Evidence of a Cyclooxygenase-related Prostaglandin Synthesis in Coral

THE ALLENE OXIDE PATHWAY IS NOT INVOLVED IN PROSTAGLANDIN BIOSYNTHESIS*

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Certain corals are rich natural sources of prostaglandins, the metabolic origin of which has remained undefined. By analogy with the lipoxygenase/allene oxide synthase pathway to jasmonic acid in plants, the presence of (8R)-lipoxygenase and allene oxide synthase in the coral *Plexaura homomalla* suggested a potential metabolic route to prostaglandins (Brash, A. R., Baertschi, S. W., Ingram, C.D., and Harris, T. M. (1987) *J. Biol. Chem.* 262, 15829–15839). Other evidence, from the Arctic coral *Geremia fruticosa*, has indicated a cyclooxygenase intermediate in the biosynthesis (Varvas, K., Koljak, R., Järving, I., Pehk, T., and Samel, N. (1994) *Tetrahedron Lett.* 35, 8267–8270). In the present study, active preparations of *G. fruticosa* have been used to identify both types of arachidonic acid metabolism and specific inhibitors were used to establish the enzyme type involved in the prostaglandin biosynthesis. The synthesis of prostaglandins and (11R)-hydroxyeicosatetraenoic acid was inhibited by mammalian cyclooxygenase inhibitors (indomethacin, aspirin, and tolfenamic acid), while the formation of the products of the 8-lipoxygenase/allene oxide pathway was not affected or was increased. The specific cyclooxygenase-2 inhibitor, nimesulide, did not inhibit the synthesis of prostaglandins in coral. We conclude that coral uses two parallel routes for the initial oxidation of polyenoic acids: the cyclooxygenase route, which leads to optically active prostaglandins, and the lipoxygenase/allene oxide synthase metabolism, the role of which remains to be established. An enzyme related to mammalian cyclooxygenases is the key to prostaglandin synthesis in coral. Based on our inhibitor data, the catalytic site of this evolutionary early cyclooxygenase appears to differ significantly from both known mammalian cyclooxygenases.

Natural A-, E-, and F-type prostaglandins, as well as atypical prostanoids such as clavulones and punaglandins, have been detected in different corals (reviewed in Ref. 1). The most important representative in the field is the Caribbean gorgonian *Plexaura homomalla*, which contains the highest known concentration of prostanoids in Nature. The content of PGA₂¹ and PGE₂ esters in *P. homomalla* amounts to 2–3% of dry weight (2, 3). There have been proposals over the years that biosynthesis of prostaglandins in coral may occur by a route totally different from the vertebrate cyclooxygenase pathway (4–6). Currently, despite the intensive studies and widespread occurrence of prostaglandins in corals, the mechanism of biosynthesis of these compounds has remained unresolved. This is mainly attributable to the inability of the coral preparations studied to form prostaglandins in vitro (4–7).

*P. homomalla* and other Caribbean corals readily metabolize exogenous arachidonic acid via the (8R)-lipoxygenase pathway (6–8). The product, (8R)-HPETE, is further converted into an allene oxide, a highly unstable epoxide (7, 9). As one of the key properties of allene oxides is their ability to undergo cyclization to give cyclopentenene rings, this compound appeared to be a promising intermediate in the potential lipoxygenase pathway from arachidonic acid to prostaglandins (Scheme 1, left side) (6, 7, 10). This type of transformation has been observed in plants where allene oxide is an intermediate of biosynthesis of the five-membered carbon ring of the growth hormone, jasmonic acid (11). In preparations of *P. homomalla*, however, the enzymatic cyclization of allene oxide to prostaglandins has not been realized (6–8). In 1993, we reported that the Arctic soft coral *Geremia fruticosa* (Ooctocorallia, Alcyonacea, Nephtheidae) contains optically active natural prostaglandins PGD₂, PGB₂, PFG₂, and 15-keto PGF₂ₐ (12). The content of prostaglandins of the Arctic coral (0.008% of dry weight) is several orders of magnitude lower than that of *P. homomalla*, but *G. fruticosa* has a significant ability to convert the exogenous arachidonic acid into optically active prostaglandins in vitro (12). Our further work led to the isolation of the prostaglandin endoperoxide intermediate, PGS₂, from *G. fruticosa* incubations (13). It is notable that it was the 15-hydroperoxy compound PGS₂, not PH₂, that accumulated in short incubations even in the presence of peroxydase reducing substrates. The nature of the enzymes involved in this pathway is the subject of the present study.

