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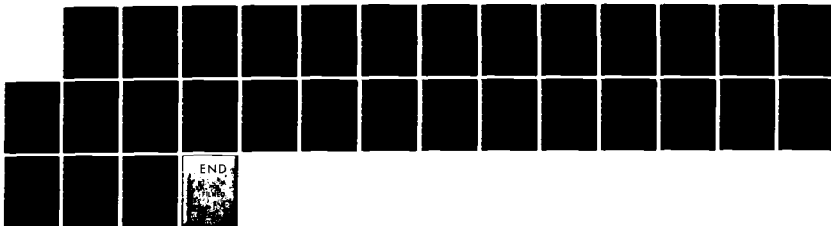
THE SYNTHESIS AND EVALUATION OF 15-KETO-PGB1 ANALOGUES  
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CHEMISTRY G L NELSON 01 MAR 83 N00014-80-C-0117

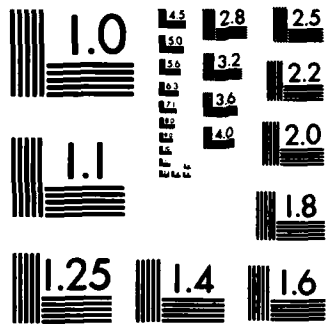
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TECHNICAL REPORT NO. 3

The Synthesis and Evaluation of 15-Keto-PGB<sub>1</sub> Analogues

by

George L. Nelson, Ph.D.  
Saint Joseph's University  
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1 March 1983

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20. ABSTRACT (Continue on reverse side if necessary and identify by block number) The reaction pathway for the oligomerization of 15-dehydro-PGB1, the PGBx precursor, has been determined to proceed by a Michael addition pathway involving multiple reaction sites. The resultant complexity of such oligomeric mixtures precludes the isolation of individual components from the complex PGBx mixture. As an alternative, procedures were developed to provide less complex mixtures in which oligomers as small as dimers and trimers exhibited activities comparable to PGBx. The dimers were isolated and fully characterized. "Blocked" 15-dehydro-PGB1 derivatives were also developed.		

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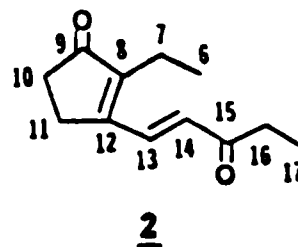
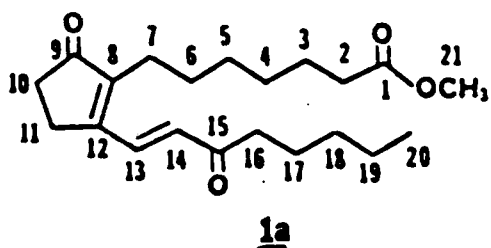
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## I. OBJECTIVES

Prolonged base treatment of 15-dehydro-prostaglandin B<sub>1</sub>, **1a**, results in the conversion to a complex mixture termed PGBx. A number of unique *in vitro* and *in vivo* activities have been described for PGBx which appear to indicate an ability to prevent or restore damage on a cellular level resulting from oxygen deprivation. The detailed structural characteristics of the PGBx mixture remain unclear in spite of the efforts of a number of research groups.

The objective of contract N00014-80-C-0117 for the period covered by Technical Report No. 3 remains the elucidation of the complex structural aspects of the PGBx mixture and the chemical pathway of formation. Detailed structural information is essential to the determination of mode of action leading to the unique *in vitro* and *in vivo* activities observed for this complex mixture. Knowledge of the reaction pathway leading to PGBx formation is required for the design of modified prostaglandin precursors for the preparation of less complicated mixtures that would be more suitable as the basis of a therapeutic agent.



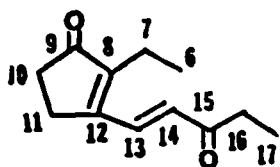
## II. SUMMARY

We have previously demonstrated that the oligomerization of the structurally less complicated 15-dehydro-PGB<sub>1</sub> analog **2** under mild conditions results in the formation of a lower molecular weight oligomeric mixture containing dimer through octamer components. Through the isolation and characterization of individual dimer components, it was established that the initial oligomer forming reaction was taking place via a Michael addition pathway involving multiple reaction sites. As a consequence of such a reaction pathway, the resulting oligomeric mixtures rapidly become very complex as each additional unit of analog **2** is incorporated into the oligomeric chain. Additional support for the operation of this pathway has been provided by investigation of the complex trimer and tetramer components of the oligomerization mixture.

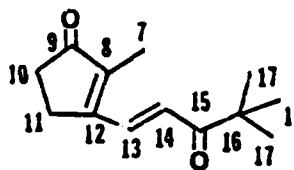
An investigation of the oligomerization pathway of 15-dehydro-PGB<sub>1</sub>, 1, was also undertaken to insure that a similar reaction pathway was operable. This investigation has confirmed that the oligomerization of 15-dehydro-PGB<sub>1</sub> also proceeds through a Michael addition pathway involving multiple reaction sites in a manner analogous to the analog 2. As a result of this investigation, we are convinced that the direct isolation and elucidation of individual active components of the complex mixture termed PGB<sub>x</sub> is not a feasible approach and that a complete characterization of PGB<sub>x</sub> in the classical sense is not possible.

Having established the basis for the exceedingly complex nature of the PGB<sub>x</sub> mixture, our investigations became focused on the development of structurally less complicated oligomeric mixtures retaining the unique *in vitro* and *in vivo* activities of PGB<sub>x</sub>. Our initial approach incorporated the mild oligomerization conditions developed from the previous mechanistic investigations to prepare low molecular weight oligomeric mixtures (principally dimer-tetramer). With the precursor 15-dehydro-PGB<sub>1</sub> methyl ester, such mixtures were inactive since the methyl ester functionality was retained in the oligomer under the very mild conditions of oligomerization. This problem was circumvented by the preparation of 15-dehydro-PGB<sub>1</sub> free acid, 1b, as the precursor for use under the mild oligomerization conditions. Under mild conditions, the free acid 1b could be converted into low molecular weight mixtures which were readily separable into oligomeric components such as dimers, trimers, etc. by size exclusion chromatography. Although the dimer component proved inactive, the trimer and tetramer components exhibited *in vitro* activities in the mitochondrial assay on the same order or higher than "standard PGB<sub>x</sub>". This provided for the first time low molecular weight oligomer components as small as trimers that exhibited *in vitro* mitochondrial activity comparable to that of PGB<sub>x</sub>.

Attempts to isolate individual active trimer components proved less successful. The initial separation of the single addition (Type 1) and the double addition (Type 2) trimers was readily accomplished by reverse phase HPLC. The isolation of pure individual Type 1 and Type 2 trimers in any significant amounts proved considerably more difficult. During the course of the isolation, the level of complexity, due to stereochemical factors, of even the trimer component was becoming evident from the parallel analog studies. Since this level of complexity precluded the direct isolation of the individual trimers in pure form, this approach was abandoned. Instead, the introduction of structural modifications into the precursor was undertaken in order to limit the number of possible reaction sites.



