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BIOLOGICAL ACTIVITY OF METHYL 7-METHYL-JASMONATES

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Key Word Index—*Solanum tuberosum*; Solanaceae; *Bryonia dioica*; Cucurbitaceae; *Glycine max*; Leguminosae; *Avena sativa*; Gramineae; methyl 7-methyl-jasmonates; methyl jasmonate; bioassay.

Abstract—Stereochemically-locked *cis*- and *trans*-7-methyl derivatives of methyl jasmonate were found to have low biological activity in several assays, including that with *Bryonia dioica*. This suggests that the introduction of the locking methyl group at position 7 considerably lowers affinity for the jasmonate receptor, presumably owing to a steric effect.

INTRODUCTION

Jasmonic acid (**1**) and the corresponding methyl ester (**2**) are endogenous signalling compounds involved in plant reactions to stresses such as wounding and pathogen attack. These α,β-disubstituted-cyclopentanones are biosynthesized from α-linolenic acid, apparently as the *cis*-stereoisomer [1]. However, isolated and synthetic jasmonate exists with the α- and β-substituents mainly (*ca* 95%) in the more thermodynamically stable *trans*-configuration. We have attempted to determine which of the stereoisomers is responsible for the observed biological activities of jasmonic acid by synthesizing analogues that cannot be interconverted by enolization. Recently, we described the synthesis and preliminary biological evaluation of the *cis*- and *trans*-7-methyl derivatives of methyl jasmonate (**3**) and (**4**) [2]. In this work, we examined the derivatives for induction of tendril coiling in shoots of *Bryonia dioica* exposed to airborne compound. In this paper, we describe a more detailed quantitative assessment of the bioactivity of these derivatives using a number of jasmonate-responsive systems.

RESULTS AND DISCUSSION

The assay of the airborne activity of methyl jasmonate for the induction of tendril coiling has been described by Falkenstein *et al.* [3]. Owing to differences in volatility between derivatives of differing $[M]^+$, this bioassay cannot give accurate quantitative data on their relative bioactivity. However, our initial experiments indicated that methyl 7-methyl-7-epi-jasmonate (**3**) was active and that the *trans*-isomer (**4**) was not [2]. In order to quantify this activity, we needed to examine the relative activities of **2**–**4** in other well characterized jasmonate responses. The 7-methyl epimers were prepared and purified by silica gel flash chromatography as described previously [2]. These new samples of **3** and **4** were further purified by preparative HPLC. Both showed little activity in the airborne induction of tendril coiling in *Bryonia dioica*. This is in contrast to our previous observation that the *cis*-isomer (**3**) had activity in this assay [2]. A possible explanation for our initial observations of bioactivity in our original sample of **3** was contamination with methyl jasmonate. Methyl jasmonate impurities were difficult to distinguish from methyl 7-methyl-jasmonate (**4**) by NMR. GC–mass spectroscopic analysis revealed traces of methyl jasmonate in our original preparation of **3**. This arises from incomplete reaction in the alkylation step, and co-purification of the starting materials through the rest of the synthetic sequence. Purification by preparative HPLC was necessary to ensure complete purity of **3**.

Further investigation of the effects of **2**–**4** on *Bryonia* tendrils was carried out with cut tendrils floating in Petri dishes containing solutions of the jasmonate derivatives, as described by Falkenstein *et al.* [3]. The results were inconclusive as far as **3** and **4** were concerned, but methyl jasmonate at 100 μM was clearly active. One problem that we encountered with this assay was reproducibility, with even some untreated cut tendrils tending to coil, presumably owing to a response to wounding or mechanical shock. Thus, we found it difficult to assess compounds of low to medium activity in this assay.

