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THE CONVERSION OF SCOPOLETIN-4-^{14}C INTO SCOPOLIN AND FABIATRIN IN TOBACCO SEEDLINGS*

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Abstract—Time studies were made on the fate of scopoletin-4-^{14}C taken up through the roots of whole tobacco seedlings. Of the total radioactivity recovered in one set of scopoletin-4-^{14}C continuous feeding experiments, a range of 43–51% (av. 46%) was found to be in the 'soluble' portion of the roots and shoots. At one period (after 4 hr of continual feeding of scopoletin-4-^{14}C), about 91% of the radioactivity in the 'soluble' fraction was found to be present as scopoletin-7-glucoside (scopolin); less than 3% as scopoletin; and approximately 5% as fabiatrin. The percentage of the total recovered radioactivity contributed by the 'soluble' compounds decreased with time, whereas the percentage of the radioactivity due to the 'insolubles' increased with time. The results also suggest that some of the radioactive scopolin (from scopoletin-4-^{14}C not converted at an early stage to 'insoluble' material) may be converted with more time to other 'soluble' compounds and possibly also to some 'insoluble' material. In the roots, fabiatrin was formed slowly from scopolin. The half-life of scopolin was found to be 16 days. Fabiatrin had a slower turnover rate than did scopolin. No measurable amount of fabiatrin was found in the shoots. When a lower starting concentration of scopoletin-4-^{14}C was fed to the seedling in continuous feeding experiments, a larger percentage of the radioactivity appeared in the 'insoluble' fraction of tobacco roots. A hypothesis based on compartmentation of metabolic and vacuolar pools is proposed.

INTRODUCTION

ALTHOUGH numerous reports have appeared concerning scopoletin (6-methoxy-7-hydroxy-coumarin) and its most commonly occurring glycoside, scopolin (7-glucoside of scopoletin), in plants, the metabolic fate of these compounds in whole plants has not been completely elucidated. Some possibilities may be postulated from earlier results. For example, Sargent and Skoog[1] isolated and identified a 7-gentiobioside of scopoletin from the primary root of tobacco. Runeckles[2] reported that a complex polyphenol which he had isolated from flue-cured tobacco was comprised of equimolecular amounts of scopolin, rutin, and chlorogenic acid. Winkler[3] obtained two unknown derivatives of scopolin from maleic hydrazide-treated tobacco. On hydrolysis, each compound yielded scopoletin, glucose, and a fragment identified tentatively as shikimic acid. Sargent and Skoog[4] reported that under conditions which favor growth, scopolin could be converted in tissue cultures of tobacco pith and callus into a non-fluorescent substance called *X*, which may be utilized in the growth process, producing cell wall materials. Steck[5] found that 42% of the radioactivity of scopoletin-3-^{14}C fed to mature leaves of flowering tobacco plants had been incorporated

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[1] J. A. SARGENT and F. SKOOG, *Physiol. Plantarum* **14,** 504 (1961).
[2] V. C. RUNECKLES, *Arch. Biochem. Biophys.* **102,** 354 (1963).
[3] B. C. WINKLER, Ph.D. dissertation, University of Oklahoma (1967).
[4] J. A. SARGENT and F. SKOOG, *Plant Physiol.* **35,** 934 (1960).
[5] W. STECK, *Can. J. Biochem.* **45,** 1995 (1967).

after three days into a water-soluble fraction. Loewenberg[6] used randomly labeled phenylalanine as the precursor in the biosynthesis in tobacco callus of labeled scopoletin, which was then used to produce labeled scopolin and resulted in radioactivity associated with certain protein and cell-wall fractions. Loewenberg's results indicated that only a very small fraction of the externally supplied radioactive phenylalanine was converted to scopoletin and scopolin and that most of the phenylalanine was converted to other compounds.

In our investigations, we undertook studies of the fate of supplied scopoletin-4-^{14}C in tobacco seedlings, rather than in tissue cultures or in older, flowering plants. Position 4 in the coumarin ring was chosen for inserting the radioactivity in that such synthesis could be accomplished, and the radioactive carbon atom would likely remain attached to the aromatic ring of scopoletin during metabolism.

