Chemoenzymatic Synthesis of Some Macrocyclic C_{13}-Lactones

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Oxidation of linoleic acid using immobilised soybean lipoxygenase-1 gave (13*S*)-13-hydroxyoctadeca-9,11-dienoic acid **1**: lactonisation and Diels–Alder reactions involving this hydroxy acid have been explored.

The preparation of (13*S*,9*Z*,11*E*)-13-hydroxyoctadeca-9,11-dienoic acid [(13*S*)-HODE] **1** has attracted considerable attention. Linear syntheses of this biologically interesting molecule[1] have been superceded by convergent approaches to the target structure, as a result of work in these laboratories[2] and elsewhere.[3] Now we show that (13*S*)-HODE **1** is most conveniently obtained by the action of soybean lipoxygenase on linoleic acid followed by sodium borohydride reduction. With multigram quantities of the diene **1** made available in this way, cyclisations and some cycloadditions have been investigated.

Soybean lipoxygenase-1 was immobilised over a period of 60 h in buffer with oxirane acrylic beads.[4] The resin was washed to remove unbound protein. A suspension of the immobilised enzyme in oxygenated aqueous buffer converted linoleic acid into (13*S*,9*Z*,11*E*)-13-hydroperoxyoctadeca-9,11-dienoic acid.[5] This hydroperoxide was not isolated but, after removal of the immobilised enzyme by filtration, was reduced with sodium borohydride to give, after chromatography, pure (13*S*)-HODE **1** in a reproducible 70% yield on a 0.8 g scale. The recovered immobilised enzyme was re-used at a later date and, while the reaction rate was reduced, the overall yield of (13*S*)-HODE was maintained.

The hydroxy acid **1** was cyclised in 40% yield (using di-2-pyridyl disulphide, triphenylphosphine, xylenes, 145 °C, 20 h)[6] to afford the macrocyclic lactone **2** contaminated with small quantities (*ca.* 15%) of other unsaturated compounds. Reaction of this lactone with 4-phenyl-1,2,4-triazoline-3,5-dione (1.6 equiv. of dienophile in ethyl acetate, room temperature for 0.5 h) was highly face-selective and gave only the Diels–Alder adduct **3** (70%). The assignment of structure to this cycloadduct was aided by single-crystal X-ray data (Fig. 1).* Methanolysis of compound **3** gave the hydroxy ester **4**.

Reaction of (13*S*)-HODE **1** with the above triazolinedione gave two cycloadducts **5** and **6** in excellent yield (90%) and in a 2:1 ratio favouring *anti*-addition. The major product **5** was cyclised to give a macrocyclic lactone **7** that was different (by NMR spectroscopy) to the lactone **3** and yet produced the hydroxy ester **4** on methanolysis. This result together with NOE

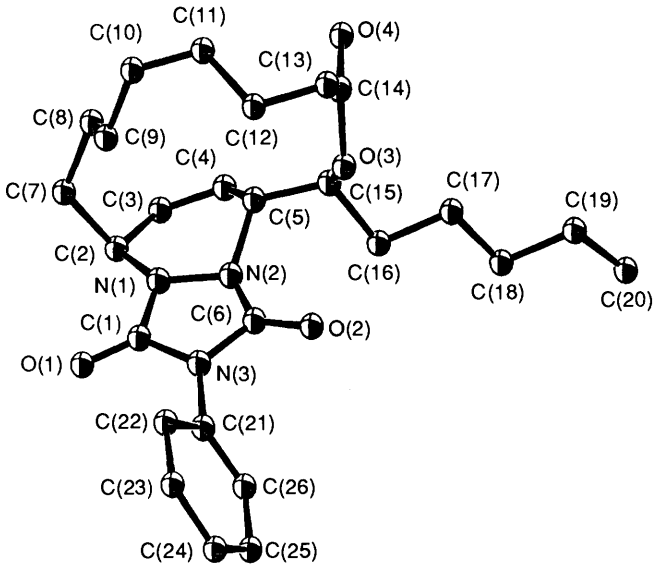
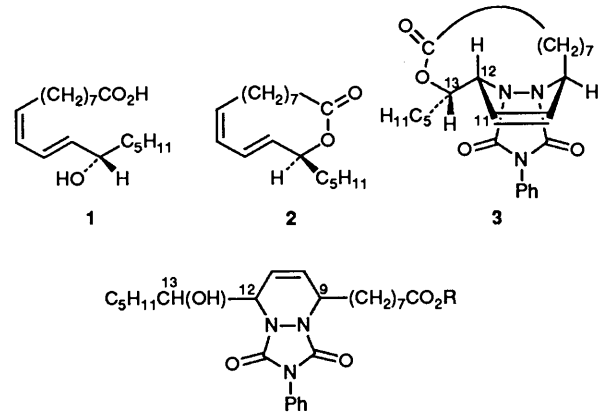
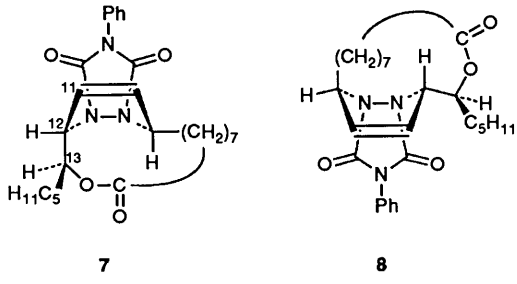


Fig. 1 Crystal structure of compound **3**



4 (*S*)C-9, (*R*)C-12, (*S*)C-13, R = Me
5 (*S*)C-9, (*R*)C-12, (*S*)C-13, R = H
6 (*R*)C-9, (*S*)C-12, (*S*)C-13, R = H
9 (*R*)C-9, (*S*)C-12, (*S*)C-13, R = Me



studies suggested that the lactones **3** (NOE: 13-H/12-H, 4.1%; 13-H/11-H, 10.4%) and **7** (NOE: 13-H/12-H, 4.9%; 13-H/11-H, 0%) were rotamers and that the absolute configuration of **5** was as indicated.

