*C-18*	*C-19*	*C-20*
PGB_2	0.59 (1)			0.24 (8)	0.20 (23)	0.19 (6
11-Deoxy-PGE_1	0.66 (1)		0.35 (1)	0.30 (8)	0.25 (20)	0.24 (1
11-Deoxy-15-epi-PGE_1	0.68 (1)	0.43 (1)	0.40 (1)	0.34 (8)	0.28 (12)	
11-Deoxy-20-ethyl-15-methyl-PGE_1	0.62 (1)				0.43 (5)	0.42 (3
11-Deoxy-20-ethyl-15-methyl-15-epi-PGE_1	0.62 (1)				0.44 (2)	0.42 (6
11-Deoxy-PGE_2	0.54 (1)			0.32 (20)	0.25 (26)	0.24 (14
11-Deoxy-$PGF_{1\alpha}$	0.62 (1)			0.28 (3)	0.23 (4)	
11-Deoxy-15-epi-$PGF_{1\alpha}$	0.66 (1)		0.36 (2)	0.32 (17)	0.27 (26)	0.25 (1
11-Deoxy-20-ethyl-15-methyl-$PGF_{1\alpha}$	0.63 (1)					
11-Deoxy-20-ethyl-15-methyl-15-epi-$PGF_{1\alpha}$	0.63 (1)					
11-Deoxy-$PGF_{1\beta}$	0.56 (1)		0.33 (1)	0.25 (2)	0.20 (5)	0.20 (1
11-Deoxy-15-epi-$PGF_{1\beta}$	0.62 (1)			0.28 (13)	0.23 (16)	0.22 (4
PGE_1	0.31 (1)			0.27* (5)	0.23* (7)	
PGE_2	0.32			0.28* (7)	0.24* (8)	
$PGF_{2\alpha}$	0.22			0.22* (5)	0.19* (5)	
$PGF_{2\beta}$	0.17 (1)					

* R_F in eluent II.

occur. In the prostaglandins of the A series these modifications are accompanied by reduction of the Δ^{10} double bond.

The E prostaglandins appeared to be surprisingly stable in the procedures described above, provided that they were handled quickly and stored at −20°.

DISCUSSION

In order to identify the molecule on the basis of the experimental results, it is first necessary to establish the main characteristics of the molecule and then to study, in detail, the structural features in the various parts of the molecule. Details of this procedure are indicated below.

Type of molecule

Mass spectrometry. The mass spectrum provides information concerning the type and size of the molecule, chain length, type of ring and numbers and locations of the hydroxyl, keto and carboxyl groups and double bonds. The known spectra of the derivatized starting prostaglandins can serve as a first reference file for the identification of the microbial conversion products. Molecular transformations are distinguished by shifts of the characteristic peaks in the spectrum. Thus, a shift of the molecular ion peak makes it clear whether the transformation consists of hydroxylation, chain degradation or reduction of a double bond. 11-Deoxy-$PGF_{1\alpha}$, for example, has a molecular ion peak of m/e 498 (*cf.* Fig. 3). After microbial conversion, the value m/e 586 indicates hydroxylation (Fig. 4), while m/e 470 denotes chain degradation to a dinor compound and m/e 558 is due to a combination of hydroxylation and chain

degradation. Where the mass spectra of the usual derivatives do not give unequivocal information, derivatization to trimethyl-d_9-silyl ethers or methyl-d_3-esters may be useful (*cf.*, Fig. 8).

Gas chromatography and thin-layer chromatography. The extent to which the C and R_F values are changed may give a clue to the modification. For example, a sharp decrease in the C value, combined with a moderate decrease in lipophilicity, indicates chain degradation to tetranor compounds. An increase in the C value and a lower R_F is observed in the case of hydroxylation.

Site of modification

Mass spectrometry. (a) Modifications in the C-8 side-chain. The intact chain has very faint features, such as a slight contribution to *m/e* 143 $\{[(CH_2)_6COOCH_3]^+\}$ and an $[M-143]^+$ peak, which is very small or sometimes even not observable. Usually, the peaks $[M-143-n\,(CH_3)_3SiOH]^+$ (n = 1,2,3,4), in which consecutive losses of $(CH_3)_3SiOH$ occur, are observable.

