Reactivity of Lysine Moieties toward an Epoxyhydroxylinoleic Acid Derivative: Aminolysis versus Hydrolysis

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Epoxyols are generally accepted as crucial intermediates in lipid oxidation. The reactivity of *tert*-butyl (9*R**,10*S**,11*E*,13*S*)-9,10-epoxy-13-hydroxy-11-octadecenoate (**11a,b**) toward lysine moieties is investigated, employing N^2-acetyllysine 4-methylcoumar-7-ylamide (**12**) as a model for protein-bound lysine. The prefixes *R** and *S** denote the relative configuration at the respective stereogenic centers. Independent synthesis and unequivocal structural characterization are reported for **11a,b**, its precursors, and *tert*-butyl (9*R**,10*R**,11*E*,13*S*)-10-({5-(acetylamino)-6-[(4-methyl-2-oxo-2*H*-chromen-7-yl)amino]-6-oxohexyl}amino)-9,13-dihydroxy-11-octadecenoate (**13a**−**d**). Reactions of **11a,b** and **12** in 1-methyl-2-pyrrolidone (MP) and MP/water mixtures at pH 7.4 and 37 °C for 56 days show formation of the aminols **13a**−**d** to be favored by an increased water content. The same trend is observed for hydrolytic cleavage of **11a,b** to *tert*-butyl (*E*)-9,10,13-trihydroxy-11-octadecenoate (**14**) and *tert*-butyl (*E*)-9,12,13-trihydroxy-10-octadecenoate (**15**). Under the given conditions, aminolysis proceeds via an S_N2 substitution, in contrast with the S_N1 process for hydrolysis. In the MP/water (8:2) incubation, 15.8% of **12** has been transformed to **13a**−**d** and 10.5% of **11a,b** hydrolyzed to the regioisomers **14** and **15** after 8 weeks, respectively. Aminolysis of α,β-unsaturated epoxides by lysine moieties therefore is expected to be an important mode of interaction between proteins and lipid oxidation products.

Keywords: *Lipid peroxidation; lysine model reactions; epoxyhydroxylinoleic acid; epoxide ring cleavage; aminol formation*

INTRODUCTION

Among the products of lipid hydroperoxide decomposition are nonvolatile fatty acid derivatives with oxygenated functional groups, such as hydroxyl, ketone, and epoxide (Gardner, 1989). Formation of epoxyols from fatty acid hydroperoxides either is metal-catalyzed (Dix and Marnett, 1985; Hamberg, 1975; Gardner et al., 1974) or can proceed enzymatically. The nonenzymatic pathway predominantly leads to epoxides with *trans* configuration. This is due to the fact that metal ions or metalloproteins readily decompose (*E*)-α,β-(*Z*)-γ,δ-unsaturated hydroperoxides by a homolytic route giving alkoxy radicals which attack the α-carbon to close a *trans*-configured epoxide ring with concomitant formation of an allylic radical. This process may, for example, finally transform (9*Z*,11*E*)-13-hydroperoxy-9,11-octadecadienoic acid (13-LOOH) into (10*E*,12*R**,13*R**)-12,13-epoxy-9-hydroxy-10-octadecenoic acid and (9*E*,12*R**,13*R**)-12,13-epoxy-11-hydroxy-9-octadecenoic acid. Thus, 13*S*-LOOH is in part converted by hemoglobin to (10*E*,12*S*,13*S*)-12,13-epoxy-9-hydroxy-10-octadecenoic acid (Hamberg, 1975). Such epoxides derived from alkoxy radicals are optically active and are formed as diastereoisomers of (9*R*,*S*)-hydroxy derivatives (Van Os et al., 1982). Graveland (1970) unraveled an enzymatic oxidation pathway for linoleic acid in wheat flour/water suspensions. Successive action of lipoxygenase and a water-insoluble "factor Y", which is adsorbed on gluten, transforms linoleic acid to a mixture of (9*R**,10*S**,11*E*)-9,10-epoxy-13-hydroxy-11-octadecenoic acid (**1**, see Figure 1) and (10*E*,12*R**,13*S**)-12,13-epoxy-9-hydroxy-10-octadecenoic acid (**2**); these products have *cis* configuration at the epoxide ring. The possibility that the epoxide is derived from oxygen addition to the (*Z*)-double bond γ,δ to the hydroperoxy group was strengthened by both Graveland (1973) and Heimann et al. (1973). In the case of the broad bean (*Vicia faba* L.), studies with $^{18}O_2$-labeled 13-LOOH showed that the epoxide ring in **1** is formed by either inter- or intramolecular oxygen transfer from the hydroperoxide through action of an epoxygenase (Hamberg and Hamberg, 1990).

Epoxyols have also been characterized as metabolites of linoleic acid hydroperoxides, generated by a prostaglandin endoperoxide synthase from adult or fetal blood vessels (Funck and Powell, 1983). Analogous structures have been isolated as decomposition products of 13-LOOH from a reaction with carp intestinal acetone powder (Hata et al., 1986). Incubation of arachidonic acid and its 12-hydroperoxy derivative with a rat lung preparation (Pace-Asciak et al., 1983) affords (5*Z*,9*E*,14*Z*)-11,12-epoxy-8-hydroxy-5,9,14-eicosatrienoic acid (**3**) and (5*Z*,8*E*,14*Z*)-11,12-epoxy-10-hydroxy-5,8,14-eicosatrienoic acid.

Epoxides thus represent crucial intermediates in the metabolism of polyunsaturated fatty acids, both in foodstuffs and under physiological conditions. Because an epoxy function vicinal to an olefinic double bond is smoothly hydrolyzed, aminolysis, for example, by the ϵ-amino function of a lysine moiety, may likewise be envisaged. In this context, Pokorny et al. (1966) have reported that both 9,10-epoxystearic acid and the re-

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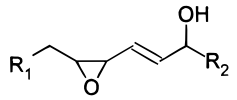

1: R_1 = -$(CH_2)_6$-COOH; R_2 = -$(CH_2)_4$-CH_3

2: R_1 = -$(CH_2)_3$-CH_3; R_2 = -$(CH_2)_7$-COOH

3: R_1 = -CH=CH-$(CH_2)_4$-CH_3; R_2 = -CH_2-CH=CH-$(CH_2)_3$-COOH

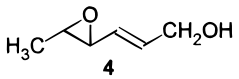

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Figure 1. Structural formulas of (9*R**,10*S**,11*E*)-9,10-epoxy-13-hydroxy-11-octadecenoic acid (**1**), (10*E*,12*R**,13*S**)-12,13-epoxy-9-hydroxy-10-octadecenoic acid (**2**), (5*Z*,9*E*,14*Z*)-11,12-epoxy-8-hydroxy-5,9,14-eicosatrienoic acid (**3**), and (2*E*,4*R**,5*R**)-4,5-epoxy-2-hexen-1-ol (**4**).

spective methyl ester are bonded to albumin without, however, characterizing the structure of the addition product.

Among proteinogenic amino acids, lysine and histidine moieties are the most susceptible to attack by lipid oxidation products. Nielsen et al. (1985) reported a significant loss of lysine (up to 71%) and histidine (up to 57%) in a whey protein/methyl linolenate ($C_{18:3}$)/water model system. Steinbrecher (1987) has found that 32% of the lysine residues of apolipoprotein B are modified by lipid peroxide decomposition products from human low-density lipoprotein (LDL). Due to the innate complexity of these processes, few of the products formed from lipid oxidation derivatives and amino acid moieties have so far been structurally characterized (Zamora and Hidalgo, 1995; Zamora et al., 1995; Gardner, 1979).

Recently, we have shown that (2*E*,4*R**,5*R**)-4,5-epoxy-2-hexen-1-ol (**4**), a structural model for the γ-hydroxy-α,β-unsaturated epoxides of linoleic and arachidonic acid, reacts with the ϵ-amino group of lysine side chains to form aminol compounds (Lederer, 1996). We have now synthesized the *tert*-butyl ester of **1**, *tert*-butyl (9*R**,10*S**,11*E*,13*S*)-9,10-epoxy-13-hydroxy-11-octadecenoate (**11**, see Figure 3) and reacted it with N^2-acetyllysine 4-methylcoumar-7-ylamide (**12**). Formation of the aminol *tert*-butyl (9*R**,10*R**,11*E*,13*S*)-10-({5-(acetylamino)-6-[(4-methyl-2-oxo-2*H*-chromen-7-yl)-amino]-6-oxohexyl}amino)-9,13-dihydroxy-11-octadecenoate (**13a**−**d**) is shown to be a competitive process to direct hydrolysis of **11**.

