NORNICOTINE AS A PRECURSOR OF NICOTINE IN *NICOTIANA* PLANTS

T. C. Tso[1]

(Received Aug. 31, 1962)

"One should see the doughnut as a whole, not the hole of a doughnut."

—L. F. Chao, 1960, visiting Beltsville

The conversion of nicotine to nornicotine in *Nicotiana* plants (Mothes, 1959) is well known. The mechanism of such conversion has been demonstrated to be through transmethylation (Bose *et al.*, 1956) or oxidative demethylation (Stepka and Dewey, 1961). It was believed (Dawson, 1945; Il'in, 1948) that nicotine had to be translocated from its origin in the root to the leaf to be converted to nornicotine.

There are two main types of alkaloid-producing commercial tobaccos—nicotine type and nornicotine type—based on the principal alkaloid composition of the matured leaf. Most of the reported investigations involving the nicotine-nornicotine conversion were conducted with nornicotine-type plants (Dawson, 1945; Mothes, 1959). Discussions of such experimental findings were mostly on the assumption that all nornicotine is formed at the expense of nicotine. Despite the fact that in nornicotine-type plants nicotine conversion is the principal source of nornicotine, nornicotine is, nevertheless, formed independently from nicotine and is formed in the root *in situ* as demonstrated by experiments employing N^{15} (Tso and Jeffrey, 1957).

In this paper data showing that nornicotine is a precursor of nicotine are presented. Portions of the results of the same experiment concerning general synthesis of alkaloids are published elsewhere (Tso and Jeffrey, 1962).

Materials and Methods

Nicotine-type plants, *Nicotiana rustica* L. var. *brasilia*, were used in this experiment. They were grown in nutrient solution until the flowering stage. These plants were decapitated immediately before transferring to a nutrient

(1) U. S. Department of Agriculture, Agricultural Research Service, Crops Research Division, Beltsville, Maryland.

solution containing tritiated water to accelerate the formation of labeled alkaloids.

The original H^3 activity of the nutrient solution was 1.41×10^6 counts/sec/ml. A total volume of 10 liter was used. Decapitated plants, two in a set, were grown in this solution for various periods (30 min., 2.5 hr., 20 hr., 30 hr., and 70 hr.). One plant from each set was assayed 30 minutes after exposure and the other after 16 days of further growth in nutrient solution without added H^3.

Alkaloids were extracted, separated, and purified as previously described (Tso and Jeffrey, 1962). The H^3 activity was measured with a Tri-carb liquid scintillation spectrometer, using toluene-absolute ethanol (450:50) solvent system with POPOP and PPO as scintillators.

Results and Discussion

As in an earlier study with tritiated water (Tso, 1962), fast H^3 incorporation into test plants was observed in the first 2.5 hours, indicating a possible replacement of regular plant water by tritiated water. H^3 activity was also detected in mixed alkaloids isolated from plants assayed 30 minutes after 1-minute exposure. This fast incorporation is very likely through incorporation of H^3 into an exchangeable position of alkaloid molecule (Moses and Calvin, 1959) or through transmethylation of nicotine (Dewey *et al.,* 1954).

The H^3 activities of nicotine and nornicotine, expressed as counts/sec/mmole, isolated from plants after various length of exposure to H^3, are shown in Table 1. In the case of samples assayed 30 minutes after exposure, the specific activity of nornicotine was always higher than that of nicotine from the same plant; however, the contrary was true if similar were assayed after allowing 16 additional days' growth in nutrient solutions without added H^3.

Table 1.—*H^3 activity (counts/sec/mmole) found in nicotine and nornicotine*

Treatment	Nicotine	Nornicotine
30 min. after exposure of:		
30 min.	9.75×10^2	2.03×10^3
2.5 hr.	2.03×10^3	2.70×10^3
20 hr.	1.07×10^4	1.30×10^4
30 hr.	2.48×10^4	3.00×10^4
70 hr.	2.85×10^4	3.23×10^4
16 days after exposure of:		
30 hr.	1.25×10^4	4.12×10^3
70 hr.	2.00×10^4	3.75×10^3

The activity of H^3 in all alkaloids increased with exposure time. However, the H^3 activity in nornicotine was always higher than in nicotine from the same plant, but the difference became gradually smaller as the the time of exposure became longer. This ratio, expressed as specific activity of nicotine to nornicotine, is shown in Figure 1. As the exposure time increased from 30 minutes to 2.5, 20, 30, and 70 hours, the ratio increased from .48 to .75, .82, .83, and .88. It gradually approached but never reached a ratio of 1.0. However, when the test plants were allowed to grow an additional 16 days in regular nutrient solution, the same nicotine/nornicotine specific activity ratios from 30- and 70-hour exposed plants were 3.03 and 5.33 respectively, indicating a much higher H^3 activity in nicotine than in nornicotine.

