^{13}C NMR SPECTRA OF BIOLOGICALLY ACTIVE COMPOUNDS.

I. α-HOMOLOGS OF 11-DEOXYPROSTAGLANDIN E_1

G. A. Tolstikov, L. M. Khalilov,
A. A. Panasenko, F. A. Valeev,
and M. S. Niftakhov

UDC 543.422.25:547.915

The ^{13}C NMR spectra of a number of α-homologs of 11-deoxyprostaglandin E_1 have been investigated and a stereochemical assignment has been made of the diastereomeric pairs of isomers with lengths of the α-chain from seven to twelve carbon atoms. The most informative characteristics for the separation of the classes of epimers have been determined; these are differences in the chemical shifts of the C_{13} and C_{15} carbon atoms.

At the present time, exceptionally great attention is being devoted to the study of the "structure–activity" relationship in a number of biologically active compounds. It is generally known that the spectrum of biological activity of a compound depends not only on the presence in the molecule of fragments responsible for a definite type of activity but also on the stereochemical features of the individual parts of the molecule. Thus, among the α and β epimers of the prostaglandins differing only by the spatial arrangement of the 15-OH group the more active are the 15α epimers [1]. Consequently, in the performance of a successful correlation of the "structure–activity" relationship one must use analytical methods permitting the individuality and fine differences among diastereomeric compounds to be taken into account. One of the useful approaches to the investigation of the structures of biologically active compounds the their synthetic analogs is the use of the ^{13}C NMR method [2-5].

In the present paper the results of a study of the ^{13}C NMR spectra of a number of α-homoanalogs of 11-deoxyprostaglandin E_1 are given, a relationship is found between the values of the chemical shifts (CSs) of the carbon atoms on the configuration of the C_{15} hydroxy group, and a stereochemical assignment of the 15α and 15β isomers is made.

Table 1 gives the values of the ^{13}C NMR CSs of homologs of 11-deoxy-PGE_1 studied by other workers (I-III) [5] and by ourselves (IV-XIV) with numbers of carbon atoms in the α-chains of from seven to twelve. For convenience of comparing the spectral characteristics, the numbering of the carbon atoms of the initial 11-deoxy-PGE_1 (n = 0) has been reained as shown in the scheme given below. The remaining carbon atoms of the α-chain are numbered according to the increase in the chain length (C_{21}-C_{25}).

The assignment of the signals in the spectra of the prostanoids studied was made in accordance with literature information for 11-deoxyprostaglandin E_1, and also on the basis of an analysis of the spectra on an SM-4 minicomputer [6]. The CSs calculated by additive schemes show satisfactory agreement with the experimental CSs.

For a comparative analysis of the spectral characteristics of the 15α and 15β epimers Table 1 also gives the difference between the CSs of the corresponding signals. As was to be expected, the greatest changes in the CSs are observed for the carboxylic, C_1, and the carbonyl, C_9, carbon atoms, and the C_{12}-C_{15} atoms of the ω-chain. It must be mentioned that the variations in the difference in the CSs of the carboxylic and carbonyl atoms are irregular for different pairs of epimers, amounting to from −0.70 to +0.70 ppm. The observed changes are cause by the conditions of the medium and the concentration dependences of the CSs of carbon atoms attached to oxygen atoms by multiple bonds [4].

Characteristic features permitting the spectral identification of the 15α and 15β epimers of a concrete prostaglandin are the CSs of the carbon atoms located between the C_{12} and C_{15} chiral centers. In the series of epimeric pairs of prostaglandins that we have studied –

Institute of Chemistry, Bashkir Branch, Academy of Sciences of the USSR, Ufa. Translated from Khimiya Prirodnykh Soedinenii, No. 3, pp. 315-319, May-June, 1985. Original article submitted June 26, 1984.

0009-3130/85/2103-0293$09.50

TABLE 1. ^{13}C Chemical Shifts of Homologs of 11-Deoxyprostaglandin E_1 (11-Deoxy-PGE_1, n = 0-5) (δ, ppm, $CDCl_3$, TMS)

