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THE SYNTHESIS OF 16,16-DIMETHYL-15-KETO-PROSTAGLANDIN B_1 OLIGOMERS. THE CHEMICAL STRUCTURES OF DIMERS.

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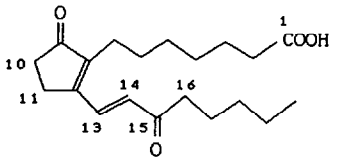
Summary: Chemical structures of dimers of 16,16-dimethyl-15-keto-PGB_1 synthesised by Michael addition were established from spectral data.

Introduction

Biologically active oligomeric prostaglandins synthesized by Michael reaction from 15-keto-PGB_1 **1** by B.D.Polis et al., firstly termed as PGB_x, consisted of six to eight prostaglandin subunits with total M_w between 2200 and 2800 [1]. PGB_x has been shown to maintain oxidative phosphorylation during hypotonic degradation in aged rat liver mitochondria[2]. It also stimulates the release of Ca^{2+} from the fragmented sarcoplasmic reticulum and heart mitochondria[3]. It has been reported that *in vivo* PGB_x provides the protection of animals following the cardiac[4], cerebral[5] and spinal[6] ischemia, and hypoxia[7]. The obvious medical potentialities of PGB_x cannot be tested in humans without adequate knowledge of its chemical structure. However, the biologically active oligomers were proved too complicated for complete interpretation of their n.m.r. spectra. Therefore structure elucidation studies of dimers were undertaken[8] and the results used for further interpretation. G.L.Nelson et al.[8c,8e] have reported, that six dimers are formed from **1** by base catalyzed Michael addition in which two nucleophilic (C-10 and C-16) and two electrophilic (C-13 and C-14) sites of **1** are active. The first two dimers, a diastereomeric pair, are formed by the addition of the C-10 enolate of **1** to C-14' of a second unit of **1**. The next two, a diastereomeric pair, arise from the addition of the C-10



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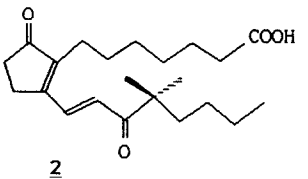

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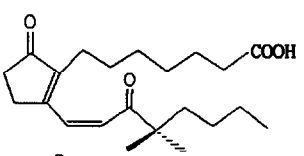
enolate to C-13' of a second unit. The fifth and the sixth, the double addition dimers, result from the addition of the C-16 enolate of 1 to C-13' and C-14' of a second unit, respectively, to form a new enolate which internally cyclizes to form a cyclopentanone ring by addition to C-14 of the original unit. In order to decrease the number of structural isomers of dimers, the blocking of the C-16 nucleophilic site by methyl groups was undertaken[9]. It was demonstrated, that oligomers synthesized from 2 also provide protection from the loss of oxidative phosphorylation in isolated rat liver mitochondria[10] and the Ca^{2+} ionophoric activity[9]. However, the elucidation of chemical structures of 2 dimers was not yet published.

Results and Discussion

The base-catalysed Michael addition applied to 2 afforded crude oligomerization product which consists of oligomers up to pentamer. The crude product was separated into mono-, di-, tri-, tetra- and pentamer fractions by size-exclusion chromatography on Sephadex G-50[11]. The monomer fraction (15% overall yield from 2) was separated by preparative HPLC on silica gel into two fractions: unreacted 2[12] and its 13-Z isomer 3 with the



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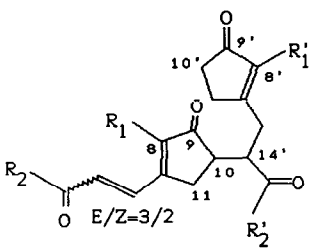
ratio 3:2 respectively.