2



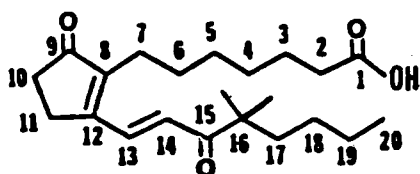
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The "C-16 blocked" analog 3 was designed as a precursor with a reduced number of reaction sites. Since the C-16 carbon in 3 is fully substituted, enolate formation is not possible at that site. This eliminates the formation of all Type 2 (double addition) oligomer formation. In addition, the increased steric bulk at C-16 from the additional substitution was expected to favor oligomer formation at C-13 over C-14. Finally the use of the mild oligomerization conditions was expected to provide low molecular weight, i.e. principally dimer-tetramer, oligomeric mixtures.

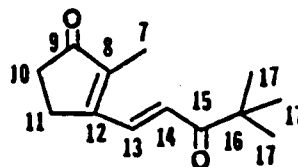
The reaction of the blocked analog 3 under mild conditions resulted in the conversion to an oligomeric mixture that could readily be separated into dimers, trimers, tetramers and some higher oligomers. The success of this approach was immediately evident upon examination of the dimer component. Only three dimers were present and, when characterized, indicated; 1) the absence of any C-16 enolate derived oligomer from Type 2 addition and 2) the attack of the C-10 enolate was preferentially taking place at the less hindered C-13 acceptor site. The oligomerization of the "blocked" analog 3 indicated the realization of our major goals: the formation of a lower molecular weight oligomeric mixture in which one of the nucleophilic reaction pathways was eliminated completely, while a differential reactivity in the acceptor sites was simultaneously introduced.

Several other advantages that were derived from the use of the blocked analog 3 became evident during the course of the investigation of the dimer component. An increased ease in the HPLC separation of the individual dimer components was readily apparent due to the decreased number of components. With this increased availability, the individual dimers become attractive as intermediates of "known structure" for conversion to higher oligomers. Based on this availability, methods for the conversion of individual isolated dimers to trimer and tetramer mixtures of considerably lower complexity were developed.

In addition, the substituents introduced for purposes of blocking the C-16 enolate formation provided a marked increase in the crystallinity of the oligomers. As a result, the Type 1 dimers could be obtained in crystalline form and the crystal structures were determined in collaboration with Dr. George DeTitta of the Buffalo Medical Foundation. This information permits the previously unavailable correlation of proton and carbon-13 NMR spectral parameters with actual stereochemistry in the Type 1 dimers. This correlation provides the foundation for a full stereochemical assignment of the structurally more complex trimers.



4



3

As the potential of the "blocked" analog 3 became evident, a program was undertaken to develop a synthetic route to the corresponding "blocked" prostaglandin, 16,16-dimethyl-15-dehydro-PGB1 4. The initial synthesis was successful and provided enough material for a preliminary evaluation of the chemistry and activity of oligomeric mixtures derived from this "blocked" 15-dehydro-PGB1 derivative. The oligomerization of 4 was carried out under mild conditions and resulted in oligomeric mixtures similar to those obtained from the "blocked" analog 3. The dimer, trimer and tetramer components were readily separated by size exclusion chromatography. Analysis of the dimer component indicated a similarity in pathway to that of the blocked analog 3 although there may be an even greater enhancement of the reactivity of the C-13 over the C-14 acceptor site due to a greater steric hindrance in the approach to C-14 in the "blocked" prostaglandin derivative. The preliminary evaluation of mitochondrial activity by Dr. T. Devlin of Hahnemann Medical College indicated an activity comparable to that of PGBx and the structurally more complicated oligomeric components derived from 15-dehydro-PGB1 free acid 1b.

### III. CONCLUSIONS

Based on studies of model systems, a "blocked" prostaglandin precursor has been developed that can be converted to oligomeric mixtures that are significantly less complicated than those derived from the PGBx precursor 15-dehydro-PGB1. The oligomeric mixtures derived from the "blocked" prostaglandin analog 4 retain a comparable level of activity in the protection of oxidative phosphorylation. The generation of less complicated mixtures was accomplished by modification of 15-dehydro-PGB1 in such a way that the formation of C-16 enolate derived Type 2 oligomer is completely eliminated. The structural modifications also result in a significant enhancement of the reactivity of the C-13 acceptor site over that of the C-14 site. The net result is a major reduction in the complexity of the resulting oligomeric mixture due to the reduction in the number of multiple reaction sites operable in the Michael addition pathway leading to oligomer chain formation. Furthermore, the investigation of the oligomerization of the "blocked" analog 3 has resulted in the establishment of a critical relationship between stereochemistry and the proton and carbon-13 nmr parameters for the single addition Type 1 dimers. This relationship, coupled with the development of procedures for the conversion of dimers of known structure into trimer and tetramer mixtures of considerably lower complexity, provides the greatest potential opportunity in the history of the PGBx program to provide mixtures of greatly reduced complexity in which components as low in molecular weight as trimers exhibit mitochondrial activities comparable to or greater than PGBx.

### IV. PROJECTED INVESTIGATIONS

The synthetic route to the "C-16 blocked" prostaglandin derivative, 16,16-dimethyl-15-dehydro-PGB1, 4, will be refined to allow the preparation of 4 in amounts sufficient for both chemical and biological investigations. Structural studies of trimers and possibly tetramers derived from 3 and 4 will be carried out to provide individual oligomers of established structure for a full range of biological evaluation by Dr. T. Devlin of Hahnemann Medical College. These collaborative investigations may provide the first real insight into the mechanism on a molecular level leading to an understanding of the unique *in vitro* and *in vivo* activities attributed to a range of prostaglandin derived oligomers. As in the past, parallel investigations will be carried out in an effort to provide a structurally simple non-prostaglandin precursor for conversion to oligomeric mixtures of lower complexity that still retain the unique *in vitro* and *in vivo* biological activities associated with prostaglandin oligomers.

## V. BACKGROUND

The term PGBx (i.e. an unknown "X" derived from a prostaglandin B, "PGB") has been rather loosely applied to the reaction mixtures derived from extensive base (hydroxide) treatment of a variety of prostaglandin B precursors<sup>1</sup>. On some occasions, the term PGBx refers to the crude reaction product while at other times to the most active fraction derived from size exclusion chromatography of the crude reaction product. Over the last few years, the term PGBx has come to denote a complex mixture derived from treatment of 15-dehydro-prostaglandin B1 (15-dehydro-PGB1) methyl ester, 1a, with 1 N ethanolic potassium hydroxide<sup>2</sup>. In the discussion that follows, the term "PGBx" will refer to the reaction mixture that results from the treatment of 15-dehydro-PGB1 methyl ester, 1a, with 1 N ethanolic potassium hydroxide for 4 hours at 80 degrees. The term "standard PGBx" will refer to the most active fraction derived from size exclusion chromatography of the crude reaction mixture.

A number of unique *in vitro* and *in vivo* activities have been demonstrated for certain fractions, i.e. standard PGBx, of the complex mixture derived by ethanolic potassium hydroxide treatment of 15-dehydro-PGB1. Standard PGBx protects against the loss of phosphorylating activity during aging *in vitro* of rat liver mitochondria<sup>3-4</sup> and functions as a potent "water soluble" ionophore<sup>5-7</sup> which stimulates the release of  $Ca^{++}$  from sarcoplasmic reticulum and heart mitochondria<sup>5</sup>. *In vivo*, standard PGBx facilitates and significantly increases survival after what otherwise would be lethal episodes of myocardial ischemia in monkeys<sup>8</sup> and restores nervous system function in dogs after otherwise fatal hypoxia<sup>9-10</sup>. Standard PGBx also provides a significant measure of protection against the severest forms of cardiac ischemia in various isolated segments of canine heart<sup>11</sup> and protects isolated anoxic rat heart<sup>12</sup>. PGBx appears to represent a class of structures which possess a unique ability to prevent or restore damage on a cellular level due to oxygen deprivation although the mechanism of this action remains unclear. Such unique properties strongly suggest potential future application in treatment of incidents of cerebral and myocardial ischemia and as a therapeutic agent for hemorrhagic traumatized combat casualties.

