gest: $Lys_{2.05}Orn_{1.05}Glu_{1.99}Thr_{1.90}Ala_{4.05}Phe_{1.00}His_{0.95}Met_{1.02}Asp_{0.96}$-(Ser + Gln)$_{4.90}$. The [Ser$^6$,Orn$^{10}$]-S-peptide gave, after recombination in a 1:1 molar ratio with S-protein, a 40% active partially synthetic ribonuclease.

**Lysylglutamylthreonylalanylalanylprolyllysylphenylalanylglutamylornithylglutaminylhistidylmethionylaspartylserylserylthreonylserylalanylalanine (3G, Pro$^6$ 1–20).** The condensation of 3D, Pro$^6$ 1–12 (0.335 g, 0.174 mmol), with 3E, 13–20[13] (0.306 g, 0.348 mmol, as monoacetate, trihydrate), by the azide procedure was carried out as described above for 3G, Ser$^6$ 1–20, and gave the partially protected [Pro$^6$,Orn$^{10}$]-S-peptide (3F, Ser$^6$ 1–20; 0.264 g, 57%). Treatment of the crude product with anhydrous TFA (2.5 ml), purification on Amberlite CG 50 and on Sephadex G-25, followed by lyophilization gave the pure [Pro$^6$,Orn$^{11}$]-S-peptide (3G, Pro$^6$ 1–20; 0.053 g, 25%), $[\alpha]^{23}D$ $-97.5 \pm 1°$ ($c$ 0.117, water); single ninhydrin- and Pauly-positive component on paper electrophoresis at pH 1.9, 3.5, 6.4, and 9.5; amino acid ratios in acid hydrolysate: $Lys_{2.00}Glu_{2.95}Thr_{1.95}Ala_{3.80}Orn_{1.00}Phe_{1.05}His_{1.05}Pro_{0.95}$-$Met_{0.97}Asp_{1.05}Ser_{2.95}$; amino acid ratios in AP-M digest: $Lys_{1.97}$-$Glu_{1.95}Thr_{2.00}Ala_{3.90}Orn_{0.95}Phe_{0.97}His_{0.97}Pro_{0.97}Met_{1.00}Asp_{0.95}$(Ser + Gln)$_{4.05}$. The [Pro$^6$,Orn$^{10}$]-S-peptide gave, after recombination in a 1:1 molar ratio with S-protein, a 15% active partially synthetic ribonuclease.

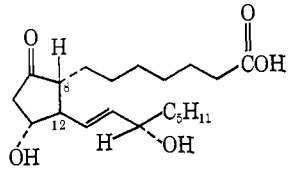
**Acknowledgment.** The authors wish to thank Dr. E. Celon for carrying out the microanalyses, Professor J. Steigman for reading the manuscript, and Mr. U. Anselmi and Mr. D. Stivanello for their skillful technical assistance.

# Communications to the Editor

## Isolation and Characterization of a New Prostaglandin Isomer

*Sir:*

Prostaglandins are biosynthesized from C-20 polyunsaturated fatty acids when the latter are incubated in a variety of mammalian tissue homogenates;[1] for example, prostaglandin $E_1$ ($PGE_1$) (**1**) has been obtained from all-*cis*-8,11,14-eicosatrienoic acid. In addition to $PGE_1$, the corresponding 9$\alpha$-hydroxy compound, prostaglandin $F_1\alpha$ ($PGF_1\alpha$), has also been obtained from the same C-20:3 acid,[2] and recently an isomer of $PGE_1$, 11-dehydro-$PGF_1\alpha$, has been isolated as the main product when a purified microsomal enzyme fraction from sheep seminal vesicular glands was employed for the conversion.[3] We wish to report the isolation and structure determination of a new isomer of $PGE_1$, namely 8-isoprostaglandin $E_1$ (**2**). This acid is of special interest in view of its chemical instability and significant biological properties. Relative to $PGE_1$ this new isomer has only very low activity in stimulating smooth muscle contraction *in vitro* or in