The dimers fraction (13% overall yield from 2) was also separated by preparative HPLC on silica gel into four fractions which were distributed

as: 23% **4**, 22% **5a**, 24% **5b** and 31% **6**. SIMS measurements of dimers fraction with glycerine, glycerine -NaCl and glycerine - $NaHCO_3$ liquid matrixes were carried out[13]. The abundant ions at *m/z* 725(M+H), 747(M+Na), 769(M+2Na-H) and 791(M+3Na-2H) show that dimers possess three acidic hydrogens, two carboxylic and one presumably at C-10. From the carbon chemical shifts of **2**, 13,14-dihydro-16,16-dimethyl-15-keto-PGB_1 and previous results on dimers of **1**[8], the chemical shifts of bridging CH-groups can be estimated. For C-10 - C-14' bond C-10 must resonate at ~46 ppm and C-14' at ~43 ppm. For C-10 - C-13' bond C-14' has to be shifted to higher fields to about 37 ppm. These key features determine the structure of two diastereoisomers of C-10 - C-13' dimer **5a-b** and one diastereoisomer of C-10 - C-14' dimer **4**. The presence of another diastereoisomer of C-10 -C-14' dimer was determined as a minor component in the fraction of **5a**. Each of the above mentioned isomers consists of the mixture of 13-E and 13-Z stereoisomers with the E isomer predominating in the ratio 3/2. This result is confirmed by ^1H



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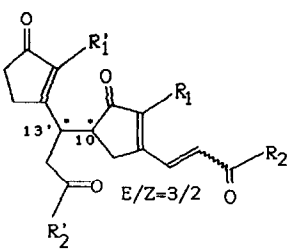
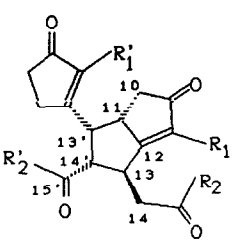


5a-b



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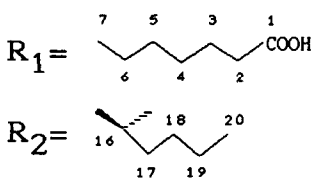

n.m.r. spectra which show isolated double bonds with different $^3J_{HH}$ values: 15,5 Hz for E and 12,4 Hz for Z isomers. The **6** (single diastereomer) has no ethylenic protons and has four saturated CH-carbons. It's structure was determined on the basis of ^1H and ^{13}C n.m.r. spectra and 2D ^1H -^1H and ^1H - ^{13}C chemical shift correlations. Examination of two correlation diagrams reveals only following carbon-carbon bond connectivities of cycles: 10-11, 11-13', 13-14, 13-14', 13'-14' and 10'-11'. This is in full agreement with

the proposed structure. The formation of substituted bicyclo[3.3.0]octenone sceleton is also supported by the carbon chemical shifts of various 2-substituted bicyclo[3.3.0]oct-1(2)-en-3-ones[14]. Interproton coupling constants between H-11 - H-13'(10,3 Hz), H-13' - H-14' (8.0 Hz) and H-13 - H-14' (5.9 Hz) support the given configuration of **6**. Judging the structure of **6** it is obvious that **6** arises from the addition of C-11 carbanion of **2** to C-13' of a second unit leading to C-14' enolate which cyclizes by

Table 1. Carbon Chemical Shifts of 16,16-dimethyl-15-keto prostaglandin B_1 dimers[a]

Carbon No	**4**		**5a**		**5b**		**6**
	E	Z	E	Z	E	Z	
8	147.3	143.0	147.4	142.8	147.2	143.1	141.7
9	207.7	207.9	208.7	208.9	208.7	208.9	208.3
10	46.2	46.1	45.7	46.0	45.3	45.7	41.2
11	b	b	b	b	b	b	49.1
12	160.7	165.8	158.7	164.3	157.9	162.4	180.9
13	134.4	134.0	134.8	134.1	134.2	134.1	38.0
14	126.8	129.0	126.2	128.3	126.0	128.9	41.6
15	204.1	206.4	203.9	206.0	204.0	206.6	212.4
16	46.9	47.3	46.8	47.2	46.9	47.2	47.4
8'	142.4	142.2	142.2	141.8	142.3	142.1	137.0
9'	209.7	210.0	209.7	210.1	210.1	210.0	209.3
10'	34.1	33.9	34.1	34.2	34.1	34.2	33.9
11'	b	b	30.9	33.0	31.6	33.7	25.6
12'	169.7	170.6	172.1	173.0	172.4	172.9	170.0
13'	b	b	36.9	36.7	37.4	37.1	53.8
14'	42.8	43.2	37.5	36.8	37.6	37.6	54.4
15'	216.2	215.3	213.3	213.5	212.8	213.1	215.9
16'	48.3	48.1	47.7	47.6	47.4	47.4	47.4

[a] in $CDCl_3$, δ_{TMS}=77.0. Chemical shifts of other carbon atoms are close to those of **2**

[b] overlapping resonances at about 28 ppm were not assigned

addition of C-14' to C-13 resulting in the formation of a bicyclo[3.3.0]octenone ring. Carbon chemical shifts of essential carbon atoms are presented in the Table 1.

