preparation of novel epothilone derivatives. On the other hand, the necessary exchange of the 15-O-silyl protective groups is a clear disadvantage. As to the biological role of the epoxide in epothilone B (**1**), it appears doubtful that it is opened under physiological conditions because of the high stability of the oxirane. Rather, the epoxide may interact with the receptor unchanged or may be used in an intramolecular hydrogen bridge with the 3-OH function to generate a favorable conformation.[9]

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[1] a) Isolation of the epothilones: a) G. Höfle, N. Bedorf, K. Gerth, H. Reichenbach (GBF), DE-B 4138042, **1993** [*Chem. Abstr.* **1993**, *120*, 52841]; b) antitumor activity: D. M. Bollag, P. A. McQueney, J. Zhu, O.Hensens, L. Koupal, J. Liesch, M. Goetz, E. Lazarides, C. M. Woods, *Cancer Res.* **1995**, *55*, 2325; c) review of the chemistry and biology of the epothilones: K. C. Nicolaou, F. Roschangar, D. Vourloumis, *Angew. Chem.* **1998**, *110*, 2120; *Angew. Chem. Int. Ed.* **1998**, *37*, 2014.

[2] a) D.-S. Su, A. Balog, D. Meng, P. Bertinato, S. J. Danishefsky, Y.-H. Zheng, T.-C. Chou, L. He, S. B. Horwitz, *Angew. Chem.* **1997**, *109*, 2178; *Angew. Chem. Int. Ed. Engl.* **1997**, *36*, 2093; b) K. C. Nicolaou, N. Wissinger, J. Pastor, S. Ninkowic, F. Sarabia, Y. He, D. Vourloumis, Z. Yang, T. Li, P. Giannakakou, E. Hamel, *Nature* **1997**, *387*, 268.

[3] a) D.-S. Su, D. Meng, P. Bertinato, A. Balog, E. J. Sorensen, S. J. Danishefsky, Y. H. Zheng, T.-C. Chou, L. He, S. B. Horwitz, *Angew. Chem.* **1997**, *109*, 775; *Angew. Chem. Int. Ed. Engl.* **1997**, *36*, 757; b) K. C. Nicolaou, S. Ninkovic, F. Sarabia, D. Vourloumis, Y. He, H. Vallberg, M. R. V. Finlay, Z. Yang, *J. Am. Chem. Soc.* **1997**, *119*, 7974; c) D. Meng, P. Bertinato, A. Balog, D.-S. Su, T. Kamenecka, E. J. Sorensen, S. J. Danishefsky, *J. Am. Chem. Soc.* **1997**, *119*, 10073; d) S. A. May, P. A. Grieco, *Chem. Commun.* **1998**, 1597; e) D. Schinzer, A. Bauer, J. Schieber, *Synlett* **1998**, 861; f) A. Ballog, C. Harris, K. Savin, X.-G. Zhang, T.-C. Chou, S. J. Danishefsky, *Angew. Chem.* **1998**, *110*, 2821; *Angew. Chem. Int. Ed.* **1998**, *37*, 2675; g) J. Mulzer, A. Mantoulidis, E. Öhler, *Tetrahedron Lett.* **1998**, *39*, 8633; h) J. D. White, R. G. Carter, K. F. Sundermann, *J. Org. Chem.* **1999**, *64*, 684.

[4] review: H. C. Kolb, M. S. VanNieuwenzhe, K. B. Sharpless, *Chem. Rev.* **1994**, *94*, 2483.

[5] *ent*-**15**: W. Oppolzer, D. Dupuis, G. Poli, T. M. Raynham, G. Bernardinelli, *Tetrahedron Lett.* **1988**, *29*, 5885.

[6] W. Oppolzer, G. Poli, *Tetrahedron. Lett.* **1986**, *27*, 4717.

[7] a) Review: J. Gorzynski Smith, *Synthesis* **1983**, 629; epoxides as intermediates in natural product syntheses: b) Monensin: G. Schmid, T. Tukuyama, K. Akasaka, Y. Kishi, *J. Am. Chem. Soc.* **1979**, *101*, 259; c) lonomycin A: D. A. Evans, A. M. Ratz, B. E. Huff, G. S. Sheppard, *J. Am. Chem. Soc.* **1995**, *117*, 3448; d) brevetoxin: K. C. Nicolaou, *Angew. Chem.* **1996**, *108*, 644; *Angew. Chem. Int. Ed. Engl.* **1996**, *35*, 589. Regarding the stability of "early" epoxides in total synthesis see: e) E. J. Corey, B. B. Snider, *J. Am. Chem. Soc.* **1972**, *94*, 2549; f) E. J. Corey, J. P. Dittami, *J. Am. Chem. Soc.* **1985**, *107*, 256; g) D. A. Evans, A. S. Kim, R. Metternich, V. J. Novack, *J. Am. Chem. Soc.* **1998**, *120*, 5921.

