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SHORT COMMUNICATION

MICRO DETERMINATION OF SCOPOLETIN IN *NICOTIANA TABACUM**

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Key Word Index—*Nicotiana tabacum*; Solanaceae; scopoletin determination; GLC and electron capture.

Abstract—A GLC method for quantitative analysis of tobacco for scopoletin (as free scopoletin and the glycoside, scopolin) utilizes tritium foil electron capture detection of as little as 100 ng scopoletin. Polyphenols are directly extracted with *n*-propanol, and hydrolyzed to produce scopoletin. After silylation the TMS-scopoletin is separated on 10% OV-101 with *p*-coumaric acid as internal standard.

INTRODUCTION

SCOPOLETIN, a phenolic coumarin widely distributed in the plant kingdom[1] and present in tobacco leaf was qualitatively determined in mixtures of authentic compounds by GLC with flame ionization detection.[2,3] In analyses for caffeic acid and quercetin in plants, electron capture detection has the advantages compared to flame ionization detection of higher sensitivity, lower background and no need for preliminary purification.[4] Our objective herein was to develop a sensitive, rapid GLC assay for scopoletin in tissue.

RESULTS AND DISCUSSION

Preliminary tests showed: (1) scopolin refluxed 1 hr in *n*-propanol saturated with dry HCl gas was hydrolyzed and yielded an equivalent amount of scopoletin; (2) TMS-scopoletin chromatographed satisfactorily on a 10% OV-101 column; and (3) TMS-scopoletin was five times more sensitive to electron capture than to flame ionization detection.

Samples of scopoletin, caffeic acid *n*-propyl ester, and *p*-coumaric acid were silylated and chromatographed according to conditions given in Experimental. Relative retentions (r) of TMS-scopoletin, TMS-caffeic acid *n*-propyl ester (present on chromatograms of samples analyzed) and the internal standard as TMS-*p*-coumaric acid are given in Table 1. A tobacco sample spiked with scopolin, and a tobacco tumor sample were analyzed for scopoletin moieties. Chromatograms of an air-cured Burley 21 'low-phenolic' tobacco containing synthetic scopolin and tobacco tumor tissue (excised from *Nicotiana suaveolens* × *N. langsdorfii*, 4*n*) showed peaks corresponding to TMS-scopoletin. The lower detection limits at 75 V were approx. 100 ng scopoletin (and 50 ng caffeic acid).

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[1] T. ROBINSON, *The Organic Constituents of Higher Plants*, 2nd Edition, pp. 57 and 59, Burgess, Minneapolis, Minn. (1967).
[2] T. FURUYA and H. KOJIMA, *J. Chromatog.* **29**, 382 (1967).
[3] E. D. PELLIZZARI, CHIEN-MEI CHUANG, J. KUC' and E. B. WILLIAMS, *J. Chromatog.* **40**, 285 (1969).
[4] R. A. ANDERSEN and T. H. VAUGHN, *J. Chromatog.* **52**, 385 (1970).

A calibration curve for scopoletin moieties (as scopolin) was plotted. Tobacco samples prepared from Burley 21 air-cured leaf with non-detectable endogenous scopoletin and scopolin, spiked with synthetic scopolin; 100 mg samples contained 0–4 mg scopolin and each was carried through the entire analytical method. Aliquots of extracts equivalent to 25·0 μg sample with up to 1·00 μg scopolin were injected. There was a linear relationship between the amounts of equivalent scopoletin and corresponding peak areas.

TABLE 1. RELATIVE RETENTION (r) OF TRIMETHYLSILYLATED SCOPOLETIN AND CAFFEIC ACID n-PROPYL ESTER ON 10% OV-101*

Component	r
p-Coumaric acid	1·00
Scopoletin	0·29
Caffeic acid n-propyl ester	1·40

* GLC operating conditions: Electron capture detector 75 V, column temp. 190°, inlet, detector and outlet temps. of 220°, 215° and 220°, respectively, and argon carrier gas flow rate 100 cm³/min. Retention time of TMS-p-coumaric acid internal standard: 0·08 min.

