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Cyclization of natural allene oxide in aprotic solvent: formation of the novel oxylipin methyl *cis*-12-oxo-10-phytoenoate

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Abstract

Allene oxide, (9*Z*,11*E*)-12,13-epoxy-9,11-octadecadienoic acid (12,13-EOD), was prepared by incubation of linoleic acid (13*S*)-hydroperoxide with flaxseed allene oxide synthase (AOS) and purified (as methyl ester) by low temperature HPLC. Identification of pure 12,13-EOD was substantiated by its UV and ^{1}H NMR spectra and by GC–MS data for its methanol trapping product. The methyl ester of 12,13-EOD (but not the free carboxylic acid) is slowly cyclized in hexane solution, affording a novel cyclopentenone *cis*-12-oxo-10-phytoenoic acid. Free carboxylic form of 12,13-EOD does not cyclize due to the exceeding formation of macrolactone (9*Z*)-12-oxo-9-octadecen-11-olide. The spontaneous cyclization of pure natural allene oxide (12,13-EOD) into *cis*-cyclopentenone have been observed first time.

Keywords: Allene oxide synthase; Allene oxides; Cyclization; Cyclopentenone; Oxylipins; NMR

1. Introduction

Oxylipins, the products of diverse metabolic pathways of polyenoic fatty acid oxidation, play important physiological roles both in plants and animals. Allene oxide synthases (AOS; EC 4.2.1.92), which are cytochrome P450s of CYP74A subfamily, belong to the key enzymes of the plant lipoxygenase cascade (Grechkin, 1998; Tijet and Brash, 2002). The products of AOS are unstable allene oxide fatty acids. These oxylipins present great interest as metabolic precursors of physiologically active cyclopentenones, particularly of plant signalling mediators 12-oxo-10,15-phytodienoic acid (**6**), 7-*epi*-jasmonic acid (**7**) and other jasmonoids (Hamberg and Gardner, 1992; Kessler et al., 2004) (Fig. 1). Being short-lived compounds, allene oxides undergo conversions in two competing ways: hydrolysis and cyclization. Cyclization may occur spontaneously (Grechkin, 1998; Tijet and Brash, 2002) or enzymatically (Ziegler et al., 1999). The mechanistic details of allene oxide cyclization still remain largely unrevealed.

Previously the natural allene oxides were isolated (Hamberg, 1987; Brash et al., 1988) and their ^{1}H NMR spectra were recorded (Brash et al., 1988).

Abbreviations: AOS, allene oxide synthase; 13-HPOD, (9*Z*,11*E*,13*S*)-13-hydroperoxy-9,11-octadecadienoic acid; 12,13-EOD, (9*Z*,11*E*,13*S*)-12,13-epoxy-9,11-octadecadienoic acid; 12,13-EOT, (9*Z*,13*S*,15*Z*)-12,13-epoxy-9,11,15-octadecatrienoic acid; 12-oxo-PEA, 12-oxo-10-phytoenoic acid; 12-oxo-PDA, (15*Z*)-12-oxo-10,15-phytodienoic acid; GC–MS, gas chromatography–mass spectrometry

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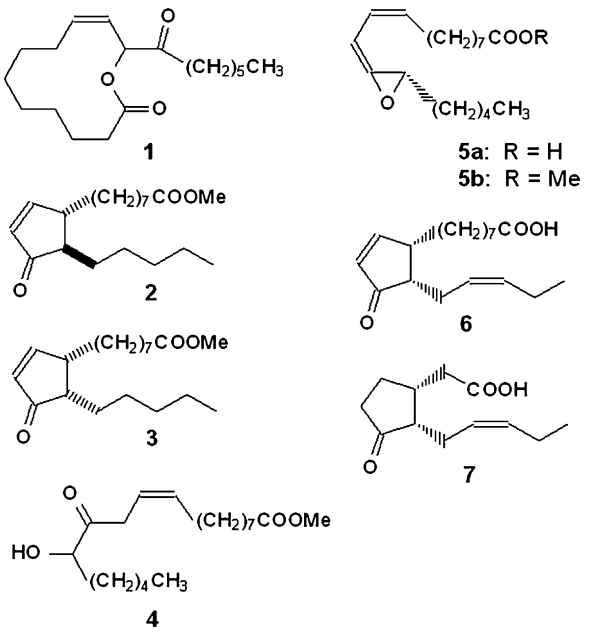

Fig. 1. The structural formulae of mentioned oxylipins.

