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Biochemistry

Arachidonic acid metabolism in polymorphonuclear leukocytes*: Unstable intermediate in formation of dihydroxy acids

(polyunsaturated fatty acid/lipoxygenase/isotopic oxygen/reaction mechanism)

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ABSTRACT **An unstable intermediate was detected in the transformation of arachidonic acid into 5,6-dihydroxyicosatetraenoic acids (two isomers) and 5,12-dihydroxyicosatetraenoic acids (three isomers) in rabbit peritoneal (glycogen-induced) polymorphonuclear leukocytes. Addition of 10 vol of methanol, ethanol, or ethylene glycol to short-term incubations (30–45 sec) led to the formation of the corresponding 12-*O*-alkyl derivatives of the 5,12-dihydroxy acids. The time for 50% disappearance of the intermediate (37°C), as measured by formation of 5-hydroxy-12-*O*-methylicosatetraenoic acids (two isomers) upon trapping with methanol, was about 1 min in live cell preparations (pH 7.4) and about 4 min in water/acetone (1:1), pH 7.4. At pH 6.0 or below, the hydrolysis of the intermediate was too rapid to be measured by the method employed. Data supporting both enzymatic and nonenzymatic hydrolysis of the intermediate into dihydroxy acids are presented. Incubation of the cells with arachidonic acid under an atmosphere of $^{18}O_2$ led to incorporation of ^{18}O into the 5,6-dihydroxy acids and 5,12-dihydroxy acids only at C-5. The 5-hydroxyicosatetraenoic acid was also labeled at C-5. Considering the chemical reactivity of the intermediate and the structures of the derivatives obtained, it is proposed that the intermediate is 5(6)-oxido-7,9,11,14-icosatetraenoic acid.**

In a previous report (1), we described the structure of the major metabolite of arachidonic acid in rabbit peritoneal polymorphonuclear leukocytes (PMNL) as (5*S*)-hydroxy-6,8,11,14-icosatetraenoic acid, a compound that accounted for 5–15% of added substrate. More recently we reported (2) the structure of the second most abundant metabolite of arachidonic acid in leukocytes as (5*S*,12*R*)-dihydroxy-6,8,10,14-icosatetraenoic acid (see Fig. 5, compound **III**). Later some other minor metabolites were identified: (5*S*,12*R*)-dihydroxy-(*E*,*E*,*E*,*Z*)-6,8,10,14-icosatetraenoic acid (compound **I**), (5*S*,12*S*)-dihydroxy-(*E*,*E*,*E*,*Z*)-6,8,10,14-icosatetraenoic acid (compound **II**), and 5,6-dihydroxy-7,9,11,14-icosatetraenoic acids (compounds **IV** and **V**, two diastereoisomers).§

This paper reports studies on the mechanism of formation of dihydroxy acid metabolites of arachidonic acid in PMNL.

MATERIALS AND METHODS

Materials and procedures used were essentially the same as described (2) unless otherwise indicated.

For measurement of incorporation of ^{18}O from molecular oxygen, 90 ml of phosphate-buffered saline was introduced in a 250-ml three-necked flask. This flask was evacuated (by using a water pump) with stirring for 10 min at 37°C and then cooled to 5°C. $^{18}O_2$ was introduced into the flask (from a 100-ml ampule), which was brought to atmospheric pressure with N_2. The cells (3.5×10^9) were injected into the flask in a suspension in 10 ml of phosphate-buffered saline. The mixture was warmed up to 37°C and 6 mg of arachidonic acid (in 100 μl of ethanol) was added. After 45 sec, the reaction was stopped by addition of 5 ml of 1 M HCl. Two minutes later this mixture was neutralized and poured into 150 ml of methanol. After ether extraction, the sample was fractionated by chromatography.