The spectrum of a compound containing a Δ^5 chain has a very small *m/e* 141 peak and, as a rule, relatively small $[M-141-n\,(CH_3)_3SiOH]^+$ peaks. In addition, its $[M-87]^+$ and $[M-101]^+$ ions are instructive (see inserts in Figs. 11 and 12).

In the C-8 chain, microbial conversion sometimes occurs as degradation to, *e.g.*, 2,3-dinorprostaglandins. Instead of $[M-143-n\,(CH_3)_3SiOH]^+$ ions, $[M-115-n\,(CH_3)_3SiOH]^+$ ions are then formed. In the spectra of dinor-Δ^5 compounds, $[M-113]^+$ and the ions derived from it occur, as do small amounts of $[M-87-n\,(CH_3)_3SiOH]^+$ ions in 2,3,4,5-tetranor structures (Fig. 9).

(b) Modifications in the ring. Information about the intact 11-deoxy-ring alone is very scanty. However, much information can be gained from the fragments containing that ring plus the C-8 side-chain.

With those prostaglandins of the 11-deoxy-F series in which there is an unchanged C-8 side-chain, such a fragment may make a major contribution to the peak *m/e* 297.

The localization of the relevant fragment was performed in the following way. The peak is shifted to *m/e* 355 for the silyl ester, to *m/e* 269 for the dinorprostaglandins, to *m/e* 241 for the tetranorprostaglandins and to *m/e* 306 for the trimethyl-d_9- silyl derivatives. No shift was observed on transition to 15-methyl-, 20-ethyl- and 17-, 18-, 19- and 20-hydroxyl compounds (Figs. 3, 4 and 10). A proposed structure for this fragment is illustrated in Fig. 13.

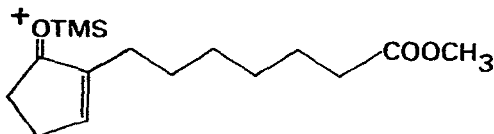

Fig. 13. Structure of ion of *m/e* 297.

On introduction of an 11α-OTMS group into the ring, a shift to *m/e* 385 occurs, as is observed in $PGF_{1\alpha}$. For $PGF_{2\alpha}$ the peak shifts to *m/e* 383. The presence of a 9,11-dihydroxy system can also be postulated from the stabilization of a fragment to a very intense ion at *m/e* 217 (ref. 9) and a rearrangement ion at *m/e* 191 (refs. 12 and 13).

In the 11-deoxy-E prostaglandins, the ion at *m/e* 222 (see Figs. 7 and 8) represents the intact ring plus intact C-8 chain minus CH_3OH. Hydroxylation in the ring causes the peak to shift to *m/e* 310, but this peak is not clearly observable in all E prostaglandins. A clear indication of the presence of an 11-hydroxyl group is given by the peak at *m/e* 133, which occurs in all E prostaglandins.

In the prostaglandins of the B_2 series, the ion at *m/e* 162 (Fig. 12) is of interest for the identification of the intact ring, including the methoxime group. Presumably it has a highly stabilized bicyclic structure in which the second ring contains the atoms C-6, -7, -13 and -14 (see Fig. 14).

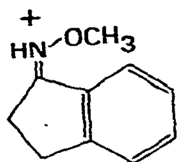

Fig. 14. Structure of ion of *m/e* 162.

On transition to ethoxime derivatives[14], there is a shift to *m/e* 176. The spectra of the derivatives of prostaglandins of the B_2 series lack this peak in a special instance, when isomerization occurs probably to Δ^{13}-*cis* compounds[15,16]. It is obvious that the bicyclic structure cannot be formed easily in these compounds. On isomerization to A_2 prostaglandins, the *m/e* 162 peak also disappears.