MATERIALS AND METHODS

Materials. Ultrapure water, obtained from a Milli-Q 185 Plus apparatus (Millipore, Eschborn, Germany), HPLC grade methanol, and HPLC grade acetonitrile were used for LC. For preparative HPLC, solvents were deaerated by flushing with helium. Linoleic acid, lipoxygenase I (EC 1.13.11.12; ∼9 units/mg; 1 unit generates 1 μmol of 13*S*-LOOH/min) from soybean, 1,1′-carbonyldiimidazole, 1,8-diazabicyclo[5.4.0]-7-undecene (DBU), *m*-chloroperoxobenzoic acid, *p*-toluenesulfonic acid, (−)-(1*R*)-menthyl chloroformate, and 1-methyl-2-pyrrolidone were purchased from Fluka (Neu-Ulm, Germany). Pb(IV) tetraacetate was obtained from Merck (Darmstadt, Germany).

Preparation of (9*Z*,11*E*,13*S*)-13-Hydroxy-9,11-octadecadienoic Acid (7), According to the Method of Maguire et al. (1997). Linoleic acid (925 mg, 3.3 mmol) was dissolved in a sodium borate buffer solution (470 mL; 0.1 M, pH 9.0) under nitrogen atmosphere and cooled to 0 °C in an ice bath. Soybean lipoxygenase I (89 mg) was added, a gentle stream of oxygen was bubbled through the solution via a Pasteur pipet, and the mixture was stirred for 30 min. Progress of the reaction was monitored by UV spectroscopy at 234 nm (absorption maximum of the conjugated diene chromophore in **6**). The mixture was allowed to warm to room temperature, sodium borohydride (1.2 g, 31.8 mmol) added, and the solution stirred for 1 h. The pH was adjusted to 3 with HCl (7.7 N), the emulsion was extracted with diethyl ether (4 × 150 mL), the organic layer was dried with anhydrous Na_2SO_4 and evaporated to dryness, and the residue was purified on a silica gel column [6 (height) × 3 (i.d., ∅) cm, hexane/diethyl ether/glacial acetic acid 83:30:1, v/v/v]. Fractions (6 mL each) were tested for **7** by TLC (R_f = 0.12, solvent mixture as above). From the respective fractions, the solvent was stripped off and the residue dried in high vacuum, yielding **7** (695 mg, 2.34 mmol, 71.1%). For ^{1}H and ^{13}C NMR ($CDCl_3$) see Table 1. ^{1}H NMR and UV data are fully consistent with those in references Gargouri and Legoy (1997) and Maguire et al. (1997); CI-GLC-MS (silylated compound, program A): t_R = 22.83 min, *m*/*z* (relative intensity) 441 (4, $[M + H]^+$), 425 (16), 351 (100), 261 (33), 243 (20), 173 (20), 98 (18), 73 (53).

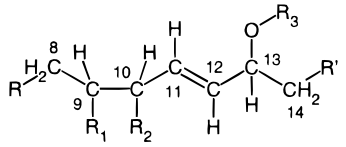
(9*Z*,11*E*,13*S*)-13-(Acetoxy)-9,11-octadecadienoic Acid (8). Compound **7** (695 mg, 2.34 mmol) was dissolved in pyridine (1 mL), acetic anhydride (2 mL) was added, and the solution was kept at room temperature for 17 h. The mixture was poured on ice (80 mL), stirred for 1 h, and, after pH adjustment to 3 (1 N H_2SO_4), extracted with CH_2Cl_2 (3 × 20 mL). The organic layer was washed with H_2SO_4 (1 N, 20 mL) and water (acidified with H_2SO_4, pH ∼2, 3 × 20 mL), dried with anhydrous Na_2SO_4, and evaporated to dryness, and the residue was dried in high vacuum, yielding **8** (700 mg, 2.07 mmol, 88.2%). For ^{1}H and ^{13}C NMR ($CDCl_3$) see Table 1. CI-GLC-MS (silylated compound, program A): t_R = 23.92 min, *m*/*z* 351 (100, $[M + H]^+ - CH_3COOH$), 279 (21), 261 (63), 243 (38), 177 (12), 75 (13).

***tert*-Butyl (9*Z*,11*E*,13*S*)-13-(Acetoxy)-9,11-octadecadienoate (9).** Compound **8** (700 mg, 2.07 mmol) was reacted with 1,1′-carbonyldiimidazole (335 mg, 2.07 mmol) in dimethylformamide (DMF; 2.2 mL) at 40 °C for 4 h. *tert*-Butyl alcohol (313 mg, 4.22 mmol) and DBU (315 mg, 2.07 mmol) were added, and the reaction mixture was flushed with nitrogen and kept at 40 °C for 22 h. The mixture was diluted with CH_2Cl_2 (35 mL) and washed successively with HCl (3 N, 10 mL) and aqueous K_2CO_3 (0.72 M, 10 mL). The K_2CO_3 solution was extracted with CH_2Cl_2 (4 × 10 mL), the combined organic layers were evaporated to dryness, and the residue was purified on a silica gel column (8 × ∅ 3 cm, hexane/ethyl acetate 19:1, v/v). Fractions (6 mL each) were tested for **9** by TLC (R_f = 0.22, solvent mixture as above). From the respective fractions the solvent was stripped off and the residue dried in high vacuum, yielding **9** (185 mg, 0.47 mmol, 22.7%). For ^{1}H and ^{13}C NMR ($CDCl_3$) see Table 1. CI-GLC-MS (program A): t_R = 24.20 min, *m*/*z* 335 (7, $[M + H]^+ - CH_3COOH$), 279 (100), 261 (6), 243 (3).

***tert*-Butyl (9*R**,10*S**,11*E*,13*S*)-13-(Acetoxy)-9,10-epoxy-11-octadecenoate (10a,b).** Compound **9** (185 mg, 0.47 mmol) was dissolved in diethyl ether (2 mL) and cooled to 0 °C in an ice bath. *m*-Chloroperoxobenzoic acid (210 mg, ∼55%, ∼0.67 mmol) was added and the mixture kept at 0 °C for 24 h. Progress of the reaction was monitored by TLC [mobile phase, hexane/ethyl acetate 19:7 (v/v); R_f = 0.90 (**9**), 0.80 (**10a,b**); detection, spray reagent 5% H_2SO_4 in anhydrous ethanol/10 min at 120 °C]. The mixture was diluted with diethyl ether (30 mL) and extracted with aqueous NaOH (2.5 M, 3 × 20 mL) and water (3 × 10 mL); negative starch iodine reaction in the washing water indicated complete removal of peroxides. The organic layer was stripped off and the residue dried in high vacuum, yielding **10a,b** (146 mg, 0.36 mmol, 77%). For ^{1}H and ^{13}C NMR ($CDCl_3$) see Table 1. CI-GLC-MS (program A): t_R = 26.17 min, *m*/*z* 351 (5, $[M + H]^+ - CH_3COOH$), 295 (98), 277 (100), 185 (4), 75 (14).