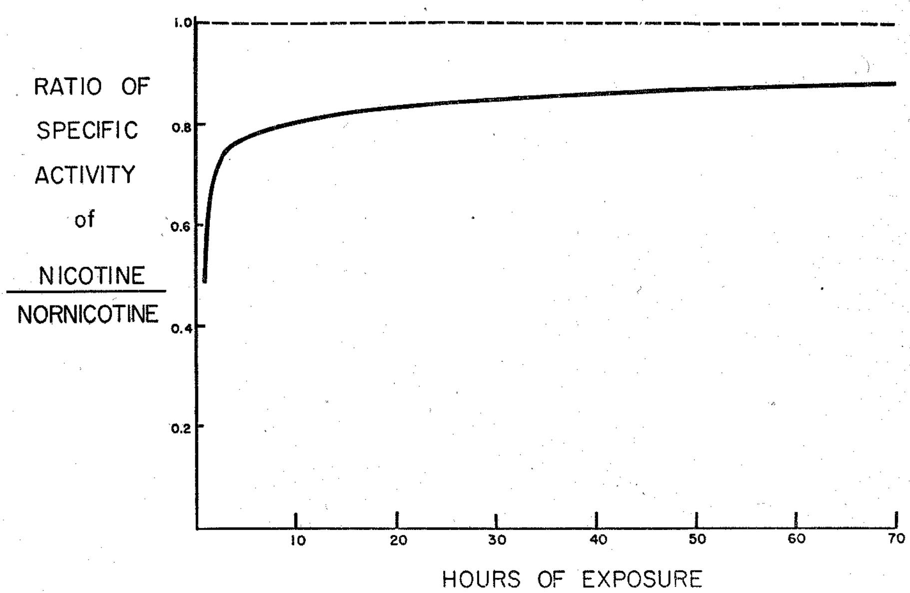

Fig. 1. Ratio of specific activity of nicotine to nornicotine from plants after various length of H^3 exposure.

These results can best be explained on the hypothesis that nornicotine is a precursor of nicotine. When experimental plants were exposed to an H^3 medium, H^3 was incorporated into the nornicotine molecule either through ion-exchange or by other not yet defined pathways. The longer a plant is exposed to H^3 medium, the more H^3 is incorporated into the nornicotine molecule. This H^3 nornicotine is then converted to H^3 nicotine. Under such circumstances there is a continued increase of H^3 activity in both nornicotine and nicotine, but the H^3 activity in the product (nicotine) is always lower than in the precursor (nornicotine), although the difference gradually becomes less.

When the supply of H^3 was stopped by returning the exposed plants to the regular nutrient solution, new nornicotine was formed with precursor(s) of diluted H^3 labeling. The degree of H^3 dilution is gradually increased as time progresses. Under this process the H^3 activity in nicotine becomes and remains higher than in nornicotine.

Further changes of this activity ratio beyond the observed period are to be expected. Such changes may result from transmethylation, incorporation into an exchangeable position, alkaloid interconversion, and participation of alkaloids in the general metabolic pool. The total H^3 activity in alkaloids will gradually decrease, but the specific activity in nicotine will always be higher than that in nornicotine.

These results confirm earlier findings with N^{15} incorporation into alkaloids (Tso and Jeffrey, 1957) which suggested that nornicotine was a precursor of nicotine. Kisaki and Tamaki (1961), using an entirely different approach in studying the phytochemistry of tobacco alkaloids, reached the same conclusion. It also appeared sound chemically to assume that a comparatively simpler molecule (nornicotine) is a precursor of its methylation product (nicotine).

A proposed scheme on the pathway of nornicotine-nicotine conversion is shown in Figure 2. In nicotine-type plants, nicotine originated from methylation of nornicotine, while in nornicotine-type plants a portion of the nornicotine is formed through direct synthesis and most of the nornicotine is a conversion product of (nornicotine-originated) nicotine through oxidative demethylation or transmethylation.

