Biosynthesis of Prostaglandin D_2, 15-Ketoprostaglandin E_2, and Hydroxy Fatty Acids by Ram Seminal Vesicle Microsomes

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Abstract □ At high arachidonic acid concentrations (164 μM) and without exogenous cofactors, ram seminal vesicle microsomes produced prostaglandin E_2 and two less polar products, identified as prostaglandin D_2 and 15-ketoprostaglandin E_2. The ratio of the biosynthetic products formed depended on the exogenous cofactor and on the arachidonic acid concentration. At high arachidonic acid concentrations (>150 μM), tryptophan, phenol, and glutathione stimulated prostaglandin E_2 formation, but each affected the formation of the other prostaglandins differently. Ascorbic acid markedly stimulated hydroxy fatty acid formation. GLC–mass spectral analysis of the hydroxy fatty acid fraction indicated the presence of 11-hydroxy-5,8,12,14-eicosatetraenoic acid, 15-hydroxy-5,8,11,13-eicosatetraenoic acid, and 12-hydroxy-5,8,10-heptadecatrienoic acid. At low arachidonic acid concentrations (30 μM), glutathione still stimulated prostaglandin E_2 biosynthesis, but the other cofactors stimulated 6-ketoprostaglandin $F_{1\alpha}$ and hydroxy fatty acid formation.

Keyphrases □ Prostaglandin D_2—biosynthesis, ram seminal vesicle microsomes, dependence on arachidonic acid, cofactors □ 15-Ketoprostaglandin E_2—biosynthesis, ram seminal vesicles, dependence on arachidonic acid, cofactors □ Seminal vesicles—microsomes, prostaglandin synthesis, arachidonic acid metabolism □ Microsomes—seminal vesicles, prostaglandin synthesis, arachidonic acid metabolism

Ram seminal vesicle microsomes convert arachidonic and eicosatrienoic acids into prostaglandins of the D, E, and F series (1–5). Recently, it was reported (6) that, at low substrate to enzyme ratios and in the absence of reduced glutathione, the major prostaglandin synthesized by the ram seminal vesicle cyclo-oxygenase from arachidonic acid was 6-ketoprostaglandin $F_{1\alpha}$. When the substrate concentration was increased to 50 μM, only traces of 6-ketoprostaglandin $F_{1\alpha}$ were formed, the major products being prostaglandin E_2, prostaglandin $F_{2\alpha}$, and two unidentified less polar products.

This paper reports experimental data that characterize these unknowns as prostaglandin D_2 and 15-ketoprostaglandin E_2. The effect of exogenous cofactors on the ratio of biosynthetic products was also examined.

EXPERIMENTAL

Materials—Radioactive 1-^{14}C-arachidonic acid[1] (60 mCi/mmole), unlabeled arachidonic acid[2] (Grade 1), reduced glutathione[2], L-epinephrine[2], serotonin[2], methemoglobin[2], prostaglandin E_2[3], prostaglandin D_2[4], 15-ketoprostaglandin E_2[4], and frozen ram seminal vesicles[5] were used. All solvents were reagent grade and were redistilled.

Silicic acid[6] was used for column chromatography. Precoated analytical silica gel plates[7] were used for TLC separation. Radioactivity was assayed in a scintillation spectrometer[8] after the addition of 10 ml of Bray's solution. The radiochromatogram was assayed on an autoscanner[9].

Mass spectra were recorded on a mass spectrometer[10] with an ion source temperature of 220° and an electron potential of 70 ev. GLC–mass spectral analyses were carried out in a gas chromatograph–mass spectrometer[11] equipped with a data system[12]. The column, 1.83 m, was 3% SE-30 with temperature programming from 200 to 250° at 6°/min; the electronic energy was kept at 70 ev. UV spectra were recorded on a recording spectrophotometer[13].

PMR spectra (δ value) were recorded on a spectrometer[14] in deuterochloroform. All chemical shifts were recorded relative to internal tetramethylsilane. 15-Hydroperoxyarachidonic acid was prepared according to the method of Hamberg and Samuelsson (7) and purified by column chromatography, using hexane–ether as the solvent.