(c) Modification in the C-12 side-chain. The intact chain is characterized by the intense peak $[M-71-n\,(CH_3)_3SiOH]^+$, *m/e* 199 and 173 (ref. 13). Hydroxylation in the C-16/20 part of the aliphatic chain is demonstrated in the mass spectrum by the presence of an $[M-159]^+$ ion $(M-C_5H_{10}OSi(CH_3)_3)$ instead of an $[M-71]^+$ ion $(M-C_5H_{11})$[17,18] (see Figs. 3 and 4). In the spectra of 20-ethylprostaglandins, the ion $[M-187]^+$ occurs instead of $[M-99]^+$ (see Fig. 10). These characteristic peaks are very intense in the spectra of most 11-deoxyprostaglandins except in those of the B series. They are small in the spectra of all prostaglandins containing an 11-hydroxyl group, but in the E series they are intense in the first-eluting isomer and rather small in the second.

Further evidence pointing to hydroxylation in the C-16/20 part of the aliphatic chain is provided by a peak at *m/e* 197 instead of 199. Hydroxylation in the 15-methyl compounds results in a peak at *m/e* 211 instead of 213 and in the 15-methyl-20-ethyl analogues in a peak at *m/e* 239 instead of 241 (see Fig. 10).

An accurate determination of the site of hydroxylation is possible on the basis of peaks resulting from α-cleavage relative to the introduced $(CH_3)_3SiO$- group. These are *m/e* 103 (Fig. 12); *m/e* 117 and a contribution to $[M-15-n \times 90]^+$ (Fig. 8); *m/e* 131 and $[M-29-n \times 90]^+$ (Fig. 4); *m/e* 145 and $[M-43-n \times 90]^+$ (Fig. 11); and *m/e* 159 and $[M-57-n \times 90]^+$ for 20-, 19-, 18-, 17- and 16-hydroxyl compounds, respectively. In the 20-ethyl compounds 20-, 19- and 18-hydroxylation is characterized by *m/e* 131 etc., 145 etc. and 159 etc., respectively (Fig. 10).

It follows that when the spectral features are used in a uniform identification system, the site of hydroxylation may best be classified by counting down from the terminal carbon atom.

In addition to these characteristic peaks, there are those which, although less intense, are representative of the site of hydroxylation. Their structures have not yet been fully elucidated, however. Thus, there is a small peak at m/e 142 on ω-hydroxylation (Fig. 12). On $\omega-1$ hydroxylation there is an extra contribution to the peak at m/e 143 (see Figs. 7 and 8) and peaks at m/e 144 and 157 are representative of $\omega-2$ hydroxylation (Figs. 4 and 5).

Gas chromatography. For the identification of a limited group of related compounds such as microbial hydroxylation products of certain prostaglandins, sufficient information can usually be gained from the GC C values. It appears that the C value has a specific increment for each type of modification in the molecule. This increment seems to be nearly independent of the type of starting prostaglandin. In this way, the site of hydroxylation can be established on the basis of an increase in C value over that of the starting prostaglandin, as illustrated in the increment columns in Table I. Even if there are several modifications in one molecule, the system remains fully additive. The C value, for example, for the second isomer of the derivative of 19-hydroxy-13,14-dihydro-11-deoxy-PGE_1 is tabulated as 26.43. Calculation starting from 11-deoxy-PGE_1 and using the mean increment values 0.24 and 2.18 for the reduction of the Δ^{13} double bond and the introduction of the 19-hydroxyl group, respectively, gives $C = 26.42$.

Practice demonstrated that for normal identification purposes it is sufficient to use a set of mean increment values as given in Table III. With this table, the C value of any compound mentioned in Table I can be calculated starting from any other prostaglandin. The calculated values are in excellent agreement with the measured values. By comparing the C values of various series of prostaglandins, it is possible to calculate increments, *e.g.*, for the transitions between those series or for chain lengthening and shortening, respectively. The C value for the second isomer of the derivative of 19-OH-PGA_2 is calculated from that for 19-OH-PGE_2: $26.9-p = 26.0$ (p refers to the increment for the transition A $\rightarrow$ E series: see Table III). The measured value is 26.10.

