Synthesis and Biological Activity of Stereochemically Locked Derivatives of Jasmonic Acid

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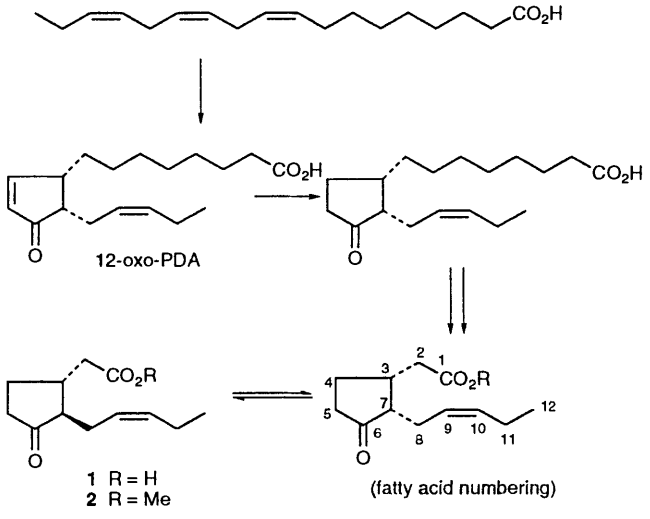
Racemic 7-methyljasmonic acid **6** and 7-methyl-7-*epi*-jasmonic acid **7** have been synthesised. Bioassay of these derivatives for induction of tendril coiling in *Bryonia dioica* demonstrated that the observed biological activity of jasmonic acid is due to the minor 7-*epi*-isomer.

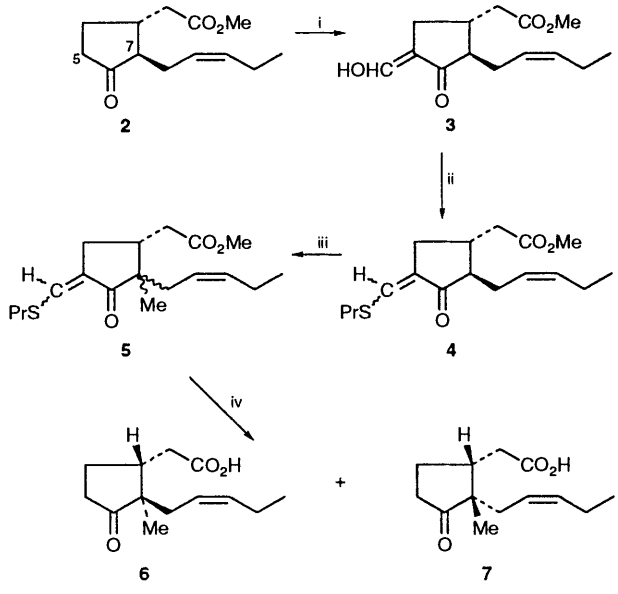
Jasmonic acid **1** and the corresponding methyl ester **2** are naturally occurring compounds which appear to have important roles as signalling compounds in several aspects of plant development, as well as mediation of phytoalexin production in response to fungal attack.[1,2] They are biosynthesised from linolenic acid by the action of a lipoxygenase/cyclase[3] yielding 12-oxo-phytodienoic acid (12-oxo-PDA) which then undergoes reduction and conversion to the jasmonic acid skeleton *via* a number of β-oxidation steps[4] (Scheme 1). This mechanism



Scheme 1

demands that the biosynthetic product has the *cis*-arrangement of the side-chains about the cyclopentane ring, *i.e.* that 7-*epi*-jasmonic acid is the initial product of the oxidative cascade. However, isolated natural jasmonic acid has equilibrated to the more stable *trans*-arrangement (actual equilibrium for isolated and synthetic jasmonic acid equals 95 : 5, *trans* : *cis*. It is unclear whether this equilibration occurs *in vivo* or during extraction. It is also not proven whether the observed biological activities residue in either the *cis*, *trans* or both isomers, although it is suspected that the *cis*-isomer is the physiologically active.[5] However, epimerisation of the applied compound during the biological assessment cannot be ruled out. The provision of derivatives of jasmonic acid that cannot be interconverted by enolisation will be important in defining the stereochemical arrangements of the side-chains for biological activity. In this paper we describe the synthesis and biological activity of both 7-methyljasmonic acid **6** and 7-methyl-7-*epi*-jasmonic acid **7**, derivatives within which the side-chains are stereochemically locked.

