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METHODS OF ANALYZING PROSTAGLANDINS

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In recent years, the number of publications devoted to the prostaglandins (PGs) and of patents and investor's certificates connected with this comparatively recently discovered class of natural physiologically active compounds have been constantly rising. A large number of reviews have been devoted to the prostaglandins, but only in some of them [1-6] are methodical approaches to the analysis of PGs considered, and there are no special reviews including all the analytical methods for their investigation.

The aim of the present paper is to cover in condensed form all methods of analyzing PGs.

Method of Analyzing the Physiological Activity of the Prostaglandins

For a long time, the only method of determining the physiological activity of the PGs and their amounts in tissues under investigation was the use of their capacity for acting on the smooth musclature (stomach, intestine, large intestine, uterus), the blood pressure, etc. [1]. All methods of determining PGs using isolated sections of the smooth musculature have a low specificity for the individual types of PGs, but are fairly sensitive and permit them to be detected in concentrations of $1 \cdot 10^{-8}$ to $50 \cdot 10^{-8}$ g/ml (rat uterus) and less than 10^{-9} g/ml. More recently, a series of biochemical methods has been developed of which the following must be mentioned:

a) The enzymatic method, consisting in the oxidation of the 15(S) alcoholic hydroxyl of a PG with purified prostaglandin-15(S) dehydrogenase (from porcine lung) in the presence of NAD^+ and the spectrometric detection of the NADH formed [7, 8] (sensitivity about $3.5 \cdot 10^{-10}$ g). The method is not specific for individual types of PGs and is inapplicable to PGB;

b) a method of determining the change in the amount of c-AMP in the cells of the mouse ovary under the action of PGs and their analogs [9], based on determining the amount of labeled c-AMP [10, 11] formed on the incubation of the ovary tissue with [8^{14}-C]adenine;

c) a method of determining the affinity (capacity for binding) of the compound under investigation with prostaglandin receptors — rat lipocytes; the results are expressed in the number of nanograms of test compounds displacing 1 ng of labeled PGE per binding section [12].

A radioimmunological method is also distinguished by very high sensitivity (10^{-10} g) [13-17], but the antibodies to the prostaglandins obtained in this way do not possess selectivity, which interferes with the identification of the individual types of PGs.

Chromatographic Methods of Separating and Identifying the Prostaglandins

Methods of separating the PGs have been described in special reviews [3, 5] and they are considered in fairly great detail in other reviews [2, 4]. In working with the PGs,

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their metabolites, and derivatives (methyl ethers, acetates of the methyl ethers, etc.) many workers use mainly TLC on silica gel for analytical purposes. In a number of publications the results are given of a systematic study of the chromatographic behavior of PGs and their derivatives [18-21].

In many investigations [18-26] a large number of solvent systems have been used, of which the most common are the following: chloroform–methanol–water (80:10:0.5); benzene–dioxane (5:4); hexane–diethyl ether (1:1); ethyl acetate–methanol–water (8:2:5) and (160:25:10), upper phase; benzene–dioxane–acetic acid (20:20:1); and chloroform–methanol–acetic acid–water (90:10:1:0.8).

Revealing agents used are 50% sulfuric acid (followed by heating to 200-300°C), a 10% methanolic solution of phosphotungstic acid (followed by heating to 100°C), iodine vapor, and a 1% solution of vanillin or copper acetate in 15% phosphoric acid (followed by heating to 80-90°C). In the last two cases, the PGEs are colored yellow, and PGFs pale blue [2]. The sensitivity of these methods is about 10^{-6} g. The separation of components differing in degree of unsaturation is achieved by impregnating silica gel with 3-20% of silver nitrate [18, 19, 22, 23, 26-28].