From the chemical structure of **6**, one can see that it does not have double bonds (acceptor site in Michael reaction) in side chains. This leads to supposition that: a) trimers are formed from the monoaddition dimers **4**, **5a** and **5b** by the addition of **2** to corresponding dimer; b) dimer **6** does not take part in the formation of trimers. The data presented on Fig.1 show the

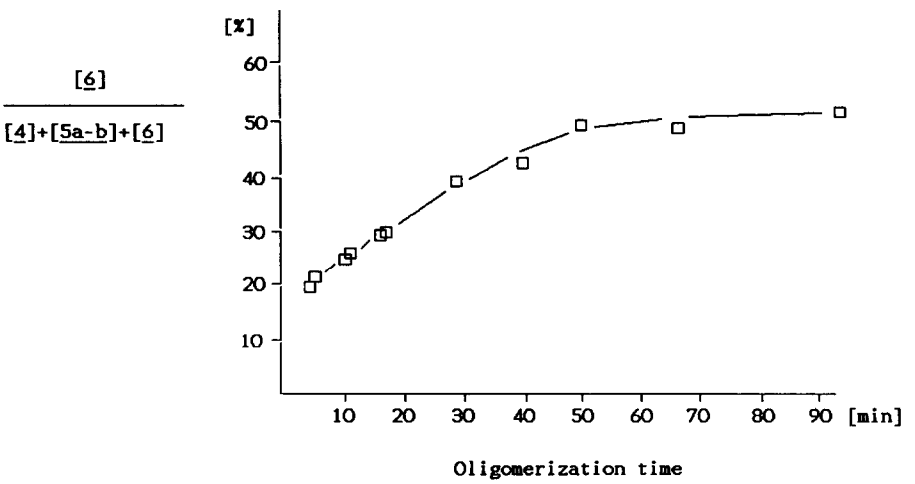

Fig.1 Increase of the yield of dimer 6 in the dimers fraction during the oligomerization reaction

increase of concentration of **6** in the dimer fraction from 20% at the beginning of the reaction to 55% at the steady state during the oligomerization reaction. At the same time the ratio between the monoaddition dimers **4**, **5a** and **5b** was constant and was measured to be approx. 1:1:1.

The blocking of the nucleophilic site by methyl groups at C-16 in the prostaglandin molecule leads to the decrease of the number of structural isomers in the Michael addition reaction. However, the new addition between C-11 and C-13' was found (resulting in the formation of **6**). Under the alkaline conditions of the Michael reaction the isomerisation of the 13,14(E)-double bond was observed so that all compounds containing the 13,14-double bond were separated as mixtures of their E/Z isomers.

Recently, high biological activity of the trimer of **2** (M_w=1087.5) was demonstrated[10].

Experimental

^{13}C n.m.r. spectra were recorded on a Bruker AM-500 spectrometer in $CDCl_3$ solution. The chemical shifts are reported in TMS scale relatively to $CDCl_3$ (δ_{TMS}=77.0 ppm). I.r. spectra were recorded on a Specord 71 IR spectrophotometer and u.v. spectra on a Specord M40. Ethanol, *iso*-Propanol and *n*-Hexane were distilled before use. Ethyl acetate was treated with sat. Na_2CO_3 solution and dried with anhydrous $CaCl_2$ before distillation. Synthesis of **2** was described in [12].

