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TOTAL SYNTHESIS OF 12-(S)-10-HYDROXY-TRANS-11,12-EPOXYEICOSA-5,9,14-(Z)-TRIENOIC ACIDS, METABOLITES OF ARACHIDONIC ACID IN MAMMALIAN BLOOD PLATELETS

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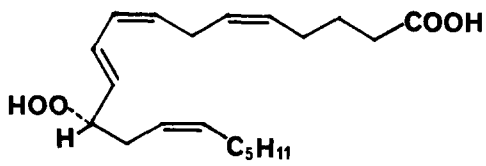
Summary: The two isomeric hydroxy-11,12-epoxides produced from arachidonic acid in mammalian blood platelets have been identified by comparison with totally synthetic substances as the C(10)-diastereomers of 2.

Blood platelets play a crucial role in hemostasis and in many circulatory disorders. Their activity is regulated in part by metabolites of arachidonic acid, the most carefully studied of which are those of the cyclooxygenase pathway especially thromboxane A_2 and prostaglandins (PG's) E_2, $F_{2\alpha}$, and H_2. Although 12-lipoxygenation (12-LO) is also an important pathway for arachidonate oxidation in platelets, little is known of the biochemistry or biological effects of the substances of this family. The physiological actions of the primary metabolite 12-(S)-hydroperoxy-5,8,14-(Z)-,10-(E)-eicosatetraenoic acid (12-HPETE) (1) and the corresponding alcohol (12-HETE),[1] are at present obscure, although 1 has been reported to stimulate leukotriene biosynthesis in human blood leukocytes.[2] Gas-chromatographic-mass spectrometric (GC-MS) analysis has provided evidence for the formation from arachidonate (presumably via 1) in blood platelets of two isomeric 10-hydroxy-11,12-epoxyeicosatrienoic acids and a number of trihydroxyeicosatrienoic acids,[4] but these substances have not been thoroughly characterized. In this note we report the total synthesis of the two C(10) diastereomers of 12-(S)-10-hydroxy-trans-11,12-epoxyeicosa-5,9,14-(Z)-trienoic acid (2) and their correlation with the native epoxy acids from mammalian platelets. The ready availability of the synthetic substances should expedite the study of the biological significance of these epoxides and their biochemical progeny.[5] Another possible oxirane metabolite of 12-HPETE has been produced by total synthesis,[6] but so far has not been detected in biological systems, i.e., 11,12-EPETE,[6] the 11,12-analog of leukotriene A_4.



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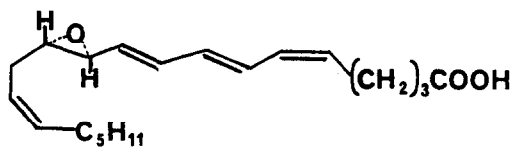
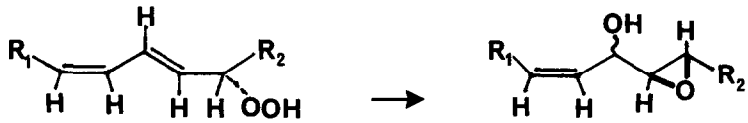


11,12-EPETE

The choice of the 12-(S)-trans-epoxide 2 as the target for synthesis was made on the assumption that the isomeric platelet epoxides originate from 12-HPETE by the known lipoxygenase-catalyzed isomerization of trans allylic hydroperoxides to trans epoxy alcohols[7] according to the following scheme:

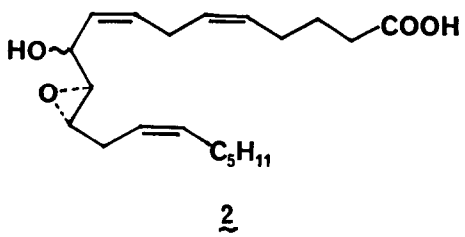


The synthesis of 2 was carried out starting with 5,8-nonadiynoic acid[8] which was transformed into cis-5-nonen-8-ynoic acid (3) by the sequence: (1) reaction with 2 equiv. of ethylmagnesium bromide in ether at 0° followed by treatment with a solution of iodine (2 equiv.) in tetrahydrofuran (THF) at -78° for 3 hr. to give 9-iodo-5,8-nonadiynoic acid (100%);[9] (2) reaction with 4 equiv. of disiamylborane in THF at 0° for 1 hr. and 20° for 4 hr. followed by dilution with one-half volume of acetic acid and stirring at 0° for 8 hr. to afford 80% of 9-iodo-5,8-nonadienoic acid; and (3) reaction with 4 equiv. of lithium diisopropylamide in THF at -78° for 3 hr. to give 80% of 3. The 9-lithio derivative of lithium cis-5-nonen-8-ynoate was prepared in THF-hexane from 3 by reaction with 2 equiv. of n-butyllithium at -45° for 1 hr. and treated with a solution of 2-(R),3-(S)-epoxy-cis-5-undecenal (4)[10] (1 equiv.) in THF at -78° for 1.5 hr. Extractive isolation, treatment of the crude product with ethereal diazomethane and chromatography on silica gel afforded 83% of a mixture of two C(10) diastereomers of 8,9-dehydro 2 methyl ester, hydrogenation of which (1 atm. H_2, Lindlar catalyst, THF-triethylamine) afforded cleanly (>95%) two C(10) diastereomers of 2 methyl ester.[11] These were easily separated by HPLC on a Waters Assoc. μ-Porasil column (30 x 0.4 cm.) using 0.5% iso-propyl alcohol-hexane (retention vols. 29.4 and 38.4 min.).

These synthetic hydroxy esters were compared with the methyl esters of the two isomeric hydroxy epoxides formed from arachidonate in washed blood platelets (horse). Each of the synthetic C(10) diastereomers was identical with one of the naturally derived isomers by HPLC, thin layer chromatography, and GC-MS, the GC-MS comparisons being made on both the trimethylsilyl (TMS) ether

methyl ester and t-butyldimethylsilyl (TBDMS) ether methyl ester derivatives. The HPLC comparisons were carried out using a Partisil PAC column (normal phase), the naturally derived material being detected by ^{3}H counting and the synthetic diastereomers of 2 methyl ester by GC-MS single ion detection (m/e 269). The native and synthetic diastereomers of 2 methyl ester showed tlc R_f values of 0.49 and 0.52 using silica gel plates with 3:1 ether-hexane for elution. The GC retention times and mass spectra of the natural and synthetic TMS ether-methyl esters of 2 were identical for all four samples (M^+ 422, base peak = 269). The diastereomeric TBDMS ether-methyl esters of 2 (both natural and synthetic) were resolved by GC (17.6 and 17.3 min. elution times at 230 °C) and showed no M^+ but peaks at m/e = 407, 324, 311, 267 and 221.

In view of the correspondence of synthetic and native samples in the above physical measurements we conclude that the two arachidonate derived C_{20} hydroxy epoxides from blood platelets are the two C(10) diastereomers of 2, which are now easily available by synthesis.[12]



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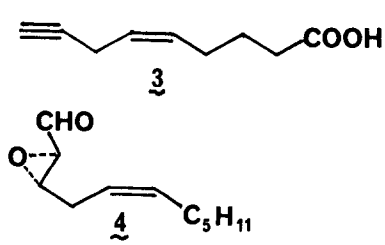

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References and Notes

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9. Satisfactory proton magnetic resonance, infrared and mass spectrometric data were obtained for each synthetic product.

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11. The 5,6- , 8,9-diacetylene corresponding to 2 methyl ester was also synthesized, but it was found that Lindlar reduction of this intermediate was difficult to control.

12. This research was assisted financially by grants from the National Science Foundation and the National Institutes of Health.

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