[8] G. Höfle, N. Glaser, M. Kiffe, H. J. Hecht, F. Sasse, H. Reichenbach, *Angew. Chem.* **1999**, *111*, 2090; *Angew. Chem. Int. Ed.* **1999**, *38*, 1971.

[9] M. Wang, X. Xia, Y. Kim, D. Hwan, J. M. Jansen, M. Botta, D. C. Liotta, J. P. Snyder, *Org. Lett.* **1999**, *1*, 43.

Identification of Toxic 2,4-Decadienal in Oxidized, Low-Density Lipoprotein by Solid-Phase Microextraction

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9-Hydroxy-10,12-octadecadienoic acid (9-HODE) induces the liberation of interleukin-1β (IL-1β) together with α,β-unsaturated aldehydes, especially 2,4-decadienal, from macrophages.[1] IL-1β in turn stimulates the proliferation of smooth muscle cells.[2, 3] This process is regarded as being connected to atherogenesis,[1] since particularily high levels of IL-1β were detected in atherosclerotic plaques.[4] 2,4-Decadienal was detectable only in trace quantities after copper(II) ion induced air oxidation of low-density lipoprotein (LDL).[1] In addition, this detection required long separation procedures and preparation of the 2,4-dinitrophenylhydrazone derivative.[1, 5]

A detection method which needs neither sample procession nor derivatization is solid-phase microextraction (SPME).[6, 7] It also avoids the formation of artifacts by handling. Electron impact mass spectrometry (EI-MS) is excellent for the characterization of α,β-unsaturated aldehydes. We used the combination of SPME/EI-MS to obtain insight into the events proceeding in the artificial oxidation of LDL: Blood samples were withdrawn from volunteers and LDL was isolated immediately.[9] The LDL thus obtained was oxidized by air after addition of catalytic amounts of $CuSO_4$. Samples were collected in time intervals and analyzed by GC/MS. The measurement of the compounds present is achieved by determining the total ion current. Such a chromatogram is reproduced in Figure 1.

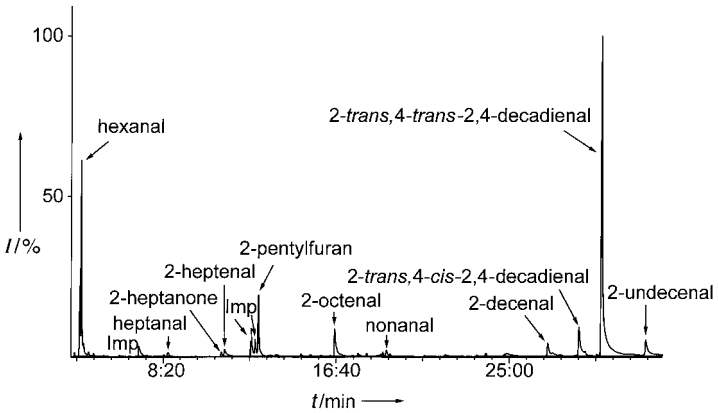

Figure 1. Reconstituted ion current chromatogram (RIC) of a LDL sample after 24 h oxidation with 50 μM $CuSO_4$ solution at 37 °C.

LDL contains different amounts of individual antioxidants which are consumed first. Therefore, some time (lag-time) is required before lipid peroxidation starts.[10] This lag-time also

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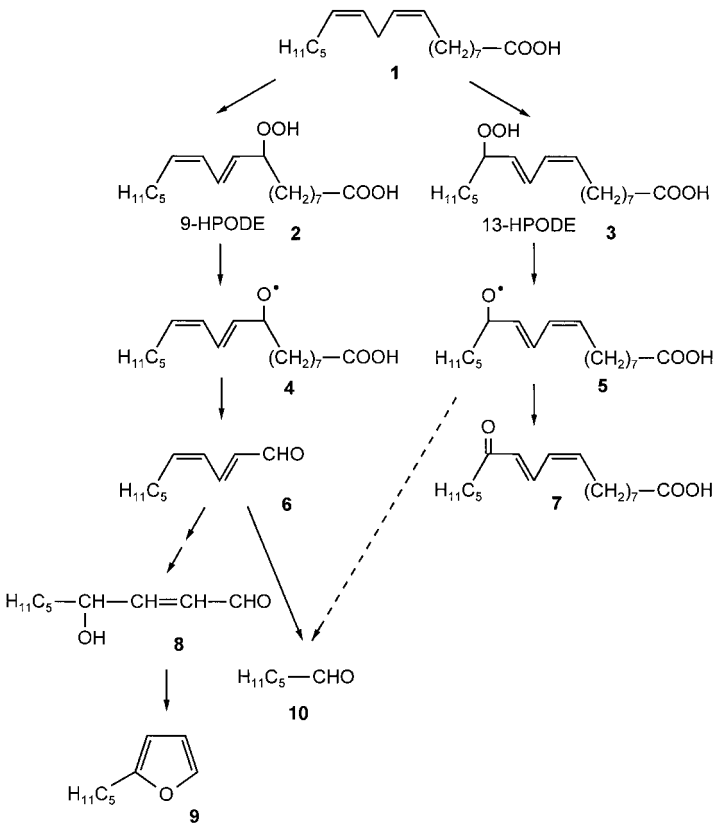