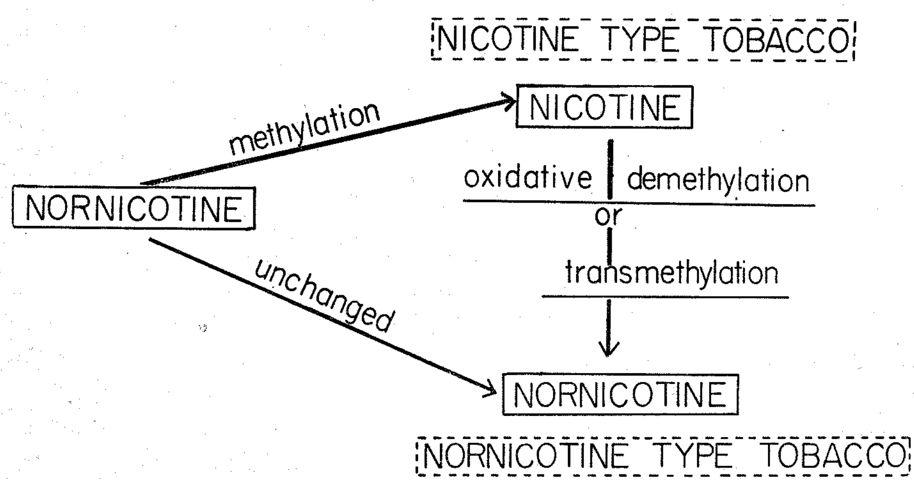

Fig. 2. Proposed pathways of nicotine-nornicotine interconversion in different types of tobacco plants.

This interpretation is by no means intended to rule out other biological pathways of nicotine formation from various precursors. The evidence presented, however, strongly indicates that methylation of nornicotine is by far the most prominent way of nicotine formation.

Summary

The precursor-product relation between nornicotine and nicotine is demonstrated by growing *Nicotiana rustica* L. var. *brasilia* plants in nutrients solution containing tritiated water for various periods. As long as there was a continuous supply of H^3, the H^3 activity in nornicotine was always higher than that of nicotine. This situation was reversed when the exposed plants were grown for an additional period without further H^3 supply. These findings suggest that nornicotine is a precursor of nicotine. A pathway of nornicotine-nicotine relation is proposed for interpreting the alkaloid formation in nicotine-type and nornicotine-type plants.

煙草植物中的 Nornicotine 是 Nicotine 的前驅體

左 天 覺

以 *Nicotiana rustica* L. var. *brasilia* 培養於含有 H^3 的溶液中。作者見不問培養時間長短如何，Nornicotine 中的 H^3 activity 是常較 Nicotine 中者爲高；但在以植物移置於不含有 H^3 的培養液中後，則 Nicotine 中的 H^3 activity 是較 Nornicotine 中者爲高。作者據此推測 Nornicotine 是 Nicotine 的前驅體，卽 Nicotine 是由 Nornicotine 經 Methylation 而形成。至不含有 Nicotine 的 Nornicotine type 的植物，作者推測其 Nornicotine 或係由於所謂前驅體的 Nornicotine 未經變形，或係由 Nicotine 經 Oxidative demethylation 或 Transmethylation 而形成。(摘要)

References

BOSE, B. C., H. N. DE, and M. SULTAN. Biogenesis of alkaloids in tobacco plants. II. Transmethylation of nicotine and nornicotine in *Nicotiana tabacum* and *Nicotiana glauca*. India J. Med. Res. **44:** 91-97, 1956.

DAWSON, R. F. An experimental analysis of alkaloid production in *Nicotiana:* The origin of nornicotine. Am. J. Botany **32:** 416-423, 1945.

DEWEY, L. J., R. U. BYERRUM, and C. D. BALL. The origin of the methyl group of nicotine through transmethylation. J. Am. Chem. Soc. **76:** 3997-3999, 1954.

IL'IN, G. S. The interrelationship among the chief tobacco alkaloids. Biokhimiya **13:** 193-196, 1948.

KISAKI, T., and E. TAMAKI. Phytochemical studies on the tobacco alkaloids. III. Observations on the interconversion of DL-nicotine and DL-nornicotine in excised tobacco leaves. Arch. Biochem. & Biophys. **94:** 252-256, 1961.

MOSES, V., and M. CALVIN. Photosynthesis studies with tritiated water. Biochimica et Biophysica Acta **33:** 297-312, 1959.

MOTHES, K. Über neue Arbeiten zur Biosynthese der Alkaloide. I. Pharmazie **14:** 121-132 und II. Ibid. 177-190, 1959.

STEPKA, W., and L. J. DEWEY. Conversion of nicotine to nornicotine in harvested tobacco: Fate of the methyl group. Plant Physiol. **36:** 592-597, 1961.

TSO, T. C. Some novel concepts on the biosynthesis and biogenesis of tobacco alkaloids. Bot. Bul. Academia Sinica **3:** 61-71, 1962.

TSO, T. C., and R. N. JEFFREY. Studies on tobacco alkaloids. II. The formation of nicotine and nornicotine in tobacco supplied with N^{15}. Plant Physiol. **32:** 86-92, 1957.

TSO, T. C., and R. N. JEFFREY. Biochemical studies on tobacco alkaloids. V. The incorporation of H^3 in alkaloid biosynthesis. Arch. Biochem. Biophys. **97:** 4-8, 1962.