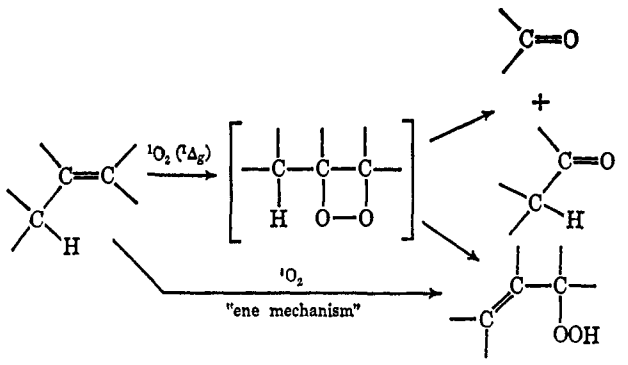


We are continuing our investigations on these reactions in the hope that we may be able to resolve this problem.

(21) Alfred P. Sloan Fellow.

William Fenical, David R. Kearns, Phillip Radlick[21]
Department of Chemistry, University of California
Riverside, California 92502
Received March 18, 1969

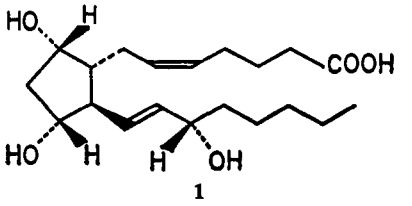
The Structure of a Urinary Metabolite of Prostaglandin $F_{2\alpha}$ in Man

Sir:

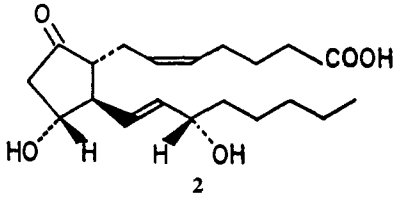
The main urinary metabolite of prostaglandin $F_{2\alpha}$[1,2] (**1**) in the guinea pig was recently identified as 5α,7α-dihydroxy-11-ketotetranorprostanoic acid (**12**). Studies on the metabolism of prostaglandin E_2[3] (**2**) in man led to the identification of a dicarboxylic acid as the major urinary metabolite[4] (**3**). We now wish to report the structure of a urinary metabolite (**4**) of prostaglandin $F_{2\alpha}$ (**1**) in man. [9β-^{3}H]Prostaglandin $F_{2\alpha}$[2] (35 μg, 200 μCi/μmole) was injected intravenously into female subjects. The urinary excretion of radioactive material was completed in about 5 hr; 85–95% of the administered radioactivity had then been excreted. To obtain larger amounts of the urinary metabolites, unlabeled prostaglandin $F_{2\alpha}$ was administered to female subjects by intravenous infusion at a rate of 4–12 μg/min for several hours.[5] Urine was collected from the beginning of the infusion to 5 hr after the administration of prostaglandin $F_{2\alpha}$ was completed. The urine thus obtained was added to the urine containing the tritium-labeled metabolites, and samples of this pool were processed as described below.

The urine was acidified to pH 3 and extracted four times with ethyl acetate and subsequently with butanol. About 50% of the urinary radioactivity was extracted with ethyl acetate and 45% with butanol. The ethyl acetate extract was purified by reversed-phase partition chromatography.[6] Two peaks of radioactivity (I, 110–180 ml of effluent, and II, 180–240 ml of effluent) appeared. The material in peaks I and II was esterified with diazomethane and purified by reversed-phase partition chromatography. Chromatography of the esterified material in peak I gave two peaks of radioactivity, *viz.* Ia (30–40 ml of effluent) and Ib (42–58 ml of effluent). The methyl ester of metabolite II was eluted with 40–53 ml of effluent.

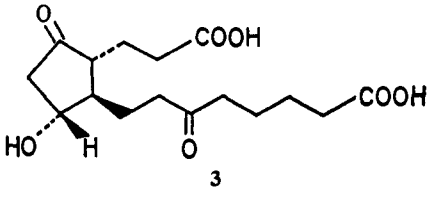
Metabolite Ib (**5**) was further purified by silicic acid chromatography (eluted with ethyl acetate–benzene 60:40) and subsequently converted into four derivatives for glpc and mass spectrometry: acetate **6**, trimethylsilyl ether **7**, O-methyloxime (methoxime) acetate **8**, and methoxime trimethylsilyl ether **9**.[7] Deuterated trimethylsilyl ether derivatives were also prepared using trimethylchlorosilane-d_9 (**10**, **11**).[8] The derivatives **13**, **14**, **15**, and **16** were prepared[2] for use as references in the analysis by glpc–mass spectrometry.