Scheme 1. Processes occuring in the oxidation of linoleic acid (**1**, from LDL samples) with air/Cu^{2+} ions.

depends on the amount of added copper ions. It amounted in our experiments to 2–5 h. Immediately after the start of oxidation the only main oxidation product is 2-*trans*,4-*cis*-2,4-decadienal (**6** in Scheme 1). Additional products, for example, 2-*trans*,4-*trans*-2,4-decadienal, hexanal (**10** in Scheme 1, and 2-pentylfuran (**9** in scheme 1), are detectable merely in trace amounts at this time. Within 2 h the peak corresponding to 2-*trans*,4-*trans*-2,4-decadienal becomes the main peak, and also the peaks corresponding to hexanal (**10**) and 2-pentylfuran (**9**) increase considerably in intensity. After 12 h these peaks are several times larger than that of 2-*trans*,4-*cis*-2,4-decadienal. In addition, small peaks are recognized which correspond to 2-octenal, 2-decenal, and 2-undecenal (the two latter compounds are oxidation products of oleic acid). After 24 h the chromatogram reproduced in Figure 1 was obtained.

After oxidation for 36 h 2-*trans*,4-*trans*-2,4-decadienal was still recognizable as the main peak, nevertheless the amounts of hexanal (**10**) and 2-pentylfuran (**9**) had increased further. After five days the product spectrum had changed a great deal: 2-*trans*,4-*trans*-2,4-decadienal was detectable in trace amounts only, the main peaks corresponded to **9** and **10**. Many additional peaks were also recognized. Since adsorption of compounds at the SPME fibre is not proportional to the quantity present, the intensity ratio recognized in successive runs do not reflect a quantitative picture of the compounds present. Nevertheless—according to model experiments—half quantitative deductions are possible, which allow insight into the kinetics of lipid peroxidation (LPO). Such measurements have been previously impossible or were at least not possible in such a mild and simple way with other methods. The observation of 2-*trans*,4-*trans*- 2,4-decadienal as the main peak in these experiments suggests that this compound is subjected to a fast degradation or reaction with other compounds present in LDL. In order to confirm this speculation the compounds detected in the chromatograms were subjected to a renewed oxidation in their pure form by air/Cu^{2+} ions.

The degradation of 2-*trans*,4-*trans*-2,4-decadienal, a well known fat aroma product, by air has already been investigated by Schreier et al.[11] They obtained 2-octenal, hexanal (**10**), 4-hydroxy-2-nonenal (4-HNE; **8** in Scheme 1), and 2-pentylfuran (**9**). We detected the same products in the above mentioned oxidation of LDL with Cu^{2+}/air, 4-HNE (**8**) admittedly only in trace quantities. However, **8** was detectable in larger amounts after derivatization with pentafluorobenzylhydroxylamine hydrochloride.[12] This result can be explained since 4-HNE is relatively polar and polar compounds are much less prone to adsorption than unpolar aldehydes on the SPME fibre used by us. When pure *trans*-4-hydroxy-2-nonenal was stirred in air with copper(II) ions the main peak was found to correspond to 2-pentylfuran (**9**). In contrast, 4-HNE remained unchanged on stirring for a long time in the absence of bivalent metal ions. This result demonstrates that traces of bivalent metal ions catalyze the transformation of **8** to **9**.

