tends to overload the column, and the nicotine interferes with the much smaller nornicotine peak. Determination of the area of the latter is then difficult. A practical value for the limiting ratio of nicotine to nornicotine because of this effect is about 20–30 to 1. However, this problem could probably be overcome by using more sensitive detecting devices, such as those based on ionization phenomena. A much more dilute solution could then be used and the broadening of the nicotine peak due to column overloading in the present work would be avoided. No experience with tobacco samples having a large nornicotine to nicotine ratio has yet been obtained.

Of more than passing interest is the use of gas chromatography to obtain sizable amounts of pure alkaloid, notably nornicotine. The columns used here are sufficiently selective to eliminate undesired volatile alkaloids, and with the use of a wide-diameter column processing of individual samples up to 0.5 gram has been realized. No significant reduction in resolution was noted with use of large columns. It has thus become possible to procure, with little difficulty, nornicotine from an appropriate tobacco, such as *N. sylvestris*, *N. glutinosa*, or cherry red *N. tabacum*, in a state free from other alkaloids. In a similar manner, pure anabasine can be obtained from a source such as *N. glausa*. Such operations would be even more feasible with the use of gas chromatographs specially designed for preparative scale work at high temperatures.

Acknowledgment

Cooperation of the Research Laboratories of the Liggett and Myers Tobacco Co., and The American Tobacco Co. is gratefully acknowledged. Samples of cherry red *N. tabacum* and *N. sylvestris* were kindly supplied by J. A. Weybrew, North Carolina State College.

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Received for review February 27, 1961. Accepted May 18, 1961. Taken from a thesis submitted by N. A. Pappas in partial fulfillment of the requirements for the A.M. degree, Duke University. The National Tobacco Board of Greece provided N. A. Pappas with financial aid in this study.

MOLECULAR STRUCTURE AND ACTIVITY

Effect of Alpha-Methoxylation and Nitrogen Acetylation on Absorption and Translocation of a Plant Regulator, Methyl Indole-3-acetate

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Alpha-methoxylation of several plant growth–regulating chemicals enhanced their absorption and translocation by plants. Alpha-methoxylation has now been extended to methyl indole-3-acetate, a growth regulator of the indole type; absorption and translocation of this regulator were also enhanced. Alpha-methoxylation together with nitrogen acetylation, however, resulted in greater translocatability than did methoxylation alone.

Substitution of a methoxy group for one of the hydrogen atoms of the alpha-carbon of several plant regulators of the phenylacetic acid type resulted in compounds that were more readily absorbed and translocated than phenylacetic acid itself (*3*). In addition, some of these methoxy derivatives were exuded from roots of plants in biologically detectable amounts (*4*, *5*). Absorption and translocation of regulators of the carbamate type were enhanced by similar structural modifications (*2*).

This article describes similar enhancement of absorption and translocation of methyl 3-indoleacetate, through alpha-methoxylation, and the effect of nitrogen acetylation on absorption and translocation of this indole compound.

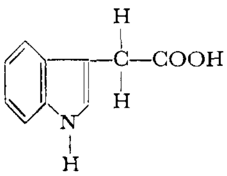
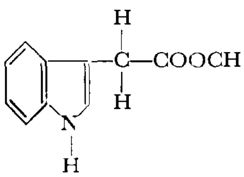
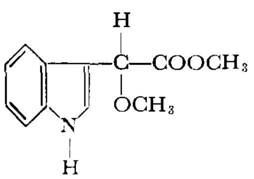
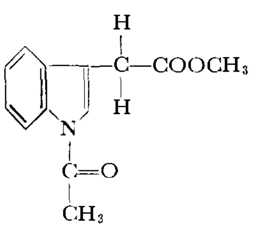
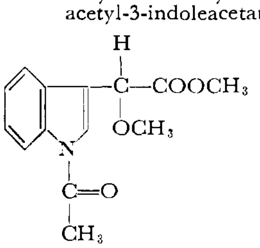
Methods

