(11) W. A. Lathan, W. J. Hehre, L. A. Curtiss, and J. A. Pople, *J. Am. Chem. Soc.*, **93**, 6377 (1971).
(12) The recent work of Schlegel and Wolfe (H. B. Schlegel and S. Wolfe, unpublished results) should be noted. These authors have calculated fundamental vibrational frequencies of methanol and methylamine in good agreement with experiment by evaluating the energy derivatives at the observed rather than the theoretical equilibrium geometry. We thank Professor Wolfe for communicating these results to us prior to publication.
(13) There is, of course, a long history of correlations between force constants and geometrical parameters. For a discussion, see L. Pauling, "The Nature of the Chemical Bond", 2nd ed, Cornell University Press, Ithaca, N.Y., 1948, p 171.

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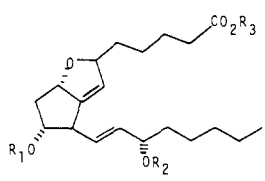
August 24, 1977

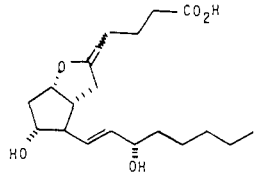
Concerning the Chemical Identity of 6,9α-Oxido-11α,15α-dihydroxyprosta-7(8),(*E*)13-dienoic Acid

Sir:

In 1971, Pace-Asciak and Wolf[1] reported the isolation of two novel prostanoic acid derivatives during the biosynthetic conversion of arachidonic acid into prostaglandins by rat stomach homogenates. The major product was characterized as 6,9α-oxido-11α,15α-dihydroxyprosta-7(8),(*E*)13-dienoic acid (**1**), primarily on the basis of mass spectrometric evidence of several derivatives and products derived from oxidative ozonolysis. A minor product, identified as an isomer of **1**,



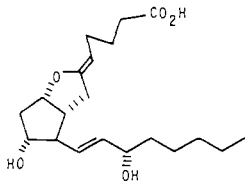
1 $R_1 = R_2 = R_3 = H$
6 $R_1 = R_2 = TMS$; $R_3 = CH_3$



2

6,9α-oxido-11α,15α-dihydroxyprosta-5(6),(*E*)13-dienoic acid (**2**) was postulated as an intermediate in its formation.

Recently, Vane and his co-workers[2,3] reported the conversion of prostaglandin (PG) endoperoxides PGG_2 and PGH_2 by pig and rabbit aortic microsomes to a substance that inhibits platelet aggregation and causes relaxation of blood vessel walls, which they termed PGX. Its chemistry was elucidated by Johnson et al.,[4,5] who assigned to it structure **3**, 6,9α-oxido-11α,15α-dihydroxyprosta-(*Z*)5,(*E*)13-dienoic acid, and renamed it prostacyclin or PGI_2 (**3**). In aqueous and/or acidic media, **3** is readily hydrated to 6-keto-$PGF_{1\alpha}$ (hemiketal form, **4**). While 6-keto-$PGF_{1\alpha}$ (**4**) has been detected in various



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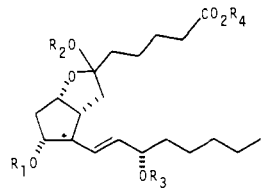
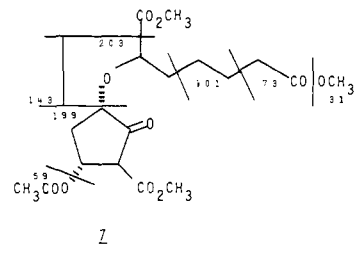