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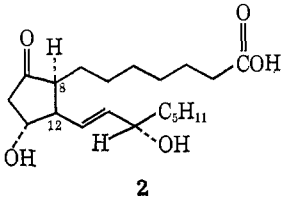


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causing an acute vasodepression in rats,[4] but is of the same order of potency as $PGE_1$ in its antilipolytic effects and in its inhibition of platelet aggregation.[4] Conditions for the large-scale bioconversion of all-*cis*-8,11,14-eicosatrienoic acid and extraction and purification of $PGE_1$ have been described earlier.[5] Mother liquors (20.9 g) from the crystallization of $PGE_1$ obtained biosynthetically were chromatographed on an Amberlyst-15 (silver form) ion-exchange column.[5] The fraction (11.0 g) containing both $PGE_1$ and 8-iso-$PGE_1$ was further fractionated on a column containing acid-washed silica gel (E. Merck AG., 0.05–2 mm). Solvents containing an increasing concentration of ethyl acetate in benzene were used for elution. Crystallization of a fraction eluted just prior to $PGE_1$ from an ethyl acetate–hexane mixture yielded 955 mg of material[6] which had a slightly faster mobility than $PGE_1$ on silica gel thin layers using system A IX[7] for development. The infrared spectrum (Nujol) of 8-iso-$PGE_1$ showed evidence for the presence of the same functional groups as in $PGE_1$[8] but was clearly different from the spectrum of $PGE_1$ in the fingerprint region. The mass spectra of the 8-iso-$PGE_1$ and its methyl ester were identical with the corresponding spectra of $PGE_1$ and $PGE_1$ methyl ester.[8] When 8-iso-$PGE_1$ was treated at 37° for 1 hr with 1 $N$ sodium hydroxide a material was obtained that was identical with prostaglandin $B_1$ by thin layer chromatography and mass spectrometry (as the methyl ester) and in the sign and shape of its optical rotatory dispersion curve between 310 and 600 m$\mu$.

The ORD spectrum of 8-iso-$PGE_1$ is nearly the mirror image of that of $PGE_1$ which has a negative Cotton effect curve.[8] As the most important determinant of the sign of the Cotton effect in cyclopentanones seems to be the stereochemistry of substituents $\alpha$ to the ketone, this suggests that a change has occurred at the C-8 position to give a $\beta$-oriented carboxy side chain (*i.e.*,

(1) D. A. van Dorp, R. K. Beerthuis, D. H. Nugteren, and H. Vonkeman, *Biochim. Biophys. Acta*, **90**, 204 (1964); S. Bergstrom, H. Danielsson, and B. Samuelsson, *ibid.*, **90**, 207 (1964); S. Bergstrom, L. A. Carlson, and J. R. Weeks, *Pharmacol. Rev.*, 20, 1 (1968).

(2) F. P. Kupiecki, *Life Sci.*, **4**, 1811 (1965).

(3) W. E. Lands, E. Granstrom, and B. Samuelsson, *J. Biol. Chem.*, in press.

(4) J. R. Weeks, N. C. Sekhar, and F. P. Kupiecki, *Pharmacologist*, in press. For recent reviews of the biological activity of the prostaglandins, see S. Bergstrom, *Science*, **157**, 382 (1967); S. Bergstrom, L. A. Carlson, and J. R. Weeks, *Pharmacol. Rev.*, 20, 1 (1968).

(5) E. G. Daniels and J. E. Pike, Abstracts, Symposium on Prostaglandins, Worcester Foundation, 1967, to be published.

(6) Material which was pure by other criteria sometimes exhibited a melting point range of about 70–88°. Slow heating on a Kofler microscope hot stage showed the presence of several crystal polymorphs melting and resolidifying over this range. Some samples from ethyl acetate–Skellysolve B melted cleanly at 87–88° without previous change.

