A stereochemical requirement for the coupling reaction was suggested by the examination of the reaction of 2,2′-dilithiobiphenyl with triphenylsulfonium salt. The reaction involves initial ligand exchange to produce 2-(2′lithiobiphenyl)diphenylsulfonium salt followed by ring closure to VI. For steric and electronic reasons, the biphenylyl group would prefer the diequatorial position. If equatorial–equatorial (ee) coupling occurred, the products would be biphenylene and diphenyl sulfide, whereas apical–equatorial (ae) coupling leads to VII. The formation of VII as the exclusive product derived from VI is indicative of preference for ae coupling.

These reactions are strikingly similar to the reactions of pentavalent phosphorus, lending further support to the above interpretations.[9]

Acknowledgment. We wish to express our thanks to the National Institutes of Health for support of this work.

(9) D. Seyferth, T. Fogel, and J. K. Heeren, *J. Am. Chem. Soc.*, **88**, 2207 (1966), and references therein.
(10) Alfred P. Sloan Foundation Fellow.
(11) National Institutes of Health Predoctoral Fellow.

Barry M. Trost,[10] Ronald LaRochelle,[11] Robert C. Atkins[11]
Department of Chemistry, University of Wisconsin
Madison, Wisconsin 53706
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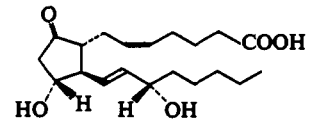
The Structure of the Major Urinary Metabolite of Prostaglandin E_2 in Man

Sir:

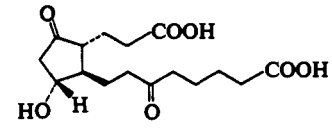
A urinary metabolite of prostaglandin E_2[1] (**1**) in the guinea pig was recently identified as 5β,7α-dihydroxy-11-ketotetranorprostanoic acid.[2] We now report the structure of the major urinary metabolite (**2**) formed from prostaglandin E_2 (**1**) in man.

[17,18-$^3H_2$]Prostaglandin E_2[3] (5.8 μg, specific activity 420 μCi/μmol) was injected intravenously into male subjects. Of the injected radioactivity, about 50% could be recovered in the urine during the first 5 hr and less than 3% during the following 12 hr. The first portion of radioactive urine was added to about 10 l. of urine, and 1-l. samples of this pool were processed as described below.

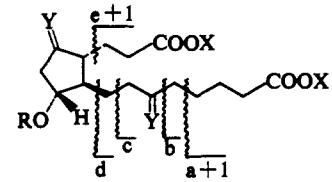
After acidification of the urine, 75–85% of the radioactivity could be extracted with three portions of ethyl acetate. This extract was subjected to reversed-phase partition chromatography.[4] The material in the main peak of radioactivity (200–300 ml of effluent) was treated with diazomethane or diazoethane and was again subjected to reversed-phase partition chromatography.[4] The dimethyl ester **3** (114–156 ml of effluent) and the diethyl ester **4** (168–216 ml of effluent) were then purified by silicic acid chromatography (eluted with ethyl acetate–benzene, 60:40). The esters were subsequently converted into O-methyloxime derivatives and subjected to trimethylsilylation or acetylation. The methods used to prepare the derivatives **5–8** have been described previously.[5,6] Reduction of **3** with sodium borohydride in methanol yielded **9**, from which the triacetate **10** was prepared by treatment with acetic anhydride–pyridine. Reduction of **3** with sodium borodeuteride yielded **11**, which was acetylated to afford **12**.



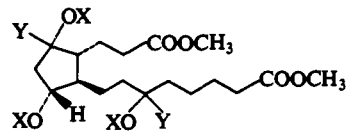
1



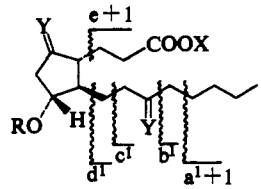
2



3, X = CH_3; Y = O; R = H
4, X = C_2H_5; Y = O; R = H
5, X = CH_3; Y = CH_3ON; R = CH_3CO
6, X = CH_3; Y = CH_3ON; R = $Si(CH_3)_3$
7, X = CH_3; Y = C^2H_3ON; R = $Si(CH_3)_3$
8, X = C_2H_5; Y = CH_3ON; R = $Si(CH_3)_3$



9, X = H; Y = H
10, X = CH_3CO; Y = H
11, X = H; Y = ^2H
12, X = CH_3CO; Y = ^2H



13, X = H; Y = O; R = H
14, X = CH_3; Y = CH_3ON; R = CH_3CO
15, X = CH_3; Y = CH_3ON; R = $Si(CH_3)_3$
16, X = C_2H_5; Y = CH_3ON; R = $Si(CH_3)_3$

7α-Hydroxy-5,11-diketotetranorprostanoic acid (**13**) and the derivatives **14**, **15**, and **16** were prepared[5] for use as references in the analysis by gas–liquid partition chromatography–mass spectrometry.