4 $R_1 = R_2 = R_3 = R_4 = H$
5 $R_1 = R_2 = R_3 = TMS$; $R_4 = CH_3$

tissues[6-8] after incubation with arachidonic acid, **1** appears to be confined to the rat stomach[1] and sheep seminal vesicle[9] systems. Incubation of [1-^{14}C]arachidonic acid with rat stomach homogenates in our laboratory[10] afforded [1-^{14}C]-6-keto-$PGF_{1\alpha}$ as the major product, but no **1** was detected. Further, it was reported[4] that the mass spectrum of the trimethylsilyl derivative of the hemiketal form of 6-keto-$PGF_{1\alpha}$ methyl ester (**5**) was found to be identical with that of the trimethylsilyl derivative of 6,9α-oxido-11α,15α-dihydroxyprosta-7(8),(*E*)13-dienoic acid methyl ester (**6**). These results raise considerable uncertainty as to the existence of **1**. In view of the potential physiological importance of natural prostacyclin-type compounds, we decided to reexamine the significant chemical evidences, used in establishing the proposed structure **1**, which is the subject of this communication.

One may suspect that the proposed structure for **1** had been erroneously assigned and that **1** may instead be **4**, since the mass spectra of the two derivatives **5** and presumed **6** were found to be identical. However, it should be emphasized that **1** and **2** had never been isolated in their pure forms; instead, they were always obtained as a mixture termed fraction A.[1] Thus, the value of the mass spectral data of derivatives of fraction A is in considerable doubt. The location of the double bonds in **1** was deduced from the results of the oxidative ozonolysis of the methyl ester and diacetate derivative of fraction A. Gas chromatography of the oxidative products after methylation gave two major fragments. One of these was identified as methyl α-acetoxyheptanoate; the other product showed a molecular ion at *m/e* 402 (M^+) with principal fragments at 371 (M − 31), 329 (M − 73), 311 (M − 60 + 31), 302 (M − (101 − H)), 297 (M − (73 + 32)), 242 (M − (60 + (101 − H))), 210 (242 − 32), 200 (M − (203 − H)), 199 (M − 203), 182 (M − ((2 × 60) + (101 − H))), 143, and 111, and was assigned the structure **7**. These data are clearly not



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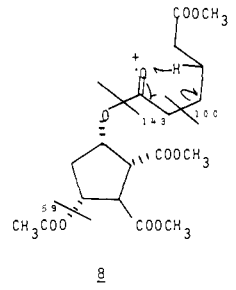
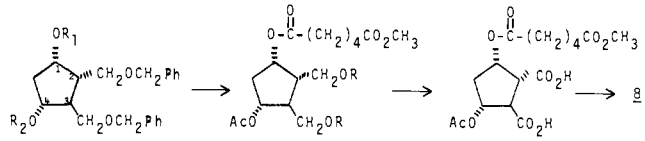


8

in accord with the supposition that **1** is simply the hemiketal form of 6-keto-$PFG_{1\alpha}$. However, a careful analysis of these mass spectral data reveals that an alternative structure **8** more suitably fits the fragmentation pattern. In particular, the loss of 100 was not adequately explained by Pace-Asciak and Wolf,[1] but one can readily envisage this fragment to be derived from a McLafferty rearrangement of the ester grouping resulting in β cleavage as shown in **8**. Also, the base peak, 143, can best be rationalized via the common α cleavage of the ester.

To test the validity of this hypothesis, we undertook the synthesis of **8** using the following sequence of reactions:



10 $R_1 = R_2 = Ac$
11 $R_1 = R_2 = H$
12 $R_1 = H$; $R_2 = Ac$
13 $R_1 = Ac$; $R_2 = H$

14 $R = CH_2Ph$
15 $R = H$

16

Deacetylation of **10**[11] using 2 N methanolic NaOH afforded **11** in quantitative yields. Compound **11** (25 mg) was reacted with a mixture of acetic anhydride–pyridine–ether (0.1:0.1:1.0 mL) for 2 days at 25 °C to yield two monoacetates **12** and **13**[12] and **11** in a ratio of 4:1:5. We assumed that the less hindered hydroxyl group at C-4 in **11** was preferentially acetylated. Also, chemical (CH_3OH-Et_3N-H_2O (2:1:1)) and microbial (*Aspergillus repens*) hydrolyses of **10** gave exclusively **13**, con-