***tert*-Butyl (9*R**,10*S**,11*E*,13*S*)-9,10-Epoxy-13-hydroxy-11-octadecenoate (11a,b).** Compound **10a,b** (146 mg, 0.36 mmol) was dissolved in MeOH (4.2 mL), aqueous K_2CO_3 (1.2

Table 1. ^{1}H and ^{13}C NMR Data of Compounds 7−11 and 14 (0.1 M, in $CDCl_3$)[a]



7, 8 R = -$(CH_2)_6$-COOH

9-11, 14 R = -$(CH_2)_6$-COO-$C(CH_3)_3$

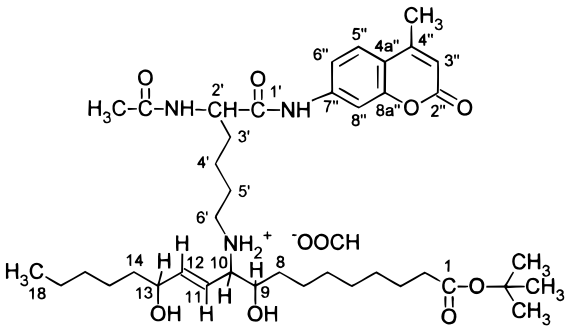
R' = -C_4H_9

	7	**8/9**	**10a,b**	**11a,b**	**14**
R_1	⇒ C_9	⇒ C_9	O	O	OH
R_2	⇒ C_{10}	⇒ C_{10}			OH
R_3	H	CO−CH_3	CO−CH_3	H	H
			^{1}H NMR δ		
$C(CH_3)_3$		−/1.44	1.44	1.44	1.44
2-H_2	2.34	2.44/2.20	2.20	2.20	2.20
3-H_2	1.62	1.66/≈1.6	≈1.6	≈1.6	≈1.6
(4−7; 15−17)-H_{14}	1.31 br	1.30 br	1.30 br	1.31 br	1.30 br
CO−CH_3		2.05	2.05		
8-H_A	2.17	2.17	≈1.5	1.45	≈1.45
8-H_B	2.17	2.17	≈1.5	1.57	≈1.45
9-H	5.44	5.46	3.06	3.07	3.46
10-H	5.97	5.94	3.384, 3.385[b]	3.41	3.94
11-H	6.48	6.50	5.56, 5.58[b]	5.54, 5.55[b]	5.71
12-H	5.66	5.56	5.83, 5.84[b]	5.947, 5.950[b]	5.83
13-H	4.17	5.28	5.272, 5.266[b]	4.15	4.15
14-H_A	1.51	1.58	≈1.5	1.52	≈1.55
14-H_B	1.57	1.66	≈1.7	1.81	≈1.55
18-H_3	0.89	0.88	0.88	0.89	0.89
			J (Hz)		
^3J(8-$H_{A/B}$, 9-H)	7.7	7.6	≈6	5.5, 6.7[d]	*e*
^3J(9-H, 10-H)	10.9	10.9	4.3	4.3	6.1
^3J(10-H, 11-H)	11.0	11.0	7.0	7.5	6.5
^3J(11-H, 12-H)	15.2	15.2	15.6	15.6	15.6
^3J(12-H, 13-H)	6.9	7.6	6.9, 6.5[c]	6.3	5.9
^3J(13-H, 14-$H_{A/B}$)	6.4	6.2	6.7	6−7	6.5
^4J(8-$H_{A/B}$, 10-H)	1.9	1.7			
^4J(9-H, 11-H)	1.3	1.0			
^4J(10-H, 12-H)	0.6	0.8	0.8	0.7	1.1
^4J(11-H, 13-H)	1.1	1.1	1.1, 0.9[c]	1.2	1.2
^5J(9-H, 12-H)		1.0			
			^{13}C NMR δ		
C(**C**H_3)$_3$		−/28.1	28.1	28.1	28.1
C$(CH_3)_3$		−/79.9	79.9	80.0	80.0
C-1	179.5	169.6/173.3	173.2	173.3	173.4
C-2	34.0	35.3/35.6	35.6	35.6	35.6
C-3	24.6	24.2/25.1	25.1	25.0	25.1
CO−CH_3		170.5	170.2		
CO-**C**H_3		21.4	21.3		
C−(4−7)	28.84, 28.88, 28.91, 29.4	28.8/−, 29.0/29.1 × 2, 29.1/29.2, 29.5	26.3, 29.0, 29.2 × 2	(26.24,26.26),[b] 28.9, 29.16, 29.21	25.5, 28.9, 29.1, 29.3
C-8	27.6	27.7	27.7	27.8	32.9
C-9	132.8	133.6	58.84, 58.91[b]	58.90, 58.92[b]	75.4
C-10	127.9	127.6	56.14, 56.22[b]	56.51, 56.55[b]	74.6
C-11	125.9	128.0	126.9, 127.3[b]	124.6	129.5
C-12	135.7	131.0	134.5	139.7	136.4
C-13	73.0	74.9	73.7, 73.9[b]	72.2	72.1
C-14	37.2	34.5	34.22, 34.32[b]	37.10, 37.18[b]	37.2
C-15	25.1	24.8	24.7	25.0	25.1
C-16	31.8	31.6	31.5	31.7	31.7
C-17	22.6	22.5	22.5	22.6	22.6
C-18	14.1	14.0	14.0	14.0	14.0

[a] δ, chemical shift for the indicated hydrogen/carbon; *J* (Hz), coupling constant between the indicated protons. Hydrogen/carbon assignment has been validated by ^{1}H,^{1}H−COSY, ^{1}H,^{13}C−COSY, and ^{13}C-DEPT techniques. [b] Hydrogen/carbon chemical shifts for the respective two diastereoisomers. [c] Coupling constants for the respective two diastereoisomers. [d] Coupling constants between 10-H and the diastereotopic protons 8-$H_{A/B}$. [e] Coupling constant cannot be determined, due to overlapping multiplets.

M, 1.8 mL) added, and the mixture vigorously stirred at room temperature for 4 h. Progress of the reaction was monitored by TLC [mobile phase: hexane/ethyl acetate 19:7 (v/v); R_f = 0.80 (**10a,b**), 0.42 (**11a,b**); detection as described for **10a,b**]. The mixture was diluted with water (10 mL) and extracted with diethyl ether (3 × 30 mL). The organic layer was washed with water (2 × 30 mL), dried with anhydrous Na_2SO_4, and evaporated to dryness, yielding **11a,b** (116 mg, 0.31 mmol, 88.5%). For ^{1}H and ^{13}C NMR ($CDCl_3$) see Table 1. CI-GLC-MS (silylated compound, program A): **11a**, t_R = 25.05 min, *m*/*z* 441 (3, $[M + H]^+$), 439 (10, $[M - H]^+$), 367 (100), 351 (18), 295 (65), 277 (87), 185 (13), 105 (9), 73 (7); **11b**, t_R = 25.13 min, *m*/*z* 439 (7, $[M - H]^+$), 367 (85), 351 (21), 295 (71), 277 (100), 185 (11), 105 (7), 73 (8).

Table 2. ^{1}H and ^{13}C NMR Data of Compounds 13a−d (0.1 M, in DMSO-d_6)[a]



