COMMUNICATION

First synthesis of phosphatidylcholine and cholesteryl derivatives bearing an unsaturated aldehyde residue †

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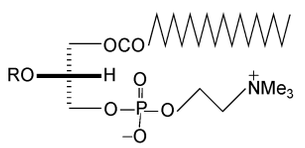
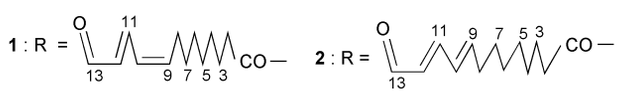
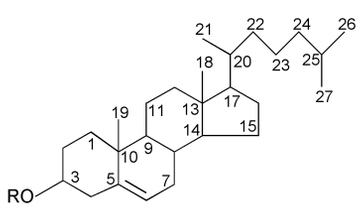
The first synthesis of phosphatidylcholine and cholesteryl esters bearing a shortened acyl chain with a 2,4-dienal terminal is described.

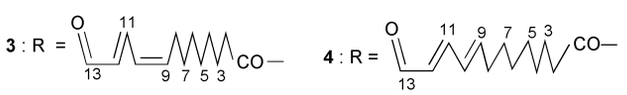
Much attention has recently been paid to lipids containing an oxidatively shortened fatty acyl chain with a carbonyl terminal, the so-called core aldehydes, since their reactivity with biological nucleophiles such as the amino group gives them important biological implications. For example, phospholipid and cholesteryl ester core aldehydes modify membrane proteins and lipoproteins, and contribute in various ways to atherogenesis.[1] Various platelet activating factor (PAF)-like activities of phosphatidylcholine (PC) core aldehydes have also been documented.[1,2] The core aldehydes that have so far been employed in previous studies are esters of saturated simple aldehyde acids such as 5-oxopentanoic and 9-oxononanoic acids. In biological systems, however, the oxidative degradation of polyunsaturated acyl groups in lipids should also produce unsaturated core aldehydes. Tokumura *et al.* recently identified a 13 carbon dienal-containing PC as a major product of the 15-lipoxygenase catalyzed oxidation of PCs under hypoxic conditions, and this reaction is likely to occur *in vivo*.[2] The biological activities of this kind of PC and cholesteryl core aldehydes should therefore be examined, especially in view of the fact that the role of 12/15 lipoxygenase in atherogenesis has recently attracted much attention.[3] It has also been suggested that the difficulty in detecting such unsaturated core aldehydes from biological systems might be due to their stronger interactions with protein side chains than carbonyl groups alone.[4] Indeed, their Schiff base adducts should be stabilized by the conjugation of their diene system with the imine double bond. Besides, they might also form Michael type adducts with thiol and amino groups. Thus, the characterization of such adducts will be important for understanding the fate and effects of these aldehydes in biological systems. For these studies, the availability of large amounts of unsaturated core aldehydes in a pure state is vital. Here we report the preparation of such PC and cholesteryl esters, compounds **1**–**4**.

During the homolytic decomposition of PC hydroperoxides, the formation of PC core aldehyde instead of other products seems to be greatly favoured by the accompanying formation of an allyl radical upon β-scission of the alkoxy radical.[5] We thus anticipated that in the absence of oxygen, the homolytic decomposition of ω-6 hydroperoxides of *n*-3 fatty acids (Scheme 1) might give preparatively useful amounts of ω-oxo fatty acids, which could then be esterified to lysophosphatidylcholine (lysoPC) or cholesterol.









Although fatty acid hydroperoxides decompose extremely fast at 50 °C,[6] we could not achieve considerable decomposition of 13-hydroperoxy-octadecatrienoic acid (HPOT) under a nitrogen atmosphere even at 60 °C, prompting us to use Fe^{2+} to effect the formation of the requisite alkoxy radicals according to the Fenton reaction (eqn. (1)).

$$ROOH + Fe^{2+} \longrightarrow RO\cdot + OH^- + Fe^{3+} \qquad (1)$$

This reaction afforded a mixture of some decomposition products including the desired 13-oxotrideca-9,11-dienoic acid (OTDA) as the major product, and 13-hydroxyoctadecatrienoic acid. Unfortunately, these latter two compounds could not be separated from each other by silica gel chromatography. Therefore as shown in Scheme 2, HPOT obtained from α-linolenic acid by the action of soybean lipoxygenase was first converted to methyl ester **5**, which was then cleaved by $FeSO_4 \cdot 7H_2O$ at 60 °C to give a mixture of methyl OTDA isomers **6** and **7** in 51% isolated yield. This yield was impressive since the decomposition of fatty acid hydroperoxides has not generally been associated with such a level of selectivity for any of the possible products. The structures of compounds **6** and **7** were confirmed by EI MS and ^{1}H NMR.[7] Stereochemical assignment was based on the coupling constants of 11 and 15 Hz for the *cis* and *trans* double bonds respectively in compound **6**. For compound **7**, however, the coupling constant for H-9 and H-10 could not be

