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BIOMIMETIC SYNTHESIS OF LEUKOTRIENE A

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Summary - A biogenetically patterned conversion of 1 into 2 is described. This transformation has been found to be non-stereospecific with respect to the geometry of the newly generated double bond at C-9(10).

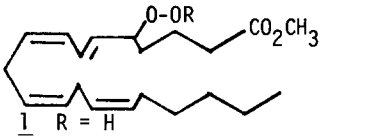
Leukotriene A (LTA), 5(S)-trans-5,6-oxido-7,9-E-11,14-Z-eicosatetraenoic acid[1] was shown to be an unstable pivotal biosynthetic precursor of leukotriene B[2] (LTB), 5(S),12-(R)-dihydroxy-6,14-Z-8,10-E-eicosatetraenoic acid[3] and the slow reacting substances[4] [SRS-GSH (LTC), SRS-Cys-Gly (LTD) and SRS-Cys (LTE)]. In turn, LTA[5] is enzymically derived from arachidonic acid via the intermediate, 5(S)-hydroperoxy-6-E-8,11,14-Z-eicosatetraenoic acid [(S)-5-HPETE].

Recently, we developed a biomimetic conversion[6] of (±)5-HPETE methyl ester[7] (1) into (±)LTA methyl ester (2), a key chemical intermediate in the synthesis[1,8] of slow reacting substances. This method entailed the conversion of 1 to its mesylate, 3 and the selective abstraction of a C-10 proton by a hindered base to afford 2. This transformation has not only allowed the preparation of 2 from arachidonic acid via a relatively short reaction sequence, but also has provided a useful chemical model for further mechanistic study. We now report on the stereochemical fidelity of this interesting chemical transformation, which formally may be construed as a 1,7-elimination process.

Reaction of 1 (0.7 mmol) in CH_2Cl_2 (12 ml) with methanesulfonyl chloride (0.75 mmol) and dicyclohexylmethylamine (DCMA) (2.8 mmol) at -78°C for 1 hr afforded besides some polar products and 4 (27 mg) [UV (hexane) 276 (ε 23,000], two isomeric epoxides, which were separated by HPLC[9]. The less polar epoxide (20 mg) was identical [UV (268.5, 279.5, 292), pmr, m.s. retention time on HPLC] to an authentic sample of 2[8b]. Reaction of 2 with glutathione and triethylamine in methanol[1] followed by ester cleavage gave two sulfur linked glutathione conjugates, which were separated by HPLC[10]. Both 5(S)-hydroxy-6(R)-glutathionyl-7,9-E-11,14-Z-eicosatetraenoic acid (LTC) and 5R,6S-LTC exhibited UV maxima at 280 nm (ε 40,000) with shoulders at 270 and 290 nm. The more polar epoxide (46 mg) showed UV maxima at 269 (s), 279.5 (ε 40,000) and 291 (s)[11]. Its physical properties (HPLC and pmr) were in good agreement with the 7E,9,11,14Z isomer[12a] (5). Furthermore, 5 rearranged at room temperature via a 1,7-hydrogen shift to the known tetraene isomer, 6 [UV 280 (s), 291, 304, 318.5 nm)[12a,b]; m/e 332 (M^+), 301 ($M-OCH_3$), 231 [$M-(CH_2)_3CO_2CH_3$], 189 [$M-\overset{O}{\overset{\|}{C}}H\overset{O}{\overset{\|}{C}}H(CH_2)_3CO_2CH_3$], 149, 143, 131, 101. Treatment of 5 with GSH and triethylamine in methanol followed by ester cleavage gave 9-Z-LTC and 5R,6S-9-Z-LTC, which were separated by HPLC[10]. Both products exhibit UV maxima at 280 nm (ε 40,000)[13] and readily undergo a 1,7-hydrogen migration at room temperature to give their corresponding tetraene isomers [280 (s), 292, 306, 322 nm].

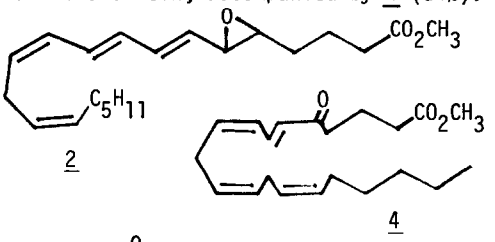
In contrast to published observations[14], our results show that the biomimetic conversion of

3 to 2 via this 1,7-elimination process is not stereospecific with respect to the configuration of the newly generated double bond at C-9(10). We repeated this conversion using the triflate, 7 and 1,2,2,6,6-pentamethylpiperidine (PMP) at -110°C according to the reported procedure[14] and again observed a mixture of 2 and 5 (29% yield) in a ratio of 1:2, accompanied by 4 (14%).

