12-Oxo-LTB$_4$, a Key Pivotal Intermediate in LTB$_4$ Metabolism

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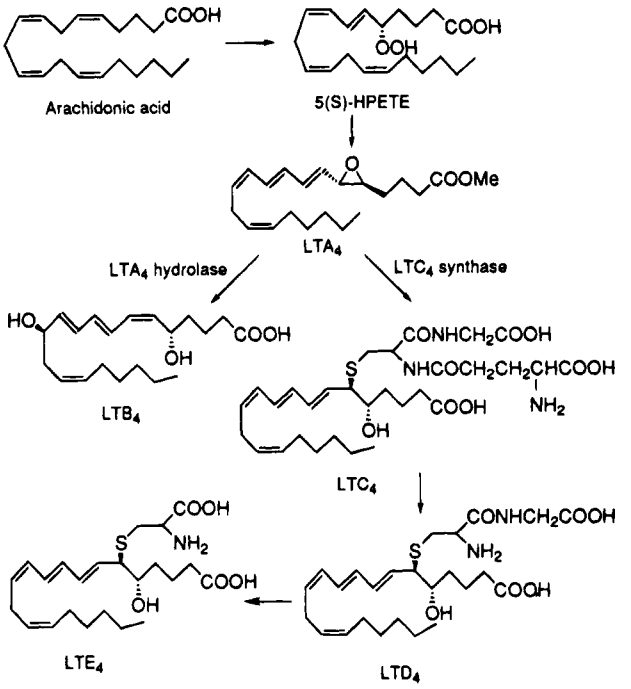
The first total synthesis of the very unstable and elusive biochemical intermediate 5(*S*)-hydroxy-12-oxo-6(*Z*),8(*E*),10(*E*),14(*Z*)-eicosatetraenoic acid (12-oxo-LTB$_4$) (**2**) has been accomplished. Incubation of the synthetic 12-oxo-LTB$_4$ (**2**) with human keratinocytes produced 5(*S*),12-dihydroxy-6-glutathionyl-7(*E*),9(*E*),14(*Z*)-eicosatrienoic acid (c-LTB$_3$) and 5(*S*),12-dihydroxy-6-cysteinylglycyl-7(*E*),9(*E*),14(*Z*)-eicosatrienoic acid (d-LTB$_3$), two new intriguing mediators which have been identified recently during the incubations of LTB$_4$ with keratinocytes. We have also confirmed the identity of 12-oxo-LTB$_4$ prepared from porcine leukocytes by comparison with the synthetic material and identified the formation in these incubates of the 6-*trans*-isomer of 12-oxo-LTB$_4$.

Introduction

The 5-LO-mediated transformation of arachidonic acid yields two products, 5-hydroperoxyeicosatetraenoic acid (5-HPETE) and 5(*S*),6(*R*)-*trans* 5,6-epoxy-7,9-*trans*-11,-14-*cis*-eicosatetraenoic acid (LTA$_4$). The enzyme is unusual in the sense that it does mediate the two steps, the oxygenation of arachidonic acid at the 5-position and the dehydration to yield the LTA$_4$, Scheme 1.

A great deal of work has been done by us and other groups on the 5-LO and its translocation mechanism.[1] LTA$_4$ is a substrate to two enzymes. The first, LTA$_4$ hydrolase, produces 5(*S*),12(*R*)-dihydroxy-6,14-*cis*-8,10-*trans*-eicosatetraenoic acid (LTB$_4$) a potent chemotactic factor for proinflammatory cells such as polymorphonuclear leukocytes (PMNL) and is implicated in the pathology of psoriasis, inflammatory bowel diseases, and arthritis; the second, LTC$_4$ synthase, only very recently identified, isolated, sequenced and cloned,[2] produces 5(*S*)-hydroxy-6(*R*)-glutathionyl-7,9,11-*trans*-14-*cis*-eicosatetraenoic acid (LTC$_4$), 5(*S*)-hydroxy-6(*R*)-cysteinylglycyl-7,9,11-*trans*-14-*cis*-eicosatetraenoic acid (LTD$_4$), and 5(*S*)-hydroxy-6(*R*)-cysteinyl-7,9,11-*trans*-14-*cis*-eicosatetraenoic acid (LTE$_4$), which are potent smooth muscle constrictors in the lung and vasculature. The peptido leukotrienes are implicated in asthma as the main bronchoconstrictive agents and rhinitis by vasoconstriction of the microvasculature. LTB$_4$ and LTC$_4$ exert their biological effects through dedicated high affinity receptors. Drugs inhibiting the formation of LTC$_4$ and LTB$_4$

Scheme 1



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(1) (a) Samuelsson, B.; Rouzer, C. A.; Matsumoto, T. *Adv. Prostaglandin, Thromboxane Leukotriene Res.* **1987**, *17*, 1. (b) Miller, D. K.; Gillard, J. W.; Vickers, P. J.; Sadowski, S.; Leveille, C.; Mancini, J. A.; Charleson, P.; Dixon, R. A. F.; Ford-Hutchinson, A. W.; Fortin, R.; Gauthier, J. Y.; Rodkey, J.; Rosen, R.; Rouzer, C.; Sigal, I. S.; Strader, C. D.; Evans, J. F. *Nature* **1990**, *343*, 278. (c) Dixon, R. A. F.; Diehl, R. E.; Opas, E.; Rands, E.; Vickers, P. J.; Evans, J. F.; Gillard, J. W.; Miller, D. K. *Nature* **1990**, *343*, 282. (d) Gillard, J. A.; Ford-Hutchinson, A. W.; Chan, C.; Charlson, S.; Denis, D.; Foster, A.; Fortin, R.; Leger, S.; McFarlane, C. S.; Morton, H.; Piechuta, D.; Riendeau, D.; Rouzer, C. A.; Rokach, J.; Young, R.; MacIntyre, D. E.; Peterson, L.; Bach, T.; Eiermann, G.; Hopple, S.; Humes, J.; Hupe, L.; Luell, S.; Metzger, J.; Meurer, R.; Miller, D. K.; Opas, E.; Pacholok, S. *Can. J. Physiol. Pharmacol.* **1989**, *67*, 456. (e) Rouzer, C. A.; Ford-Hutchinson, A. W.; Morton, H. E.; Gillard, J. W. *J. Biol. Chem.* **1990**, 1436.

(2) (a) Nicholson, D. W.; Klemba, M. W.; Rasper, D. M.; Metters, K. M.; Zamboni, R. J.; Ford-Hutchinson, A. W. *Eur. J. Biochem.* **1992**, *209*, 725. (b) Nicholson, D. W.; Ali, A.; Vaillancourt, J. P.; Calaycay, J. R.; Mumford, R. A.; Zamboni, R. J.; Ford-Hutchinson, A. W. *Proc. Natl. Acad. Sci. U.S.A.* **1993**, *90*, 2015. (c) Lam, B. K.; Penrose, J. F.; Freeman, G. J.; Austen, K. F. *Proc. Natl. Acad. Sci. U.S.A.* **1994**, *91*, 7663. (d) Welsch, D. J.; Creely, D. P.; Hauser, S. D.; Mathis, K. J.; Isakson, P. C. *Proc. Natl. Acad. Sci. U.S.A.* **1994**, *91*, 9745.

