

fewer upper lines of deposition (compare Fig. 3 and Fig. 1B). The maximum inhibition for *B. subtilis* occurs in the region of the translucent zone.

To concentrate the bacteriostatic substance still further, the extract was placed in a beaker so that the evaporation area was less and the evaporation slower than in a dish. This method of evaporation produced a longer zone of translucency on the filter strips and increased the zone of inhibition.

When the solvent and extracted substance were evaporated to about 20 cc, a milky-tan precipitate and a dark brown-red substance were produced. Evaporation filter strips of each showed that the milky-tan substance contributed to the upper lines of the previous strips, whereas the dark brown-red substance formed the translucent zones. Both of these strips proved to contain a bacteriostatic substance.

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The Growth Regulating Properties of Some β -Naphthylalanines^{1, 2, 3}

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The growth regulating activity of *dl*- α -alanine and β -indolylalanine (1) and β -alanine (2) has been reported. Since tryptophane (β -indolylalanine) is a precursor to 3-indoleacetic acid in plants (3), it was of interest to study the effect of substituting naphthyl radicals for the indolyl group in tryptophane. Several naphthyl derivatives of alanine were studied and found to have unusual growth regulating properties.

The relative activity of the β -naphthylalanines as possible growth regulators was determined by employing a biological test. Alamercury (4) has demonstrated that cucumber seeds during germination are very sensitive to 3-indoleacetic acid, the inhibition of root growth being proportional to the concentration of 3-indoleacetic acid used. Accordingly, cucumber seeds of the variety Marketer were germinated on filter paper in Petri dishes. The seeds were first grown on filter paper impregnated with 0.05 *M* KH_2PO_4 buffer solutions for 24 hr and then treated with the desired chemicals to a concentration of 100 ppm. Ten seeds were placed in each dish and each treatment was replicated three times. The temperature of the containers was kept at 25° C and observations were made at the end of 120 hr for growth and abnormal

development. The length of the main root of the seedling was used as an index of plant response (Table 1).

TABLE 1. The effect of various β -naphthylalanines upon the root growth and the respiratory quotient of germinating cucumber seeds.

Compound*	Root length in mm after 5 days	$\mu\text{l O}_2/100 \text{ mg of tissue/hr}^\dagger$	$\mu\text{l CO}_2/100 \text{ mg of tissue/hr}^\dagger$	RQ
Control	83.0	36.4	31.0	0.852
1-Naphthaleneacetic acid	2.0	26.4	33.7	1.278
β -1-Naphthylalanine	9.0	44.1	41.7	0.945
Glycyl- β -1-naphthylalanine	14.0	39.6	37.4	0.945
β -3-Thianaphthylalanine	15.0	49.6	50.4	1.013
Carbobenzoxylglycyl- β -1-naphthylalanine	44.0	34.7	29.5	0.850
Carbobenzoxylglycyl- β -2-naphthylalanine	42.0	40.5	33.6	0.830
β -2-Naphthylalanine	3.0	39.7	33.5	0.844

* Concentration of chemicals was 100 ppm.

† Determinations made after 48 hr of treatment.

The β -naphthylalanines produced abnormal modification of root growth, besides causing inhibition of growth of the main root. In many instances root growth was characterized by the production of a

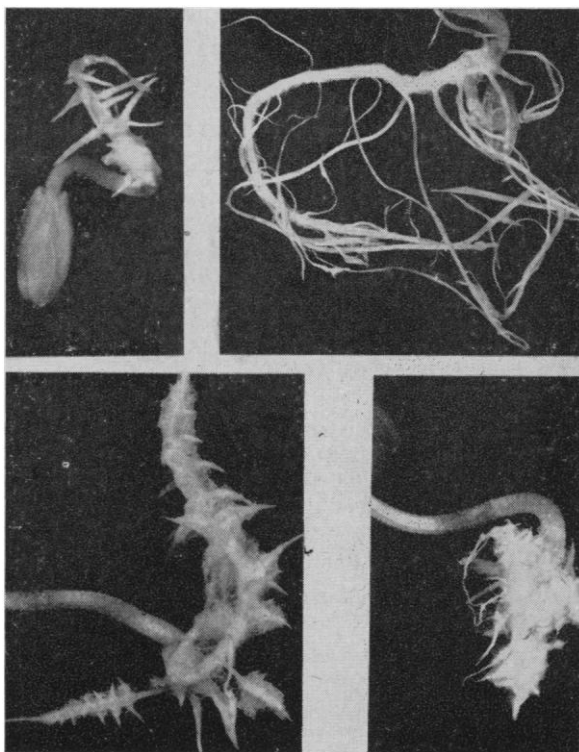


FIG. 1. Modification of root growth in cucumber seedlings induced by various naphthyl- β -alanines.