Bean plants of the Pinto variety (*Phaseolus vulgaris* L.) with primary leaves approximately 4 cm. across and trifoliolate leaves folded in the terminal buds were selected for uniformity. A standard mixture of indole-3-acetic acid was prepared for comparative purposes as follows: 2 mg. of the acid was placed in a microbeaker and dissolved in 0.25 ml. of 95% ethyl alcohol. The microbeaker containing the solution was placed in 3.75 ml. of water mixed with sufficient ethyl alcohol to give a final concentration of 8% of alcohol. Forty milligrams of Tween 20 was then dissolved in the resulting solution to make the final concentration of growth regulator 0.05%, alcohol 8%, and Tween 20 1%. The related indole compounds under investigation were prepared in a similar way to give molecular concentrations equivalent to the concentration of indole-3-acetic acid in the standard mixture. Leaf treatments were made by application of 0.01 ml. of the preparation containing 5 μg. of indole-3-acetic acid or a molar equivalent amount of the related compounds as a narrow band across the upper surface of each primary leaf near the petioles of five plants. The liquid was distributed over the surface of each leaf with a glass rod (*1*). Treatment in this manner resulted in application of a total of 10 μg. of a compound to each plant.

In stem treatments, 0.01 ml. of the indole-3-acetic acid or related compounds was applied as a band approximately 1 cm. wide around the first internodes of each of five test plants. The mixture was confined to a designated area of stem surface by lanolin rings that extended around the stem (*1*). Response to leaf or stem treatments was evident as stem curvatures. These were measured and expressed as degrees.

Derivatives of indole-3-acetic acid studied were methyl 3-indoleacetate, mehyl *N*-acetyl-3-indoleacetate, methyl α-methoxy-3-indoleacetate, and methyl α - methoxy - *N* - acetyl - 3 - indoleacetate. One experiment involving the acetylated acetate, both with and without methoxylation, was carried out followed by two additional experiments in which these two indole compounds, together with the remaining ones listed above, were used.

Table I. Stem Curvature after Application of Indoleacetic Acid and Equal Molar Concentrations of Related Indole Compounds to Primary Leaves and Stems of Bean Plants

		Degrees of Curvature	
Compound	Structure	Leaf treatment	Stem treatment
I Indole-3-acetic acid		11	84
II Methyl 3-indoleacetate		8	56
III Methyl α-methoxy-3-indoleacetate		76	92
IV Methyl *N*-acetyl-3-indoleacetate		10	22
V Methyl α-methoxy-*N*-acetyl-3-indoleacetate		111	93

Results and Discussion

Indole-3-acetic acid and its methyl ester did not induce an appreciable amount of stem curvature when applied to the leaves of bean plants (I, II, Table I). Since both of these compounds induced marked curvature when applied directly to the stems, it is apparent that these regulators (or active metabolites of them) were not translocated from the leaves to the stems in biologically active amounts.

Substitution of a methoxy group for one hydrogen atom associated with the α-carbon greatly increased translocatability of the unacetylated ester (leaf treatment, II, III). Using a dosage equivalent to 5 μg. of indoleacetic acid, the magnitude of the response (expressed as degrees of curvature) was much greater than that induced by the parent compound, indole-3-acetic acid, when translocation of the stimulus from leaves to stems was involved. With stem applications where translocation was not a major factor, response to the methoxy-substituted ester and to indole-3-acetic acid was approximately equal. Alpha-methoxylation also increased the growth-regulating activity and translocatability of the acetylated ester (IV, V).

At the dosage level used, methoxy substitution, together with acetylation of the indole nitrogen, resulted in stem curvature (expressed as degrees) that was much greater than that induced by the unsubstituted ester or that of the parent acid (I, II, and V) when translocation of the stimulus from the leaf to the stem was involved. Unlike α-methoxyphenylacetic acid which exudes from roots, there was no evidence that these new methoxy-substituted compounds exuded from roots in biologically detectable amounts (*5*).

The nature of the effect of α-methoxylation on absorption and translocation of indole-type regulators is not known, but introduction of the methoxy group may have blocked or reduced degradation and subsequent inactivation of the compound within the plant, thus making possible translocation of the intact regulator from the leaves to the responsive stem tissues.

In summary, the methoxy-substituted compounds used were absorbed and these or metabolites of them were translocated more readily as plant regulators than were the compounds without the methoxy group added. Alpha-methoxylation together with nitrogen acetylation enhanced absorption and translocation of the methyl indole-3-acetate entity even more than did methoxylation alone.

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Received for review April 17, 1961. Accepted May 26, 1961.