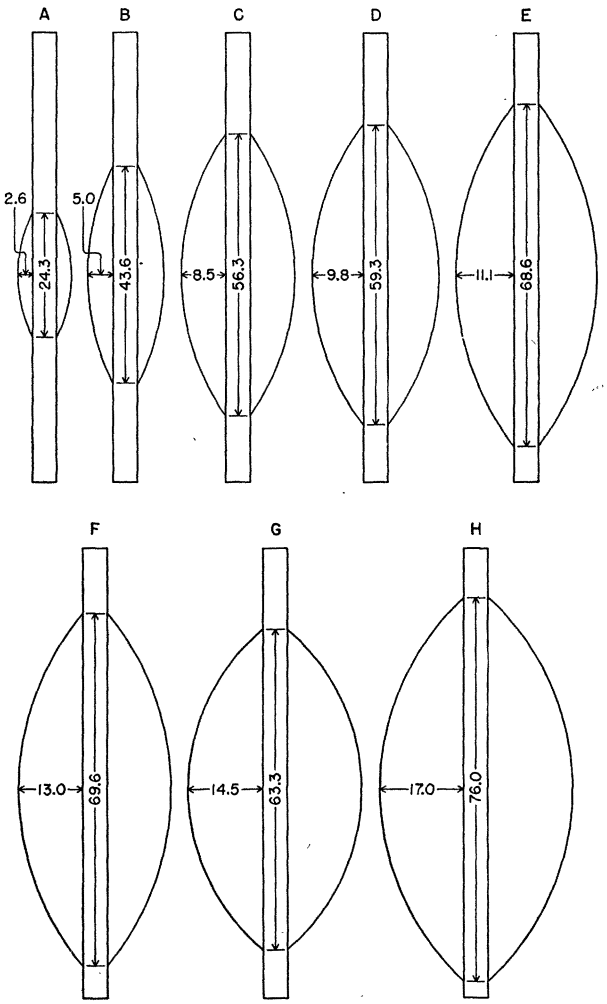

FIG. 1. Diagrams of the average maximum zones of inhibition around extract-impregnated filter paper (from Wyoming coal—Hanna Basin). A. *A. niger* in 24 hours; B. *A. terreus* in 24 hours; C. *M. verrucaria* in 33 hours; D. *V. albo-atrum* in 43 hours; E. *G. cingulata* in 33 hours; F. *C. ulmi* in 33 hours; G. *C. trifolii* in 43 hours; H. *E. fagacearum* in 74 hours.

for the eight fungi. The measurements are the averages of the widths and lengths of three replications when inhibition was at a maximum. *A. niger* was inhibited least, *E. fagacearum* most. Inhibition of the other fungi lay between these extremes. The greatest width of inhibition usually occurred about 27 mm above the part of the chromatograph that had been submerged. At this point there was a band in the chromatograph which appeared translucent when placed in front of a light.

Because of differences among the test fungi in time required for spore germination and in rate of growth of hyphae, different lengths of time, for example, 24 hr for *A. niger*, 74 hr for *E. fagacearum*, were required for the maximum, or first, area of inhibition to become defined. Such differences no doubt influenced the size of the regions of inhibition, as the slower fungi would afford opportunity for wider diffusion of the fungistatic material. Once established, however, the inhibition area usually remained constant for 12 or more hours, after which invasion from the outer margin occurred, *A. niger* advancing 1 mm, *A. terreus* 21 mm, in 54 hr.

The effect of the extract was fungicidal on *E. fagacearum* and *C. trifolii*, but fungistatic on the other six fungi. This difference in effect may have been due, also, to the rate of spore germination, as the spores of *E. fagacearum* and *C. trifolii* required longer periods for germination and were therefore exposed longer to the extract than were the spores of the other fungi.

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A Bacteriostatic Substance Extracted from the Vitrain Ingredient of Coal[1]

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A substance obtained from vitrain (bright bands in banded coal) can prevent the growth of *Bacillus subtilis*, as reported by Evans (*1, 2*). He termed this substance vitricin. To extract the substance, Evans covered finely ground vitrain with methanol. Strips of Whatman No. 1 filter paper were suspended about ½ in. into the mixture of methanol and coal, and the active material, vitricin, was carried along with the solvent in this modified ascending paper chromatograph method.

A bacteriostatic substance has been extracted from American coals using the method proposed by Evans and by other methods and with other solvents. This substance has been extracted (see Figs. 1, 2) from lignite, subbituminous coal, and high volatile bituminous coal of C, B, and A rank (classification of coal according to the degree of metamorphism from lignite to anthracite). The bacteriostatic action of extracts from high volatile A rank coal is slight, and entirely absent from one sample of the Buck Mountain anthracite. It would seem, therefore, that either the substance is absent or it cannot be extracted from higher rank coal, at least with present methods.