At the start of our investigation, relatively little from a chemical viewpoint had been conclusively established concerning the structural details of PGBx or the chemistry involved in the formation of the active site(s). Earlier descriptions of PGBx as a stable free-radical prostaglandin polymer<sup>3</sup> or even as a polymer derivative of prostaglandin B have been demonstrated to be incorrect upon closer inspection<sup>1,13-14</sup>. PGBx has been generally characterized on the basis of spectral data as a complex

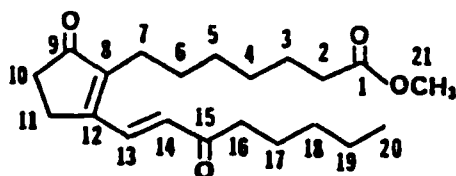
mixture of closely related oligomers formed by an initial reaction at the 13,14-unsaturation of 15-dehydro-PGB1 with the retention of the overall prostaglandin skeleton<sup>1</sup>. Recent attempts by a number of research groups to resolve this complex oligomeric mixture into individual components retaining activity have proven unsuccessful precluding a more definitive structural assignment. The lack of any definitive structural detail has hindered the development of a more detailed understanding of the unique biological properties associated with this material.

As an alternative approach to the direct structural elucidation of PGBx, our present program (N00014-80-C-0117) was undertaken in an effort to define the chemical pathway of oligomerization. Our initial investigation was based on structurally simpler analogs of 15-dehydro-PGB1, the precursor to PGBx.

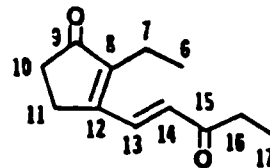
## V. RESULTS AND DISCUSSION

Many of the problems associated with the direct structural elucidation of the complex mixture termed PGBx appeared to be related to the inherent complexity of oligomeric mixtures in which the unit is a 20-carbon prostaglandin.

The analog 2 contains substantially fewer carbons than 15-dehydro-PGB1, 1, the PGBx precursor, but the essential conjugated cyclopentenone functionality of 1 is retained in 2 leading to the expectation of similar reaction pathways.



1a R = CH<sub>3</sub>  
b R = H



2

### Model Studies Related To Analog 2.

Treatment of the analog 2 with ethanolic potassium hydroxide under very mild conditions resulted in the conversion to an oligomeric mixture containing dimer through octamer components. The individual oligomer components, i.e. dimers, trimers, etc., were separated by size exclusion chromatography. The dimer component was further separated by HPLC into six individual dimers referred to as Dimers 1-6 in the following discussion. The structural assignments of the individual dimers, based on a detailed consideration of spectroscopic data, are given in Figure 1.

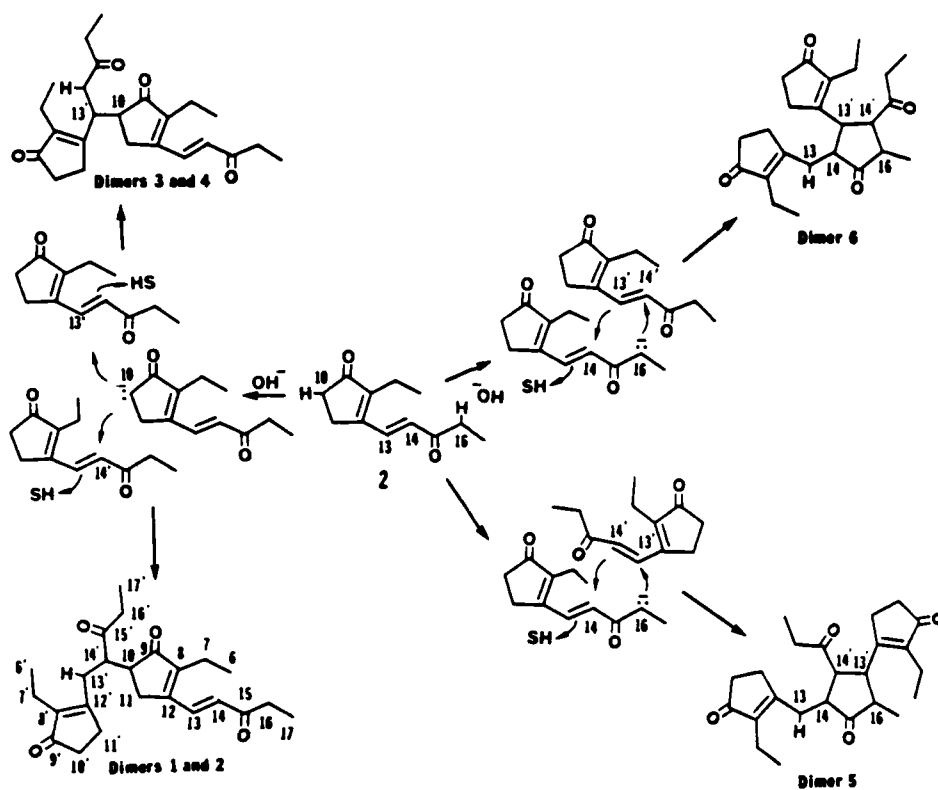


Figure 1

The assignment of the dimer structures provides for the first time an insight into the chemical pathway of oligomerization and an understanding of the exceptional complexity of the oligomeric mixture that results. 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 the analog 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 1-4, derived from C-10 enolate addition, retain a residual 13,14-unsaturation through which further oligomerization can proceed in a similar manner. Dimers 5 and 6, resulting from C-16 enolate initiated double addition, lack a 13,14-unsaturation required for further oligomerization and represent terminal reaction products. The formation of higher oligomers, e.g. dimers to trimers, proceeds in a stepwise fashion by enolate addition to a residual 13,14-unsaturation.

The reaction pathway for oligomer chain formation, as derived from the dimer studies of analog 2, has major implications concerning the structural complexity of PGBx. This is evident when considering the sequence: dimers  $\rightarrow$  trimers  $\rightarrow$  tetramers. Trimers are produced from both C-10 and C-16 enolate addition to either C-13' or C-14' of the residual 13,14-unsaturation of Dimers 1-4 as illustrated in Figure 2 in the case of Dimers 1 and 2. The addition of the C-10 enolate of 2 to either C-13' or C-14' of Dimers 1 and 2 gives rise to two structurally isomeric trimers, each having 4 chiral centers. Such trimers, hereafter referred to as Type 1 trimers, retain a residual 13,14-unsaturation which is required for enolate addition to form the next higher oligomer. In contrast, the C-16 enolate addition to either C-13' or C-14' of Dimers 1 and 2 by the double addition pathway leads to two structurally isomeric trimers, each having 6 chiral centers (Figure 2). Such trimers, referred to as Type 2 trimers, lack the residual 13,14-unsaturation necessary for further chain growth.

