NITROGENOUS AND PHENOLIC COMPOUNDS OF NICOTIANA PLANTS

II. Selective Incorporation of Aromatic Amino Acids in Phenolic Compounds of Tumorous Nicotiana Hybrids

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Scopoletin (6-methoxy-7-hydroxy coumarin) and scopolin (7-glucoside of scopoletin) levels are higher in the tumorous tissue of 2N hybrid (*N. glauca* Grah. × *N. langsdoffii* Weinm.), than in those of either parent (Tso *et al* 1964). The levels of chlorogenic acid in these plants, however, vary only slightly. The range in aromatic amino acid content of the F_1 hybrid plant differs widely among various organs (Tso *et al* 1962). The phenylanine content in the leaf tissue, nodal meristems, and new stem tumors of this hybrid, was trace, 9, and 110 ppm, respectively. The tyrosine content for the same plant parts was trace, 7, and 35 ppm, respectively. These results suggest that aromatic amino acids exercise a special influence on the level of phenolics, especially coumarin derivatives in the tumorous tissue of *Nicotiana* hybrids.

It is generally accepted that the major biosynthetic pathway leading to phenolic compounds in higher plants is by way of deamination of phenylalanine and tyrosine (Neish 1964) or through transcinnamic acid and p-coumaric acid. The incorporation of p-coumaric acid to form chlorogenic acid was demonstrated in leaf disks of *Nicotiana tabacum* var. Delcrest (Runeckles 1963b). The metabolism of ferulic acid to form the coumaric glucoside, scopolin, was also reported for tobacco leaf tissue (Runeckles 1963a).

In this paper we report investigations on the relative incorporation of phenylalanine and tyrosine into scopoletin, scopolin, and chlorogenic acid in leaves and in tumorous tissue of F_1 hybrid (*N. glauca*×*N. langsdorffii*). Such information will enrich our understanding of the relationship among nitrogenous and phenolic fractions in cultivated tobacco plants (Tso *et al* in press).

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Materials and Methods

F_1 hybrids of *N. glauca*×*N. langsdorffii* were grown in the greenhouse, in 7-inch pots of soil or in 10-liters of nutrient solution, as described previously (Tso *et al* 1962). When these plants reached a height of about 18″, proper measures were taken to induce and accelerate tumor formation in designated areas. This was accomplished by a number of ways, such as breaking the midrib of a leaf, removing the plant top or axillary buds, scratching the stem surface. Injury which causes membrane rupture initiates tumor formation. Figures 1 and 2 illustrate the tumorous plant parts. Tumors also formed spontaneously without artificial induction in plants of this particular F_1 hybrid, but primarily in the post-floral stages of growth, and then the location of the tumors is random.

L-Phenylalanine-1-C^{14} with a specific activity of 24.3 mc/mM and L-tyrosine-U-C^{14} with 297 mc/mM, were used for these experiments. U-C^{14} glucose was also used as reference material. Two feeding methods were adapted. One method was for detached leaves with midrib tumors, in which labeled material was supplied by immersing the petiole in the solution. Another method was used for intact plants, in which labeled material was supplied through the stem by wicks connected to a small vial containing labeled compounds. In either case, total feeding time was less than 2 hours, including the infusion of washing liquid following the labeled material.

Plant material was extracted with ethanol, and sparation of phenolic compounds was made by paper chromatography, as described previously (Tso *et al* in press). Radioactvity was measured with a Tri-Carb* scintillation spectrometer using a toluenabsolute ethanol system (450:50) with POPOP (100 mg) and PPO (1.5 g) as the scintillator. An Actigraph* radiochromatogram scanning system was used for measurement of the relative activity of individual compounds separated on paper chromatograms before elution.

Results and Discussion

During several preliminary tests, L-Phenylalanine-1-C^{14} or L-tyrosine-U-C^{14} was supplied to aged tumors, including axillary-tumors and plant-tip tumors which were over six weeks old. In either case, C^{14} labeling was evenly distributed in tumorous and surrounding non-tumorous tissue, indicating the absence of selectivity. In another test, feeding was conducted to a plant mass which was at an early stage of differentiating into tumors and leaves. Again no significant difference in C^{14} distribution was observed. These results appeared to suggest that the age or stage of tumor development is a major

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factor; either too old or too young a tumorous tissue is not active for phenolics biosynthesis in *Nicotiana* tumors.