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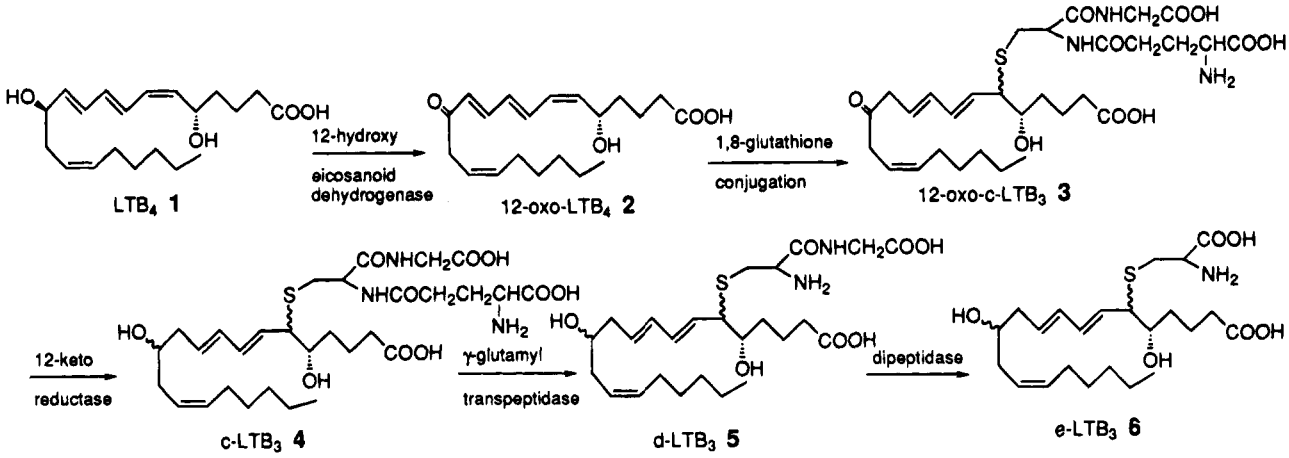
Scheme 2



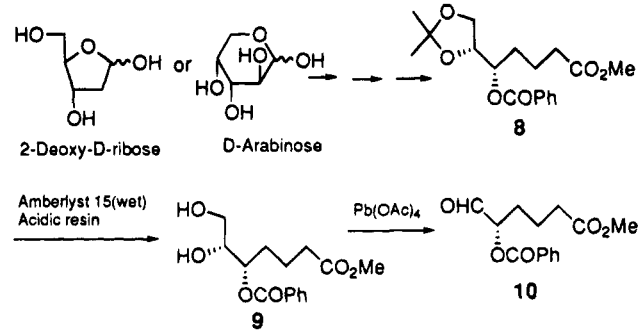
are presently in advanced phase clinical trials. The two enzymes are localized in separate cells (with rare exceptions, such as the macrophages) and perform separate physiological functions and exert different pathological effects. Recently, however, we have shown in a preliminary report[3] that this situation may be changing and that the two branches of the 5-LO pathway may be intertwined. A proposal was put forward explaining the formation of **4**–**6** from LTB$_4$, Scheme 2.

This result was arrived at by incubating radioactive LTB$_4$ with keratinocytes, and the identity of the products **4**–**6** were determined by HPLC and mass spectrometric techniques. Compounds **2** and **3** are logical intermediates for the formation of **4**. As can be seen from the Scheme 2, compound **4** is a structural hybrid of LTB$_4$ and LTC$_4$. It has a C$_{20}$ skeleton and carbon 1–10 fragment is identical to LTC$_4$. Earlier structure–activity studies show that this is the critical part for the LTC$_4$ biological activity. For example, LTC$_2$, a structural analog of LTC$_4$ in which the double bonds at 11 and 14 are saturated, has smooth muscle contracting activity similar to that LTC$_4$.[4] On the other hand, carbons 1–5 and 12–20 are identical to LTB$_4$, notwithstanding the unknown stereochemistry at C$_{12}$. It is still too early to tell if these molecules are going to display the biological activity of both LTC$_4$ and LTB$_4$. We found this new pathway not only fascinating, but also bearing a direct relationship to the handling of the design of drugs to interfere with the two branches of the 5-LO pathway. In addition to its implication in this new pathway of metabolism of LTB$_4$ in keratinocytes, **2** has been identified in subcellular fraction of porcine leukocytes as a product of a specific dehydrogenase enzyme.[5]

We embarked on a synthetic program, the purpose of which was to identify the missing components **2** and **3** in the proposal presented in Scheme 2, secure the original structural assignment of all intermediates, determine their stereochemistry, and study their biological properties and the interaction of these molecules on the LTB$_4$ and LTC$_4$ receptor-mediated assays.

We decided on a synthetic strategy which would mimic the anticipated biochemical events as shown in Scheme 2. We selected the dehydro-LTB$_4$ **2** as our first target.

Scheme 3



From there we will in time proceed synthetically and biochemically to unravel this pathway in keratinocytes first and then in other inflammatory cells.

Results

We suspected compound **2** to be extremely unstable. We had just completed the synthesis of 12-oxo-ETE (12-KETE)[6] (**7**), and we found it to be the most unstable molecule we have had to handle in leukotriene chemistry.

We figured that adding an extra conjugated double bond as in **2** would only increase the instability of the molecule. Our synthetic approach is outlined in Schemes 3–5. From the outset we judged the protection of the carbonyl group a must and the removal of the protecting group to be the last step in the synthesis. The dithiane (Scheme 4) was selected as starting material because of the ready availability and the various options to introduce functionality and deprotection.

We selected the aldehyde synthon **10** as the precursor of the stereogenic portion of the molecule **2** and it was synthesized from **8**, as shown in Scheme 3. At first we prepared compound **8** from 2-deoxy-D-ribose[7] and subsequently from the cheaper D-arabinose.[8] We originally

(3) Wheelan, P.; Zirrolli, J. A.; Morelli, J. G.; Murphy, R. C. *J. Biol. Chem.* **1993**, *268*, 25439.
(4) Klotz, P.; Foucaud, B.; Goeldner, M. P.; Hirth, C. G. *J. Org. Chem.* **1993**, *58*, 1076.
(5) (a) Powell, W. S.; Gravelle, F. *J. Biol. Chem.* **1989**, 5364. (b) Powell, W. S. *Biochem. Biophys. Res. Commun.* **1987**, *145*, 991.
(6) Wang, S. S.; Rokach, J.; Powell, W. S.; Dekle, C.; Feinmark, S. J. *Tetrahedron Lett.* **1994**, *35*, 4051.
(7) Guinodon, Y.; Zamboni, R.; Lau, C. K.; Rokach, J. *Tetrahedron Lett.* **1982**, *23*, 739.
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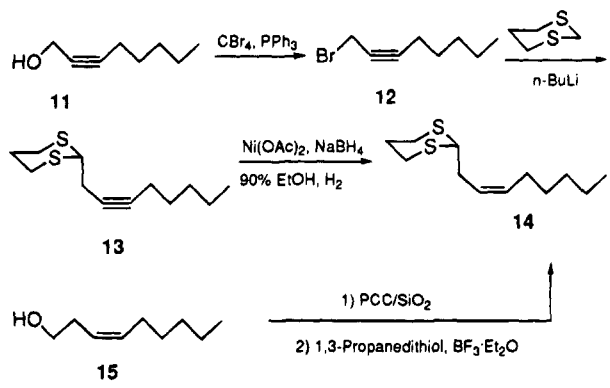
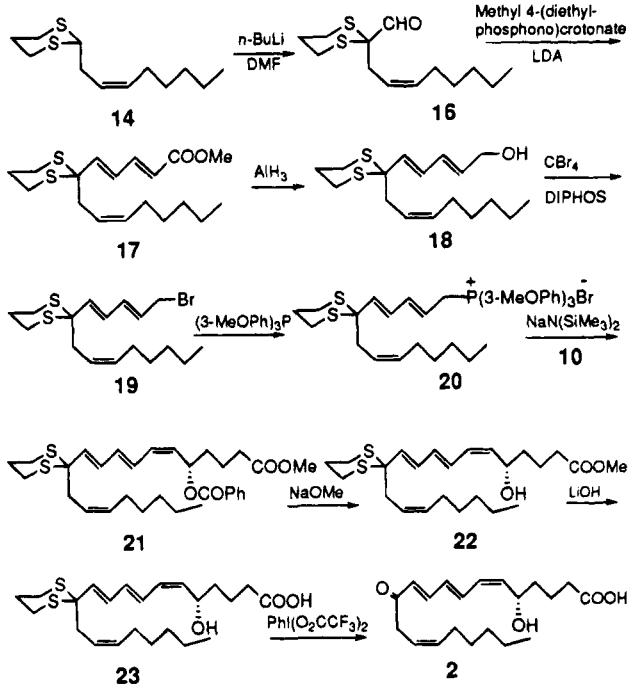
Scheme 4