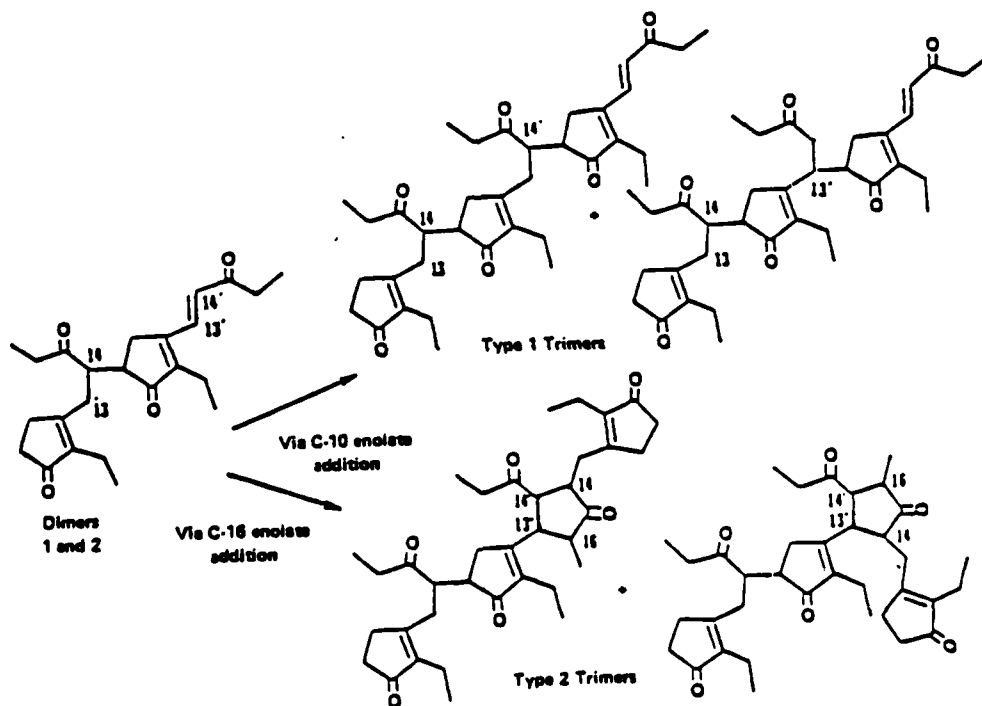
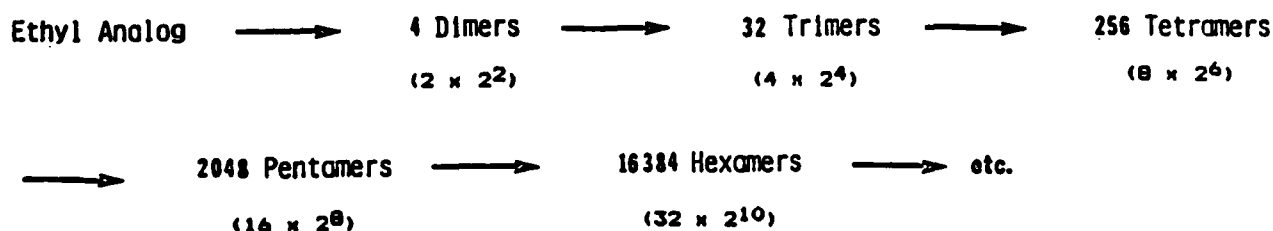


Figure 2

It is when the complexity arising from multiple reaction sites is considered along with the stereochemical consequences of the formation of two new chiral centers for each unit added to the oligomeric chain, that the complexity of a mixture such as PGB<sub>x</sub> becomes fully evident. Considering only the formation of Type 1 oligomers, i.e. those retaining a residual 13,14-unsaturation required for further chain growth, the expected complexity increases exponentially with each new unit added to the oligomer chain. For example, the single addition dimers (Dimers 1-4, Type 1) could be converted to 4 structurally isomeric trimers, each having 4 chiral centers. Each of the structurally isomeric Type 1 trimers could exist as 8 pairs ( $2^4$  possible stereoisomers) of enantiomers. The Type 1 trimers in turn could lead to 8 structurally isomeric Type 1 tetramers, each having 6 chiral centers. Each of the structurally isomeric tetramers could exist as 32 pairs ( $2^6$  possible stereoisomers) of enantiomers. The Type 1 tetramer in turn could be converted to 16 structurally isomeric Type 1 pentamers, each having 8 chiral centers. Each of the structurally isomeric pentamers could possibly exist as 128 pairs ( $2^8$  possible stereoisomers) of enantiomers. In summary, considering only oligomer chain formation by C-10 enolate addition to either the C-13 or C-14 acceptor sites coupled with the creation of two new chiral centers for each unit added, analog 2 could lead to 4 pairs ( $2 \times 2^2$ ) of Type 1 enantiomeric dimers, the dimers to 32 pairs ( $4 \times 2^4$ ) of Type 1 enantiomeric tetramers, the tetramers to 2048 pairs ( $16 \times 2^8$ ) of Type 1 enantiomeric pentamers, the pentamers to 16,384 pairs ( $32 \times 2^{10}$ ) of Type 1 enantiomeric hexamers, etc. (Figure 3).

### Higher Oligomers

Consequences of multiple reaction sites: (SINGLE ADDITION ONLY)



PGB<sub>x</sub>: Estimated to be in the hexamer - octamer range

Figure 3

The actual reaction mixture would be considerably more complex due to the presence of the Type 2 (double addition) oligomers derived from the C-16 enolate addition to either C-13 or C-14. A similar analysis of the possible number of Type 2 C-16 enolate derived stereoisomers reveals a similar level of complexity;  $2 \times 2^4$  dimers,  $4 \times 2^6$  trimers,  $8 \times 2^8$  tetramers,  $16 \times 2^{10}$  hexamers, etc. It becomes evident when considering this level of potential complexity that even if only a fraction of the possible stereoisomers are formed in appreciable yields, the direct structural elucidation of PGB<sub>x</sub>, estimated to be in the hexamer-octamer range<sup>2</sup>, does not represent a feasible approach.

### Oligomerization of 15-Dehydro-PGB<sub>1</sub>

An investigation of the oligomerization pathway of 15-dehydro-PGB<sub>1</sub>, 1, was also undertaken to ensure that a similar reaction pathway was operable. A mild oligomerization of 15-dehydro-PGB<sub>1</sub> (1) has been carried out to give an oligomeric mixture similar to that derived from analog 2. The lower molecular weight oligomers such as dimers, trimers, and tetramers were separated by size exclusion chromatography. The dimer fraction was separated into 6 individual dimers which corresponded structurally to Dimers 1-6 derived from analog 2 indicating a similar reaction pathway in dimer formation (Figure 4).