Enzymatic Transformation of 1-^{14}C-Arachidonic Acid—Ram seminal vesicle microsomes were prepared as described elsewhere (8) and were freeze dried and stored at −78° until used. Enzymatic incubations

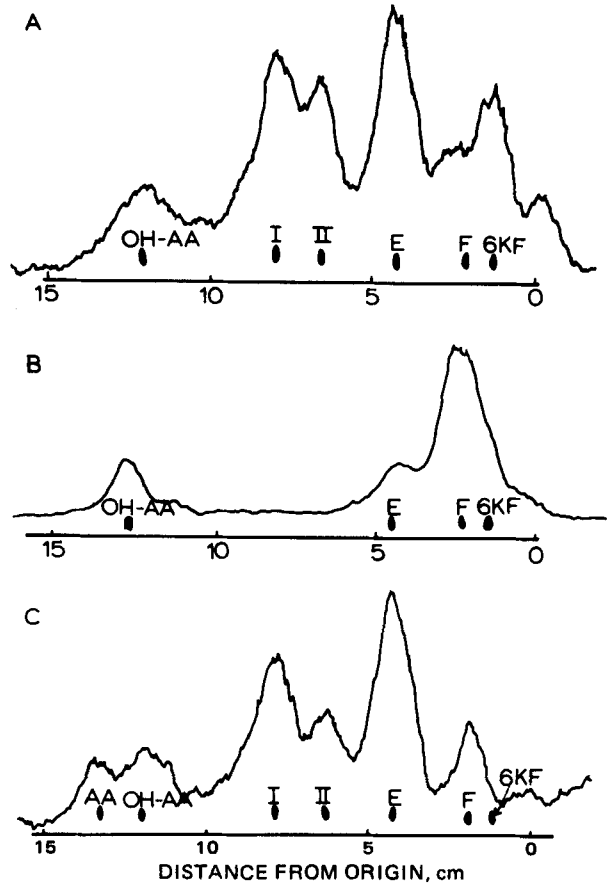

Figure 1—*Radiochromatographic profiles of 0.2 μCi of 1-^{14}C-arachidonic acid (164 μM) metabolism by ram seminal vesicle microsomes. Key: A, ethyl acetate extract; B, ethyl acetate extract plus sodium borohydride; and C, incubation mixture consisting of 15-hydroperoxyeicosa-5,8,11,13-tetraenoic acid (100 μM) and 0.2 μCi of 1-^{14}C-arachidonic acid (164 μM).*

[1] Amersham/Searle Co., Arlington Heights, Ill.
[2] Sigma Chemical Co., St. Louis, Mo.
[3] G. D. Searle Co., Chicago, Ill.
[4] Miles Laboratories, Madison, Wis.
[5] Roth Products, Gwynedd, Pa.
[6] Mallinckrodt 2847, 100 mesh.
[7] Brinkmann 0.25 F0254 (EM).
[8] Packard model 2002.
[9] Vangard model 930.
[10] AEI MS-9.
[11] Varian MAT 112.
[12] SS 100C.
[13] Cary model 14.
[14] Varian EM 390.

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Table I—Effect of Cofactors on Product Distribution at High Arachidonic Acid Concentration [a] (164 μM)

Cofactor	Concentration	Percent of Products Formed						Radioactivity in Products, %
			Prostaglandin					
		Hydroxy-Fatty Acids	15-Keto-E_2	D_2	E_2	$F_{2\alpha}$	6-Keto-$F_{1\alpha}$	
None	—	10.0	21.4	13.9	26.5	10.2	10.6	93
Glutathione	20 μM	0	22.1	0	48.1	0	0	70
Ascorbic acid	1 mM	35.1	0	8.4	16.8	21.0	18.5	99.8
Phenol	1 mM	13.7	0	15.0	42.7	3.6	22.2	97
L-Tryptophan	1 mM	14.4	0	13.4	38.3	7.1	23.4	96.6
L-Epinephrine	1 mM	19.6	4.1	9.4	23.8	17.2	22.6	96.7
Serotonin	1 mM	19.7	0	17.1	37.8	5.2	2.6	82.4
Methemoglobin	8 μM	8.8	19.8	13.9	30.3	7.2	20.0	99

[a] The reaction mixture contained ram seminal vesicle microsomes (10 mg) dissolved in 1.0 ml of 0.1 M tromethamine buffer. The cofactors were added at the concentrations indicated, and the reaction was initiated by the addition of 0.2 μCi of 1-^{14}C-arachidonic acid containing 50 μg of unlabeled arachidonic acid. Products were extracted and analyzed as described.

were carried out at 25° in 1.0 ml of pH 7.5 tromethamine buffer containing 10 mg of lyophilized enzyme powder (3.85 mg of protein by biuret), 50 μg of arachidonic acid, 0.2 μCi of 1-^{14}C-arachidonic acid, and cofactors when necessary. The reaction was initiated by the addition of arachidonic acid and was terminated after 20 min by the addition of 25 μl of 1.0 N HCl (final pH 3.0).