Further experiments were conducted with L-phenylalanine-1-C^{14}. A total of 10 uc material was fed with a wick through stem to an intact solution-cultured tumorous plant on which the tumors were 2–3 weeks old. After 24 hours exposure to C^{14}-phenylalanine, the plant was harvested and divided into five portions. Some parts were further separated into leaf, tumor, and stem fractions, and each fraction was extracted and measured for C^{14} activity. Results are shown in Table 1. In examining these data, one should bear in mind that the stem was the direct feeding and translocation organ, and therefore maintained a higher C^{14} activity. In the area immediately above the feeding site, the leaf petiole was actually in contact with the wicks, which may explain a slightly higher C^{14} activity in this leaf than in tumor in this particular portion of the experimental plant. In the other three sections, including the bottom, the area below feeding point, and the top of the plant, C^{14} activity was considerably higher in the tumors than in leaves. Since tumorous tissues are known to contain high concentrations of coumarin compounds, such distribution differences may indicate a selective incorporation of C^{14}-phenylalanine into tumors to serve as precursors for coumarin formation.

Table 1. *C^{14} Recovery From F_1 (N. glauca×N. langsdorffii) 24 Hours After Phenylalanine Feeding*

	dpm/g. fresh weight		dpm/g. fresh weight
Root	1.50×10^3	Area Above Feeding	
Bottom		Leaf	2.20×10^4
Leaf	2.28×10^3	Tumor	1.67×10^4
Tumor	4.98×10^3	Stem (Feeding Location)	2.80×10^4
Stem	4.02×10^3	Top	
Area Below Feeding		Leaf	5.58×10^3
Leaf	2.28×10^3	Tumor	5.88×10^3
Tumor	6.90×10^3	Stem	7.56×10^3
Stem	9.66×10^3		

In another experiment, a leaf with a 2-week old midrib tumor (Fig. 1) was detached and 10 uc L-Phenylalanine-1-C^{14} was fed through the petiole. L-tyrosine-U-C^{14}, 10 uc, was fed to another leaf in the same way. Plant material was thus extracted 7 hours after feeding. These experiments were repeated twice and the average results are shown Table 2. It is clearly indicated that tyrosine and probably its metabolites were distributed in a systematic way, decreasing from the petiole, the area of feeding, to the tip of the

leaf blade. There was no selective accumulation in the midrib tumor which was located in the center of the experimental leaf. Such a distribution was similar to that of glucose-U-C^{14}, which was used as a reference.

Table 2. *C^{14} Activity Recovered From Leaf Containing Mid-Rib Tumor, 7 Hours After Petiole Feeding With Tyrosine-U-C^{14} or Phenylalanine-1-C^{14}*

	C^{14} Activity (dpm/g. fresh tissue)		C^{14} Activity (dpm/g. fresh tissue)
Tyrosine Feeding Results		Phenylalanine Feeding Results	
Petiole (feeding area)	5.86×10^5	Petiole (feeding area)	3.63×10^5
Base of leaf blade	1.90×10^5	Base of leaf blade (below tumor)	2.88×10^5
Middle of leaf blade (below tumor)	1.05×10^5	Tumor	6.51×10^5
Tumor	3.49×10^4	Middle of leaf blade (above tumor)	4.45×10^5
Tip of leaf blade (above tumor)	3.43×10^4	Tip of leaf blade	6.27×10^4

The distribution of phenylalanine and its metabolites, however, show selective accumulation in the tumor itself, as did the intact plant test. The C^{14} activity in the tumor tissue was much higher than in tissues from the surrounding leaf blade area, as well as that of the petiole at which the material was applied.

The recovery of C^{14}, originating from tyrosine and phenylalanine in the plant tissue, was 4.5% and 8.1%, respectively, and in "phenolic" fraction was 1.57% and 2.14%, respectively.

Phenolic compounds, including scopoletin, scopolin, chlorogenic acid, and "others" were separated. The compounds described here as "others" include minute quantities of rutin, quinic acid, ferulic acid, caffeic acid, quercetric and several other unknown fluorescent materials. The C^{14} activity of each compound or group of compounds was determined. The results are expressed as percentage of C^{14} activity in total phenolics, as shown in Table 3. The chlorogenic acid from tyrosine-fed-samples comprised 41.9% of the total C^{14} activity in leaf tissue, but only 16.7% in tumorous tissue. In the phenylalanine-fed-samples, chlorogenic acid had 67.4% of the total C^{14} activity in leaf tissue, and 38.8% in tumorous tissue. The reverse is true for the C^{14} activity of scopoletin and scopolin; there was a lower C^{14} incorporation in leaf tissue and a higher incorporation in tumorous tissue, in both tyrosine and phenylalanine fed samples. The total amount of C^{14} incorporation, either in the leaf or the tumor, into scopoletin, scopolin, and chlorogenic acid, is much higher in phenylalanine-fed samples than in tyrosine-fed ones.