The synthetic route is outlined in Scheme 2. Alkylation at



Scheme 2 *Reagents and conditions:* i, KH, HCO_2Me, THF; ii PrSH, pyTs, benzene; iii KH, MeI, THF, room temp.; iv NaOH, aq. MeOH, reflux 3 h

the more hindered side of the cyclopentanone was best accomplished by use of a blocking group. Treatment of commercial racemic methyl jasmonate with potassium hydride and methyl formate gave the 5-formyl-derivative **3** in high yield. This compound was treated immediately with propanethiol–pyridinium toluene-*p*-sulfonate under azeotropic distillation in benzene to give the 5-propylthiomethylidene derivative **4** in 72% overall yield from compound **2** after flash chromatography. The blocked derivative was alkylated cleanly and rapidly by potassium hydride–methyl iodide to give essentially an equal mixture of the epimeric 7-methyl compounds **5**, although full interpretation of the ^1H NMR spectrum was complicated by the presence of geometrical isomers within the propylthiomethylidene group. Deblocking and ester hydrolysis were carried out simultaneously by sodium hydroxide in refluxing aqueous methanol to give a 1 : 1 mixture of 7-methyljasmonic acid **6** and 7-methyl-7-*epi*-jasmonic acid **7** as revealed by NMR and GC-MS analysis. The removal of the blocking group under these relatively mild conditions was surprising because this reaction is normally performed in high boiling solvents (*e.g.* diethylene glycol) with extended reaction times. The isomers are poorly resolved on silica gel either as the free acids or as their methyl esters. However, repeated chromatography of the free acids successfully yielded samples of the pure isomers.

The structures of compounds **6** and **7** were determined by

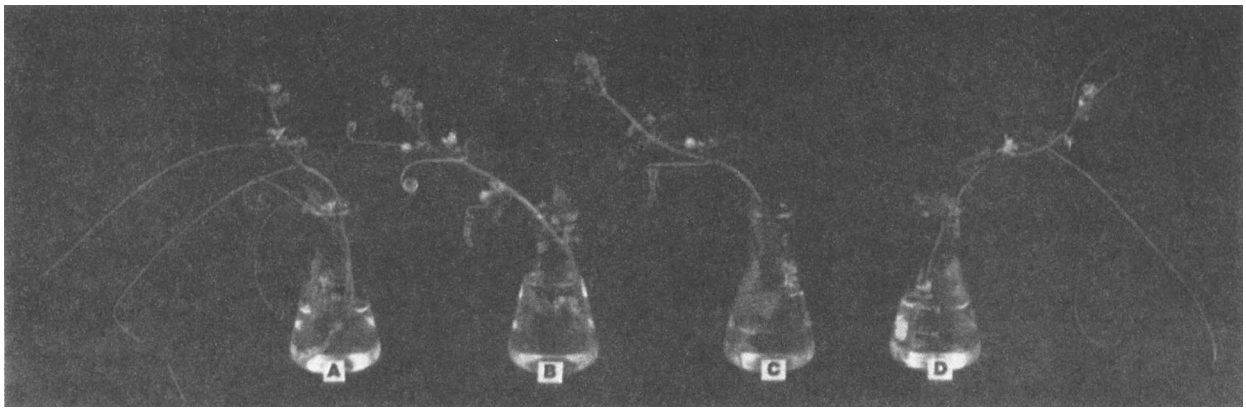


Fig. 1 *Effects of airborne* jasmonates (5×10^{-8} mol dm^{-3}) on tendril coiling of *Bryonia dioica*: A, control; B, methyl jasmonate **2**; C, methyl 7-methyl-7-*epi*-jasmonate (**7**, methyl ester); D, methyl 7-methyljasmonate (**6**, methyl ester).