The reaction mixture was extracted with 2.0 ml of ethyl acetate; the organic layer was dried over sodium sulfate and evaporated with a nitrogen stream. The total recovery of radioactivity was generally 60%. The residue was redissolved in ethyl acetate, and a portion of this solution was spotted on TLC plates. They were developed twice in the organic phase of ethyl acetate–isooctane–acetic acid–water (11:5:2:10) and scanned for radioactivity.

The percent incorporation of radioactivity into each product was determined by the cut and weigh method. Radioactive peaks on the chromatogram corresponding to the products were traced with tracing paper, cut out, and weighed. The total weight of all peaks represented 100% of the sample. The weight of each peak was divided by the total weight and multiplied by 100 to obtain the percentage of that product present.

Isolation of Compounds I and II—Ram seminal vesicle microsomes (11–3.94 g of protein) were suspended in 1.1 liters of 0.1 M, pH 7.5 tromethamine buffer. The enzymatic reaction was initiated by the addition of 55 mg of arachidonic acid containing 47 μCi of 1-^{14}C-arachidonic acid. After 20 min at 25°, the incubation mixture was acidified with hydrochloric acid to pH 3.0 and extracted with ethyl acetate (4 × 250 ml). The combined ethyl acetate extracts were washed with water (3 × 250 ml) and dried over sodium sulfate. Approximately 44% of the radioactivity was recovered.

The residue was chromatographed over 10 g of silicic acid–diatomaceous earth (85:15); the column (1 × 23 cm) was eluted with benzene–ethyl acetate (8:2), with gradually increasing ethyl acetate concentrations. Fractions (8 ml) were collected. Fractions 2–14 contained arachidonic acid and hydroxy fatty acids (20.7% of radioactivity), fractions 15–22 consisted of I (14.7%), fractions 56–60 consisted of II (5.5%), fractions 64–74 afforded prostaglandin E_2 (24.8%), fractions 74–82 gave prostaglandin $F_{2\alpha}$ (7.56%), and fractions 104–107 yielded 6-ketoprostaglandin $F_{1\alpha}$ (4.4%).

Fractions 15–22 were combined and further purified by chromatography over an LH-20 column (1 × 36 cm). The column was eluted with methylene chloride–benzene–methanol (10:10:1), and 6-ml fractions were collected. Fractions 9–13 (82% radioactivity) were pooled and evaporated to dryness. The residue was methylated with diazomethane and further purified over a silica gel (10 g) column (1 × 23.5 cm). The column was eluted successively with benzene–ethyl acetate (100 ml of 95:5, 200 ml of 7:3, and 200 ml of 1:1), and 6-ml fractions were collected. Fraction 28, consisting of the pure methyl ester of I (23% of the added radioactivity), was used for NMR and mass spectrometric analyses.

Fractions 56–60, consisting of II, were similarly purified.

Hydroxy Fatty Acid Isolation—Ram seminal vesicle microsomes (24 g) were suspended in 2.4 liters of 0.1 M, pH 7.5 tromethamine buffer. Ascorbic acid (1 mM) was added. The enzyme reaction was initiated by the addition of 120 mg of arachidonic acid containing 32 μCi of 1-^{14}C-arachidonic acid. After incubation at 25° for 20 min with gentle shaking on a Dubnoff shaker, the reaction mixture was extracted in the same manner as that for 15-ketoprostaglandin E_2 and prostaglandin D_2. The total recovery of radioactivity was 47.3%.

This material was chromatographed over 30 g of silicic acid–diatomaceous earth (85:15), and the column was eluted with the following solvent systems: 50 ml of benzene–ethyl acetate (8:2), 250 ml of benzene–ethyl acetate (7:3), and a gradient system consisting of 300 ml of benzene–ethyl acetate (7:3) in the mixing chamber and 300 ml of ethyl acetate in the reservoir chamber. Fractions (6.5 ml) were collected.

