Synthesis of (±)-(10-^{2}H, 11-^{2}H$_2$, 12-^{2}H$_3$)Jasmonic Acid

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(±)-(10-^{2}H, 11-^{2}H$_2$, 12-^{2}H$_3$)Jasmonic Acid, Plant Growth Regulator, Synthesis, Mass Spectra

(±)-(10-^{2}H, 11-^{2}H$_2$, 12-^{2}H$_3$)Jasmonic acid has been synthesized starting from racemic jasmonic acid *via* 2-formylmethyl-3-methoxycarbonylmethylcyclopentanone by reaction with (1-^{2}H, 2-^{2}H$_2$, 3-^{2}H$_3$)propylidene-triphenylphosphoran followed by sponification of the labelled methyl jasmonate.

Introduction

Jasmonic acid (JA) and methyl jasmonate (JA-Me, **1**) are naturally occurring substances in plants. They are representatives of a group of plant growth regulators [1]. The exact quantification of JA in plant materials is of great interest for further studies on transport, metabolism and endogenous importance of JA. Using the GC/MS-SIM method deuterium-labelled JA should be a good internal standard. Therefore this report describe a simple way for the preparation of (10-^{2}H, 11-^{2}H$_2$, 12-^{2}H$_3$)JA. MS-spectrum is compared to that of JA.

Material and Methods

(1-^{2}H$_2$, 2-^{2}H$_2$, 3-^{3}H$_3$)Iodopropane (99,8% pure) was obtained from Isocommerz Handelsgesellschaft mbH, Leipzig, F.R.G. and (±)-methyl jasmonate from Firmenich SA, Geneva, Switzerland.

Ozonolysis

To (±)-methyl jasmonate (5 g, 22 mmol) in 100 ml CH_2Cl_2 O_3 was introduced at −15 °C until the color of the mixture became pale. An excess of ozone was flushed of with N_2 and the solution slowly added to a stirred suspension of Zn powder (7 g) in acetic acid (20 ml) at about 20 °C. Filtration of the mixture, treatment with water, sodium bicarbonate and drying with anhydrous sodium sulfate gave the crude aldehyde. Further purification was done by column chromatography (3.5×90 cm) on silica gel (Merck, 0.063–0.2 mm) and solvent mixture *n*-hexane : ethyl acetate : acetic acid (30 : 65 : 5). Aldehyde containing fractions were evaporated giving 2 g (46%) of **2.**

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Phosphonium salt

(1-^{2}H$_2$, 2-^{2}H$_2$, 3-^{3}H$_3$)Propyltriphenylphosphonium iodide was prepared according to [2]. Triphenylphosphin (2.62 g, 10 mmol), (1-^{2}H$_2$, 2-^{2}H$_2$, 3-^{3}H$_3$)-iodopropane (1.77 g, 10 mmol), 1 g K_2CO_3 in 7 ml CH_3CN were refluxed for 48 h. The mixture was filtered and after addition of ether the iodide crystallized. Recrystallization from $CHCl_3$/ether gave 2.1 g phosphonium salt (49%, Fp. 201–202 °C).

Wittig reaction

Phosphonium salt (2.1 g) was suspended in 15 ml dry THF, stirred and 1.7 ml of 1.6 M lithiumbutyl in *n*-hexane was added slowly at −30 °C. The so prepared deep yellow solution of propylidene-phosphorane was added to 0.44 g aldehyde **2** in 15 ml dry THF at −30 °C and further stirred for 15 min. The mixture was pured into 5 N HCl and extracted with ether. After washing with $NaHCO_3$ solution and drying with Na_2SO_4 evaporation of ether the residue was chromatographed on silica gel with *n*-hexane and an increasing gradient of ethyl acetate.