Scheme 5



prepared the octenyl dithiane **14**, as shown, from hydroxy acetylene **11**, and have now more conveniently made it in a two-step, one-pot procedure in 60% yield from commercial alcohol **15** as shown in Scheme 4. Intermediate **13** will be useful when it becomes necessary to prepare radioactive precursors. Aldehyde **16** was prepared in 76% yield by formylation of **14** with dimethylformamide (DMF) (Scheme 5). The diene ester **17** was prepared *via* a Wittig reaction with commercial methyl 4-(diethylphosphono)crotonate in 92% yield. The reduction of the ester group with AlH_3 was clean, proceeding in high yield, with no contamination of double bond reduction products. The preparation of diene bromide **19** was tricky and all attempts to isolate the pure bromide **19** resulted in extensive decomposition. The combination of the high reactivity of the allyl bromide and the presence of sulfur in the molecule is responsible for the instability of this intermediate. We reacted the crude bromide **19** as soon as it was prepared with the triarylphosphine reagent. The diene phosphonium bromide **20** was obtained in 88% yield from the alcohol. It was stable and could be purified by column chromatography. The condensation step of **20** with aldehyde **10** proceeded smoothly to give **21** in 66% yield; 7% of the *trans* isomer **24** was also obtained. The use of the tris(3-methoxyphenyl)phosphonium bromide **20** is a good alternative in the Wittig condensation, considering that the best result we obtained with the most commonly used triphenylphosphonium bromide reagent was a 2:1 *cis:trans* ratio. We found that the two-step hydrolysis to yield **22** and then **23** was preferable to the one-step procedure. Minute amounts of unidentified byproducts seem to be produced during the one-step procedure. The deprotection of the dithiane group in **23** using bis(trifluoroacetoxy)iodobenzene[9] gave a 55% yield of **2** which was purified by normal phase HPLC to afford the desired pure target molecule. Electrospray ionization mass spectrum (ESI MS) showed *m/z* 333.2 (M − 1), which confirmed the molecular weight of **2**. The stereochemistry of the double bonds was assigned as 6*Z*,8*E*,10*E* from the coupling constants for the corresponding olefinic protons supported by decoupling experiment ($J_{10,11}$ = 15.5 Hz, $J_{8,9}$ = 14.5 Hz and $J_{6,7}$ = 11.0 Hz). The comparison of the coupling constants of **2** with those of parent compound **23** as well as precursors **22** and **21** indicated that the 6*Z*,8*E*,10*E* stereochemical geometry of the parent triene compound was retained in trienone **2**.

Along with the major product **2**, a certain amount of the 6-*trans* isomer **26** is always formed in an approximate *cis* to *trans* ratio of 5:1. The reverse phase chromatography we first used did not separate the two isomers. However, we developed normal phase HPLC conditions (4.5% 2-propanol, 0.1% acetic acid in hexane) which afforded excellent separation of the two isomers. The use of normal phase HPLC required a low concentration of acetic acid. At this point, the product is 100% pure and can be stored for a reasonable amount of time at −20 °C (a few days) and used for analytical purposes as such. However, in order to isolate the material on a preparative scale from the HPLC eluate, neutralization with triethylamine (TEA) was necessary. Complete decomposition results if the eluate is not treated with TEA before evaporation. The organic solvents (hexane, 2-propanol) were evaporated and the residue passed through a reverse phase flash column chromatography (C_{18} Bed 55-105 μM) 5 × 1 cm, eluting the product with ethanol. The pure 12-oxo-LTB_4 (**2**) can be kept in ethanol water at −20 °C for several weeks.

Incubation of the synthetic 12-oxo-LTB_4 (**2**) (1.9 μM) with cultured human keratinocytes resulted in the formation of two metabolites arising from initial glutathione conjugation of 12-oxo-LTB_4. These metabolites, c-LTB_3 (5(*S*),12-dihydroxy-6-glutathionyl-7(*E*),9(*E*),14(*Z*)-eicosatrienoic acid) (**4**) and d-LTB_3 (5(*S*),12-dihydroxy-6-cysteinylglycyl-7(*E*),9(*E*),14(*Z*)-eicosatrienoic acid) (**5**) were identified previously during incubation of keratinocytes with LTB_4.[3] Both c-LTB_3 and d-LTB_3 were identified in the present study by known retention times during reverse phase HPLC analysis and by the unique ultraviolet spectrum with λ_{max} at 232 nm and extended absorption to 270 nm. Additionally, the structures of these metabolites were confirmed by negative ion ESI MS which yielded mass spectra identical to those previously observed by negative ion fast-atom bombardment analysis. ESI MS analysis of the c-LTB_3 metabolite resulted in an observed molecular ion at *m/z* 642. CID of this ion produced ions at *m/z* 272 and at *m/z* 254 which arise from fragmentation of the glutathione moiety.[10] ESI MS analysis of d-LTB_3 revealed a molecular ion at *m/z* 513.

(9) Stork, G.; Zhao, K. *Tetrahedron Lett.* **1989**, *30*, 287.
(10) Rafferty, M. J.; Thorne, G. C.; Orkiszewski, R. S.; Gaskell, S. J. *Biomed. Environ. Mass Spectrom.* **1990**, *19*, 465.

CID of this ion resulted in observed ions at *m/z* 143, which is related to fragmentation of the cysteinylglycine moiety, and at *m/z* 229, $[C_5H_7CH(SH)CH(OH)(CH_2)_3CO_2^-]$. From these experiments it is clear that these unusual metabolites of LTB_4 formed by human keratinocytes involve 12-oxo-LTB_4 as the biochemical intermediate as postulated.

Porcine leukocytes were incubated with LTB_4 and 200 000*g* subcellular fractions extracted. HPLC analysis shows the presence of two peaks in approximately 1:1 ratio which were identified by comparison with authentic synthetic samples as 12-oxo-LTB_4 (**2**) and 12-oxo-6-*trans*-LTB_4 (**26**). It is the first report on the formation of **26** from biological sources.

Discussion

Synthesis of 12-oxo-LTB_4 (**2**) was designed to achieve two main objectives. The first was to check the proposal shown in Scheme 2 and verify if it is indeed the intermediate in the formation of c-LTB_3, a very unusual and intriguing metabolite, which is a hybrid between LTC_4 and LTB_4. The second is to confirm and ascertain the structure and stereochemistry of the 12-oxo-LTB_4 previously identified from biological sources.

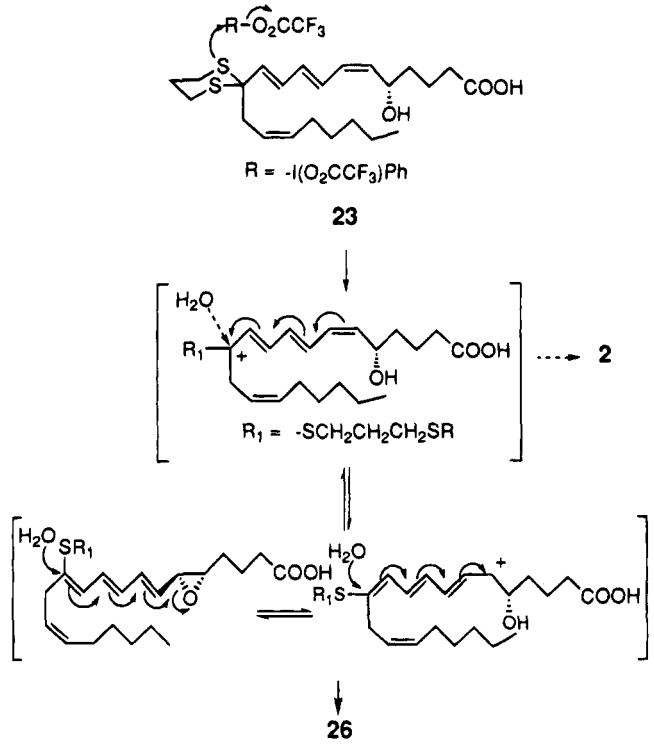
As was predicted, the deprotection of the carbonyl group in **23** to yield **2** proved to be by far the most challenging step in the synthesis and deserves some comment. A variety of reagents, reaction conditions, and times were used (oxone, chloramine T, nitrosylsulfinic acid, ceric ammonium nitrate, methyl iodide, 1,3-dibromo-5,5-dimethylhydantoin, NBS/barium carbonate, $NBS/AgNO_3$, $NCS/AgNO_3$), all of which were unsuccessful in obtaining the desired **2**. Recovery of products was monitored by UV expected to be in the 315–320 regions.[5,11] Acidic and basic workup conditions are detrimental to the product. The conditions which we used to deprotect 12-KETE dithiane,[6] NBS or NCS, $AgNO_3$ in acetone–water for a very short exposure time (between 15 and 45 s at −10 to −20 °C), resulted in decomposition products when applied to **23**.