RESULTS

Labeling Experiments with $^{18}O_2$. Gas chromatographic/mass spectrometric analyses of the trimethylsilyl (Me_3Si) ether methyl ester derivatives of the hydrogenated compounds **I, II, III, IV,** and **V** and 5-hydroxy acid showed that the percentage of incorporation of ^{18}O into the six metabolites was respectively 72, 72, 66, 75, 75, and 68%, and that all compounds were labeled with a single atom of ^{18}O. It is likely that this small variation in the percentage of incorporation of ^{18}O in the different metabolites is due to isotopic dilution by metabolites present in the cell suspension prior to incubation under an atmosphere of $^{18}O_2$. The mass spectrometric analyses also allowed unambiguous determination that the six metabolites carried the ^{18}O atom at C-5. The ion at mass to charge ratio m/e 203 [Me_3Si-O^+=CH—$(CH_2)_3$—$COOCH_3$], which contains carbons C-1 to C-5 and is common to the mass spectra of the Me_3Si ether methyl ester derivatives of the hydrogenated metabolites (see ref. 2 for the mass spectrum of this derivative of compound **I, II,** or **III**), was in all cases shifted to m/e 205 in a percentage equal to the total ^{18}O content of the molecule, whereas the ion containing the oxygen atom at C-12 (compounds **I, II,** and **III**)—i.e., m/e 215 [Me_3SiO^+=CH—$(CH_2)_7$—CH_3]—contained 100% ^{16}O atom. The ion containing the oxygen atom at C-6 in compound **IV**—i.e., m/e 299 [Me_3SiO^+=CH—$(CH_2)_{13}$—CH_3]—was accompanied by an ion at m/e 301 of low abundancy (about 5%). It was not conclusively established that this was due to the presence of ^{18}O in the ion. The same ion (m/e 299) from compound **V** contained 100% ^{16}O. These data clearly indicated that the hydroxyl groups at C-5 in compounds **I, II, III, IV,** and **V** and in the 5-hydroxy-icosatetraenoic acid are derived from molecular oxygen.

This was in agreement with data obtained on the incorporation of ^{18}O at C-12 in compounds **I, II,** and **III** when PMNL were incubated in an $H_2{}^{18}O$-enriched buffer.

Trapping with Alcohols. The labeling experiments described above suggested that other nucleophiles such as alcohols might react with an intermediate in the formation of compounds **I** to **V** and yield monohydroxymono-*O*-alkyl derivatives of ara-

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Abbreviations: PMNL, polymorphonuclear leukocytes; HPLC, high-pressure liquid chromatography; RP-HPLC, reversed-phase HPLC; Me_3Si, trimethylsilyl.

* This is the fifth paper in a series on this subject; the first, second, and third papers are refs. 1, 2, and 7, respectively, and the fourth paper is in press.§

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§ Borgeat, P. & Samuelsson, B. (1979) *J. Biol. Chem.* **254**, in press.

chidonic acid. Fig. 1*A* shows the pattern of compounds formed when rabbit PMNL were incubated 30 sec with arachidonic acid prior to addition of 10 vol of methanol. In Fig. 1*C* the pattern of compounds formed in a similar incubation terminated by acidification of the mixture to pH 3 is shown. The chromatogram in Fig. 1*A* clearly shows the presence of a pair of compounds that are absent in Fig. 1*C*. Fig. 1*B* shows the pattern of compounds formed when rabbit PMNL were incubated 30 sec with arachidonic acid prior to addition of 10 vol of ethanol. The chromatogram shows the presence of a pair of compounds that appear at longer elution time than the compounds formed upon methanol trapping, and that are also absent in Fig. 1*C*. Addition of 8 vol of ethylene glycol to 30-sec incubations of PMNL with arachidonic acid also led to the formation of a pair of compounds (with retention times very close to the retention time of compound **III** in RP-HPLC, data not shown). These data confirmed that a compound produced upon incubation of PMNL with arachidonic acid can react with alcohols to form new derivatives of arachidonic acid. The data further indicated that the alcohols compete with water for reaction with the same intermediate, because the formation of the derivatives coincided with the disappearance of compounds **I, II, IV,** and **V** (Fig. 1).

For structural work larger amounts of the arachidonic acid derivatives formed by trapping with methanol were prepared as described in legend to Fig. 1 (for sample *A*), but on a larger scale. Four incubations (100 ml each) were carried out with a total of 14×10^9 cells and 24 mg of substrate. The silicic acid column chromatography was done by using the following solvents in order: diethyl ether/hexane, 15:85 and 35:65 vol/vol, and ethyl acetate. RP-HPLC was also performed as described in the legend to Fig. 1, but at a solvent flow of 0.3 ml/min to improve the separation of the two compounds of interest. The compounds isolated were treated with diazomethane and further purified by silicic acid HPLC on a μPorasil column (3.9 × 300 mm, from Waters Associates, Milford, MA) with 10-μm particles and isopropanol/hexane, 4:100 vol/vol as solvent (1 ml/min) and an ultraviolet spectrophotometer as detector (280 nm). About 15 μg of each of the two derivatives of arachidonic acid were obtained from silicic acid HPLC (0.06% yield).