Fractions containing methyl jasmonate (**3**) were collected and evaporated. Methanol (2 ml) and 1 N NaOH (5 ml) were added and the mixture saponified at 60 °C for 2 h. The free acid was recuperated and further chromatographed as described above giving 158 mg (43%) of (±)-(10-^{2}H, 11-^{2}H$_2$, 12-^{2}H$_3$)JA (**4**).

Gas chromatography/mass spectrometry

Combined GC/MS was achieved under the following conditions: 80 eV mass spectrometer; steel column (1.5 m×2 mm) containing 3% OV 225 on Gaschrom Q (100–120 mesh); column temp. 180 °C; He 17 ml/min; R_t of methyl esters: **1** = 3.6 min, **3** = 3.6 min, 7-iso-**1** = 4.2 min, 7-iso-**3** = 4.2 min.

MS of **3:** *m/z* (rel. int.) 230 $[M]^+$ (43%), 212 $[M-H_2O]^+$ (6), 199 $[M-OCH_3]^+$ (20), 157 $[M-CH_2COOMe]^+$ (72), 156 $[M-C_5H_2{}^2H_6]^+$ (52), 138 (30), 96 $[C_8H_6{}^2HO]^+$ (48), 95 $[C_6H_7O]^+$ (43), 84 $[C_5H_6{}^2HO]^+$ (100), 83 $[C_5H_7O]^+$ (46).

Conditions for capillary GC were: column (50 m×320 μm) containing PB-1 (film thickness 0.2 μm), column temp. 140 °C, 2 ml/min N_2; R_t: **1** = 19.07 min, **3** = 18.75 min, 7-iso-**1** = 21.74 min, 7-iso-**3** = 21.36 min.

Results and Discussion

Modification of a given natural compound is a rapid tool for synthesizing derivatives. Here we report a short synthesis of deuterium-labelled jas-

monic acid (**4**), starting from racemic methyl jasmonate (±)-JA-Me (**1,** Fig. 1). Ozonolysis of (±)-JA-Me in dichlormethane at temperatures higher than −20 °C followed by reductive cleavage of the ozonide gave 2-formylmethyl-3-methoxycarbonylcyclopentanone (**2**) [3, 4], which could be purified by column chromatography.

The aldehyde **2** reacts rapidly with (1-^{2}H, 2-$^2H_2$, 3-$^2H_2$)propylidene-triphenylphosphoran in THF prepared from the ylide from (1-$^2H_2$, 2-$^2H_2$, 3-$^2H_2$)propyltriphenylphosphonium iodide. Under the used conditions the newly formed double bond is *cis*-configurated [4]. A parallel reaction with unlabelled ylide prepared from 1-bromopropane gave a better yield and a higher purity of the crude product. The free acid **4** was obtained by saponification of **3.** The final product was methylated with diazomethane and characterized by capillary GC and combined GC/MS. In capillary GC deuterium-labelled JA-Me showed 95% **3** (*trans*-situated side chains) and 5% 7-iso-**3** (*cis*-situated side chains) (Fig. 2).

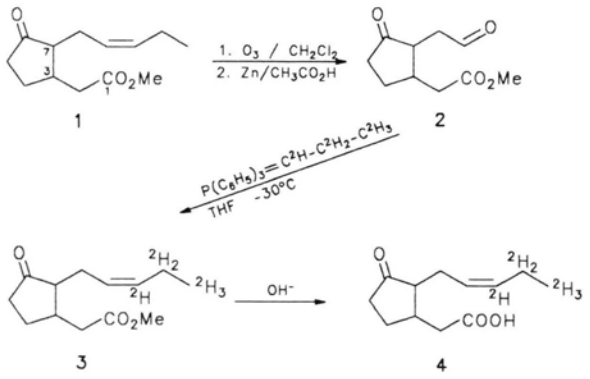

Fig. 1. Synthesis of (±)-(10-^{2}H, 11-$^2H_2$, 12-$^2H_3$)jasmonic acid.