In some cases [29-32] a number of variants of paper chromatography has been used successfully for the separation of the PGs. For the preparative separation of the PGs, together with TLC wide use is made of column chromatography on silica gel. Thus, for example, by using as the eluting systems mixtures of benzene and ethyl acetate [28], cyclohexane–ethyl acetate–methanol [2], and hexane–ethyl acetate–acetone [32] it is possible to achieve the separation of mixtures of PGs of the A, B, E, and F series. However, this method is not very effective for separating the individual PGs. For this purpose wide use is made of reversed-phase partition chromatography [19, 29, 30, 33]. For example, $PGF_{2\alpha}$ and $PGF_{3\alpha}$ can be separated by chromatography on the hydrophobic Hyflo Supercel [24, 28, 31, 34]. A similar separation takes place in the gel filtration of the PGs of the lipophilic Sephadex LH-20 [35].

Elution with heptane–chloroform–ethyl acetate (10:10:1) permits the satisfactory separation of PGAs and PGBs from PGEs and PGFs, and less effectively, the individual PGs of the E and F series.

The impregnation of silica gel with silver nitrate makes it possible to separate individual PGs with different numbers of double bonds in the form of their methyl ethers. The column separation of the individual PGs differing in degree of unsaturation is also performed with the aid of ion-exchange chromatography on Amberlite-15 (Ag^+) [36, 63].

The development of methods for analysis and separation of PGs by high-pressure chromatography appears extremely promising.

By using UV detectors it is possible to chromatograph the PGs of groups A, B, and C [37]. Morozowich and Douglas [38] separated the prostaglandin of other groups in the form of their p-nitrophenyl benzoates [38]. In all probability, investigations in this field will develop in parallel with the improvement of instruments and, in particular, methods of detection.

To detect the PGs and the metabolites and degradation products the widest use is made of GLC, as a rule in combination with mass spectrometry [5, 23, 30, 39]. The conditions for the GLC of the prostaglandins have been described in most detail in the reviews [2, 4, 5]. A necessary condition for the GLC of all types of PGs is their conversion into the most convenient derivatives for analysis, which permits: a) the elimination of degradation on the column; b) an improvement in separation; and c) a decrease in adsorption. This is achieved by converting the carboxy group into the methyl or trimethylsilyl ester; the hydroxy groups into methyl or trimethylsilyl ethers, acetates, or trifluoroacetates; and the keto groups sometimes into methoxy groups. In the case of PGFs with the cis configuration of the 9,11-hydroxy groups, their butyl ethers are also used [44]. For all types of derivatives the most effective and widely used are columns with 0.5-3.0% of silicones SE-30, OV-1, OV-17, and QF-1 on Gas Chrom P.

In addition to qualitative information concerning the mixture undergoing analysis, GLC has been used to determine the quantitative composition of PGs in various natural tissues [39, 50, 53]. Highly sensitive methods of analysis have been developed using flame-ionization detectors [39] (down to 10^{-8} g) and electron capture detectors [50, 53] (10^{-9} g).

The combined GLC–MS method is also used for the quantitative analysis of the PGs [41, 43, 48, 54].

Combining in itself high sensitivity and great informative value (identification of the components of complex mixtures), the chromato–mass spectrometric method appears to be the best of the existing methods for the analysis of the PGs.

The most sensitive of the GLC–MS methods for determining the amounts of PGs ($0.25 \cdot 10^{-9}$ to 10^{-9} g) is the method using the Multiple-Ion Analyzer [43, 48]. Its essence is as follows. A certain (comparatively large) amount of a labeled standard (a deutero-PGE) is introduced into the mixture to be analyzed before [43] or after [48] the isolation. The mixture is silylated [48] (or some other derivative such as the acetate [43] is obtained) and is subjected to chromato–mass-spectrometry. After the repeated focusing of any pair of peaks corresponding to the ion of the compound under investigation and its deutero analog (from the standard), the ratio of the areas and peaks is calculated and, from this, the amount in the mixture of the component under investigation.