	13a,b	13c,d		13a,b	13c,d
		^{1}H NMR δ			
$C(CH_3)_3$	1.36	1.39	4′-H_2	1.35	1.35
2-H_2	2.13	2.15	5′-H_2	1.50	1.50
8-H_2	1.27	1.25	6′-H_A	2.60	2.69
9-H	3.33	3.36	6′-H_B	2.45	2.52
10-H	2.97, 2.99[b]	3.02, 3.03[b]	−N**H**$COCH_3$	8.31	8.37, 8.38[b]
11-H	5.32	5.29	−NHCO**C**H_3	1.85	1.88
12-H	5.68	5.68	3″-H	6.27	6.26
13-H	3.94	3.91	4″-C**H**$_3$	2.38	2.40
14-H_2	1.35	1.40	5″-H	7.72	7.71
18-H_3	0.80	0.81	6″-H	7.51	7.53
2′-H	4.39	4.39	7″-NH	10.62, 10.64[b]	10.68, 10.70[b]
3′-H_A	1.68	1.70	8″-H	7.80	7.81
3′-H_B	1.57	1.61	$HCOO^-$	8.34	8.39
		J (Hz)			
^3J(9-H, 10-H)	7.5	7.2	^3J(2′-H, 3′-H_B)	8.5	9.0
^3J(10-H, 11-H)	9.2	9.5	^3J(5″-H, 6″-H)	8.7	8.7
^3J(11-H, 12-H)	15.5	15.5	^4J(11-H, 13-H)	1.0	1.0
^3J(12-H, 13-H)	5.5	6.3	^4J(3″-H, 4″-CH_3)	1.3	1.3
^3J(2′-H, NH)	7.5	7.5	^4J(6″-H, 8″-H)	2.1	2.1
^3J(2′-H, 3′-H_A)	5.7	5.6			
		^{13}C NMR δ			
C(**C**$H_3)_3$	27.7	27.7	C-4′	22.9	22.9
C$(CH_3)_3$	79.2	79.2	C-5′	25.8	25.8
C-1	172.2	172.2	C-6′	45.1	44.9
C-2	34.7	34.7	−NH**C**OCH_3	169.4	169.5
C-(3−7; 15, 16)	24.3, 24.5, 24.8, 28.3 28.6, 28.9, 31.2	24.46, 24.52, 24.7, 28.3 28.6, 28.9, 31.2	−NHCO**C**H_3	22.3	22.3
C-8	33.3	33.4	C-2″	159.9	159.9
C-9	70.9	70.5	C-3″	112.2	112.2
C-10	65.05, 65.12[b]	65.11, 65.16[b]	C-4″	153.6[c]	153.0[d]
C-11	124.9	124.8	−CH_3 (4″)	17.9	17.9
C-12	140.2	140.7, 141.0[b]	C-4a″	114.9	114.9
C-13	69.81, 69.86[b]	70.29, 70.33[b]	C-5″	125.8	125.8
C-14	37.0	37.0	C-6″	115.2	115.2
C-17	22.0	22.1	C-7″	142.2	142.3
C-18	13.7	13.8	C-8″	105.6	105.7
C-1′	171.7	171.8	C-8a″	153.0[c]	153.6[d]
C-2′	53.5	53.6	$HCOO^-$	164.8	165.9
C-3′	31.5	31.5			

[a] δ, chemical shift for the indicated hydrogen/carbon; J (Hz), coupling constant between the indicated protons. Hydrogen/carbon assignment has been validated by ^{1}H,^{1}H-COSY, ^{1}H,^{13}C-COSY, and ^{13}C-DEPT techniques. [b] Hydrogen/carbon chemical shifts for the respective two diastereoisomers. [c,d] Assignment may have to be reversed.

***tert*-Butyl (9*R**,10*R**,11*E*,13*S*)-10-({5-(Acetylamino)-6-[(4-methyl-2-oxo-2*H*-chromen-7-yl)amino]-6-oxohexyl}-amino)-9,13-dihydroxy-11-octadecenoate (13a−d).** A mixture of **11a,b** (120 mg, 0.33 mmol) and **12** [130 mg, 0.38 mmol; prepared according to the method of Lederer (1996)] in 1-methyl-2-pyrrolidone (0.5 mL) was kept at 100 °C for 48 h. The solution was diluted with eluent (5.5 mL) and purified by preparative HPLC. Fractions at t_R = 13.5 and 14.2 min were collected and the methanol was removed in vacuo. The aqueous layer was lyophilized, yielding **13a,b** (42.2 mg, 0.056 mmol, 16.8%) and **13c,d** (84.3 mg, 0.111 mmol, 33.7%) as the respective formate salts. For ^{1}H and ^{13}C NMR ($CDCl_3$) see Table 2. UV and fluorescence characteristics are identical with those of compound **2** in Lederer (1996). HPLC-DAD: t_R = 30.87 min (**13a,b**), 31.12 min (**13c,d**). FAB−HRMS (*m*-nitrobenzyl alcohol): *m*/*z* 714.4694 $[M + H]^+$ (714.4693, calcd for $C_{40}H_{64}N_3O_8$).

Hydrolysis of 11a,b and Pb(IV) Tetraacetate Cleavage of the Resulting Triols *tert*-Butyl (*E*)-9,10,13-Trihydroxy-11-octadecenoate (14) and *tert*-Butyl (*E*)-9,12,13-Trihydroxy-10-octadecenoate (15). Compound **11a,b** (40 mg, 0.11 mmol) was dissolved in THF (1.6 mL), *p*-toluenesulfonic acid (0.1 N, 0.4 mL) added, and the mixture stirred at room temperature for 1 h. Progress of the reaction was monitored by TLC [mobile phase, hexane/ethyl acetate 1:1 (v/v); R_f = 0.78 (**11a,b**), 0.15, 0.12 (**14, 15**); spray reagent, (1) as described for **10a,b** and (2) for specific detection of **14** and **15**, (a) 1% $Pb(Ac)_4$ in toluene (w/v) and (b) 0.05% fuchsine in MeOH (w/v)].

The mixture was diluted with water (10 mL) and the pH adjusted to 8 with solid $NaHCO_3$. The solution was extracted with CH_2Cl_2 (4 × 10 mL), and the organic phase was dried with anhydrous Na_2SO_4 and evaporated to dryness. The residue was purified on a silica gel column [6 × ∅ 0.8 cm, hexane/ethyl acetate 1:1 (v/v)]. Fractions (1.5 mL each) were collected and tested by TLC (see above). Two groups of isomers could be isolated for **14** and **15**; however, no complete separation of the regioisomers could be achieved. The respective fractions yielded (I) (R_f = 0.15, 6.8 mg, 0.018 mmol) and (II) (R_f = 0.12, 6.4 mg, 0.017 mmol); both products contained **14** and **15** in 3:1 (I) and 2:1 (II) ratio, respectively. For ^1H and ^{13}C NMR ($CDCl_3$) for the major diastereoisomer of **14**, data obtained from product I, see Table 1. CI-GLC-MS (silylated compounds, program A): **14**, t_R = 23.02 min, *m/z* 603 (5; $[M + H]^+$), 513 (4), 439 (68), 367 (100), 349 (19), 317 (78), 301 (4), 277 (24), 245 (98), 155 (37), 109 (28), 89 (17), 73 (69); t_R = 23.11 min, *m/z* 603 (4; $[M + H]^+$), 513 (3), 439 (81), 367 (100), 349 (17), 317 (94), 301 (5), 277 (26), 245 (80), 155 (33), 109 (26), 89 (16), 73 (83); t_R = 23.40 min, *m/z* 603 (4; $[M + H]^+$), 513 (3), 439 (81), 367 (94), 351 (20), 317 (87), 301 (7), 277 (26), 245 (100), 155 (39), 109 (31), 89 (22), 73 (93). **15**, t_R = 23.35 min, *m/z* 603 (2; $[M + H]^+$), 513 (4), 439 (82), 429 (11), 413 (10), 367 (71), 357 (25), 349 (15), 317 (68), 277 (18), 245 (63), 173 (38), 155 (17), 105 (13), 83 (25), 73 (100); t_R = 23.50 min, *m/z* 603 (1; $[M + H]^+$), 513 (4), 439 (89), 429 (30), 413 (15), 367 (70), 357 (39), 349 (15), 317 (33), 277 (24), 245 (22), 173 (50), 155 (15), 105 (15), 83 (36), 73 (100).

Products I and II (6 mg each) were dissolved in diethyl ether (1 mL), and $Pb(Ac)_4$ (13 mg, 0.025 mmol) was added. The mixtures were kept at 0 °C for 1 h and at room temperature for 2 h, diluted with diethyl ether (25 mL), and extracted successively with water (3 × 5 mL), aqueous NaOH (0.25 M, 5 mL), and water (3 × 5 mL). The organic layer was dried with anhydrous Na_2SO_4 and analyzed by GLC-FID (program B) and GLC-MS (program B). CI-GLC-MS: **16**, t_R = 19.80 min, *m/z* 157 (100; $[M + H]^+$), 139 (48), 121 (39), 97 (39), 93 (28), 81 (31); **17**, t_R = 23.73 min, *m/z* 155 (100; $[M + H]^+$ − *tert*-butyl alcohol), 137 (6), 109 (5); **18**, t_R = 6.29 min, *m/z* 101 (19; $[M + H]^+$), 83 (100); **19**, t_R = 31.51 min, *m/z* 211 (100; $[M + H]^+$ − *tert*-butyl alcohol), 193 (7).