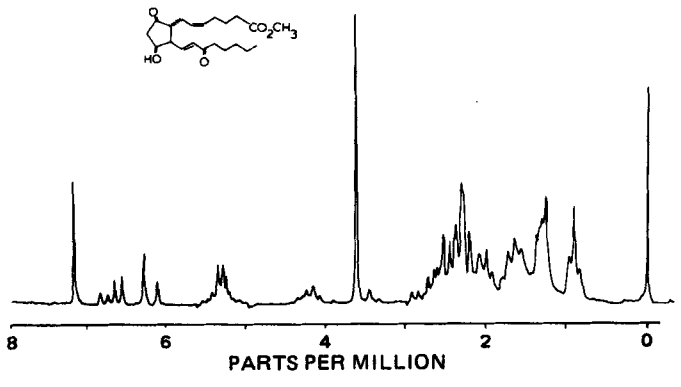

Figure 2—^1H-*NMR spectrum of I methyl ester.*

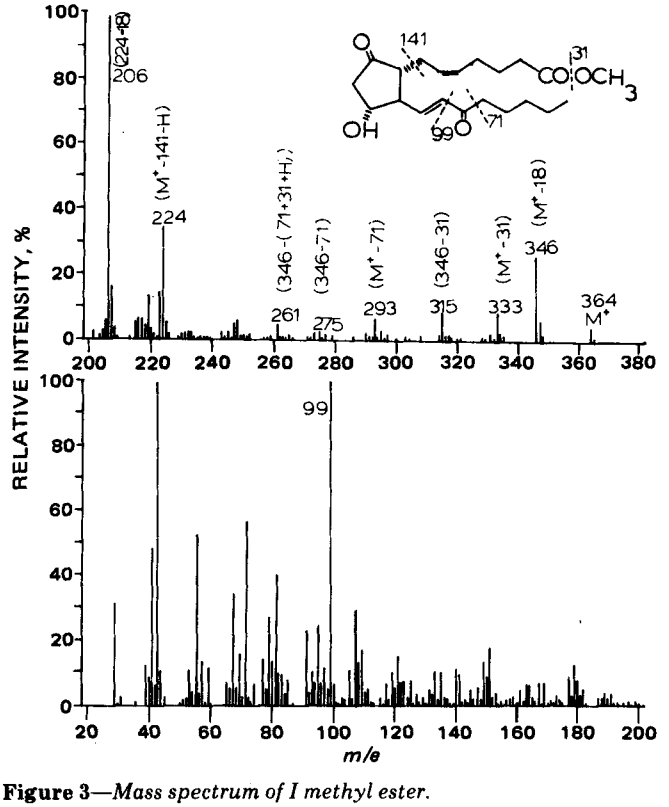

Figure 3—*Mass spectrum of I methyl ester.*

Table II—Effect of Cofactors on Product Distribution at Low Arachidonic Acid Concentration [a] (30 μM)

Cofactor	Concentration	Percent of Products Formed						Radioactivity in Products, %
			Prostaglandin					
		Hydroxy Fatty Acids	15-Keto-E_2	D_2	E_2	$F_{2\alpha}$	6-Keto-$F_{1\alpha}$	
None	—	17.7	14.9	19.9	28.1	8.9	7.8	97.3
Glutathione	20 μM	4.8	0	3.4	91.8	0	0	100
Ascorbic acid	1 mM	34.2	4.7	6.4	8.7	19.8	26.2	100
Phenol	1 mM	18.5	0	24.3	31.7	7.7	7.1	89.3
L-Tryptophan	1 mM	17.6	6.5	14.2	19.0	10.3	30.8	98.4
L-Epinephrine	1 mM	23.4	0	20.5	25.0	20.3	10.8	100
Serotonin	1 mM	21.6	0	24.4	30.4	11.5	7.8	95.7
Methemoglobin	8 μM	15.7	13.6	21.9	32.2	7.2	6.0	96.7

[a] The reaction mixture was the same as that described in Table I except that 10 μg of unlabeled arachidonic acid was used.

Fractions 6–20, containing 25.75% of radioactivity, were pooled and rechromatographed over an LH-20 column (1.8 × 24.5 cm). The column was eluted with benzene–methylene chloride (6:4), and 4-ml fractions were collected. Fractions 45–95 (28% of radioactivity), containing 11-hydroxy- and 15-hydroxyeicosatetraenoic acids and 12-hydroxy-5,8,10-heptadecatrienoic acid, were methylated with diazomethane and used for GLC–mass spectral analysis.

RESULTS

When 1-^{14}C-arachidonic acid at a concentration greater than 150 μM was exposed to ram seminal vesicle microsomes, several radioactive products were formed (Fig. 1A). Besides those products corresponding in mobilities to prostaglandin E_2, prostaglandin $F_{2\alpha}$, and 6-ketoprostaglandin $F_{1\alpha}$, two compounds (I and II) less polar than prostaglandin E_2 were produced. When a portion of the organic extract of this reaction mixture was treated with sodium borohydride, the radioactive chromatographic profile of the products was markedly changed (Fig. 1B). The radioactive peaks corresponding to I and II disappeared, the prostaglandin E_2 peak was considerably reduced, and there was a radioactivity increase in the prostaglandin $F_{2\alpha}$ region. These observations suggested that both I and II possessed ketonic functions, which, upon reduction with sodium borohydride, afforded compounds similar in polarity to prostaglandin $F_{2\alpha}$.

