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THE BASE PROMOTED OLIGOMERIZATION OF A 15-DEHYDRO-PGB$_1$ ANALOG: STRUCTURAL INSIGHTS INTO THE COMPLEX OLIGOMERIC MIXTURE TERMED PGB$_X$

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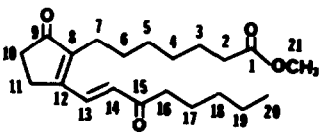
Summary: The base promoted oligomerization of the 15-dehydro-PGB$_1$ analog 2 takes place by a Michael addition pathway involving multiple nucleophilic and acceptor sites.

Currently, the term PGBx refers to a complex mixture derived from treatment of 15-dehydro-PGB$_1$ methyl ester (1) with 1 M ethanolic KOH.[1] A number of unique properties have been demonstrated for PGBx such as the *in vitro* protection of oxidative phosphorylation in isolated degenerated mitochondria[2] and potent ionophoretic function in the release of Ca^{++} from heart mitochondria.[3] *In vivo*, significant increases in survival after otherwise lethal episodes of myocardial ischemia in monkeys[4] and hypoxia in dogs[5] have been reported. PGBx has been generally described as a complex mixture of closely related oligomers formed by the initial reaction at the 13,14-unsaturation of 1 with retention of the overall prostaglandin skeleton.[6] Attempts by a number of research groups to resolve this complex mixture into individual components retaining activity have been unsuccessful. Our efforts have been directed towards the elucidation of the oligomerization pathway of 1 by study of structurally simpler analogs such as 2.[7] We wish to report here on the nature of the initial oligomerization of 2 and the implications relative to the structural complexity of the PGBx mixture.



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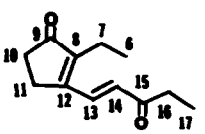

2

Treatment of 2 with 1 M ethanolic KOH at room temperature resulted in an immediate reaction with a new 238 nm UVmax replacing the 296 nm absorption of 2. When the reaction mixture was quenched immediately after addition of the KOH, the crude product obtained after extraction with ethyl acetate contained 20-25% of unreacted 2.[8] Analysis of the crude product by field desorption mass spectrometry (FDMS) indicated an oligomeric mixture of the formula $(C_{12}H_{16}O_2)_n$ with n = 1-8.[9] Separation of the oligomeric mixture into dimer, trimer, tetramer, pentamer, and hexamer-octamer fractions, as confirmed by FDMS analysis of each fraction,[9] was accomplished by size exclusion chromatography on Sephadex LH-20 using CH_3OH as solvent.

HPLC analysis of the dimer fraction (Zorbax Sil, 40% EtOAc/C_6H_{12}) revealed six major components which are referred to in the following discussion, in order of increasing retention times, as Dimers 1-6.[10] Separation of the six dimers was effected using a 10 mm x 25 cm Li-Chrosorb column with 30% EtOAc/C_6H_{12}. A molecular formula of $C_{24}H_{32}O_4$, i.e. $(C_{12}H_{16}O_2)_2$, was established for each of the six dimers by HRMS measurement of the molecular ion.[11]

Two distinctly different dimer types were indicated by the spectral data. Dimers 1-4 exhibited UVmax at 296 and 238 nm and conjugated C=C IR absorptions at 1585 and 1640 cm^{-1}.[12] Dimers 5 and 6 had a single UVmax at 238 nm, a conjugated C=C IR absorption at 1640 cm^{-1}, and a new absorption at 1750 cm^{-1} characteristic of a cyclopentanone C=O. Whereas Dimers 1-4 exhibited an intense fragment ion at m/e 192, corresponding to $C_{12}H_{16}O_2$, only a weak m/e 192 ion was present in Dimers 5 and 6. In the 360 MHz ^1H NMR spectra of Dimers 1-4, absorptions corresponding to a 13,14-unsaturation and four distinct methyl triplets were present. In contrast, Dimers 5 and 6 exhibited no olefinic protons, three methyl triplets, and a methyl doublet. In the ^{13}C NMR spectra of Dimers 1-4, two methine carbons were evident indicating the formation of a single C-C bond. The presence of four methine carbons in Dimers 5 and 6 along with a carbonyl carbon at 214 ppm indicated the formation of two C-C bonds leading to a cyclopentanone ring. This data taken with a detailed consideration of ^1H and ^{13}C NMR data led to the structural assignment of Dimers 1-6 found in Figure 1.[13-15]

