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Efficient syntheses of (10*E*,12*Z*,15*Z*)-9-oxo- and (9*Z*,11*E*,15*E*)-13-oxo-octadecatrienoic acids; two stress metabolites of wounded plants

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Abstract—Configurationally pure 9-oxo-10*E*,12*Z*,15*Z*- and 13-oxo-9*Z*,11*E*,15*E*-octadecatrienoic acid are available from linolenic acid via regioselective functionalisation using lipoxygenases from soybean or tomato at specific pH conditions. Reduction of the resulting hydroperoxides followed by oxidation of the resulting allylic alcohols with Bobbitt's reagent yields the configurationally pure but labile ketotrienoic acids **4** and **5** without concomitant isomerisation.

1. Introduction

Oxylipins (oxidised fatty acid derivatives) play an important role in plant biology as signal molecules for defence gene expression.[1] The biosynthetic pathway towards these bioactive compounds starts with lipoxygenases which add molecular oxygen to pentadienyl-units of unsaturated fatty acids (linoleic- and, predominantly linolenic acid) yielding hydroperoxides in a regio- and stereospecific fashion. Products of hydroperoxide transformations typically comprise α-ketols, γ-hydroxy-enones, epoxyalcohols and triols as their hydrolysis products.[2] The hydroperoxides 13-HPOTE **2** and 9-HPOTE **3** serve as precursors for a multitude of oxidation- and fragmentation products that may act as selective signals for different defensive pathways or represent themselves as defensive compounds acting against invading (micro)organisms. The most prominent terminal product of 13-HPOTE is jasmonic acid (JA), a master switch controlling plant secondary metabolism.[3] In wounded leaves of *Arabidopsis thaliana* the hydroperoxides **2** or **3** are, among other transformations, reduced to the corresponding allylic alcohols followed by oxidation to the reactive oxotrienoic acids 13-KOTE **4** and 9-KOTE **5**.[4]

Both compounds accumulate in leaves of *A. thaliana* soon after wounding or infection with *Pseudomonas syringae* and were shown to induce characteristic defence reactions.[4]

2. Results

To unravel the complexity of such signalling pathways along with their characteristic molecules, an access to configurationally defined octadecanoids such as **4** and **5** and other stress metabolites is of great importance. Of particular value are enzymatic methods, since they allow a direct and regioselective functionalisation of the intact skeleton of readily available unsaturated fatty acids.[5] For example, linolenic acid can be selectively functionalised at C-9 or C-13 using either a 13-LOX from soybean or a 9-LOX from tomato.[6–8] The resulting hydroperoxides are readily reduced to configurationally stable allylic alcohols, but their oxidation to the *E*,*Z*-ketotrienoic acids **4** or **5** is not trivial and is often accompanied by excessive isomerisation to the *E*,*E*-isomer. For example, oxidation of the allylic alcohol **7** (Scheme 2; also known as didehydrochoriolic acid)[9] derived from **3** with MnO_2 resulted in a low yield of a complex mixture comprising the ketoacid **5** only as minor compound. Decomposition of fatty acid hydroperoxides by Fe(III) provides an alternative access, but the complexity of the product spectrum is not satisfying and requires extensive purification protocols.[10] Previous approaches to establish such a structural element in eicosanoids, e.g. 5-oxo-ETE (5-oxo-6*E*,8*Z*,11*Z*,14*Z*-eicosatetraenoic acid),[11] utilised a dithianyl-protected building block which was oxidatively deprotected after elaboration of the fatty acid backbone. However, even mild deprotection methods resulted in significant isomerisation of the labile *E*,*Z*-configuration of the α,β-unsaturated carbonyl moiety.[11] Here we report a direct and efficient process, utilising regioselective functionalisation of linolenic acid by lipoxygenases from soybean and tomato, followed by reduction and oxidation of the resulting allylic alcohols with Bobbitt's reagent (4-acetylamino-2,2,6,6-tetramethylpiperidine-1-oxoammonium

Keywords: Bobbitt's reagent; hydroperoxides; linolenic acid; oxylipins; 9-KOTE; 13-KOTE; lipoxygenases.