Calculations in many steps also give good results. The C value for derivatized 18-OH-$PGF_{2\alpha}$, for example, is calculated from 20-OH-11-deoxy-15-epi-$PGF_{1\beta}$ (as a theoretical example) via $25.5-a-v-r-l+c = 25.2$ (measured value = 25.11). The increments for the 20-ethyl compounds cannot be fitted into the system unless the site of hydroxylation is established by counting down from the ω-C atom. An example is the calculation of the C value for derivatized 20-OH-11-deoxy-15-methyl-20-ethyl-$PGF_{1\alpha}$ using that for 20-OH-11-deoxy-PGE_1 as a starting point: $27.2-a-s+i+h+c = 26.2$ (measured value = 26.20). The importance of calculating a C value lies in providing additional evidence for identification when the mass spectrum gives no conclusive answer, *e.g.*, in the spectra of incompletely resolved GC peaks.

For some of the prostaglandins mentioned here, retention indices[10,19] and increments[20,21] have been reported in the literature. The conversion of these indices into C values using the experimentally determined equation

$$C = 0.01\,(\text{retention index} - 430)$$

for the C range 18–26 showed good agreement with our results.

TABLE III

INCREMENTS IN *C* VALUES

Type of modification		*Index*	*Increment (C units)*
Introduction of ω-OH		a	+3.1
Introduction of ($\omega-1$)-OH		b	+2.2
Introduction of ($\omega-2$)-OH		c	+1.9
Introduction of ($\omega-3$)-OH		d	+1.4
Introduction of ($\omega-4$)-OH		e	+1.1
Degradation to dinor		f	−1.9
Degradation to tetranor		g	−3.7
Introduction of 20-ethyl		h	+1.9
Introduction of 15-methyl		i	0.0
Introduction of 13,14-dihydro		j	+0.2
Introduction of 10,11-dihydro		k	+0.1
Introduction of 5,6-dihydro		l	+0.1
Transition	A* → B series	m	+1.6
Transition	B → 11-deoxy-E* series	n	−1.5
Transition	11-deoxy-E* → E* series	o	+0.8
Transition	E* → A* series	p	−0.9
Transition	E* → F_α series	q	−1.4
Transition	F_α → 11-deoxy-F series	r	−1.1
Transition	11-deoxy-F → 11-deoxy-E* series	s	+1.7
Transition	F_α → F_β series	t	−0.6
Transition	11-deoxy F_α → 11-deoxy F_β series	u	0.0
Transition	to 15-epi series	v	+0.1
Transition	between *syn*- and *anti*-isomers	w	0.5
Transition	methyl → silyl ester	x	+0.5

* Second isomers.

Distinction between the isomers at C-9

Mass spectrometry. The spectra of the derivatives of the F_α prostaglandins show obvious differences in relative peak intensity on comparison with those of the corresponding 9β-isomers. A quantitative approach to the intensity data of specific peaks appeared to be a good means for the discrimination of these isomers. As during the fairly slow MS scan over the GC peak the ion current changes markedly, intensities need to be expressed relative to that of a nearby reference peak.

A diagnostic peak in the spectra of the derivatives of the 11-deoxy-F prostaglandins with an unchanged C-8 side-chain occurs at *m/e* 297 (see Figs. 3—5, 10 and 13). The peak at *m/e* 323 which occurs in the spectra of all of these prostaglandins provides a useful reference and is fairly constant in relative intensity. For 14 different prostaglandins containing a 9α-$(CH_3)_3SiO$ group the intensity ratio I_{297}/I_{323} ranges from 5 to 20. The peak at *m/e* 297 is very intense, irrespective of any hydroxylation in the C-16/20 part of the aliphatic chain. For 10 compounds containing a 9β-$(CH_3)_3SiO$ group, this ratio is 3–4 (Figs. 4 and 5). It appears that the range in which the ratios for the 9α-isomer are found does not overlap with that for the 9β-isomers. Moreover, it appears that for each pair of isomers the intensity ratio for the 9α-isomer is about five times larger than in the corresponding β-compound.

