Quantitative Analysis for Scopolin and Scopoletin in Flowers of Tobacco Plants Treated With 2,4-Dichlorophenoxyacetic Acid

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Fults and Johnson (1950) observed that certain plants, including tobacco, when sprayed with 2,4-dichlorophenoxyacetic acid (2,4-D) showed a substantial increase in the amount of scopoletin (7-hydroxy-6-methoxycoumarin) present. Scopolin (7-glucoside of scopoletin), however, was not reported. Dieterman et al. (1964) proved that scopolin especially, and to a very much lesser degree scopoletin, increase in concentration in tobacco leaves, midveins, stems, and roots after the tobacco plant has been sprayed with 2,4-D. The present paper reports the results obtained on analysis of flowers of tobacco following 2,4-D treatment.

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EXPERIMENTAL

Seeds of *Nicotiana tabacum*, One-Sucker variety, were planted on sterile quartz sand and watered every day with 400 ml of complete solution (Machlis and Torrey, 1956). The seeds were germinated in a large growth chamber under the following conditions: 16 hr of light/day; temperature, 27 C in the light period and 10 C in the dark period. The light intensity was approximately 700 ft-c. Seedlings were transplanted to large crocks of sterile quartz sand approximately one month after planting. One seedling was placed in each crock. The plants started to flower at an age of about 84 days. Eleven days later, the plants to be treated were sprayed with a solution of 2,4-D until the solution ran off the leaves. The 2,4-D solution consisted of 1 g of 2,4-D dissolved in approximately 1 liter of water with 5 g of Carbowax 1500 added. The pH was adjusted to 7.0 with ammonium hydroxide. Two drops of Tween 20 were added, and the solution was adjusted to exactly 1 liter with additional water. The controls were sprayed with a solution containing all of the above except the 2,4-D. The plants were harvested at weekly intervals beginning at age 102 days and continuing until age 130 days. Plants were harvested in the morning after they had been under illumination for approximately 2 hr. The flowers were removed, immediately weighed, and plunged into boiling methyl alcohol for 5 min. The flower tissue was further subjected to an exhaustive extraction procedure which yields crude extracts containing essentially all of the phenolic compounds present in the original tissue (Wilson, Dunlap, and Wender, 1968). In this procedure, the plant tissue was ground thoroughly with the suspending methyl alcohol in a Waring Blendor and transferred to a Soxhlet extraction thimble. The filtrate plus extensive washings with isopropyl alcohol-water azeotrope (87.9:12.1 w/w) and isopropyl alcohol-benzene-methyl alcohol-water (2:1:1:1 v/v/v/v) were collected. The residue was then extracted in a Soxhlet extractor for 24 hr with isopropyl alcohol azeotrope and finally for 24 hr with pure isopropyl alcohol. The combined filtrate, washings, and extracts were concentrated *in vacuo* on a rotary evaporator.

The quantities of scopolin and scopoletin in the samples were then determined by a procedure involving thin-layer chromatography for initial separation of the coumarins from the crude tobacco tissue extracts and fluorimetry for their quantitative determination after removal from the thin-layer (Winkler et al., 1968). Samples of the crude tobacco extracts and standard scopolin and scopoletin solutions were spotted quantitatively on thin layers of Polyamide Woelm (Alupharm Chemicals, New Orleans, La.). These were developed for 40 min in benzene-methyl alcohol-98% formic acid (4:1:0.1, v/v/v) in chambers lined with filter paper to improve saturation. The developed chromatograms were dried, and the scopolin and scopoletin zones (R_f values: 0.75 and 0.85, respectively) were located under long-wavelength, ultraviolet light and were outlined by dotting the surface of the absorbent with a dissecting needle. A blank zone of approximately the same size was also outlined. The outlined polyamide zones were then removed quantitatively from the thin layers by means of microvacuum cleaners, and the adsorbed coumarin compound in each individual vacuum cleaner was eluted directly into a 5-ml volumetric flask with warm methyl alcohol. After cooling and equilibration, the volume was adjusted to exactly 5 ml.

Determination of the fluorescence of each scopolin and scopoletin solution was made with a Turner Fluorometer, model 110. Quantities of scopolin and scopoletin in the sample solutions were determined by comparing fluorescence intensities of the sample and those of standard solutions.

Results on a typical set of flowers from 2,4-D-treated tobacco plants are shown in Table I.

TABLE I. SCOPOLIN AND SCOPOLETIN IN FLOWERS FROM TOBACCO PLANTS TREATED WITH 2,4-DICHLOROPHENOXYACETIC ACID.

Days after treatment	Scopolin (μg/fresh tissue)		Scopoletin (μg/fresh tissue)	
	Control	Treated	Control	Treated
8	55	163	1.3	4.0
14	53	170	1.7	3.7
21	69	325	2.0	7.1
28	52	144	2.5	3.2
35	107	180	4.9	6.4

The scopolin content of the flower tissue from tobacco plants treated with 2,4-D was substantially higher, in every case, than the scopolin content of the corresponding control.

The scopolin concentration remained fairly close to the same value at 14 days as at 8 days after 2,4-D treatment. This was also the case with controls at 14 vs. 8 days. In the treated flowers, however, this was followed by a very sharp increase, reaching a maximum on the 21st day. The increase in the controls was not so large. This was followed by a substantial decrease in the scopolin content of the flowers on the 28th day. During the fifth week, the scopolin content of both the controls and the treated flowers again increased.

Changes in scopoletin concentration in treated flowers were in general in the same direction as those for scopolin, but considerably less in amount.

SUMMARY

Flowers from tobacco plants treated with 2,4-dichlorophenoxyacetic acid were shown to contain a substantially higher concentration of scopolin than did flowers from control plants grown under the same conditions, except that the latter did not receive 2,4-D treatment. Scopoletin concentration also increased in treated flowers relative to controls, but the actual concentrations were considerably less than those of scopolin.

ACKNOWLEDGEMENTS

This work was supported in part by grants from the National Science Foundation and by the Council for Tobacco Research — U. S. A.

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