† Electronic Supplementary Information (ESI) available: experimental data for compounds **1**–**9**. See http://www.rsc.org/suppdata/p1/b2/b206231n/

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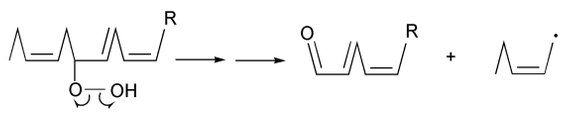
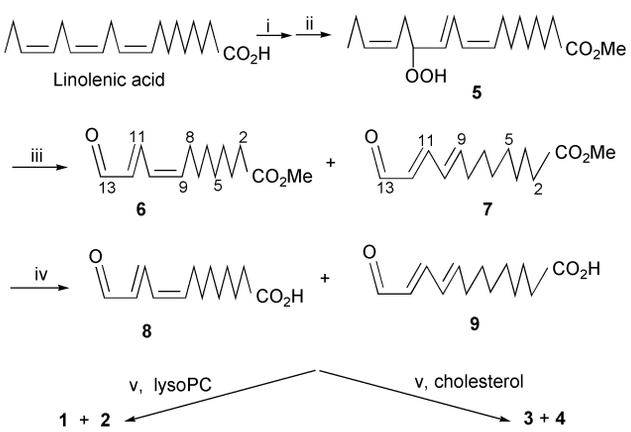
Table 1 Dependence of the total yield and the ratio of methyl OTDA isomers **6** and **7** on reaction time at 60 °C[a]

Time/min	Yield (%)	**6** : **7** ratio
10[b]		1 : 0
30	51	3 : 1
40	46	1 : 1
60	38	1 : 4

[a] 60 °C gave better results than lower temperatures. [b] ^{1}H NMR revealed that a little of compound **6** had formed, but it could not be separated from the remaining hydroperoxide.



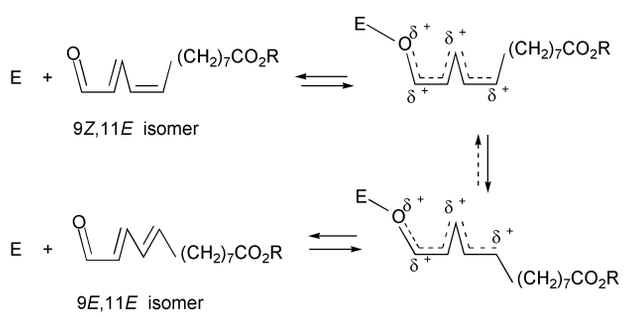
Scheme 1 Formation of ω-oxo fatty acid and allylic radical by the homolytic decomposition of ω-6 hydroperoxides of *n*-3 fatty acids.



Scheme 2 *Reagents and conditions*: i. soybean lipoxygenase, O_2, borate buffer (pH 9), 6 h; ii. diazomethane; iii. $FeSO_4 \cdot 7H_2O$, EtOH, 60 °C, N_2; iv. lipase PS (Amano), H_2O, rt, 3 h; v. DCC, DMAP, EtOH-free $CHCl_3$, rt, 20 h.

calculated because their signals overlapped. Nevertheless, the coupling constant for its H-11 and H-12 was 15 Hz like in compound **6**, indicating that its H-9 and H-10 must be in a *trans* relationship.

As shown in Table 1, both the total yield and the isomeric ratio were found to depend on the reaction time. From these results it is clear that compound **7** was formed from **6**. We propose that this isomerization was due to electrophilic attack on the carbonyl oxygen of **6** as shown in Scheme 3, since both Fe^{2+}



Scheme 3 Proposed mechanism for double bond isomerization in the unsaturated oxo fatty acid derivatives.

and Fe^{3+} which are present in the reaction mixture (eqn. (1)) are Lewis acids. That the conversion of **7** back to **6** was unfavourable should be due to greater stability of the former than the latter. Additional isomerization was observed during the DCC-mediated esterification reaction (*vide infra*). This might also be explained, at least in part, by the mechanism presented in Scheme 3, with DCC acting as the electrophile, since the extent of the isomerization was found to depend on the amount of DCC used.

