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(8-*R*)- AND (8-*S*)-HEPOXILIN A_3. ASSIGNMENT OF CONFIGURATION AND CONVERSION TO BIOLOGICALLY ACTIVE CONJUGATES WITH GLUTATHIONE.

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Summary: The determination of absolute configuration of (8-*R*)-hepoxilin A_3 (**2**) (more polar methyl ester) and (8-*S*)-hepoxilin A_3 (**3**) (less polar methyl ester) is reported along with the synthesis of the biologically active 11-*R* glutathione thiol conjugates **4** and **5** which may function as important neuromodulators.

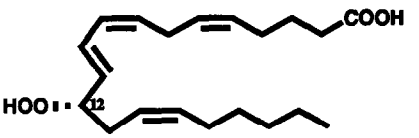
Arachidonic acid, the precursor of prostanoids (11-lipoxygenation), leukotrienes (5-lipoxygenation), and lipoxins (5- and 15-lipoxygenation), is converted in many kinds of cells and tissues to a number of more oxygenated compounds by an initial 12-lipoxygenation (12-LO) step which forms (12-*S*)-hydroperoxyeicosa-5,8,14(*Z*)-10(*E*)-tetraenoic acid (12-*S*-HPETE) (**1**). Among the most interesting of these products of the 12-LO pathway are the C(8)-diastereomeric 11,12-epoxides **2** and **3** which have been called hepoxilin A_3 (HxA_3). The 8-epimers of HxA_3 have been found to enhance the release of insulin from rat pancreatic islets,[2] to modulate synaptic neurotransmission,[3] and to raise cytosolic Ca^{++} levels in human neutrophils.[4] In addition HxA_3 is formed by *Aplysia* neurons and causes slow hyperpolarization.[5] A total synthesis of the two 8-epimers of HxA_3, which has greatly facilitated biological research, was described by us in 1984,[6] but configurations at C(8) were not assigned. In this note we report the clarification of C(8) stereochemistry in the two epimers of HxA_3 and also the synthesis of the corresponding 11-*R* glutathione thiol conjugates (**4** and **5**). The synthetic conjugates have already been utilized to demonstrate that these compounds are produced in homogenates of rat brain hippocamus and that they cause membrane hyperpolarization and changes in postsynaptic potential and spike threshold in rat hippocampal CA1 neurons at concentrations as low as 20 nM.[7] The ready availability of synthetic **4** and **5** will accelerate studies on the biological roles of these new eicosanoids, both as neuromodulators and cell regulators.

The two C(8) epimeric HxA_3 methyl esters were synthesized as previously described[6] and separated by chromatography on silica gel[6] to afford the more polar epimer (R_f 0.33 using 85 : 15 benzene-ether containing 1% triethylamine and three developments) and the less polar epimer (R_f 0.38, same system). Acetylation of each methyl ester using acetic anhydride-pyridine-4-*N*,*N*-dimethylaminopyridine in dichloromethane at 23°C for 1 h afforded the corresponding acetate in > 99% yield. Treatment of each

acetoxy methyl ester with excess ozone in dichloromethane at -78°C for 5 min and reduction at -78°C with a solution of sodium borohydride in ethanol afforded a mixture of alcohols which was acetylated as described above. Thin layer chromatographic separation afforded from each epimer a triacetate of butan-1,2,4-triol (> 95% yield) which was then analyzed to determine absolute configuration by high performance liquid chromatography (HPLC) using a Daicel 4.6 x 250 mm OD chiral column[8] with 95 : 5 hexane-isopropyl alcohol as eluant, a flow rate of 1.0 ml/min, and ultraviolet detection at 215 nm. Under these conditions the measured retention times for authentic samples of (2*R*)- and (2*S*)-butan-1,2,4-triol triacetates (prepared by acetylation of the corresponding triols which were purchased from Aldrich Chemical Co.) were found to be 10.5 and 12.6 min, respectively. Since the more polar hepoxilin A_3 methyl ester afforded only triacetate of retention time 10.5 min and the less polar hepoxilin A_3 methyl ester gave triacetate of retention time 12.6 min, these HxA_3 methyl esters must be **2** and **3**, respectively. The determination of the complete stereochemistry of **2** and **3** will be helpful in further research on the hepoxilin pathway.