Another problem which has complicated the recovery of the target molecule **2** is the fact that we had to use the free acid **23** and not its ester, which could have simplified the isolation and purification procedure substantially. We decided against this approach since basic conditions necessary to hydrolyze the ester of **2** would cause immediate enolization of the ketone to yield a pentaene conjugated system, scrambling of the 6- and 14-*cis*-double bonds and possible movement of the 14-olefin into conjugation with the ketone. In fact an attempted hydrolysis of the methyl ester of **2** using lithium hydroxide resulted in destruction of the product. HPLC analysis of the hydrolysates did not show any of the expected free acid **2** and instead showed numerous unidentifiable polar peaks.

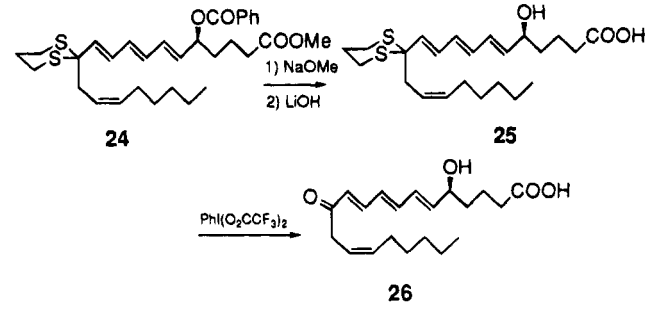
The formation of the 6-*trans* compound **26** in the deprotection step cannot be entirely prevented. We cannot exclude the possibility that it is partly formed during the hydrolysis of the dithiane functional group as an inherent part of the deprotection mechanism, such as shown in Scheme 6.

The *trans* isomer **26** has the same UV, λ_{max} 315 (hexane, 2-propanol, acetic acid), as the *cis* isomer **2**.

Scheme 6



Scheme 7



Because of this fact and since slight changes in column and HPLC solvent conditions will affect the retention time, it is recommended that synthetic standards be used for ascertaining the identity of 12-oxo-LTB_4 (*vide infra*). In order to obtain larger amounts of **26** for characterization purposes and for our biochemical studies and future biological evaluation, we used the pure intermediate **24** and hydrolyzed the dithiane group as described for the *cis* isomer **23**, Scheme 7.

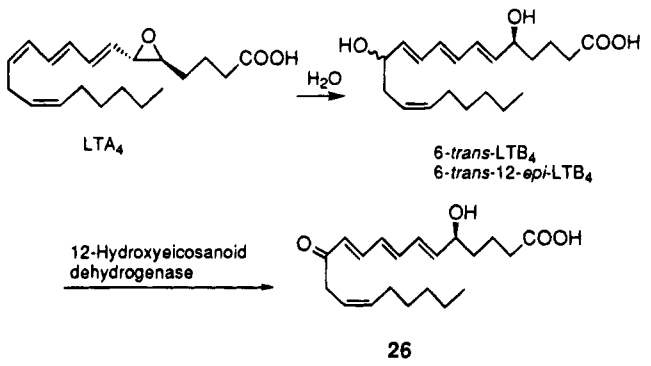
12-Oxo-eicosanoids are formed biologically by porcine neutrophils as intermediates in the conversion of LTB_4, 6-*trans* isomers of LTB_4, and 12-HETE to their 10,11-dihydro metabolites.[12,13] Although the major metabolite of LTB_4 by these cells would be expected to be the 6-*cis* isomer **2**, 12-oxo-6-*trans*-LTB_4 (**26**) was also detected in the present study by normal phase-HPLC after incubation of LTB_4 with microsomal fractions from porcine PMNL in the presence of NAD. It is not clear at this point whether the 6-*trans* compound was formed during the incubation itself, or was formed later during the manipulation of the sample. Further experiments will be required to clarify this point. In any event, 12-oxo-

(11) Yokomizo, T.; Izumi, T.; Takahashi, T.; Kasama, T.; Kobayashi, Y.; Sato, F.; Taketani, Y.; Shimizu, T. *J. Biol. Chem.* **1993**, *268*, 18128.

(12) Wainwright, S.; Powell, W. S. *J. Biol. Chem.* **1991**, *266*, 20899.

(13) Powell, W. S.; Gravelle, F. *Biochim. Biophys. Acta* **1990**, 1044, 147.

Scheme 8



26

6-*trans*-LTB_4 would be expected to be formed by oxidation of the naturally occurring isomers of LTB_4, 6-*trans*-LTB_4 and 12-*epi*-6-*trans*-LTB_4, by 12-hydroxyeicosanoid dehydrogenase,[5] Scheme 8. Although it is too early to speculate, the possibility exist that the 6-*trans* isomer **26** formed biologically is a reflection of the amount of LTA_4 present in the cells.

The synthesis of 12-oxo-LTB_4 is the first step in an effort to elucidate the biochemical steps of a novel metabolic pathway of LTB_4 in keratinocytes. It has allowed us so far to establish its intermediacy in the formation of the unique LTB_4 metabolites, c-LTB_3 (**4**) and d-LTB_3 (**5**). It has also allowed us to confirm the stereochemistry at carbon 5 which is as shown in Scheme 2 and is the same as the one in LTB_4. The stereochemistry of metabolites **4** and **5** at carbons 6 and 12 is still to be determined. Glutathione transferases add glutathione moiety onto a variety of substrates for detoxification purposes and are not usually stereoselective. A special glutathione-transferring enzyme, the LTC_4 synthase is selective for LTA_4 (Scheme 1). The nature of the enzyme coupling glutathione with 12-oxo-LTB_4 at carbon 6 and the stereochemistry at this center are not known. Whether the keratinocytes have a specific glutathione-S-transferase for this conjugation reaction as is the case for LTC_4 synthase[2] or if conjugation with glutathione is catalyzed by glutathione-S-transferases of broader specificity[14] is not yet known. Equally the stereochemical outcome of the ketoreductase reduction of the 12-oxo group is not known. The very high degree of structural analogy between the newly discovered metabolites, c-LTB_3 and d-LTB_3, and LTC_4 and LTD_4, warrants investigation into their biological properties, in particular following some recent reports on the involvement of LTC_4 and LTD_4 in the pathology of skin diseases. In addition to their proliferative effect on keratinocytes,[15] LTC_4 and LTD_4 have been shown to be potent mitogens for cultured human neonatal melanocytes.[16] LTC_4 also stimulates melanocyte migration *in vitro*[17] and causes growth stimulation of human adult melanocytes, loss of contact inhibition with formation of structures resembling tumor spheroids. Taken together such transformations of the melanocytes are thought to be precancerous and can eventually lead to melanomas.[18] UV exposure, one of the most common environmental causes of skin cancer, is known to be proinflammatory and to cause the release of eicosanoids, including the peptido leukotrienes LTC_4 and LTD_4.

This recent discovery of proinflammatory, proliferative, and potentially precancerous properties of LTC_4 and LTD_4 in the skin highlights the importance we attribute to c-LTB_3 and d-LTB_3 and to the study of their biological properties. Progress is ongoing in the biological evaluation of 12-oxo-LTB_4 (**2**), its 6-*trans* isomer **26**, as well as in the synthesis of the different isomers of c-LTB_3 and d-LTB_3.

Experimental Section

Reagents and Methods. Unless stated otherwise, all reagents and chemicals were obtained from commercial sources and used without further purification.

^1H and ^{13}C NMR spectra were recorded on a Bruker 360 MHz spectrometer in $CDCl_3$, C_6D_6, CD_3OD, or CD_3COCD_3; J values are given in hertz. All reactions were monitored by thin-layer chromatography carried out on 0.25 mm E. Merck silica gel plates (60F-254) using UV light, 5% ethanolic *p*-anisaldehyde or 10% ethanolic phosphomolybdic acid, and heat as developing agent. E. Merck 270-400 mesh silica gel was used for flash column chromatography. All reactions were carried out under an inert (nitrogen or argon) atmosphere with dry, freshly distilled solvents under anhydrous conditions unless otherwise noted. Yields refer to chromatographically and spectroscopically (^1H NMR) homogeneous materials, unless otherwise stated. The assignments of chemical shifts for the olefinic protons were supported by decoupling experiments. HPLC analyses and separations were carried out using a Waters 600 E pump with a Waters 994 programmable photodiode array variable wavelength UV detector. Analytical HPLC used Waters or spherisorb 5 μM silica (3.9 mm × 300 mm) column for normal-phase (NP) conditions and Waters μ bondpack C-18 columns for reverse-phase (RP) conditions. All HPLC columns were equipped with guard columns, and HPLC solvents were filtered through Waters (0.45-μm) filters.