Synthesis and separation of oligomers. To a mixture of 1.6 g of **2** in 100 ml ethanol under argon 100 ml of 2M KOH was added. The mixture was stirred for 25 min at r.t., then 105 ml 2M HCl was added, ethanol was removed by vacuo and residue was extracted three times with 200 ml of ethyl acetate. The extracts were washed with brine followed by the extraction of oligomers with 120 ml of 50 mM borate buffer, pH 8.9. The oligomers were separated repeatedly (10×160 mg) on a Sephadex G-50 column (150×3.0 cm) with 50 mM borate buffer, pH 8.9 as an eluent to afford 266 mg monomeric fraction, 216 mg dimers, 275 mg trimers, 205 mg tetramers and 124 mg pentamers. Details of separation and identification see in [11].

Preparative HPLC separation of **3** and dimers **4**, **5a-b** and **6**. The LKB HPLC system was used: 2150 HPLC Pump and 2140 Rapid Spectral Detector with automated data handling. The Separon Si600 250×9 mm column was used. Chromatographic conditions: sample size 10 mg, flow rate 3.0 ml/min, benzene : *iso*-propanol : water = 97.9 : 2.06 : 0.04 for monomeric fraction and *n*-hexane : *iso*-propanol : water = 92: 6.72 : 0.28 for dimeric fraction as eluents were used .

From 266 mg of monomeric fraction, 130 mg of **2** and 86 mg of **3** were separated. **2**: UV λ_{max}=296 nm (*n*-hexane) ε=24900; **3**: UV λ_{max}=285 nm (*n*-hexane) ε=14600. MS(70 eV) (m/z, r.i.): 363(2.7;M+1$^+$), 362(3.2;M$^+$), 319(0.8;M-$C_3H_7^+$), 305(9.5;M-$C_4H_9^+$), 263(5.2;M-$C(CH_3)_2C_4H_9^+$), 233(100; M-$C_6H_{12}COOH^+$), 129(2;$C_6H_{12}COOH^+$), 99(13;$C(CH_3)_2C_4H_9^+$), 57(80;$C_4H_9^+$), 43(27;$C_3H_7^+$). ^{13}C n.m.r C_1(s)178.4, C_2(t)34.3, C_3(t)24.5, C_4(t)28.8, C_5(t)29.1, C_6(t)28.2, C_7(t)23.5, C_8(s)144.1, C_9(s)207.2, C_{10}(t)33.7, C_{11}(t)27.7, C_{12}(s)163.9, C_{13}(d)134.0, C_{14}(d)129.1, C_{15}(s)202.4, C_{16}(s)47.4, C_{17}(t)39.7, C_{18}(t)26.8, C_{19}(t)23.3, C_{20}(q)13.9, C_1'(q)24.1, C_1''(q)24.3

From 216 mg of dimeric fraction, 27 mg **4**, 25 mg **5a**, 27 mg **5b** and 36 mg **6** were separated. **4**: UV λ_{max}=238 (ε=14300) and 296 nm (ε=24900) (*n*-hexane); **5a**: UV λ_{max}=238 (ε=17000) and 296 nm (ε=24900) (*n*-hexane); **5b**: UV λ_{max}=238 (ε=19000) and 296 nm (ε=24900) (*n*-hexane); **6**: UV λ_{max}=243 (ε=26000). See Table 1 for spectral data.

Determination of **6** in the dimers fraction. The oligomerization reaction was started by addition of 1.8 ml 2 M KOH to the mixture of 19 mg of **2** in 1.8 ml ethanol. The reaction mixture was stirred under argon at 22°C. The 0.3 ml of reaction medium was transferred into the 1 ml 0.5 M HCl at appropriate times. Each sample was extracted with 1 ml of benzene. In order to separate dimers from other reaction products the benzene extract was subjected to flash chromatography (Separon Si VSK 10 μm 150×10 mm column, 5 ml/min *n*-hexane:*iso*-propanol:water = 88:11.5:0.5). The dimeric fraction was concentrated by vacuo. The contents of **4**, **5a-b** and **6** were measured by using HPLC (Separon SGX 5μm 300×3 mm column, 0.6 ml/min *n*-hexane:*iso*-propanol:water = 91.5:8.24:0.26, detection with LKB 2140 UV Rapid Spectral Detector at 210-320 nm).