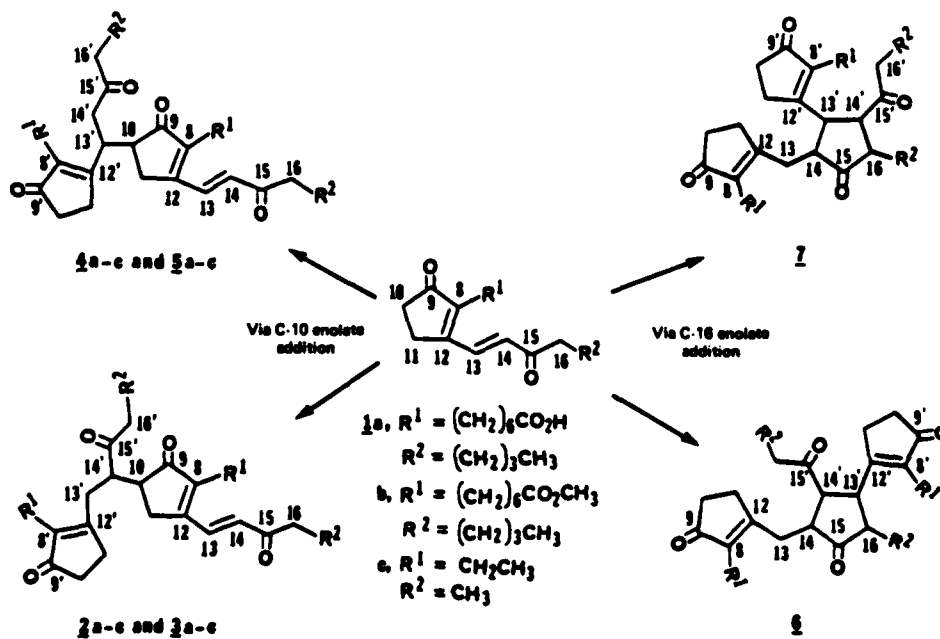


Figure 4

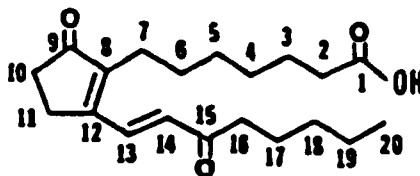
The trimer fractions from both 15-dehydro-PGB1 and analog 2 were analyzed and, although very complex, could be separated into a mixture of Type 1 and Type 2 trimers. A similar observation of both Type 1 and Type 2 tetramers was made for the more complex tetramer fractions. Of major significance, mitochondrial activities in the protection of oxidative phosphorylation comparable to or even higher than PGBx were observed for oligomers, as low in molecular weight as trimers, derived from the mild oligomerization of 15-dehydro-PGB1.

### Less Complicated Oligomeric Mixtures

Recognizing the extremely complex nature of PGBx, alternative approaches to less complicated oligomeric mixtures that retained the activity characteristics associated with PGBx were explored. The investigations were designed to take advantage of two critical pieces of information derived from the analog studies: 1) the oligomerization reaction is very rapid but can be controlled by use of very mild oligomerization conditions and 2) the oligomerization reaction precedes by Michael addition through multiple nucleophilic and acceptor sites.

### Oligomerization of 15-Dehydro-PGB1 Under Mild Conditions

The initial approach to the formation of less complicated oligomeric mixtures from 15-dehydro-PGB1 methyl ester involved the use of the mild reaction conditions developed for the analog 2. The oligomerization took place under mild conditions to give oligomeric mixtures consisting of principally dimer-tetramer components. However, under such mild conditions, the ester functionality was retained in the oligomeric mixture resulting, as would be anticipated from earlier work<sup>1</sup>, in a product that was inactive in the protection of oxidative phosphorylation. In an effort to avoid the problem completely, an alternative synthesis of 15-dehydro-PGB1 free acid **1b** was developed.

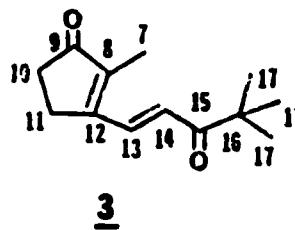
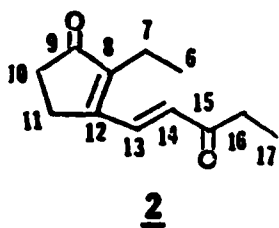


**1b**

The oligomerization of 15-dehydro-PGB1 as the free acid under mild conditions resulted in the conversion to an oligomeric mixture consisting primarily of dimer-tetramer components formed as the free acid. Separation of the mixture by size exclusion chromatography followed by analysis of the oligomer components by T. Devlin of Hahnemann Medical College indicated that, although the dimers had somewhat lower activity, trimer and higher components exhibited activities on the same order or higher than PGBx. Attention was then focused on the trimer component which was separated into Type 1 and Type 2 (cf. above) trimer fractions. Initial work indicated that the Type 1 trimer was the major component and appeared to generally exhibit higher activity. The Type 1 trimer mixture was further fractionated and the initial assay results appeared to indicate that activity was distributed throughout and did not reside with a particular stereoisomer or structure type. An attempt was made to separate this Type 1 trimer fraction into individual components but this proved to be very difficult as would be anticipated from a consideration of the predicted complexity of the Type 1 trimer fraction based on the analog studies. At this stage of the investigation the results of a parallel study of a "blocked" analog became known and led to a shift of focus to the investigation of "blocked" 15-dehydro-PGB1 derivatives.

#### Model Studies of Blocked Prostaglandin Derivatives

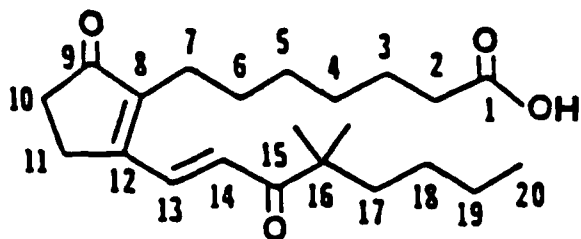
In a parallel investigation, the goal of less complicated oligomeric mixtures was approached by attempting to reduce the number of available reaction sites in the Michael addition pathway. The initial approach involved the complete elimination of the C-16 enolate pathway while simultaneously introducing some degree of reactivity differential in the acceptor sites. This approach is illustrated in the use of the "C-16 blocked" analog 3. Since the C-16 carbon in analog 3 is fully substituted, enolate formation is not possible at that site so that all oligomers previously derived from C-16 enolate addition in analog 2 are eliminated. Simultaneously, the increased steric demand of the C-16 site serves to provide an increased steric hinderance to the approach of the sterically demanding C-10 enolate at the C-14 acceptor site. The use of the previously developed mild oligomerization conditions was expected to provide low molecular weight, i.e. principally dimer-tetramer, oligomeric mixtures.



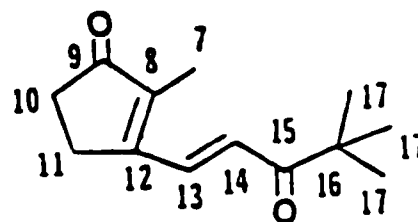
The reaction of the "blocked" analog 3 under mild conditions resulted in the conversion to an oligomeric mixture that could readily be separated into dimers, trimers, etc. The success of this approach was immediately evident upon examination of the dimer component. Only three dimers were present and, when characterized, indicated; 1) the absence of any C-16 enolate derived product of the Type 2 and 2) the attack of the C-10 enolate was preferentially taking place at the less hindered C-13 acceptor site. The oligomerization of the "blocked" analog 3 indicated the realization of our major goals: the formation of a lower molecular weight oligomeric mixture in which one of the nucleophilic pathways was eliminated completely while a differential reactivity in the acceptor sites was simultaneously introduced.

Several other advantages that were derived from the use of the blocked analog 3 became evident during the course of the investigation of the dimer component. An increased ease in the HPLC separation of the individual dimer components was readily apparent due to the decreased number of isomers formed; i.e. three dimers rather than the six obtained from the analog 2. With this increased availability, the individual dimers become attractive as intermediates of "known structure" for conversion to higher oligomers. Based on this availability, methods for the conversion of individual dimers into trimer and tetramer mixtures of much lower complexity were developed. In addition, the substituents introduced for purposes of blocking the C-16 enolate formation provided a marked increase in the crystallinity of the dimers. As a result, the Type 1 dimers could be obtained in crystalline form and the crystal structures were determined in collaboration with Dr. George DeTitta of the Buffalo Medical Foundation. This information permits the previously unavailable correlation of proton and carbon-13 NMR parameters with established stereochemistry in the Type 1 dimers. This correlation provides the foundation for a full stereochemical assignment of the structurally more complex trimers.