A main degradation path of 2-*trans*,4-*cis*-2,4-decadienal (**6**) leads to hexanal (**10**). Its generation has been previously deduced by cleavage of 9-*cis*,11-*trans*-13-hydroperoxy-9,11-octadecadienoic acid (13-HPODE; **3** in Scheme 1),[13] although this process seemed doubtful for energetic reasons.[14, 15] 10-*trans*,12-*cis*-9-Hydroperoxy-10,12-octadecadienoic acid (9-HPODE; **2** in Scheme 1) and 13-HPODE (**3**) are generated in comparable amounts by LPO of linoleic acid (**1** in Scheme 1). Cleavage of **2** generates 2-*trans*,4-*cis*-2,4-decadienal (**6**). Thus the enormous prevalence of **6** over hexanal (**10**) immediately after the start of LPO demonstrates that **10** is generated directly by cleavage of small amounts of **3** only.

In conclusion the following sequence of events is deduced: First linoleic acid (**1**) is transformed by oxidation to 9-HPODE (**2**) and 13-HPODE (**3**; Scheme 1).[14, 15] Cleavage of the peroxyl bond in **2** by copper(II) ions generates the radical **4**, which then decomposes to 2-*trans*-4-*cis*-2,4-decadienal (**6**). In contrast **3** reacts to form radical **5** and further to 9-*cis*,11-*trans*-13-oxo-9,11-tridecadienoic acid (**7**). The latter (polar compound) is not detectable by the SPME method used by us.

In the presence of peroxyl radicals **6** undergoes an analogous Baeyer–Villiger oxidation[16] and is epoxidized. Opening of the epoxide ring finally generates 4-HNE (**8**), which is then cyclized to 2-pentylfuran (**9**). Otherwise **6** is cleaved in the course of a retroaldol reaction after addition of water[17] to form hexanal (**10**). The cyclization of 4-HNE (**8**) to 2-pentylfuran (**9**) described in Scheme 1 is thus similar to a method used for the synthesis of trisubstituted furans.[18]

α,β-unsaturated aldehydes are starting materials for Michael reactions. 2-*trans*,4-*trans*-2,4-Decadienal, which has a higher toxicity than 4-HNE (**8**),[19] is expected, as a result of

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its larger conjugated system, to react faster than **8**. Indeed, 2-*trans*,4-*trans*-2,4-decadienal reacts quickly with glutathione to give a large number of products.[20] It is, therefore, understandable that this compound so far escaped detection in blood samples of atherosclerotic patients.

LPO processes are increased by tissue injury, for example, in the course of a myocardial infarction.[21] As a consequence, it is expected that the early oxidation processes described in this paper should also occur in vivo.[22]

Experimental Section

LDL was isolated according to Leiss et al.,[9] or was obtained commercially (Fluka). LDL, obtained from 3–5 mL serum, was dissolved in 1 mL of phosphate buffer (0.1M, pH 7.4) in a GC vial fitted with a stirring bar. 1 mL of a 10 or 100 μM aqueous solution of $CuSO_4$ was added. The vial was closed with a septum, and the mixture stirred at 37 °C. The septum was penetrated by the SPME device and the 100 μm fibre (polydisiloxane, PDMS) half submerged in the solution. After 2 h, 3 h, 4 h, 5 h, 6 h, 10 h, 12 h, 1 d, 2 d, 4 d, 7 d the fibre was removed from the vial and directly introduced into the inlet of the GC/MS instrument (MAT 95 mass spectrometer (Finnigan), EI ion source, 70 eV, HP 5890 series II gas chromatograph, fused silica gel column from J&W Scientific, DB 05, 30 m × 0.32 mm, carrier gas H_2, splitless, injector temperature 280 °C, temperature program: 5 min isothermic at 40 °C, then 3 °C min^{-1} up to 300 °C). The usual flushing of the septum by purge gas prevents detection of compounds with high volatility. In order to avoid this, flushing of the septum was shut down for 30 s when the PDMS fibre was introduced. Use of a special, narrow GC liner tube allowed adjustment of the peak form of the chromatograms and resulted in a considerable improvement of the quality of the mass spectra.

Model oxidations with pure 2,4-decadienal and 4-hydroxy-2-nonenal were carried out in a similar manner as those of LDL samples.