Not surprisingly, free oxo acids **8** and **9** could not be obtained from their esters **6** and **7** by either basic or acidic hydrolysis, owing to the reactivity of the unsaturated carbonyl groups under such conditions. Fortunately, lipase PS (from *Pseudomonas* sp., Amano Seiyaku Co., Ltd.) was found to effectively perform the hydrolysis, affording **8** and **9** in 80% yield and with retention of the isomeric ratio.

Although all previously reported syntheses of core aldehydes involved protecting the aldehyde function of the oxo acid as a dimethyl acetal prior to the DCC–DMAP mediated esterification,[8] we found that such a protection–deprotection sequence could be avoided. Thus in the presence of DCC and a catalytic amount of DMAP, a mixture of compounds **8** and **9** were esterified to lysoPC prepared as previously described,[9] affording core aldehydes **1** and **2** in 90% yield. As mentioned above, some double bond isomerization occurred during this reaction, and it was found to depend on the amount of DCC used. For example, with 1 equivalent of DCC, a 2 : 1 mixture of acids **8** and **9** gave a 3 : 2 mixture of core aldehydes **1** and **2**, which ratio remarkably changed to 1 : 9 when 1.2 equivalents of DCC was used. ‡ Purification by chromatography on silica gel ($CHCl_3$–MeOH–H_2O, 60 : 30 : 1) and subsequent reverse phase HPLC on an ODS column (MeOH–$CHCl_3$–H_2O, 100 : 4.5 : 5) gave **1** and **2** with high purity as resinous solids whose structural integrity was confirmed by MS and ^{1}H NMR.[10] However, their separation was not achieved under these conditions. §

Similarly, the esterification of oxo acids **8** and **9** to cholesterol afforded core aldehydes **3** and **4**,[11] which were obtained as iridescent solids in 87% yield after careful column chromatography on silica gel (hexane–EtOAc, 95 : 5). Fortunately, their separation was also achieved thereby. Their purity was confirmed by analytical scale HPLC on an inertsil column eluted with hexane–chloroform (8 : 2).

The first successful synthesis of these compounds described herein sets the stage for studies on their biological activities and biologically relevant chemistry, and provides reference compounds for their possible identification in biological systems. This method should also be applicable to the synthesis of other core aldehydes having a longer ω-oxo acyl group, whose facile formation *in vitro* has previously been reported.[5]

Experimental

General

Apart from the preparation of compounds **6** and **7** from compound **5**, all reactions in the present synthesis were conducted in the presence of a trace amount of the free-radical scavenging antioxidant 2,6-di-*tert*-butyl-*p*-hydroxytoluene (BHT). All reactions were conducted under a nitrogen atmosphere. Column chromatography was done on silica gel 60.

Preparation of aldehyde esters 6 and 7

To ethanol (200 ml) maintained at 60 °C were added methyl ester **5** (600 mg, 1.8 mmol), and $FeSO_4 \cdot 7H_2O$ (495 mg, 1.8 mmol). This mixture was stirred vigorously for 30 minutes, after which a trace of BHT was added, followed by solvent evaporation *in vacuo* and column chromatography (hexane–EtOAc, 9.3 : 0.7), affording a mixture of aldehyde esters **6** and **7** (218 mg, 51%). Characterization data are given in ref. 7 below.

Preparation of oxo acids 8 and 9

A mixture of esters **6** and **7** (200 mg, 0.84 mmol) and lipase PS (200 mg, 6000 units) in water (4 ml) was stirred at rt for 4 hours, followed by extraction with ethyl acetate. The organic phase was washed with water and dried over Na_2SO_4. Subsequent solvent evaporation *in vacuo* and column chromatography (hexane–EtOAc, 4 : 1) afforded oxo acids **8** and **9** (150 mg, 80%). ^{1}H NMR was similar to that for esters **6** and **7**, but lacking the signal at 3.66 ppm. HR-MS (EI): Found, 244.1400 *m*/*z* (M^+, $C_{13}H_{20}O_3$ requires 244.1412).

Acknowledgements

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‡ Protection of the aldehyde group is not expected to eliminate double bond isomerization because the deprotection step involves acidic conditions.

§ The composition of such core aldehydes *in vivo* is not known, but besides possible isomerization of the (*Z,E*)-isomer as presented above, both isomers are expected to form directly from the corresponding nonenzymatically-produced (*Z,E*) and (*E,E*)-hydroperoxides.