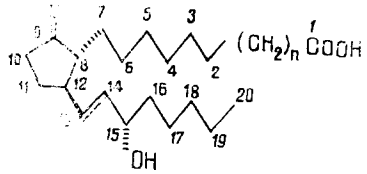
Compound	$\delta C_\alpha - \delta C_\beta$	C_1	C_2	C_3	C_4	C_5	C_6	C_7	C_8	C_9	C_{10}	C_{11}	C_{12}
I. 11-Deoxy-PGE_1, 15 S(α)OH, n = 0		177,5	34,0	24,7	28,8	29,3	26 6	27.7	54 6	219,7	37,3	28,0	45,6
II. 11-Deoxy-PGE_1, 15 R(β)OH, n) 0		177,4	34,0	24,5	28,6	29,1	26,4	27,6	54 6	219,7	37,3	28,0	45,6
	δI—δII	0,1	0,00	0,2	0,2	0 2	0,2	0,1	0,0	0.0	0,0	0,0	0,0
III. Ethyl ester of 8-iso-11-deoxy-PGE_1, $n=0$		173,7	34,0	24,6	28 6	29,0	26,6	25,1	53,5	219,6	35,1	26,9	42,2
IV. 11-Deoxyhomo-PGE_1, 15S(α)OH, n = 1		177,97	24,51	29 21	28,49	28,49	26 27	27,54	54,67	219,30	37,30	28,07	45,46
V. 11-Deoxyhomo-PGE_1, 15R(β)OH, n = 1		178,52	24,51	29,34	28,69	28,69	26,47	27,52	54,47	218,81	37,24	27,93	45,43
	δIV—δV	−0,55	0,0	−0,13	−0,20	−0,20	−0,20	−0,02	0 20	0.58	0,06	0,14	0 03
VI. 11-Deoxydihomo-PGE_1, 15S(β)OH, n = 2		178,99	28,98	29,15	29,15	29,73	26,76	27,71	54 67	220 04	37,31	27,94	45,53
VII. 11-Deoxydihomo-PGE_1, 15R(α)OH, n = 2		179,18	29,05	29,21	29,51	29,86	26,86	27,84	54 70	220,07	37,27	27,97	45,37
	δVI—δVII	+0,71	−0,07	−0,06	−0,36	−0,13	−0,10	−0,13	−0,03	−0 03	0,04	−0 03	0,16
VIII. 11-Deoxytrihomo-PGE_1, 15S(β)OH, n = 3		178,89	29,15	29,28	29,28	29,70	26,76	27,71	54,67	219,98	37,31	27,94	45,53
IX. 11-Deoxytrihomo-PGE_1, 15R(β)OH, n = 3		179,25	29,05	29,24	29,24	29,73	26,76	27,74	54,67	220,04	37,24	27,97	45,40
	δVIII—δIX	−0 36	0,10	0,04	0.04	−0,03	−0,00	−0,03	0 0	− 0.06	0,07	− 0,03	0,13
X. 11-Deoxytetrahomo-PGE_1, 15S(α)OH, n = 4		178,89	29,34	29,34	29,34	29,844	26,81	27,78	54,70	219,89	37,34	27,96	45 58
XI. 11-Deoxytetrahomo-PGE_1, 15S(β)OH, $n=4$		179,25	29,37	29,37	29,14	29,83	26,80	27,81	54,70	220,15	37,24	27,97	45,37
	δX—δXI	−0 36	0,03	0,03	0,20	0,01	0,01	− 0,03	0 00	− 0 26	0,10	− 0,01	0 21
XII. 11-Deoxytetrahomo-PGE_1, 8-iso, n = 4		179,25	29,34	29,34	29,34	29,84	27,96	25,44	53,85	218,18	35,43	27 37	42,41
XIII. 11-Deoxypentahomo-PGE_1, 15S(α)OH, $n=5$		178,56	29,29	29,29	29,29	29 78	26,80	27,75	54 66	219,81	37,36	27,96	45,56
XIV. 11-Deoxypentahomo-PGE_1, 15R(α)OH, n = 15		179,25	29,42	29,47	29,53	29 85	26 87	27 87	54,70	219,95	37,33	27,99	45,41
	δXIII—δXIV	−0,69	−0,23	−0,18	−0,24	− 0,07	−0,07	−0,12	− 0.04	− 0.14	0,03	−0,03	0,15
XV. 3-trans,23-trans-Diene-PGE_3, 11-deoxy-pentahomo, 15S(α)OH, n = 5		177,59	31,76	129,90	130,13	32,77	26,76	27,71	54,60	220,15	37,24	27,94	45,53
XVI. 3-trans,23-trans-Diene-PGE_3, 11-deoxy-pentahomo, 15R(β)OH, n = 5	δXV—δXVI	178,08	31,72	129,83	130,19	32,70	26 73	27 61	54 57	220,15	37 24	27,94	45,40
	δXVI_{calc}	−0,49	+0,04	0,07	−0,06	0,07	0,03	0,10	0,03	0.0	0,0	0,0	0,13
		—	32,98	131,40	131,40	32,98	28,23	26,55	58,25	—	40,34	26,18	41,11