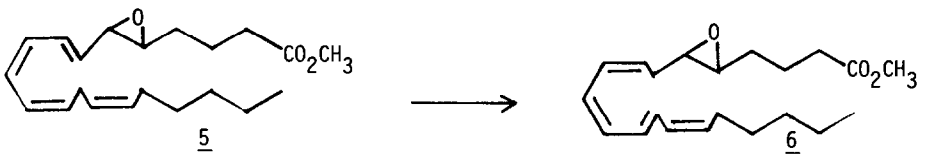


1 R = H

3 R = SO_2CH_3 7 R = SO_2CF_3



2 4



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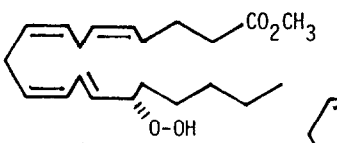
A series of experiments were conducted with a view to securing a more favorable ratio of 2 to 5 and to reducing the amount of 4 formed. The results of Table 1 indicate that the stereochemical outcome of this elimination process may be influenced to a considerable degree by the choice of leaving group and solvent. Even after numerous attempts, we have been unsuccessful in transforming 1 into 2 exclusively. However, we found that by carrying out the reaction at a higher dilution using CH_2Cl_2-ether as solvent, the ratio of 2 to 5 may be raised to ca. 1:1 and formation of 4 reduced considerably (entry 5). It is noteworthy that in all these experiments, 5,6-cis-oxido-7,9-E-11,14-Z-eicosatetraenoic acid methyl ester [5(6)-cis-LTA methyl ester][8b] was not detected in the reaction mixtures.

Four new dihydroxy acids were recently isolated from a human leukocyte preparation after incubation with arachidonic acid.[15] They were characterized as 14,15-dihydroxy-5,8,10,12-eicosatetraenoic acid (2 isomers) and 8,15-dihydroxy-5,9,11,13-eicosatetraenoic acid (2 isomers). It was proposed that these compounds originated from 14,15-oxido-5,8,10,12-eicosatetraenoic acid (14,15-LTA), which in turn was derived from 15-hydroperoxyeicosatetraenoic acid (15-HPETE), via an elimination process analogous to the biosynthesis of LTA from 5-HPETE.



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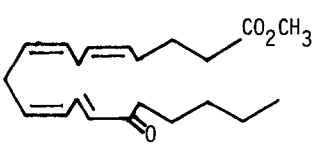

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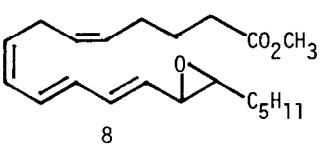

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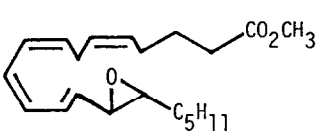
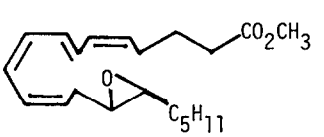


11



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Because 14,15-LTA could be in principle a precursor to another important family of physiologically-active sulfur-linked peptide conjugates, a similar synthesis of 8 from 9[16] was reported.[17] These authors claimed that 9 was transformed stereospecifically in 40% yield into

Table 1. Effect of solvent and leaving group on product distribution

Entry	Method[a]	Solvent	Products (yield %)[b] 4	2	5
1	A	CH_2Cl_2	12	8	20
2	A	ether	22	3	3
3	A	CH_2Cl_2/ether (1:1)	17	7	10
4	B	CH_2Cl_2/ether (1:1)	14	10	19
5	B[c]	CH_2Cl_2/ether (1:1)	7	13	15
6	B[c,d]	CH_2Cl_2	8	6	24
7	B[c]	ether	10	7	14

[a]Method A: the reaction mixture contained 0.03 mmol of 1, MeSCl (0.033 mmol), DCMA (0.12 mmol) in 1 ml of solvent at -78°C; Method B: the contents were 0.03 mol of 1, 0.06 mmol of $(CF_3SO_2)_2O$ and 0.18 mmol of PMP in 0.2 ml of solvent at -110°C according to reference 16. [b]Yields of 2 and 5 were estimated from their UV (λ_{max} = 280 nm, ε = 40,000) and 4 was measured from UV 276 nm assuming ε = 23,000. [c]0.15 mmol of PMP and 1 ml of solvent were used. [d]Reaction conducted at -78°C instead of -110°C.

8, accompanied by 10.[17] However, under the same reaction conditions we have found that 9 gave a mixture of two isomeric epoxides 8 and 11 which were easily separated by HPLC[18]. The 5,8,10Z-12E isomer[19], 11, (23%) UV_{max} 268 (s), 279 (ε 40,000), 291.5 nm, was formed preferentially to the 5,8Z-10,12E isomer, 8, (8%) UV_{max} 269 (s), 279.5 (ε 40,000), 290 nm, consistent with the stereochemical result we observed for the analogous transformation of 1. The former epoxide, 11 may also be readily distinguished from 8 by its characteristic pmr pattern[12a] in the olefinic region and its rearrangement to the tetraene, 12. The 15-ketone, 10 (35%) UV_{max} 274 nm (ε 23,000), arising from simple 1,2-elimination was also formed in substantial quantity.