The ultraviolet spectra of the methyl ester of both compounds showed three main bands λ_{max}^{MeOH} = 268 nm ($\epsilon \approx 56{,}000$ M^{-1} cm^{-1}), 280 nm (≈44,000), and 258 nm (≈42,000) indicating three conjugated double bonds. This is in agreement with the reported ultraviolet spectrum of β-elaeostearic acid, CH_3—$(CH_2)_3$—$(CH\overset{E}{=}CH)_3$—$(CH_2)_7$—COOH, which has a λ_{max}^{EtOH} = 268 nm (≈58,000) and two other absorption bands at 279 nm (≈45,000) and 258 nm (≈42,000) (3). The infrared spectrum (in CS_2) showed a band *inter alia* at 996 cm^{-1}, in agreement with a conjugated triene consisting of three *trans* double bonds (β-elaeostearic acid in CS_2, 995 cm^{-1}) (3).

Gas chromatographic analysis of the Me_3Si ether derivatives of the methyl esters of both compounds showed a peak with equivalent chain length C-25.1 (3% OV-210) or C-24.3 (1% SE-30). The mass spectra of the compounds were similar. The mass spectrum of the Me_3Si ether methyl ester of the first compound eluted in RP-HPLC is given in Fig. 2. Ions were present at *m/e* 436 (parent ion M), 421 (M − 15), 404 (M − 32), 389 [M − (32 + 15)], 325 [M − 111, loss of $\cdot CH_2$—CH=CH—$(CH_2)_4$—CH_3], 293 [M − (32 + 111)], 235 [M − (111 + 90)], 203 [M − (111 + 90 + 32)] and Me_3SiO^+=CH—$(CH_2)_3$—$COOCH_3$], 189, 171 (203 − 32), 161, 159 (probably Me_3SiO^+=CH—CH=CH—OCH_3 from a rearrangement), 143, 133 (probably Me_3SiO^+=CH—OCH_3 from a rearrangement), 129, 113, 109, 73, and 71. Several of these ions appeared in the mass spectra of the Me_3Si ether derivatives of the methyl esters of compounds **I, II,** and **III** (the mass spectra of these three compounds are practically identical; see ref. 2 for mass spectrum of compound **III**)—i.e., *m/e* 404, 293, 203, 189, and 171. Other ions were shifted by 58 units of lower *m/e* values—i.e., 436, 421, 325, 235, 159, 133, and 71. Because a shift by 58 units corresponded to the difference between the molecular weights of $Me_3SiO\cdot$ and $CH_3O\cdot$ (89 and 31, respectively), it seemed likely that the compounds are mono-*O*-methyl de-