In vertebrates, prostaglandins are synthesized by prosta-
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Two Potential Pathways of PG synthesis in Coral

Lipoxygenase

COOH

11

Cyclooxygenase

COOH

11

Allene Oxide Synthase

COOH

COOH

COOH

COOH

COOH

COOH

COOH

COOH

Prostaglandins

PG E2

PG D 2

PG F 2a

SCHMME 1

amidc, methoxylamine hydrochloride, and pyridine were obtained from Serva. The coral G. fruticosa was collected in the Gulf of Kandalaksha at a depth of 25–30 m and water temperature about 4 °C. The collected coral was frozen in liquid nitrogen within 5 min, transported on dry ice, and stored below −70 °C.

Enzyme Preparation

The acetone powder of G. fruticosa was prepared by homogenization of 20 g of frozen coral with a Whirler blender for 1 min at full speed in cold acetone (−20 °C). The mixture was centrifuged at 3000 × g at 4 °C for 5 min, the supernatant was decanted, and the procedure was repeated three times on the residual solids. The skeletal elements were removed from the mixture by swirling and decanting with cold acetone. The fine solids obtained were filtered and dried in a stream of argon at room temperature. The yield was 1–1.5 g of powder. The powder was divided into aliquots and stored under argon at −70 °C. The enzyme preparations remained active for several months.

Incubations—15 mg of the coral acetone powder was blended with 3 ml of 50 mM Tris buffer, pH 9, and preincubated for 5 min. The reaction was initiated with 0.4 µCi [14C]arachidonic acid (final concentration, 100 µM) in ethanol solution. Incubations were performed at room temperature for 10 min, after which the incubation mixture was treated with SnCl2 (2 mg/ml), acidified, and the reaction products were extracted with three portions of ethyl acetate. When hydroperoxy compounds were studied, SnCl2 was omitted. The combined extracts were washed with brine, dried over potassium sulfate, and evaporated at reduced pressure to dryness. The residue was subjected to TLC analysis, and the radioactivity was monitored. The total recovery of radioactivity was about 60%.

To trap the intermediates a mild reducing agent (SnCl2; final concentration, 0.5 mM) was added to the incubation mixture directly before the substrate. Incubations in the presence of SnCl2 were performed in 50 mM Tris buffer, pH 8.

For inhibition studies, the coral acetone powder was preincubated at room temperature for 5 min with various amounts of inhibitors added in a few microliters of ethanol. An equal amount of ethanol was added to the control. The reaction was initiated with 0.3 µCi of [14C]arachidonic acid (final concentration, 50 µM). The incubation was carried out for 10 min, followed by treatment with SnCl2. The products were extracted with ethyl acetate and analyzed by TLC, and the radioactivity was monitored. All experiments were carried out in triplicate.

Chromatographic Methods

Thin Layer Chromatography—TLC was performed using precoated plates (Silica Gel 60, 0.25 mm) from E. Merck (Darmstadt, Germany). A solvent system (A) of benzene:dioxane:acetic acid (10:5:0.25, v/v/v) for separation of polar products and a solvent system (B) of diethyl ether:hexane:acetic acid (3:3:0.05, v/v/v) for separation of nonpolar products were used. For the product quantification, the TLC plates were cut into zones and extracted with methanol, and the radioactivity was measured with a liquid scintillation counter. Authentic eicosanoid standards used as markers were visualized with an anisaldehyde spray reagent.

High Performance Liquid Chromatography—For identification of the mono- and dihydroxy acids formed, products were separated initially by RP-HPLC using a Beckman Ultrasphere 5-µm ODS column (25 × 0.46 cm) and the solvent system of methanol:water:acetic acid (50:20:0.01, v/v/v) at a flow rate of 1 ml/min. The UV spectra and the chromatograms were recorded between 215 and 235 nm. The samples were studied with a liquid scintillation counter. Authentic eicosanoid standards were used as markers. The UV spectra were measured on a Hitachi M 80-B spectrophotometer operated at 200 nm. Samples were run on an RSL fused silica capillary column (10 m × 250 µm) and the solvent system of hexane:isopropyl alcohol:acetic acid (100:20:0.1, v/v/v) at a flow rate of 1 ml/min.