(1) Prostaglandin E_2 is the trivial name for 11α,15-dihydroxy-9-keto-prosta-5-*cis*,13-*trans*-dienoic acid.
(2) M. Hamberg and B. Samuelsson, *Biochem. Biophys. Res. Commun.*, **34**, 22 (1969).
(3) E. Änggård, K. Grēen, and B. Samuelsson, *J. Biol. Chem.*, **240**, 1932 (1965).
(4) Reversed-phase partition chromatography of ethyl acetate extracts of urine was carried out using columns of 45 g of hydrophobic Hyflo SuperCel and solvent system D supplemented with acetic acid (*cf.* A. Norman, *Acta Chem. Scand.*, **7**, 1413 (1953), and ref 5). Reversed-phase partition chromatography of **3** and **4** was performed with columns of 9 g of hydrophobic Hyflo SuperCel and solvent system F-58 supplemented with acetic acid (*cf.* ref 5).
(5) M. Hamberg, *European J. Biochem.*, **6**, 135 (1968).
(6) $C^2H_3ONH_2 \cdot HCl$ was synthesized by treating $HON(SO_3K)_2$ (F. Raschig, *Ber.*, **40**, 4580 (1907)) with C^2H_3I.

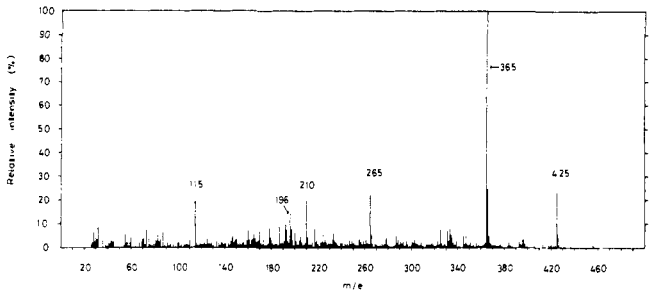


Figure 1. Mass spectrum of **5**.

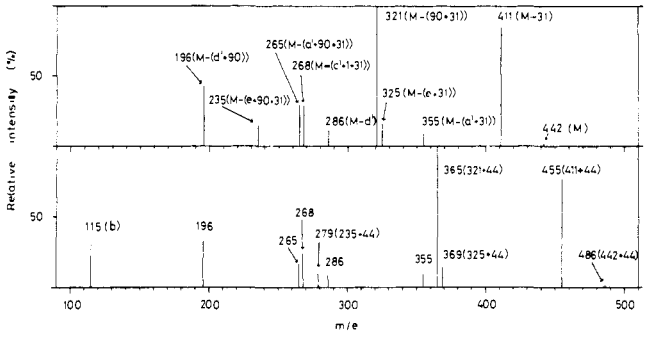


Figure 2. Prominent ions of the mass spectra of **15** (upper mass spectrum) and **6** (lower mass spectrum). The base peaks have been reduced to one-third of the original intensities.

Some information on functional groups in **2** was obtained by gas–liquid partition chromatographic analysis of various derivatives (see Table I). These experiments showed that the difference between the retention times of **5** and **6** (*C* 0.5) was identical with the difference between the retention times of **14** and **15** (*C* 0.5), thus indicating the presence of one hydroxyl group in the metabolite. The presence of two carboxyl groups in **2** was suggested by the finding that the difference between the retention times of **8** and **6** (*C* 1.2) was twice the difference between the retention times of **16** and **15** (*C* 0.6). In addition the mass spectra of **10** and **12** (obtained by reduction with borohydride and borodeuteride, respectively) showed base peaks [M − (3 × 60)] at *m/e* 306 (**10**) and 308 (**12**), thus indicating the presence of two keto groups in **2**.