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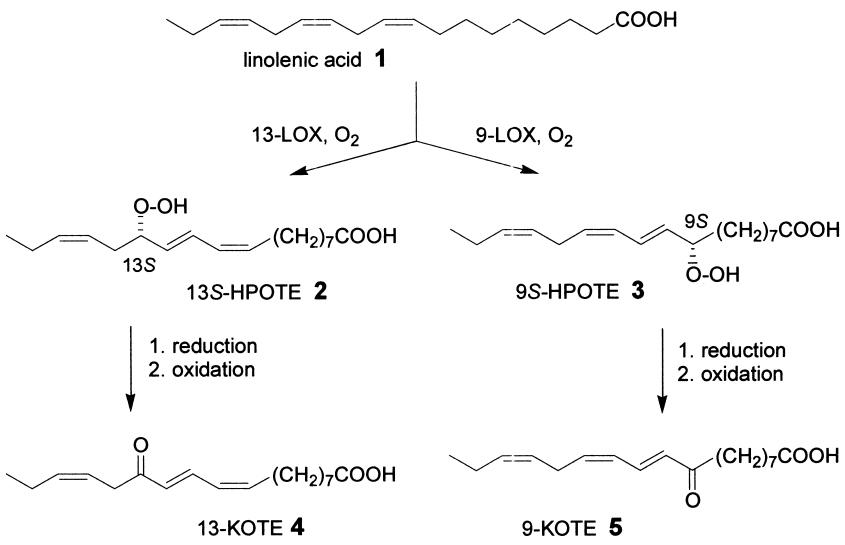
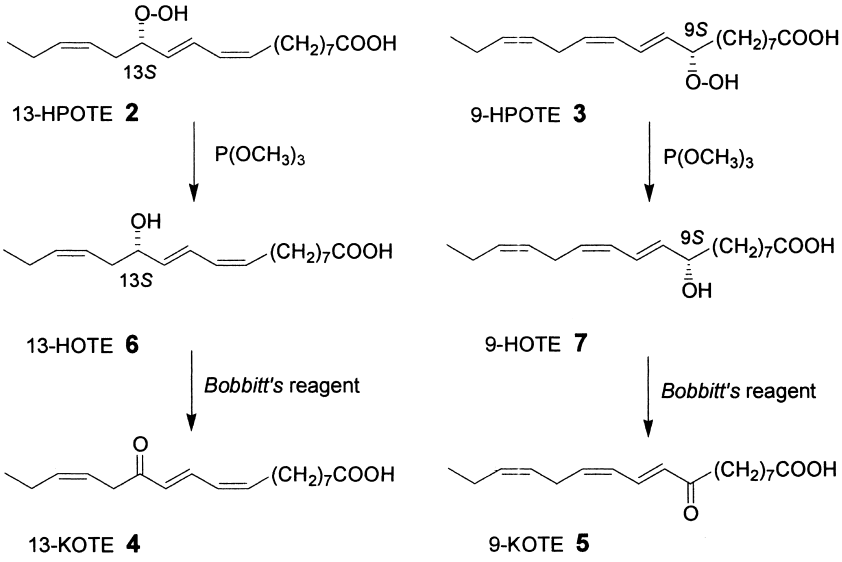


Scheme 1.



Scheme 2.

perchlorate)[12] to give the title compounds **4** and **5** without concomitant isomerisation.

Analogous to Scheme 1, linolenic acid **1** is regioselectively functionalised at C(13) by using a commercial lipoxygenase from soybean at pH 9 in borate buffer to generate 13-HPOTE **2**.[7,8] On the other hand, stirring of linolenic acid with a homogenate of tomatoes in a phosphate buffer (pH 5.7) along with simultaneous aeration yielded 9-HPOTE **3** without concomitant formation of 13-HPOTE.[8] The crude hydroperoxides were isolated by extraction and aliquots were directly reduced to the corresponding alcohols **6** or **7** (see Scheme 2) by treatment with $P(OCH_3)_3$.[13] Stirring of the alcohols with 1 equiv. of Bobbitt's reagent in CH_2Cl_2 in the presence of silica gel afforded the keto-trienoic acids **4** or **5** in good yield (ca. 70%) and essentially free from unwanted *E*,*E*-isomers.

The keto acids **4** and **5**, as well as the two hydroxy acids **6** and **7**, were tested for their ability to induce volatile biosynthesis in freshly cut plantlets of the Lima bean.[14,15] While the two keto acids **4** and **5** failed to induce volatile biosynthesis in the Lima bean, the two hydroperoxides **2** and **3** triggered a weak emission of volatiles.