It appears that in tissue of this F_1 hybrid there is an active system for the incorporation of aromatic amino acids into chlorogenic acid in leaf tissue, and into coumarin compounds in the tumor tissues. At the same time, a more

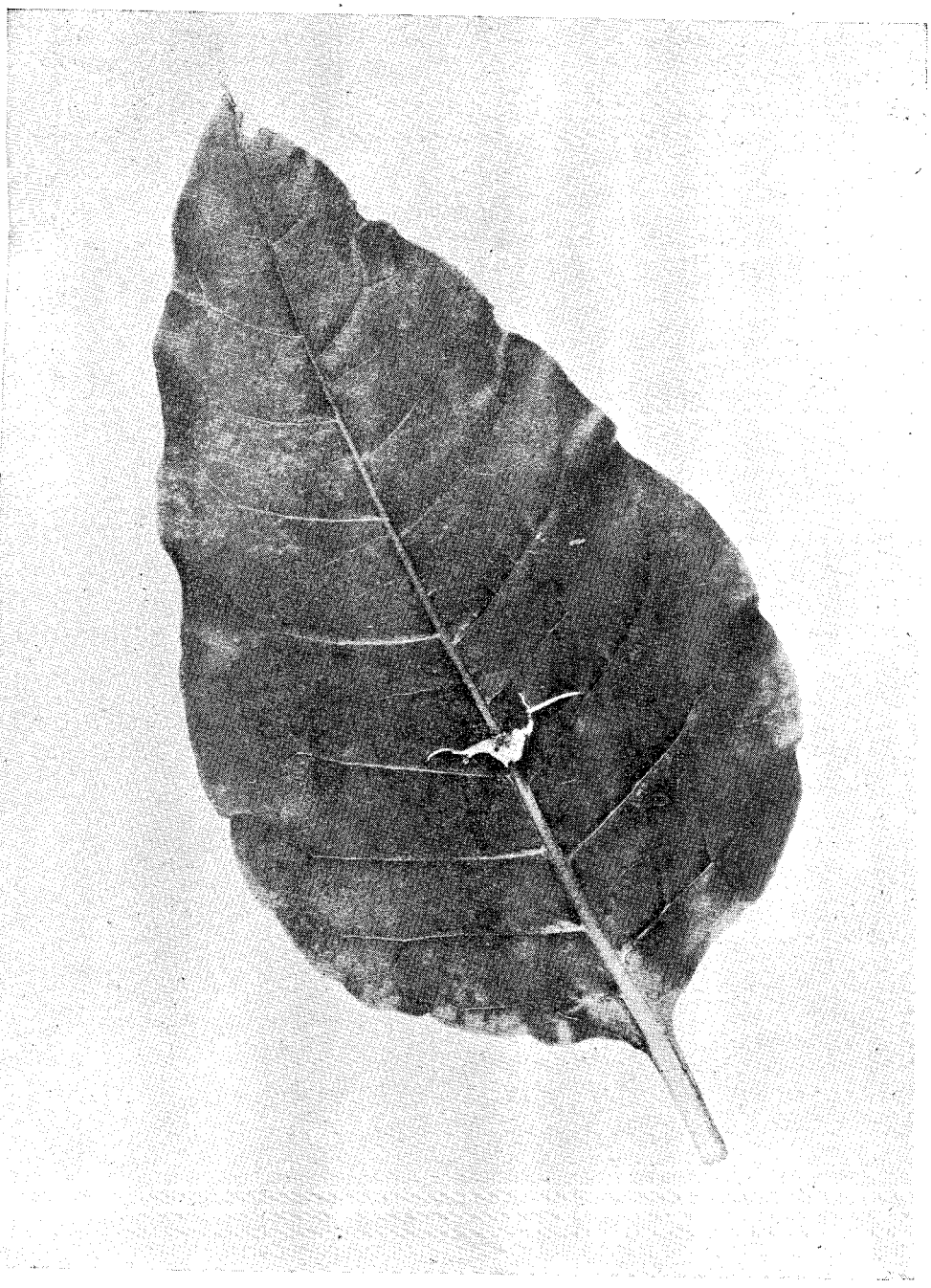

Figure 1. Detached leaf from F_1 (*N. glauca* × *N. langsdorffii*) plant showing midrib tumor.

