

SHORT COMMUNICATION

MONOTERPENES: RELATIONSHIP BETWEEN
STRUCTURE AND INHIBITION OF GERMINATION

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Abstract—Of a group of structurally and metabolically related monoterpenes, the ones most toxic toward radish seeds (*Raphanus sativus*) were those having a ketone functional group, camphor and pulegone. All the monoterpenes stimulated germination at low concentrations and inhibited it at increasing concentrations.

INTRODUCTION

VOLATILE monoterpenes have been shown to be toxic in the vapor phase toward vascular plants by the work of Dässler and Zentsch¹ and of Muller and Hauge.² The relationship between chemical structure and toxicity, and the relevant quantitative relationships have not been established. For this purpose, the radish seed (*Raphanus sativus*) was chosen as the test organism because of its relatively rapid response and the ease of making large numbers of simultaneous tests.

RESULTS AND DISCUSSION

The level of germination of untreated seeds at 48 hr was 92 per cent. Since the monoterpenes of lesser toxicity would not completely repress germination at saturation levels in air, the standard of reduction to one-half of the control level (i.e. 46 per cent germination) was used as the reference level (Table 1). HCN was used as an arbitrary reference compound.

TABLE 1. CONCENTRATION IN AMBIENT AIR WHICH YIELDS A 50 PER CENT REDUCTION IN GERMINATION OF RADISH SEEDS (*Raphanus sativus*) AT 48 hr

Compound	Chemical function	$\mu\text{moles/l}^*$
HCN	—	5.8 ± 0.3
(+)-Camphor	Ketone	3.3 ± 1.2
(-)-Camphor	Ketone	3.1 ± 0.8
(+)-Pulegone	Ketone	1.5 ± 0.9
(-)-Borneol	Alcohol	21 ± 0.8
1,8-Cineole	Ether	78 ± 4.6
Limonene	Diene	45 ± 1.3
α -Phellandrene	Diene	57 ± 2.2
p-Cymene	Aromatic	51 ± 5.8
α -Pinene	Monoene	30 ± 1.9
β -Pinene	Monoene	170 ± 11

* Standard error of four triplicate determinations.

¹ VON H.-C. DÄSSLER and W. ZENTSH, *Pharmazie* **14**, 111 (1959).

² W. H. MULLER and R. HAUGE, *Bull. Torrey Botany Club* **94**, 182 (1967).

(+)-Pulegone was nearly 4 times as toxic as was HCN, and (+)- and (-)-camphor were nearly twice as toxic. These compounds were an order of magnitude more toxic than any of the other monoterpenes tested, indicating the activity of the ketone functional group. The other compounds generally appeared to have toxicities comparable to their ease of being metabolically altered to a ketone.

Borneol, which would require a single step, is next in order, followed by α -pinene, limonene and α -phellandrene—each of which would require at least a two-step biological oxidation to yield a ketone. A more complex route must be postulated for cineole and β -pinene with no reactive site on the ring. Only the aromatic compound, cymene, is anomalous. The fact that there is no significant difference between (+)- and (-)-camphor indicates there is little effect of ring conformation on toxicity.

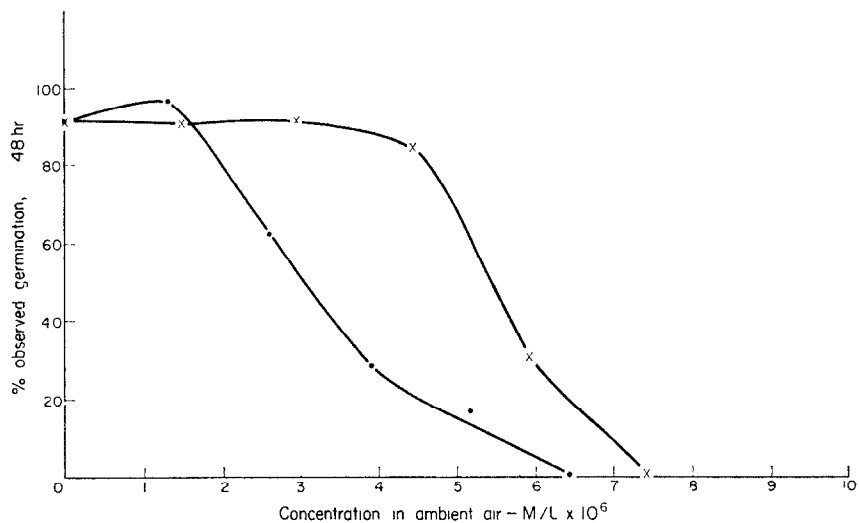


FIG. 1. DOSE-RESPONSE CURVES OF THE EFFECT OF (+)-CAMPHOR (•—•) AND HCN (×—×) ON GERMINATION AT 48 hr OF RADISH SEED (*Raphanus sativus*). POINTS SHOWN ARE MEANS OF TWELVE DETERMINATIONS. ALL DIFFERENCES ARE STATISTICALLY SIGNIFICANT.

All of the monoterpenes studied were capable of stimulating germination at concentrations lower than those which were inhibitory. Figure 1 is the dose-response curve for (+)-camphor, and similar-shaped curves were given by the other compounds. Although a stimulation of germination from 92 per cent to approximately 98 per cent is not large, in all cases it was statistically significant.

It should be noted that the toxicity evoked by monoterpenes upon the seeds of vascular plants was severe. Comparison with HCN points to this conclusion. Further, it is somewhat specific. A comparison of the concentrations required to inhibit seeds of vascular plants, and that which will inhibit bacteria, showed that the bacteria require concentrations of two orders of magnitude greater to show an observable effect.³

³ G. M. WARD and J. G. NAGY, Report to the National Science Foundation, Bacterial Inhibition by the Essential Oils of Sagebrush. Grant No. G. B. 1507 (1966).

EXPERIMENTAL

Monoterpenes were obtained from commercial sources and further purified either by sublimation or distillation at reduced pressure. Radish seeds were Red Globe variety obtained from Burpee Seed Company, San Francisco, California. Germination tests were conducted in covered plastic containers, $4.5 \times 4.5 \times 2$ cm. Seeds were germinated on filter paper cut to fit the container and moistened with 1.5 ml water. Test materials were added by pipetting them into plastic bottle caps and placing these in the container. Concentrations were calculated from the amount of materials added, their vapor pressures, and the volume of the container. Concentrations of selected runs were checked by means of gas chromatography. A 10 ft by $\frac{1}{4}$ in. column of 15 per cent Reoplex-400 on 60-80 mesh Gas-Chrom P was used. In all cases, the determination was within 5 per cent of the calculated concentration.

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