A substance extracted from a Pleistocene wood and two modern woods prevents the growth of *B. subtilis*. Whether this substance is the same as that obtained from coal is not known.

Initially, Evans' method was employed for the extraction of the substance from vitrain of Illinois Herrin (No. 6) coal. A bacteriostatic substance was adsorbed on paper strips, as shown in Fig. 1D. The

[1] Published with permission of the Chief, Illinois State Geological Survey, Urbana.

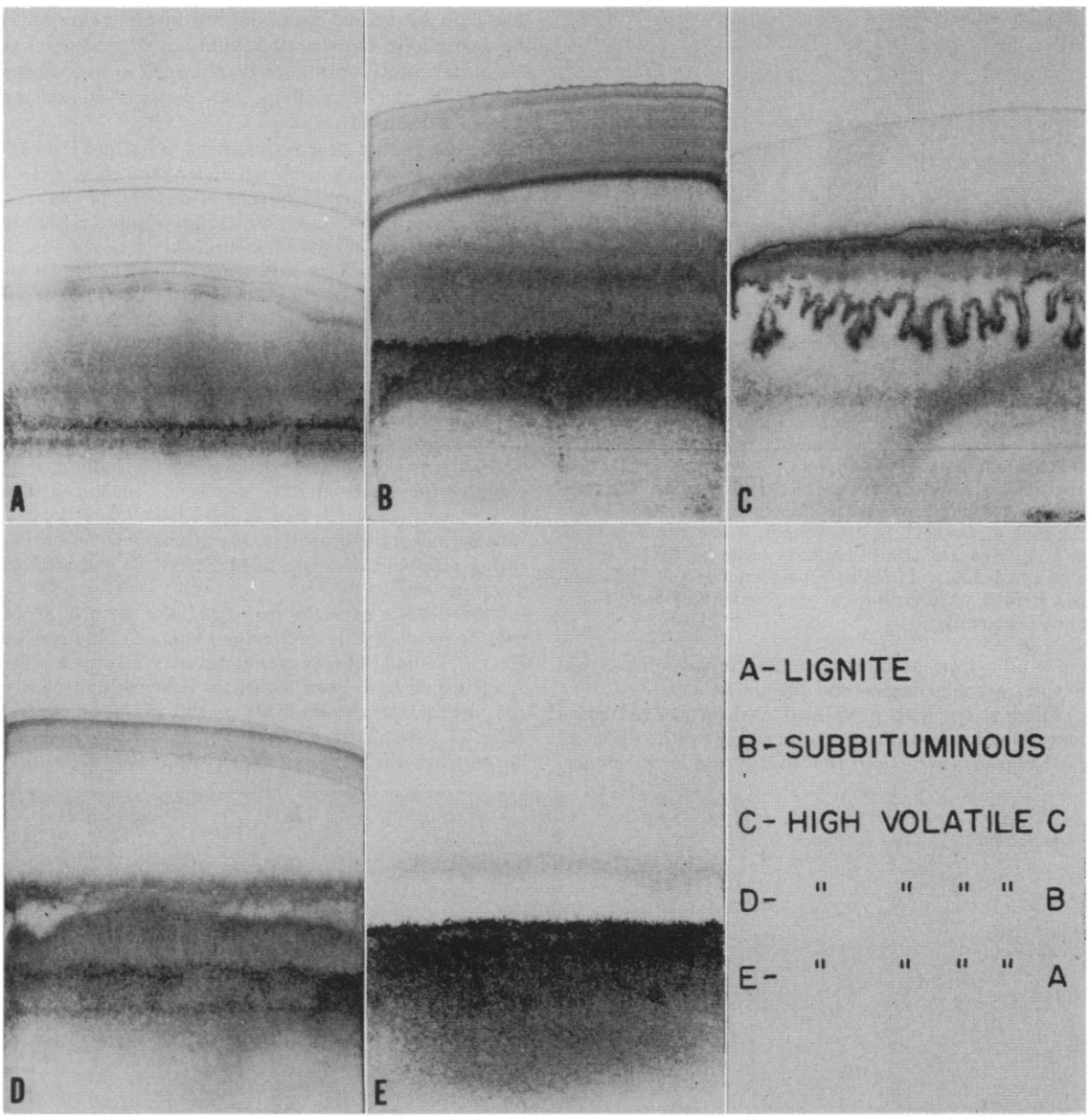

FIG. 1. Strips of filter paper 2¼ in. wide containing substance extracted from vitrain by the simple evaporation method. Methanol was used as the solvent. The filter strips are arranged according to the rank of the coal from which the vitrain was obtained.