1



2



3

The retention times of the four derivatives of metabolite Ib (**6**, **7**, **8**, and **9**) found on glpc analysis were converted into *C* values[7] and are listed in Table I. The difference between the retention times of the acetates and the trimethylsilyl ethers (between **6** and **7**, $C = 1.0$; between **8** and **9**, $C = 1.1$) indicated the presence of two hydroxyl groups in the metabolite.[4]

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

Derivative	*C* value	Derivative	*C* value
6	24.9	**13**	21.9
7	23.9	**14**	20.9
8	24.9	**15**	21.9
9	23.8	**16**	20.9

In the mass spectrum of **8**, the ion with the highest *m/e* value was found at *m/e* 471. This corresponds to the molecular ion of a C-16 dicarboxylic acid containing two acetoxy groups and one methoxime group.

(1) Prostaglandin $F_{2\alpha}$ is the trivial name for 9α,11α,15-trihydroxyprosta-5(*cis*),13(*trans*)-dienoic acid.
(2) E. Granström and B. Samuelsson, submitted for publication.
(3) Prostaglandin E_2 is the trivial name for 11α,15-dihydroxy-9-ketoprosta-5(*cis*),13(*trans*)-dienoic acid.
(4) M. Hamberg and B. Samuelsson, *J. Am. Chem. Soc.*, **91**, 2177 (1969).
(5) The infusion of prostaglandin $F_{2\alpha}$ to healthy female subjects (20–30 years) was carried out by Dr. M. Bygdeman.
(6) Ethyl acetate extracts of urine were purified by reversed phase partition chromatography using solvent system D containing acetic acid[7] and columns of 27 g of hydrophobic Hyflo Super-Cel. Reversed-phase partition chromatography of the methyl esters of the metabolites was performed with columns of 4.5 g of hydrophobic Hyflo Super-Cel and solvent system F-50 (moving phase: methanol–water (150:150, v/v); stationary phase: chloroform–heptane (45:5, v/v)).
(7) M. Hamberg, *European J. Biochem.*, **6**, 135 (1968).
(8) K. Gréen, submitted for publication.

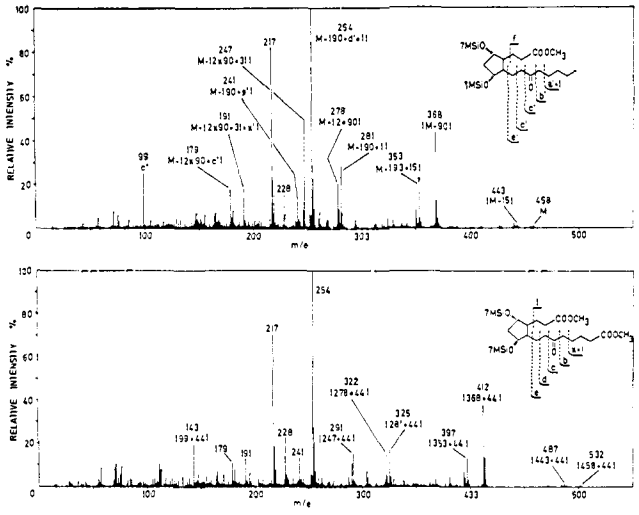


Figure 1. Mass spectra of **14** and **7**: upper spectrum, **14**; lower spectrum, **7**.