TABLE 1 (continued)

Compound	$\delta C_\alpha - \delta C_\beta$	C_{13}	C_{14}	C_{15}	C_{16}	C_{17}	C_{18}	C_{19}	C_{20}	C_{21}	C_{22}	C_{23}	C_{24}	C_{25}	Literature
I. 11-Deoxy-PGE_1, 15 S(α)OH, n = 0		133,0	134.1	72,8	37,7	25,1	31,8	22.6	14,0						[5]
II. 11-Deoxy-PGE_1, 15 R(β)OH, n = 0		132.7	134.0	72,7	37,7	25,1	31,8	22 6	14,0						[5]
	δI—δII	1,3	0 1	0,1	0,0	0,0	0,0	0 0	0.0						
III. Ethyl ester of 8-iso-11-deoxy-PGE_1, n = 0		128,9	135.2	72,4	37,2	24,9	31,5	22.4	14,0	60,0	13 8				[5]
IV. 11-Deoxyhomo-PGE_1, 15S(α)OH, n = 1		133,45	133.82	72,75	37 66	25,03	31.69	22 58	14,00	33 72					
V. 11-Deoxyhomo-PGE_1, 15R(β)OH, n = 1		133,23	133.98	72,58	37,47	25,07	31.69	22,55	13,97	33,78					
	δIV—δV	0,22	−0.16	0,17	0,19	−0,04	0,0	0,03	0,03	−0,06					
VI. 11-Deoxydihomo-PGE_1, 15S(α)OH, n = 2		133,60	133,85	72,95	37,70	25,13	31.76	22,62	14,03	34,04	24,71				
VII. 11-Deoxydihomo-PGE_1, 15R(α)OH, n = 2		133,20	133,75	72 50	37,73	25,07	31.76	22,62	14,03	34 02	24,71				
	δVI—δVII	0,40	0.10	0,36	− 0 03	0,06	0,0	0,0	0.0	0,02	0,0				
VIII. 11-Deoxytrihomo-PGE_1, 15S(α)OH, n = 3		133 71	133.84	72.95	37,70	25,10	31,72	22,62	14,00	33,98	24.71	29 02			
IX. 11-Deoxytrihomo-PGE_1, 15R(β)OH, n = 3		133 22	133,78	72,62	37.73	25,07	31,72	22 62	14 03	33,98	24,64	28 92			
	δVIII—δIX	0.49	0 06	0,33	0.0	0.0	0,0	0,0	0,03	0 0	0 07	0,10			
X. 11-Deoxytetrahomo-PGE_1, 15S(α)OH, n = 4		133 82	133.91	72,99	37,71	25,12	31,75	22,63	14,04	33,98	24,71	29,02	29,20		
XI. 11-Deoxytetrahomo-PGE_1, 15R(β)OH, n = 4		133,19	133,72	72,58	37,73	25,03	31,72	22,62	14,00	34 04	24,67	28,98	29,34		
	δX—δXI	0,63	0,19	0 41	−0,2	0,09	0 03	0 01	0,01	−0,06	0,04	0.04	- 0,13		
XII. 11-Deoxytetrahomo-PGE_1, 8-iso, n = 4		129.75	134 95	73,10	37,71	25,12	31,75	22,63	14,04	33,98	24,71	29,02	29,20		
XIII. 11-Deoxypentahomo-PGE_1, 15S(α)OH, n=5		133,60	133,91	72,94	37,71	25,13	31,75	22 61	14,02	33,92	24 67	29,00	29,14	29,29	
XIV. 11-Deoxypentahomo-PGE_1, 15R(β)OH n =5		133,23	133,82	72,62	37,73	25,06	−0 01	22,61	14,02	34 01	24,71	29,03	29.17	29,34	
	δXIII —δXIV	+0,46	0,09	0,32	−0,02	0,07	31,76	—0 0	0,0	- 0,09	−0,04	−0,03	−0,03	- 0 05	
XV. 3-trans,23-trans-Diene-PGE_3, 11-deoxy-pentahomo, 15S(α)OH, n = 5		133,56	133,82	72,82	37.66	25,13		22.62	14,07	34,14	27,35	131,11	128,21	32,47	
	δXV—δXVI	133,13	133,75	72,55	37,70	25,07	31,72	25 59	14,00	34,07	27,42	131,21	128,11	32 51	
XVI. 3-trans,23-Diene-PGE_3, 11-deoxy-pentahomo, 15R(β)OH, n = 5	δXVI calc	+0,43	0,07	0,27	−0,03	0,06	0.04	0,03	0,07	0.07	−0,07	−0 10	−0,10	−0.04	
		133,80	134,80	74,23	37,98	23,73	32,41	22,66	13,85	34,85	28,66	132,90	129,90	32,98	