3. Conclusions

In summary, by using the well established regioselective functionalisation of linolenic acid with lipoxygenases and final oxidation of intermediary allylic alcohols to the keto acids, we have established an efficient and versatile route to the configurationally pure ketotrienoic acids **4** and **5** which can be used for elicitation studies in plants. It is to be expected that analogous sequences may also be useful for the synthesis of highly unsaturated keto acids in the field of eicosanoids.

4. Experimental

4.1. General

All reactions were run in flame-dried glassware under argon. Solvents and reagents were dried prior to use. ^{1}H and ^{13}C NMR: chemical shifts of ^{1}H (500 MHz) and ^{13}C NMR (125 MHz) are given in ppm (δ) downfield relative to TMS as internal standard. Thin layer chromatography was performed with silica gel plates. Column chromatographic separations were performed on silica gel (Merck, Kieselgel 60; 230–400 mesh). Soybean lipoxygenase was obtained from Sigma–Aldrich, D-82024 Taufkirchen, Germany. The configurational purity of the products was established by gas chromatography on a capillary column, Econo-cap© EC-5 (SE 54), 15 m×0.25 mm (Alltech, Deerfield), under temperature programmed conditions. Free acids and alcohols were derivatised with diazomethane and MSTFA prior to analysis.

4.1.1. (13*S*,9*Z*,11*E*,15*Z*-Hydroxyoctadeca-9,11,15-trienoic acid (6). Linolenic acid (1.0 g, 3.6 mM) was emulsified by sonication in a borate buffer (600 mL, 0.1 M, pH 9.0). To the rapidly stirred emulsion (20°C) was added lipoxygenase from soybean (0.1 g, 5.3 U). After a few minutes the cloudy solution became clear and stirring was continued for 1 h followed by addition of dil. HCl (10 mL, 2 M). The crude hydroperoxide was extracted with $CHCl_3$ (3×500 mL) and dried using anhydrous Na_2SO_4, followed by removal of solvent at 20°C. Purification was achieved by chromatography on silica gel using hexane/ethyl acetate/acetic acid (600:400:1) for elution. Yield: 0.27 g (39%). An aliquot of the hydroperoxide (60 mg, 0.20 mM) was reduced to the alcohol **6** by stirring for 10 min at room temperature with $P(OCH_3)_3$ (100 μL, 0.816 mM) in CH_2Cl_2 (8 mL). Solvents and excess reagent were removed in vacuo and the alcohol was purified by chromatography on silica gel using the same solvent as before. Yield: 0.029 g (53%). The spectroscopic data of the compounds were in agreement with the literature.[9]

4.1.2. (9*S*,10*E*,12*Z*,15*Z*-Hydroxyoctadeca-10,12,15-trienoic acid (7). Tomatoes from the local market (1 kg) were homogenized in a phosphate buffer (600 mL, 0.1 M, pH 5.7) with simultaneous aeration. Then, linolenic acid **1** (1.0 g, 3.6 mM), previously emulsified by sonication (30 min) in the same buffer (300 mL) containing Tween 20 (1 mL), was added. Efficient stirring with simultaneous aeration was maintained for 4 h and then the reaction was stopped by addition of 0.2 M HCl (20 mL). The crude hydroperoxide was extracted with ether (3×800 mL). Separation of the phases was forced by centrifugation at 2500 rpm. Solid material accumulating at the interphase was extracted with ether (3×100 mL) and the combined extracts dried (anhydrous Na_2SO_4). Removal of solvent at 20°C afforded 0.73 g (65%) of the crude hydroperoxide **3**. An aliquot of 0.10 g was purified by preparative thin layer chromatography using a ternary solvent mixture of hexane/ethyl acetate/acetic acid (600:400:1) for elution. Yield: 0.058 g (58%). The hydroperoxide (58 mg, 0.19 mM) was reduced to the alcohol **7** by stirring for 10 min at room temperature with $P(OCH_3)_3$ (100 μL, 0.816 mM) in CH_2Cl_2 (8 mL). Solvents and excess of reagent were removed in vacuo and compound **7** was purified by chromatography on silica gel using the same solvent as before. Yield: 0.029 g (53%). Spectroscopic data of the products obtained were in agreement with the literature.[16]