Incubations of *tert*-Butyl (9*R,10*S**,11*E*,13*S*)-9,10-Epoxy-13-hydroxy-11-octadecenoate (11a,b) with N^2-Acetyllysine 4-Methylcoumar-7-ylamide (12) at pH 7.4 and 37 °C.** Compound **12** (70 mg, 0.20 mmol) was dissolved in water (10 mL), the pH adjusted to 7.4 with H_3PO_4 (0.1 N), and the solution lyophilized, yielding **12**·H_3PO_4 (87 mg). Compounds **12**·H_3PO_4 (11.1 mg; 0.025 mmol) and **11a,b** (18.4 mg; 0.05 mmol) were dissolved in (A) 1-methyl-2-pyrrolidone (MP), (B) MP/water (9:1, v/v), (C) MP/water (8:2, v/v), and (D) MP/water (1:1, v/v) (0.5 mL each) and the mixtures kept at 37 °C under nitrogen atmosphere for 56 days; incubation D was stirred vigorously. In regular intervals aliquots (5 μL) of each incubation were diluted with MeOH (1 mL) and 10 μL was injected into the HPLC system.

Quantification of *tert*-Butyl (*E*)-9,10,13-Trihydroxy-11-octadecenoate (14) and *tert*-Butyl (*E*)-9,12,13-Trihydroxy-10-octadecenoate (15) in Incubations A−D. Incubations A−D were diluted with water (10 mL) and extracted with CH_2Cl_2 (3 × 10 mL). The solutions were filled to a final volume of 50 mL with CH_2Cl_2 and divided into two portions, and the solvent was stripped off. *N*,*O*-Bis(trimethylsilyl)acetamide (BSA, 150 μL) was added to one aliquot, the mixture was kept at room temperature for 2 h, and the volume was filled up to 100 mL with toluene. One microliter was injected into the GLC-FID system (program A). The other aliquot was dissolved in diethyl ether (1 mL), $Pb(Ac)_4$ (20 mg, 0.038 mmol) was added, and the solution was kept at 0 °C for 1 h and at room temperature for 2 h and diluted with diethyl ether (25 mL). Further workup and analysis of compounds **16**−**19** followed the procedure given above.

Spectra. Ultraviolet (UV) spectra were measured with a Perkin-Elmer Lambda 2 (Überlingen, Germany). ^1H and ^{13}C nuclear magnetic resonance (NMR) spectra were recorded on Bruker (Karlsruhe, Germany) AC-250/ARX-500 spectrometers at 250/500 and 63/126 MHz nominal frequency, respectively. Chemical shifts are given in δ relative to Me_4Si (tetramethylsilane) as internal standard; coupling constants *J* are given in hertz. Liquid secondary-ion high-resolution mass spectra (SIMS-HRMS, analogous to FAB-HRMS) were obtained on a Finnigan MAT 95 (Bremen, Germany).

Polarimetry. Optical rotation was determined on a Perkin-Elmer 241 polarimeter.

Analytical High-Performance Liquid Chromatography (HPLC). The analytical HPLC system comprised an HP1100 autosampler, HP1100 gradient pump, HP1100 diode array detector (DAD), and HP1046A fluorescence detector (FLD) module (Hewlett-Packard, Waldbronn, Germany). For data acquisition and processing, the HP Chem Station (rev A 04.02) software was used. The column (Bischoff, Leonberg, Germany) used was a Nucleosil C_{18}, 5 μm, 100 Å (column, 250 × 4 mm; guard column, 10 × 4 mm); flow rate = 0.8 mL/min; injection volume = 10 μL; MeOH/phosphate buffer (0.01 M, pH 7.4) gradient, % MeOH [*t* (min)] 5(0), 100(35−40), 5(50−60); DAD detection wavelengths = 230 and 326 nm; spectral bandwidth (SBW) = 4 nm, ref 500 nm (SBW 100 nm); FLD λ_{ex} = 326 nm, λ_{em} = 390 nm.

Preparative HPLC Purification. The preparative HPLC system consisted of a Knauer (Berlin, Germany) 64 liquid chromatograph combined with an A0293 variable-wavelength detector and a Kronlab HPLC column (Nucleosil C_{18}, 7 μm, 100 Å, column 250 × 20 mm, guard column 50 × 20 mm); flow rate = 10 mL/min; eluent, MeOH/NH_4HCOO buffer (0.01 M, pH 4.0) 80:20 (v/v); injection volume = 0.75 mL; detection wavelength = 230 nm. Solutions were filtered (membrane filter, 0.45 μm) before preparative HPLC.

Gas−Liquid Chromatography (GLC). GLC-FID was run on a Carlo Erba (Hofheim, Germany) HRGC 5160 instrument equipped with an on-column (OC) injector and a flame ionization detector (FID). The operating conditions were as follows: carrier gas, hydrogen; carrier linear velocity, 62 cm/s at 100 °C; oven temperature programs, (A) 100 °C, raised at 10 °C/min to 200 °C, raised at 3 °C/min to 280 °C with 5 min isothermal at 280 °C, and (B) 5 min at 35 °C, raised at 8 °C/min to 270 °C with 5 min isothermal at 270 °C; FID temperature, 300 °C; capillary column, Supelco PTE 5 (Steinheim, Germany), 30 m × 0.32 mm i.d.; film thickness, 0.3 μm.

GLC-Mass Spectrometry Analysis. GLC-MS was performed using a Finnigan MAT (Bremen, Germany) Ion Trap 800 equipped with a Perkin-Elmer (Überlingen, Germany) 8420 gas−liquid chromatograph. GC conditions were as follows: carrier gas, helium; carrier linear velocity, 28 cm/s at 100 °C; oven temperature programs, (A) 150 °C, raised at 6 °C/min to 290 °C with 15 min isothermal at 290 °C, and (B) 5 min at 40 °C, raised at 8 °C/min to 270 °C with 10 min isothermal at 270 °C; split injector and transfer line temperature, 290 °C, split ratio, 1:30; capillary column, PVMS 54 (Perkin-Elmer), 30 m × 0.25 mm i.d.; film thickness, 0.25 μm. MS conditions were as follows: positive MeOH−CI mode; ion source temperature, 220 °C.

Liquid Chromatography. Silica gel 60 F_{254} (Merck, Darmstadt, Germany) was used for thin-layer chromatography (TLC) and silica gel (63−200 μm) (Baker, Gross-Gerau, Germany) for column chromatography.

Lyophilization. A Leybold-Heraeus (Köln, Germany) Lyovac GT 2 was applied.

RESULTS AND DISCUSSION

Because no independent synthesis is reported for either (9*R**,10*S**,11*E*)-9,10-epoxy-13-hydroxy-11-octadecenoic acid (**1**) or its *tert*-butyl ester **11** in the literature, we elaborated such a protocol, starting from linoleic acid (**5**, see Figure 2). Compound **5** was oxidized by soybean lipoxygenase I to (9*Z*,11*E*,13*S*)-13-hydroperoxy-9,11-octadecadienoic acid (**6**, 13*S*-LOOH) and reduced in situ with sodium borohydride to (9*Z*,11*E*,-13*S*)-13-hydroxy-9,11-octadecadienoic acid (**7**), which is also designated (+)-coriolic acid (Maguire et al., 1991,