TABLE 2. SCOPOLETIN CONTENT IN VARIOUS TOBACCO LEAF SAMPLES

Sample	% Scopoletin (mean ± average deviation)
Burley 21, mature, field-grown	None detected
F.C. 402, immature leaves harvested 4 weeks after transplant to field	0·083 ± 0·004
N.C. 95, mature, field-grown, flue-cured	0·248 ± 0·004

Precision was determined by four individual analyses for scopoletin on each of three tobacco leaf samples which represented low, intermediate and high phenolic contents (Table 2); means and average deviations are given. For comparison purposes, one flue-cured tobacco sample was assayed by GLC and a quantitative, two-dimensional paper chromatographic-spectrophotometric method for scopoletin (as scopoletin and scopolin).[5] Results were 0·030% and 0·034% scopoletin moieties by the GLC and paper chromatographic methods, respectively. Analytical time is shorter for the GLC than for the chromatographic analysis. Results for scopoletin reflect free scopoletin and the glycoside scopolin[4] in tobacco and this GLC method cannot distinguish among them, whereas methods utilizing PC or TLC can.

EXPERIMENTAL

Reagents. All chemicals were reagent grade unless otherwise specified. The sources or handling of acetonitrile, n-propyl alcohol, HCl gas, bis(trimethylsilyl)trifluoroacetamide (BSTFA), and caffeic acid were the same as previously described.[4] Scopoletin and p-coumaric acid were obtained commercially; scopolin was synthesized as previously described.[6]

Analytical method. Weigh 100 mg freeze-dried powdered tobacco into a flask, add 15 ml n-propanol and reflux 45 min. Filter through Whatman No. 1 paper and collect filtrate and several n-propanol washings. Pass dry HCl gas through the solution until saturated, then reflux for 1 hr. Take to dryness on a rotary evaporator. Add p-coumaric acid as internal standard in sufficient amount to provide a satisfactory peak height; suitable injection amounts are 25–100 ng p-coumaric acid. Add 1·80 ml acetonitrile and 0·20 ml BSTFA. Cover flask and swirl contents to effect complete solution. Transfer most of the contents rapidly into an acylation tube which is then sealed. Heat at 150° for 1 hr. Cool to room temp., open the tube and inject a known amount (1·0–3·0 μl) into the GLC.

[5] S. J. SHEEN and J. CALVERT, *Tob. Sci.* **XIII,** 10 (1969).
[6] R. A. ANDERSEN and J. A. SOWERS, *Phytochem.* **7,** 293 (1968).

GLC. A Packard Model 7821 gas chromatograph was used with a Packard Model 810 electron capture detector with tritium foil as the ionizing source. The chromatograms were obtained on a Photovolt Microcord Model 44 recorder at a chart speed of 10 in./hr. A glass column packed with 10% OV-101 silicone stationary phase on 80–90 mesh Anakrom AS was used at 190°. The detector voltage was 75. Other operating conditions were the same as those previously described for caffeic acid moieties,[4] i.e. inlet, detector and outlet temperatures of 220°, 215° and 220°, respectively, and argon carrier gas flow rate of 100 cm^3/min. *p*-Coumaric acid was used as the internal standard. The peak areas were measured by triangulation at retention times established with TMS-scopoletin. Retention times were calibrated by determining: (a) the relative peak positions of TMS-scopoletin, TMS-caffeic acid *n*-propyl ester and TMS -*p*-coumaric acid under the conditions specified for the 10% OV-101 column. Peak areas of silylated scopoletin were compared with standard curve peak areas of various amounts of scopolin or scopoletin added to a tobacco sample which had non-detectable amounts of scopoletin moieties; these samples were carried through the entire analytical method.

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