In order to define more clearly the activity of the 7-methylated isomers, they were assayed in potato tuberization, senescence promotion and inhibition of cell division assays as described previously [4]. The results are shown in Figs 1–3. Methyl jasmonate is known to be as active as the natural agonists, tuberonic acid and its glycoside, in the induction of tubers in potato shoots [5]. It has also

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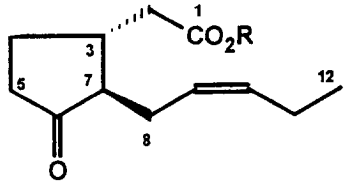

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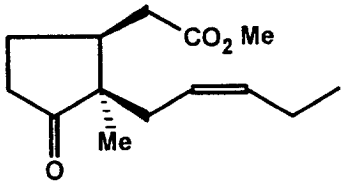
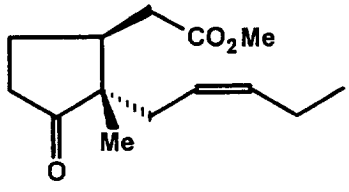


(3)



(4)

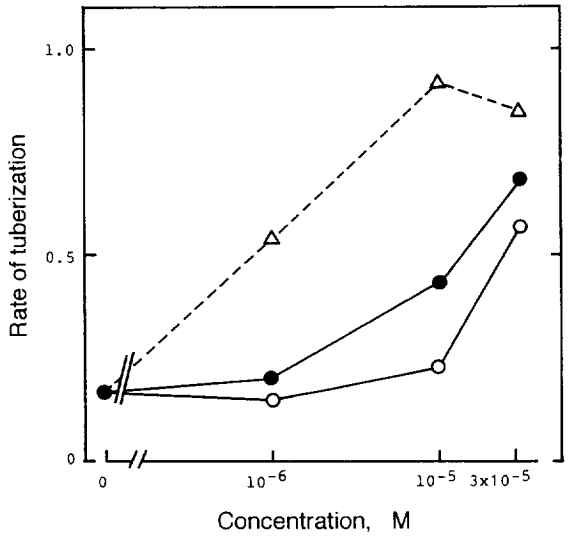


Fig. 1. Tuber-inducing activities of methyl jasmonate (**2**) (Δ), methyl 7-methyl-7-epi-jasmonate (**3**) (●) and methyl 7-methyl jasmonate (**4**) (○). Single-node segments of etiolated potato shoots were cultured aseptically on the test medium that contained 2% sucrose, for three weeks. The rate of tuberization was calculated as the number of tuberized laterals divided by the total number of emerged laterals.

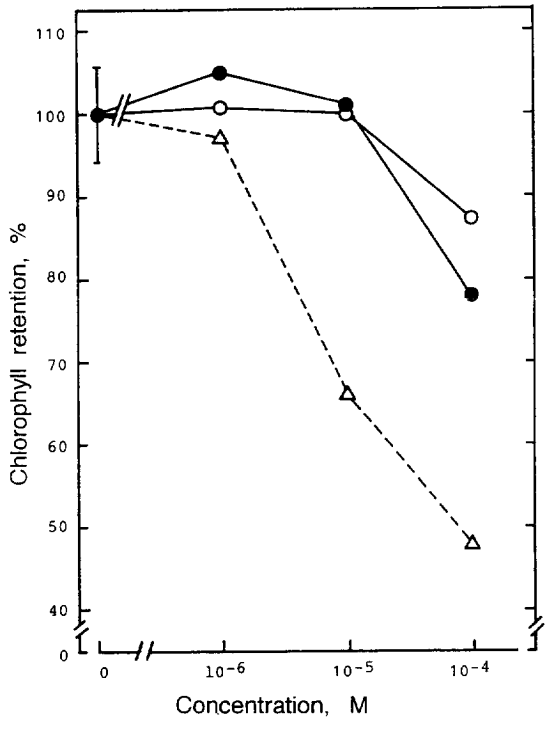


Fig. 2. Effects of methyl jasmonate (**2**) (Δ), methyl 7-methyl-7-epi-jasmonate (**3**) (●) and methyl 7-methyl-jasmonate (**4**) (○) on senescence of oat leaves in the presence of 10^{-6} M benzyl adenine (see [7]). Chlorophyll remaining in the leaves was extracted with hot 80% ethanol 3 days after treatment and the amount was measured as absorbance at 665 nm. The value of the control represents a mean ± SE (n = 5). SE for the other points were similar.