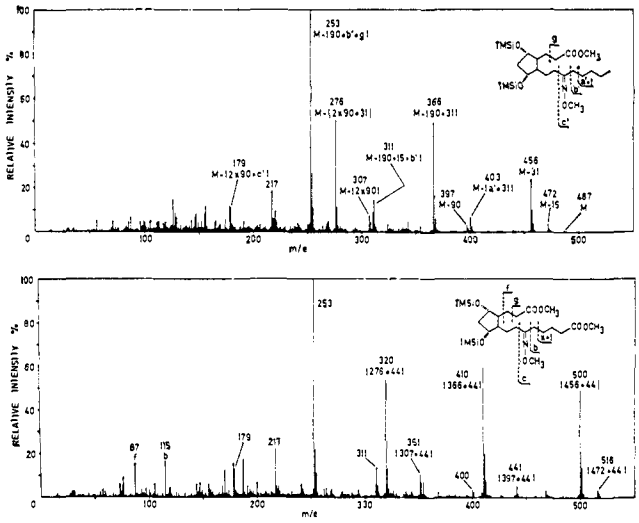


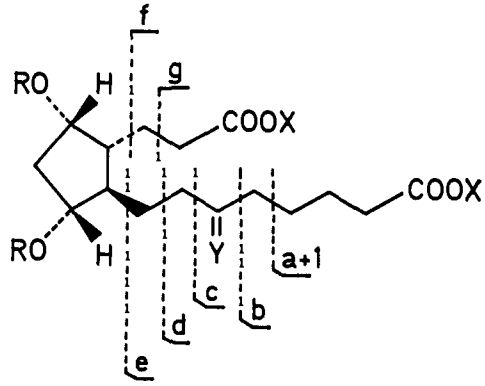
Figure 2. Mass spectra of **16** and **9**: upper spectrum, **16**; lower spectrum, **9**.

Ions of high intensities were found at *m/e* 440 (M − 31), 412 (M − 59), 371 (M − a), 352 (M − (59 + 60)), 320 (M − (2 × 60 + 31)), 312 (M − (59 + a)), 264 (M − (2 × 60 + f)), 220 (M − (2 × 60 + 31 + a)), 200 (e), 187 (d + l), 179 (M − (2 × 60 + c)), 172 (c), 115 (b), and 100 (a). The mass spectrum of **15** showed prominent ions at *m/e* 427 (M), 396 (M − 31), 371 (M − a′), 368 (M − 59), 312 (M − (59 + a′)), 308 (M − (59 + 60)), 276 (M − (2 × 60 + 31)), 220 (M − (2 × 60 + 31 + a′) and M − (2 × 60 + f)), 206 (M − (59 + 60 + b′ + 31)), 179 (M − (2 × 60 + c′)), 156 (e′), 128 (c′), and 87 (f). Several ions of these two mass spectra thus appeared at the same *m/e* values, whereas all fragments containing the ω-carbomethoxy group of **8** were found at an *m/e* value 44 units higher than in the mass spectrum of **15**. The presence of an additional carboxyl group in **4** as compared with **12** was in accordance with the difference in retention time (*C* = 2.9–3.0; *cf.* ref 9) of derivatives **6** and **13**, **7** and **14**, **8** and **15**, and **9** and **16** (Table I).

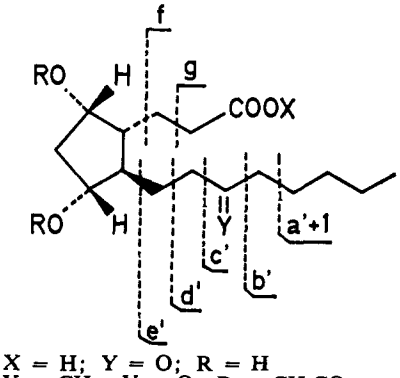
The use of **12** as a reference in the mass spectrometric analysis is illustrated in Figure 1 where the mass spectra of **14** and **7** are shown. All ions that have been formed by eliminations of the carbomethoxy group at C-16 in **7** had the same *m/e* values as those formed by the corresponding fragmentations in **14**. Other ions, formed by removal of 15, 90, (90 + 15), (2 × 90), (90 + f), (2 × 90 + 31), as well as the molecular ions, differed in 44 mass units. The ion at *m/e* 143 (c) in the mass spectrum of **7** corresponds to the ion at *m/e* 99 in the mass spectrum of **14** (α cleavage with respect to the keto group at C-11, $CH_3(CH_2)_4C{=}O$).

The mass spectrum of the deuterium-labeled derivative **10** supported the interpretation of the mass spectrum of **7**. All ions assigned structures containing one and two trimethylsilyl ether groups were shifted 9 and 18 units, respectively, upward in the spectrum of **10**. The ions found at *m/e* 487 (M − 15) and 397 (M − (90 + 15)) in the spectrum of **7** were shifted to *m/e* 502 (M − 18) and 403 (M − (99 + 18)).