NMR spectroscopy; [^{1}H–^{1}H] and [^{1}H–^{13}C] COSY spectra allowed assignment of all protons in both compounds. The *cis*-structure **7** was assigned to the more polar of the isomers by NOE difference spectroscopy. Irradiation at the 7-methyl resonance (δ 1.05) gave a large enhancement to the 3-H signal (δ 2.28), as well as to one of the protons of the 8-CH_2 group (δ 2.08) and the 9-H (δ 5.26) resonance. Conversely, for the less polar *trans*-isomer **6** irradiation of the 7-methyl signal (δ 0.89) gave no NOE to the 3-H signal (δ 2.54) but showed enhancement of the 2-CH_2 (δ 2.22 and 2.59), 4α-H (δ 1.54), one of the 8-CH_2 protons (δ 2.28) and 9-H (δ 5.21). Molecular modelling (NEMESIS) of both isomers gave energy-minimised structures in which the predicted intramolecular distances (< 2.9 Å for all the above) agreed with the observed NOEs.

Biological assessment of the derivatives was carried out by analysing the free-coiling induced in tendrils of *Bryonia dioica* by airborne compound as described by Falkenstein *et al.*[6] For this, the derivatives were converted to their methyl esters with diazomethane. After 16 h exposure, tendrils on cuttings in chambers containing methyl jasmonate **2** or methyl 7-methyl-7-*epi*-jasmonate (**7**, methyl ester) had fully coiled whereas tendrils on cuttings in chambers containing methanol only or methyl 7-methyljasmonate (**6**, methyl ester) were unaffected and remained straight (Fig. 1). This clear-cut result demonstrates unequivocally that the *cis*-arrangement of the side chains in jasmonates is necessary for biological activity and that in synthetic (and isolated) jasmonate only the minor isomer is active. Because of probable differences in volatility between methyl jasmonate and compounds **6** or **7** this assay is not strictly quantitative and further assays are planned to completely characterise the relative activities of **7** and methyl jasmonate as well as resolution of **7** and testing of the enantiomers.

Experimental

Methyl 5-Propylthiomethylidenejasmonate **4**.—To a stirred suspension of KH (1.34 g of 35% oil dispersion, washed with hexane) in tetrahydrofuran (THF) (100 cm^3), under N_2, was added 18-crown-6 (10 mg) and methyl jasmonate **2** (2.5 g in THF; 5 cm^3). After 15 min the solution was cooled to 0 °C, and methyl formate (1.03 cm^3) was added. The solution was allowed to warm to room temperature. During this time the solution darkened and effervescence occurred. After 0.5 h, water was added dropwise to destroy excess KH, and the resulting solution acidified with conc. HCl, before extraction with ethyl acetate to give the formyl derivative **3** (2.8 g) as a yellow oil; δ_H(400 MHz, $CDCl_3$) 0.97 (t, *J* 7.5, 12-H_3), 3.69 (s, OMe), 5.34 (m, 9-H), 5.47 (m, 10-H) and 7.19 (br, s, HOC*H*=).

The formyl derivative **3** (2.8 g) in dry benzene (100 cm^3) containing pyridinium toluene-*p*-sulfonate (10 mg) and propanethiol (5 cm^3) was refluxed using Dean–Stark apparatus for 2 h. After removal of the solvent, flash chromatography on silica gel (ethyl acetate–hexane) gave the 5-propylthiomethylidene derivative **4** (2.5 g); δ_H (400 MHz, $CDCl_3$) 0.95 and 1.01 (2 t, *J* 7.5, 12-H_3 and CH_3CH_2CH_2S–), 1.72 (sextet, *J* 7.5, $CH_3CH_2CH_2$S–), 2.83 (t, *J* 7.5, $CH_3CH_2CH_2$S–), 3.70 (s, OMe), 5.31 (m, 9-H), 5.44 (m, 10-H) and 7.43 (t, *J* 2.5, PrSC*H*=).