RESULTS AND DISCUSSION

Continuous-Feeding of Scopoletin-4-^{14}C

The results of the determination of the radioactivity of scopolin, fabiatrin, and scopoletin and other fractions prepared from the tobacco seedlings, as well as the specific activity of scopolin and fabiatrin, after a continuous feeding experiment involving sco-

TABLE 1. ACTIVITIES OF SCOPOLIN, SCOPOLETIN, AND FABIATRIN IN THE SOLUBLE FRACTION AT VARIOUS TIMES DURING THE CONTINUAL FEEDING OF SCOPOLETIN-4-^{14}C TO TOBACCO SEEDLINGS

Tissue, compound, and parameter	Time (hr) of uptake					
	1	4	11	28	50	74
Roots						
Activity in cpm ($\times 10^{-4}$)						
Total 'soluble'	4·4	11	26	55	73	88
Scopolin	4·1	10	22	41	49	61
Scopoletin	0·4	0·3	0·5	0·6	0·9	1·2
Fabiatrin	0·2	0·6	1·0	2·5	3·6	6·6
Total 'insoluble'	7·1	16	33	61	94	140
Percentage cpm in 'soluble' fraction of total cpm recovered						
Scopolin	93	91	85	75	67	69
Scopoletin	9·1	2·7	1·9	1·1	1·2	1·4
Fabiatrin	4·5	5·4	3·8	4·5	4·9	7·5
Other 'solubles', unidentified (by difference)	(−6·6)	0·9	9·3	19	27	22
Specific activity (μCi/μmol)						
Scopolin	1·2	2·6	2·6	3·0	3·1	3·0
Scopoletin	2·6	3·4	3·2	3·5	3·2	3·2
Fabiatrin	1·6	2·5	2·7	2·8	3·0	2·9
Shoots						
Activity in cpm ($\times 10^{-4}$)						
Total 'soluble'	1·0	1·9	2·4	10	12	19
Scopolin	0·7	1·2	1·4	5·9	7·8	10
Percentage cpm of radioactivity absorbed						
Scopolin	68	61	56	58	67	54

The concentration of the original scopoletin-4-^{14}C was 1·94 × 10^{-4} M and specific activity was 3·5 μCi/μmol.

[6] J. R. LOEWENBERG, *Phytochem.* **9**, 361 (1970).

poletin-4-^{14}C, are shown in Table 1. In this experiment. 6 groups of 4 plants each of the tobacco seedlings were left in a nutrient solution containing scopoletin-4-^{14}C (concentration, $1{\cdot}94 \times 10^{-4}$ M; specific activity, 3·5 μCi/μmol) for the specified length of time. This concentration gave sufficient radioactivity for measurement, yet did not inhibit the growth of the seedlings.[7]

'Soluble' in this paper shall refer to that portion of the plant tissue which dissolved in one or more of the following solvent systems (hot), when used in sequence: *iso*PrOH–H_2O azeotrope (22:3); *iso*PrOH–H_2O (1:1); *iso*PrOH–C_6H_6–MeOH–H_2O (2:1:1:1, called 'IBMW'); and finally, *iso*PrOH–H_2O azeotrope again. The several extracts were combined to produce the fraction called 'soluble'.

It is obvious from the data in Table 1 that most of the scopoletin-4-^{14}C found in the 'soluble' portion of the tobacco seedling roots was present as the 7-glucoside, scopolin. For the three identified, individual coumarins measured, actual counts per minute definitely increased with time, under our experimental conditions, although radioactivity in scopoletin increased with time much less than the radioactivity in scopolin and fabiatrin. The specific activity of scopolin and fabiatrin increased slightly after the first 4 hr. On the other hand, the percentage of the total radioactivity present in the 'soluble' fraction that was there as scopolin (in both roots and shoots) and in much lesser amount that was present as scopoletin (in roots), definitely decreased with time. No fabiatrin and only trace amounts of scopoletin were found in the shoots.

These results suggest that some radioactive scopolin (from scopoletin-4-^{14}C not converted at an early stage to 'insoluble' material) may be converted with time to other 'soluble' compounds—and possibly also to some 'insoluble' material. The data could also be interpreted to indicate that some of the radioactive scopoletin was being converted in a larger proportion, with time, to 'soluble' compounds other than scopolin, or that both of the above may be occurring.

The percentage of the total 'soluble' radioactivity present due to one or more compounds in the roots, other than scopolin, fabiatrin, and scopoletin, definitely increased—at least in the period studied (50 hr).