It was previously reported (6) that addition of 15-hydroperoxyarachidonic acid (100 μM) to the incubation medium inhibited the formation of 6-ketoprostaglandin $F_{1\alpha}$ but did not affect the formation of prostaglandin E_2, I, and II. This observation suggests that I and II were not derived from the prostacyclin pathway. This observation was confirmed by evidence that 15-hydroperoxyarachidonic acid did not significantly inhibit the formation of I and II (Fig. 1C).

To establish conclusively the chemical structures of I and II, the arachidonic acid-derived products were resolved by silicic acid column chromatography. The PMR spectrum of the I methyl ester (Fig. 2) showed characteristic prostaglandin signals: a pair of doublets centered at δ 6.76 (1H, $J_{12,13}$ = 7.8 Hz, C-13H), a doublet at δ 6.3 (1H, $J_{13,14}$ = 16 Hz, C-14H), a multiplet at δ 5.39 (2H, vinylic protons at C-5 and C-6), and a multiplet at δ 4.25 (1H, C-11H). The mass spectrum of the I methyl ester (Fig. 3) gave prominent ions at *m/e* 364 (M^+), 346 (M − 18), 333 (M − 31), 315 (346 − 31), 293 (M − 71), 275 (346 − 71), 261 [346 − (71 + 31 + H)], 224 [M − (141 − H)], and 206 (224 − 18). The UV spectrum of the I methyl ester (Fig. 4) exhibited a maximum at 228 nm (ε 11,000) characteristic of an α,β-unsaturated ketone.

These spectral data are consistent with the supposition that the chemical structure of I is either 15-ketoprostaglandin E_2 or its regioisomer, 15-ketoprostaglandin D_2. However, when the I methyl ester was treated with 0.1 *N* NaOH, a compound with a chromophore at 500 nm (ε 22,400) was formed. This experiment clearly established that I is 15-ketoprostaglandin E_2 (9), because the presence of 15-ketoprostaglandin D_2 would have resulted in the formation of a stable chromophore at 415 nm (10).

In a similar fashion, II was identified as prostaglandin D_2 (11, 12). The NMR (Fig. 5) and mass (Fig. 6) spectra as well as the chromatographic behavior of the II methyl ester were identical to those of the authentic sample of prostaglandin D_2 methyl ester.

The ratio of the products formed by ram seminal vesicle microsomes had been shown to be dependent on the arachidonic acid concentration (6). Thus, it appeared of interest to examine the effect of cofactors on biosynthesis at high (Table I) and low (Table II) substrate concentrations. At high arachidonic acid concentrations (164 μM), the addition of tryptophan and phenol reduced the formation of 15-ketoprostaglandin E_2 and prostaglandin $F_{2\alpha}$ whereas synthesis of prostaglandin E_2 and 6-ketoprostaglandin $F_{1\alpha}$ was enhanced. On the other hand, reduced glutathione favored the biosynthesis of prostaglandin E_2 and 15-ketoprostaglandin E_2, but the overall conversion of arachidonic acid to these products was reduced (70%) compared to control (93%).

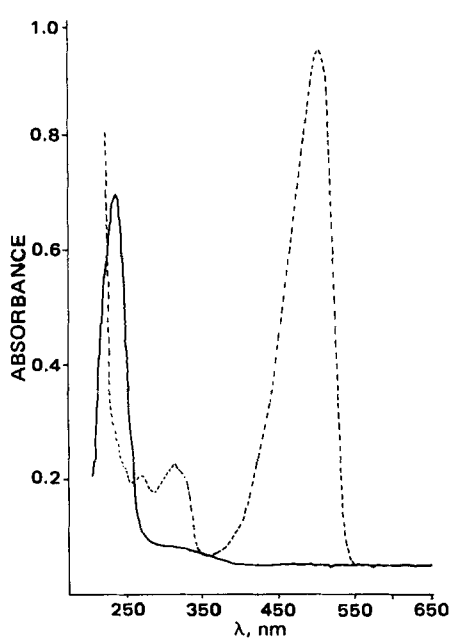

Figure 4—*Alkali effect of I absorption properties. Key: —, 0.01 mg of I/ml of methanol; and ---, after 5 min in 0.1* N *NaOH.*