The biosynthesis of the peptidic leukotriene LTC_4 from LTA_4 by enzymic coupling to the thiol function of glutathione[9] and the important biological activity of LTC_4 suggested that the synthesis and study of analogous conjugates of HxA_3 with glutathione might be fruitful. Toward this end displacement reactions of the methyl esters of **2** and **3** with a glutathione derivative were investigated. The conversion of **2** and **3** separately to the conjugates **4** and **5**, respectively, was achieved by the following sequence: (1) reaction of 2.0 equivalents of *N*-trifluoroacetylglutathione dimethyl ester with either methyl ester **2** or **3** in 4 : 1 methanol-triethylamine at 80°C for 24 h under an atmosphere of argon to give a mixture of the C(11) (*R*) thioether conjugate (a single isomer) and two (*R* and *S*) C(9) thioether conjugates (80% yield) in a ratio of 1 : 1.5, respectively; (2) chromatographic separation of the C(11) and C(9) thioether conjugates using preparative thin layer chromatography on silica gel plates with 45 : 45 : 10 ethyl acetate-hexane-methanol for development (R_f values 0.37 for the C(11) and 0.44 and 0.46 for the C(9) conjugates, respectively);[10] and (3) saponification with 0.15 M potassium carbonate in 3 : 1 water-methanol at 23°C for 24 h under argon in the dark, separately for the C(11) and C(9) thioether conjugates. In this way the pure C(11) (*R*) thioether **4** was obtained from **2** and the diastereomer **5** was prepared from **3**.[11] Similarly the C(9) glutathione conjugates with **2** and **3**, thioethers **6** and **7** respectively, were obtained separately as a mixture of C(9) epimers.

Structural assignment to the C(11) and C(9) conjugates was possible by ^1H NMR spectroscopy of the 8-deuterated conjugates. These were synthesized from 8-deuterated **2** and **3**, which in turn were prepared by reduction of the corresponding 8-ketones[6] with sodium borodeuteride. In the ^1H NMR spectra of the *N*-trifluoroacetyl trimethyl ester derivatives of 8-deutero **6** and 8-deutero **7**, the C(12) carbinyl proton (C*H*OH) occurs at 4.61 δ and is obviously allylic, whereas in the corresponding derivatives of 8-deutero **4**



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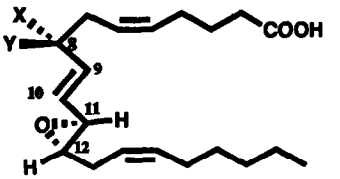
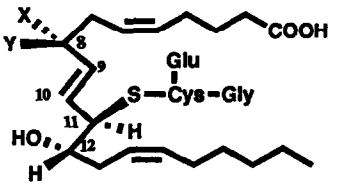


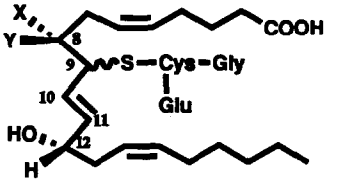
2 X = OH, Y = H (8*R*)

3 X = H, Y = OH (8*S*)



4 X = OH, Y = H

5 X = H, Y = OH



6 X = OH, Y = H

7 X = H, Y = OH

and **5**, the C(12) carbinyl proton is clearly not allylic and is considerably upfield (3.7 - 3.8 δ). The proton of the -C*H*-S-glutathione subunit is allylic in each case and is located at *ca.* 4.5 ppm. Desulfurization of the *N*-trifluoroacetyl trimethyl ester derivatives of 8-deuterated **6** and **7** (Raney Ni in ethanol at 23°C) occurs without migration of the 10,11-double bond to give a 5,10,14-triene in which the C(12) carbinyl proton shows an NMR peak at 4.15 δ characteristic of an allylic carbinyl proton. Desulfurization of the *N*-trifluoroacetyl trimethyl ester derivatives of 8-deuterated **4** and **5** also proceeds without transposition of the allylic double bond to form a 5,9,14-triene in which the C(12) carbinyl proton shows an NMR peak at 3.78 δ, as expected for a non-allylic alcohol. This demonstration that desulfurization using Raney nickel in ethanol occurs without double bond transposition is an interesting result of these structural studies.

Hepoxilin A_3 glutathione conjugates **4** and **5** are produced when the precursor hepoxilins **2** and **3** are incubated with homogenates of rat brain hippocamus,[7] as indicated in the introduction. Studies are underway to provide more detail with regard to the biosynthesis and biological activity of these interesting new eicosanoids in other tissues and systems.[12]

References and Notes

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9. See "The Leukotrienes, Chemistry and Biology," L. W. Chakrin and D. M. Bailey. (Academic Press, New York, 1984), pp. 13-101.
10. The following HPLC retention times were determined for the products of coupling of the methyl esters of **2** or **3** with *N*-trifluoroacetylglutathione dimethyl ester using a DuPont C_{18} Zorbax ODS column (4.6 x 250 mm) with UV detection at 215 nm: (1) from **2** (75 : 25 : 0.01 CH_3OH-H_2O-HOAc as eluant): C(11) conjugate 13 min; C(9) conjugates (9*R* + 9*S*) 14.5, 15.5 min. (2) from **3** (70 : 30 : 0.01 CH_3OH-H_2O-HOAc as eluant): C(11) conjugate 30 min; C(9) conjugates (9*R* + 9*S*) 34.4, 37.2 min.
11. The following HPLC data were obtained for **4** and **5** under the above conditions[10] with 70 : 30 : 0.01 CH_3OH-H_2O-HOAc as eluant: for **4**, 10 min (position isomeric C(9) conjugates **6** (9*R* + 9*S*), 6.0 and 9.8 min); for **5**, 12.8 min (position isomeric C(9) conjugates **7** (9*R* + 9*S*), 7.2 and 9.8 min).

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