EXPERIMENTAL PROCEDURES

Materials and Reagents

Unlabeled arachidonic acid and biosynthetic prostaglandin standards were obtained as a generous gift from Kevelt (Tallinn, Estonia). [14C]Arachidonic acid was purchased from Amersham Pharmacia Biotech. [14C]Arachidonic acid was purchased from Amersham Pharmacia Biotech; it was diluted with unlabeled arachidonic acid to the specific activity needed. Racemic HETE standards were prepared by vitamin E-controlled autoxidation (21). The (8R,15S)-diHETE standard was prepared by incubation of arachidonic acid with the acetone powder of the coral P. homomalla followed by incubation of the resulting (8R)-HETE with soybean lipoxigenase (6). Toluenesulfonic acid and nimesulide were bought from Cayman Chemical. Aspirin, indomethacin and SnCl2 were purchased from Sigma. N,O-Bis-(trimethylsilyl)trifluoroacetamide was obtained from Sigma. N,O-Bis-(trimethylsilyl)trifluoroacetamide was obtained from Sigma.

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RESULTS

Metabolism of Arachidonic Acid by G. fruticosa

[14C]Arachidonic acid was metabolized by G. fruticosa acetone powder to a complex mixture of oxygenated compounds. The products were separated by TLC in solvent systems A and/or B, followed by monitoring of the radioactivity. Typically, the reaction product pattern obtained with solvent system A consisted of the four well separated polar bands (about 19–25% of recovered radioactivity) co-migrated with natural mammalian prostaglandin standards and gave color reactions with anisaldehyde spray reagent characteristic of the corresponding prostaglandins (Fig. 1A). The prostaglandins PGD<sub>2</sub>, PGE<sub>2</sub>, PGF<sub>2α</sub>, and 15-keto PGF<sub>2α</sub> formed from the exogenous arachidonic acid with the G. fruticosa acetone powder were characterized earlier by HPLC, GC-MS, 13C NMR, and optical rotation measurements in comparison with authentic standards (12). The main nonpolar products 8-KETE, 8-HPETE, and 8-hydroxy-9-ketoicosatetraenoic acid (20–22%), and unchanged arachidonic acid (30–35%) unresolved in the first solvent system were separated using solvent system B (Fig. 1B). When [14C]arachidonic acid was incubated with the coral acetone powder in the presence of a mild reducing agent to trap peroxy intermediates to the corresponding hydroxy compounds, TLC analysis with solvent system A showed three main bands (Fig. 1C). These products were completely separated by RP-HPLC (see Fig. 2 and text below). When identical oxygenations were carried out using a heat-inactivated coral enzyme (at 80 °C for 40 min), no detectable radioactive products were obtained.

Identification of Lipoxgenase Products

8-HPETE—In order to detect hydroperoxides, the TLC plates eluted in solvent system B were sprayed with the ferrous thiocyanate spray reagent. One compound with RF 0.52 showed a positive reaction. This compound was scraped from the plate, extracted with methanol and reduced with NaBH<sub>4</sub> to get a slightly more polar product. The UV spectra of both unreduced and reduced compound had λ<sub>max</sub> at 235 nm, which is typical of a conjugated diene chromophore. The identity of the reduced compound as 8-HPETE was confirmed by GC-MS analysis of the methyl ester Me<sub>3</sub>Si ether derivative. The mass-spectrum had characteristic ion fragments at m/z 406, 243 (C<sub>1</sub>–C<sub>8</sub>), 355 (M<sup>+</sup>), 375 (M<sup>+</sup>), and a molecular ion at m/z 406.