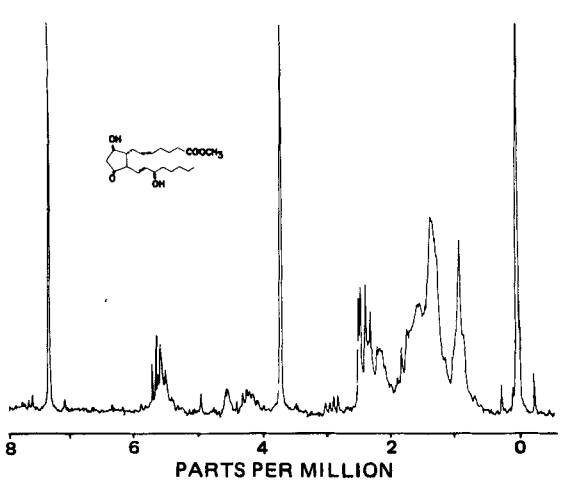

Figure 5—^{1}*H-NMR spectrum of II methyl ester.*

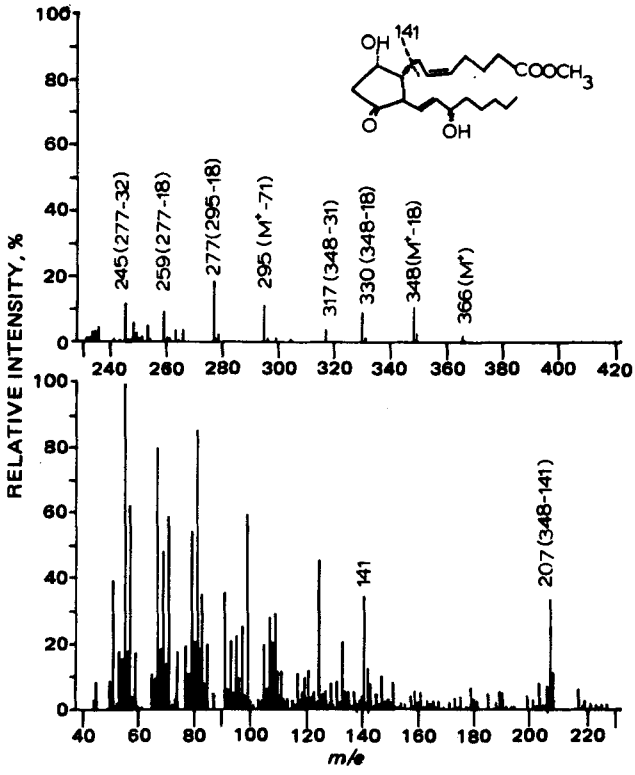

Figure 6—*Mass spectrum of II methyl ester.*

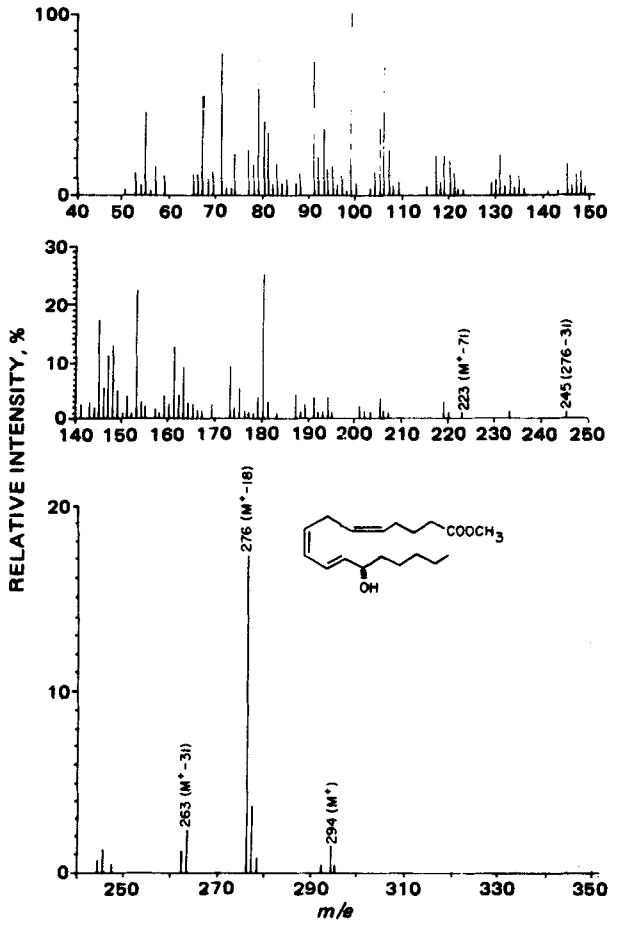

Figure 7—*Mass spectrum of 12-hydroxy-5,8,10-heptadecatrienoic acid methyl ester.*