Of the total radioactivity recovered in this set of experiments, a range of 43–51% (av. 46%) was found to be in the 'soluble' portion (roots plus shoots). The observation that the ratio of radioactivity between the 'soluble' and 'insoluble' portions varies only slightly with time suggests that the radioactive scopoletin is partitioned between soluble and insoluble material soon after it is taken up or at least in the shortest time period studied (1 hr). The 'soluble' portion of the roots exhibited 4·4–11 times as much radioactivity as found in the 'soluble' portion of the shoots during the length of time the seedlings were left in the radioactive scopoletin solution. It also appears that a substantial part of the radioactivity in the shoots resulted from translocation of radioactive scopoletin, scopolin, or some derivative of one or both from the roots to the shoots within the time intervals used for analysis—including significant amounts within the first hour.

Pulse-Feeding of Scopoletin-4-^{14}C

Previous unreported pulse-feeding experiments in this laboratory, using tritium labeled scopoletin, had indicated that much of the scopolin—when formed in relatively large amount—was metabolized slowly. Therefore, an experiment was conducted over a period of 16 days. Scopoletin-4-^{14}C, at a concentration of $1{\cdot}94 \times 10^{-4}$ M and a specific activity

[7] F. A. Einhellig, E. L. Rice, P. G. Risser and S. H. Wender, *Bull. Torrey Bot. Club* **97**, 22 (1970).

of 3·5 μCi/μmol was taken up by tobacco seedlings for 28 hr. At the end of each time period—0·3, 4, 10 and 16 days of metabolizing the radioactive scopoletin—a group of 4 plants was washed, fixed, and extracted as described in the Experimental. The growth of the plants which had absorbed the scopoletin-4-^{14}C appeared essentially the same as that of the controls, and the fresh weight of both groups more than quadrupled during the 16 days of the experiment.

If one plots, from the data of Table 2, the log of the radioactivity of root scopolin versus days, a straight line results. The biological half-life of the radioactive scopoletin portion of scopolin under the experimental conditions used was calculated from the slope of the curve to be approximately 16 days. In the roots, the radioactivity in scopolin as

TABLE 2. ACTIVITIES OF SCOPOLIN, SCOPOLETIN, AND FABIATRIN IN THE 'SOLUBLE' FRACTIONS AT VARIOUS TIMES AFTER CESSATION OF FEEDING (28-hr CONTACT) OF SCOPOLETIN-4-^{14}C TO TOBACCO SEEDLINGS

Tissue, compound, and parameter	Days after removal of precursor			
	0·3	4	10	16
Roots				
Activity in cpm ($\times 10^{-4}$)				
Total 'soluble'	47	39	36	31
Scopolin	34	29	23	17
Scopoletin	0·8	0·7	0·8	0·7
Fabiatrin	2·6	4·2	5·9	4·8
Ratio: Scopolin/Fabiatrin	13	6·9	3·9	3·5
Percentage cpm of total 'soluble'				
Scopolin	72	74	64	55
Scopoletin	1·7	1 8	2·2	2·2
Fabiatrin	5·5	11	16	15
Zone 'D'	16	13	12	8 8
Other 'solubles', (by difference) unidentified	5·8	0·2	5·8	19
Percentage cpm of total recovered radioactivity				
Total 'soluble'	50	41	39	30
Total 'insoluble'	50	59	61	70
Specific activity (μCi/μmole)				
Scopolin	2·8	2·5	1·8	1·1
Fabiatrin	2·8	2·8	2·4	2·0
Shoots				
Activity in cpm ($\times 10^{-4}$)				
Total 'soluble'	13	11	7·5	5·0
Scopolin	6·5	6·0	5·4	—

The concentration of the original scopoletin-4-^{14}C was 1·94 $\times$ 10^{-4} M, and specific activity was 3·5 μCi/μmol.

compared to that in fabiatrin, at various time periods, was as follows: 8 hr after removal of the precursor, 13 times as much radioactive scopolin as fabiatrin was present; after 16 days, with both an increase in fabiatrin radioactivity and a decrease in scopolin, there was only 3·5 times as much radioactivity present in scopolin as in fabiatrin (Table 2). These data indicate that scopolin is the precursor of fabiatrin. Also, since the specific activity of root fabiatrin decreased at a slower rate than did the specific activity of root scopolin, fabiatrin apparently is metabolized further at a rate slower than scopolin in the roots.

The percentage of the total recovered radioactivity contributed by the 'soluble' compounds in this set of experiments also decreased with time, whereas the percentage of the radioactivity due to the 'insolubles' increased with time. Thus, one or more compounds of the 'soluble' portion were apparently being converted to 'insoluble' material.

