the injection was made intraperitoneally, the lesion results from an amount of the enzyme spreading about the trauma produced by the insertion and removal of the needle.

(5) The introduction of a suitable amount of arginase into the system buffers the arginine of the body, and, when into the tissues directly, inhibits the action of this substance in normal cell metabolism.

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Antibiotic Compound Isolated From the Lichen *Ramalina reticulata*

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Experiments conducted during the period July 1945–January 1947 showed that a crystalline substance isolated from *Ramalina reticulata* had *in vitro* antibiotic activity against a variety of gram-positive organisms and some acid-fast bacteria, including *Mycobacterium tuberculosis hominis*, but not against a number of different gram-negative organisms tested. *In vivo* tests with mice infected with pneumococcus gave negative results, while similar tests with tuberculous guinea pigs showed significant retardation of the progress of the disease (*6*).

Addition of base did not yield simple solutions of the lichen acid, and on the basis of this observation V. C. Barry suggested that the compound isolated might be usnic acid (*1*), shown to be a common constituent of many lichens.

The comparative studies described below were made with a sample of usnic acid received from V. C. Barry and isolated from *Cladonia sylvatica* [L] Harm. emend. Sandst.

The empirical formula (*5*) for usnic acid is given as $C_{18}H_{16}O_7$ [C: 62.79, H: 4.65]. The compound isolated from *Ramalina reticulata* gave the analytical figures [C: 62.88, H: 4.61], as previously reported, in excellent agreement with the above empirical formula. As doubt existed concerning the purity of the parent compound, an attempt was made to prepare a methoxyl derivative with diazomethane. It did not crystallize, but upon distillation in a molecular still gave analytical data [C: 65.75, H: 5.26, OCH_3: 9.50] which fitted the empirical formula $C_{16}H_{14}O_6$ (*6*). However, as the fractionally distilled methoxyl derivative was not crystalline, its homogeneity was also questionable.

A study was made of the homogeneity of the parent compound isolated from *Ramalina reticulata* by the method of countercurrent distribution (*4*). Sixty mg. of the material was distributed in a 24-tube countercurrent distribution machine, using a system containing 20 per cent cyclohexane and 80 per cent benzene as the upper layer and 10 per cent water and 90 per cent methanol as the lower layer, the two phases having first been equilibrated with each other before being used for the distribution. The volumes of the two phases were 12 cc. (upper phase) and 8 cc. (lower phase), giving an operational distribution ratio, K, of 0.41. In Fig. 1 is shown a 24-transfer distribution pattern of the substance. The concentration in each tube was determined by absorption at 284 mμ using the Beckman spectrophotometer. From the nearly complete correspondence with the theoretical curve it can be concluded that the material is apparently homogeneous, and therefore the empirical formula can safely be derived on the basis of the analysis of the original compound isolated rather than the doubtful methoxyl derivative.

The molecular weight of the compound as determined from the methoxyl content in the methoxyl derivative and by an alkali acetone titration of the parent compound was about 310.

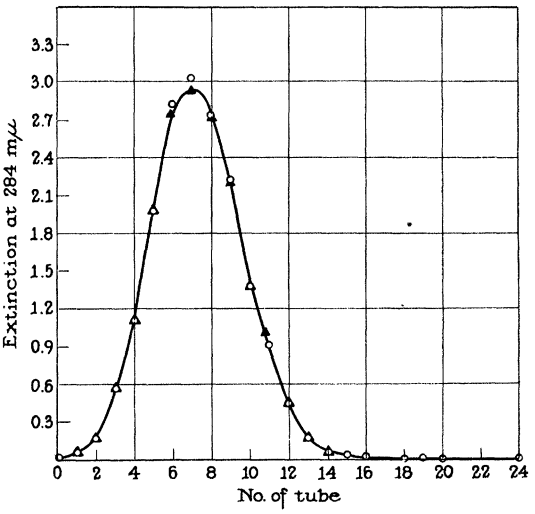

FIG. 1

This value favors a compound with an empirical formula $C_{16}H_{14}O_6$ (M., 302) rather than $C_{18}H_{16}O_7$ (M., 344) (*6*). However, since the acetone titration method employed can be considered valid only to within 5–10 per cent, the value 344 could still be considered possible as the molecular weight for the compound isolated from *Ramalina reticulata*. The coincidental similarity of the molecular weights as determined by the two methods first led the authors to consider the lower empirical formula. The later homogeneity study performed on the parent compound and the additional evidence given below now favors $C_{18}H_{16}O_7$ as the more probable value.

Usnic acid has been reported to melt in the range of 191–205° (*3, 7*). The compound isolated from *Ramalina reticulata* was reported to melt at 193–194° and showed a melting point similar to the compound received from V. C. Barry and isolated from *Cladonia sylvatica*. The melting point of the mixture of the two compounds showed no depression.

The ultraviolet absorption spectrum of the compound from *Ramalina reticulata* (Fig. 2) was similar to that of the compound isolated from *Cladonia sylvatica*. The absorption maxima are at 226–230 mμ and at 284 mμ.

The optical rotation for the compound from *Ramalina reticulata* in chloroform was $[\alpha]_D^{25} = +498°$, which corresponds to the value +495° reported for d-usnic acid (*4, 5*). The compound isolated from *Cladonia sylvatica* gave an $[\alpha]_D^{20} = -20.8°$ in chloroform and was considered to be the racemate of usnic acid with a small admixture of the l-form (*2*).