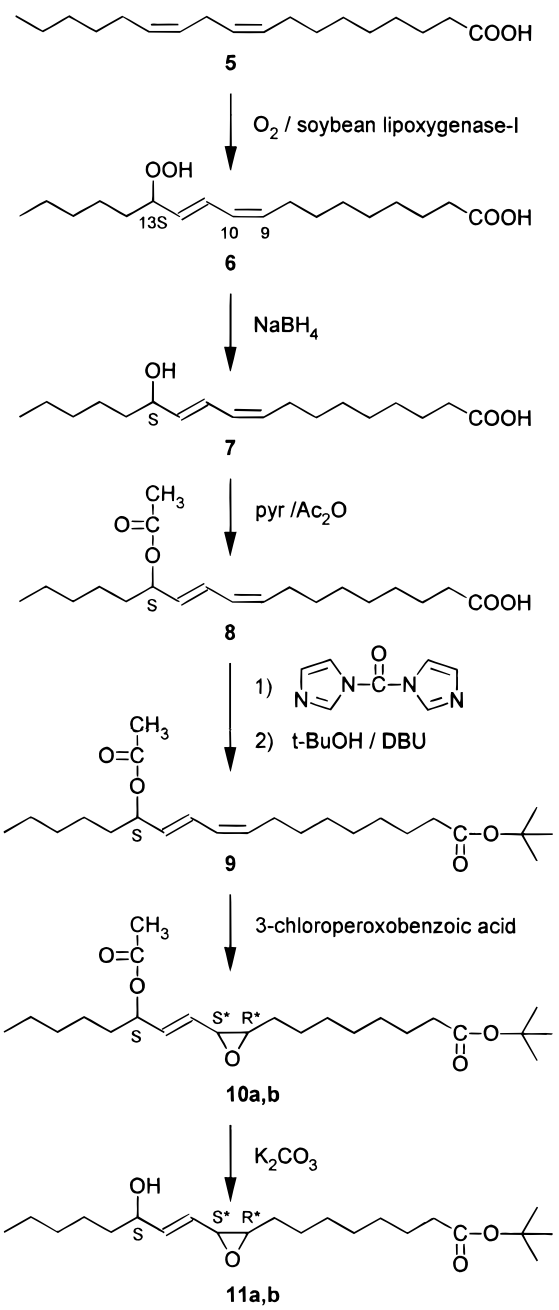


Figure 2. Reaction pathway for the synthesis of *tert*-butyl (9*R**,10*S**,11*E*,13*S*)-9,10-epoxy-13-hydroxy-11-octadecenoate (**11a,b**).

1997; Gargouri and Legoy, 1997). The 13-OH group of **7** was acetylated to avoid interference in the subsequent esterification step and also to direct the epoxidation to the γ,δ-double bond relative to C-13. Such a derivatization procedure has already been successfully employed for the synthesis of (2*E*,4*R**,5*R**)-4,5-epoxy-2-hexen-1-ol (**4**) from (2*E*,4*E*)-2,4-hexadien-1-ol (Lederer, 1996). (9*Z*,11*E*,13*S*)-13-(Acetoxy)-9,11-octadecadienoic acid (**8**) was transformed to its *tert*-butyl ester **9** according to a method by Ohta et al. (1982). The *tert*-butyl group was chosen for protection of the COOH function because this ester is stable under mildly basic conditions as well as toward aminolysis. It is thus unaffected during saponification of the 13-acetoxy protective group, and amide formation with lysine side chains can be definitely ruled out. Epoxidation of **9** with 3-chloroperoxobenzoic acid (Prilezhaev reaction) yielded *tert*-butyl

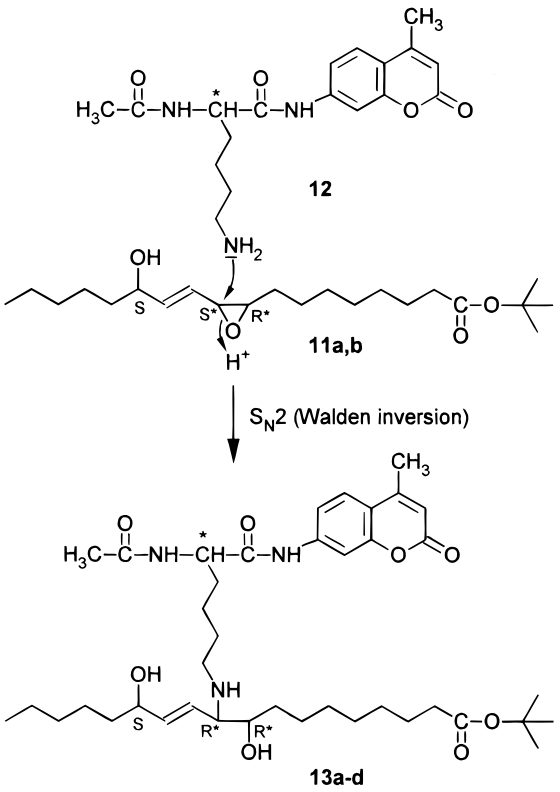


Figure 3. Reaction scheme for the formation of *tert*-butyl (9*R**,10*R**,11*E*,13*S*)-10-({5-(acetylamino)-6-[(4-methyl-2-oxo-2*H*-chromen-7-yl)amino]-6-oxohexyl}amino)-9,13-dihydroxy-11-octadecenoate (**13a**−**d**) from *tert*-butyl (9*R**,10*S**,11*E*,13*S*)-9,10-epoxy-13-hydroxy-11-octadecenoate (**11a,b**) and N^2-acetyllysine 4-methylcoumar-7-ylamide (**12**).

(9*R**,10*S**,11*E*,13*S*)-13-(acetoxy)-9,10-epoxy-11-octadecenoate (**10**) as a pair of diastereoisomers **10a** and **10b**. Because the regioisomeric *tert*-butyl (9*Z*,11*R**,12*R**,-13*S*)-13-(acetoxy)-11,12-epoxy-9-octadecenoate was formed only in negligible amounts, no chromatographic purification of **10a,b** on silica gel was necessary; this slightly acidic stationary phase causes partial hydrolysis of the epoxide ring and substantially decreases the yield of **10**. The acetate group was cleaved with potassium carbonate in methanol/water, and the structure of *tert*-butyl (9*R**,10*S**,11*E*,13*S*)-9,10-epoxy-13-hydroxy-11-octadecenoate (**11a,b**), as for all precursors, was unequivocally characterized by ^{1}H, ^{13}C NMR, and MS (see below).

For an in-depth study of the reaction of **11** with lysine moieties, we prepared N^2-acetyllysine 4-methylcoumar-7-ylamide (**12**). This lysine derivative incorporates a fluorophore and thus allows direct and sensitive HPLC monitoring of the reaction (Lederer, 1996). The synthesis of *tert*-butyl (9*R**,10*R**,11*E*,13*S*)-10-({5-(acetylamino)-6-[(4-methyl-2-oxo-2*H*-chromen-7-yl)amino]-6-oxohexyl}-amino)-9,13-dihydroxy-11-octadecenoate (**13a**−**d**, see Figure 3) from **11** and **12** was carried out at 100 °C in 1-methyl-2-pyrrolidone. The progress of the reaction was monitored by HPLC, showing almost quantitative turnover within 48 h. Two products could be isolated by preparative HPLC in a 1:2 ratio. FAB−HRMS analysis gave a quasimolecular ion ($[M + H]^+$) at *m*/*z* 714.4694 for both compounds, corresponding to an elemental composition of $C_{40}H_{64}N_3O_8$. Thus, the products obtained represent 1:1 adducts of **11** and **12**. The structure of the isomers was definitely characterized as **13a**−**d**; that is, four diastereoisomers have been formed from which two

pairs can be separated chromatographically (see Figure 3).

As shown for the formation of N^2-acetyl-N^6-[(*E*)-1,5-dihydroxyethyl-2-hexen-4-yl]lysine 4-methylcoumar-7-ylamide from **4** and **12** (Lederer, 1996), nucleophilic attack of the amine to the epoxide occurs exclusively in allylic position and strictly follows an S_N2 mechanism. This also seems to hold for the reaction of **11a,b** and **12**. An S_N1 process, as a mechanistic alternative, would proceed via the allylic carbocation, which then could be attacked by the amine at either C-10 or C-12. This pathway would by necessity yield two regioisomers, probably with a ratio far from 1:1. Because not even traces of positional isomers could be detected for **13**, an S_N1 mechanism can definitely be excluded. The absence of a compound resulting from an allylic shift likewise rules out an S_N2' mechanism as the third possible route. The preference for nucleophilic attack at C-10 rather than at C-9 is probably due to resonance stabilization of the S_N2 transition state vicinal to a double bond. With the S_N2 mechanism established, formation of the four diastereoisomeric structures **13a**−**d** can be rationalized only as follows: The stereochemistry of the oxirane ring in **11** has unequivocally been proven as *cis*; the relative configuration consequently must be 9*R**/10*S**. In the course of an S_N2 process, with Walden inversion, the ring cleavage products **13a**−**d** then are formed as 9R*/10R*; that is, the two possible absolute configurations are enantiotopic. If the other two stereogenic centers in **13a**−**d** at C-13 and C-2′ (for the numbering see Table 2) were both (*S*)-configured, one pair of diastereoisomers only would result. Therefore, racemization must have occurred at C-13*S* and/or C-2′*S* during the respective synthesis of **11** and/or **12**. Chiral analysis of both educts clearly proves compound **12** to have suffered racemization, whereas the C-13 stereogenic center in **11** has retained the (*S*)-configuration of its precursor 13*S*-LOOH (see Structural Assignment).