been demonstrated that applied *cis*-methyl jasmonate is more active than *trans*-methyl jasmonate in this assay [4]. However, *trans*-methyl jasmonate is not inactive in this system. The activities of the synthetic 7-methyl derivatives, **3** and **4** are shown in Fig. 1; both compounds are less active than synthetic methyl jasmonate (*trans*: *cis*, 19:1). Note that the differences would be more

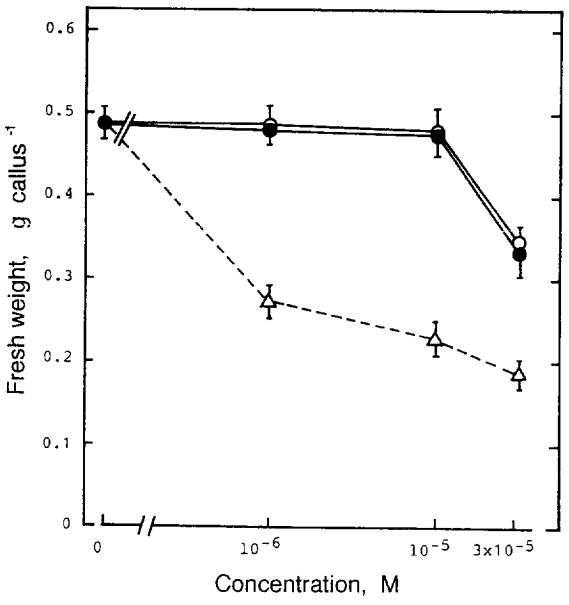


Fig. 3. Effects of methyl jasmonate (**2**) (Δ), methyl 7-methyl-7-epi-jasmonate (**3**) (●) and methyl 7-methyl-jasmonate (**4**) (○) on growth of soybean callus induced by 3×10^{-8} M zeatin riboside. Fresh weight of callus was measured after 4 weeks. Each value represents a mean ± SE ($n = 9$).

pronounced using pure *cis*-methyl jasmonate as a control [4]. The activity of the *cis*-7-methyl derivative (**3**) is a little higher than that of the *trans*-7-methyl compound **4**. In the presence of these compounds, tubers were induced on the laterals of the elongated shoots, rather than sessile tuber formation which is characteristic of a strong induction [4, 6].

Compounds **3** and **4** were of low activity in both oat leaf senescence and growth of soybean callus assays (Figs 2 and 3). There is no significant difference between **3** and **4** in these two assays. This is perhaps to be expected as these systems appear to show preference to the *R*-configuration at C-3, irrespective of the relative orientation of the C-7 side-chain.

In conclusion, it appears that the stereochemically-locked 7-methyl-derivatives of methyl jasmonate are of low activity in most bioassays. Very little discrimination between *cis*- and *trans*-forms was observed. It is apparent that the bioactivity previously observed in tendril coiling of *B. dioica* for airborne *cis*-locked compound (**3**) was a result of contaminating methyl jasmonate. The addition of the methyl group at C-7 apparently introduces enough steric bulk to severely reduce receptor binding in all of these systems, even though previous results indicated that the receptors in these systems may have different affinities for various stereoisomers [4]. This, of course, assumes that all the compounds are taken up by the plant material at the same rate and also are not differentially metabolised. The question of which of the stereoisomers of jasmonic acid is the active form remains open. We are preparing further derivatives to test the hypothesis that it is the *cis*-form.

EXPERIMENTAL

Chemicals. Me 7-methyl-7-epi-jasmonate (**3**) and Me 7-methyl-jasmonate (**4**) were synthesized as previously described. After sepn by flash CC, **3** was purified by prep. HPLC on Econosphere silica (5 μ) (Alltech, U.K.) eluted with 1.25% isoPrOH in *n*-hexane. Both **3** and **4** were analysed by GC-MS prior to use. Racemic Me jasmonate (**2**) was a gift from Dr Ferdinand Naf (Firmenich, Geneva).

Bioassay on Bryonia dioica. Assay for airborne activity was carried out as described previously [2] by the method of ref. [3]. Soln assays on cut tendrils were carried as described in ref. [3].

Bioassays for tuber-inducing activity, oat leaf senescence and inhibition of soybean callus growth. These were carried as described in refs [4, 5].

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