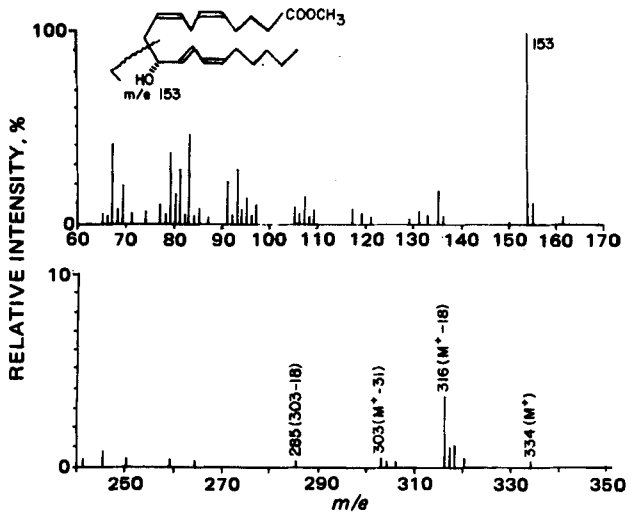

Figure 8—*Mass spectrum of 11-hydroxy-5,8,12,14-eicosatetraenoic acid methyl ester.*

Ascorbic acid markedly stimulated the formation of hydroxy fatty acids. GLC–mass spectrometric analysis of the methyl esters of this mixture revealed two major and one minor hydroxy fatty acids. They were characterized on the basis of the mass spectral data as 12-hydroxy-5,8,10-heptadecatrienoic acid methyl ester, which showed a molecular ion at *m/e* 294 (M^+) with principal fragments at 276 (M − 18), 263 (M − 31), 245 (M − 31 − 18), 223 (M − C_5H_{11}), and 207 (M − 87) (Fig. 7); 11-hydroxy-5,8,12,14-eicosatetraenoic acid methyl ester, showing prominent ions at *m/e* 334 (M^+), 316 (M − 18), 303 (M − 31), 285 (M − 31 − 18), 275 (M − 59), 153 (M − 181) [$CH_2CH{=}CHCH_2CH{=}CH(CH_3)_3CO_2CH_3$], and 135 (153 − 18) (Fig. 8); and 15-hydroxy-5,8,11,13-eicosatetraenoic acid methyl ester as the minor component, showing a molecular ion at *m/e* 334 (M^+) with fragments appearing at 316 (M − 18), 303 (M − 31), 285 (303 − 18), 263 (M − 71), and 245 (263 − 18).

At a low arachidonic concentration (30 μM), reduced glutathione greatly favored prostaglandin E_2 biosynthesis, but no 15-ketoprostaglandin E_2 was detectable. Ascorbic acid again dramatically enhanced hydroxy fatty acid synthesis but also stimulated the formation of 6-ketoprostaglandin $F_{1\alpha}$. L-Tryptophan again caused a significant increase in 6-ketoprostaglandin $F_{1\alpha}$ (Table II).

DISCUSSION

By comparing the spectral data to those of authentic samples, the two known products formed after exposure of relatively high arachidonic acid concentrations (164 μM) to ram seminal vesicle microsomes were identified as 15-ketoprostaglandin E_2 and prostaglandin D_2. The formation of these two products was not inhibited significantly by the exogenous addition of 15-hydroperoxyeicosa-5,8,11,13-tetraenoic acid to the incubation mixture, confirming the report of Cottee *et al.* (6). However, the compound originally designated by them in their thin-layer chromatograms as prostaglandin D_2 was probably 8-isoprostaglandin E_2.

Although a small quantity of 15-ketoprostaglandin E_1 had been detected in previous ram seminal vesicle microsomal incubations with eicosatrienoic acid (13), the formation of a substantial quantity of 15-ketoprostaglandin E_2 in these incubations deserves comment. Perhaps 15-ketoprostaglandin E_2 may be derived from the oxidation of the 15(S)-hydroxyl group in prostaglandin E_2 or prostaglandin H_2 by a hydroxyprostaglandin dehydrogenase. However, exposure of 1-^{14}C-prostaglandin E_2 to ram seminal vesicle microsomes under these conditions did not produce detectable 15-ketoprostaglandin E_2. Thus, the 15-keto functionality probably originated from the nonenzymatic decomposition of the 15-hydroperoxy group either at the prostaglandin G_2 or at the 15-hydroperoxyprostaglandin E_2 stage. The corresponding regioisomer, 15-ketoprostaglandin D_2, was not formed in any detectable quantities.

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p-Aminophenol Fluorescence and Determination in the Presence of Acetaminophen

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Received February 15, 1978, from the **Department of Chemistry, University of Cincinnati, Cincinnati, OH 45221*, and the ‡*Department of Chemistry, Wayne State University, Detroit, MI 48202.* Accepted for publication April 30, 1979.

