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Polyhydroxy Cyclic Ethers Formed from Tritiated Arachidonic Acid by Acetone Powders of Sheep Seminal Vesicles*

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ABSTRACT: Three novel derivatives of polyunsaturated fatty acids were isolated during the enzymatic conversion of tritiated arachidonic acid into prostaglandins by acetone powders of sheep seminal vesicles. Two were derived from exogenous tritiated arachidonic acid and are: 9(12)-oxy-8,11,15-trihydroxyeicosa-5,13-dienoic acid (I) and 6(9)-oxy-11,15-dihydroxyprosta-7,13-dienoic acid (II). The third compound, 9(12)-oxy-8,11,15-trihydroxyeicosa-13-enoic acid (III), was derived from endogenous eicosatrienoic acid. Compound II was also shown to be formed from arachidonic acid by rat stomach homogenates. The structure of I was elucidated mainly by mass spectrometry of several derivatives and of the products of oxidative ozonolysis. Compounds III and I could be converted into the same compound by catalytic reduction.

The conversion of certain polyunsaturated fatty acids into prostaglandins and their nor and homo derivatives by sheep seminal vesicles has been well documented (Bergström *et al.*, 1964; Wallach, 1965; Kupiecki, 1965; Struijk *et al.*, 1966; Hamberg and Samuelsson, 1967; Beerthuis *et al.*, 1968; Lapidus *et al.*, 1968). During our investigation of the biosynthesis of prostaglandins from tritiated arachidonic by the rat stomach, the major quantity of radioactivity converted into more polar products was found associated with a compound migrating chromatographically like prostaglandin E_2[1] (Pace-Asciak and Wolfe, 1970a). This was shown to be a novel prostanoic acid derivative containing an ether linkage between the C-6 and C-9 positions (Pace-Asciak and Wolfe, 1970b). The present work was carried out to determine whether this compound and/or others were formed in a seminal vesicle preparation known to have a high capacity to form prostaglandins. A preliminary communication has already appeared (Pace-Asciak and Wolfe, 1970c).

Experimental Section

Preparation of Acetone Powder. Seminal vesicles from sheep were obtained from Canada Packers Ltd., Montreal. The glands were dissected at the killing floor, washed with water, and immediately frozen on Dry Ice until used. When 3 kg was collected, acetone powders were prepared in 300 g (fresh weight) lots as follows. The glands were placed in a mortar filled with liquid nitrogen and pulverized by pounding with a pestle. The powdered frozen tissue was poured with liquid nitrogen into precooled acetone (4 l.) and the mixture was stirred at room temperature until a temperature of −20° was reached, then suction filtered through a Büchner funnel. The powder was again extracted with acetone (4 l.) precooled to −20°, and the mixture was stirred at room temperature until a temperature of 4° was reached. The mixture was filtered and finally 400 ml of diethyl ether was passed through the powder

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[1] The abbreviations used are: PGA_1 and prostaglandin A_1, 9-keto-15(*S*)-hydroxyprosta-10,13-dienoic acid; PGA_2 and prostaglandin A_2, 9-keto-15(*S*)-hydroxyprosta-5,10,13-trienoic acid; PGB_2 and prostaglandin B_2, 9-keto-15(*S*)-hydroxyprosta-5,8(12),13-trienoic acid; PGE_1 and prostaglandin E_1, 9-keto-11α,15(*S*)-dihydroxyprost-13-enoic acid; PGE_2 and prostaglandin E_2, 9-keto-11α,15(*S*)-dihydroxyprosta-5,13-dienoic acid; $PGF_{1\alpha}$ and prostaglandin $F_{1\alpha}$, 9α,11α,15(*S*)-trihydroxyprost-5-enoic acid; $PGF_{2\alpha}$ and prostaglandin $F_{2\alpha}$, 9α,11α,15(*S*)-trihydroxyprosta-5,13-dienoic acid; BHT, butylated hydroxytoluene.

in the Büchner funnel. The acetone and ether washed powder was air-dried for a few minutes, then placed in a desiccator, and evacuated for 5 hr. The yield of acetone powder was about 15% of the wet weight of glands.