(5*S*,6*R*)-Methyl 5-(Benzoyloxy)-6,7-*O*-isopropylideneheptanoate (8). The benzoate ester **8** was prepared in three steps using 2-deoxy D-ribose or in eight steps but using less expensive D-arabinose as starting material: ^1H NMR ($CDCl_3$) δ 1.35 (s, 6 H), 1.77 (m, 4 H), 2.37 (m, 2 H), 3.91 (t, J = 7.1 Hz, 1 H), 4.09 (t, J = 7.1 Hz, 1 H), 4.26 (q, J = 7.2 Hz, 1 H), 5.25 (m, 1 H), 7.45 (t, J = 7.1 Hz, 2 H), 7.58 (t, J = 7.3 Hz, 1 H), 8.05 (d, J = 7.6 Hz, 2 H); ^{13}C NMR ($CDCl_3$) δ 20.52, 25.17, 26.35, 30.25, 33.55, 51.44, 66.10, 73.58, 76.63, 109.64, 128.36, (2 C), 129.65, (2 C), 130.04, 133.06, 165.91, 173.53.

(5*S*,6*R*)-Methyl 5-(Benzoyloxy)-6,7-dihydroxyheptanoate (9). To a solution of acetonide **8** (6 g, 1.78 mmol) in methanol (50 mL) was added strongly acidic Amberlyst 15-(wet) ion-exchange resin (10 g), and the mixture was stirred at room temperature overnight. The resin was removed by filtration, and the filtrate was evaporated at reduced pressure to give the residue which was extracted with dichloromethane (3 × 100 mL). The combined organic layers were washed with brine (2 × 50 mL), dried over anhydrous Na_2SO_4, filtered, and concentrated *in vacuo*. The product was purified by flash column chromatography (8:2 hexane/acetone) to give the pure dihydroxy compound **9** (5.0 g, 95%): ^1H NMR (CD_3COCD_3) δ 1.60−1.90 (m, 4 H, C_3, C_4-H), 2.31 (t, J = 7.1 Hz, 2 H, C_2-H), 3.52 (m, 1 H), 3.55 (s, 3 H), 3.65 (m, 1 H), 3.71 (m, 1 H), 5.07 (m, 1 H), 7.40 (t, J = 7.6 Hz, 2 H), 7.52 (t, J = 7.0 Hz, 1 H), 7.98 (d, J = 8.2 Hz, 2 H); ^{13}C NMR ($CDCl_3$) δ 20.54, 30.02, 33.51, 51.59, 62.52, 72.73, 74.10, 128.49, 129.45, 129.75, 133.44, 167.0, 173.90.

Methyl 5(*S*)-(Benzoyloxy)-5-formylpentanoate (10). To a mixture of diol **9** (439 mg, 1.48 mmol) and anhydrous sodium carbonate (471 mg, 4.45 mmol, 3 equiv) in anhydrous dichloromethane (10 mL) was added lead tetraacetate (723 mg, 1.628 mmol, 1.1 equiv) at −40 °C. The mixture was stirred at −40 °C for 20 min and then allowed slowly to warm to −30 °C over

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a period of 10 min. The reaction mixture was then diluted with dichloromethane (10 mL) and filtered immediately through a silical gel column (10 × 1 cm) and eluted with CH_2Cl_2 (200 mL). The solvent was evaporated at reduced pressure to give the residue which was further purified by flash column chromatography (CH_2Cl_2) to give the pure benzoate aldehyde **10** (271 mg, 69%); ^{1}H NMR ($CDCl_3$) δ 1.80–2.02 (m, 4 H, C_3, C_4-H), 2.42 (t, J = 7.2 Hz, 2 H, C_2-H), 3.70 (s, 3 H, $COOCH_3$), 5.24 (dd, J = 8.0, 4.7 Hz, 1 H, C_5-H), 7.49 (t, J = 7.6 Hz, 2 H), 7.62 (t, J = 7.4 Hz, 1 H), 8.11 (d, J = 7.8 Hz, 2 H).

2-(2′(*Z*)-Octenyl)-1,3-dithiane (14). At 0 °C, pyridinium chlorochromate (3.23 g, 15.0 mmol) was added in portions to a well-stirred solution of *cis*-3-nonen-1-ol (1.42 g, 10 mmol) in methylene chloride (80 mL) which was suspended by silica gel (3.3 g) and kept at 0 °C for 8 h. After the reaction mixture was cooled to −30 °C, 1,3-propanedithiol (1.62 g, 15.0 mmol) and boron trifluoride diethyl etherate (3.7 mL, 30 mmol) were added successively. The mixture was stirred at −30 °C and allowed slowly to warm to 0 °C over a period of 2 h and then diluted with ether (100 mL) and filtered through Celite. The organic layer was washed with water (2 × 50 mL) and brine (1 × 50 mL), dried over anhydrous $MgSO_4$, filtered, and evaporated *in vacuo* to give the residue which was purified by flash column chromatography on silica gel, eluting with hexane/ethyl acetate (96:4) to give pure **14** as a colorless oil (1.38 g, 60%): ^{1}H NMR ($CDCl_3$) δ 0.84 (t, 3H); 1.30 (m, 6 H), 1.78 (m, 1 H), 2.05 (m, 3 H), 2.45 (t, J = 7.0 Hz, 2 H), 2.80 (m, 4 H), 4.01 (t, 1 H, J = 7.0 Hz), 5.40 (m, 1 H), 5.50 (m, 1 H); ^{13}C NMR ($CDCl_3$) δ 14.18, 22.65, 25.90, 27.57, 29.26, 30.65, 31.60, 33.30, 47.84, 124.47, 133.34.

2,2-(Trimethylenedithio)dec-4(*Z*)-enal (16). To a cooled (−78 °C) solution of dithiane **14** (1.7 g, 7.39 mmol) in THF (40 mL) was added n-BuLi (1.6 M, 5.1 mL, 1.10 equiv). The reaction mixture was stirred for 30 min at −78 °C and then allowed to slowly warm to room temperature and stirring continued for 2.5 h at room temperature. The reaction mixture was cooled to −78 °C, and then dimethylformamide (DMF) (4 mL) was added dropwise over a period of 10 min. The resulting mixture was stirred, allowed to slowly warm to room temperature during 2 h and stirring continued for 1 h more at room temperature. Workup consisted of the addition of saturated aqueous ammonium chloride solution (100 mL), extraction of the aqueous layer with diethyl ether (2 × 100 mL), washing the combined organic layers with brine (2 × 50 mL), drying over anhydrous Na_2SO_4, filtration, and concentration *in vacuo*. The crude product was obtained as a slightly yellow oil, which was purified by flash column chromatography (1:50 diethyl ether/hexane) to give aldehyde **16** (1.45 g, 76%): ^{1}H NMR ($CDCl_3$) δ 0.87 (t, J = 6.5 Hz, 3 H, C_{10}-H), 1.27–1.38 (m, 6 H, C_7, C_8, and C_9-H), 1.79 (m, 1 H, SCH_2CHHCH_2S), 2.05 (m, 3 H, SCH_2CHHCH_2S, and C_6-H), 2.58 (m, 4 H, $SCH_2CH_2CH_2S$), 3.01 (t, J = 12.3 Hz, 2 H, C_3-H), 5.37–5.40 (m, 1 H, C_4-H), 5.57–5.64 (m, 1 H, C_5-H), 9.06 (s, 1 H, CHO); ^{13}C NMR ($CDCl_3$) δ 14.14, 22.62, 24.36, 26.89, 27.60, 29.09, 31.57, 33.82, 57.82, 120.23, 135.38, 189.55; HREIMS calcd for $C_{13}H_{22}OS_2$ m/z = 258.1113, found 258.1114.