In another experiment, a scopoletin-4-^{14}C concentration of $5{\cdot}57 \times 10^{-5}$ M with a specific activity of 3·5 μCi/μmol was taken up by another batch of tobacco seedlings for 28 hr. At this starting concentration, 89% of total recovered radioactivity from the roots was found in the 'insoluble' fraction. At the starting scopoletin-4-^{14}C concentration of $1{\cdot}94 \times 10^{-4}$ M, the corresponding value for the same time period and same experimental conditions had been found to be 48%. Thus, at these two particular concentrations of scopoletin-4-^{14}C, when a lower starting concentration of scopoletin-4-^{14}C was used, a larger percentage of the radioactivity appeared in the tobacco root 'insoluble' fraction.

Translocation Experiment

Four tobacco seedlings, which had taken up scopoletin-4-^{14}C for 28 hr, were allowed to grow for 16 days. The leaves were separated into old and new leaves. The 'old leaves' group consisted of those leaves which had been present at the beginning of the experiment; the 'new leaves' were those which had appeared during the 16 day growth period. The specific activity of the scopolin in the old leaves was found to be about 12 times greater than in the new leaves. The radioactivity of scopolin per g of fresh weight in the old leaves was about 18 times greater than in the new leaves.

Compartmentation

One may propose a hypothesis, based on the concept of compartmentation as discussed by Oaks and Bidwell,[8] to explain the experimental data reported here. One may assume, for example, that scopolin is compartmentalized in at least one metabolic pool and in at least one vacuolar pool. Scopolin is metabolized in the metabolic pool and stored in the vacuolar pool. We assume, however, that the compartmentation is not absolute, i.e. scopolin can pass from one compartment to another.

To explain the distribution and turnover rate of these compounds, it is further proposed that when scopoletin passes through the cell wall or plasmalemma, part of it is converted into 'insoluble' polymers, including a 'lignin-like' material. The remainder of the radioactive scopoletin apparently locates in the cytoplasm where most of it appears to have been glucosylated to scopolin, for the most part probably as a detoxification reaction. The exact location of the glucosylation has not been determined; the action occurs in a very short period of time. Depending upon concentration, much of this scopolin is compartmentalized in a vacuolar pool and thus stored. Some of it, however, is slowly converted to fabiatrin and to other compounds not yet identified.

In the present study, by comparing the specific activity of the precursor scopoletin with that of scopolin in the tissues, one can calculate that most of the scopolin in the tissues was actually obtained from the supplied radioactive scopoletin. Since a relatively larger amount of scopolin became available in the treated seedlings than is 'normally' present in a healthy control tobacco seedling of comparable age and environment, one may speculate that the metabolic pool in the treated seedlings became glutted, and the 'excess' scopolin was stored in the vacuolar pool and thus exhibited a slower turnover rate. In the

[8] Ann Oaks and R. G. S. Bidwell, *Ann. Rev. Plant Physiol.* **21**, 43 (1970).

experiments of Loewenberg,[6] using phenylalanine as the precursor of scopolin, less than 1% of his total scopolin was derived from the radioactive phenylalanine. Therefore, the size of the metabolic pool in his experiments was hardly affected, and only a small part of the radioactive scopolin was shunted into the vacuolar pool, as interpreted by the speculated compartmentation concept. The vacuolar pool in his case would, therefore, have relatively little radioactive scopolin in it. When such tissues, including those in our experiments, are ground up, the scopolin from the two pools will mix, and the resulting scopolin should have a specific activity lower than the scopolin from the metabolic pool, yet higher than the scopolin from the vacuolar pool. Therefore, as was previously found by Fritig *et al.*[9] and Loewenberg,[6] the specific activity of scopoletin would be higher than that of scopolin.

As stated previously, the data from the present study indicate that when scopoletin first enters the plant some of it that has not been converted to insoluble material or compartmentalized as scopolin, is translocated, either as scopoletin, scopolin, or other derivatives of one of these. We found, however, that very little scopolin is translocated from old leaves to new leaves; therefore, the major part of this portion of the scopolin must already have found its way into storage in vacuolar pools.