Incubation Conditions. Eight separate incubations were carried out using a total of 600 mg of tritium-labeled arachidonic acid and 360 g of acetone powder. The following procedure describes one of such incubations. To tritium-labeled arachidonic acid (75 mg, 3.6×10^6 dpm/mg) in 0.5 ml of acetone in a 6-l. erlenmeyer flask 45 g of acetone powder was added and 1 l. of preoxygenated ice-cold buffer (0.05 M, KH_2PO_4–NaOH, pH 7.4) containing 0.02 M EDTA, reduced glutathione (56 μg/ml), and recrystallized hydroquinone (0.57 μg/ml). The mixture was incubated at 37° for 60 min while a gentle stream of oxygen was kept passing through the solution. Four volumes of ethanol containing a few crystals of BHT antioxidant were added to terminate the incubation, and the mixture was left stirring at room temperature for 45 min. Filtration through Whatman No. 42 filter paper gave a clear yellow filtrate which was evaporated to dryness *in vacuo*. The recovery of radioactivity was approximately 60%. The residue from all eight incubations were combined and subjected to the purification described below.

Purification of the Cyclic Ethers. The combined residues from all eight incubations were extracted with 1800 ml of chloroform–methanol (2:1, v/v) and 1 M HCl added until the upper aqueous phase became weakly acidic (pH 3.0). The chloroform layer was separated and washed twice with 125 ml of water and evaporated to dryness *in vacuo*. The residue was partitioned twice between 400 ml each of petroleum ether (bp 30–60°) and ethanol–water (2:1, v/v). The residue after evaporation of the combined ethanol layers was fractionated on a silicic acid column (150 g) by successive elution with chloroform (1600 ml), ethyl acetate (1400 ml), and methanol (1000 ml). Most of the radioactivity was in the ethyl acetate fraction which contained prostaglandins as well as the cyclic ethers. This fraction was therefore evaporated to dryness and further fractionated on another column of silicic acid by stepwise elution with increasing proportions of ethyl acetate in benzene (Hamberg and Samuelsson, 1967). The benzene–ethyl acetate (2:3, v/v) fraction was evaporated to dryness and the residue was treated with 10 ml of a solution of ethanol–aqueous 1 M KOH (1:1, v/v) for 90 min at room temperature to convert the PGE_2 formed in the biosynthesis experiment into PGB_2 (Bergström *et al.*, 1964). The optical density at 280 nm was compared to that resulting from known quantities of authentic PGE_2. The total fraction was calculated to contain 210 mg of PGE_2. The alkaline mixture was diluted with water, acidified to pH 3 with 1 M HCl, and extracted with ether. The ether phase was washed with water and evaporated to dryness. The residue obtained was chromatographed on a column of silicic acid (6 g) and eluted with the following three solvents: benzene–ethyl acetate (4:1, v/v) which eluted tritiated PGB_2, benzene–ethyl acetate (1:4, v/v) which eluted the tritiated cyclic ethers, and methanol which eluted negligible radioactivity. The fraction containing the cyclic ethers was further purified by preparative thin-layer chromatography on silica gel G developed with chloroform–methanol–acetic acid–water (90:9:1:0.65, v/v). A wide zone corresponding in R_F to authentic PGE_2 (0.3–0.4) was removed and eluted with 10% aqueous methanol. The solvent was evaporated and the residue was extracted with diethyl ether after acidification. The ether layer was washed to neutrality with water and evaporated to dryness. This residue was resolved into at least four compounds by thin-layer chromatography on silica gel impregnated with 10% silver nitrate using the AII developing system of Gréen and Samuelsson (1964) modified to contain double the amount of water, *viz.*, the upper phase of ethyl acetate–methanol–acetic acid–2,2,4-trimethylpentane–water (110:35:30:10:200, v/v). Three zones of R_F 0.7–0.8 (III), 0.45–0.55 (II), 0.35–0.45 (I) were removed. Reference standards of PGA_1 (R_F 0.97), PGE_1 (R_F 0.87), PGE_2 (R_F 0.63), $PGF_{1\alpha}$ (R_F 0.54), and $PGF_{2\alpha}$ (RF 0.25) were spotted on the lateral portion of the plate and after development were detected by spraying with a solution of 10% phosphomolybdic acid in ethanol and gentle heating. The zones mentioned above were removed, extracted with 10% aqueous methanol, evaporated to dryness, and extracted further with ether as described above. Approximately 1 mg of each compound (I–III) was obtained.