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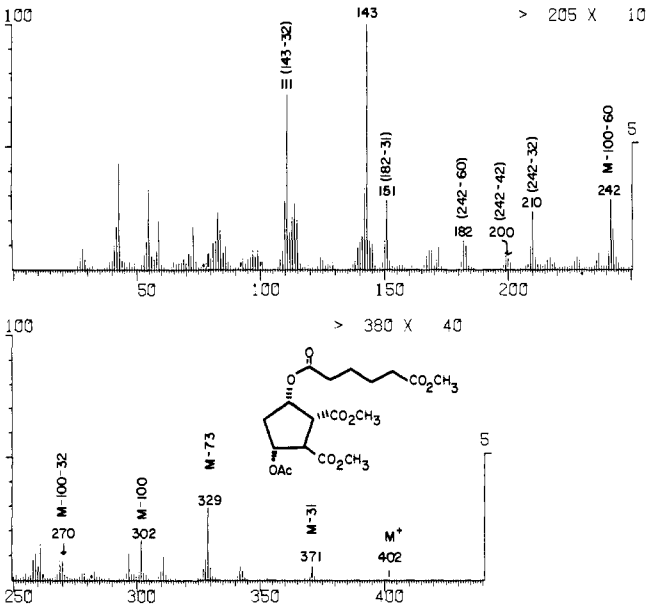
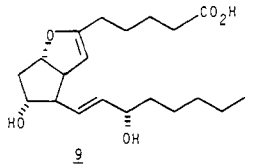


Figure 1. The mass spectrum of **8**.

sistent with the supposition that the least hindered acetoxyl group was cleaved. The monoacetate **12** was treated with an excess of adipyl chloride methyl ester in pyridine to give **14.** Hydrogenolysis (10% Pd/C) of **14** gave **15,** which upon Jones oxidation and methylation afforded **8:** NMR[13] δ 1.5–2.65 (m, 10 H), 2.06 (s, 3 H), 3.17–3.66 (m, 2 H), 3.67 (s, 3 H), 3.70 (s, 3 H), 3.73 (a, 3 H), 5.17–5.52 (m, 2 H).

It is gratifying to note that the mass spectrum of **8,** shown in Figure 1, is indeed identical with that of the oxidative ozonolysis product (after methylation) derived from the methyl ester and diacetate derivative of **1** (Figure 7a, ref 1). This result strongly indicates that the major compound in fraction A as reported by Pace-Asciak and Wolf[1] is the $\Delta^{6(7)}$ isomer, 6,9α-oxido-11α,15α-dihydroxyprosta-6(7),(*E*)13-dienoic acid (**9**).

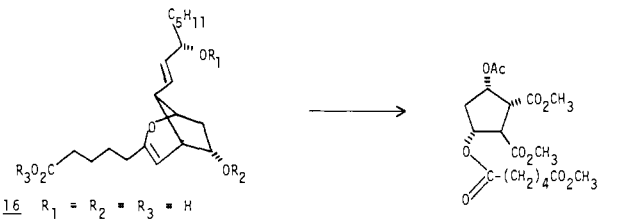


9

One can now readily reconcile the identity of the mass spectra of **5** with that of the trimethylsilyl derivative of **9** methyl ester. Also, our failure to detect the presence of **1** and that 6-keto-$PGF_{1\alpha}$ (**4**) was found to be the major product in our incubation experiments[10] are completely consistent with the anticipated chemical behavior of **9,** for the enol ether **9,** like **3,** should also undergo ready hydrolysis in aqueous and/or acidic media to **4.**

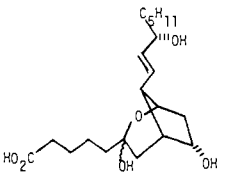
While one may readily envisage a suitable biosynthetic mechanism[14] for the origin of **9** from PGG_2 or PGH_2, one cannot preclude at this stage the possibility that **9** may be nonenzymatically derived from **4** via some yet unknown process, for the procedure[1] used in the isolation of fraction A is incompatible with the properties of **3** and **9.** However, it is also possible that **9** has thus far evaded detection in biological tissues, because, like its isomer **3,** it is unstable and readily decomposes to the common product, 6-keto-$PGF_{1\alpha}$ (**4**).