EXPERIMENTAL

Growth of tobacco seedlings. Tobacco seeds (*Nicotiana tabacum* L. cv. One Sucker) were germinated in a crock containing pure washed quartz sand which had been autoclaved overnight at 121°. After the seeds were planted, the crock was covered with a glass lid, left in the laboratory for 24 hr, and then transferred to a Percival growth chamber. The light intensity of the growth chamber was 13000 lx; the photoperiods were 16 hr light and 8 hr dark. The light/dark temp were 29·4/21·1°, resp. The seedlings were watered daily with either Fe-EDTA double strength Hoagland's nutrient solution[10] or distilled water.

When the tobacco seedlings were about 1–2 cm tall, they were transplanted to 40 ml opaque plastic vials containing a nutrient solution consisting of one part Fe-EDTA Hoagland's solution and two parts distilled water. The transplanted tobacco seedlings were left 24 hr in the laboratory and then were moved to a growth chamber. After the seedlings had grown for approximately 2 weeks in the vials, they were selected on the basis of uniform size for the designed experiments.

Feeding experiments. In one set of experiments (continuous feeding), the tobacco seedlings were transferred to vials containing known amounts of radioactive solution. The radioactive solution contained the same concentration of Fe-EDTA Hoagland's solution as the nutrient solution. Each sample group was allowed to take up and metabolize the radioactive compound for various lengths of time. The plants in each group were then washed, fixed, and extracted.

In another series of experiments (pulse-feeding), all the plants were left in the vials containing the radioactive solution for the same length of time. The roots on all the plants were washed with distilled water, and the plants were transferred to vials containing the usual non-radioactive nutrient solution (Fe-EDTA Hoagland's solution: distilled water, 1:2, v/v). The seedlings were allowed to metabolize the radioactive compound they had previously taken up for different time periods. At the end of each defined time period a sample group was washed, fixed and extracted.

Isolation and quantitative determination of compounds. The seedlings were separated into roots and shoots. The roots and shoots were weighed (fresh wt) and were fixed by boiling them in isopropyl alcohol azeotrope for 5 min. The fixed plant material was ground and extracted by the procedure of Wilson *et al.*[11] Separation of fabiatrin, scopolin and scopoletin was accomplished by descending paper chromatography. The concentration of the three coumarins was determined by fluorescence measurements.[7,12] Either the whole sample or an aliquot from the sample used in the fluorescence measurement was evaporated to dryness and counted for radioactivity.

Synthesis of scopoletin-4-^{14}C. The scopoletin-4-^{14}C was synthesized in this laboratory several years ago by A. Zane,[13] and extensively purified by column chromatography and crystallization. Intermediates in the synthesis included zinc cyanide-^{14}C, 2,4,-dihydroxy-5-methoxybenzaldehyde-1-^{14}C, and 6-methoxy-

[9] B. FRITIG, L. HIRTH and G. OURISSON, *Phytochem.* **9**, 1963 (1970).
[10] D. R. HOAGLAND and D. I. ARNON, *Calif. Agric. Exptl. Sta. Circ.* 347 (1950).
[11] J. L. WILSON, W. J. DUNLAP and S. H. WENDER, *J. Chromatog.* **35**, 329 (1968).
[12] D. E. KOEPPE, L. M. ROHRBAUGH and S. H. WENDER, *Phytochem.* **8**, 889 (1969).
[13] A. ZANE, Ph.D. dissertation, University of Oklahoma, pp. 41–44 (1963).

7-acetoxycoumarin-4-^{14}C. At the time of use, the radiochemical purity of the scopoletin-4-^{14}C was found to be at least 99%.

Measurement of radioactivity in the insoluble material. The radioactive *iso* PrOH–C_6H_6–MeOH–H_2O 'insoluble material' was oxidized to $^{14}CO_2$ which was trapped in a phenethylamine–dioxane (1:1), v/v solution.[14] 2 ml aliquots of the dioxane–phenethylamine solution were transferred to scintillation vials and counted for radioactivity.

The radioactivity was measured in a Beckman DPM-100 liquid scintillation counter using dioxane containing 5 g of 2·5-diphenyloxazole and 100 g of naphthalene per l. Corrections were made for quenching by using an external standard reference.