8-Hydroxy-9-ketoicosatrienoic Acid (α-Ketol)—This arachidonic acid metabolite was identified in the band with RF 0.44 in solvent system B. The compound was scraped from the TLC plate and extracted with methanol. An aliquot of the extract was derivatized and analyzed by GC-MS. The mass spectrum of the methyl ester Me<sub>3</sub>Si ether derivative was identical to that of the 8-hydroxy-9-ketoicosatrienonic acid formed from arachidonic acid in P. homomalla incubations (6). This mass spectrum has characteristic ion fragments at m/z 243 (C<sub>1</sub>–C<sub>8</sub>), 310 (M – 141, loss of C<sub>1</sub>–C<sub>7</sub>), 420 (M – 31), 436 (M – 15), and 451 (M<sup>+</sup>). The reduction of the keto-hydroxy compound with NaBH<sub>4</sub> led to the formation of two more polar diastereomers. The mass spectra of the methyl ester Me<sub>3</sub>Si ether derivatives of the two diastereomers gave the same prominent ions at m/z 243 (C<sub>1</sub>–C<sub>8</sub>), 345 (C<sub>1</sub>–C<sub>9</sub>), 355 (M – 141, loss of C<sub>1</sub>–C<sub>7</sub>), 481 (M – 15), and 496 (M<sup>+</sup>), which are characteristic of the spectra of the same derivative of 8,9-dihydroxyeicosatrienoic acid. The isolation of α-ketol (the major product of nonenzymatic hydrolysis of unstable allene oxide), which shared 7–9% of total radioactivity, is indicative of the occurrence of an active allene oxide synthase in G. fruticosa.

8-KETE—In addition to 8-HPETE and α-ketol, one more arachidonic acid metabolite, having about 10% of total radioactivity, was detected in the nonpolar region (RF 0.62 in solvent system B). The UV spectrum of this compound had λ<sub>max</sub> at 280 nm, which is characteristic of the conjugated dieneone. The compound was identified as 8-KETE by its conversion into HETE on treatment with NaBH<sub>4</sub>. The reduced compound...
comigrated with 8-HETE standard on TLC and had a uv absorbance maximum at 235 nm. The mass spectrum of the methyl ester Me3Si ether derivative of the reduced compound confirmed the identity as that of 8-HETE.

Identification of Peroxy Intermediates

In order to simplify product quantification in enzyme inhibition studies, the number of labeled oxygenated metabolites was diminished by the immediate reduction of the peroxyl intermediates formed with a mild reducing agent, SnCl2, to the corresponding hydroxy compounds. Additionally, the prevention of hydroperoxy acids from further transformations allowed us to isolate several minor HETEs not detectable in the absence of SnCl2. The incubation products were examined by TLC and RP-HPLC. Typical profiles of the labeled metabolites formed are shown in Figs. 1C and 2.

Prostaglandin PGF2α.—The only prominent peak in the prostaglandin region had the retention time of 5.0 min, and it was co-chromatographed with PGF2α standard. The structural and stereochemical identity of the coral PGF2α with the mammalian’s has been shown previously (12).

8,15-DiHETE.—The second peak had the retention time of 7.2 min and co-chromatographed with authentic (8R,15S)-diHETE. Furthermore, the uv spectrum of the compound from peak 2 had λmax of 268 nm and shoulders at approximately 258 and 278 nm, which were similar to those of authentic (8R,15S)-diHETE. GC-MS analysis of the methyl ester Me3Si ether derivative confirmed the identity of this compound as 8,15-diHETE. The spectra had prominent ions at 173 (C15–C20), 225 (M–181, loss of C11–C10), 335 (M–71, loss of C16–C20), and 391 (M–15). Peak 4 had identical to that of the corresponding derivative of authentic 15-HETE. Characteristic ion fragments were observed at m/z values of 173 (C15–C20); 225 (M–181, loss of C11–C10); 335 (M–71, loss of C16–C20), and 391 (M–15). Peak 4 was identified as 11-HETE, with characteristic ion fragments at m/z values of 225 (base peak, cleavage of the C10–C11 bond), 316 (M–90), 375 (M–31), 391 (M–15), and 406 (M^+). Peak 5 had a mass spectrum identical to that of the corresponding derivative of authentic 8-HETE.

The enantiomeric composition of the products was determined by chiral-phase HPLC of their methyl ester derivatives (23). The uv chromatograms monitored at 235 nm showed that 15-HETE was predominantly in the 15S conformation (93.6%), 11-HETE consisted mainly of R-enantiomer (89%), and 8-HETE was mostly R-enantiomer (96.5%) (Fig. 3).

Effect of Inhibitors on Arachidonic Acid Metabolism in G. fruticosa

The effect of typical mammalian cyclooxygenase inhibitors (aspirin, indomethacin, and tolfenamic acid) was tested against the product formation in coral G. fruticosa. Usually, 0.5 mM SnCl2 was included in the incubation mixture. Experiments with indomethacin and tolfenamic acid indicated that the influence of SnCl2 on the enzyme inhibition was not considerable.

