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Odor Thresholds of the Stereoisomers of Methyl Jasmonate

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Four stereoisomers of methyl jasmonate, methyl 3-oxo-2-(2-(Z)-pentenyl)cyclopentane-1-acetate, were isolated from a commercial sample of methyl jasmonate. Enantiomeric resolution was based on the separation of (-)-bornyl jasmonate diastereoisomers with liquid chromatography. Odor detection thresholds showed that methyl (+)-epijasmonate, (1R,2R)-(+)-methyl 3-oxo-2-(2-(Z)-pentenyl)cyclopentane-1-acetate, has the strongest odor-activity.

"Today jasmine flower oil and its synthetic substitutes are key ingredients universally employed in the manufacture of high grade perfumes...and methyl (-)-(Z)-jasmonate [is] said to be [one of] the specific carriers of the true natural jasmine fragrance." (Demole, 1982). However,

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an epimer of this compound, methyl epijasmonate is the sole form found in lemon peels (Nishida and Acree, 1984) where it seems to contribute significant odor. Furthermore, both epimers have been detected in the pheromone glands of a moth (Nishida et al., 1982) but only the epijasmonate form showed biological activity for the insect (Baker et al., 1981). These results led us to question the notion that methyl (-)-(Z)-jasmonate, (1R,2R)-(-)-methyl 3-oxo-2-(2-(Z)-pentenyl)cyclopentane-1-acetate, is the isomer with the greatest odor activity.

Commercial methyl jasmonate is a racemic mixture of four E isomers and four Z isomers. Liquid chromatogra-

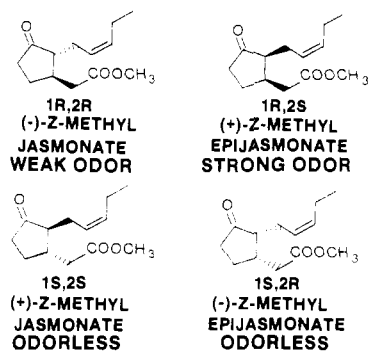


Figure 1. The four stereoisomers of methyl jasmonate are shown along with odor intensity of the neat samples measured immediately after preparation.

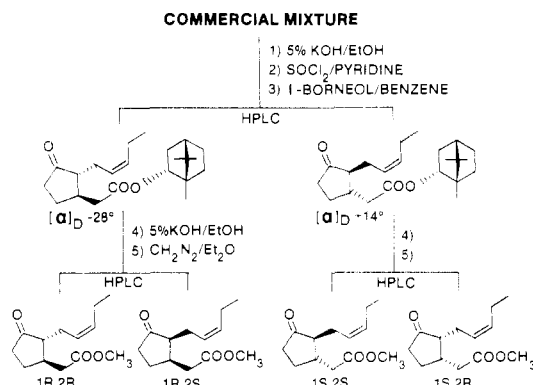


Figure 2. Strategy used to resolve the stereoisomers of (*Z*)-methyl jasmonate described in Nishida et al. (1985).

phy, synthesis, and separation of (*-*)-bornyl esters and their conversion to methyl ester was used to resolve commercial methyl jasmonate into the four *Z* stereoisomers shown in Figure 1. The odor detection thresholds determined here produce a clear picture of the relative odor-activity of these compounds.

EXPERIMENTAL SECTION

Purification and Separation of Jasmonate Isomers.

Methyl jasmonate (3.5 g from Shiono, Niitaka 5-17-75, Yodogawa-ku, Osaka, Japan) was chromatographed on a 28 mm \times 280 mm column filled with 100 g of 15% AgNO₃ on silica (Wako C-200). The column was eluted with hexane containing increasing amounts of ether. Methyl jasmonate was collected between 20% and 30% ether (yield 1.7 g). Gas chromatography on 25 m \times 0.36 mm i.d. fused silica column coated with OV101 cross-linked and bonded methyl silicone showed a mixture of methyl jasmonate, 93% (retention index 1612), and methyl epijasmonate, 7% (retention index 1642), but the absence of any (*E*)-methyl jasmonate (retention index 1596). This commercial mixture of jasmonates was then used in sensory analysis.