Preparation of Derivatives. The following derivatives and reactions were carried out as described in the preceding paper (Pace-Asciak and Wolfe, 1971): methyl esters, trimethylsilyl ethers and esters, acetates, oxidative ozonolysis, and sodium borohydride reductions. Catalytic hydrogenations were carried out in ethanol (2.5 ml) using platinum oxide (10 mg) in a microhydrogenator. Catalytic deuterations were performed with deuterium gas, deuterated ethanol, and platinum oxide.

Analytical Methods. The types of instrumentation used have been described in the preceding paper (Pace-Asciak and Wolfe, 1971).

Materials. All solvents were of the highest commercially available quality and were dried and distilled as described in the preceding publication (Pace-Asciak and Wolfe, 1971). Deuterium gas and deuterated ethanol were purchased from Merck, Sharp and Dohme.

Results

The infrared spectrum of a mixture of I–III before final purification showed only carboxyl absorption at 1700 cm^{-1} in the carbonyl region, indicating the absence of any keto groups. Other significant absorptions were observed at 3300 (OH), 2900, and 2820 (alkyl) cm^{-1}. A strong peak at 1010 cm^{-1} suggested the presence of an ether linkage. Reduction with sodium borohydride failed to give any new products confirming further the absence of keto groups in the mixture. Also since alkali treatment is involved in the purification scheme, I–III do not contain any labile functional groups such as the β-hydroxyketo group of the E prostaglandins.

Structure of I. The nuclear magnetic resonance spectrum displayed resonances at 5.55 and 5.75 ppm due to *cis*- and *trans*-olefinic protons, respectively (see PGE_2 δ 5.37 and 5.70; Ramwell *et al.*, 1968). Other protons at 4.07 (*H*COH) and 2.40 ppm (CH_2COO) were observed similar to the prostaglandins (Ramwell *et al.*, 1968). The positions of the double bonds were placed at Δ^5 and Δ^{18}, respectively, by oxidative ozonolysis of the acetylated methyl ester derivative in which dimethyl glutarate and methyl α-acetoxyheptanoate were obtained (see below). Further structural information was obtained from the mass spectra of four derivatives. The mass spectrum of the methyl ester and trimethylsilyl ether derivative (*C* value by gas chromatography equivalent to 25.24) is shown in Figure 1a. A molecular ion at *m/e* 600, 16 mass units (oxygen) greater than that of the prostaglandin $F_{2\alpha}$ derivative (*m/e* 584) is consistent with the molecular composition $C_{21}H_{33}O_3(OSi(CH_3)_3)_3$ and indicates that three oxygen functions had been silylated and one had not. To aid interpretation of the above mass spectrum it was compared to that of the trimethylsilyl ester and ether