The availability of pure 8 and 11 from arachidonic acid not only will allow us to prepare the respective C-14 sulfur-linked peptide conjugates but also to define the stereochemistry of 14,15-LTA of natural origin. These investigations along with further refinement of this biomimetic reaction to favor the formation of 8 are currently in progress.

Acknowledgments. We are indebted to Drs. M. Rosenberger and W. E. Scott of Hoffmann LaRoche for the authentic specimens of (±)LTA and (±)5(6)-cis-LTA as their methyl esters, and arachidonic acid ethyl ester, respectively. Supported in part by grant AM 09688 from the NIH.

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9. The HPLC separation was carried out using a 50 cm x 9.4 mm I.D. 10μ porasil column (Alltech) eluted with ethyl acetate:hexane:triethylamine (0.7:100:0.7) at a flow rate of 3.5 ml/min. The retention times of 4, 2 and 5 were 52, 56 and 58 min respectively. The isomer ratios (2 and 5) were estimated from their UV (λ_{max} = 280 nm, ε 40,000), and 4 was measured from UV 276 nm assuming ε 23,000.
10. This HPLC separation was carried out using a 25 cm x 4.6 mm I.D. 5 μ Nucleosil C_{18} column (Alltech) eluted with methanol:water (60:40) containing 0.01% acetic acid, pH 4.3 at a flow rate of 1 ml/min. The retention times of LTC and 5R,6S-LTC were 42 and 51 min respectively; 9-Z-LTC and 5R,6S-9-Z-LTC have retention times of 50 and 54 min respectively.
11. Although reference 1 reported the λ_{max} for the 7E,9,11,14Z isomer 5 as 266, 276, 286 nm, it was suggested by reference 12a that this compound was the 7E,9Z,11E,14Z isomer.
12. This rearrangement is of the type reported by Rokach and is characteristic of the Z,Z,E-conjugated triene unit. (a) S. R. Baker, W. B. Jamieson, S. W. McKay, S. E. Morgan, D. M. Rackham, W. J. Ross, and P. R. Shrubsall, Tetrahedron Lett., 21, 4123(1980); (b) J. Rokach, Y. Girard, Y. Guindon, J. G. Atkinson, M. Larue, R. N. Young, P. Masson, and G. Holme, Tetrahedron Lett., 21, 1485(1980).
13. Reference 1 reported the UV λ_{max} for the presumed 9-Z-LTC as 277 nm whereas our data and those of reference 12a suggest that this compound was 9-Z-11-E-LTC.
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18. The mixture was separated on an Alltech μporasil (10μ) column (50 cm x 9.4 mm I.D.) using ethyl acetate:hexane:triethylamine (0.6:100:0.6) as eluent at a flow rate of 3.5 ml/min. The retention times for 8, 11 and 10 were 39, 43.5 and 48 min respectively.
19. pmr (90 MHz) data for 11 (δ $CDCl_3$ + trace C_5D_5N): 0.89 (t, CH_2CH_3), 1.25-1.68 (m, CH_2), 2.05 (m, =C-CH_2), 2.32 (t, $CH_2CO_2CH_3$), 2.8 (m, CH-O-CH-CH_2, =C-CH_2-C=), 3.15 (m, CH-O-CH-CH_2), 3.66 (s, OCH_3), 5.38 (m, CH_2-CH=CH-CH_2), 5.8-6.55 [m, $(-CH=CH)_3$]; m/e 332 (M^+), 301 (M-OCH_3), 276 (M-$CH_2CH=CHCH_2CH_3$), 261 [M-$(CH_2)_4CH_3$], 233, 231 [M-HOCH$(CH_2)_4CH_3$], 219 [M-CH-O-CH$(CH_2)_4CH_3$], 101, 99, 71; 8 pmr (δ $CDCl_3$ + trace C_5D_5N): 0.89 (t, CH_2CH_3), 1.25-1.68 (m, CH_2), 2.05 (m, =C-CH_2), 2.32 (t, J = 7 Hz, CH_2CO_2), 2.8 (m, CH-O-CH-CH_2, =CCH_2C=), 3.15 (m, CH-O-CH-CH_2), 3.66 (s, OCH_3), 5.38 (m, CH_2CH=CH-CH_2), 5.80-6.55 [m, (-CH=CH-)$_3$]; m/e 332 (M^+), 301 (M-OCH_3), 276 (M-CH_2=CH$(CH_2)_3CH_3^{+\bullet}$), 261 [M-$(CH_2)_4CH_3$], 219 [M-CH-O-CH$(CH_2)_4CH_3$], 71, 56; 10 pmr (δ $CDCl_3$): 2.01 (m, =C-CH_2), 2.32 (t, CH_2CO_2), 2.56 (t, $COCH_2$), 2.82 (t, C-7 CH_2), 3.08 (t, C-10 CH_2), 3.66 (s, OCH_3), 5.39 (m, C-5, C-6, C-8, C-9 -CH=CH-), 5.9-6.27 (m, C-14, C-12, C-11 -CH=CH-), 7.52 (dd, J = 16 Hz, C-13 -CH=CH-).

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