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prodigious amount of root hairs. Beta-1-naphthylalanine, glycyl- β -1-naphthylalanine, β -3-thianaphthylalanine, and β -2-naphthylalanine produced the greatest inhibition of root growth of the naphthylalanines tested.

The respiratory quotient (RQ) was obtained by standard procedures (5). These studies show that the RQ of germinating cucumber seeds was increased by the naphthylalanines previously mentioned (Table 1), with the exception of β -2-naphthylalanine. The RQ of the β -1-naphthylalanine, glycyl- β -1-naphthylalanines, and β -3-thianaphthylalanine treated seed can be correlated to the growth pattern observed in the roots. These compounds produced an effect similar to that observed with 1-naphthaleneacetic acid. The RQ of the seeds treated with the carbobenzoxyglycyl- β -naphthylalanines and β -naphthylalanine was approximately the same as the control whereas the growth behavior was much less than that of the nontreated seeds.

A possible explanation for these results is that the β -2-naphthylalanines may be converted into naphthyleneacetic acid by a mechanism similar to the conversion of tryptophane to indoleacetic acid in plants.

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The Role of Lactones in Flavor Deterioration of Milk Fat^{1,2}

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At an early stage in the storage deterioration of anhydrous milk fat a coconut-like flavor defect is evident (1, 2). This defect is manifest prior to typical oxidized fat flavors. Keeney and Doan (1) have suggested that lactones formed during deterioration of the fat are responsible for the off-flavor. This lipid-associated flavor defect is considered to be a principal factor limiting the acceptability of dry whole milk for beverage purposes (2).

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Evidence from this laboratory has indicated that the flavor and odor properties of δ decalactone appear to be identical with those of the compound responsible for the coconut-like off-flavor of milk fat. A logical origin of this lactone is Δ^9 decenoic acid, a component of milk fat glycerides. This acid and Δ^9 dodecanoic and Δ^9 tetradecenoic acids occur in milk fat but apparently are absent in most other fats (3-5). Of a number of fats studied, including milk fat, lard, coconut, cottonseed, and soybean and peanut oils, only milk fat developed the off-flavor in question. A comparison of flavor deterioration in milk fat containing phospholipids and in that washed free of phospholipids showed development of the off-flavor in both media with equal facility. Extensive study also eliminated vitamin A, carotene, and other constituents of the unsaponifiable matter as possible origins of the flavor defect. Experiments involving low-temperature crystallization and hydraulic expression of milk fat demonstrated that the flavor defect develops most readily in the low-melting fraction. Hydrogenation of milk fat from an iodine value of 32 to a value of 12 completely prevented development of the off-flavor. These results are considered good evidence that the off-flavor originates from the unsaturated glycerides of milk fat. Observations on flavor deterioration of the methyl esters of oleic, linoleic, and linolenic acids dissolved in mineral oil revealed that these compounds yield only oily, tallowy types of off-flavors. However, storage deterioration of Δ^{10} undecenoic acid resulted in the production of an aroma reminiscent of the milk fat off-flavor. Grün and Wirth (4) have reported that Δ^9 decenoic acid slowly decomposes on standing and develops the odor of decalactone. This observation was confirmed. Δ^9 decenoic acid was prepared from milk fat according to the bromination-debromination method of Bosworth and Brown (3). The acid on standing several months gave rise to the odor of δ decalactone. In addition, it was noted that odor of the lactone could be developed in 48 hr by treating the decenoic acid with 70 percent sulfuric acid as shown by Grün and Wirth (4). Study of various fatty acid fractions from milk fat indicated that the C_{10} fraction is the only one that will develop the typical coconut-like aroma of dry whole milk either autocatalytically or in the presence of sulfuric acid.

Additional evidence that Δ^9 decenoic acid may be involved in the flavor defect was obtained from infrared studies of milk fat recovered from dry whole milk immediately after manufacture and after one month of storage. Among the significant spectral changes in storage was a loss in absorption in the vicinity of 3.2μ . This change can be assigned logically to a loss of the vinyl group in Δ^9 decenoic acid (6).

In order for the lactones of decanoic acid to be of significance from a flavor standpoint, they should be detectable at relatively low concentrations. Results using four taste observers showed that the γ and δ decalactones are roughly equivalent in flavor potency and can be detected at a concentration of 1 to 2 ppm in milk. The γ lactones of decanoic and dodecanoic