As the structural assignments of the monoacetates **12** and **13** are based on suggestive evidence, one may raise the possibility that the mass spectrum of presumed **8** is actually that of **18,** derived from **17** via oxidative ozonolysis followed by methylation. In turn, **16** may be nonenzymically formed from



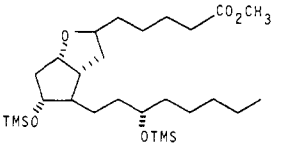
16 $R_1 = R_2 = R_3 = H$
17 $R_1 = R_2 = OAc$; $R_3 = CH_3$

18



19

another hemiketal form of 6-keto-$PGF_{1\alpha}$ (**19**) via dehydration, or biosynthesized from PGG_2 or PGH_2 as follows: heterolytic cleavage of the cyclic endoperoxide to yield an oxonium ion at C-11, which attacks the 5(6) double bond resulting in the formation of a 6,11α-oxido ring and a carbonium ion at C-5; a 1,2-hydride shift followed by proton abstraction from C-7 to afford **16.** However, this interpretation appears less likely in view of our observation that the mass spectrum of **20**[15] was



20

found to be identical with that reported for the trimethylsilyl ether and methyl ester derivative of fraction A after hydrogenation (Figure 5, ref 1). Experiments are currently in progress to further clarify these issues.[16]

Acknowledgments. The authors are grateful to Dr. Geraint Jones of ICI for supplying us with generous quantities of the synthetic intermediate, **10,** Dr. Harold Kluender of Miles Laboratories for the methyl esters of 6-keto-$PGF_{1\alpha}$ and PGI_2, and Mr. Gary Girdaukas for the mass spectral analyses.

References and Notes

(1) C. Pace-Asciak and L. S. Wolfe, *Biochemistry,* **10,** 3657 (1971).
(2) S. Moncada, R. J. Gryglewsky, S. Bunting, and J. R. Vane, *Nature,* **263,** 663 (1976).
(3) R. J. Gryglewsky, S. Bunting, S. Moncada, K. J. Flower, and J. R. Vane, *Prostaglandins,* **12,** 685 (1976).
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(5) R. A. Johnson, F. H. Lincoln, J. L. Thompson, E. G. Nidy, S. A. Mizsak, and U. Axen, *J. Am. Chem. Soc.,* **99,** 4182 (1977).
(6) C. R. Pace-Asciak and G. Rangaraj, *Biochim. Biophys. Acta,* **486,** 579 (1977).
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(8) W. Dawson, J. R. Boot, A. F. Cockerill, D.-N. B. Mallen, and D. J. Osborn, *Nature,* **262,** 699 (1976).
(9) C. Pace-Asciak, *Biochemistry,* **10,** 3664 (1971).
(10) The incubation conditions were the same as those described in ref 1, except the reaction was terminated by acidification of mixture to pH 1.5. Under these conditions, all the PGI_2 is decomposed to **4,** but one would expect **1** to remain unchanged. On the assumption that the chromatographic behavior of **1** and PGI_2 are very similar, we were unable to detect any radioactive product, on TLC corresponding in mobility to authentic PGI_2 methyl ester after methylation of our crude incubation mixture extract.
(11) G. Jones, R. A. Raphael, and S. Wright, *J. Chem. Soc., Perkin Trans. 1,* 1676 (1974).
(12) The isomeric monoacetate, **13,** was also carried through these similar sequence of reactions to yield the corresponding isomer, **18;** its mass spectrum was found to be very similar (but not identical) to that of **8.**
(13) NMR (δ) spectra were taken on a Varian EM 390 spectrometer in $CDCl_3$ solution using Me_4Si as internal standard. Mass spectra were obtained with a Model 1015 Finnigan quadrupole mass spectrometer equipped with a 6000 data syatem using direct probe introduction with an ion source temperature of 170 °C, electron potential of 70 eV, and an ionizing current