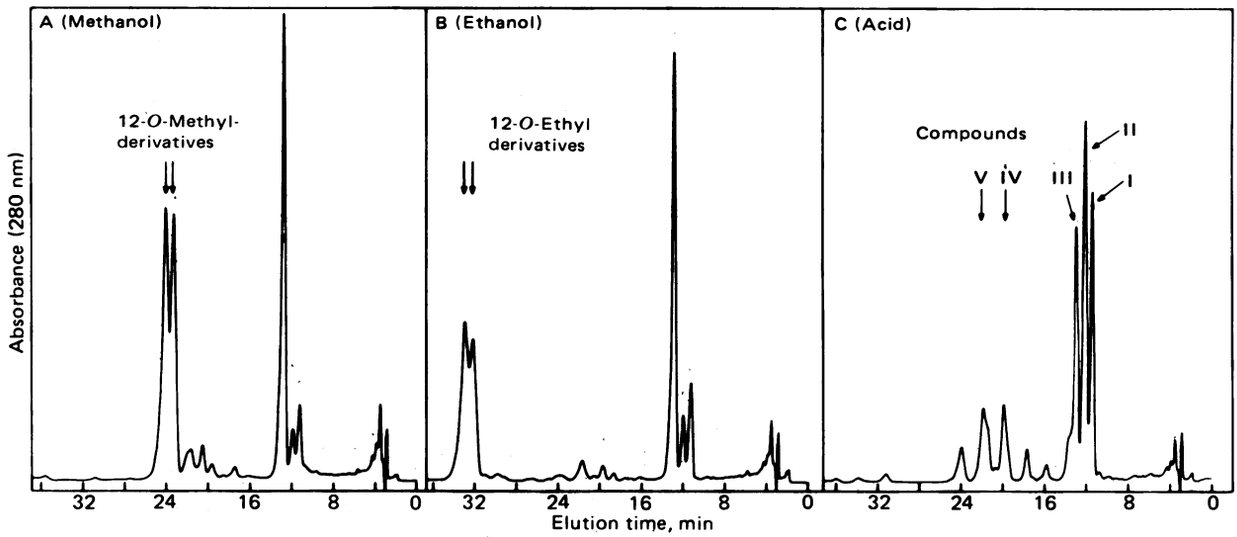

FIG. 1. Reversed-phase high-pressure liquid chromatography (RP-HPLC) chromatograms of the products obtained upon addition of 10 vol of methanol (*A*), 10 vol of ethanol (*B*), or 1 ml of 1 M HCl (*C*) to 5 ml of a suspension of PMNL (35×10^6/ml) incubated 30 sec with arachidonic acid (0.166 mM) at 37°C in phosphate-buffered saline. The pH of the alcohol used for trapping was previously lowered to 1 (HCl). Two minutes after stopping the reactions, the three samples (pH ≈ 3) were neutralized. The excess of alcohol in samples *A* and *B* was evaporated under reduced pressure until 10–15 ml was left in the flasks. Methanol (7.5 ml) was added to sample *C* and the three samples were extracted with ether. The samples were fractionated by silicic acid column chromatography using 1 g of Silicar CC-4 (Mallinckrodt) and 30 ml of the two following solvents: diethyl ether/hexane, 20:80 vol/vol, and ethyl acetate, successively. The ethyl acetate fraction was evaporated and dissolved in 50 μl of methanol. Aliquots (4 μl) of each sample were analyzed by RP-HPLC: column (250 × 4.6 mm) packed with Nucleosil C_{18}, 5 μm particles (packing purchased from Macherey-Nagel, Düren, West Germany); solvent, methanol/H_2O, 75:25 vol/vol, + 0.01% acetic acid; conditions, 1 ml/min, 1200 pounds/inch^2 (8.3 MPa) and 25°C; detector ultraviolet spectrophotometer, 280 nm and 0.04 absorbance unit at full scale deflection.

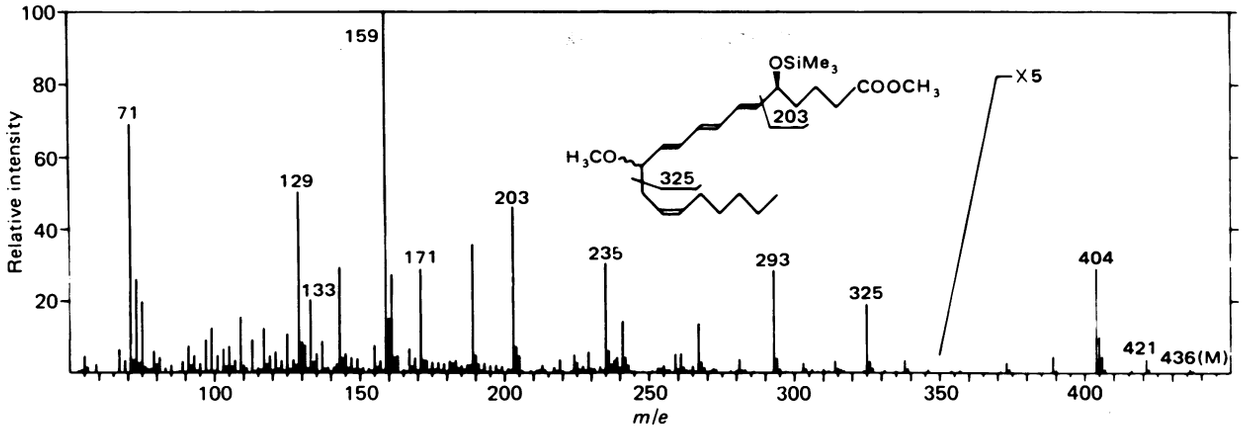

FIG. 2. Mass spectrum of the Me_3Si ether derivative of the methyl ester of 5-hydroxy-12-*O*-methyl-6,8,10,14-icosatetraenoic acid (ionizing energy 22.5 eV).

rivatives of compounds **I** and **II**. The mass spectra of the [$^2H_9$]Me_3Si ether derivatives of the methyl esters of the compounds were in agreement with the structures given for the ions above. About 2 μg of the methyl esters of each compound were subjected to catalytic hydrogenation. The mass spectra of the Me_3Si ether methyl ester derivatives of the hydrogenated compounds (equivalent chain lengths C-23.9 on 3% OV-210 and C-23.4 on 1% SE-30, for both compounds) were identical and showed ions at *m/e* 397 (M − 47), 343 [M − 101, loss of $\cdot CH_2—(CH_2)_2—COOCH_3$], 331 [M − 113, loss of $\cdot CH_2—(CH_2)_6—CH_3$], 311 [M − (101 + 32)], 241 [M − (113 + 90)], 221 [M − (101 + 90 + 32)], 209 [M − (113 + 90 + 32)], 203 [base peak, $Me_3SiO^+{=}CH—(CH_2)_3—COOCH_3$], 171 (203 − 32), and 157 [$CH_3O^+{=}CH—(CH_2)_7—CH_3$]. These data indicated that the hydroxyl groups of both compounds were located at C-5 and the *O*-methyl groups at C-12.