## Investigation of Blocked Prostaglandin Derivatives



4

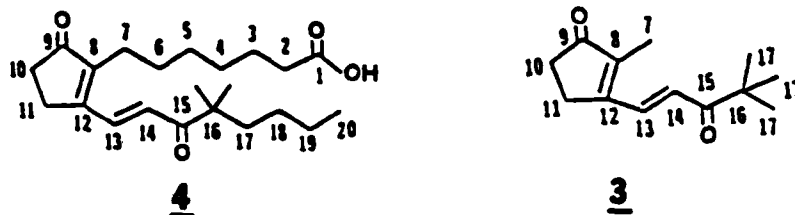


3

As the potential of the "blocked" analog 3 became evident, a program was undertaken to develop a synthetic route to the corresponding "blocked" prostaglandin 16,16-dimethyl-15-dehydro-PGB1 (4, Figure 3). The initial synthesis was successful and provided enough material for a preliminary evaluation of the chemistry and activity of oligomeric mixtures derived from this "blocked" 15-dehydro-PGB1 derivative. The oligomerization of the "blocked" prostaglandin derivative 4 was carried out under mild conditions and resulted in oligomeric mixtures similar to those obtained from the "blocked" analog 3. The dimer, trimer and tetramer components were readily separated from the oligomeric mixture by size exclusion chromatography. Analysis of the dimer component indicated a similarity in pathway to that of the "blocked analog" 3 although there appears to be an even greater enhancement of the reactivity of the C-13 acceptor site relative to the C-14 due an increased steric hinderance in the approach to C-14 in the "blocked" prostaglandin 4. The preliminary evaluation of mitochondrial activity by Dr. T. Devlin indicated an activity comparable to that of PGBx and the corresponding structurally more complicated oligomeric component derived from oligomerization of 15-dehydro-PGB1 free acid (1b) under mild conditions.

## VI. EXPERIMENTAL

### A. Synthesis



The synthesis of the "blocked" analog 3 was accomplished by modification of the synthetic sequence previously developed in our laboratory for the preparation of analog 2.<sup>13</sup> HPLC separations were developed using normal phase chromatography.

The "blocked" prostaglandin 4 was prepared by modification of the synthetic sequence previously developed for 15-dehydro-PGB1 free acid (1a).<sup>2</sup> HPLC separations were carried out using reverse phase chromatography.

### B. Oligomerization of 15-Dehydro-PGB1 (1) Under Mild Conditions: Dimer Formation.

Treatment of 1a with 0.05 M ethanolic KOH (8.3 mg/ml) for 90 minutes gave a crude reaction product from which the dimer component was separated by chromatography on Sephadex LH-20 (CH<sub>3</sub>OH).<sup>15</sup> After treatment with diazomethane, the dimer fraction was separated by HPLC on two 10 mm x 25 cm LiChrosorb columns in series (35-45% EtOAc/C<sub>6</sub>H<sub>12</sub>) into six components.<sup>16</sup> A molecular formula of C<sub>42</sub>H<sub>64</sub>O<sub>8</sub>, i.e. (C<sub>21</sub>H<sub>32</sub>O<sub>4</sub>)<sub>2</sub>, was determined for each component by HRMS measurement of the molecular ion.<sup>17</sup>

Two distinctly different dimer types were indicated by the spectral data. Dimers 2b-5b exhibited UV<sub>max</sub> at 296 and 238 nm and conjugated C=C IR absorptions at 1585 and 1640 cm<sup>-1</sup> whereas dimers 6b and 7b had a single UV<sub>max</sub> at 238 nm and a conjugated IR absorption at 1640 cm<sup>-1</sup>.<sup>18</sup> Dimers 2b-5b exhibited a strong fragment ion at m/e 348, i.e. C<sub>21</sub>H<sub>32</sub>O<sub>4</sub>, while only a weak m/e 348 fragment ion was present in dimers 6b and 7b.<sup>17</sup> The structural assignments of the individual dimers follow from the previously established structures of the dimers derived from the analog 1c and are given in Figure 1.<sup>14</sup> This correspondence is evident from a comparison of <sup>13</sup>C and <sup>1</sup>H chemical shifts of dimers derived from 1a and 1c listed in Tables 1 and 2.<sup>20,21</sup>

Dimers 2a-7a are derived from 1a via Michael addition in which two nucleophilic (C-10 and C-16) and two acceptor (C-13 and C-14) sites are active as indicated in Figure 4. Dimers 2a and 3a, a diastereomeric pair, are formed by the addition of the C-10 enolate of 1a to C-14' of a second unit of 1a. Dimers 4a and 5a, a second diastereomeric pair, arise from the addition of the C-10 enolate of 1a to C-13' of a second unit. Dimer 6a arises from the initial addition of the C-16 enolate of 1a to C-13' of a second unit leading to a new enolate which internally cyclizes by addition of C-14' to C-14 resulting in the formation of a cyclopentanone ring with linkages at 16-13' and 14-14'. In a similar manner, dimer 7a results from the initial addition of the C-16 enolate to C-14' followed by the internal cyclization by addition of C-13' to C-14 giving rise to a cyclopentanone ring with 16-14' and 14-13' linkages. The single addition dimers 2a-5a retain a residual 13,14-unsaturation while dimers 6a and 7a, resulting from double addition, lack the residual 13,14-unsaturation necessary for further oligomerization.

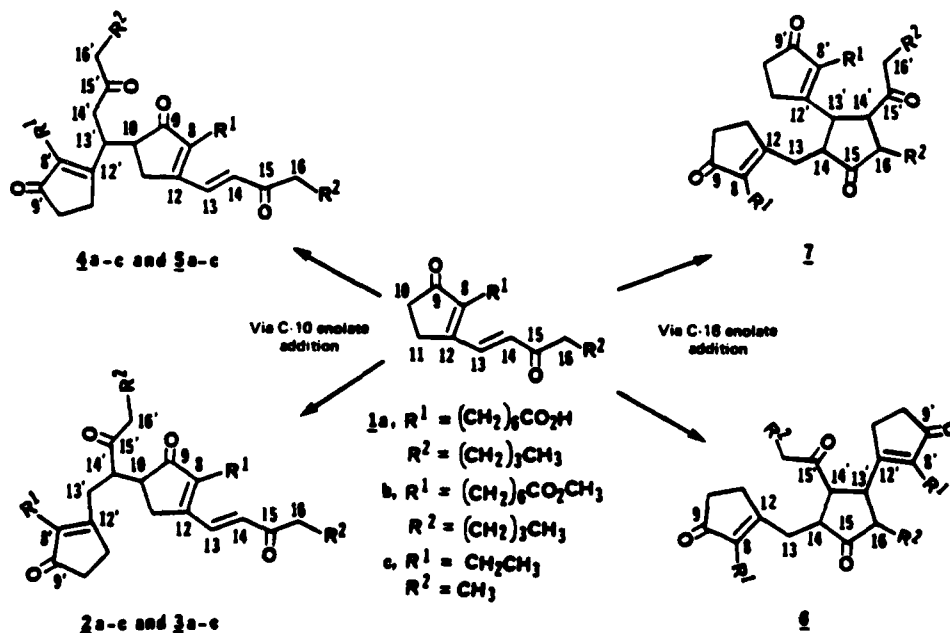


Figure 4

Table 1. A Comparison of  $^{13}\text{C}$  NMR Chemical Shifts<sup>a</sup> of Dimers 2-7 derived from 15-Dehydro-PGB<sub>1</sub> (1a) and the Analog 1c.<sup>10</sup>