7-Methyljasmonic Acid **6** *and 7-Methyl*-7-epi-*jasmonic Acid* **7**.—To a stirred suspension of KH (0.56 g of 35% oil dispersion, washed with hexane) in THF (75 cm^3), under N_2, was added 18-crown-6 (5 mg) and the 5-propylthiomethylidene derivative **4** (1.45 g) in THF (5 cm^3). After 10 min the solution was cooled to 0 °C and freshly distilled methyl iodide (338 mm^3) was added. After 0.5 h a thick precipitate had formed and the reaction was worked up as above. The alkylated product **5** (1.42 g) was dissolved in methanol–2 mol dm^{-3} aqueous sodium hydroxide (1:1, 140 cm^3) and refluxed overnight. The methanol was removed under reduced pressure and the solution acidified with conc. HCl. Recovery in ethyl acetate and initial flash chromatography (hexane–ethyl acetate) gave a 1:1 mixture of compounds **6** and **7** (452 mg). Repeated flash chromatography and preparative TLC was used to obtain pure samples of each isomer.

7-Methyljasmonic acid **6**. (Higher R_f) (Found: M^+, 224.1424. $C_{13}H_{20}O_3$ requires *M*, 224.1412); δ_H(400 MHz, $CDCl_3$) 0.89 (s, 7-CH_3), 0.95 (t, *J* 7.5, 12-H_3), 1.54 (m, 4α-H), 2.04 (m, 11-H_2), 2.15 and 2.38 (both m, 5-H_2), 2.18 and 2.28 (both m, 8-H_2), 2.22 and 2.59 (both m, 2-H_2), 2.25 (m, 4-Hβ), 2.54 (m, 3-H), 5.21 (m, 9-H) and 5.48 (m, 10-H); δ_C(100 MHz, $CDCl_3$) 14.26 (C-12), 17.75 (7-CH_3), 20.76 (C-11), 25.45 (C-4), 33.59 (C-8), 35.36 (C-2), 37.40 (C-5), 39.34 (C-3), 51.27 (C-7) 123.42 (C-9), 135.44 (C-10), 178.97 (C-1) and 219.0 (C-6).

7-Methyl-7-epi-*jasmonic acid* **7** (Lower R_f) (Found: M^+, 224.1418. $C_{13}H_{20}O_3$ requires *M*, 224.1412); δ_H(400 MHz, $CDCl_3$) 0.97 (t, *J* 7.5, 12-H_3), 1.05 (s, 7-CH_3), 1.71 (m, 4α-H), 2.01 (m, 11-H_2), 2.05 and 2.08 (both m, 8-H_2), 2.16 (m, 4β-H), 2.22 and 2.25 (both m, 5-H_2), 2.28 (m, 3-H), 2.38 and 2.61 (both m, 2-H_2), 5.26 (m, 9-H) and 5.47 (m, 10-H); δ_C(100 MHz, $CDCl_3$) 14.22 (C-12), 20.74 (C-11), 20.85 (7-CH_3), 25.04 (C-4), 29.74 (C-8), 34.73 (C-3), 36.14 (C-5), 43.98 (C-3), 51.12 (C-7), 122.93 (C-9), 134.87 (C-10), 178.65 (C-1) and 221.29 (C-6).

Bioassay.[6]—Shoots of *Bryonia dioica*, having two or more straight lateral tendrils, were collected locally, stood in glucose–KCl soluion[6] and placed inside glass chambers (10 dm^3). After the plant material had equilibrated for 30 min the methyl esters (0.5 μmol) were applied, in methanol solution (50 mm^3), to

cotton wool plugs inside the chambers, avoiding contact with the plants. After 16 h tendril coiling was assessed and the plants were removed from the chambers for photography.

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