Figure 2 shows the mass spectra of **16** and **9**. The same difference in 44 mass units for all ions containing the ω-carbomethoxy group of **9** was seen here, *e.g.*, M − 15 (*m/e* 472 and 516), M − 31 (*m/e* 456 and 500), M − (90 + 31) (*m/e* 366 and 410), and M − (2 × 90 + 31) (*m/e* 276 and 320). The ions formed by removal of fragments containing this carbomethoxy group



4, X = H; Y = O; R = H
5, X = CH_3; Y = O; R = H
6, X = CH_3; Y = O; R = CH_3CO
7, X = CH_3; Y = O; R = $Si(CH_3)_3$
8, X = CH_3; Y = CH_3ON; R = CH_3CO
9, X = CH_3; Y = CH_3ON; R = $Si(CH_3)_3$
10, X = CH_3; Y = O; R = $Si(C^2H_3)_3$
11, X = CH_3; Y = CH_3ON; R = $Si(C^2H_3)_3$



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

appeared at the same *m/e* values as those formed by the corresponding cleavage in **16**, *e.g.*, *m/e* 400 (M − (a + 31)), 311 (M − (90 + 15 + b)), 253 (M −

(9) M. Hamberg and B. Samuelsson, *J. Biol. Chem.*, **242**, 5344 (1967).

(90 + b + g)), and 179 (M − (2 × 90 + c)). The interpretation of the spectrum of **9** was supported by the fragmentation pattern obtained in the mass spectrum of **11**. A shift upward by 9 or 18 mass units was found here for all ions interpreted to contain one or two trimethylsilyl ether groups, respectively.

Metabolite **4** of prostaglandin $F_{2\alpha}$ (**1**) corresponds to metabolite **3** formed from prostaglandin E_2[4] (**2**) and differs only in the functional group at C-5. These two metabolites can be visualized to be formed by dehydrogenation of the alcohol group at C-15, reduction of the Δ^{13} double bond,[2,4,10] two steps of β oxidation,[2,4,7] and ω oxidation.[4] Studies on the structures of the remaining urinary metabolites of prostaglandin $F_{2\alpha}$ in man are in progress in this laboratory.

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

(10) E. Änggård, K. Gréen, and B. Samuelsson, *J. Biol. Chem.*, 240, 1932 (1965).

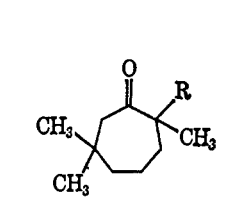
Elisabeth Granström, Bengt Samuelsson
Department of Medical Chemistry
Royal Veterinary College, Stockholm, Sweden
Received March 24, 1969

A Total Synthesis of Culmorin

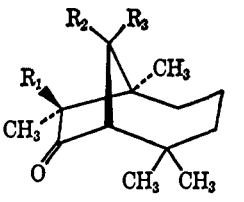
Sir:

The compact and rigid polycyclic ring systems associated with sesquiterpenes related to longifolene and longiborneol present a formidable challenge to total synthesis. The problems involved in rational construction of members of this family are typified by culmorin, a mold metabolite first isolated by Ashley, *et al.*, in 1937[1] and shown recently by Barton and Werstiuk in an elegant degradation study to possess structure **7**.[2] We report herein a rational total synthesis of culmorin which confirms the original structural assignment and which establishes a possible general synthetic pathway to related sesquiterpenes. The approach is based on construction of an intermediate bicyclo[4.2.1]nonane derivative appropriately substituted for introduction of remaining ring skeletal features and functionality.[3]

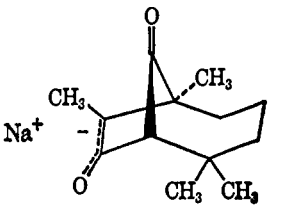
Treatment of tetrahydroeucarvone (**1a**)[4] with sodium hydride in glyme and alkylation of the resulting sodium enolate with 2-chloro-3-pentene[8] produced keto olefin



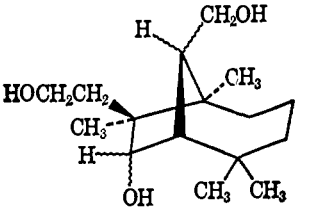
1a, R = H
b, R = CH(CH$_3$)CH=CHCH$_3$
c, R = CH(CH$_3$)CO$_2$C$_2$H$_5$