C #	2b	2c	3b	3c	4b	4c	5b	5c	6b	6c	7b	7c
C-8	147.6 <sup>s</sup>	148.9 <sup>s</sup>	147.1 <sup>s</sup>	148.4 <sup>s</sup>	147.6 <sup>s</sup>	148.9 <sup>s</sup>	147.4 <sup>s</sup>	148.7 <sup>s</sup>	142.2 <sup>s</sup>	143.5 <sup>s</sup>	142.0 <sup>s</sup>	143.6 <sup>s</sup>
C-8'	142.5 <sup>s</sup>	143.7 <sup>s</sup>	142.9 <sup>s</sup>	144.1 <sup>s</sup>	142.5 <sup>s</sup>	143.8 <sup>s</sup>	142.0 <sup>s</sup>	143.4 <sup>s</sup>	144.7 <sup>s</sup>	146.6 <sup>s</sup>	145.0 <sup>s</sup>	146.6 <sup>s</sup>
C-9	208.8 <sup>s</sup>	207.5 <sup>s</sup>	207.5 <sup>s</sup>	208.2 <sup>s</sup>	208.4 <sup>s</sup>	208.3 <sup>s</sup>	207.7 <sup>s</sup>	208.0 <sup>s</sup>	208.6 <sup>s</sup>	208.8 <sup>s</sup>	209.0 <sup>s</sup>	208.5 <sup>s</sup>
C-9'	208.9 <sup>s</sup>	208.9 <sup>s</sup>	208.7 <sup>s</sup>	208.7 <sup>s</sup>	208.8 <sup>s</sup>	208.8 <sup>s</sup>	208.7 <sup>s</sup>	208.4 <sup>s</sup>	207.9 <sup>s</sup>	208.0 <sup>s</sup>	208.1 <sup>s</sup>	207.8 <sup>s</sup>
C-10	<u>45.9<sup>d</sup></u>	<u>46.1<sup>d</sup></u>	<u>46.6<sup>d</sup></u>	<u>46.6<sup>d</sup></u>	<u>46.1<sup>d</sup></u>	<u>46.1<sup>d</sup></u>	<u>45.3<sup>d</sup></u>	<u>45.6<sup>d</sup></u>	34.3 <sup>t</sup>	34.4 <sup>t</sup>	33.9 <sup>t</sup>	34.2 <sup>t</sup>
C-12	189.6 <sup>s</sup>	189.0 <sup>s</sup>	189.3 <sup>s</sup>	188.8 <sup>s</sup>	188.8 <sup>s</sup>	188.2 <sup>s</sup>	187.9 <sup>s</sup>	187.4 <sup>s</sup>	168.8 <sup>s</sup>	168.6 <sup>s</sup>	168.8 <sup>s</sup>	168.6 <sup>s</sup>
C-12'	168.8 <sup>s</sup>	168.3 <sup>s</sup>	168.2 <sup>s</sup>	167.7 <sup>s</sup>	170.8 <sup>s</sup>	170.5 <sup>s</sup>	171.8 <sup>s</sup>	171.2 <sup>s</sup>	166.8 <sup>s</sup>	168.3 <sup>s</sup>	166.2 <sup>s</sup>	167.7 <sup>s</sup>
C-13	133.2 <sup>d</sup>	133.1 <sup>d</sup>	133.4 <sup>d</sup>	133.2 <sup>d</sup>	133.3 <sup>d</sup>	133.1 <sup>d</sup>	133.3 <sup>d</sup>	133.2 <sup>d</sup>	31.0 <sup>t</sup>	31.0 <sup>t</sup>	31.2 <sup>t</sup>	30.4 <sup>t</sup>
C-13'					<u>37.1<sup>d</sup></u>	<u>37.2<sup>d</sup></u>	<u>37.2<sup>d</sup></u>	<u>37.6<sup>d</sup></u>	<u>48.2<sup>d</sup></u>	<u>48.2<sup>d</sup></u>	<u>46.9<sup>d</sup></u>	<u>46.2<sup>d</sup></u>
C-14	131.3 <sup>d</sup>	131.0 <sup>d</sup>	131.2 <sup>d</sup>	130.9 <sup>d</sup>	131.2 <sup>d</sup>	130.9 <sup>d</sup>	130.8 <sup>d</sup>	130.6 <sup>d</sup>	<u>56.2<sup>d</sup></u>	<u>56.2<sup>d</sup></u>	<u>56.3<sup>d</sup></u>	<u>57.5<sup>d</sup></u>
C-14'	<u>49.6<sup>d</sup></u>	<u>49.6<sup>d</sup></u>	<u>49.1<sup>d</sup></u>	<u>49.0<sup>d</sup></u>					<u>50.4<sup>d</sup></u>	<u>50.4<sup>d</sup></u>	<u>51.3<sup>d</sup></u>	<u>50.7<sup>d</sup></u>
C-15	199.8 <sup>s</sup>	200.0 <sup>s</sup>	199.7 <sup>s</sup>	200.0 <sup>s</sup>	199.5 <sup>s</sup>	200.0 <sup>s</sup>	199.8 <sup>s</sup>	200.0 <sup>s</sup>	214.6 <sup>s</sup>	214.7 <sup>s</sup>	214.7 <sup>s</sup>	214.5 <sup>s</sup>
C-15'	210.7 <sup>s</sup>	211.2 <sup>s</sup>	210.7 <sup>s</sup>	211.1 <sup>s</sup>	208.8 <sup>s</sup>	208.8 <sup>s</sup>	209.5 <sup>s</sup>	209.1 <sup>s</sup>	209.0 <sup>s</sup>	209.3 <sup>s</sup>	209.7 <sup>s</sup>	208.6 <sup>s</sup>
C-16									<u>49.0<sup>d</sup></u>	<u>49.0<sup>d</sup></u>	<u>52.1<sup>d</sup></u>	<u>47.7<sup>d</sup></u>

a) The chemical shifts ( $\delta$ ,  $\text{CDCl}_3$ ) of carbons directly involved in dimer bond formation are underlined.

Table 2. A Comparison of  $^1\text{H}$  NMR Chemical Shifts<sup>a</sup> of Dimers 2-7 derived from 15-dehydro-PGB<sub>1</sub> (1a) and the Analog 1c.<sup>10</sup>

H #	2b	2c	3b	3c	4b	4c	5b	5c	6b	6c	7b	7c
H-10	2.82	2.86	2.83	2.88	2.78	2.78	2.57	2.60				
H-13'					3.77	3.70	3.52	3.52	3.20	2.04	3.29	3.35
H-14'	3.43	3.42	3.50	3.52					2.88	2.91	3.04	3.00
H-14									3.12	3.20	2.58	2.68
H-16									2.40	2.40	2.77	2.72

a) The chemical shifts ( $\delta$ ,  $\text{CDCl}_3$ ) of protons attached to carbons directly involved in dimer bond formation are included in Table 2.

### C. Mitochondrial Assays

Evaluation of the degree of protection and inhibition in the restoration of oxidative phosphorylation in isolated mitochondria was carried out by Dr. T. Devlin of Hahnemann Medical College, Philadelphia, PA.