4.1.3. (9*Z*,11*E*,15*Z*)-13-Oxooctadeca-9,11,15-trienoic acid (4). A well stirred solution of the hydroxy acid resulting from reduction of **2** (23 mg, 0.08 mM) in CH_2Cl_2 (300 μL) was treated at room temperature with Bobbitt's reagent (0.027 g, 0.09 mM) and silica gel (5 mg) to catalyse the oxidation. Stirring was continued for 30 min followed by removal of solvent in a gentle stream of argon. The crude residue was taken up in hexane/ethyl acetate/acetic acid (1:1:0.1%, v/v/v) and purified by column chromatography on silica gel using the same solvent. Yield: 13.3 mg (58%). ^{1}H NMR (500 MHz, $CDCl_3$) δ: 0.92 (t, J=7.50 Hz, 3H), 1.15–1.4 (m, 8H), 1.50–1.62 (m, 2H), 2.02 (m, 2H), 2.20–2.33 (m, 4H), 3.24 (d, J=6.91 Hz, 2H), 5.43–5.59 (m, 2H), 5.75–5.90 (m, 1H), 6.05 (t, J=11.3 Hz, 1H), 6.13 (d, J=15.3 Hz, 1H), 7.42–7.5 (dd, J=11.6, 15.3 Hz, 1H). ^{13}C NMR (125 MHz, $CDCl_3$) δ: 13.9, 20.9, 24.6, 27.2, 28.3, 28.6, 29.0, 29.4, 33.9, 40.1, 120.5, 126.9, 128.6, 135.3, 137.7, 142.8, 179.3, 198.7. MS (EI, 70 eV) m/z: 292 ($M^{+\cdot}$, 7), 224 (16), 223 (100), 149 (29), 135 (10), 123 (12), 109 (15), 107 (17), 97 (14), 95 (27), 83 (17), 81 (64), 79 (19), 69 (24), 67 (30), 57 (23), 55 (45). IR (KBr, neat): 3019, 2931, 2856, 1706, 1665, 1627, 1586, 1457, 1412, 1272, 1189, 996 cm^{-1}. HR-MS m/z calcd for $C_{18}H_{28}O_3$ 292.2038, found 292.2039.

4.1.4. (10*E*,12*Z*,15*Z*)-9-Oxooctadeca-10,12,15-trienoic acid (5). A well stirred solution of the hydroxy acid resulting from reduction of **3** (8.7 mg, 29.7 μM) in CH_2Cl_2 (300 μL) was treated at room temperature with Bobbitt's reagent (10 mg) and silica gel (5 mg) to catalyse the oxidation. Stirring was continued for 30 min followed by removal of solvent in a gentle stream of argon. The crude residue was taken up in hexane/ethyl acetate/acetic acid (1:1:0.1%, v/v/v) and purified by column chromatography on silica gel. Yield: 6.0 mg (69%). ^{1}H NMR (500 MHz, $CDCl_3$) δ: 0.92 (t, J=7.48 Hz, 3H), 1.15–1.35 (m, 6H), 1.47–1.61 (m, 4H), 2.03 (m, 2H), 2.28 (t, J=7.48 Hz, 2H), 2.5 (t, J=7.48 Hz, 2H), 3.0 (t, J=7.48 Hz, 2H), 5.25 (m, 1H), 5.4 (m, 1H), 5.79 (m, 1H), 6.06 (t, J=11.4 Hz, 1H), 6.12 (d, J=15.21 Hz, 1H), 7.45 (dd, J=11.6, 15.21 Hz, 1H). ^{13}C NMR (125 MHz, $CDCl_3$) δ: 14.2, 20.6, 24.2, 24.5, 24.6, 26.5, 29.0, 29.1, 34.0, 41.0, 125.2, 126.8, 129.6, 133.3, 136.8, 140.2, 179.9, 201.1. IR (KBr, neat): 3015, 2962, 2928, 2856, 1695, 1620, 1589, 1468, 1408, 1306, 1226, 1196, 1113, 1068, 992, 966 cm^{-1}. MS (EI, 70 eV) m/z: 292 ($M^{+\cdot}$, 75), 274 (17), 223 (64), 171 (74), 149 (55), 135 (17), 125 (46), 121 (51), 107 (50), 97 (38), 95 (46), 93 (46), 83 (49), 81 (54), 79 (77), 77 (32), 69 (23), 67 (36), 55 (100). HR-MS m/z calcd for $C_{18}H_{28}O_3$ 292.2038, found 292.2037.

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