In the 2,3-dinorprostaglandins, the C-8 side-chain is shorter and the diagnostic and reference peaks are shifted by 28 a.m.u. The intensity ratio I_{269}/I_{295} is $>$ 10 for the α-isomer and 4–5 for the β-isomer. In the 2,3,4,5-tetranor compounds, I_{241}/I_{267} =

1–3 for the 9β-isomer. The 9α-isomer lacks this type of fragmentation pattern due to δ-lactone formation[22].

In prostaglandin derivatives with an extra OTMS group in the ring, the diagnostic peak is shifted to *m/e* 385. Using *m/e* 411 as a reference peak, the intensity ratio I_{385}/I_{411} is 18 for $PGF_{1\alpha}$ and 4 for $PGF_{1\beta}$. Although the significant peaks are less intense, the same phenomena are observed as mentioned above for the 11-deoxy-prostaglandins.

Gas chromatography. The *C* values of the two C-9 isomers of the 11-deoxy-F prostaglandins appear to be equal in measurements performed in the same period of time. When an 11α-$(CH_3)_3SiO$ group is also present in the molecule, the *C* value of the 9β-isomer appears to be 0.6 *C* unit smaller than that of the corresponding 9α-isomer; it is 23.6 for $PGF_{1\alpha}$ and 23.0 for $PGF_{1\beta}$ (Table I).

Thin-layer chromatography. This technique also permits the differentiation between the 9α- and the corresponding 9β-hydroxyl compounds in the group of 11-deoxy-F prostaglandins. The R_F value of the α-isomer always exceeds that of the corresponding 9β-structure (Table II).

Distinction between the isomers at C-15

Thin-layer chromatography. The isomers at C-15 can only be distinguished in a TLC analysis. In general, the R_F values of the 15-S-hydroxyl compounds are lower than those of the corresponding 15-epi compounds (Table II). In 15-methylprostaglandins the two isomers have the same R_F value in the eluent systems used.

Distinction between the syn- and anti-isomers of the methoxime

Mass spectrometry. The mass spectra of the two isomers (see Results) show obvious differences in relative peak intensity, which permits the recognition of the isomer concerned. In these spectra ions X of the type $[M-15]^+$, $[M-71]^+$ or $[M-159]^+$ are accompanied by pairs of ions $[X\text{-}30]^+$ and $[X\text{-}32]^+$. The intensity ratio I_{X-32}/I_{X-30} for the first-eluting GC peak is larger than that for the second (Figs. 6 and 7). Often, this ratio is > 1 for the first and < 1 for the second peak. Interference, may occur, however, and is caused by another fragmentation pathway, leading to the same *m/e* values.

As a consequence these criteria may help to establish the order of elution of isomers. From this the presence of 8-isoprostaglandins may be concluded, which are assumed to be eluted in the reverse order of isomers[23].

Overall analysis

Various methods and techniques for the analysis of prostaglandins have been presented. When a starting prostaglandin has a known structure and physical characteristics, a specific decrease in the R_F value after microbial conversion may suggest hydroxylation but the site of substitution cannot be determined exactly by TLC alone. The *C* value may give a clue to the site of hydroxylation but MS is required in order to establish this unambiguously.

With unknown prostaglandins, all three techniques are necessary, as together they permit a complete identification in many instances. Two compounds may have the same *C* and R_F values and yet may have different mass spectra. This occurs with, *e.g.*, 19-OH-11-deoxy-$PGF_{1\alpha}$ and 19-OH-11-deoxy-15-epi-$PGF_{1\beta}$. The mass spectrum

alone is not always sufficient for unequivocal identification, *e.g.*, for the derivatives of 11-deoxy-$PGF_{1\alpha}$ and its 15-epimer. Moreover, these compounds have identical *C* values and differ only in their R_F values. Also, a single GC peak may give rise to a spectrum which, on comparison with the file, turns out to be a combination of two or more spectra.

CONCLUSION

Rules and criteria for identification such as compound-independent C increment values were established, which appear to hold for a large variety of prostaglandins.

A novel tool based on MS peak intensity ratios for the differentiation between 9α and 9β-OH or *syn*- and *anti*-isomers is provided. Examples indicate that a field, which in a recent review article[24] was mentioned as being unexplored, has now been opened up.

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