uppermost lines were light brown and extended for 7 mm. Below this region was a tan zone and a darker brown zone; at the bottom of the strip were deposited fine particles of coal. These filter strips were cut into segments 3/16 in. wide and placed on the surface of nutrient agar (pH 7.3) previously inoculated with *B. subtilis* and incubated at 25° C.[2] Tests were conducted in triplicate plates. A clear area or zone of inhibition occurred in all three Petri dishes and was measured at 24 hr. The maximum clear area of the first tests was 4.5 mm wide and 60 mm in length. In some later experiments, measurements of the zone of inhibition were also made at 48 and 72 hr. In many of these the zone of inhibition was about 1 mm narrower and 1–2 mm shorter after 72 hr.

Besides methanol some other solvents were tested: acetone, absolute ethanol, benzol, pyridine, amyl alcohol, butyl acetate, and ether. With all these solvents, the filter paper adsorbed a coloration. Acetone extracts gave results comparable with those of methanol, absolute ethanol somewhat less, and amyl alcohol only a

[2] The author wishes to express his appreciation to Norman C. Schenck and J. C. Carter of the Illinois State Natural History Survey for determination of the potency of the bacteriostatic substance obtained from vitrain.

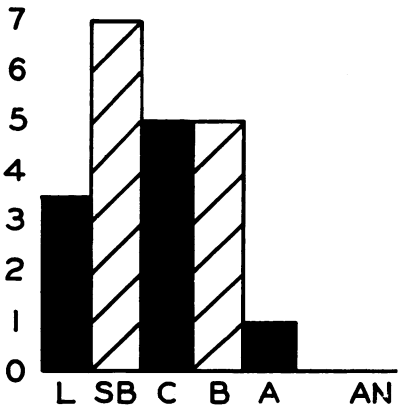

FIG. 2. Average width of inhibition on either side of a 3/16-in. strip of filter paper containing a bacteriostatic substance extracted by methanol from vitrain. The simple evaporation method was used and *B. subtilis* was the test organism. Measurements were taken after 24 hr. The numbers to the left represent millimeters, the letters denote the rank of the coal. L, lignite; SB, subbituminous; C, B, and A, high volatile bituminous coals of C, B, and A rank; and AN, anthracite. Subbituminous and high volatile B rank vitrain samples (diagonal lines) were tested most frequently.

trace of a zone of inhibition. The other solvents did not extract a bacteriostatic substance.

Filter strips with prominent dark brown-red zones were obtained while experimenting with different methods of extracting the bacteriostatic substance. The dark brown-red zones did not give a corresponding increase in the size of inhibition, suggesting that the bacteriostatic substance is contained in the colored portion of the filter strip, but perhaps is not the colored substance.

The most satisfactory equipment to extract the bacteriostatic substance consisted of a pyrex glass sample container with a capillary tube at one end of the container. This sample container was attached to a 1000-cc vacuum flask by means of a cork. Air was allowed to enter the vacuum flask through a 4-mm glass tube inserted in the cork of the flask. Glass wool was placed in the container between the sample and the capillary tube to screen out vitrain particles.

A finely ground sample of vitrain weighing 41.5 g was placed in the container and covered with a solvent (acetone or half acetone and half methanol) which was drained through the sample with the aid of a vacuum. The solvent containing the extracted substance was rerun several times and fresh solvent added each time to replace that which had evaporated. When the solvent became dark brown, it was poured into an evaporating dish with the suspended filter strip and allowed to evaporate.

These latter extracts prevented the growth of *B. subtilis* only slightly better than extracts obtained by Evans' method. They were, however, vastly better for fungi which have been tested by Schenck and Carter (*3*). Filter strips obtained by this vacuum method (Fig. 3) have a dark brown to red zone which is translucent when the strip is viewed in a strong light, and