Tolftenamic acid was found to be the most potent inhibitor of prostaglandin synthesis among the drugs tested. The IC50 values were estimated to be 0.45 μM in the presence of the reducing agent and 0.65 μM when SnCl2 was omitted. 5 μM tolfenamic acid decreased the prostaglandin formation by 90% in both cases. The IC50 values for indomethacin were determined to be between 10 and 20 μM. Dose-response curves for inhibition of coral enzymes by tolfenamic acid and indomethacin are shown in Fig. 4. A and B, respectively. The irreversible cyclooxygenase inhibitor, aspirin, was found to inhibit the prostaglandin forming activity in coral with the IC50 values of 0.6 mm. As in case of mammalian cyclooxygenases, aspirin inhibited the coral enzyme in a time-dependent manner. For inhibition of the enzyme with 200 μM aspirin, the half-life (t1/2) was approximately 25 min; with 300 μM aspirin, t1/2 was about 10 min. The dose-response curves and the time course for inhibition of prostaglandin synthesis by aspirin are shown in Fig. 4.
C and D, respectively. Furthermore, NSAIDs blocked the (11R)-HETE synthesis in a dose-dependent manner to 50% of initial activity. The amount of (15S)-HETE was too low to determine whether this product was inhibited or not, but the accumulation of 15-HETE following aspirin treatment of the coral enzyme was not observed. The selective inhibitor of PGHS-2, nimesulide, was found to be a poor inhibitor of prostaglandin catalyzing enzyme in coral and did not affect the prostaglandin synthesis even at very high concentrations up to 1 mM. In side by side incubations, these high concentrations of nimesulide completely blocked the activity of mammalian PGHS-1 (ram seminal vesicle cyclooxygenase) for which it is a less potent inhibitor than it is for PGHS-2. The (8R)-lipoxygenase/allene oxide synthase pathway was not blocked by NSAIDs. Otherwise, the formation of 8-HETE even increased with increasing concentrations of NSAIDs (Fig. 4, A–C).

DISCUSSION

The results of this study show that the acetone powder of G. fruticosa converts exogenous arachidonic acid into a complex mixture of oxygenation products. One dominating group of metabolites is formed via the (8R)-lipoxygenase/allene oxide synthase and the other via the cyclooxygenase pathway. This is the first report of both biosynthetic capabilities in the same organism. Products of the (8R)-lipoxygenase pathway consist of (8R)-HPETE, 8-KETE, and α-ketol. When incubations were performed in the presence of a mild reducing agent, only (8R)-HETE was formed, confirming that 8-KETE and α-ketol are formed from 8-HPETE as a common hydroperoxide intermediate. Notably, although the co-incubation with a reducing agent blocked the allene oxide pathway (α-ketol synthesis), it did not eliminate the prostaglandin biosynthesis in the same incubations. We also isolated and identified 8-HETE as an endogenous component of G. fruticosa. Thus, G. fruticosa is different from P. homomalla and the other gorgonians where none of these lipoxygenase products have been detected in fresh coral extracts. Otherwise, the occurrence of 8-HETE and 8-HEPE in the other Gersemia species, Gersemia rubiformis, has been documented (24).

Unlike P. homomalla and all the other corals studied before, G. fruticosa has a unique ability to convert exogenous arachidonic acid into optically active prostaglandins in vitro. The typical mammalian prostaglandins, PGE2, PGD2, PGF2α, and 15-keto PGF2α, isolated and identified in fresh coral extracts, were also synthesized from labeled arachidonic acid in a high total yield of 19–25%. Neither PGA2 nor its derivatives were detected in the incubation mixtures or coral extracts. The esters of PGA2 are the most prominent prostaglandins in P. powder was preincubated at room temperature for 5 min with various amounts of inhibitors added in a few microliters of ethanol. An equal amount of ethanol was added to the control. The reaction was initiated with 0.3 μCi of [14C]arachidonic acid (final concentration, 50 μM). The incubation was carried out for 10 min, followed by treatment with SnCl2. The products were extracted with ethyl acetate and analyzed by TLC as described in Fig. 1. When the inhibition of intermediates was studied, SnCl2 (final concentration, 0.5 mM) was added into the incubation mixture. Values are means ± S. D., n = 3. ●, total PGs formed in incubations without the reducing agent; ■, PGA2; ▲, (11R)-HETE; ◇, (8R)-HETE formed in incubations with the reducing agent. Panel A, the effect of tolfenamic acid on the formation of PGs and HETEs. Panel B, the effect of indomethacin on the formation of PGs and HETEs. Panel C, the effect of aspirin on the formation of PGs and HETEs. Panel D, time course for inhibition of the PG formation by aspirin. The coral acetone powder was incubated at room temperature with or without aspirin. At the indicated moments, aliquots were removed and assayed for the PG formation. The residual PG forming activity is presented as a percentage of control (no aspirin) for each time point; ○, 200 μM aspirin; □, 300 μM aspirin.