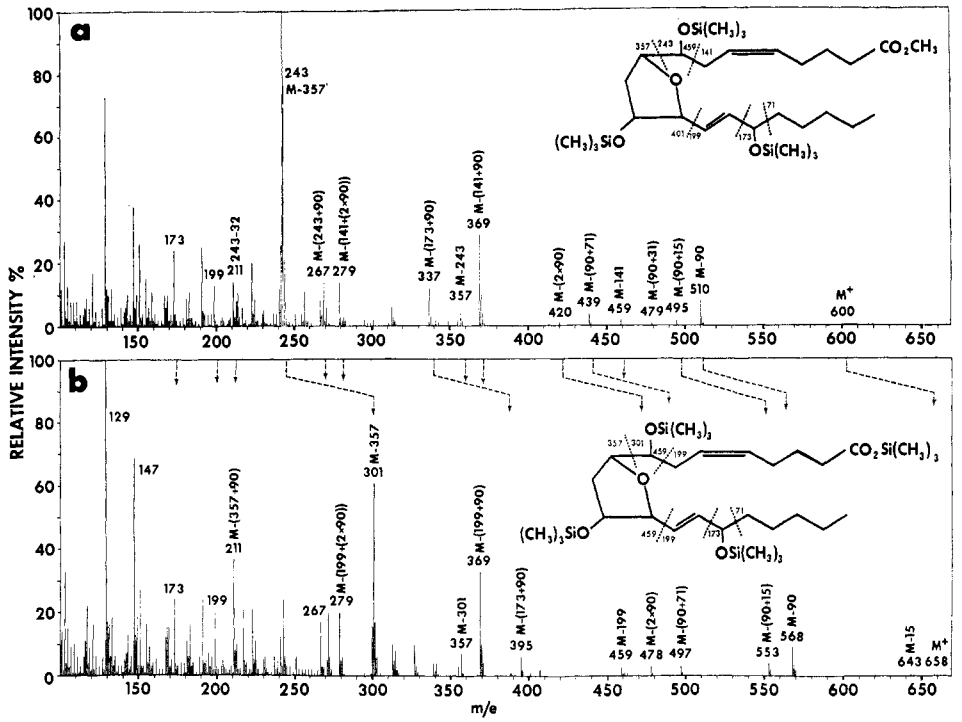

FIGURE 1: Mass spectra of the trimethylsilyl ether derivative of I (a) methyl ester and (b) trimethylsilyl ester.

derivative (*C* value 26.24) shown in Figure 1b. The molecular ion is shifted to *m/e* 658. Fragments containing the ester group are now displaced by 58 mass units over the corresponding fragments in the methyl ester derivative. Such fragments appear in the methyl ester derivative (Figure 1a) at *m/e* 600 (M^+), 510 (M − 90; loss of Me_3SiOH), 495 (M − (90 + 15); loss of Me_3SiOH and $\cdot CH_3$), 439 (M − (90 + 71); loss of Me_3SiOH and C_5H_{11}), 420 (M − (2 × 90)), 337 (M − (173 + 90); loss of $Me_3SiOCHC_5H_{11}$ and Me_3SiOH), and 243 ($Me_3SiOCHCH_2CH{=}CH(CH_2)_3CO_2CH_3$). Fragments involving loss of the ester group appear in both spectra at *m/e* 459, 369, 357, 279, 267, 199, 191, 173, 147, and 129. Ions of structural significance arise from loss of 71 and 173 from both spectra, 141 from the methyl ester, and 199 from the trimethylsilyl ester derivatives. Loss of 71 is due to the loss of C_5H_{11}, *viz.*, C-16 to C-20 fragment. Loss of 173 can be interpreted as a loss of $Me_3SiOCHC_5H_{11}$, *viz.*, C-15 to C-20 side chain. Confirmation of the presence of this fragment was also obtained from oxidative ozonolysis (see below). Very good evidence for the location of a hydroxyl group at the C-8 position was obtained from the very intense ion at *m/e* 243 in the methyl ester derivative (Figure 1a). Since this ion is displaced to *m/e* 301 in the trimethylsilyl ester derivative (Figure 1b), it must contain the ester group. Through oxidative ozonolysis a Δ^5 double bond has already been located. A further extension of this C-6 moiety by $Me_3SiOCHCH_2$ would satisfy the above requirements of 243. The fragment at *m/e* 243 is therefore $(Me_3SiOCHCH_2CH{=}CH(CH_2)_3COOCH_3)^+$ which is displaced by 58 mass units to *m/e* 301 in the trimethylsilyl ester derivative. This therefore places a hydroxyl group in the C-8 position. Fragments arising from the loss of 243 in the methyl ester or 301 in the trimethylsilyl ester derivatives (*m/e* 357) also appear in the spectra. Furthermore, cleavage at C-7,8 (141) α to the carbon bearing the trimethylsilyloxy group at C-8 in the methyl ester derivative also takes place producing fragments at *m/e* 459 (M − 141) and 369 (M − (141 + 90)) arising from the loss of the C-1 to C-7 fragment. A corresponding loss of 199 takes place in the trimethylsilyl ester derivative. Two hydroxyl groups have therefore now been located at the C-8 and C-15 positions leaving one further hydroxyl group and one oxygen atom to be placed in the remaining positions: 9, 10, 11, or 12. The intensity of the ion at *m/e* 243 in the methyl ester derivative (base peak) indicates a very favorable cleavage of the C-8,9