About 10 μg of each compound was converted into the menthoxycarbonyl derivative and subjected to oxidative ozonolysis. The products were analyzed by gas chromatography/mass spectrometry as described (2). From both samples, a compound corresponding to the menthoxycarbonyl derivative of dimethyl-2*L*-hydroxyadipate was eluted. These data indicated that both compounds had the *S* configuration at C-5 and supported the presence of a double bond at Δ^6 (as indicated by mass spectrometric analysis). It is assumed that the fourth double bond has retained the original position and geometry (Δ^{14}, *cis*) from arachidonic acid. Although the absolute configuration at C-12 has not been determined, the isomerism of the two compounds is most likely explained by epimerism at C-12 (because other forms of isomerism have been ruled out by spectrophotometric and mass spectrometric analyses). The two derivatives of arachidonic acid (formed upon trapping with methanol) are thus (5*S*)-hydroxy-12-*O*-methyl-(*E*,*E*,*E*,*Z*)-6,8,10,14-icosatetraenoic acids, epimers at C-12—i.e., the 12-*O*-methyl derivatives of compounds **I** and **II**.

Derivatives of arachidonic acid formed upon trapping with ethanol (Fig. 1*B*) and ethylene glycol (not shown) were also obtained (as a pair of isomers) in similar experiments. Ultraviolet spectrophotometric and gas chromatographic/mass spectrometric analyses were in complete agreement with the identities of the compounds isolated from ethanol trapping experiments as the 12-*O*-ethyl derivatives of compounds **I** and **II** [chain length equivalent for the Me_3Si ether methyl ester derivatives, C-24.6 (1% SE-30) and C-25.2 (3% OV-210)].

Time Course Studies. Experiments were performed to evaluate the stability of the intermediate in aqueous medium. Fig. 3 shows the time course of formation of the intermediate after addition of substrate to a suspension of PMNL. The concentration of the compound reached a maximum within 45 sec of incubation and then decreased rapidly to 30–35% of its maximal value in about 4 min. The disappearance of this residual amount of intermediate was very slow: at 30 min, about

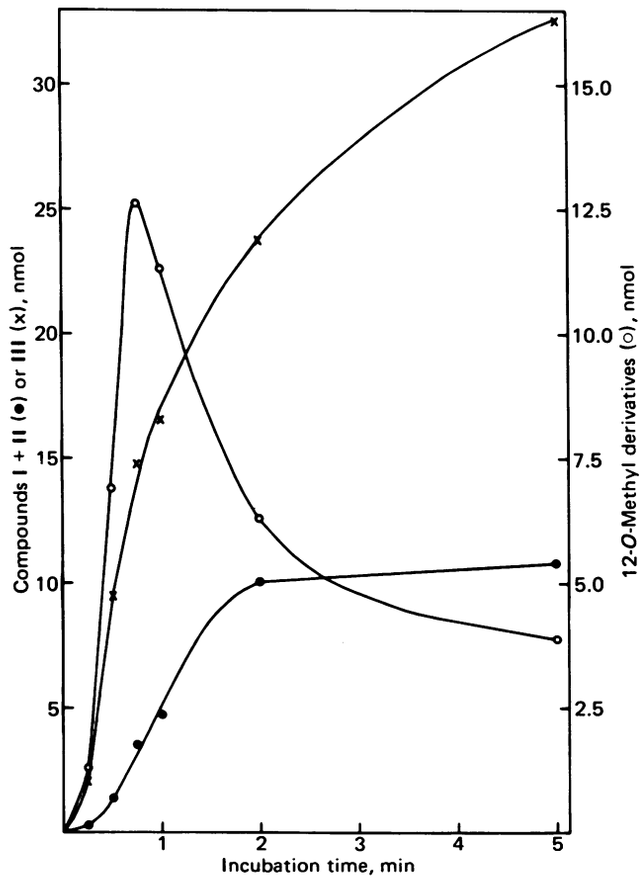

FIG. 3. Time course of the formation of compounds **I** + **II** (●), and compound **III** (x), and time course of the formation and disappearance of the unstable intermediate, measured as 12-*O*-methyl compounds **I** and **II** (○) in PMNL incubated with arachidonic acid. Suspensions of PMNL (5 ml) were incubated for different times before addition of 10 vol of methanol (previously acidified to pH 1). Two minutes after addition of the methanol the samples were neutralized. Two micrograms of prostaglandin B_2 were added to each sample (before any purification procedure) as an internal standard to allow absolute quantitation of the compounds by RP-HPLC. The preparation and RP-HPLC analyses of the samples were done as described in the legend to Fig. 1.