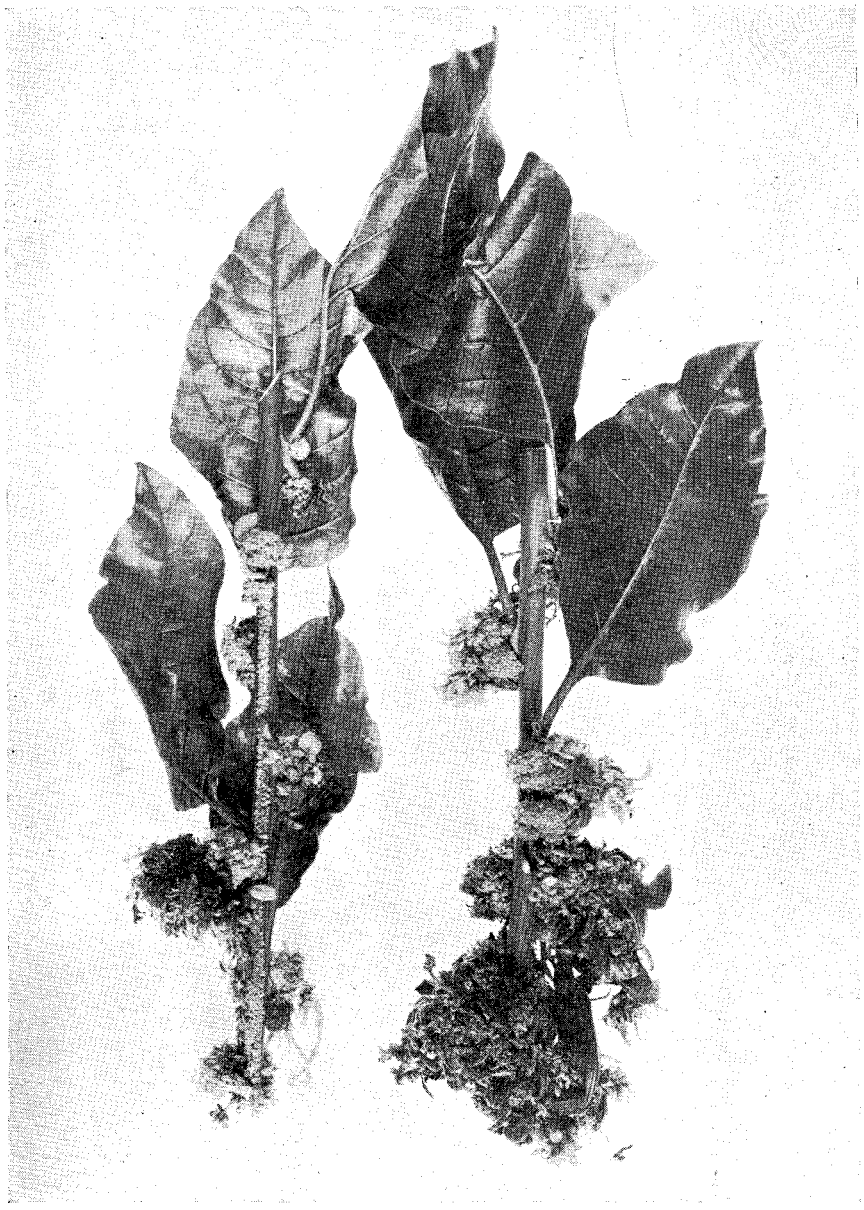

Figure 2. Sections from a plant of F_1 (*N. glauca*×*N. langsdorffii*) showing tumorous tissues.

Table 3. *Relative Distribution (%) of C^{14} Activity 7 Hours After Feeding With Tyrosine-UL and Phenylalanine-1-C^{14}*

	Tyrosine Fed	Phenylalanine Fed		Tyrosine Fed	Phenylalanine Fed
In Leaf Tissue			In Tumor Tissue		
Scopoletin	—	7.6	Scopoletin	25.7	18.9
Scopolin	25.7	14.4	Scopolin	9.3	30.0
Chlorogenic Acid	41.9	67.4	Chlorogecni Acid	16.7	38.8
Others	32.4	10.5	Others	48.3	12.3

effective deamination system seems available for phenylalanine than for tyrosine.

Summary

L-Phenylalanine-1-C^{14} and L-tyrosine-U-C^{14} were supplied to tumorous and non-tumorous plant materials of the F_1 hybrid *N. glauca*×*N. langsdorffii*. In leaf tissue, these aromatic amino acids were more favorably incorporated into chlorogenic acid than other phenolic compounds, whereas in the tumors there was preferential incorporation into scopoletin and scopolin. Phenylalanine is more effectively utilized than tyrosine for the formation of scopoletin, scopolin, and chlorogenic acid.

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