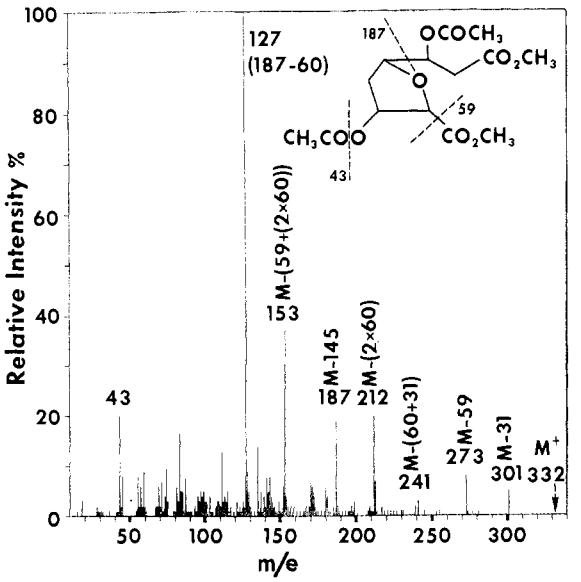

FIGURE 2: Mass spectrum (20 eV) of the ring component (*C* value 16.25) from the oxidative ozonolysis of the methyl ester and triacetate of I.

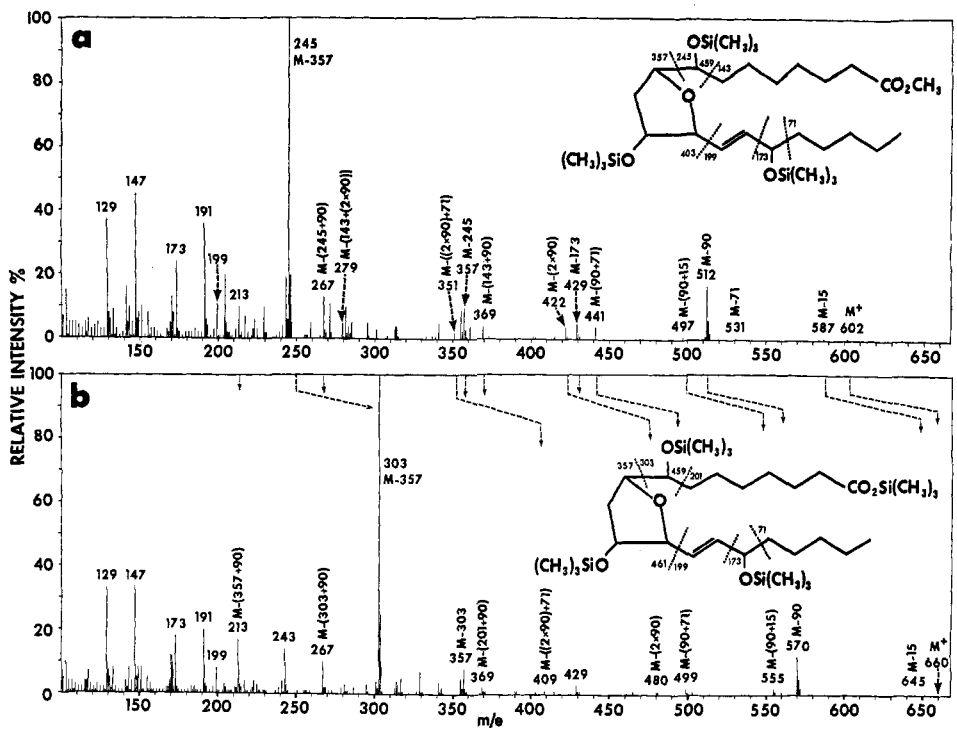

FIGURE 3: Mass spectra of the trimethylsilyl ether derivative of III (a) methyl ester and (b) trimethylsilyl ester.