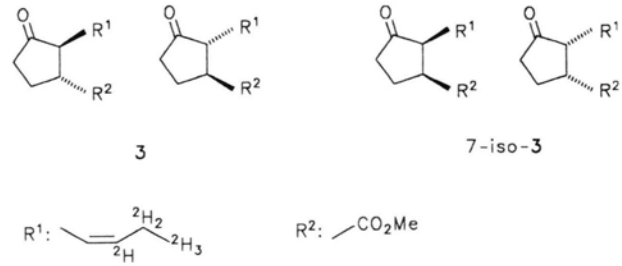

Fig. 2. Composition of product isomers.

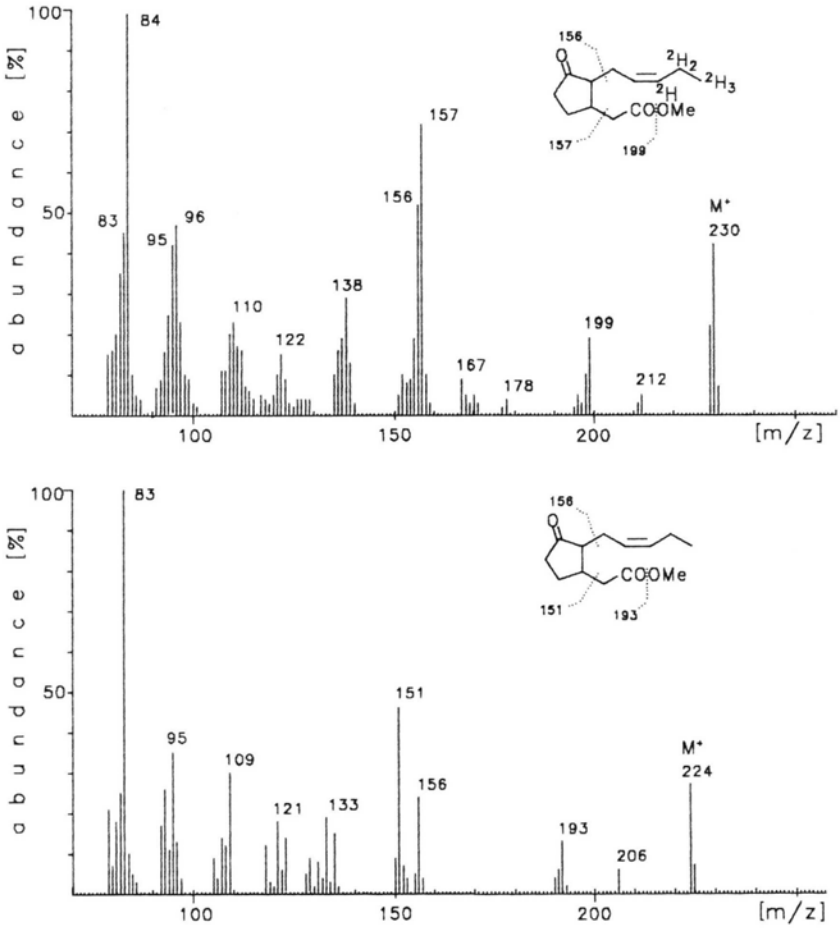

Fig. 3. Mass spectra of deuterium-labelled methyl jasmonate and methyl jasmonate (JA-Me).

In the mass spectrum of **3** fragments at m/z 230, 212, 199, 157 indicated the deuterium-labelled side chain compared to those of JA-Me (**1**) with fragments 224, 206, 193, 151, respectively (Fig. 3). Fragments at 96 and 84 stem from the cyclopentanone part of **3.** Compared to those of **1** they showed one unit more, surely, due to a partial Mc Lafferty rearrangement [5].

Retention times of **1** and **3** differ slightly in the capillary GC, because its differences in molecular weights.

For quantification of jasmonic acid by GC/MS-SIM ions at m/z 230 or 157 of the labelled compound and ions 224 or 151 of non-labelled one, respectively, are recommended.

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