Abstract □ The spectrophotometric and fluorometric properties of the aminophenols and of several compounds related to *p*-aminophenol were examined. A direct spectrofluorometric method for *p*-aminophenol determination at trace levels in methanol was developed and evaluated for the effect of inner filtering by acetaminophen. The method was applied to the determination of *p*-aminophenol as an impurity in acetaminophen and acetaminophen-containing tablets.

Keyphrases □ *p*-Aminophenol—analysis, spectrofluorometry, as impurity in acetaminophen tablets □ Acetaminophen—tablets, spectrofluorometric analysis of *p*-aminophenol as impurity □ Spectrofluorometry—analysis, *p*-aminophenol as impurity in acetaminophen tablets

Most methods for determining *p*-aminophenol (I) as an impurity in acetaminophen (II) involve spectrophotometric measurement of a chromophore developed *via* reaction with an appropriate reagent, either before or after chromatographic separation from II (1–10). In many of these methods, the chromophore has not been identified (4–10). Other methods involve nonaqueous titrations with perchloric acid (11) or polarographic analysis (12, 13).

To date, only an indirect fluorometric procedure for the determination of I in the presence of II has been reported (14). The fluorometric method involves a lengthy prior derivatization with benzylamine in alkaline solution. The fluorophore has not been identified.

This paper introduces a direct spectrofluorometric method for the determination of I either alone or as an impurity in II or tablets containing II. Due to overlapping UV absorption bands of these compounds, a critical evaluation of the inner filter effect was necessary. The effect of other aminophenols on the determination of I is less critical, but the possibility of positive interference in mixtures exists.

EXPERIMENTAL[1]

Solvents—Since most "spectro" quality solvents are not of suitable purity, all fluorescence work was done with purified alcohols, except where indicated. Purified alcohols were easily prepared from analytical reagent grade anhydrous alcohols by percolating each through a column packed with 30–40 cm of 40–80-mesh activated coconut charcoal[2]. The first portion (~50 ml) of the eluate from each column was highly contaminated with fluorescent impurities and was discarded. After the initial 50 ml, each column produced 5–10 liters of purified solvent before the packing was replaced.

n-Propanol did not purify as well as ethanol or methanol. Spectroquality chloroform always was used except where the chloroform fluorescence blank exceeded 3 in 100 units of full scale. To prepare fluorescent grade solvent from spectro or analytical reagent grade reagent, the chloroform was first vigorously extracted with a small volume (~10:1 v/v) of 0.45 *M* NaOH. The chloroform was recovered, washed twice with small volumes (~10:1 v/v) of water, and filtered through chloroform-wet filter paper. The resulting chloroform was suitable for spectrophotometry or spectrofluorometry.

Solutions of I—Crystals of I[3] decompose on standing and were available as black granules. Compound I was easily sublimed to gray crystals under ~4 torr at 180°; a second sublimation under similar conditions yielded white crystals with a 186–188° melting-point range (15). Alcohol or chloroform solutions, 0.01–0.001 *M*, were prepared fresh daily prior to use.

***o*-Aminophenol**—The *o*-aminophenol[3] deteriorated to black granules on standing. It was easily sublimed to pale-red crystals under ~4 torr at ~150°; a second sublimation under similar conditions yielded pale-yellow needles with a 170–174° melting-point range (16). Standard solutions were prepared fresh daily prior to use.

***m*-Aminophenol**—The *m*-aminophenol[4] was used without further purification. Although the *m*-aminophenol is somewhat more stable in alcoholic solution than the other aminophenols, standard solutions had to be prepared fresh daily.

Acetaminophen[5], Phenacetin[6], Aniline[7], Acetanilid[8], and Phenol[9]—These chemicals were used to prepare solutions without further purification.

Working Standards for Solvent Studies—All standard solutions of drugs (and related compounds) were prepared by dissolving an appropriate amount of the drug in the desired solvent and volumetrically diluting to the correct molarity. Compound I was sparingly soluble in chloroform. Standards of I in chloroform were prepared by volumetric dilutions of a concentrated methanolic I standard to the correct molarity

[1] Absorption spectra were taken on a Beckman DK-2A spectrophotometer. All fluorescence spectra were taken on a Perkin–Elmer MPF-2A spectrofluorometer.

[2] Fisher 50–200 mesh.
[3] Eastman practical grade.
[4] NF sample courtesy of Parke, Davis.
[5] NF sample courtesy of Wyeth Laboratories.
[6] American Pharmaceutical Co. USP grade.
[7] Mallinckrodt practical grade.
[8] Matheson, Coleman and Bell practical grade.
[9] Baker Chemical Co. practical grade.

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