Several milligrams of purified methyl jasmonate dissolved in hexane were chromatographed in a 300 mm \times 8 mm i.d. column filled with 5- μ m silica (Nucleosil 100-5, Nagel) and developed with 12% ethyl acetate in hexane. Center cuts of peaks detected by refractive index were collected and concentrated within 4 h of their sensory analysis and their purity was determined by gas chromatography on methyl silicone.

Resolution of the Jasmonate Isomers. The strategy used to resolve the stereoisomers of (*Z*)-methyl jasmonate shown in Figure 2 is described in detail elsewhere (Nishida et al., 1985). It was based on the stereoselective synthesis and separation of bornyl jasmonate diastereoisomers from the (*Z*)-methyl jasmonates isolated from the commercial

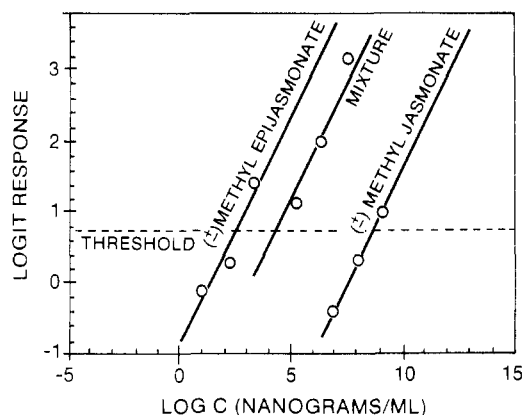


Figure 3. Triangle test data for (i) the commercial mixture of jasmonates, (ii) the purified racemic methyl jasmonate, and (iii) methyl epijasmonate are plotted as logit response vs. log concentration. The thresholds are taken as the intersections of the ED_{2/3} line with the fitted lines.

Table I. ORD Data Obtained for the Methyl Jasmonate Stereoisomers Used in This Study

compd	$[\alpha]_D^{30}$, deg
(+)-methyl jasmonate	+70
(-)-methyl jasmonate	-68
(+)-methyl epijasmonate	+58
(-)-methyl epijasmonate	-50

preparation. The purity of the four isolates is best demonstrated by the ORD data summarized in Table I.

Determination of Odor Thresholds. Triangle tests were used to determine odor thresholds. Samples to be tested were dissolved in 95% ethanol (rectified grain spirit) and added along with enough ethanol to make a ratio of 10 μ L to 20 mL distilled water. Samples were prepared in 50-mL bottles and covered with petri dishes. The blank sample contained 10 μ L of ethanol and 20 mL water. The panel, consisting of five members of the Institute, were presented with the six combinations AAO, AOA, OAA, OOA, OA0, and A00 (where A = added jasmonate and 0 = blank). Panel members were presented the samples in increasing concentration, they indicated the odd sample in each set, and for each concentration the proportion of correct responses, *P*, was determined. The linear regression of the logit transformed response, $\log(P/(1-P))$, on log concentration was calculated by iterative weighted least squares (Nelder and Wedderburn, 1972). Threshold concentrations were taken as concentrations yielding fitted values equal to $\logit^{2/3}$ (ED_{2/3} in Figure 3).

RESULTS AND DISCUSSION

The methods used to resolve the stereoisomers of methyl jasmonate yielded samples with high optical purity (Nishida et al., 1985) and smelling them revealed equally high sensory purity. Among the four stereoisomers of methyl jasmonate isolated, only (+)-methyl epijasmonate had a strong and characteristic odor (Figure 1). This isomer is always present in preparations of its 2-epimer (*-*)-methyl epijasmonate (Kaiser and Lamparsky, 1974) and, as we will show here, it is responsible for the odor usually associated with the 2-epimer (Demole and Stoll, 1962). Indeed, the weak odor we observed in (*-*)-methyl jasmonate may have been caused by a small amount of epimerization. However, the absence of any characteristic jasmonate-like odor in (*-*)-methyl epijasmonate and (+)-methyl jasmonate demonstrates a considerable chiral specificity in the human odor perception of methyl jasmonates.