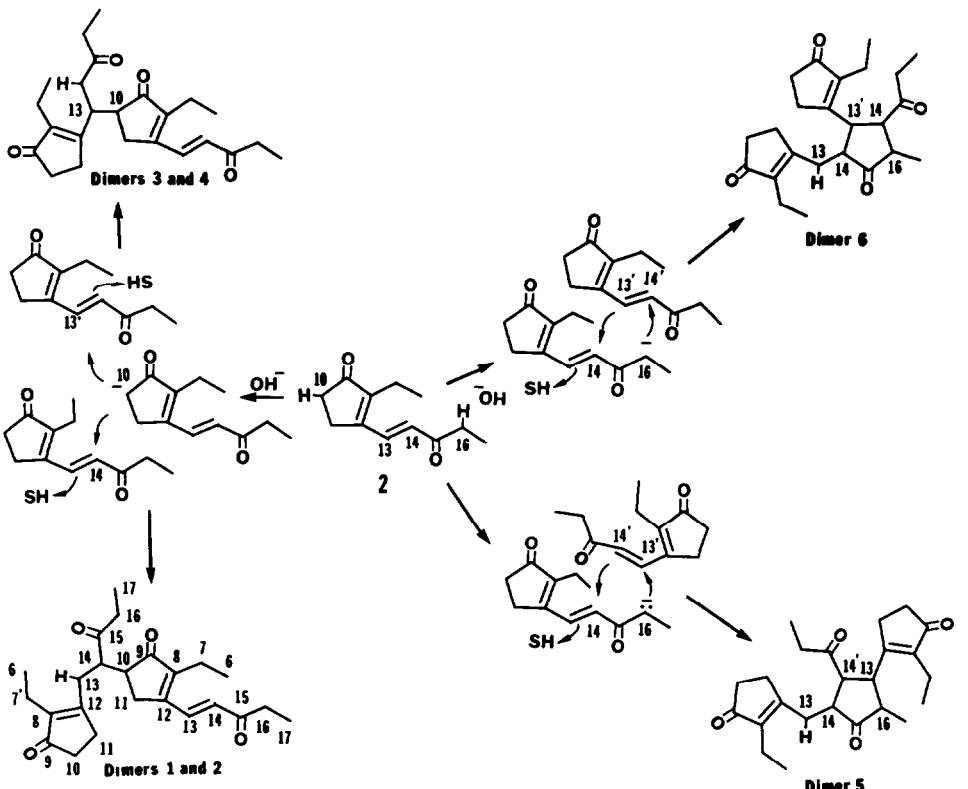

FIGURE 1

Dimers 1-6 are formed by base catalyzed Michael addition in which two nucleophilic (C-10 and C-16) and two acceptor (C-13 and C-14) sites of 2 are active (Figure 1). The presence of multiple reaction sites coupled with the formation of two new chiral centers for each new bond formed results in the formation of a complicated mixture of structural isomers further complicated by the presence of closely related stereoisomers. Dimers 1 and 2, a diastereomeric pair, are formed by the addition of the C-10 enolate of 2 to C-14' of a second unit of 2. Dimers 3 and 4, a second diastereomeric pair, arise from the addition of the C-10 enolate to C-13' of a second unit. Dimers 5 and 6, the double addition dimers, result from the addition of the C-16 enolate of 2 to C-13' and C-14', respectively, of a second unit to form a new enolate which internally cyclizes to form a cyclopentanone ring by addition to C-14 of the original unit. Dimers 1-4 retain a residual 13,14-unsaturation through which further oligomerization can proceed in a similar manner. Dimers 5 and 6, which lack this unsaturation, do not appear to undergo further oligomerization.[10]

Providing the next higher oligomer arises from the addition of the C-10 enolate of 2 to the residual 13,14-unsaturation, the oligomeric mixtures formed rapidly become very complex.[16] Since each of the Dimers 1-4 could give rise to two pairs of diastereomeric trimers, a total of 16 closely related trimers retaining a 13,14-unsaturation are possible.[17] In turn, the 16 trimers could give rise to 64 tetramers, the 64 tetramers to 256 pentamers, the 256 pentamers to 1056 hexamers, etc.; with the 13,14-unsaturation required for further chain growth being retained in each case. Based on such a projection, the direct structural elucidation of PGBx, for which the most active fraction has been estimated to be in the hexamer-octamer range,[6] would not appear to be feasible. The marked lack of success over the last several years by groups attempting direct isolation and structural elucidation of individual components of the PGBx mixture supports this conclusion. We have now confirmed that the prostaglandin 1 undergoes oligomerization in the same manner as 2, providing additional support for the analog 2 as an appropriate model.[18]

We remain convinced that the most appropriate approach to the structural elucidation of the very complex oligomeric mixtures derived from prostaglandins such as PGBx will be in the determination of the general reaction pathway through study of structurally simpler analogs. We will report in the near future on detailed structural characteristics of trimers derived from the prostaglandin 1 and related analogs.