Methyl 6,6-(Trimethylenedithio)-2(*E*),4(*E*),8(*Z*)-tetradecatrienoate (17). To a cooled (−78 °C) stirred solution of methyl 4-(diethylphosphono)crotonate (2.13 g, 9.02 mmol) in THF (40 mL) was added LDA (1.5 M, 5.32 mL, 7.98 mmol) dropwise under argon. The reaction mixture was allowed slowly to warm to −30 °C and then a solution of aldehyde **16** (1.45 g, 5.61 mmol) in THF (40 mL) was added dropwise over a period of 10 min. The reaction mixture was then allowed to warm to room temperature and was stirred at room temperature overnight. The resulting mixture was poured on saturated aqueous ammonium chloride solution (100 mL) and extracted with diethyl ether (2 × 100 mL). The combined organic extract was washed with brine (1 × 50 mL), dried over anhydrous Na_2SO_4, filtered, and concentrated *in vacuo*. The product was purified by flash column chromatography (1:9 EtOAc/hexane) to give the diene ester **17** (1.75 g, 92%) as a colorless thick oil: ^{1}H NMR ($CDCL_3$) δ 0.88 (t, J = 7.0 Hz, 3 H, C_{14}-H), 1.21–1.40 (m, 6 H, C_{11}, C_{12}, and C_{13}-H), 1.86 (m, 1 H, SCH_2CHHCH_2S), 2.06 (m, 3 H, SCH_2CHHCH_2S, and C_{10}-H), 2.61 (d, J = 7.4 Hz, C_7-H), 2.65–2.71 (m, 2 H, $SCHHCH_2CHHS$), 2.82–2.90 (m, 2 H, $SCHHCH_2CHHS$), 3.77 (s, 3 H, COOMe), 5.37 (m, 1 H, C_8-H), 5.58 (m, 1 H, C_9-H), 5.96 (d, $J_{2,3}$ = 15.4 Hz, C_2-H, 1 H), 6.14 (d, $J_{5,4}$ = 15.1 Hz, 1 H, C_5-H), 6.58 (dd, $J_{4,5}$ = 15.1 Hz, $J_{4,3}$ = 11.1 Hz, C_4-H), 7.38 (dd, $J_{3,2}$ = 15.4 Hz, $J_{3,4}$ = 11.2 Hz, C_3-H); ^{13}C NMR ($CDCl_3$) δ 14.03, 22.54, 25.0, 27.15, 27.50, 29.10, 31.51, 39.52, 51.60, 54.37, 121.40, 121.52, 130.72, 134.43, 143.53, 145.29, 167.29; HREIMS calcd for $C_{18}H_{28}O_2S_2$ m/z = 340.1530, found 340.1526.

6,6-(Trimethylenedithio)-2(*E*),4(*E*),8(*Z*)-tetradecatrien-1-ol (18). To a cooled (0–5 °C) solution of diene ester **17** (1.75 g, 5.14 mmol) in THF (30 mL) was slowly added aluminum hydride (0.5 M in THF, 11.3 mL, 5.65 mmol) dropwise under argon. The reaction mixture was stirred for 1 h at 0–5 °C and then quenched by the addition of a 1:1 mixture of THF:H_2O (5 mL) and extracted with diethyl ether (3 × 100 mL). The combined organic extract was washed with brine (2 × 50 mL), dried over anhydrous Na_2SO_4, filtered, and concentrated *in vacuo*. The product was purified by flash column chromatography (19:1 EtOAc/hexane) to afford the diene alcohol **18** (1.355 g, 85%) as a colorless thick oil: ^{1}H NMR ($CDCl_3$) δ 0.88 (t, J = 7.1 Hz, 3 H, C_{14}-H), 1.27–1.38 (m, 6 H, C_{11}, C_{12}, and C_{13}-H), 1.88 (m, 1 H, SCH_2CHHCH_2S), 1.98–2.1 (m, 3 H, SCH_2CHHCH_2S, and C_{10}-H), 2.59 (d, J = 7.2 Hz, 2 H, C_7-H) 2.64–2.70 (m, 2 H, $SCHHCH_2CHHS$), 2.88 (m, 2 H, $SCHHCH_2CHHS$), 4.23 (t, J = 5.2 Hz, 2 H, C_1-H), 5.40 (m, 1 H, C_8-H), 5.56 (m, 1 H, C_9-H), 5.74 (d, $J_{5,4}$ = 14.9 Hz, C_5-H), 5.91 (dt, $J_{2,3}$ = 14.9 Hz, $J_{2,1}$ = 5.8 Hz, C_2-H), 6.35 (dd, $J_{3,2}$ = 14.9 Hz, $J_{3,4}$ = 10.7 Hz, C_3-H), 6.48 (dd, $J_{4,5}$ = 14.9 Hz, $J_{4,3}$ = 10.6 Hz, C_4-H); ^{13}C NMR ($CDCl_3$) δ 13.98, 22.48, 25.11, 27.06, 27.55, 29.07, 31.45, 39.65, 54.51, 63.17, 122.10, 130.20, 132.50 (2 C), 133.83, 136.63. HREIMS calcd for $C_{17}H_{28}OS_2$ m/z = 312.1581, found 312.1575.

[6,6-(Trimethylenedithio)-2(*E*),4(*E*),8(*Z*)-tetradecatrien-1-yl]-tris(3-methoxyphenyl)phosphonium Bromide (20). To a cooled (0 °C) magnetically stirred solution of diene alcohol **18** (1.35 g, 4.32 mmol) and carbon tetrabromide (1.718 g, 5.1 mmol, 1.2 equiv) in dry CH_2Cl_2 (60 mL) was slowly added ethylene bis(diphenylphosphene) (1.891 g, 4.75 mmol, 1.1 equiv) under argon. The reaction mixture was stirred for 20 min at 0 °C and then diluted with dry CH_2Cl_2 (100 mL), and the resulting solution of the labile diene bromide was quickly filtered through a small silica gel column into a flask containing a solution of tris(3-methoxyphenyl)phosphine (4.0 g) in dry CH_2Cl_2 (80 mL). The reaction mixture was stirred for 24 h at room temperature and then concentrated *in vacuo*. The phosphonium salt was purified by flash column chromatography with 9:1 methylene chloride/methanol to afford to the pure phosphonium salt **20** (2.75 g, 88%) (overall yield from alcohol **18**) as a light yellow-colored fluffy solid: ^{1}H NMR ($CDCl_3$) δ 0.84 (t, J = 7.5 Hz, 3 H, C_{14}-H), 1.20–1.40 (m, 6 H, C_{11}, C_{12}, and C_{13}-H), 1.76 (m, 1 H, SCH_2CHHCH_2S), 2.01 (m, 3 H, SCH_2CHHCH_2S, and C_{10}-H), 2.51 (d, J = 7.1 Hz, 2 H, C_7-H) 2.64–2.70 (m, 2 H, $SCHHCH_2CHHS$), 2.88 (m, 2 H, $SCHHCH_2CHHS$), 3.88 (s, 9 H, 3 × OMe), 5.00 (dd, J = 15.5, 7.3 Hz, 2 H), 5.26 (m, 1 H, C_8-H), 5.51 (m, 2 H, C_2, C_9-H), 5.71 (d, $J_{5,4}$ = 15.0 Hz, C_5-H), 6.30 (dd, $J_{4,5}$ = 15.0 Hz, $J_{4,3}$ = 10.8 Hz, C_4-H), 6.68 (m, 1 H, C_3-H), 7.3–7.4 (m, 9 H, ArH), 7.5 (m, 3 H, ArH); FAB MS m/z = 647 (M-Br).

Methyl 5(*S*)-(Benzoyloxy)-12,12-(trimethylenedithio)-6(*Z*),8(*E*),10(*E*),14(*Z*)-eicosatetraenoate (21). To a cooled (−93 °C) stirred solution of phosphonium salt **20** (1.25 g, 1.72 mmol) in THF (9 mL) was quickly added sodium hexamethyldisilazide (1 M, 1.05 mL, 1.05 mmol) dropwise under argon, and to the red resulting solution was immediately added aldehyde **10** (280 mg, 1.076 mmol) in THF (4 mL). The reaction mixture was stirred for 3 min and HMPA (2.3 mL) was added. The reaction mixture was stirred for 30 min at −93 °C and then allowed to warm slowly to −20 °C. It was then quenched by the addition of a 1:1 mixture of THF/H_2O (1 mL) and extracted with diethyl ether (3 × 75 mL). The combined extract was washed with cold water (3 × 25 mL), dried over anhydrous Na_2SO_4, filtered, and concentrated under reduced pressure to afford the crude product which was filtered through a column of silica (1:1 diethyl ether/hexane) to remove most of the tris(3-methoxyphenyl)phosphine oxide. A light yellow-colored oil (601 mg) was obtained. NP HPLC analysis