A study of the adsorption spectrum in the infrared region was made by Konrad Dobriner, of Memorial Hospital, New

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York City, and at the American Cyanamid Company. Both substances showed absorption bands similar in frequency and shape at 40 points. The correspondence of these two compounds at so many frequencies would leave little doubt as to their identity.

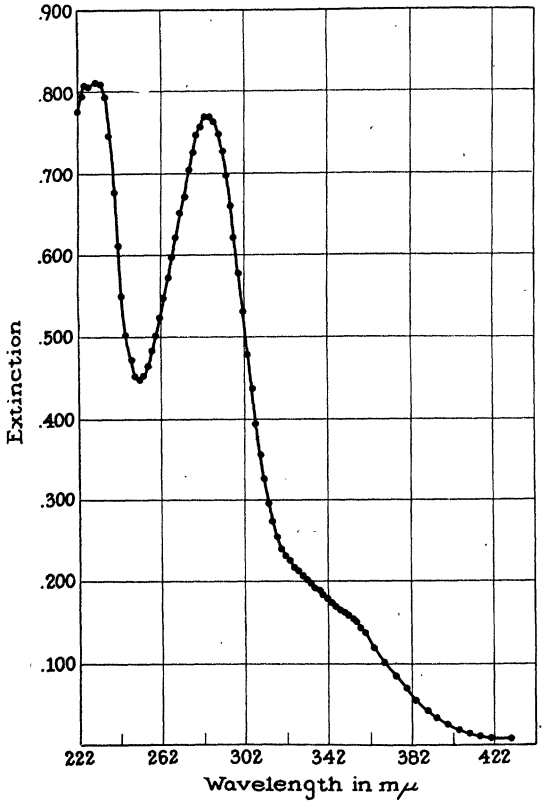

FIG. 2

Further investigations made by X-ray diffraction analysis of the two compounds by J. D. Bernal and I. Fankuchen indicated identical crystal structures. A more detailed account of their studies will be published elsewhere.

The results previously published (*6*) on *in vitro* inhibition of growth of various bacteria by the compound from *Ramalina reticulata* agree in general with those of Stoll and co-workers (*7*). Inhibition of gram-negative organisms is not obtained except at relatively high concentrations, while gram-positive organisms are inhibited by low concentrations. Human and bovine tubercle bacilli are inhibited by low concentrations, but the concentration required for inhibition of the avian strains of tubercle bacilli is higher.

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Relative Growth Rates of Bean and Oat Plants Containing Known Amounts of a Labeled Plant-Growth Regulator (2-Iodo^{131}-3-Nitrobenzoic Acid)

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Succulent dicotyledonous plants are generally more sensitive to growth-regulating substances than are most plants of the monocotyledonous type (*2*), yet very little is known regarding the factors responsible for this difference in sensitivity. In recent experiments with the growth-regulating substance 2-iodo^{131}-3-nitrobenzoic acid (INBA) labeled with radioiodine, bean plants were found to absorb and translocate this compound more readily than barley plants (*3*), and a new tool was provided for observing still other differences in the way some dicotyledonous and monocotyledonous plants respond to growth regulators of this type.[1]

The present experiments were undertaken to determine, in part, whether the difference in sensitivity of plants, such as bean and oat, to such a growth regulator as INBA can be accounted for on the basis of a quantitative difference in their ability to absorb and translocate the compound. By applying radioactive INBA in varying amounts to the older leaves of bean and oat plants and then measuring the radioactivity of the young leaves that developed subsequently, it has been possible to compare the rate of growth of the two plant types when the young leaves of each contain equal concentrations of the growth regulator. Additional information which bears directly on this problem has also been obtained by studying the translocation of INBA in a third monocotyledonous plant, namely, corn seedlings.

The compound 2-iodo^{131}-3-nitrobenzoic acid was synthesized by diazotizing 3-nitroanthranilic acid and treating the reaction product with radioiodine 131.[2] A water dispersion of the compound was prepared by first dissolving the required amount in a small quantity of a commercial detergent (Tween-20) and then adding sufficient water to make a 4 per cent solution of the detergent in the final mixture. Measured amounts of the dispersion thus obtained were added to measured amounts of a 4 per cent solution of detergent in distilled water in order to make a series of clear, aqueous dispersions containing, respectively, 3.13, 6.25, 12.50, and 25.00 μg. of INBA/0.01 ml.

Bean and oat seedlings were grown from seed in potted soil under greenhouse conditions. Bean seedlings selected for uni-

[1] In the paper referred to it was tentatively concluded on the basis of the indirect evidence then available that INBA was absorbed and translocated as such in the plant. Since this work was published, it has been proved conclusively by the isolation of pure INBA from the stem and bud tissue of bean plants treated with this compound, that INBA is absorbed, translocated, and accumulated, at least in the bean plant, in the form of the intact molecule. While these experiments will be reported elsewhere, this fact is mentioned here to obviate the necessity for considering that a possible degradation product of INBA might be responsible for the results reported in this paper.

[2] Radioiodine was obtained through the Isotopes Branch ,Manhattan District, Oak Ridge, Tennessee.