bond, strongly suggesting another oxygen (or hydroxyl) substituent at the C-9 position. The appearance of a fragment at *m/e* 199 (C-13 to C-20 side chain) further suggests an oxygen atom at the C-12 position. If a hydroxyl group was located at the C-12 position, then cleavage of the C-11,12 bond would be expected as shown in the cleavage of the C-8,9 bond already discussed to produce a fragment arising from loss of 301 in the methyl ester derivative. This does not take place. Therefore the oxygen atom at C-12 must be involved in something other than a hydroxyl or carbonyl group. Mechanistically, introduction of the Δ^{13} double bond and hydroxyl group at C-15 from arachidonic acid are preceded in the biosynthesis of prostaglandins by a hydroperoxidation at C-11 (see Discussion). Since one hydroxyl group and one oxygen atom still have to be placed in three out of four positions, *viz.*, 9, 10, 11, and 12, a hydroxyl group at C-11, and an oxygen ether linkage between C-9 and C-12 satisfy both the mass spectral and mechanistic requirements. Further confirmation of the above mass spectral interpretations were obtained with derivatives catalytically reduced with hydrogen and deuterium gas. The mass spectrum of the hydrogenated methyl ester trimethylsilyl ether derivative (*C* value 25.56) showed a molecular ion of very low intensity at *m/e* 604 consistent with addition of four hydrogen atoms. The corresponding deuterated derivative had a molecular ion at *m/e* 608 consistent with addition of four deuterium atoms. The base peak at *m/e* 243 in the unsaturated derivative (Figure 1a) already discussed above and attributed to $(Me_3SiOCHCH_2CH{=}CH(CH_2)_3COOCH_3)^+$ was shifted to *m/e* 245 in the hydrogenated derivative and *m/e* 247 in the deuterated derivative further confirming the presence of one double bond in this fragment. Furthermore, loss of 245 in the hydrogenated derivative and loss of 247 in the deuterated derivative give rise to fragments at *m/e* 359 and 361, respectively, accompanied by further losses of 90 (Me_3SiOH) and 2 × 90 from these ions.

Oxidative ozonolysis of the methyl ester and triacetate of I gave dimethyl glutarate, α-acetoxyheptanoate (identified by comparison of their mass spectra to authentic samples), and a third compound with a retention time corresponding to a *C* value of 16.25. This compound was subjected to gas chromatography–mass spectrometry. The recorded mass spectrum (Figure 2) showed fragments of high intensity at 301 (M − 31; loss of $\cdot OCH_3$), 273 (M − 59; loss of $CH_3OOC\cdot$), 241 (M − (60 + 31); loss of CH_3CO_2H and $\cdot OCH_3$), 212 (M − (2 × 60)), 187 (M − 145; loss of $CH_3CO_2CHCH_2CO_2CH_3$), 153 (M − (59 + (2 × 60)), and 127 (M − (145 + 60)) consistent with the expected substituted tetrahydrofuran structure (Figure 2).

Structure of II. The material isolated from the zone of R_F 0.45–0.55 in the final preparative argentation thin-layer chromatography contained one compound which had identical chromatographic properties with 6(9)-oxy-11,15-dihydroxyprosta-7,13-dienoic acid isolated from the rat stomach (see Pace-Asciak and Wolfe, 1971). The mass spectra of both compounds as the trimethylsilyl ether and methyl ester and as the trimethylsilyl ester derivatives were identical in all respects.

Structure III. The nuclear magnetic resonance spectrum of III showed only resonance at 5.77 ppm due to the Δ^{13} double bond. The mass spectrum of the hydrogenated methyl ester and trimethylsilyl ether derivative was identical to that of the hydrogenated methyl ester and trimethylsilyl derivative of I, indicative of the same basic structure. The mass spectrum of the methyl ester and trimethylsilyl ether derivative of III (*C* value 25.48) showed a molecular ion at *m/e* 602 further confirming the presence of only one double bond in comparison to I (compare Figure 3a and Figure 1a). The absence of a double bond at Δ^5 in III was apparent upon comparison of both spectra. The base peak at *m/e* 243 in I (Figure 1a) already shown to be $(Me_3SiOCHCH_2CH{=}CH(CH_2)_3COOCH_3)^+$ appears at *m/e* 245 in III (Figure 3a) indicating that the double bond at Δ^5 is saturated in III. These fragments appear at *m/e*

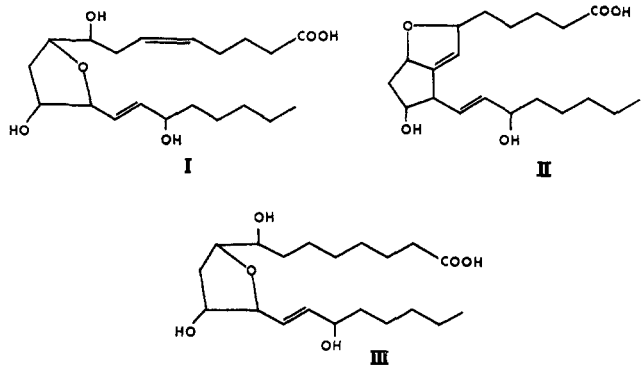

FIGURE 4: Proposed structures of three minor oxygenated products of arachidonic and eicosatrienoic acid formed by acetone powders of sheep seminal vesicles.