indicated that it was a 9:1 *Z*:*E* mixture (Spherisorb μ-porasil 4.6 × 250 mm column, eluent hexane/ethylacetate 9:1, flow rate 1 mL/min; retention time of *Z*-isomer 9.39 min, retention time of *E*-isomer 11.8 min). The purification of the product was carried out by flash column chromatography (24:1 hexane/EtOAc) to give the pure *Z*-isomer **21** (386 mg, 66.2%) (yield based on the aldehyde used): ^{1}H NMR ($CDCl_3$) δ 0.85 (t, *J* = 6.5 Hz, 3 H, C_{20}-H), 1.26 (m, 6 H, C_{17}, C_{18}, and C_{19}-H), 1.64–1.89 (m, 5 H, C_3, C_4-H and SCH_2CHCH_2S), 2.09 (m, 3 H, SCH_2CHCH_2S, and C_{16}-H), 2.36 (t, *J* = 7.0 Hz, 2 H, C_2-H), 2.56 (d, *J* = 7.2 Hz, C_{13}-H) 2.60–2.66 (m, 2 H, $SCHCH_2$-CHS), 2.80–2.95 (m, 2 H, $SCHCH_2CHS$), 5.33–5.60 (m, 3 H, C_6, C_{14}, C_{15}-H)), 5.76 (d, *J* = 15.0 Hz, 1 H, C_{11}-H), 5.91 (m, 1 H, C_5-H), 6.17 (t, *J* = 11.1 Hz, 1 H, C_7-H), 6.35 (dd, $J_{9,8}$ = 14.7 Hz, $J_{9,10}$ = 10.9 Hz, 1 H, C_9-H), 6.55 (dd, $J_{10,11}$ = 15.0 Hz, $J_{10,9}$ = 10.8 Hz, 1 H, C_{10}-H), 6.70 (dd, $J_{8,9}$ = 14.6 Hz, $J_{8,7}$ = 11.6 Hz, 1 H, C_8-H), 7.41 (t, *J* = 7.5 Hz, 2 H, OCO*Ph*), 7.53 (t, *J* = 6.4 Hz, 1 H, OCO*Ph*), 8.02 (dd, *J* = 7.1, 1.4 Hz, 2 H, OCO*Ph*); ^{13}C NMR ($CDCl_3$) δ 14.03, 20.60, 22.54, 25.16, 27.14, 27.60, 29.14, 31.50, 33.66, 34.26, 39.88, 51.55, 54.77, 70.61, 122.07, 127.62, 128.30, 128.90, 129.59, 130.37, 131.73, 132.89, 133.25, 133.86, 134.43, 138.07, 165.90, 173.58; HREIMS calcd for $C_{31}H_{42}O_4S_2$ *m/z* = 542.2526, found 542.2527. From the more polar fractions pure *E*-isomer **24** (42 mg) (7.2%) was also obtained. ^{1}H NMR ($CDCl_3$) δ 0.86 (t, *J* = 6.5 Hz, 3 H, C_{20}-H), 1.25–1.36 (m, 6 H, C_{17}, C_{18}, and C_{19}-H), 1.73–1.90 (m, 5 H, C_3, C_4-H and SCH_2CHCH_2S), 2.00 (m, 3 H, SCH_2CHCH_2S, and C_{16}-H), 2.37 (t, *J* = 7.1 Hz, 2 H, C_2-H), 2.59 (d, *J* = 7.1 Hz, C_{13}-H), 2.59 (dt, *J* = 10.4, 3.7 Hz, 2 H, $SCHCH_2CHHS$), 2.86 (t, *J* = 11.7 Hz, 2 H, $SCHCH_2CHHS$), 5.38–5.75 (m, 2 H), 6.62–6.55 (m, 4 H), 7.45 (t, *J* = 7.5 Hz, 2 H, OCO*Ph*), 7.56 (t, *J* = 6.4 Hz, 2 H, OCO*Ph*), 8.03 (dd, *J* = 7.1, 1.4 Hz, 1 H, OCO*Ph*); ^{13}C NMR ($CDCl_3$) δ 14.03, 20.60, 22.54, 25.17, 27.14, 27.61, 29.13, 31.51, 33.63, 33.89, 37.79, 51.57, 54.79, 74.74, 122.07, 128.24, 129.59, 130.38, 131.19, 131.92, 132.38, 132.94 (2 C), 133.18, 133.95, 137.31, 165.79, 173.66; HREIMS calcd for $C_{31}H_{42}O_4S_2$ *m/z* = 542.2526, found 542.2524.

5(*S*)-Hydroxy-12,12-(trimethylenedithio)-6(*Z*),8(*E*),10-(*E*),14(*Z*)-eicosatetraenoic Acid (23). Sodium methoxide (1 mL, 25 wt % in methanol) was added to a solution of benzoate ester **21** (34 mg, 0.064 mmol) in methanol (1.5 mL) at 0 °C and then the reaction mixture was stirred for 10 min at room temperature. After acidification with aqueous 5% KH_2PO_4 buffer (pH 4.3), the organic material was extracted with ethyl acetate (3 × 10 mL). The combined ethyl acetate extract was washed with cold water (1 × 10 mL) and brine (1 × 10 mL), dried over anhydrous Na_2SO_4, and filtered and the solvent evaporated under reduced pressure to afford the crude hydroxy methyl ester product **22**: ^{1}H NMR (C_6D_6) δ 0.86 (t, *J* = 7.0 Hz, 3 H, C_{20}-H), 1.20–1.80 (m, 12 H), 2.05 (m, 4 H), 2.22 (m, 2 H), 2.58 (m, 2 H), 2.84 (d, *J* = 7.0 Hz, C_{13}-H), 3.31 (s, 3 H, COOMe), 4.32 (m, 1 H, C_5-H) 5.32 (t, *J* = 9.8 Hz, 1 H), 5.59 (dt, *J* = 10.8, 7.3 Hz, 1 H), 5.75 (dt, *J* = 10.8, 7.1 Hz, 1 H), 5.86 (d, *J* = 15.0 Hz, 1 H), 5.96 (t, *J* = 11.1 Hz, 1 H), 6.20 (dd, *J* = 14.6, 11.0 Hz), 6.51 (dd, *J* = 14.6, 11.7 Hz, 1 H), 6.77 (dd, *J* = 15.1, 10.8 Hz, 1 H); ^{13}C NMR (C_6D_6) δ 13.99, 20.86, 22.66, 25.20, 26.98 (2 C), 27.71, 29.26, 31.53, 33.56, 36.86, 40.17, 50.67, 55.00, 67.28, 122.70, 128.11, 129.37, 133.38, 133.45, 133.58, 134.89, 138.08, 173.01]. The crude product **22** was dissolved in THF (2.7 mL) and 1 M LiOH (0.9 mL) was added, and then the reaction mixture was stirred for 10 min at room temperature, acidified with aqueous 5% KH_2PO_4 (pH 4.3), and extracted with ethyl acetate (3 × 10 mL). The combined ethyl acetate extract was washed with cold water, dried over anhydrous Na_2SO_4, and filtered and the solvent evaporated under reduced pressure to afford the hydroxy acid which was purified by flash column chromatography over silica gel using 1:9 MeOH/CH_2Cl_2 to give the pure hydroxy acid **23** (24 mg, 88%): ^{1}H NMR (CD_3OD) δ 0.95 (t, *J* = 7.0 Hz, 3 H, C_{20}-H), 1.25–1.80 (m, 10 H), 2.03 (m, 4 H), 2.30 (m, 2 H), 2.56 (d, *J* = 6.9 Hz, 2 H, C_{13}-H), 2.67 (m, 2 H), 2.87 (m, 2 H), 4.60 (m, 1 H, C_5-H) 5.40 (m, 1 H, C_{14}-H), 5.41 (t, *J* = 10.0 Hz, 1 H, C_6-H), 5.48 (m, 1 H, C_{15}-H), 5.77 (d, *J* = 14.8 Hz, 1 H, C_{11}-H), 6.12 (t, *J* = 11.0 Hz, 1 H, C_7-H), 6.37 (dd, *J* = 14.5, 11.0 Hz, 1 H, C_9-H), 6.55 (dd, *J* = 14.9, 11.0 Hz, 1 H, C_{10}-H), 6.62 (dd, *J* = 14.0, 12.0 Hz, 1 H, C_8-H); ^{13}C NMR (CD_3OD) δ 14.66, 22.28, 23.83, 26.68, 28.33 (2 C), 28.76, 30.48, 32.87, 35.12, 38.20, 40.99, 55.73, 68.38, 123.94, 129.48, 130.72, 134.47, 134.65, 134.76, 135.75, 138.75, 177.81; ESI MS *m/z* = 423.3 (M − 1).