The informal sniffing of neat chemicals can be confounded by adaptation and saturation (Koster, 1975). This

Table II. Odor Thresholds of Methyl Jasmonate Stereoisomers and Their Mixtures

compd	odor threshold, ng/mL	
	obsd	predicted
commercial mixture	90	a
(±)-methyl jasmonate	5700	large
(±)-methyl epijasmonate	13	6
(+)-methyl epijasmonate	3	3
(-)-methyl jasmonate	>70	large

^a Prediction based on threshold for the commercial mixture (ratio of 93:7 for jasmonate-epijasmonate) and the assumption that only (+)-methyl epijasmonate has odor.

problem was observed during the sensory analysis of methyl jasmonate as it eluted from a gas chromatograph (Acree et al., 1984a,b). In contrast, methods used to determine odor detection thresholds (Koster, 1975) are less subject to distortions of this type. Such methods involve preliminary estimates of threshold values followed by the collection of dose-response data in the neighborhood of the threshold where saturation and adaptation is unlikely to occur. Unfortunately, the analysis of compounds with very high thresholds, as expected for three of the isomers show in Figure 1, requires large samples at high purity. Furthermore, the spontaneous epimerization of jasmonates requires they be analyzed as soon as they are prepared. Sensory experiments were therefore conducted which did not require the direct analysis of all four isomers.

Initially, a commercial mixture of jasmonates was analyzed. The commercial material is abundant and presumably near epimeric equilibrium. Gas chromatographic analysis showed the commercial mixture to be 93% (±)-methyl jasmonate and 7% (±)-methyl epijasmonate. Assuming that all of the odor in the commercial mixture was derived from epijasmonate, we estimated its threshold and planned the dose-response experiment accordingly. The concentration range chose for the analysis of (±)-methyl jasmonate was based on the assumption that it contained 0.1% of (±)-methyl epijasmonate. Figure 3 shows a plot of the logits vs. log concentration for the three experiments.

A least-squares fit of the data from each experiment to a linear model produced curves of similar slope but different intercept. Therefore, we fitted these data to a linear model constrained by a common slope and computed the threshold from the intersection of these fitted lines with the logit $2/3$ line. The latter was used on the assumption that there is a $1/3$ probability of guessing correctly when no detectable difference exists (Salo, 1970). Plots of the experimental data and the fitted lines are shown in Figure 3. The threshold value of 5700 ng/mL obtained for (±)-methyl jasmonate can be explained by the presence of only 0.2% (±)-methyl epijasmonate (threshold 13 ng/mL). Therefore, we conclude that the (-)- and (+)-methyl jasmonate contribute almost nothing to the odor of the commercial mixture.

The separate analysis of (+)- and (-)-methyl jasmonate was not possible given the amount of material we had prepared. Furthermore, the absence of any jasmonate-like odor in the neat sample of (+)-methyl jasmonate leads us to conclude that its threshold is very large. However, we did analyze the enantiomers of methyl epijasmonate in the neighborhood of 6 ng/mL and the resulting dose-response data were fitted with the common slope produced for the plots in Figure 3. The value obtained for (-)-methyl epijasmonate (>70 ng/mL) and the absence of any odor in this sample neat indicates an infinite threshold for this isomer too. The threshold obtained for (+)-methyl epijasmonate was in agreement with prediction based on the threshold determined for the commercial mixture as shown in Table II. Therefore, we conclude that the odor of methyl jasmonate (*Z* isomers) is entirely due to the presence of (+)-methyl epijasmonate.

ACKNOWLEDGMENT

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Registry No. (1*R*,2*R*)-(-)-(*Z*)-methyl jasmonate, 1211-29-6; (1*R*,2*S*)-(+)-(*Z*)-methyl jasmonate, 95722-42-2; (1*S*,2*S*)-(+)-(*Z*)-methyl jasmonate, 78609-06-0; (1*S*,2*R*)-(-)-(*Z*)-methyl jasmonate, 62653-86-5; bornyl jasmonate (isomer I), 95647-06-6; bornyl jasmonate (isomer II), 95722-41-1.

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