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7 *Compound* **6**: R_f = 0.31 (hexane–EtOAc, 9 : 1); ^{1}H NMR (500 MHz, $CDCl_3$): δ 1.31 (6H, m, 4-, 5-, 6-H), 1.45 (2H, m, 7-H), 1.59 (2H, m, 3-H), 2.20–2.30 (4H, m, 2-, 8-H), 3.66 (3H, s, -OCH_3), 6.00 (1H, m, 9-H), 6.14 (1H, dd, *J* 7.9 and 15.2, 12-H), 6.25 (1H, t, *J* 11.4, 10-H), 7.44 (1H, dd, *J* 11.4 and 15.2, 11-H), 9.61 (1H, d, *J* 7.9, 13-H); HR-MS (EI): Found, 238.1567 *m/z* (M^+, $C_{14}H_{22}O_3$ requires 238.1569); *Compound* **7**: R_f = 0.27 (hexane–EtOAc, 9 : 1); ^{1}H NMR (500 MHz, $CDCl_3$): δ 1.31 (6H, m, 4-, 5-, 6-H), 1.45 (2H, m, 7-H), 1.59 (2H, m, 3-H), 2.20–2.30 (4H, m, 2-, 8-H), 3.66 (3H, s, -OCH_3), 6.07 (1H, dd, *J* 7.9 and 15.3, 12-H), 6.16–6.34 (2H, m, 9-, 10-H), 7.08 (1H, dd, *J* 10.1 and 15.3, 11-H), 9.52 (1H, d, *J* 7.9, 13-H); HR-MS (EI): Found, 238.1567 *m/z* (M^+, $C_{14}H_{22}O_3$ requires 238.1569).

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10 *Compound* **1**: ^{1}H NMR (500 MHz, $CDCl_3$): δ 0.87 (t, 3H, ω-CH_3), 1.18–1.35 (34H, m, CH_2 × 17), 1.45 (2H, m, 7′-H), 1.60 (4H, m, $OCOCH_2$ CH_2 × 2), 2.20–2.30 (6H, m, 8′-H, $OCOCH_2$ × 2), 3.35 (9H, s, NMe_3), 3.79 (2H, m, OCH_2CH_2N), 3.94 (2H, m, CH_2OP), 4.11 (1 proton of $OC\underline{H}_2CH(OR)CH_2OP$), 4.31(2H, m, OCH_2-CH_2N), 4.38 (1H, d, 1 proton of $OC\underline{H}_2CH(OR)CH_2OP$), 5.19 (1H, br s, $OCH_2C\underline{H}(OR)CH_2OP$), 6.00 (1H, m, 9′-H), 6.14 (1H, dd, *J* 7.9 and 15.2, 12′-H), 6.26 (1H, t, *J* 11.4, 10′-H), 7.44 (1H, dd, *J* 11.4 and 15.2, 11′-H), 9.61 (1H, d, *J* 7.9, 13′-H); ES MS: Found, 730.6 *m/z*; HR-FAB MS: Found, 730.5032 *m/z* ($C_{39}H_{72}NO_9P$ + H^+ requires 730.5023); *Compound* **2**: same as for **1** except: 6.07 (1H, dd, *J* 7.9, 15.3, 12′-H), 6.16–6.34 (2H, m, 9′, 10′-H), 7.08 (1H, dd, *J* 10.1 and 15.3, 11′-H), 9.52 (1H, d, *J* 7.9, 13′-H).

11 *Compound* **3**: R_f = 0.44 (hexane–EtOAc, 9 : 1); ^{1}H NMR (500 MHz, $CDCl_3$): δ 0.5–2.6 (57H, CH_3 × 5, CH_2 × 18, CH × 6), 4.59 (1H, m, 3-H), 5.38 (1H, m, 6-H), 6.00 (1H, m, 9′-H), 6.14 (1H, dd, *J* 7.9 and 15.2, 12′-H), 6.26 (1H, t, *J* 11.4, 10′-H), 7.44 (1H, dd, *J* 11.4 and 15.2, 11′-H), 9.61 (1H, d, *J* 7.9, 13′-H); HR-FAB MS: Found, 593.4932 ($C_{40}H_{64}O_3$ + H^+ requires 593.4933); *Compound* **4**: R_f = 0.35 (hexane–EtOAc, 9 : 1); ^{1}H NMR same as for **3** except: δ 6.07 (1H, dd, *J* 7.9 and 15.3, 12′-H), 6.16–6.34 (2H, m, 9′-, 10′-H), 7.08 (1H, dd, *J* 10.1 and 15.3, 11′-H), 9.52 (1H, d, *J* 7.9, 13′-H).