Polyhydroxylated By-products of the Enzymatic Conversion of Tritiated Arachidonic Acid into Prostaglandins by Sheep Seminal Vesicles

By C. PACE-ASCIAK* and L. S. WOLFE

(*Donner Laboratory of Experimental Neurochemistry, Montreal Neurological Institute, McGill University, Montreal, Canada*)

Summary In the biosynthesis of prostaglandins by sheep seminal vesicles, the prostaglandin E fraction after treatment with alkali to convert PGE_2 into PGB_2 contained four major compounds still migrating on t.l.c. in the PGE region: (I), (II), and (IV) were derived from triatiated arachidonic acid and (III) was derived from endogenous eicosatrienoic acid.

THIS report describes the structures of two new trihydroxytetrahydrofuran derivatives (I) and (III), one new trihydroxytetrahydropyran derivative (II), and a novel prostanoic acid derivative (IV) obtained during the well-documented enzymatic conversion of arachidonic acid into PGE_2.[1,2] These products were obtained after incubating at 37° for 20 min in an oxygen atmosphere an acetone powder

Pertinent physical data of isolated products

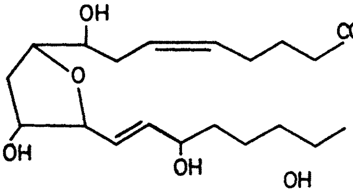
N.m.r. (p.p.m.)	(I)	(II)	(III)
Olefinic protons	5·55 (*cis*) 5·75 (*trans*)	5·55 (*cis*) 5·75 (*trans*)	— 5·75 (*trans*)

Mass spectra *m/e* (% of base peak at 73 in parentheses)

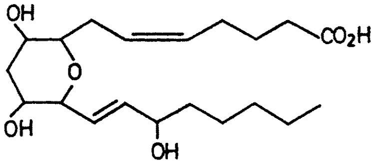
	(I)		(II)		(III)	
	a	b	a	b	a	b
M^+	658(0·3)	600(0·3)	658(1·3)	600(0·6)	660(0·2)	602(0·6)
$M - 90$	568(3·9)	510(2·4)	568(0·8)	510(0·3)	570(5·9)	512(6·5)
$M - (90 + 71)$ ..	497(0·9)	439(1·0)	497(1·1)	439(0·5)	499(1·1)	441(1·7)
$M - 199$	459(1·3)	401(0·1)	459(2·7)	401(3·8)	461(0·2)	403(0·3)
$M - (141 + 90)$ ..	—	369(8·8)	—	369(2·9)	—	371(0·3)
$M - (143 + 90)$ ..	—	367(0·2)	—	367(0·5)	—	369(1·4)
$M - [199 + (2 \times 90)]$	279(3·9)	221(1·8)	279(30·1)	221(10·6)	281(3·7)	223(2·3)
$C_1 \rightarrow C_8$	301(16·1)	243(20·0)	301(3·7)	243(1·8)	303(44·5)	245(36·4)
$C_{15} \rightarrow C_{20}$	173(6·4)	173(5·8)	173(11·9)	173(0·2)	173(8·5)	173(9·1)

a = trimethylsilyl ether and ester derivative; b = trimethylsilyl ether and methyl ester derivative.

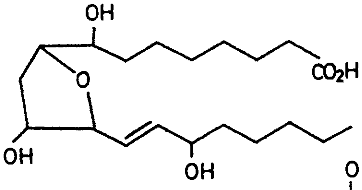
(3 kg fresh tissue) of sheep seminal vesicles and 600 mg of [5,6,8,9,11,12,14,15-$^3H_8$]arachidonic acid ($3\cdot6 \times 10^6$ dpm/mg, 97+% radiochemical purity) in phosphate buffer (pH 7·4, 0·05 M) containing 20 mM EDTA and glutathione and hydroquinone.[3] The products were purified on columns of silicic acid and t.l.c. plates of silica gel G and finally by argentation t.l.c. (10%). About 1 mg of each compound was obtained. Compounds (I), (II), and (IV) contained tritium label and (III) was unlabelled. The structural assignments are consistent with n.m.r. and mass spectra of the trimethylsilyl ethers of the methyl ester and trimethylsilyl ester derivatives (see Table). Oxidative ozonolysis of the acetate methyl ester derivatives of (I) and (II) gave dimethyl glutarate and methyl α-acetoxyheptanoate after methylation of the reaction mixture with ethereal diazomethane. The structure of (III) was derived by direct comparison of its mass spectrum with that of (I) (see Table) in which a molecular ion two units higher was observed in the mass spectrum of (III). The fragment containing the $C_1 \rightarrow C_8$ side chain confirmed the absence of the 5,6-double bond in (III). Compound (IV) was identical with the compound isolated by us from rat stomach homogenates after incubation with arachidonic acid (see ref. 4).