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References and Notes

1. B. D. Polis, S. Kwong, G. L. Nelson and H. W. Shmukler, Physiol. Chem. Phys., 11, 109 (1979). The term PGBx has previously been applied to complex mixtures derived by very vigorous KOH treatment of PGB_1, cis-PGB_1, 13,14-dehydro-PGB_1, PGA_1 and PGE_1; e.g. see reference 6.

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7. For convenience, the numbering of analog 2 follows that of the prostaglandin convention.

8. In actual practice, the degree of oligomerization is more conveniently controlled by treatment with .005 M ethanolic KOH for 1-2 minutes at room temperature.

9. Field desorption mass spectrometry (FDMS) data were determined by R. Cotter of the Middle Atlantic Mass Spectrometry Laboratory, the Johns Hopkins University School of Medicine, Baltimore, MD.

10. The overall percentage of Dimers 1-4 relative to Dimers 5-6 decreases with increasing reaction time. The distribution of Dimers 1-4 (ca. 38% 1, 26% 2, 19% 3 and 16% 4) and Dimers 5-6 (ca. 1:1) remains essentially constant with increasing reaction time.

11. High resolution mass spectrometry (HRMS) measurements of Dimers 5-6 were determined by D. T. Terwilliger of the Mass Spectrometry Laboratory, the University of Pennsylvania, Philadelphia, PA.

12. Analog 2 exhibits UVmax at 296 nm and a conjugated C=C absorption in the IR at 1585 cm^{-1} while 13,14-dihydro-2 has a UVmax at 238 nm and a conjugated C=C IR absorption at 1640 cm^{-1}.

13. The ^{13}C NMR spectra of Dimers 1-6 were determined by M. Mutter, McNeil Pharmaceutical, Spring House, PA. Chemical shift assignments (ppm, $CDCl_3$) of those carbons involved in oligomer bond formation and which appear as doublets in the off resonance decoupled spectra are as follows: Dimer 1; C-10 46.1 and C-14' 49.6; Dimer 2, C-10 46.6 and C-14' 49.0; Dimer 3, C-10 46.1 and C-13' 37.2; Dimer 4, C-10 45.6 and C-13' 37.6; Dimer 5, C-14 56.2, C-16 49.0, C-13' 48.2 and C-14' 50.4; Dimer 6, C-14 57.5, C-16 47.7, C-13' 46.2 and C-14' 50.7.
The 360 MHz ^{1}H NMR spectra were determined by G. McDonald, Middle Atlantic NMR Facility, University of Pennsylvania, Philadelphia, PA. Chemical shift assignments (δ, $CDCl_3$) for protons on those carbons involved in oligomer formation are as follows: Dimer 1, H-10 2.86 and H-14' 3.42; Dimer 2, H-10 2.88 and H-14' 3.52; Dimer 3, H-10 2.78 and H-13' 3.70; Dimer 4, H-10 2.60 and H-13' 3.52; Dimer 5, H-14 3.20, H-16 2.40, H-13' 3.04 and H-14' 2.91; Dimer 6, H-14 2.66, H-16 2.72, H-13' 3.35 and H-14' 3.00. The assignment of overlapping resonances was facilitated by selective ^{1}H-^{1}H decoupling.

14. The structural assignment of Dimer 6 was confirmed and the cyclopentanone stereochemistry established as all trans by a X-ray crystallographic determination by G. T. DeTitta, the Medical Foundation of Buffalo, Buffalo, NY. Details will be reported elsewhere.

15. As part of this research program samples of analog 2 were supplied to K. Biemann, Mass. Inst. of Tech., Cambridge, MA. Prof. Biemann independently arrived at the same structural assignments as our double addition Dimers 5 and 6. He observed another dimer component, which corresponds to our Dimers 1-4, that underwent further rapid oligomerization but was not characterized. Private communication.

16. The actual oligomeric mixtures formed would be even more complex due to a double addition type product derived from the C-16 enolate addition. The lack of a residual 13,14-unsaturation in this type of product would preclude conversion to a higher oligomer. See also reference 17.

17. The trimer component has been isolated and found to be very complex. The major fraction of trimer component retains a residual 13,14-unsaturation and is formed by C-10 enolate addition to Dimers 1-4. The remaining trimer component lacks a residual 13,14-unsaturation and results from C-16 enolate addition to Dimers 1-4 via the double addition pathway.

18. Prostaglandin 1 gives a similar distribution of oligomers to that of analog 2. Six dimers, which correspond to Dimers 1-6 of 2, were isolated and characterized. Details will be reported separately.

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