FIG. 4. Effect of tolfenamic acid, indomethacin, and aspirin on the arachidonic acid metabolism in G. fruticosa. The coral acetone
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The highest concentrations of nimesulide (1 mM) were employed. which could still be a factor in the crude coral preparations to the protein binding of the inhibitors in our experiments, those reported for purified mammalian cyclooxygenases. This means that arachidonic acid is transformed to diHETE by a subsequent attack of these two lipoxygenases. This scheme is supported by the finding that indomethacin and tolfenamic acid did not affect the formation of diHETE, thus excluding the involvement of the PGHS enzyme in the oxygenation at C-15. It was shown that 15-HPETE and 15-HETE are poor substrates for the P. homomalla (8R)-lipoxygenase (37). Thus, a plausible version is that the (8R)-HETE accumulated in high concentration in the presence of SnCl₂ is further oxidized by the (15S)-lipoxygenase to give the diHETE product. Alternatively, 8,15-diHETE could be formed via the double oxygenation of arachidonic acid by (8R)-lipoxygenase. This is analogous to reactions of soybean lipoxygenase (38), except that in this case both hydroxy groups would have the R-stereocchemistry. The occurrence of several lipoxygenase activities with different biological functions has been reported in various marine invertebrates. P. homomalla is known to synthesize minor 11-HETE (10), but no 15-HETE formation has been found in this coral. A low 15-HETE synthesizing activity has been detected in Pseudoplexaura porosa in addition to its main (8R)-lipoxygenase activity (39).

In summary, we conclude that two distinct fatty acid metabolic routes exist in coral, i.e. both lipoxygenase and cyclooxygenase enzymes are involved in the oxidative metabolism of arachidonic acid. Prostaglandin biosynthesis proceeds similarly to that of mammals via the cyclooxygenase pathway, except that the coral enzyme exhibits very low peroxidase activity. The characterization of the coral enzyme is in progress; in polymerase chain reaction experiments with the coral cDNA using primers based on highly conserved mammalian PGHS sequences, we have detected a PGHS-related sequence with about 50% amino acid identity to each mammalian PGHS-1 and PGHS-2, confirming the existence of an enzyme related to mammalian cyclooxygenases. The allene oxide pathway is not involved in the formation of coral prostaglandins. Nonetheless, there remains the possibility that some of the prostanooids found in other species of coral such as clavulones of Clavularia viridis, could be formed via the lipoxygenase/allene oxide pathway. There remain many interesting possibilities for further study of biosynthetic pathways from arachidonic acid to unusual cyclic oxylipins in coral.