To probe whether and to which extent **13** is formed under mild reaction conditions and in the presence of water, compounds **11a,b** (100 mM) and **12** (50 mM) were incubated in MP and MP/water mixtures at pH 7.4 and 37 °C for 8 weeks. Due to the poor solubility in these mixtures, buffer salts such as phosphate or borate cannot be used directly to hold the pH stable; therefore, an aqueous solution of **12**, as the free base, was adjusted to pH 7.4 with orthophosphoric acid and lyophilized, and the resulting phosphate salt **12**·H_3PO_4 was employed for the incubations. Progress of the reaction was monitored by HPLC equipped with both a DAD and an FLD. Fluorophore-labeled **12** allows for a straightforward correlation between the HPLC peak areas of the lysine educt and eventual reaction products. The time-dependent formation of *tert*-butyl (9*R**,10*R**,11*E*,-13*S*)-10-({5-(acetylamino)-6-[(4-methyl-2-oxo-2*H*-chromen-7-yl)amino]-6-oxohexyl}amino)-9,13-dihydroxy-11-octadecenoate (**13a**−**d**) was observed, and the results are shown in Figure 4. In pure MP, conversion to **13** is <1% after 56 days. Product formation is favored significantly, however, when water is added to the reaction mixture. At the end of the reaction period, 5.8 and 15.8% of **12** have been converted to **13** in incubations with 10 and 20% (v/v) water, respectively. The higher conversion rate, correlating with an increased solvent polarity, corresponds to Ingold's rules for solvent effects in nucleophilic substitution (Ingold, 1969). The initial state for an S_N2 reaction of an amine with an epoxide, both

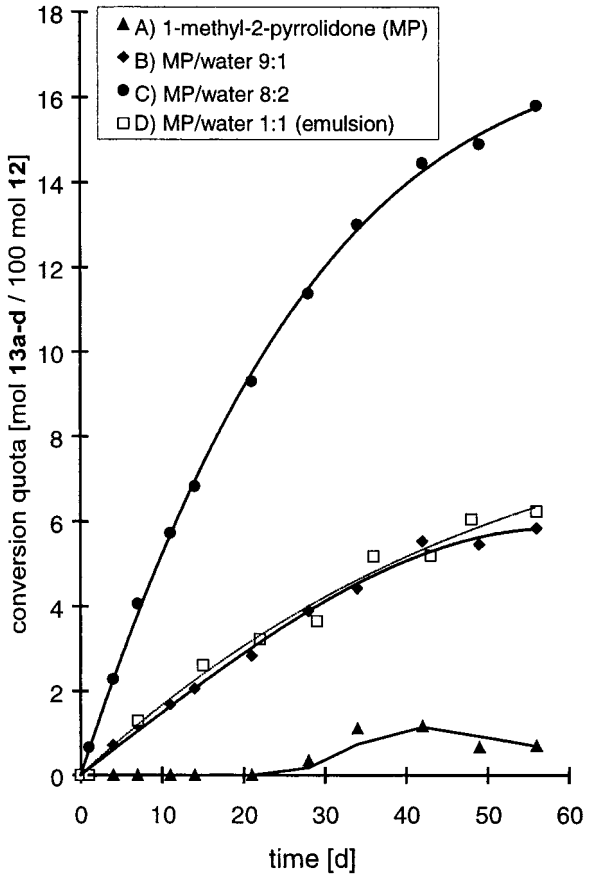


Figure 4. Time course for the formation of **13** at pH 7.4 and 37 °C from 100 mM **11** and 50 mM **12**, depending on the water content of the incubation mixture.

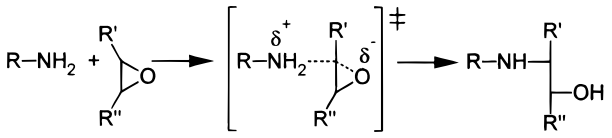


Figure 5. S_N2 mechanism for the cleavage of an epoxide by an amine [charge-type II according to the classification of Ingold (1969)].

of which are neutral, is less polar than the transition state (see Figure 5); Ingold has classified this case as charge-type II. Consequently, a higher solvent polarity stabilizes the transition state more than the initial state and thus enhances the exothermicity of the reaction. Unfortunately, at water contents >20% (v/v) **11a,b** is no longer completely dissolved but forms emulsions. Therefore, the incubation in 1:1 MP/water cannot be directly compared to the others. The conversion graph for this reaction mixture coincides with that for 9:1 MP/water (see Figure 4).

Because formation of **13** is favored in the presence of water, comparison of the aminolysis and the competing hydrolysis of **11** is of interest. We have therefore extracted the incubation mixtures with CH_2Cl_2 at the end of the reaction period and determined the triol content by GLC analysis of the respective trimethylsilyl derivatives. *tert*-Butyl (*E*)-9,10,13-trihydroxy-11-octadecenoate (**14**) and *tert*-butyl (*E*)-9,12,13-trihydroxy-10-octadecenoate (**15**) were synthesized from **11a,b**, as authentic standards, in a 0.1 N *p*-toluenesulfonic acid/THF mixture (see Figure 6). The acid-catalyzed hydrolysis proceeds via an S_N1 mechanism, yielding two regioisomers with the stereochemical correlation at C-9

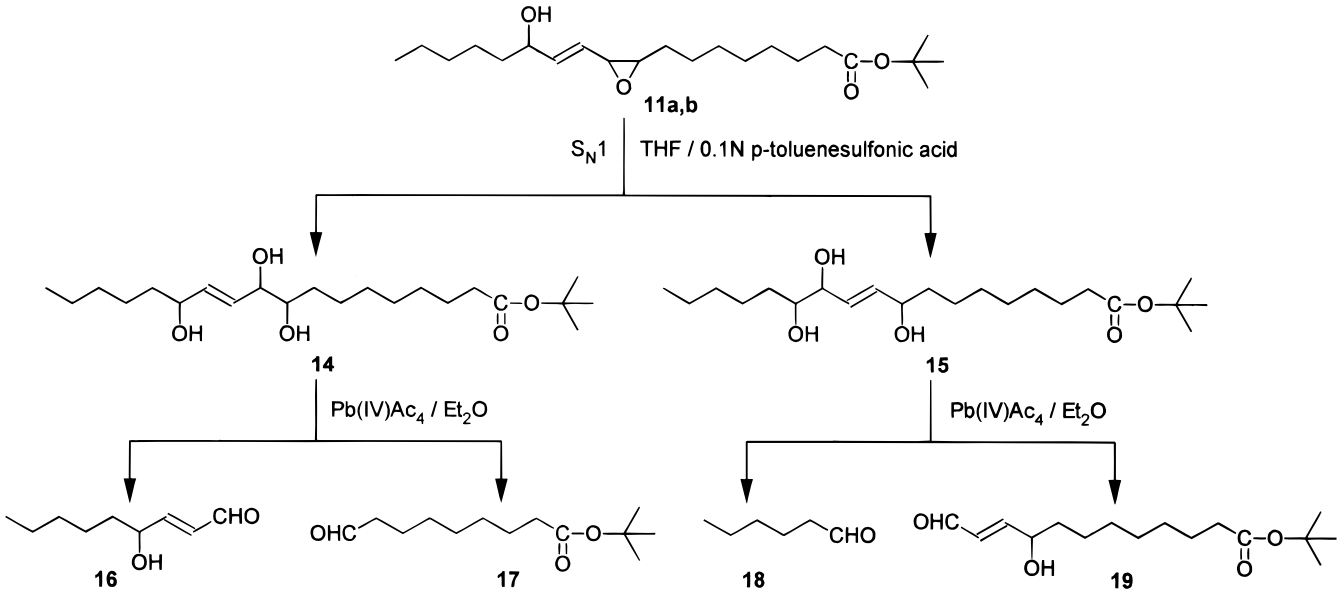


Figure 6. Acid-catalyzed hydrolysis of **11a,b**, yielding *tert*-butyl (*E*)-9,10,13-trihydroxy-11-octadecenoate (**14**) and *tert*-butyl (*E*)-9,12,13-trihydroxy-10-octadecenoate (**15**), with (*E*)-4-hydroxy-2-nonenal (**16**) and *tert*-butyl 9-oxononanoate (**17**) or hexanal (**18**) and *tert*-butyl (*E*)-9-hydroxy-12-oxo-10-dodecenoate (**19**) being formed by subsequent Pb(IV) tetraacetate cleavage of the triol **14** or **15**.