Isolation and identification of fabiatrin. An IBMW extract of tobacco roots was evaporated to dryness and redissolved in a volume of water equal to the fresh wt of the roots. The aqueous solution was extracted three times with EtOAc, and the EtOAc was discarded. Three vol. of EtOAc–pyridine (2:1) were added to the aqueous solution. A small layer, containing 'tars' was discarded. The remaining solution was evaporated to dryness and the residue dissolved in 25 ml H_2O. The aqueous solution was chromatographed in a 6 cm dia. column packed to a depth of 30 cm with Polyclar AT in water. The column was eluted with water. The blue fluorescent zone of fabiatrin eluted before a larger blue fluorescent zone of scopolin. The column fractions, which contained the fabiatrin were evaporated to a small volume and applied as a 31 cm streak to a number of 46 × 57 cm Whatman No. 3 MM chromatography sheets which were developed in BAW (4:1:2). The appropriate zone was cut out and eluted with 5% MeOH. The material was further purified by paper chromatography using *iso* PrOH–HCO_2H–H_2O (5:0·1:95) and *n*-BuOH–pyridine–H_2O (14:3:3). A column packed with Polyclar AT in C_6H_6–MeOH (19:1) was used as the last purification step.

A portion of the purified material was dissolved in water and equal amounts were pipetted into 15 ml pear-shaped flasks and evaporated under reduced pressure to dryness. To each flask, 2 ml of either 1 N HCl, 0·1 N HCl or H_2O were added. The three flasks were heated for 1 hr on a steam bath. The acid was removed by a flash evaporator, and a small amount of isopropyl alcohol azeotrope was added to each flask. The unknown samples were spotted on Avicel SF thin-layer plates along with reference samples of scopolin, scopoletin, glucose, xylose, and a number of other monosaccharides. The plates were developed in EtOAc–pyridine–H_2O (2:1:2). Both the 1 N HCl sample and the 0·1 N HCl sample had three fluorescent spots, while the H_2O sample had only one. The fluorescent spots were circled and the plate was sprayed with aniline-oxalate reagent.[15] Two, sometimes three spots, could be seen when the sprayed plates were heated at 105° for 10 min. Enzymic hydrolysis of the suspected fabiatrin was accomplished by spraying the purified fabiatrin band, which had been streaked on 3 MM Whatman chromatography paper, with 0·1% emulsin (0·1 M acetate buffer, pH 5·3). The paper was kept warm and moist for 2 hr and developed in the BAW for 13 hr.

The R_fs, fluorescent color in UV light, and the color reaction after spraying with aniline-oxalate reagent indicated the presence in the hydrolysate of scopoletin, scopolin, glucose, xylose, and a disaccharide which appeared to be primeverose. The results are consistent with the identification of this glycoside as fabiatrin, the β-primeveroside (6-(β-D-xylosyl)-β-D-glucopyranosyl) derivative of scopoletin.[16] The UV spectrum and m.p. (226°) determinations also fitted previously reported values for fabiatrin.[1,16]

Zone 'D'. Radioactive root extracts containing the 'soluble' fraction were streaked on Whatman No 1 chromatography paper. After development in *iso*BuCOMe–HCO_2H–H_2O (14:3:2), different sections of the paper were counted for radioactivity. Zone 'D' (a zone between scopolin and chlorogenic acid) was the only zone, other than the fabiatrin and scopolin zones, to have significant amounts of radioactivity. Material from this zone was chromatographed on a 1·5 cm, dia. column packed to a depth of 22 cm with Polyclar AT in C_6H_6–MeOH (97:3).

The column was eluted with C_6H_6–MeOH (19:1), and 3·5 ml fractions were taken. Aliquots were counted for radioactivity, and seven radioactive peaks were observed. The fractions from the most radioactive peak were hydrolysed with 1 N HCl and an aliquot of the hydrolysate was chromatographed on a column packed with Sephadex G-10. One large and one small radioactive peaks were seen. The remainder of the hydrolysate was chromatographed on a Polyclar AT column packed in MeOH–H_2O (7:3) and eluted with the same solution. Non-radioactive scopoletin was used as a marker. Two small radioactive peaks plus a major peak were observed. The major peak was eluted just after the scopoletin peak and was observed to be non-fluorescent under UV light. Possible relationship of this compound to the non-fluorescent 'X' compound suggested by Sargent and Skoog[4] has not been determined.

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[14] W. G. DUNCOMBE and T. J. RISING, *Analyt. Biochem.* **30**, 275 (1969).
[15] S. M. PARTRIDGE, *Biochem. Soc. Symp.* **3**, 52 (1950).
[16] D. N. CHAUDHURY, R. A. HOLLAND and A. ROBERTSON, *J. Chem. Soc.* **151**, 1671 (1948).

Key Word Index—Nicotiana tabacum; Solanaceae; tobacco; biosynthesis; scopolin; fabiatrin.