Physicochemical Methods for the Structural Analysis of the PGs (Spectral Characteristics)

The most exhaustive and reliable information concerning the structure of a compound being analyzed and, in particular, a PG, can be obtained only with the aid of NMR, UV, and IR spectroscopy, ORD, CD, and mass spectrometry, and x-ray structural analysis. Tables 1-4 give the spectral characteristics of their analogs and derivatives.

In spite of the large amount of information of all the methods mentioned, we must dwell briefly on the possibilities of NMR and, in particular, ^{13}C NMR. Thus, ^{13}C NMR has shown the "half-chair" conformation for the cyclopentane ring of PGF with four pseudoequatorial substituents, and differences (in the chemical shifts) have been found between carbon atoms 9 and 11, 8 and 12, and 15R and 15S [60]. Large differences are observed on comparing the spectra obtained in $CDCl_3$ and in aqueous solution [60], which is due to the formation of prostaglandin micelles in the latter [62].

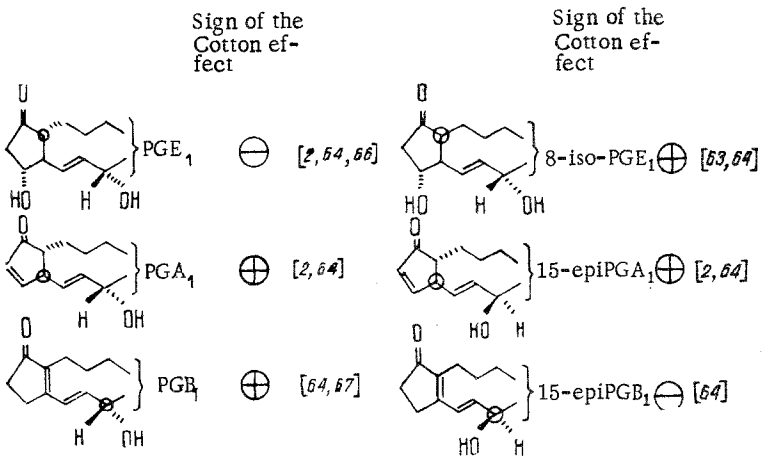
Differences are also observed in the chemical shifts of the protons at carbon atoms 8, 12, 13, and 14 carbon atoms of PGE_1 and of 8-iso-PGE_1 [63]. To determine the configuration of carbon atom 15, Schneider et al. [64] used the method of Dale et al. [65] consisting in the production of the (+)-α-methoxy-α-trifluoromethylphenyl acetates and the analysis of the PMR and ^{17}F NMR spectra: the signals of the fluorine nuclei and of the protons at carbon atom 13 of PGB_2 differ in their chemical shifts from the signals of the 15-epi-PGB_2.

In a number of cases great success has been achieved in the study of the stereochemistry of the PGs by using their optical activity [63, 64, 66-68]. In the case of the carbonyl-containing prostaglandins (cyclopentanones) given below, the stereochemistry of the asymmetric centers mentioned determines the sign of the Cotton effect in the ORD and CD curves (Scheme 1).

TABLE 1. Characteristics of the IR and UV Absorption Spectra of Some Prostaglandins [2]

Compound (mp, °C)	IR, ν_{max}, cm^{-1}				UV	
	C=O in the cyclopentane ring	C=O in COOH	conjugated C=C	trans-C=C	EtOH λ_{max} (nm)	ε
PGE_1 (115–117)	1726	1699	—	964 or/and 980	—	—
PGE_2 (68–69)	1728	1703	—	965	—	—
$PGF_{1\alpha}$ (102–103)	—	1697	—	975	—	—
$PGF_{1\beta}$ (128)	—	1695 (1730[a])	—	970	—	—
$PGF_{2\alpha}$ (93–94)	—	1697	—	977 or/and 968	—	—
PGA_1 (42–44)	1719	1703	1585	962 or/and 979	217	10900
PGB_1 (70–71)	1668	1697	1635	970	278	28650
			1595			

a) Methyl derivative.