of 250 μA. All synthetic intermediates gave NMR and mass spectra consistent with the assigned structures.

(14) This entails the heterolytic fission of the cyclic endoperoxide (PGG_2 or PGH_2) resulting in an electron-deficient oxygen at C-9 which in turn attacks the 5(6) double bond with the formation of a 6,9α-oxy ring and a carbonium ion at C-5. Instead of losing a proton from C-6 to form PGI_2, a 1,2- or possibly a 1,3-hydride shift takes place to yield a carbonium ion at either C-6 or C-7, respectively, which upon loss of a proton from C-7 or C-6 affords **9**.

(15) Tetrahydro-PGI_2 methyl ester was prepared by hydrogenation of PGI_2 methyl ester over 10% Pd/C in ethanol containing 1% Et_3N for 1 h at 25 °C.

(16) This work was supported by NIH Grant AM 09688.

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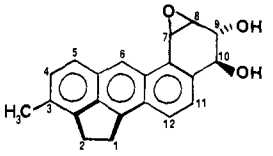
Metabolic Formation of 1,9,10-Trihydroxy-9,10-dihydro-3-methylcholanthrene: a Potential Proximate Carcinogen from 3-Methylcholanthrene

Sir:

3-Methylcholanthrene (MC), first synthesized by Fieser[1] in 1935, is one of the most potent carcinogenic polycyclic aromatic hydrocarbons.[2] The carcinogenicity of MC, like that of other polycyclic aromatic hydrocarbons, is believed to be due to its oxidative metabolism to one or more reactive species.[3-5] To date, several oxygenated metabolites of MC have been identified,[6] but the structure(s) of the chemically reactive, ultimate carcinogenic metabolite(s) of MC has yet to be determined. Previous studies from these laboratories have concentrated on the identification of the ultimate carcinogens from the substituted, alternant hydrocarbons. Data obtained with derivatives of benzo[*a*]pyrene, benzo[*a*]anthracene, and chrysene either indicated or, in some cases, proved that dihydrodiols on benzo rings with "bay-region" double bonds[7] are proximate carcinogens and that diol epoxides of these dihydrodiols are ultimate carcinogenic forms.[8] These results have led to the formulation of the bay-region theory, which postulates that epoxides which form part of a bay region on angular, saturated benzo rings, should have high chemical reactivity and biological activity for electronic reasons.[9] If this theory is applied to the substituted polycyclic aromatic hydrocarbon MC, the diol epoxide[10] predicted to have the highest chemical reactivity is that shown below:



Such a metabolite could form by hydration of MC 9,10-oxide to MC-9,10-dihydrodiol followed by epoxidation of the 7,8 double bond. To investigate this possibility, we have examined the oxidative metabolism of MC by the cytochrome P-450 dependent rat liver monooxygenase system.

In a preliminary study of the metabolism of [^{14}C]-MC by liver microsomes from immature, male Long-Evans rats,[6a] 1-hydroxy-MC (57%), 2-hydroxy-MC (16%), and 2-keto-MC (9%) were found to account for >80% of the total metabolites (4% total metabolism). Only trace amounts of metabolites (<3%) could be identified as dihydroxylated species, mainly *trans*-MC-11,12-dihydrodiol and 1,2-dihydroxy-MC. Similar product ratios were obtained for incubations with microsomes from MC-treated rats in which 25% of the substrate was metabolized.