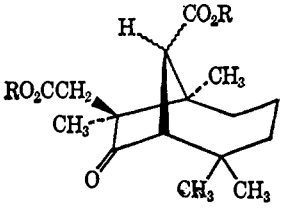
2a, R$_1$ = H; R$_2$, R$_3$ = =O
b, R$_1$ = H; R$_2$ = CH$_3$; R$_3$ = OH
c, R$_1$ = H; R$_2$, R$_3$ = =CH$_2$
d, R$_1$ = CH$_2$CO$_2$C$_2$H$_5$; R$_2$, R$_3$ = =CH$_2$



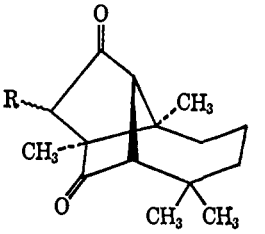
3



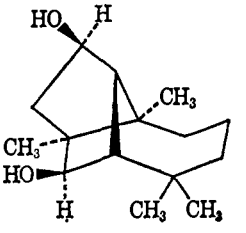
4



5a, R = H
b, R = CH$_3$



6a, R = CO$_2$C$_2$H$_5$
b, R = H



7

1b[9] [λ_{max}^{film} 3.31, 5.97, 10.35 (–CH=CH–), and 5.90 μ (CO), $\delta_{TMS}^{CCl_4}$ [10] 4.7–5.2(m, –C*H*=C*H*–)] in 86% yield as a mixture of epimers. Assignment of gross structure **1b** is based on results of an extensive study of base-catalyzed alkylation and acylation of tetrahydroeucarvone.[11] The latter investigation has established that substitution takes place exclusively at the α-methine position as shown by the appearance in the nmr spectra of a series of homogeneous substitution products of an AB quartet arising from methylene protons α to the ketone carbonyl group. The same type quartet appears in the spectrum of tetrahydroeucarvone. Lemieux–von Rudloff oxidation[12] of **1b** and conventional esterification (HCl–C_2H_5OH) of the resulting keto acid led to keto ester **1c** [λ_{max}^{film} 5.76 (ester CO) and 5.88 μ (ketone CO); $\delta_{TMS}^{CCl_4}$ 4.04 and 4.12 (overlapping quartets, –OC*H*$_2$CH$_3$, J = 7.2 cps)] as an epimeric

(1) J. N. Ashley, B. C. Hobbs, and H. Raistrick, *Biochem. J.*, 31, 385 (1937).

(2) (a) D. H. R. Barton and N. H. Werstiuk, *Chem. Commun.*, 30 (1967); (b) D. H. R. Barton and N. H. Werstiuk, *J. Chem. Soc., C*, 148 (1968).

(3) A pioneering achievement in total synthesis in this family of natural products was the recent preparation of longifolene: E. J. Corey, M. Ohno, R. B. Mitra, and P. A. Vatakencherry, *J. Am. Chem. Soc.*, 86, 478 (1964).

(4) Tetrahydroeucarvone is conveniently prepared by catalytic hydrogenation of eucarvone,[5] which in turn is available from carvone.[6] Carvone has been synthesized previously by several routes.[7]

(5) (a) O. Wallach, *Ann.*, 381, 51 (1911); (b) Y. Naves and P. Ardizio, *Helv. Chim. Acta*, 32, 329 (1949).

(6) (a) A. Baeyer, *Chem. Ber.*, 27, 810 (1894); (b) E. J. Corey and H. J. Burke, *J. Am. Chem. Soc.*, 78, 174 (1956).

(7) For reviews of early synthetic work, see: (a) J. L. Simenson, "The Terpenes," Vol. I, 2nd ed, Cambridge University Press, London, 1953; (b) P. de Mayo in "The Chemistry of Natural Products," Vol. II, K. W. Bentley, Ed., Interscience Publishers, Inc., New York, N. Y., 1959.

(8) J. Baudrenghien, *Bull. Sci. Acad. Roy. Belg.*, 15, 53 (1929); *Chem. Abstr.*, 23, 4196 (1929).

(9) (a) Compositional analyses of all new substances reported herein, including epimeric mixtures, were consistent with assigned structures. (b) Synthetic intermediates described in this report are all racemic. The configurational series depicted is that of natural culmorin.[2]

(10) Unless indicated otherwise, all nmr spectra were recorded at 60 Mcps.

(11) B. W. Roberts and S. C. Welch, unpublished results.

(12) (a) R. U. Lemieux and E. von Rudloff, *Can. J. Chem.*, 33, 1701, 1710 (1955); (b) E. von Rudloff, *ibid.*, 33, 1714 (1955).