Scheme 1

The large number of investigations in the field of the mass-spectrometric fragmentation of the PGs is explained by the considerable advantage of this method: the production of the maximum amount of information on the structure using minute amounts of the substance [2, 19, 20, 29, 31, 41, 43, 45-47, 52, 54-59, 69-82].

As a rule, the volatile derivatives mentioned above that are used in GLC are subjected to analysis.

The main fragmentation pathways common for all derivatives of all types of PGs are as follows:

a) elimination of one of the two or three ROH molecules (R = H, Me, Ac, TFAc, TMS);

b) splitting out of a R'O radical (R' = Me, Et, TMS);

c) splitting out of alkyl radicals including the C_{16}-C_{20} and C_1-C_7 (sometimes C_1-C_6) carbon atoms (frequently with the migration of a hydrogen atom).

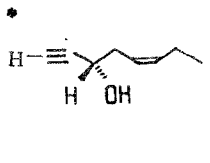
TABLE 2. PMR Characteristics of Some Prostaglandins

Compound	Solvent	Chemical shift (δ, ppm)	Area of the signal	Proton
PGE_1	DMFA	5,65 (m)	2	13,14
		4,08 (t)	2	11,15
		1,8—2,6	5	2,8,10
		0,89	3	20
PGE_2	$CDCl_3$	5 70 (t)	2	13,14
		5,37 (t)	2	5,6
		4,00—4,12	2	11,15
		5,95	2	1,C—OH
PGE_3 methyl ether	$CDCl_3$	5,5—5,8	2	13,14
		5,2—5,5	4	5,6,17,18
		3,8—4,4	2	11,15
		3,61	3	21
$PGF_{1\alpha}$	Acetone-d_6	5,50	2	13,14
		3,75—4,3	3	9,11,15
		4,74	4	1,C—OH
		0,88	3	20
$PGF_{2\alpha}$	DMF	5,4—5,5	6	5,6,13,14
		3,9—4,0	3	9,11,15
		0,87	3	20
PGA_1	$CDCl_3$	5,62 (m)	2	13,14
		7,49 (dd)	1	11
		6,61 s	2	C—OH,1
		6,17 (dd)	1	10
		4,12	1	15
		3,23	1	12
		2,34	2	2
		2,08	1	8

TABLE 3. ^{13}C Chemical Shifts of Some Prostaglandins and Their Analogs and Synthetic Intermediates Relative to TMS* [60, 61]