In light of the somewhat surprising result that dihydrodiols represent only trace metabolites from MC, the possibility that 1-hydroxy-MC, the major primary oxidative metabolite of the hydrocarbon, might function as a dihydrodiol precursor was examined. Since the chromatographic mobilities of such 1-hydroxydihydrodiols were unknown, a highly purified monooxygenase system from rat liver[11] was used to study the metabolism of [1-^{3}H]-1-hydroxy-MC. Addition of homogeneous epoxide hydrase[12] to this system would result in the formation of dihydrodiols at the expense of phenols which are formed by isomerization of arene oxides. Such an experiment (Figure 1) revealed the presence of four dihydrodiols among the metabolites of 1-hydroxy-MC. These metabolites are not formed by the purifed monoxygenase system in the absence of epoxide hydrase (Figure 1).

To isolate sufficient amounts of these dihydrodiols for structure elucidation studies, liver microsomes were prepared from 200 immature, male Long-Evans rats which had been pretreated with MC to induce metabolism and were incubated with 0.51 mmol of racemic [1-^{3}H]-1-hydroxy-MC at 37 °C for 30 min.[13] In this study, 60% of the substrate was metabolized, and the two major dihydrodiol peaks (i.e., peaks a and b in Figure 1) accounted for 23 and 8% of the total metabolites which emerge from the column as distinct peaks.[14] Dihydrodiols a and b were isolated by preparative HPLC as follows: 200 μL of an 8-mL stock solution in THF was injected onto a

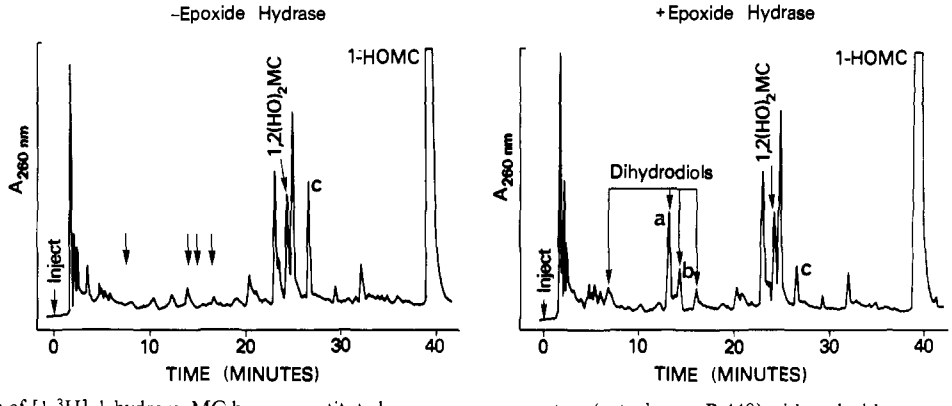


Figure 1. Metabolism of [1-^{3}H]-1-hydroxy-MC by a reconstituted monooxygenase system (cytochrome P-448) with and without epoxide hydrase. Analysis by HPLC was performed on a Du Pont Zorbax ODS column (6.2 mm × 25 cm) which was eluted with a linear gradient of 40–99% acetonitrile in water over a period of 59 min after a 1-min delay at a constant flow rate of 2.0 mL/min. [1-^{3}H]-1-Hydroxy-MC (8.4 μCi/μmol, 80 nmol in 0.1 mL acetone) was incubated with cytochrome P-448 (0.5 nmol), cytochrome *c* reductase (100 U), dilauryl phosphatidyl choline, phosphate buffer (200 μmol, pH 7.0), and $MgCl_2$ (6 μmol) in a total volume of 2 mL at 37 °C for 10 min. Incubations were performed either with or without epoxide hydrase (125 μg, 637 U/mg). The major dihydrodiols (peaks a and b) represented 10.5% of the total metabolites at 15% conversion of the substrate in the presence of epoxide hydrase. The dihydrodiols form mainly at the expense of the phenol containing peak c.

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