However, there was no published data on cyclization of isolated pure allene oxides. The present paper is concerned with the first experimental observation of cyclization of the methyl ester of allene oxide fatty acid, (9*Z*,11*E*,13*S*)-12,13-epoxy-9,11-octadecadienoic acid (**5b**, 12,13-EOD) into *cis*-cyclopentenone **3** in aprotic solvent. This observation adds an important missing feature to allene oxide properties. The product of cyclization, the methyl ester of *cis*-12-oxo-10-phytoenoic acid (**3**, 12-oxo-PEA) is a novel compound. Only a *trans* isomer of 12-oxo-PEA (**2**) has been detected before (Hamberg and Hughes, 1988; Grechkin and Hamberg, 2000; Grechkin et al., 2002).

2. Materials and methods

2.1. Materials

Linoleic acid and soybean lipoxygenase type V were purchased from Sigma. Linoleic acid 13-hydroperoxide, (9*Z*,11*E*,13*S*)-13-hydroperoxy-9,11-octadecadienoic acid (13-HPOD) was obtained by incubation of linoleic acid with soybean lipoxygenase and purified by normal phase HPLC.

2.2. Allene oxide synthase preparation and incubations

Flaxseed allene oxide synthase (CYP74A) preparation was obtained as described before (Grechkin et al., 2002). 13-HPOD (1 mg) was incubated with 3 ml of AOS preparation in phosphate buffer, pH 7.0 for 20 s at 0 °C. Products were extracted with 6 ml of cold (0 °C) hexane. The extract was concentrated *in vacuo* ca. five-fold without heating and reacted with diazomethane (0 °C, 3 min). Methyl esters were dissolved in cold hexane and stored at −85 °C.

2.3. Separation and purification of products

A single non-polar compound **5b** (λ_{max} = 236 nm) was detected during the HPLC analysis of methyl esters at −20 °C on a cyanopropyl phase column Separon SIX CN (5 μm, 3.2 mm × 150 mm), solvent mixture hexane–diethyl ether 999:1 (by volume), flow rate 0.4 ml/min. Product **5b** was collected and stored in hexane solution at −85 °C. After the storage for 90 days at −85 °C this solution was allowed to stay for 4 h at 23 °C. The remaining 12,13-EOD was quenched with methanol; the products were redissolved in hexane and subjected to GC–MS and HPLC analyses. These analyses have revealed that compound **5b** underwent transformation mainly into product **3**. Compound **3** was collected by micropreparative normal phase HPLC at 23 °C on two Separon SIX columns (5 μm, 3.2 mm × 150 mm), connected in series, solvent mixture hexane–isopropanol 99.2:0.8 (by volume), flow rate 0.4 ml/min.

2.4. Spectral analyses

UV spectra of isolated products were recorded with a Specol 1200 spectrophotometer (Analytic Jena, Germany). Alternatively, UV spectra of compounds being purified by HPLC were recorded on line using an RSD 2140 diode array detector and Wavescan software (LKB, Bromma, Sweden). The ^{1}H NMR spectra were recorded with Bruker Avance 600 instrument, 600 MHz, C^2HCl_3, 296 K) The NMR data and the results of methanol trapping enabled us to identify product **5b** as allene oxide, (9*Z*,11*E*)-12,13-EOD methyl ester (Medvedeva et al., 2005). GC–MS analyses were performed using a Shimadzu QP5050A mass spectrometer connected to Shimadzu GC-17A gas chromatograph equipped with an MDN-5S (5% phenyl, 95% methylpolysiloxane) fused capillary column (length, 30 m; i.d. 0.25 mm; film thickness, 0.25 μm). Helium at a flow rate of 30 cm/s was used as the carrier gas. Injections were made in the split mode using an initial column temperature of 120 °C. The temperature was raised at 10 °C/min until 240 °C. Full scan or selected ion monitoring (SIM) analyses were both performed using an ionization energy of 70 eV. The