Number of carbon atoms	E_1^a	E_2^a	E_2^b	$F_{1\alpha}^b$	$F_{1\alpha}^b$ epi	$F_{2\alpha}^b$	$F_{2\alpha}^b$	$F_{2\alpha}^c$	$F_{2\beta}^a$	$F_{2\beta}^b$	$F_{2\alpha}^a$ acetate	A_1^a acetate	A_2^b	I[d]	II[d]	III[b]	IV[b]	V[b,e]
1	176,7	177,2	174 0	174,1	174,4	176,6	—	174,3	180.3	174,0	175,9	173,2	174,0			174,3	174.4	174.3
2	33,8	33,4	33,4	34,0	34,0	33,2	36,2	33,5	33,1	33,5	32,9	34,0	33,5			33.4	33,4	33,4
3	24,5	24,6	24,7	24,9	24.7	24 5	25,1	24,9	24,5	24,9	24,4	24.5	24,8			24,9	24,9	24,9
4	29,0	26,5	26,7	29,5	29,5	26 3	26,9	26,9	26,1	26,7	26.1	28,9	26,7			26,7	26,6	26,6
5	28,6	131,0	130,8	27,8	27,8	132,8	130,3	129,4	133,2	130.2	131,1	28,6	131.0			130,0	129,8	129,8
6	26.3	126,7	129,6	27.7	27,8	128,9	129,2	129,1	127,8	128,1	127,3	26,5	126,7		103,5	128.7	128,8	128,8
7	27,4	25,2	25,2	29,1	28.9	25,1	25,7	25,5	25,1	25 5	24,4	28,2	27.4		31,6	26,2	26,1	26,.
8	54,6	54,6	53,8	50,2	50,6	51.7	48,9	50,2	51.7	52,1	47,0	51,8	49,6		46,3	52,4	52,1	52,1
9	215,2	215,4	213,9	73,0	73.5	—	71,4	72,6	—	74,5	74,0	197,1	210,3		71,6	73,0	72.7	72,7
10	45,9	46,2	46,1	43,1	43 0	42.6	42,5	42,9	41,9	42,4	38,4	132.9	133,3		42,2	42,9	42,8	42,8
11	71,6	72,0	72.0	78,1	78,6	77 2	76,4	77,7	73,7	75,1	77,6	164.7	165,0		77,5	79,1	78,8	78,8
12	54,2	53,6	54,6	56,2	56 2	55,0	54,7	55,7	55,8	56,4	51,5	51,0	52,0		43,6	44,4	44,0	44,1
13	131,9	131,7	131,4	133,2	133,2	129.1	134,1	132,9	130,0	132 9	131,9	132,3	130,2	72.6	83,9	83,5	83,0	84,0
14	136,6	136,7	136,9	135,0	135,0	135,0	135,1	135,5	135,0	135,8	129,6	130.0	135,2	85,2	85,9	86.5	86,3	86,2
15	72,9	73,2	73,0	73,2	73,2	72,9	73,4	73,2	72,9	73,2	74,0	74,1	72,4	61,7	61,9	62,3	62,6	62,6
16	36,9	37,0	37,3	37,3	37 3	36,8	36,7	37,2	36,9	37,2	33,9	34,8	37,3	35,6	36,1	36,0	38,1	38,1
17	25,0	25,2	25,2	25,2	25,2	25,1	25,1	25,3	25,1	25,3	24,4	23,8	25,1	123,3	124,2	123,3	25,0	25,0
18	31,5	31,8	31,8	31,8	31,8	31,5	31,5	31,8	31,6	31,8	31,1	31,2	31,8	135,0	134.0	135,3	31,5	31,5
19	22,5	22,7	22,6	22,6	22,6	22,4	22,5	22,6	22,4	22,6	21,1	22,2	22,6	20,8	20,8	20.8	22,6	22,6
20	13,8	14,1	14,0	14.0	14.0	13,9	13.8	14.0	14.0	14.0	13.6	13,9	14.0	14,1	14,3	14,2	14.0	14,0
21	—	—	51,5	51,4	51,4	—	—	51,6	—	51,6	—	—	51,5	—	—	51,5	51,5	51.6

*a) In $CDCl_3$—MeOH or $CHCl_3$—MeOH (10:1) at 15.08 MHz [61]. b) Methyl derivative in $CDCl_3$ at 22.63 MHz [60]; c) 0.07 M phosphate buffer (pH 7.02) [60]; d) in CCl_4—C_6D_6 (9:1); e) figures for the enantiomer 15-epi-13-dehydro-$PGF_{2\alpha}$, methyl derivative.



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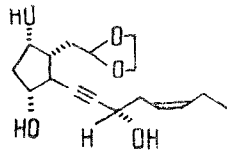

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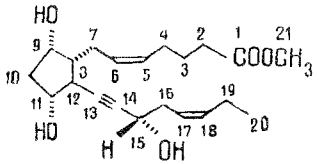

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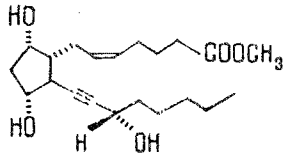

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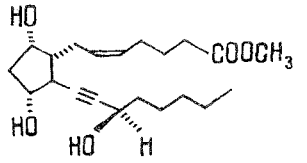

