A Prostanoic Acid Derivative Formed in the Enzymatic Conversion of Tritiated Arachidonic Acid into Prostaglandins by Rat Stomach Homogenates

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Summary The structure is reported of a new compound which behaves chromatographically like prostaglandin E_2 but is not dehydrated by alkali to PGB_2, formed during the enzymatic conversion of arachidonic acid into PGE_2 by rat stomach homogenates.

THE conversion of certain polyunsaturated fatty acids into prostaglandins by sheep seminal vesicles has been well documented.[1] We recently reported the biosynthesis of PGE_2 (I) and $PGF_{2\alpha}$ (II) from tritiated arachidonic acid by rat stomach homogenates.[2,3] During this study we found that another labelled compound chromatographically similar to (I) was formed in 6—10 times greater amounts than (I) but was not reduced by sodium borohydride or dehydrated by alkali. From scaled-up incubations of 130 rat stomachs with 100 mg of [5,6,8,9,11,12,14,15-$^3H_8$]arachidonic acid ($1{\cdot}1 \times 10^7$ dpm/mg, 97+% radiochemical purity) in 0·05M-phosphate buffer (pH 7·4) containing 20 mM EDTA, glutathione, and hydroquinone, a mixture of (I) and (IV) was isolated (incorporation 12%). Compound (IV) was separated from (I) by alkali dehydration in which (I) was converted into the less polar product (III). Purification of (IV) could then be achieved by preparative t.l.c. on silica gel G plates with and without 10% $AgNO_3$. The structure (IV) is consistent with i.r., n.m.r., and mass spectra of the following derivatives: the trimethylsilyl

ether derivative of the methyl ester (a) and trimethylsilyl ester (b). The mass spectrum of the trimethylsilyl ether methyl ester after hydrogenation (c) showed a molecular ion four mass-units greater than derivative (a) confirming the presence of two double bonds (see Table). Oxidative ozonolysis of the acetate methyl ester gave only α-acetoxyheptanoic acid.

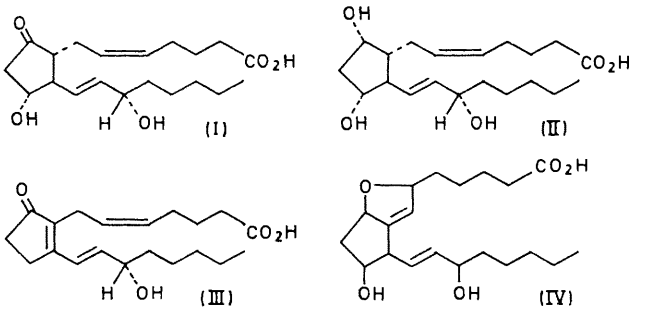
Some chemical properties of (IV)

I.r. (cm^{-1})	free acid	methyl ester
	3400(OH)	3400 (OH)
	1710 (CO_2H)	1730 (CO_2Me)
N.m.r. (p.p.m.)		methyl ester
		2·26 ($CH_2{\cdot}CO_2CH_3$)
		3·60 (CO_2CH_3)
		4·00 ($HC{\cdot}OH$)
		4·65 (olefinic protons)
		5·35 (olefinic protons)

	a	b	c
G.l.c. (C-values[5])†	24·48	25·40	24·56

Mass spectra (m/e)† significant ions	a	b	c
M^+	510	568	514
$M-71$	439	497	443
$M-90$	420	478	424
$M-115$	—	—	399
$M-(115+42)$	—	—	357
$M-173$	337	395	341
$M-[(2\times 90)+(C_1\rightarrow C_3)+18]$	225	225	—
$[C_{15}\rightarrow C_{20}]$	173	173	173

†a = trimethylsilyl ether and methyl ester.
b = trimethylsilyl ether and ester.
c = trimethylsilyl ether and methyl ester after hydrogenation.



We believe that (IV) is formed after cleavage of the cyclic endo-peroxide intermediate postulated by Hamberg and Samuelsson[1] for prostaglandin synthesis through an attack of oxygen radical at C-9 on the 5,6 *cis*-double bond followed by peroxidation at C-5, dehydration, and isomerisation. Compound (IV) was also formed in small amounts when seminal vesicle acetone powders were incubated with arachidonic acid.[4]

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