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REFERENCES

1. Gerwick, W. H., Nagle, D. G., and Proteau, P. J. (1993) Top. Curr. Chem. 167, 117–180
2. Weinheimer, A. J., and Spraggins, R. L. (1969) Tetrahedron Lett. 59, 5185–5188
3. Bundy, G. L. (1985) Adv. Prostaglandin Thromboxane Leukotriene Res. 14, 229–262
4. Corey, E. J., Nacul, H. E., Hamberg, M., and Samuelsson, B. (1980) J. Chem. Soc. Chem. Commun. 277–278
5. Corey, E. J., and Matsuda, S. P. T. (1987) Tetrahedron Lett. 28, 4247–4250
6. Brash, A. R., Baertschi, S. W., Ingram, C. D., and Harris, T. M. (1987) J. Biol. Chem. 262, 15829–15839
7. Corey, E. J., Alarcao, M., Matsuda, S. P. T., Lansbury, P. T., and Yamada, Y. (1987) J. Am. Chem. Soc. 109, 289–290
8. Corey, E. J., Matsuda, S. P. T., Nagata, R., and Cleaver, M. (1988) Tetrahedron Lett. 29, 2555–2558
9. Brash, A. R. (1989) J. Am. Chem. Soc. 111, 1891–1892
10. Brash, A. R., Baertschi, S. W., Ingram, C. D., and Harris, T. M. (1989) Adv. Prostaglandin Thromboxane Leukotriene Res. 19, 70–73
11. Hamberg, M., and Gardner, W. (1992) Biochim. Biophys. Acta 1165, 1–18
12. Varvas, K., Jarving, L., Koljak, R., Vahemets, A., Pekh, T., Muirisepp, A.-M., Lille, U., and Samel, N. (1993) Tetrahedron Lett. 34, 3643–3646
13. Varvas, K., Koljak, R., Jarving, L., Pekh, T., and Samel, N. (1994) Tetrahedron Lett. 35, 8267–8270
14. Smith, W. L., and Marnett, L. J. (1991) Biochim. Biophys. Acta 1083, 1–17
15. Smith, W. L., Garavito, R. M., and DeWitt, D. L. (1996) J. Biol. Chem. 271, 33157–33160
16. Meade, E. A., Smith, W. L., and DeWitt, D. L. (1993) J. Biol. Chem. 268, 6610–6614
17. Giesser, K., McDonald, J. J., Hauser, S. D., Rangwala, S. H., Koboldt, C. M., and Seibert, K. (1996) J. Biol. Chem. 271, 15810–15814
18. Guo, Q., Wang, L.-H., Ruan, K.-H., and Kulmacz, R. J. (1996) J. Biol. Chem. 271, 19134–19139
19. Panossian, A. G. (1987) Prostaglandins 33, 363–381
20. Beld, G. S., Bhat, S. G., Ramadoss, C. S., and Axelrod, B. (1978) J. Biol. Chem. 253, 21–23
21. Peers, K. E., and Coxon, D. T. (1983) Chem. Phys. Lipids 32, 49–56
22. Kiefer, H. C., Johnson, C. R., and Arora, K. L. (1975) Anal. Biochem. 68, 336–340
23. Brash, A. R., and Hawkins, D. J. (1990) Methods Enzymol. 187, 187–195
24. Latshev, N. A., Malyutin, A. N., Koteyv, L. S., and Bezuglov, V. V. (1988) Chem. Nat. Prod. 3, 447–448
25. Light, R. J., and Samuelsson, B. (1972) Eur. J. Biochem. 28, 232
26. Grewiss, A., and Fenical, W. (1990) Nat. Prog. 53, 222–223
27. Hamberg, M., and Hamberg, G. (1980) Biochem. Biophys. Res. Commun. 95, 1090–1097
28. Hawkins, D. J., and Brash, A. R. (1987) J. Biol. Chem. 262, 7629–7634
29. Hamberg, M., and Samuelsson, B. (1967) J. Biol. Chem. 242, 5329–5335
30. Bryant, R. W., Bailey, J. M., Schewe, T., and Rapoport, S. M. (1982) J. Biol. Chem. 257, 6050–6055
31. Hemler, M. E., Crawford, C. G., and Lands, W. E. M. (1978) Biochemistry 17, 1772–1779
32. Hubbard, W. M., Hough, A. J., Brash, A. R., Watson, J. T., and Oates, J. A. (1980) Prostaglandins 20, 431–447
33. Powell, W. S. (1992) J. Biol. Chem. 237, 9457–9464
34. Yamaja Setty, B. N., Stuart, M. J., and Walenga, W. (1985) Biochim. Biophys. Acta 833, 484–494
35. Di Marzo, V., Petrocellis, L., Gianfrani, C., and Cimino, G. (1993) Biochem. J. 295, 23–29
36. Mancini, J. A., O’Neill, G. P., Bayly, C., and Vickers, P. J. (1994) FEBS Lett. 342, 33–37
37. Song, W.-C., and Brash, A. R. (1991) Arch. Biochem. Biophys. 290, 427–435
38. van Os, C. P. A., Rijke-Schilder, G. P. M., van Halbeek, H., Verhagen, J., and Vliegenthart, J. F. G. (1981) Biochim. Biophys. Acta 663, 177–193
39. Bundy, G. L., Nidy, E. G., Epps, D. E., Mizsak, S. A., and Wnuk, R. J. (1986) J. Biol. Chem. 261, 747–751

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