10% of the amount of intermediate detected at 45 sec could still be trapped with methanol (data not shown), suggesting that part of the compound was protected from rapid hydrolysis (cf. ref. 4). This phenomenon, as well as the changing rate of synthesis of the intermediate at different times, and the presence of both enzymatic and nonenzymatic processes in the hydrolysis of the intermediate (see *Discussion*) complicate the kinetic description of the disappearance of this compound. However, assuming that the formation of the intermediate has ceased (or considerably decreased) after 45 sec of incubation, the time for 50% disappearance of the compound can be estimated to about 1 min (from Fig. 3). Fig. 3 also shows the kinetics of formation of compounds I–II and III. Compound III was formed faster and in larger amounts than compounds I and II. In these experiments the formation of the (5*S*)-hydroxyicosatetraenoic acid closely paralleled that of compound III (not shown). The concentrations of compounds IV and V were not measured.

Time course studies on the hydrolysis of the intermediate were also performed under conditions in which enzymatic activities were suppressed. Fig. 4 shows the results of an experiment in which a suspension of PMNL incubated 45 sec with arachidonic acid was rapidly mixed with 1 vol of acetone and kept at 37°C and pH 7.4 (±0.1 unit). At different times aliquots of this mixture were added to flasks containing methanol. The data indicated that the time for the disappearance of 50% of the intermediate was 4 min under these conditions. The concentrations of compounds I and II were found to increase with time and thus changed in the opposite direction to the concentration of the intermediate. The concentrations of compounds IV and V closely paralleled those of compounds I and II. The concentrations of compound III and of the 5-hydroxyicosatetraenoic acid were constant (not shown).

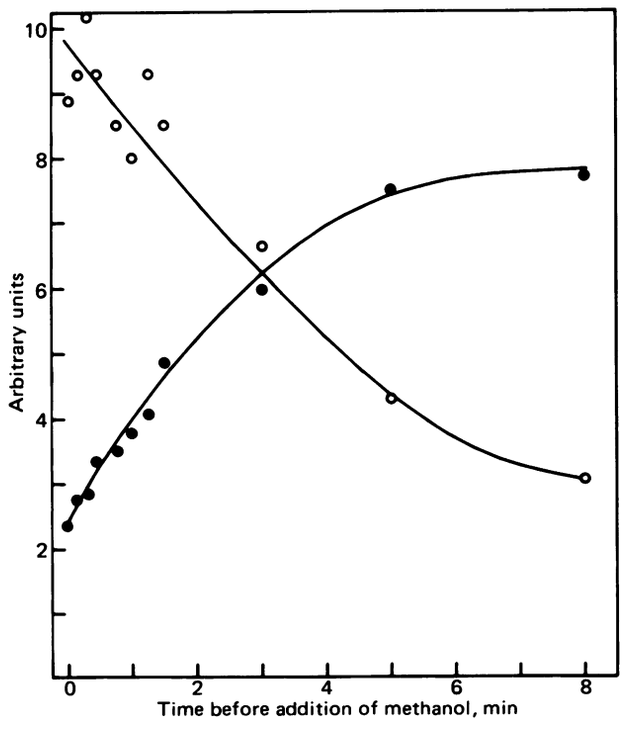

FIG. 4. Time course of the formation of compounds I, II, IV, or V (●) and of the disappearance of the intermediate measured as 12-*O*-methyl compounds I–II (○) in a mixture of water/acetone, 1:1 vol/vol, at pH 7.4 and 37°C. A suspension of PMNL (75 ml, 35 × 10^6 cells per ml) was incubated 45 sec before addition of 75 ml of acetone (previously cooled to 2°C and slightly acidified in order to give the expected pH and temperature upon mixing with the cell suspension) containing 30 μg of prostaglandin B_2 as internal standard for quantitation of the compounds by RP-HPLC. The mixture was kept at 37°C and 10-ml aliquots were added to 50 ml of methanol (previously acidified to pH 1) at different times. Two minutes after the addition of methanol the samples were neutralized. Preparation of the samples and RP-HPLC analyses were done as described in legend to Fig. 1.