## VII. REFERENCES AND NOTES

1. B. D. Polis, S. Kwong, E. Polis and G. L. Nelson. PGBx; an oligomeric derivative of prostaglandin B1: Physical, chemical and spectral properties. *Physiol. Chem. Physics*, 12, 167 (1980).
2. B. D. Polis, S. Kwong, E. Polis, G. L. Nelson and H. W. Shumkler. Studies on PGBx; A polymeric derivative of prostaglandin B1: Synthesis and purification of PGBx. *Physiol. Chem. and Physics*, 11, 109 (1979).
3. B. D. Polis, E. Polis and S. Kwong. Protection and reactivation of oxidative phosphorylation in mitochondria by a stable free-radical prostaglandin polymer (PGBx). *Proc. Natl. Acad. Sci. USA*, 76, 1598 (1979).
4. A modification of the mitochondrial assay has been developed by Dr. T. Devlin of Hahnemann Medical College. The mitochondrial assays cited in this report were determined by Dr. Devlin.
5. S. T. Ohnishi and T. M. Devlin. Calcium ionophore activity of a prostaglandin B1 derivative (PGBx). *Biochemical and Biophysical Research Communications*, 89, 240 (1979).
6. C. N. Serhan, H. M. Korchak and G. Weissmann. PGBx, a prostaglandin derivative, mimics the action of the calcium ionophore A23187 on human neutrophils. *J. Immunol.*, 80, 2020 (1980).
7. H. W. Shmukler. Cation complex formation with PGBx, a prostaglandin oligomer, as measured by fluorescence quenching. *Physiol. Chem. Physics*, 80, 558 (1981).
8. E. T. Angelokos, R. I. Riley and B. D. Polis. Recovery of monkeys after myocardial infarction with ventricular fibrillation. Effects of PGBx. *Physiol. Chem. Physics*, 12, 81 (1980).
9. G. Moss, T. Magliocchetti and R. Quarumby. Immediate restoration of central nervous system autonomic cardiopulmonary control: Survival of "lethal" cerebral hypoxia by treatment with prostaglandin Bx. *Surg. Forum*, 29, 513 (1978).
10. R. J. Kolata and B. D. Polis. Facilitation of recovery from ischemic brain damage in rabbits by polymeric prostaglandin PGBx, a mitochondrial protective agent. *Physiol. Chem. Physics*, 13, 545 (1981).

11. A. P. Walls, N. Himori and A. M. Burkman. Comparison of the effects of prostaglandin Bx (PGBx) and verapamil on changes in myocardial function that occur during ischemia. Proc. Fed. Am. Soc. Exp. Biol., April 12, 1980, Atlanta, GA.
12. S. T. Ohnishi and T. M. Devlin. Protection by PGBx of isolated anoxic rat heart. Unpublished results.
13. G. L. Nelson and G. L. Verdine. The base promoted oligomerization of a 15-dehydro-PGB1 analog: Structural insights into the complex oligomeric mixture termed PGBx. Tetrahedron Letters, 23, 1967 (1982).
14. G. L. Nelson and G. L. Verdine. The base promoted oligomerization of 15-dehydro-prostaglandin B1: Dimer formation and structural implications for a complex mixture termed PGBx. Tetrahedron Letters, 23, 991 (1983).
15. Oligomerization of 15-dehydro-PGB1 (1a) as the free acid was preferable to the methyl ester 1b since transesterification of 1b resulted in complications in the HPLC isolation of individual dimers. A reaction period of 90 minutes gave 56% unreacted monomer, 23% dimer and 21% higher oligomer that was principally trimer.
16. The overall percentage of dimers 2a-5a decreases relative to dimers 6a and 7a with increasing reaction time. The distribution of dimers 2a-5a (ca. 25% 2a, 8% 3a, 33% 4a and 34% 5a) and dimers 6a and 7a (ca. 50/50) remains essentially constant with time. The dimers 2a-7a correspond structurally to the six dimers derived from the analog 1c.<sup>14</sup>
17. High resolution mass spectrometry (HRMS) measurements of dimers 2b-7b were performed by Dr. D. T. Terwilliger and M. Davis of the Mass Spectrometry Laboratory, the University of Pennsylvania, Philadelphia, PA. We gratefully acknowledge their able assistance.
18. 15-Dehydro-PGB1 methyl ester (1b) exhibits a UV<sub>max</sub> at 296 nm and a conjugated C=C IR absorption at 1585 cm<sup>-1</sup> while 13,14-dihydro-15-dehydro-PGB1 methyl ester has a UV<sub>max</sub> at 238 nm and a conjugated C=C absorption in the IR at 1640 cm<sup>-1</sup>.
19. The structures of 5 of the 6 dimers derived from the analog 1c have been independently confirmed and the stereochemistry established by X-ray crystallographic determinations carried out by Dr. G. T. DeTitta, the Medical Foundation of Buffalo, Buffalo, NY. Personal communication.

20. The  $^{13}\text{C}$  NMR spectra of dimers 2b-7b were determined at 62.9 MHz using a Bruker WH-250 NMR instrument. We gratefully acknowledge the assistance of Dr. G. Furst of the Department of Chemistry, The University of Pennsylvania, Philadelphia, PA.
21. The  $^1\text{H}$  NMR spectra were determined at 360 MHz using a Bruker WH-360 NMR instrument at the Middle Atlantic NMR Facility, the University of Pennsylvania, Philadelphia, PA. supported by Grant NIH-RR-542. We gratefully acknowledge the assistance and suggestions of Drs. G. McDonald and D. Huang. The assignment of overlapping resonances was facilitated by selective  $^1\text{H}$ - $^1\text{H}$  decoupling experiments.

### VIII. ACKNOWLEDGEMENTS

The very capable technical assistance of the following Saint Joseph's University undergraduate students: Gregory Verdine, Jean Chmielewski, Robert Marquis, Frank Volz, Nancy Mamo and Melville Wyche III is gratefully acknowledged. It is a special pleasure to acknowledge the outstanding experimental work of Mr. Gregory Verdine which provided the basis for the structural elucidation of the individual dimer components of 15-dehydro-FGB1.

The generous assistance we have received in obtaining spectroscopic data is also gratefully acknowledged. The field desorption mass spectra of the lower molecular weight oligomeric mixtures were determined by R. Cotter of the Middle Atlantic Mass Spectrometry Laboratory, the Johns Hopkins University School of Medicine, Baltimore, MD. The high resolution mass spectrometric measurements were determined by D. T. Terwilliger and M. Davis of the Mass Spectrometry Laboratory, the University of Pennsylvania, Philadelphia, PA. The  $^{13}\text{C}$  NMR spectra were obtained at the NMR Laboratory of the Chemistry Department of the University of Pennsylvania with the assistance of Dr. George Furst. The 360 MHz  $^1\text{H}$  NMR spectra were determined at the Middle Atlantic NMR Facility, the University of Pennsylvania, with the assistance of Drs. G. McDonald and D. Huang. The X-ray crystallographic structure determinations were made by Dr. G. T. DeTitta, the Medical Foundation of Buffalo, Buffalo, NY.

I also wish to express my personal gratitude to Saint Joseph's University for a research sabbatical and to the Department of Chemistry, the University of Pennsylvania, for an appointment as Visiting Professor and the generous access to the instrumental facilities during this period. The financial support of this work by the Office of Naval Research under contract N00014-80-C-0117 is gratefully acknowledged.

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