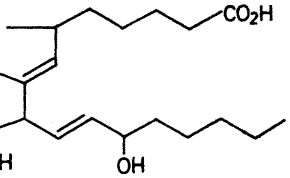
(I)



(II)

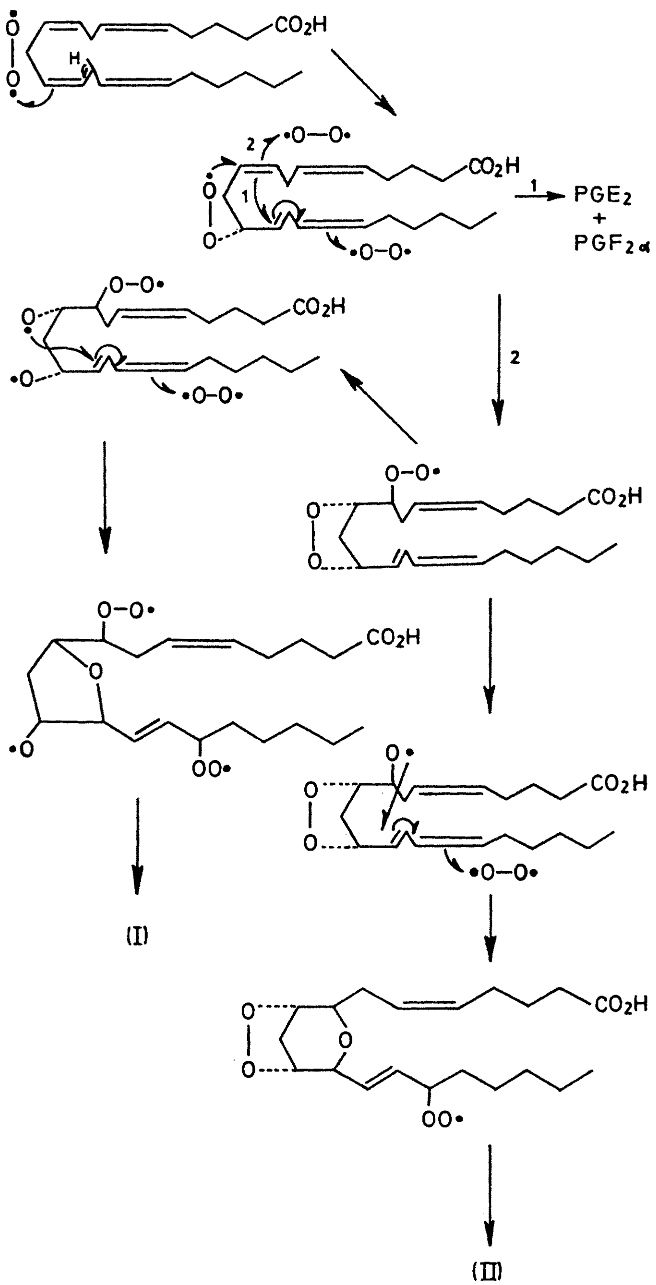


(III)



(IV)

The mechanism we suggest for the biosynthetic conversion of arachidonic acid into (I) and (II) follows in part the scheme suggested for the synthesis of prostaglandins[1] (see Scheme). A similar mechanism is envisaged for the formation of (III) from 8,11,14-eicosatrienoic acid which is present endogenously in significant amounts in sheep seminal vesicles.[5]



SCHEME

This research was supported by a grant (to L.S.W.) from the Medical Research Council of Canada.

(*Received, August 3rd*, 1970; *Com.* 1288.)

---

[1] M. Hamberg and B. Samuelsson, *J. Biol. Chem.*, 1967, **242**, 5336.
[2] S. Bergström, H. Danielsson, and B. Samuelsson, *Biochim. Biophys. Acta*, 1964, **90**, 207.
[3] C. Pace-Asciak and L. S. Wolfe, *Biochim. Biophys. Acta*, 1968, **152**, 784.
[4] C. Pace-Asciak and L. S. Wolfe, preceding communication.
[5] C. Pace-Asciak and L. S. Wolfe, unpublished observations.