301 and 303, respectively, in the trimethylsilyl ester derivatives of both compounds (Figure 1b and Figure 3b).

Discussion

The major oxygenated products of the *in vitro* transformation of arachidonic and eicosatrienoic acids by sheep seminal vesicles have been shown to be the E prostaglandins; several minor products including the F prostaglandins and monohydroxy fatty acids have also been isolated (Bergström *et al.*, 1968). The formation of the F prostaglandins has recently been shown to be enhanced in the presence of L-epinephrine (Sih *et al.*, 1970). 11-Dehydroprostaglandin $F_1\alpha$ was isolated by Granström *et al.* (1968) who attributed its formation to a loss of specificity of the enzyme complex upon storage. In this paper evidence is presented for the structures of three new minor oxygenated products of arachidonic and eicosatrienoic acids (Figure 4) formed from the incubation of acetone powders of sheep seminal vesicles. Under the experimental conditions used, these products are formed in approximately 1 mg each (about 2% of the prostaglandin E_2 formed).

A mechanism is proposed for the formation of I from exogeneous tritiated arachidonic acid (Figure 5). This compound is envisaged as formed in part by the widely accepted scheme of Hamberg and Samuelsson (1967) for the biosynthesis of prostaglandins in which it is believed that the transformation is initiated by a stereospecific abstraction of a 13-L hydrogen atom followed by hydroperoxidation at C-11. The subsequent rearrangement of the 11-hydroperoxy intermediate A along pathway 1 (Figure 5) proceeds with formation of prostaglandins after attack of the hydroperoxy radical at C-9, cyclization, double-bond isomerization, and further hydroperoxidation at C-15 (Hamberg and Samuelsson, 1967). If, however, the intermediate A rearranges according to pathway 2 with formation of intermediate B, I would be obtained following homolytic cleavage of the 9,11-peroxide (Figure 5) with subsequent cyclization, double-bond isomerization and further hydroperoxidation at C-15 as postulated for the prostaglandins. Compound III can be formed in a similar way from the eicosatrienoic acid which is present in the tissue phospholipids (Lands and Samuelsson, 1968; C. Pace-Asciak, 1968, unpublished data). Another compound which has been tentatively identified as 8(12)-oxy-9,11,15-trihydroxyeicosa-5,13-dienoic acid by mass spectrometry of several derivatives was isolated in amounts insufficient to obtain rigorous proof of the tetrahydropyran ring structure after oxidative ozonolysis. Although I may be formed by autoxidation of arachidonic

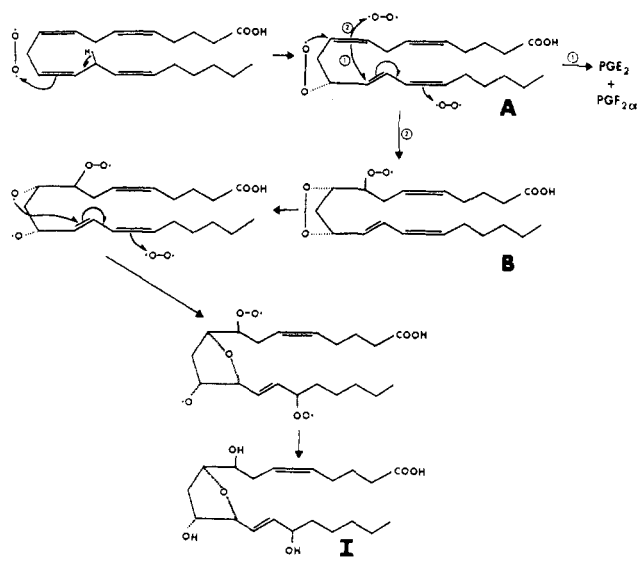

FIGURE 5: Proposed scheme for biosynthesis of I.

acid, the initial selective abstraction of a hydrogen atom is certainly enzymatic in direct analogy with that which occurs in the synthesis of prostaglandins, although the consecutive steps could be autoxidative.