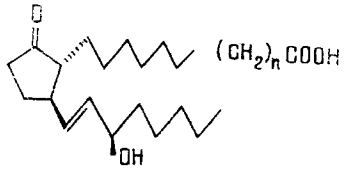
(I) and (II), (IV) and (V), (VI) and (VII), (VIII) and (IX), (X) and (XI), and (XIII) and (XIV) – the differences in the CSs of the carbon atoms mentioned on passing from the 15S(α) to the 15R(β) epimers are positive magnitudes.

This fact shows the existence of spatial interactions in the molecules of the erythro (15β) epimers for the groups and atoms bound to the C_{12} and C_{15} chiral centers which lead to a definite screening of these nuclei as compared with the CSs for threo (15α) epimers.



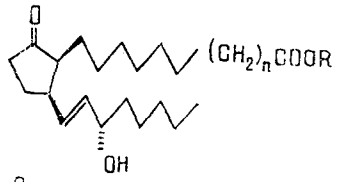
α-Homo-11-deoxyprostaglandins E_1, 15-α epimers

$n=0$ (I), 1(IV), 2(VI), 3(VIII), 4(X), 5(XIII)



α-Homo-11-deoxy-15-epiprostaglandins E_1, 15-β epimers

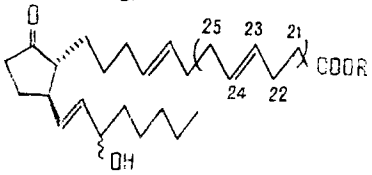
$n=0$ (II), 1(V), 2(VII), 3(IX), 4 (IX), 5 (XIV)



8-Iso-11-deoxyprostaglandins E_1

$n=0$, R = CH_2CH_3 (III)

$n=4$, R = H (XII)



15α (XV), 15β (XVI)

The most powerful characteristic of the separation of the classes of diastereomeric pairs is formed by the diamagnetic shifts of the C_{15} and C_{13} signals in the intervals from 0.2 to 1.3 ppm and from 0.1 to 0.4 ppm, respectively, for the 15β epimers as compared with the 15α epimers. It is precisely on the basis of this characteristic that the diastereosiomers (XV) and (XVI) with two double bonds in the α-chain and to some extent falling outside the series of homologues of 11-deoxy-PGE_1 were assigned. The position and the E,E configuration of the double bonds in the α-chain were shown by a calculation of the CSs using the minicomputer [6] (see Table 1).

In the spectrum of compound (X) additional weak signals were observed relating to the isomeric product (XII) with the cis arrangement of the α- and ω-chains relative to the cyclopentane ring. A similar phenomenon has been observed by Pekhk et al. [5] (compound (III)). Characteristic features of the isomeric compounds (III) and (XII) are the upfield shift of the signals of the C_8 and C_{12} atoms of the ring and the C_7 and C_{13} atoms of the α- and ω-chains through the interaction of the 1,2-cis-oriented substituents in the five-membered ring [7].

EXPERIMENTAL

^{13}C NMR spectra were recorded on a JEOL FX-90Q spectrometer (22.50 MHz). Field scanning at 6024 and 2000 Hz, resolution of the analog–digital converter 0.74 and 0.24, respectively. The samples were prepared in $CDCl_3$ (30-10 mg/ml) with TMS as internal standard. The analysis of the ^{13}C spectra was performed on an SM-4 minicomputer using a program for calculating CSs by additive schemes [6].

The synthesis of the (±)-α-homoanalogs of 11-deoxyprostaglandin E_1 that were investigated has been described in [8].

SUMMARY

The structures of diastereomeric pairs of α-homoanalogues and 11-deoxy-PGE_1 with lengths of the α-chain from seven to twelve carbon atoms have been dhown and their stereochemical assignment has been made by means of ^{13}C NMR spectroscopy. It has been shown that

the most informative characteristics of the separation of the class of epimers are differences in the CSs of the C_{13} and C_{15} carbon atoms due to stereochemical interactions of the groups of atoms at the C_{12} and C_{15} chiral centers in the 15β epimers. Characteristic features of the cis isomers of the α- and ω-chains relative to the cyclopentane ring in the homologues of 11-deoxy-PGE_1 are upfield shifts of the signals of the C_8 and C_{12} atoms of the ring and of the C_7 and C_{13} atoms of the α- and ω-chains.