Similar experiments were performed to determine the stability of the intermediate as function of pH (37°C). At pH 6 or lower, the concentration of the intermediate decreased to less than 40% of its initial value within 15 sec after mixing the cell suspensions with acetone (previously acidified). At pH 8.5 or 11.5 degradation was not noticeable after 4 min, indicating that the intermediate was more stable in alkaline solution.

These studies clearly indicated that the intermediate is very labile in live cell preparations and that its reactivity is pH dependent, the compound being more unstable under acidic conditions. Furthermore, these studies conclusively showed that the formation of compounds I, II, IV, and V was nonenzymatic because it occurred in a water/acetone mixture. The data also supported the hypothesis that compounds I, II, IV, V, and 12-*O*-methyl compounds I–II were formed by a reaction with a common intermediate because their concentrations were inversely related at all times studied (Fig. 4; see also Fig. 1).

DISCUSSION

In this paper we describe the mechanism of formation of five dihydroxy metabolites of arachidonic acid (see Fig. 5, compounds I and V) in PMNL, through a common unstable intermediate. $^{18}O_2$ labeling experiments conclusively showed that

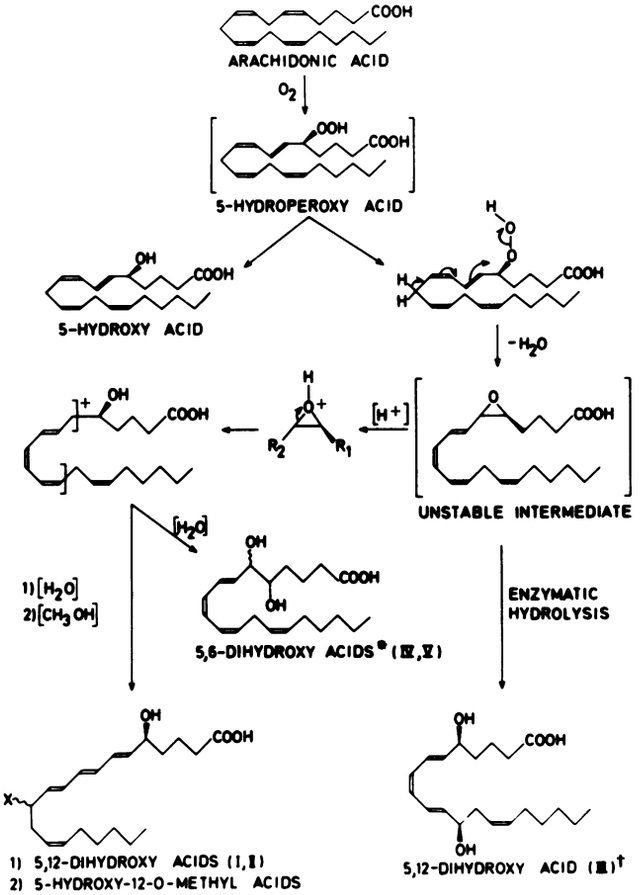

FIG. 5. Hypothetical scheme of the transformation of arachidonic acid in PMNL. Compounds between brackets have not been isolated.

* The geometry of the double bonds and the configuration of the alcohols are hypothetical.

† The position of the *cis* double bond in the conjugated triene is not known (see ref. 2).

only the oxygen atom at C-5 in the dihydroxy acids (as well as in the 5-hydroxy acid) was derived from molecular oxygen and consequently that the oxygen atoms at C-6 or C-12 were derived from water. Trapping experiments with alcohols clearly showed that a metabolite of arachidonic acid can undergo a facile reaction with nucleophiles to yield 5-hydroxy-12-*O*-alkyl derivatives. Structural analysis of the compounds formed upon addition of methanol to the intermediate revealed that the pair of derivatives produced were 12-*O*-alkyl derivatives of compounds **I** and **II** (Fig. 1).

Time course experiments performed in water/acetone mixtures (in the absence of enzymatic activity) showed that the concentration of compounds **I, II, IV,** and **V** increased with time, while the concentration of the intermediate decreased (Fig. 4). This indicated that compounds **I, II, IV,** and **V** were formed by nonenzymatic hydrolysis of the intermediate (as first suggested by the results of the trapping experiments). ^{18}O-Labeling experiments and structural similarities between compounds **I, II,** and **III** suggested that the latter was also derived from the hydrolysis of the intermediate. However, the observation that compound **III** was the major dihydroxy acid formed upon incubation of PMNL with arachidonic acid (Fig. 3), whereas it was not formed during the hydrolysis of the intermediate in water/acetone, suggested that the formation of compound **III** occurred through an enzymatic hydrolysis of the intermediate. This was strongly supported by the stereochemical purity of compound **III** (a derivative of compound **III** epimeric at C-12 was not detected) as opposed to the products of nonenzymatic reaction of the intermediate with water or alcohols, which occurred as pairs of epimers (see Figs. 1 and 5). The difference in the geometry of the conjugated triene in compound **III** (one *cis* and two *trans* double bonds) and in compounds **I, II,** and their 12-*O*-alkyl derivatives (all-*trans*) was also suggestive of two distinct mechanisms of formation. It thus appeared that the intermediate can be hydrolyzed by enzymatic and nonenzymatic reactions.