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References

1. (a) Polis,B.D.; Polis,E.; Kwong,S.W. *Proc. Natl. Acad. Sci. U.S.A.*, **1979**, 76, 1598-1602; (b) Polis,B.D.; Kwong,S.W.; Polis,E.; Nelson,G.; Shmukler,H.W. *Physiol. Chem. Phys.*, **1979**, 11, 109-123
2. (a) Shmukler,H.W.; Soffer,E.; Zawryt,M.G.; Polis,E.; Feely,W.M.; Kwong,S.F.; Cope,F.W. *Physiol. Chem. Phys.*, **1982**, 14, 445-469; (b) Shmukler,H.W.; Zawryt,M.G.; Soffer,E.; Polis,E.; Feely,W.M.; Kwong,S.F.; Cope,F.W. *Physiol. Chem. Phys.*, **1982**, 14, 471-486; (c) Devlin,T.M.; Krupinski-Olsen,R.; Uribe,S.; Nelson,G.E. *Biochem. Biophys. Res. Comm.*, **1986**, 137, 215-221
3. (a) Ohnishi,S.T.; Devlin,T.M. *Biochem. Biophys. Res. Comm.*, **1979**, 89, 240-245; (b) Uribe,S.; Villalobos-Molina,R.; Devlin,T.M. *Biophys. Res. Comm.*, **1987**, 143, 1024-1029; (c) Uribe,S.; Ohnishi,S.T.; Israelite,C.; Devlin,T.M. *Biochim. Biophys. Acta*, **1987**, 924, 87-98
4. Angelakos,E.T.; Riley,R.L.; Polis,B.D. *Physiol. Chem. Phys.*, **1980**, 12,

81-89;

5. Kolata,R.J.; Polis,B.D. *Physiol. Chem. Phys.*, **1980**, 12, 545-550
6. Jacobs,T.P.; Hallenbeck,J.M.; Devlin,T.M.; Feuerstein,G.Z. *Pharm. Res.*, **1987**, 4, 130-132
7. Polis,E.; Cope,F.W. *Aviation, Space & Environm. Med.*, **1983**, 54, 420-424
8. (a) Polis,B.D.; Polis,E.; Kwong,S.F. *Physiol. Chem. Phys.*, **1981**, 13, 531-548; (b) Toda,M.; Takaoka,S.; Konno,M.; Okuyama,S.; Hayashi,M.; Hamanaka,N.; Iwashita,T. *Tetrahedron Lett.*, **1982**, 23, 1477-1480; (c) Nelson,G.L.; Verdine,G.L. *Tetrahedron Lett.*, **1982**, 23, 1967-1970; (d) Hamanaka,N.; Toda,M.; Takaoka,S.; Hayashi,M.; Iwashita,T. *Chem. Pharm. Bull.*, **1983**, 31, 557-559; (e) Nelson,G.L.; Verdine,G.L. *Tetrahedron Lett.*, **1983**, 24, 991-994
9. Uribe,S.; Ohnishi,S.T.; Israelite,C.; Devlin,T.M. *Biochim. Biophys. Acta*, **1987**, 924, 87-98
10. (a) Devlin,T.M.; Krupinski-Olsen,R.; Uribe,S.; Nelson,G.E. *Biochem. Biophys. Res. Comm.*, **1986**, 137, 215-221; (b) Martin,I.; Männik,E.; Lille,Ü. *Eesti TA Toim., Keemia*, **1990**, 39, 57-59;
11. Martin,I.; Lille,Ü. *J.Chrom.*, **1989**, 466, 339-345
12. For synthesis and assignements of chemical shifts of 2, see Martin,I.; Anvelt,J.; Välimäe,T.; Pehk,T.; Lille,Ü. *Synth. Comm.*, **1990**, in press
13. Details of the analysis conditions see in Sadovskaya,V.L.; Rakitin,L.Yu.; Golovanova,N.K.; Kogtev,L.S.; Bezuglov,V.V.; Bergelson,L.D. *Bioorg. Khim.*, **1986**, 12, 956
14. Negishi,E.; Holmes,S.J.; Tour,J.M.; Miller,J.A.; Cederbaum,F.E.; Swanson,D.R.; Takahashi,T. *J. Am. Chem. Soc.*, **1989**, 111, 3336-3346