5(*S*)-Hydroxy-12-oxo-6(*Z*),8(*E*),10(*E*),14(*Z*)-eicosatetraenoic Acid (12-Keto-LTB$_4$) (2). A solution of dithio compound **23** (6 mg, 0.0141 mmol) in methanol/H_2O (9:1, 2 mL) and [bis(trifluoroacetoxy)iodo]benzene (9 mg, 0.0209 mmol) was stirred at room temperature for 2 min. The reaction mixture was diluted with water (20 mL), loaded on a C-18 column bed (55–105 μM, 4 × 1 cm), and washed with an additional 30 mL of water. Then 12-keto LTB$_4$ was eluted with EtOH, yield 57%. On examination by RP HPLC analysis [μ-bondpack C-18, 7.8 × 300 mm; solvent system: CH_3CN:H_2O:AcOH:EDTA (50:50:0.01:0.02%), pH adjusted to 5.6 (NH_4OH), flow rate 1 mL/min] purity was >96%, t_r 44 min (UV 315 nm). NP HPLC examination [μ-porasil, 3.9 × 300 mm, solvent system: 4.5% 2-propanol in hexane containing 0.1% AcOH] revealed a mixture of 6,7-*cis* and 6,7-*trans* isomers in the ratio of 5:1, t_r 6,7-*cis* 18 min, t_r 6,7-*trans* 25 min. Pure 12-oxo LTB$_4$ was isolated by preparative NP HPLC and acetic acid content in the eluent was neutralized with Et_3N before evaporation of solvents. The material was dissolved in water and passed through a C-18 bed (55–105 μM, 5 × 1 cm) and washed with water. The 12-oxo-LTB$_4$ was eluted with ethanol: ^{1}H NMR (CD_3OD) δ 0.89 (t, *J* = 6.8 Hz, 3 H, C_{20}-H), 1.25–1.75 (m, 10 H), 2.08 (q, *J* = 6.7 Hz, 2 H), 2.32 (t, *J* = 6.8 Hz, 2 H), 3.38 (d, *J* = 6.0 Hz, 2 H, C_{13}-H), 4.63 (m, 1 H, C_5-H) 5.56 (m, 2 H, C_{14} and C_{15}-H), 5.62 (t, *J* = 10.5 Hz, 1 H, C_6-H), 6.20 (t, *J* = 11.3 Hz, 1 H, C_7-H), 6.25 (d, *J* = 15.6 Hz, 1 H, C_{11}-H), 6.43 (dd, *J* = 14.7, 11.2 Hz, 1 H, C_9-H), 7.08 (dd, *J* = 14.6, 11.8 Hz, 1 H, C_8-H), 7.36 (dd, *J* = 15.5, 11.2 Hz, 1 H, C_{10}-H); UV (EtOH) λ_{max} 320 nm. ESI MS *m/z* = 333.2 (M − 1).

5(*S*)-Hydroxy-12-oxo-6(*E*),8(*E*),10(*E*),14(*Z*)-eicosatetraneoic Acid (26). Methyl 5(*S*)-benzoyloxy-12,12-(trimethylenedithio)-6(*E*),8(*E*),10(*E*),14(*Z*)-eicosatetraenoate (**24**) was hydrolyzed to the hydroxy acid **25** in 85% yield as described for the preparation of **23**. ^{1}H NMR (CD_3OD) δ 0.91 (t, *J* = 7.0 Hz, 3 H, C_{20}-H), 1.25–1.80 (m, 12 H), 2.03 (q, *J* = 7.0 Hz, 2 H), 2.32 (t, *J* = 6.8 Hz, 2 H), 2.57 (d, *J* = 6.9 Hz, 2 H, C_{13}-H), 2.68 (m, 2 H), 2.87 (m, 2 H), 4.11 (m, 1 H, C_5-H), 5.42 (m, 1 H), 5.47 (m, 1 H), 5.73 (d, *J* = 14.9 Hz, 1 H, C_{11}-H), 5.75 (m, 1 H), 6.31 (m, 3 H), 6.49 (m, 1 H). ESI MS *m/z* = 423.3 (M − 1). Deprotection of the dithio group as described for the preparation of **2** produced **26** in 55% yield. ^{1}H NMR (CD_3OD) δ 0.90 (t, *J* = 6.7 Hz, 3 H, C_{20}-H), 1.25–2.25 (m, 12 H), 2.32 (t, *J* = 7.2 Hz, 2 H), 3.37 (d, *J* = 6.2 Hz, 2 H, C_{13}-H), 4.16 (m, 1 H, C_5-H), 5.57 (m, 2 H, C_{14}- and C_{15}-H), 5.99 (dd, *J* = 15.2, 6.5 Hz, 1 H, C_6-H), 6.24 (d, *J* = 15.4 Hz, 1 H, C_{11}-H), 6.40 (dd, *J* = 15.6, 11.2 Hz, 1 H, C_7-H), 6.41 (dd, *J* = 14.7, 11.1 Hz, 1 H, C_9-H), 6.74 (dd, *J* = 14.7, 10.9, Hz, 1 H, C_8-H), 7.33 (dd, *J* = 15.4, 11.3 Hz, 1 H, C_{10}-H); ESI MS *m/z* = 333.2 (M − 1).

Incubation of 12-Oxo-LTB$_4$ with Keratinocytes. Human keratinocytes were isolated from neonatal foreskins and cultured as described previously.[3] At confluency, culture media was replaced with 12.5 mL of media containing 12-oxo-LTB$_4$ (8 μg). Following incubation at 37 °C for 24 h, cell supernatant was removed and stored at −70 °C. Metabolites were isolated and purified by reverse phase HPLC, employing the identical protocol used for the purification of metabolites derived from LTB$_4$ incubation with keratinocytes.[3] Negative ion ESI MS of HPLC purified metabolites were obtained using flow injection analysis on a Sciex API III+ mass spectrometer (Perkin-Elmer) with a mobile phase of water:methanol (1:1) with 0.05% triethylamine at a flow rate of 20 μL/min. The HPLC fractions containing metabolites were evaporated to dryness and reconstituted in the mobile phase at a concentration of 1 ng/μL. Aliquots (10 μL) were analyzed with spectra acquired from 50–700 atomic mass units at 1 scan/5 s. Argon was used for collision-induced dissociation (CID) and tandem mass spectrometric analyses with a collision offset energy (E_{lab}) of 20 eV.

Isolation of 12-Oxo-LTB$_4$ from Porcine PMNL. Porcine PMNL, prepared as previously described,[12] were sonicated for two periods of 10 s each. The sonicate was centrifuged at 20 000*g* for 20 min and the resulting supernatant centrifuged

at 200 000*g* for 60 min. The pellet was resuspended in PBS at a concentration equivalent to 150 million PMNL/mL and incubated with LTB$_4$ (2 μM) in the presence of NAD (1 μM) for 40 min at 37 °C. The incubation was terminated by the addition of methanol (0.5 volumes) and centrifuged, and the supernatant was extracted at neutral pH on a cartridge containing octadecylsilyl (ODS) silica as previously described.[19] 12-Oxo-LTB$_4$ was eluted from the ODS silica with methyl formate, which was removed under a stream of nitrogen. The 12-oxo-LTB$_4$ was stored in acetonitrile at −80 °C prior to analysis by HPLC.

Comparison of Biologically and Chemically Synthesized 12-Oxo-LTB$_4$ by HPLC. The retention times of both biologically-prepared and chemically-synthesized 12-oxo-LTB$_4$ (**2**) and its 6-*trans* isomer **26** obtained from the porcine leukocyte extracts were compared using a column (4.2 × 250 mm) of Econosphere silica (5 μm particle size; Alltech Associates) with hexane/2-propanol/acetic acid (95.5:4.5:0.1) as the mobile phase. The two peaks with retention times of 10.5 and 13 min cochromatographed with synthetic *cis* and *trans* isomers, respectively, confirming the identity of the biological 12-oxo-LTB$_4$ (**2**) and its 6-*trans* isomer **26**.

(19) Powell, W. S. *Prostaglandins* **1980**, *20*, 947.

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Supplementary Material Available: Copies of ^{1}H and/or ^{13}C/NMR spectra of **2**, **8**–**10**, **14**, **16**–**18**, and **20**–**26** (28 pages). This material is contained in libraries on microfiche, immediately follows this article in the microfilm version of the journal, and can be ordered from the ACS; see any current masthead page for ordering information.

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