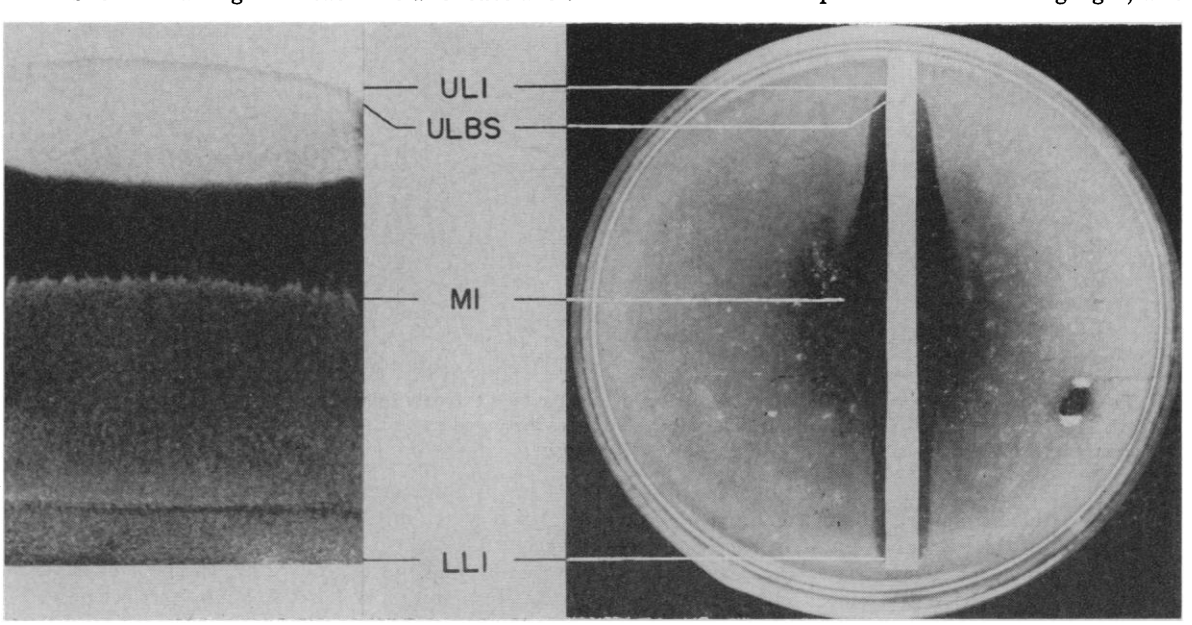

FIG. 3. Left, a strip of filter paper containing a bacteriostatic substance from vitrain from a coal of subbituminous rank by the vacuum method of extraction with acetone, and right, a portion of this same filter paper in a Petri dish containing a culture of *B. subtilis* in nutrient agar. Symbols are: ULI, upper limit of inhibition; ULBS, upper limit of extracted bacteriostatic substance; MI, maximum zone of inhibition; and LLI, lower limit of inhibition. The maximum zone of inhibition was 6.5 mm in width and 79 mm in length. The zone of inhibition appears black, and the active culture gray. The dark band to the left was 15 to 20 mm wide and was translucent when viewed in a strong light.

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.

References

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2. ———. *Ibid.* 513 (1951).
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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. Alamercery (*1*) 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	μl O_2/100 mg of tissue/hr†	μl CO_2/100 mg of tissue/hr†	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
Carbobenzoxyglycyl-β-1-naphthylalanine	44.0	34.7	29.5	0.850
Carbobenzoxyglycyl-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

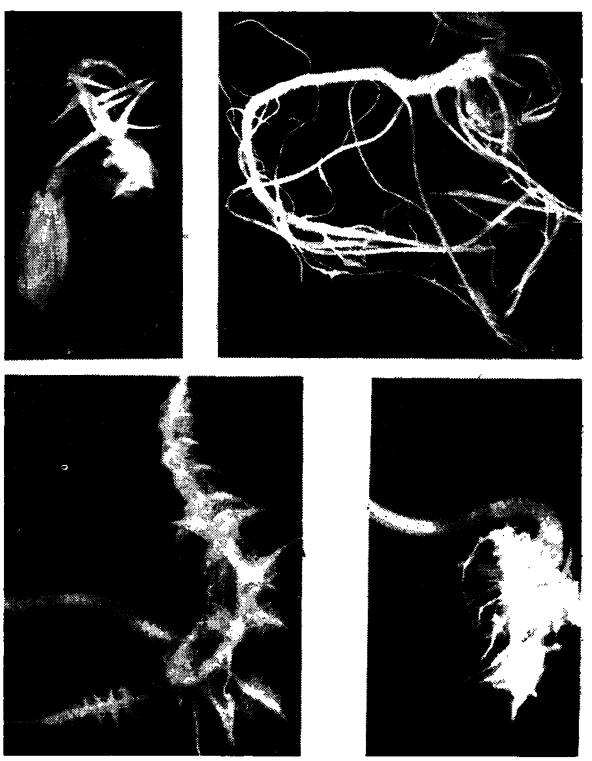

FIG. 1. Modification of root growth in cucumber seedlings induced by various naphthyl-β-alanines.

[1] Journal Article No. 1545 from the Michigan Agricultural Experiment Station, East Lansing.
[2] The β-naphthylalanines were supplied through the courtesy of Dr. Chester Stock of the Sloan-Kettering Foundation.
[3] Supported by the Horace H. Rackham Research Endowment of Michigan State College.