It is not known whether the compounds reported in this paper are formed only from the free precursor fatty acids. It has been shown that prostaglandins are not formed from fatty acids esterified to phospholipids (Lands and Samuelsson, 1968; Vonkeman and Van Dorp, 1968) but only from precursor fatty acids in the free form. May and McCay (1968) have reported a NADPH-dependent microsomal oxidation of membrane phospholipids which utilizes polyunsaturated fatty acids especially arachidonic acid. Unlike the prostaglandin system, the microsomal oxidation system utilizes polyunsaturated fatty acids esterified to phospholipids. Both systems however utilize polyunsaturated fatty acids, consume oxygen, and produce malondialdehyde. The products of the NADPH-dependent system are reported to have incorporated oxygen but so far have not been chemically characterized except that they are not prostaglandins. The similarity of the three products reported in this paper to the prostaglandins and their high oxygen content (I and III have four oxygen atoms incorporated) makes it attractive to consider the possibility that such compounds are formed during alterations of membrane phospholipids. The isolation and characterization of such polyoxygenated fatty acids in tissues could provide a more firm basis for understanding enzymatic and autoxidative modifications of membrane lipids.

Acknowledgments

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Gold(III) Complexes of Adenine Nucleotides*

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ABSTRACT: Adenine, adenosine, and adenine nucleotides (AMP, dAMP, 3′AMP, cAMP, ADP, and ATP) were allowed to react with equilibrium systems of hydroxochloroaurate ions, at several pH + pCl. Potassium salts of gold–adenine nucleotide adducts, Au·A (K), were isolated by ethanol precipitation, and analyzed for gold and phosphate contents. In mixtures containing 4 mM A and 12 mM Au ions at pH 5–8, the adducts contained 2 Au/A after 4 hr, and 3 Au/A after 24 hr. The adducts appear to be polymerized to some extent, possibly because of N → Au ← N bridges. The Au·Ade and Au·Ado adducts are very insoluble in aqueous media, whereas potassium salts of gold–adenine nucleotides are soluble to greater than 10 mM [A]. Magnesium, calcium, and manganous salts of Au·cAMP are very water soluble, whereas the corresponding salts of Au·AMP, Au·ADP, and Au·ATP are relatively insoluble, to degrees that depend on the number of phosphates and the divalent cation. The Au·A (K) adducts were allowed to react further with amino acids, related amines, and with biological stains containing primary and heterocyclic amines. The resulting amine·Au·A adducts were water soluble. In contrast, hydroxochloroaurate ions were reduced to Au(0) by amino acids, and gave insoluble amine·Au adducts with most other amines. These differences between Au·A adducts and hydroxochloroaurate ions, in reactivities toward amines and amino acids, are evidence that gold(III) is tightly bound to adenine nucleotides. Amine·Au·A adducts may contain N → Au ← N bridges between amino or heterocyclic nitrogens in the amine and adenine residues. The binding of gold(III) to adenine residues has potential applications for electron microscope studies of base sequences within isolated nucleic acids. The coupling of Au·A adducts to tissue amines, or to biological stains, has potential applications for electron microscope cytochemistry of nucleoproteins within tissue. Solubility differences between Au·Ado and potassium and divalent cation salts of gold–adenine nucleotide adducts suggest methods for electron microscope cytochemical localizations of nucleotide-dependent enzymes within tissue.

Many metals are known to bind ionically to nucleotide phosphates; others bind covalently to sugar hydroxyl groups, or coordinate with nitrogens in the base moieties (*e.g.*, Eichhorn *et al.*, 1970). Such interactions affect the secondary and tertiary structures of RNA and DNA, and the activities of polymerases, nucleases, esterases, kinases, and other nucleotide- or polynucleotide-dependent enzymes. Heavy metal interactions with nucleotides and polynucleotides are also potentially useful in physical techniques such as X-ray diffraction and electron microscopy. Beer and coworkers have studied reactions of several heavy metals with base moieties of nucleotides and nucleic acids, with a view toward determining base sequences by electron microscopy (Beer and Moudrianakis, 1962; Moudriankis and Beer, 1965; Beer *et al.*, 1966, 1970). Heavy atom derivatives of nucleotides are potentially useful for cytochemical localizations of enzymes by electron microscopy, if they can be converted by tissue enzymes

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