TABLE 1

INFLUENCE OF IAA AND CITRININ ON DEVELOPMENT OF PRIMARY ROOTS OF CUCUMBER

Citrinin	Mean length of primary root, mm*	
	No IAA	0.5 ppm IAA
0	66.5	41.2
1.0	60.5	42.9
2.5	63.5	52.0†
5.0	65.5	51.1†
10.0	56.7	47.4
25.0	51.3	44.1

* 20 seeds in each series.
† Reversal significant at 0.05 level.

ing effects of the auxin. Gliotoxin and rimocidin had no appreciable effect either alone or with IAA.

In the second method, the seedcoats of cucumber seeds were removed and the seeds were surface sterilized and planted aseptically on the surface of tubes of agar gel containing $M/30$ phosphate buffer of pH 6.0. The agar remained otherwise untreated in some cases, whereas in others IAA or antibiotics or both were added. With IAA at 0.1 ppm or less there was no apparent effect on the seedlings, but at higher concentrations there was root inhibition that increased with increases in the amount of IAA. At concentrations of 5–50 ppm IAA there was inhibition of the hypocotyl as well. Both oxytetracycline and clavacin at concentrations of 5–25 ppm reduced growth of roots and hypocotyl. When either material was used with IAA (0.5 ppm) the toxic effect was greater than that of the material without IAA. Streptomycin had little effect on seedling development either alone or in combination with IAA. As in the *Avena* section test, the effect of citrinin was opposite that of oxytetracycline. When used alone, citrinin had no apparent effect at 10 ppm, but when used with IAA (0.5 ppm) it partly reversed the inhibitive effects of IAA; the primary root was longer and laterals developed, whereas they were absent in the substrate that contained IAA alone.

In the moist chamber tests, cucumber seeds were placed in Petri dishes lined with filter paper moistened with the test solution. From 1 to 25 ppm citrinin was used in the test solution, either alone or with 0.5 ppm IAA. Measurements of roots were made after incubation in the dark at 28° C for 75 hr. Development of the primary root was apparently inhibited by the higher concentrations of citrinin used alone, but the differences were not statistically significant (Table 1). When used together with IAA, citrinin apparently partly reversed the inhibitive effect of IAA on growth of the primary root at concentrations of 2.5–25 ppm, but the reversal was statistically significant only at 2.5 and 5 ppm. Citrinin also partly reversed the inhibitive effect of IAA on development of lateral roots.

There were differences in the response of the seedlings to different concentrations of citrinin in the tests carried out in agar and in the moist chambers. For example, at 0.5 ppm IAA, development of the primary root was almost completely inhibited in the agar substrate, whereas it was fairly good in the moist chambers. These differences are ascribed to differences in the degrees of contact of the roots with the substrate under the two conditions; there was less contact of the roots with the substrate in the moist chambers.

The results provide evidence that antibiotics affect auxin action in distinctly different ways. Two types of effects received particular consideration: (1) A synergistic effect was most evident by the *Avena* section test with oxytetracycline and chloramphenicol; the effect was less pronounced with streptomycin. A suggestion of an increase in the auxin effect was noted also with these antibiotics in the seedling test, where development was inhibited. (2) The second effect was reversal or inhibition of auxin action (antiauxin) by citrinin. This effect was most evident by the seedling test, but was also indicated by the *Avena* section test.

The effects of antibiotics on auxin action may become of practical importance if antibiotics are used to control plant diseases (*2*) or to increase the rate of plant growth (*3*).

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The Odors of Optical Isomers

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The reports that certain pairs of optical antipodes display different odors have been cited as arguments against the Beck-Miles (*1*) infrared theory of olfaction in its original form. We have reinvestigated one such pair of optical isomers and have failed to confirm the original results (*2*).

The examples investigated, the *d* and *l* dimethyl *trans* hexahydrophthalates, were chosen because they

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seemed to offer the greatest possibility of freedom from chemical difficulties. All other reported instances have concerned substances isolated from natural sources, or compounds such as alcohols and olefins which are susceptible to rearrangements and other reactions which leave the purity and even the structures of the preparations in doubt. Even in the case chosen, however, which appeared to us to be the most favorable, we find that the earlier report of a difference in odor between the two isomers is in error owing to the presence of an unanticipated impurity in one of the samples.