Table I. *C* Values Found on Gas–Liquid Partition Chromatography (1% SE 30)

Derivative	**5**	**6**	**7**	**8**	**10**	**12**	**14**	**15**	**16**
C value[a]	24.5	24.0	24.0	25.2	25.2	25.2	21.6	21.1	21.7

[a] *Cf.* ref 5.

The mass spectrum of **5** is given in Figure 1. Prominent peaks are seen at *m/e* 425 (M − 31), 365 [M − (60 + 31)], 265 [M − (a + 60 + 31)], 210 [M − (c + 60)], 196 [M − (d + 60)], and 115 (b). The mass spectrum of **14** showed ions of high intensity at *m/e* 381 (M − 31), 321 [M − (60 + 31)], 265 [M − (a' + 60 + 31)], 210 [M − (c' + 60)], and 196 [M − (d' + 60)]. The fragmentation pattern of **5** was thus very similar to that of **14**, the only difference being the increase in 44 mass units of the fragments containing the carbomethoxy group of **5** instead of the methyl group of **14**.

The use of metabolite **13** as reference in the mass spectrometric analysis is further shown in Figure 2 where prominent ions of the mass spectra of derivatives **6** and **15** are given. As can be seen, in the case of **6** the molecular ion as well as ions formed by elimination of 31, e + 31, 90 + 31, and e + 90 + 31 have *m/e* values which are 44 units above corresponding ions in the mass spectrum of **15**. In **6**, elimination of fragments containing the carbomethoxy group at C-16, *viz.*, a + 31, d, c + 1 + 31, a + 31 + 90, and d + 90, yielded ions with the same molecular weights as those formed by corresponding cleavages in **15**. The ion at *m/e* 115 in the mass spectrum of **6** corresponds to the charged methyl pentanoate radical (b) which is formed by cleavage between C-11 and C-12. The interpretation of the mass spectrum of **6** was supported by the mass spectrum of the deuterium-labeled derivative **7**, in which ions containing one or two O-methyloxime groups were shifted 3 or 6 mass units upward, respectively, and by the mass spectrum of the diethyl ester derivative **8**. In the latter case, cleavage between C-11 and C-12 yielded a charged ethyl pentanoate radical (b) which gave rise to a prominent ion at *m/e* 129.

The formation of **2** from prostaglandin E_2 apparently involves four sets of reactions, *viz.*, dehydrogenation of the alcohol group in the side chain,[3] reduction of the *trans* double bond,[3] two steps of β oxidation,[7] and ω oxidation.[8] Studies on quantitative aspects of the urinary excretion of **2** are in progress in our laboratory.

Acknowledgment. This work was supported by grants from the Swedish Medical Research Council (Project No. 13X-217) and by Knut and Alice Wallenbergs Stiftelse.

(7) Prostaglandin E_2 was mainly converted into tetranorprostaglandin E_1 when incubated with a preparation of rat liver mitochondria (*cf.* ref 5).

(8) It has recently been found that prostaglandin A_1 (15-hydroxy-9-ketoprosta-10,13-*trans*-dienoic acid) is converted into a mixture of 20-hydroxyprostaglandin A_1 and 19-hydroxyprostaglandin A_1 by preparations of liver microsomes from guinea pig and man (U. Israelsson, M. Hamberg, B. Samuelsson, and T. Scherstén, unpublished observations).

Mats Hamberg, Bengt Samuelsson
Department of Medical Chemistry
Royal Veterinary College, Stockholm, Sweden
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Chemi- and Bioluminescence of Firefly Luciferin

Sir:

We recently reported a red chemiluminescent emission of firefly luciferin[1] (eq 1) and showed that it was a reasonable model for the red bioluminescence observed *in vitro* at low pH; the keto anion III was identified as the light-emitting species.[1] The normal emission from the firefly *Photinus pyralis in vivo* (and *in vitro* at physiological pH) is yellow-green (ν_{max} 17,700 cm^{-1}).[2] We now report base concentration dependent shifts in color of the chemiluminescence emitted from model systems that support excited enzyme-bound dianion VI as the species emitting the yellow-green light.

To investigate the need of enolizable hydrogens at

(1) T. A. Hopkins, H. H. Seilger, E. H. White, and M. W. Cass, *J. Am. Chem. Soc.*, **89**, 7148 (1967).

(2) H. H. Seliger and R. A. Morton in "Photophysiology," Vol. III, A. C. Geise, Ed., Academic Press, New York, N. Y., 1968, p 291.