and C-10 lost in the intermediate allylic carbocation. We could not effectively separate this complex isomer mixture. The NMR data for the major diastereoisomer of **14**, listed in Table 1, were obtained from a fraction separated by LC on silica gel, which contained ~75% **14** besides ~25% **15**. Differentiation between the regioisomers **14** and **15** is not possible on the basis of the NMR data and rests on GLC analysis of the carbaldehydes **16**−**19**, obtained by Pb(IV) tetraacetate cleavage of the isolated fraction (see Figure 6). Unequivocal identification of the fragment structures **16**−**19** by GLC-MS confirms the location of the OH groups in **14** and **15**.

GLC analyses showed, <1, 3.2, 10.4, and 20.5% of the epoxide **11** to be hydrolyzed in incubations A−D after 8 weeks, respectively. As expected, conversion to the triols correlates with the water content of the reaction mixtures. However, except for incubation D, formation of the aminol **13** is enhanced to more or less the same extent. Aminolysis therefore must be considered as an important mode of interaction between amines and α,β-unsaturated epoxides. Interestingly, both regioisomers **14** and **15** were identified from all incubations by GLC-MS analysis of the carbaldehydes **16**−**19**, after Pb(IV) tetraacetate cleavage of an aliquot of the respective incubation. This implies that hydrolysis, other than aminol formation, at least partially takes an S_N1 course at pH 7.4.

STRUCTURAL ASSIGNMENT

Special effort was invested to definitely establish the structures of compounds **11a,b** and **13a**−**d** because the mechanistic arguments rest primarily on the respective product structures. The ^1H and ^{13}C NMR chemical shifts (δ) and coupling constants (J) of **7**−**11a,b** and **14** are collected in Table 1 and those of **13a**−**d** in Table 2.

In contrast to their precursors, compounds **10** and **11** show two sets of NMR signals with almost 1:1 relative intensity for several proton and carbon sites; in Table 1, the different chemical shifts or coupling constants are marked with index *b* or *c* for **10a,b** and **11a,b**, respectively. The acetoxy group at C-13 exerts a very effective control over the regioselectivity of the epoxidation, and it seems curious that this function does not influence the diastereoselectivity for the oxirane ring formation. Therefore, we have tested whether C-13 has suffered racemization during the synthetic procedure, following a method described by Hamberg (1991). The diastereoisomers **11a,b** were reacted with (−)-(1*R*)-mentyl chloroformate and the (−)-(1*R*)-menthoxycarbonyl (MC) derivatives isolated by TLC. These were oxidized with $KMnO_4$ in glacial acetic acid at 37 °C (Hamberg et al., 1986), and the trimethylsilyl derivatives of the resulting 2-({[(−)-(1*R*)-menthoxy]carbonyl}oxy)heptanoic acids were analyzed by CI-GLC-MS. The total ion current (TIC) chromatogram shows two peaks (1:50 relative intensity) with almost identical fragmentation patterns for the diastereoisomeric MC derivatives of trimethylsilyl 2-hydroxyheptanoate. Together with GLC analyses of **9** and **10a,b** at various chiral stationary phases (not detailed under Materials and Methods) that show no loss of stereochemical purity, this result definitely proves that no racemization has occurred at the C-13 stereogenic center in the synthesis of **11a,b**. From the NMR evidence, regio- and stereoselectivity of the Prilezhaev reaction is proven unequivocally: The configurations of the double bonds $C_9{=}C_{10}$ and $C_{11}{=}C_{12}$ in compounds **7**−**9** are definitely established as (*Z*) and (*E*), respectively, by the vicinal coupling constants ^3J(9-H, 10-H) = 10.9 Hz and ^3J(11-H, 12-H) = 15.2 Hz. Because the two residual olefinic protons in **10** are coupled by 15.6 Hz, the (*Z*) double bond must have been transformed to the oxirane. This assignment was validated by correlation spectroscopy (^1H,^1H-COSY). The 4.3 Hz value for the coupling constant between 9-H and 10-H in **10** and **11** constitutes an independent proof for epoxidation of the (*Z*)-configured double bond, via a true syn-addition mechanism, yielding a *cis*-configured oxirane. For an alternative *trans* orientation, as in **4**, a ^3J value of ~2 Hz is expected (Lederer, 1996; Gardner et al., 1974) The relative configuration at C-9 and C-10 therefore can only be (*R**/*S**), and due to the stereo-

genic center at C-13, **10** and **11** thus exist each as a pair of diastereoisomers. From the structure of compound **13**, the mechanism of the nucleophilic cleavage of the epoxide ring in **11a,b** can be derived in a straightforward manner. The 9-H and 10-H resonances in **13** were assigned on the basis of the respective connectivities for the proton spin system (positions 8−14) and by ^{1}H,^{1}H-COSY. The 9-H resonance (3.33 ppm for **13a,b** and 3.36 ppm for **13c,d**; see Table 2) appears to be less shielded by 0.4 ppm relative to that of the proton at C-10 (2.97, 2.99 ppm for **13a,b**, and 3.02, 3.03 ppm for **13c,d**, respectively), clearly establishing the OH function to be located at C-9 and the amine moiety to have been introduced at C-10. An allylic shift, via an S_N2' mechanism, is likewise ruled out definitely by the spectroscopic evidence. As mentioned above, preparative HPLC of **13** allows separation of this isomer mixture into two fractions. However, a number of resonances in the spectra of each fraction, marked with the index *b* in Table 2, appear doubled, indicating that no homogeneous compounds have been isolated and that **13** exists as two pairs of diastereoisomers, **13a**−**d**. Because the (*S*)-configuration at C-13 has been firmly established, the only remaining rationale is loss of stereochemical purity of the lysine moiety. Compound **12** in fact shows no optical rotation; that is, the chiral carbon has obviously suffered complete racemization in the course of the synthetic procedure. This agrees with the 1:1 relative intensity for the doubled resonances for the diastereoisomers **13a,b** and **13c,d**, respectively. We presume racemization to have occurred during the amide formation of N^2-acetyl-N^6-(*tert*-butoxycarbonyl)-L-lysine with 7-amino-4-methylcoumarin via an imidazolide, requiring incubation for 9 days at 50 °C (Lederer, 1996). According to Staab (1962), the loss of stereochemical purity is negligible for such processes only at low temperatures (−10 °C). Hence, the configuration of **12** had erroneously been given as pure (*S*) in the literature (Lederer, 1996).

CONCLUSIONS

The findings presented above clearly demonstrate that lysine moieties, incorporated in a protein, may react with γ-hydroxy-α,β-unsaturated epoxides generated in the course of lipid oxidation. Formation of aminol structures such as **13** (Figure 3) competes with hydrolysis of the epoxide; however, the reaction rate for both processes increases with the water content [up to 20% (v/v)] in our model incubations, and the respective products are formed in comparable extent. In foodstuffs with medium or low water activity, aminolysis of α,β-unsaturated epoxides by lysine moieties therefore must be considered as an important mode of interaction between proteins and lipid oxidation products. Because formation of aminols takes place under mild conditions (pH 7.4; 37 °C), lipoproteins may be expected to be especially susceptible to such transformations in vivo, due to the proximity of the respective reaction partners and the low water activity in the hydrophobic regions of such globular proteins. Further investigations are in progress to transfer the experiences of the model study to biological systems and to either verify or disprove the hypotheses outlined here.

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