A method of synthesis for the *trans* hexahydrophthalic acid (cyclohexane-1,2-dicarboxylic acid) dimethyl esters differing from that employed by Werner and Conrad (*2*) was chosen, both for reasons of convenience and in the hope that, if accidental impurities were encountered in either method, they might differ in the two routes and lead to nonconcordant results and therefore be apprehended.

Butadiene was bubbled into molten maleic anhydride at 100–110° until absorption ceased, and the resultant *cis* 4-cyclohexene-1,2-dicarboxylic anhydride[3] was crystallized from hexane. The product, m.p. 104°, was hydrogenated in acetic acid, using platinum oxide catalyst. After removal of the catalyst and solvent, the crude saturated anhydride was dissolved in one volume of concentrated sulfuric acid and heated at 100° for 1 hr. An equal volume of water was then added, and heating continued at 150° for an additional hour. Upon cooling, the *trans* cyclohexane-1,2-dicarboxylic acid separated and was recrystallized from water, m.p. 231–232°.

The racemic acid was resolved with quinine according to the directions of Werner and Conrad (*2*). The *d*-acid quinine salt which separated first from methanol was recrystallized several times from that solvent. The acid was liberated with sulfuric acid, taken up in ether, and recrystallized twice from a minimum quantity of water, m.p. 183.5–184°.

The *l*-acid was obtained by evaporation of the mother liquors from the crystallizations and extraction with ether. It was crystallized only once from water, and had a slightly lower melting point than the *d* isomer, 178–180°.

The dimethyl esters were prepared from the acids by reaction with excess diazomethane in ether and isolated by distillation. Both esters boiled at 250° (740 mm).

The *d* dimethyl *trans* cyclohexane-1,2-dicarboxylate (hexahydrophthalate) was thus obtained as a colorless oil with a faint pleasant odor somewhat like that of spearmint. The rotation of the pure liquid in a 1 d. tube was +24.0°; d_4^{20} 1.100 : $\alpha]_D$ +21.8°. In acetone, $\alpha]_D$ +29.7° (10%).

The *l* ester as obtained in the original distillation had an odor that was considerably stronger than that of the *d* ester, and of a different character, more like that of peppermint. The rotation of the undiluted liquid was −20.6°. In acetone, $\alpha]_D$ −25.5° (10%).

The infrared spectrum of the *l* ester was almost the same as that of the *d* ester, but had an additional small maximum at 14.0 μ. The area under this band was only about 0.1% of the total area under the absorption curve from 2 μ to 15 μ. The *l* ester was therefore carefully refractionated. A small forerun (about 1% of the total) was obtained that had a strong odor and displayed a pronounced maximum at 14 μ, plus some additional lower maxima which had not appeared in the original spectrum on the *l* ester, all superimposed on the spectrum characteristic of the hexahydrophthalate. A large intermediate fraction resembling the original sample of *l* ester distilled next, followed by a small fraction which lacked the 14 μ feature. The spectrum of this highest boiling fraction was identical in every respect with that of the *d* ester. Furthermore, its odor was indistinguishable from that of the *d* ester.

The odor of the low boiling fraction was reminiscent of that of methyl benzoate. The infrared spectrum of methyl benzoate was determined, and the prominent peak at 14 μ, together with coincidence of certain other features, makes it appear likely that this is indeed the impurity present in the crude *l* ester.

Pure samples of the *d* and *l* dimethyl *trans* hexahydrophthalates have odors that are indistinguishable qualitatively and seem to be of comparable intensity. Our crude sample of the *l* ester had a noticeably different odor, but one which fits the description given by Werner and Conrad for their sample of *l* ester. The synthetic routes were different, but a common feature of both investigations was the resolution with qunine. It seems probable that a trace of benzoic acid in the quinine used was responsible in both cases for the impurity of the once-distilled *l* ester. The concentration of the impurity was estimated to be 1% or less.

We have checked cursorily one other system, that of *d*-limonene and dipentene (*d,l*-limonene). Commercial samples of these two do display different odors, but the infrared spectrum of the dipentene used (Eastman Kodak, white label) had several peaks not present in the spectrum of the *d*-limonene (Eastman Kodak, blue label). Excluding an unusual solvent effect, the dipentene sample therefore contained more than *d*- and *l*-limonene.

In view of these results, we would be reluctant to believe that any two optical isomers have different odors, unless a more carefully authenticated example should be discovered in the future. Though this tends to vitiate one of the strongest arguments against any radiation theory of olfaction, it must be pointed out that our results should not be interpreted as supporting such theories in any way.

[3] This compound has since become commercially available.

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