(7) M. Hamberg and B. Samuelsson, *J. Biol. Chem.*, **241**, 257 (1965).

(8) P W. Ramwell, J. E. Shaw, G. B. Clarke, M. F. Grostic, D. G. Kaiser, and J. E. Pike, "Progress in the Chemistry of Fats and other Lipids," R. Holman, Ed., Pergamon Press, Oxford, in press.

8α-H). The A-60 nmr spectrum of 8-iso-$PGE_1$ (in acetone-$d_6$) is clearly different from that of $PGE_1$, especially in the 13,14 vinyl proton absorption pattern at δ 5.2–5.78 and in the chemical shifts of the 8 and 12 protons.[9]

Treatment of 8-iso-$PGE_1$ with potassium acetate in ethanol at room temperature for 110 hr gave a major product with the same thin layer chromatographic properties as $PGE_1$. Isolation and crystallization gave a 70% yield of material with the same melting point (114–115.5°) and mixture melting point as $PGE_1$. A 100-Mc nmr spectrum of this substance was identical with that of $PGE_1$.

The origin of this material has been investigated. Bioconversions using 8,11,14-eicosatrienoic-1-$^{14}C$ acid as substrate showed that the yield of 8-iso-$PGE_1$ was less than 5% of that of $PGE_1$. Further, bioconversions to which $PGE_1$-$^3H$ has been added gave 8-iso-$PGE_1$ which contained more than 1% of the added tritium. Treatment of $PGE_1$ with potassium acetate in ethanol at 25° for 100 hr gave pure 8-iso-$PGE_1$ after isolation and recrystallization; the ratio of 8-iso-$PGE_1$ to recovered $PGE_1$ was 1:9. Isomerization under the same condition of $PGE_1$-$^3H$ (100 hr) and 8-iso-$PGE_1$-$^3H$ (300 hr) has confirmed that at least 10% 8-iso-$PGE_1$ is present at equilibrium. We conclude that 8-iso-$PGE_1$ arises by isomerization of $PGE_1$; whether this equilibrium is established under physiological conditions remains to be determined.

**Acknowledgment.** We thank Dr. M. F. Grostic for running the mass spectra (Atlas CH-4) and Dr. W. A. Struck and his associates for the other analytical data.

(9) A manuscript describing a complete analysis of the 100-Mc nmr spectra of $PGE_1$ and 8-iso-$PGE_1$ is in preparation (Dr. G. Slomp, private communication).

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## The Total Synthesis of Prostaglandins

*Sir:*

The total synthesis of amorphous *dl*-prostaglandin $F_1\alpha$ ($PGF_1\alpha$) and *dl*-prostaglandin $E_1$ ($PGE_1$) in impure form has been reported in a preliminary communication by Just and Simonovitch.[1] A more recent communication[2] by another group has stated that no detectable amounts of $PGE_1$ or $PGF_1\alpha$ were found on repetition of this route or various modifications of it. We wish to report our findings in this area which constitute a synthesis of crystalline *dl*-$PGE_1$ and *dl*-8-iso-$PGE_1$, involving solvolysis of bismesylates. Additionally, we have shown that intermediates described by Just and Simonovitch do produce, under modified conditions, *dl*-$PGF_1\alpha$ and *dl*-$PGF_1\beta$ methyl esters.[3]

(1) G. Just and C. Simonovitch, *Tetrahedron Letters*, 2093 (1967).
(2) K. G. Holden, B. Hwang, K. R. Williams, J. Weinstock, M. Harman, and J. A. Weisbach, *ibid.*, 1569 (1968).
(3) The "epoxide" route to 9-hydroxyprostaglandins (PGF-types) was studied collaboratively at McGill University and at The Upjohn Co. A full account of a detailed study of the experiments described in ref 1 is in preparation (authored jointly by the McGill and Upjohn groups).