Fig. 5 shows a hypothetical scheme for the transformation of arachidonic acid into the various metabolites isolated (or detected) in these studies. This scheme includes a structure of the unstable intermediate that is in agreement with the data presented—viz., 5(6)-oxido-7,9,11,14-icosatetraenoic acid. It is known that the hydrolysis of epoxides is acid catalyzed and that opening of allylic epoxides is favored at the allylic position, which is in agreement with the decreased stability of the intermediate under acid conditions and with attack of water at C-6. The formation of a carbonium ion (Fig. 5) in the mechanism of opening of this epoxide is proposed in order to explain the formation of two diastereoisomeric 5,6-dihydroxy acids (compounds **IV** and **V**) that both retained the ^{18}O atom at position C-5 (from molecular oxygen). Normally the opening of an epoxide occurs with inversion of configuration (S_N2 mechanism), which in this case would lead to formation of a single 5,6-dihydroxy acid (with ^{18}O from molecular oxygen, at C-5). An alternative explanation for the retention of the ^{18}O atom at C-5 in compounds **IV** and **V** would be that the epoxide is not stereochemically pure, which seems unlikely. The carbonium ion could also account for the formation of two of the 5,12-dihydroxy acids (compounds **I** and **II**). According to the hypothetical pathway proposed (Fig. 5), the unstable carbonium ion would react (in aqueous solution) preferentially at C-6 and C-12 to yield two pairs of epimers (compounds **I–II** and **IV–V**) which have maintained the conjugated triene structure (Fig. 1*C* illustrates the ratio of reaction of the intermediate at C-6 and C-12 under acidic conditions).

At present, little is known about the mechanism of formation of the epoxide intermediate. It is postulated that the epoxide intermediate is derived from a 5-hydroperoxyicosatetraenoic acid (not isolated). This is in agreement with the formation of large amounts of a 5-hydroxyicosatetraenoic acid, which is presumably formed by enzymatic reduction of the corresponding 5-hydroperoxy acid formed in a lipoxygenase-type reaction (1). Fig. 5 shows a hypothetical mechanism for the formation of the intermediate from the 5-hydroperoxy acid involving abstraction of a hydrogen at C-10. In this process the 5-hydroxy acid would thus be a by-product in the conversion of arachidonic acid into the epoxide intermediate (Fig. 5) in analogy with the 11-hydroxy acid and 15-hydroxy acid that are by-products in the formation of prostaglandins (5, 6). The present data do not allow the determination of the geometry of the epoxide ring and double bonds of the intermediate. It is, however, likely that the epoxide has the *S* configuration at C-5, as found in the 5,12-dihydroxy acids (2) and the 5-hydroxy acid (1). It is also conceivable that the double bonds at Δ^{11} and Δ^{14} have retained the *cis* geometry from arachidonic acid.

The concept that a biologically active compound is formed as an unstable intermediate that is subsequently hydrolyzed to a stable inactive derivative has been proposed for the thromboxanes. Thus, thromboxane A_2, which is formed by isomerization of the endoperoxide prostaglandin H_2, has a half-life of about 30 sec. It is a strong smooth-muscle stimulant and causes release of ADP and serotonin from platelets. Hydrolysis yields a stable, biologically inactive derivative, thromboxane B_2. More recently this concept has also been shown to be applicable to another endoperoxide derived product, prostacyclin, which is hydrolyzed to 6-ketoprostaglandin $F_{1\alpha}$. The present results indicate that a similar mechanism might operate in a lipoxygenase-catalyzed pathway of arachidonic acid metabolism in leukocytes. Isolation of the different enzymes involved is desirable, as are further studies on the biological significance of these transformations of arachidonic acid. It is of interest in this context that the ionophore A23187 induces synthesis of the stable end product 5,12-dihydroxyicosatetraenoic acid from endogenous arachidonic acid in human PMNL (7).

Note Added in Proof. A nomenclature was recently introduced (8): 5(6)oxido-7,9,11,14-icosatetraenoic acid, leukotriene A; compound **III**, leukotriene B.

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