The cycloadduct **6** formed the macrocyclic lactone **8** on treatment with di-2-pyridyl disulphide and triphenylphosphine in xylenes at reflux, and the lactone **8** provided the bicyclic ester **9** on treatment with sodium methoxide in methanol.

We are currently exploring the synthesis of other polysubstituted, optically active, macrocyclic lactones using this chemoenzymatic approach. For example we have found that (2*E*,9*Z*,12*Z*)-octadeca-2,9,12-trienoic acid affords (2*E*,9*Z*,11*E*)-13-hydroxyoctadeca-2,9,11-trienoic acid on treatment with the lipoxygenase and we are investigating the possibility of performing lactonisation reactions and intramolecular Diels–Alder reactions using this hydroxy trienoic acid.

Experimental

Preparation of (13S)-*HODE*.—Soybean lipoxygenase-1 (Sigma) was immobilised by adding oxirane acrylic beads (Sigma, 150 μm, 3 g) to a solution of the enzyme (66 mg, 8.844 M units) in 1 mol dm^{-3} phosphate buffer (pH 7.5; 75 ml) and storage of the suspension at 4 °C for 60 h. The beads were filtered off, washed successively with 0.1 mol dm^{-3} phosphate buffer (pH 7.5) and 0.1 mol dm^{-3} sodium borate buffer (pH 9) and suspended in the latter (600 ml). To this ice-cooled, mechanically stirred suspension was added an emulsion of linoleic acid (852 mg, 3 mmol) in ammonium hydroxide (0.019 mol dm^{-3}; 200 ml) and oxygen was bubbled through. The reaction was monitored by UV spectrometry at 234 nm (ε 23 000 l mol^{-1} cm^{-1}) and after 45 min conversion into conjugated product was estimated at 99%. The enzyme was filtered off, washed successively with 0.1 mol dm^{-3} borate buffer and 0.1 mol dm^{-3} phosphate buffer and stored in the latter for re-use. The borate filtrates were acidified to pH 3 with 5% citric acid (350 ml) and extracted with diethyl ether (3 × 300 ml). The combined organic phases were washed with water and concentrated under reduced pressure. The residue was dissolved in methanol (30 ml) and cooled in ice before the addition of sodium borohydride (1 g, 25 mmol). After being stirred for 20 min at 0 °C and then 40 min at room temperature, the reaction mixture was acidified (2 mol dm^{-3} HCl), diluted with water (50 ml) and extracted with diethyl ether (3 × 200 ml). The combined organic phases were washed successively with water and brine, dried ($MgSO_4$), filtered and concentrated under reduced pressure to afford the crude product. This was purified by column chromatography over silica using dichloromethane–diethyl ether–acetic acid (84:15:1) as eluent to give the title compound (706 mg, 77%) as a colourless oil; $[\alpha]_D^{30}$ + 9.4 10^{-1} deg cm^2 g^{-1} (*c* 1.29, $CHCl_3$); ν_{max}/cm^{-1} 1708; $\delta_H(CDCl_3)$ 6.48 (1 H, dd, *J* 15, 11‡ 11-H), 5.96 (1 H, dd, *J* 11, 11, 10-H), 5.82 (2 H, br s, OH, CO_2H), 5.65 (1 H, dd, *J* 7, 15, 12-H), 5.43 (1 H, dd, *J* 7, 11, 9-H), 4.16 (1 H, q, *J* 7, 13-H), 2.33 (2 H, t, CH_2CO_2H), 2.17 (2 H, m) and 0.8–1.8 (21 H, m).

* *Crystal data* for compound **3**. A crystal of approximate dimensions 0.2 × 0.2 × 0.2 mm was used for data collection. $C_{26}H_{35}N_3O_4$, *M* = 453.8, orthorhombic, *a* = 9.243(5), *b* = 16.156(4), *c* = 16.408(4) Å, *U* = 2449.9 $Å^3$, space group $P2_12_12_1$, *Z* = 4, D_c = 1.23 g cm^{-3}, μ(Mo-$K\alpha$) = 0.48 cm^{-1}, *F*(000) = 976. Data were measured at room temperature on a Hilger and Watts Y290 four-circle diffractometer in the range $2 \leqslant \theta \leqslant 22°$. 1743 Reflections were collected of which 995 were unique with $I \geqslant 3\sigma(I)$. Data were corrected for Lorentz and polarization effects but not for absorption.[7] The structure was solved by direct methods and refined using the SHELX[8,9] suite of programs. All atoms were treated isotropically in the final stages of convergence due to limited data. Hydrogen atoms were included at calculated positions.

Final residuals after 14 cycles of full-matrix least squares refinement were $R = R_w = 0.0879$ with unit weights. The total number of parameters varied was 133. Max. final shift/esd was 0.004, the average being 0.002. The max. and min. residual densities were 0.14 and −0.12 e $Å^{-3}$ respectively. Final fractional atomic co-ordinates and bond distances and angles together with the anisotropic temperature factors and hydrogen atom positions are available from the CCDC.† The asymmetric unit is shown in Fig. 1, along with the labelling scheme used.

† For details of the CCDC Scheme see section 5.6.3 of Instructions for Authors. Issue 1.

‡ *J* Values in Hz.

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