LITERATURE CITED

1. W. Bortmann, J. Bock, et al., in: Chemistry, Biochemistry, and Pharmacological Activity of Prostaglandins (ed. by M. Roberts and F. Scheinmann), Pergamon, Oxford (1979), p. 193.
2. G. Lukacs, F. Pirion, D. S. Gero, et al., Tetrahedron Lett., 515 (1973).
3. G. F. Cooper and I. Fried, Proc. Mat. Acad. Sci., USA, 70, 1579 (1973).
4. G. C. Levy, R. L. Lichter, and L. Nelson, Carbon-13 Nuclear Magnetic Resonance Spectroscopy, Wiley-Interscience, New York, 2nd ed. (1980), p. 264.
5. T. Pekhk, T. Vyalimyaé, H. Samel', et al., Izv. Akad. Nauk SSSR, Khim., No. 2, 85 (1982).
6. L. M. Khalilov, R. A. Sadykov, A. A. Fatykhov, et al., in: Abstracts of Lectures at the VIth All-Union Conference [in Russian], Novosibirsk (1983), p. 54.
7. M. Christl, H. J. Reich, and J. D. Roberts, J. Am. Chem. Soc., 93, No. 14, 3463 (1971).
8. A. Tolstikov, M. S. Miftakhov, F. A. Valeev, et al., Zh. Org. Khim., 19, 279 (1983).

PREPARATION OF TRITIUM-LABELED PROSTAGLANDIN E_3

V. P. Shevchenko, I. V. Fetisova,
T. Yu. Lazurkina, I. Yu. Nagaev,
and N. F. Myasoedov

UDC 546.110.23:577.15/17

Conditions for the biosynthesis of PGE_3 from eicosa-5,8,11,14,17-pentaenoic acid using an enzyme system isolated from ram seminal vesicles have been worked out. Tritium-labeled PGE_3 has been obtained in good yield with a molar radioactivity sufficient for performing many biological investigations.

The possibility of obtaining tritium-labeled prostaglandin E_2 has been shown previously [1]. Enzyme systems converting eicosa-5,8,11,14,17-pentaenoic acid into various prostaglandins of the 3 series are known [2, 3]. The aim of the present work was to obtain tritium-labeled prostaglandin E_3 (PGE_3) from [^{3}H]eicosa-5,8,11,14,17-pentaenoic acid using an enzyme system isolated from ram seminal vesicles. To optimize the conditions for the biosynthesis of PGE_3 we performed a series of experiments at various concentrations of enzyme, polyunsaturated acid, epinephrine, hemin and hydroquinone, and we also investigated the kinetics of the conversion of the polyunsaturated acid into PGE_3. In each case the yield of prostaglandin was determined after alkaline isomerization [1] and measurement of the absorption at λ 278 nm. With increases in the concentrations of polyenic acid, enzyme, and hemin and with a lengthening of the time of incubation the yield of PGE_3 rose to a definite value and then did not change further, while the curves reflecting the dependence of the yield of product on the concentrations of epinephrine and of hydroquinone had maxima. In the investigation of the stability of the enzyme with time it was found that the enzyme did not lose its initial activity for a period of two months.

As a result of the experiments performed, the following conditions were selected for biosynthesis using the enzyme system in the form of an unpurified supernatant (per 2 ml of supernatant with a concentration of 22 mg of protein/ml): 0.5 mg (1.65 μmole) of eicosa-5,8,11,14,17-pentaenoic acid, 0.56 mg (3.06 μmole) of epinephrine or 0.32 mg (2.9 μmole) of hydroquinone, 1 μg (1.5 nmole) of hemin, and 1.2 mg (3.9 μmole) of reduced glutathione, the reaction being performed at 32°C for 8 min. If 50-100 mg of eicosa-5,8,11,14,17-penatenoic acid was used in the reaction with observance of the same ratios of the other ingredients

Institute of Molecular Genetics, Academy of Sciences of the USSR, Moscow.

Translated from Khimiya Prirodnykh Soedinenii, No. 3, pp. 319-322, May-June, 1985. Original article submitted July 2, 1984.