The *cis* and *trans* isomers of 6-*exo*-(1-heptenyl)-bicyclo[3.1.0]hexan-3-one[1] (**1** and **2**) were separated on a silver nitrate impregnated silica gel column, the *trans* form being eluted with 15% ethyl acetate in Skellysolve B, the *cis* with 25% ethyl acetate. The *cis* isomer **1** on alkylation with methyl ω-iodoheptanoate gave two isomeric keto esters, **3a** and **4**; the *trans* gave, analogously, **5** and **6**. Each pair was separated by silica gel chromatography (elution with ethyl acetate–Skellysolve B mixtures) and configurations of the $C_7$ side chains were assigned on the basis of the nmr spectra of their borohydride reduction products (see below). Base-catalyzed equilibration of pure **3a** and **4** gave the same rato of 35:65 (**3a**:**4**), showing the thermodynamic preference for the β-alkylated isomer **4**.

Sodium borohydride reduction in isopropyl alcohol at 0° of the α-alkylated isomer **3a** gave alcohols **7** and **8** in the ratio 1:9, while similar reduction of **4** gave the corresponding β-alkylated isomers in nearly equal amounts. The stereochemistry of these alcohols was assigned on the basis of their nmr spectra[4] and polarity on adsorbents and was confirmed by their further transformations to the 9α- and 9β-hydroxyprostaglandins of the "F" series and the corresponding 8-iso-PGF compounds.[3]

Hydroxylation of **3a** with osmium tetroxide gave two *erythro-vic*-glycol racemates **9** and **10**, while **5** gave the *threo* pairs **11** and **12**, each pair readily separable by silica gel chromatography. In contrast, the hydroxylation of **3a** and **5** with performic acid containing sodium formate[1] was quite nonstereospecific, giving all four possible *vic*-glycol racemates (70% total yield). Each of these four glycol racemates was converted to its bismethanesulfonate, which was solvolyzed in 2:1 acetone–water at 25°. From the solovolysis products was separated *dl*-prostaglandin $E_1$ methyl ester (**13a**; the yield varied from 5 to 10% depending on the starting glycol used), mp 55–57° (*Anal.* Found: C, 68.08; H, 9.92), along with similar amounts of *dl*-15-epi-$PGE_1$ methyl ester.[5] The major products in each case were unrearranged glycol 15-monomesylates resulting from hydrolysis of only the cyclopropylcarbinyl mesylate. The synthetic *dl*-$PGE_1$ methyl ester was characterized by identity of infrared, nmr, and mass spectra with those of natural material, by identical mobility and color reactions in several thin layer chromatographic systems, and by biological activity greater than 50% of natural material in two test systems.[6]

*dl*-$PGE_1$ (**13b**) was prepared by a modification of the above route. The mixture of alcohols **7** and **8**, obtained by reduction of **3a**, was hydrolyzed to the acids. These were reoxidized with chromic acid (Jones reagent at 0°) to the acid **3b**, which was hydroxylated with performic acid to a mixture of glycol acids analogous to the methyl esters **9**–**12**. These were esterified, using dicy-

(4) Relevant chemical shifts and couplings constants of **7** and **8** were very similar to those of thujyl and neothujyl alcohols; the β-alkylated isomers were related to those of isothujyl and neoisothujyl alcohols in the same way. See M. S. Bergqvist and T. Norin, *Arch. Kemi*, **22**, 137 (1964); K. Tori, *Chem. Pharm. Bull.* (Tokyo), **12**, 1439 (1964). Complete details of the nmr analysis will be published in our full paper.
(5) This was compared with (15*R*)-$PGE_1$ methyl ester prepared from natural (15*S*)-$PGE_1$ by epimerization of the allylic alcohol function in formic acid followed by esterification.
(6) Effects on smooth muscle (gerbil colon) and lowering of blood pressure in rats were measured in the laboratory of Dr. J. R. Weeks, Pharmacology Research, The Upjohn Co.