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[1] C. E. Thomas, R. L. Jackson, D. F. Ohlweiler, G. Ku, *J. Lipid Res.* **1994**, *35*, 417–427.
[2] E. W. Raines, S. K. Dower, R. Ross, *Science* **1989**, *243*, 393–396.
[3] G. Ku, N. S. Doherty, J. A. Wolos, R. L. Jackson, L. F. Schmidt, D. Hendricks, *Am. J. Cardiol.* **1988**, *62*, 77B–81B.
[4] A. M. Wang, M. V. Doyle, D. Mark, *Proc. Natl. Acad. Sci. USA.* **1989**, *86*, 9717–9722.
[5] H. Esterbauer, K. H. Cheeseman, *Methods Enzymol.* **1990**, *186*, 407–421.
[6] C. L. Arthur, L. M. Killam, K. D. Buchholz, J. Pawliszyn, J. R. Berg, *Anal. Chem.* **1992**, *64*, 1960–1966.
[7] Z. Zhang, J. Pawliszyn, *Anal. Chem.* **1993**, *65*, 1843–1845.
[8] G. Spiteller, W. Kern, P. Spiteller, *J. Chromatogr. A* **1999**, *843*, 29–98.
[9] O. Leiss, U. Murawski, H. Egge, *J. Clin. Chem. Clin. Biochem.* **1979**, *17*, 619–625.
[10] H. Esterbauer, G. Jürgens, O. Quehenberger, E. Koller, *J. Lipid Res.* **1987**, *28*, 495–509.
[11] B. Grein, M. Huffer, G. Scheller, P. Schreier, *J. Agric. Food Chem.* **1993**, *41*, 2385–2390.
[12] F. J. G. M. Van Kuijk, D. W. Thomas, R. J. Stephens, E. A. Dratz, *Biochem. Biophys. Res. Commun.* **1986**, *139*, 144–149.
[13] H. Esterbauer, H. Zollner, R. J. Schaur in *Membrane Lipid Oxidation*, *Vol. II* (Ed.: C. Vigo-Pelfrey), CRC, Boca Raton, FL, USA, **1991**, Chap. 11, pp. 239–268.
[14] H. W. Gardner, *Free Radicals Biol. Med.* **1989**, *7*, 65–86.
[15] E. N. Frankel, *Prog. Lipid Res.* **1982**, *22*, 1–33.
[16] W. A. Pryor, N. A. Porter, *Free Radicals Biol. Med.* **1990**, *8*, 541–543.
[17] D. B. Josephson, R. C. Lindsay, *J. Food Sci.* **1987**, *52*, 1186–1218.
[18] P. Lee, H. S. Ho Kim, S. Kim, *Chem. Lett.* **1994**, *12*, 2401–2402.
[19] T. Kaneko, S. Honda, S. I. Nakano, M. Matsuo, *Chem. Biol. Interact.* **1987**, *63*, 127–137.
[20] M. Spiteller, Universität Dortmund, private communication, **1999**.
[21] A. Dudda, G. Spiteller, F. Kobelt, *Chem. Phys. Lipids* **1996**, *82*, 39–51.
[22] Note added in proof (30. December 1999): Recently A. Miralto et al., reported antiproliferative and apoptotic effects of 2-*trans*, 4-*trans*-2,4-decadienal on human carcinoma cells. (A. Miralto, G. Barone, G. Romano, S. A. Poulet, A. Ianora, G. L. Russo, I. Buttino, G. Mazzarella, M. Laabir, M. Cabrini, M. G. Giacobbe, *Nature* **1999**, *402*, 173–176.)

Oxidation of Linoleic Acid in Low-Density Lipoprotein: An Important Event in Atherogenesis**

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Half of all deaths in Germany are caused by vascular diseases,[1] which are caused in part by deposition of cholesterol-rich plaques in the arteries. Cholesterol, an essential compound in cell membranes, is transfered to the cells by the low-density lipoprotein (LDL). LDL consists of a lipid core composed mainly of cholesterol esters. This core is surrounded by a layer of phospholipids that are wrapped by a protein. Lysyl residues of that protein are recognized by receptors that are arranged in so-called coated pits at the cell surface. The entire coated pit together with receptor-bound LDL molecules is then enclosed by a protein and transported inside the cell where the LDL is separated from the receptor. The liberated LDL is digested: proteins are broken down to amino acids and cholesterol esters and phospholipids are saponified. Depending on the need for cholesterol more or less receptors are generated.[2, 3]

Oxidatively modified LDL[4, 5] is no longer recognized by the LDL receptor but is recognized by scavenger receptors of macrophages. LDL is taken up without limitation by macrophages,[2, 3] which are finally deposited as plaques. LDL is very sensitive to oxidation, which occurs by copper(II) ion catalysis in air[5, 6] or on its own in three to six months when stored at 0 °C.[7] Oxidatively modified LDL (oxLDL) is toxic[4] and the toxic components can be extracted with organic solvents.[5] Thus it was suspected that the toxic compounds might be the products of lipid peroxidation (LPO) of polyunsaturated fatty acids (PUFAs).[4] Some of these products were identified,[8, 9, 10] but most remained unknown until now.

Since LPO products occur in LDL in only trace amounts compared to other compounds their identification requires

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