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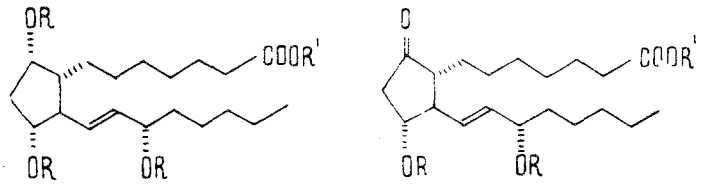
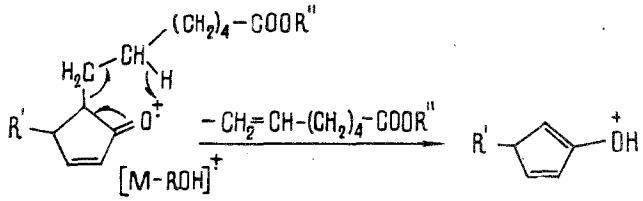


As an example we give the mass spectra of $PGF_{1\alpha}$ and its various derivatives (see Table 4).

The types of ions formed include almost all possible combinations of the cleavages mentioned. It does not appear possible to establish the true sequence of decomposition (in almost all cases) because of the absence of peaks of metastable ions.

In the case of the TMS derivatives, groups of ions formed as the result of cleavages and rearrangements in the cyclopentanone ring are also observed [56-59].

For the mass spectra of the PGs of series A and E the presence of ions formed in the McLafferty rearrangement is typical [19, 20, 31, 69-71, 79-81] (see Scheme 2).



Scheme 2

An analogous arrangement is observed in the case of O-methyl oximes of β-ketol systems – PGE_1 (as mentioned above, O-methyl oximes are obtained in order to prevent degradation on GLC) with the splitting out of a $CH_3O\cdot$ radical from the methoxy group [45].

TABLE 4. Main Ions Formed in the Mass-Spectrometric Fragmentation of $PGF_{1\alpha}$ and Its Derivatives*

Type of ion	Literature						
	[2]	[2,29]	[71]	[20,82]	[41]	[41]	[51,59]
	R=H R=H	R'=H R'=Me	R=Me R'=Me	R=Ac R'=Me	R=TFA R'=Me	R=TMS R'=Me	R=TMS R'=TMS
M^+	356 (0.1)	370 (0.7)	412 (0)	496 (0)	658 (0)	586 (1.0)	644
$[M-ROH]^+$	338 (0,3)	352 (2,0)	380 (20.0)	436 (0)	544 (1.0)	496 (60.0)	554
$[M-2ROH]^+$	320 (18.0)	334 (8.0)	348 (10)	376 (6.0)	430 (19.0)	406	464
$[M-3ROH]^+$				316 (100.0)	316 (100,0)	316	374
$[M-71\,(C_{15}-C_{20})]^+$	284	298	341 (30,0)			515 (57,0)	573
$[M-ROH-71]^+$	266 (100,0)	280 (56,0)	309 (40.0)			425 (100,0)	483
$[M-2ROH-71]^+$	249 (8.0)	263 (6.0)	277 (20,0)			335 (20,0)	393
$[M-ROH-R'O\cdot]^+$					513 (8,0)	465	
$[M-2ROH-R'O\cdot]^+$				345 (4.0)			
$[M-3ROH-129\,(C_1-C_6)]^+$				187 (12,0)			
$[M-3ROH-143\,(C_5-C_7)]^+$				173 (13,0)	173 (29,0)		
$[M-2ROH-71-143]^+$						191 (86,0)	

*The relative intensities of the ions are given in parentheses.

The methods of isolation and analysis of the prostaglandins given were developed in the course of a study of their chemical nature, biosynthesis, and metabolism, and physiological action and functions in the organism. Depending on the problem posed, certain particular methods or others are used or basically new methods come into being which present wider possibilities to investigators working in this field.

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