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A thin-layer chromatography–fluorometry method for quantitative analysis of scopolin and scopoletin in tobacco

Scopoletin (6-methoxy-7-hydroxy-coumarin) and its 7-glucoside, scopolin, occur in many plants[1]. YANG and coworkers[2] found scopoletin in the cured tobacco of cigarettes and in the smoke therefrom, and they quantitatively measured the amount present by paper chromatography and spectrophotometry[3]. Other quantitative analyses for scopoletin have been reported by various research groups[4]. This report describes an improved, relatively rapid and precise quantitative method capable of determining fractional microgram amounts of both scopolin and scopoletin in tobacco extracts. The procedure employs thin-layer chromatography for initial separation of both these coumarins from crude extracts of tobacco, and fluorometry for their quantitative determination after removal from the thin layer. Both scopolin and scopoletin can be determined from the same chromatogram, whereas many of the other published methods determine only one of these, usually scopoletin. Preliminary purification of the crude extracts of tobacco is not necessary in this new procedure, and a complete analysis of both coumarins in two unknown samples can be accomplished in less than 8 h. The method is quite specific in that the employed thin-layer chromatography procedure effectively separates scopolin and scopoletin from other major fluorescing compounds of tobacco, and the filters employed in fluorometry provide further selectivity for the desired compounds.

Experimental

Initial separation of scopolin and scopoletin from crude tobacco extracts is achieved by chromatography on thin layers of Polyamide Woelm (supplied by Alupharm Chemicals, New Orleans, La.). Plates are prepared from a slurry of 1 part polyamide and 0.1 part soluble potato starch in 9 parts methanol, using a commercial applicator adjusted to yield a layer of 250 μ thickness. One gram of polyamide is required for each 20 × 20 cm plate, and starch is included to obtain thin layers of better texture and handling qualities than are obtained with polyamide alone.

Two crude tobacco extract samples and standard scopolin and scopoletin solutions are spotted quantitatively in triplicate on each 20 × 20 cm thin layer. The quantities of sample and standards which are spotted should contain 0.2 to 5 μg scopolin and 0.01 to 0.5 μg scopoletin in order to obtain best results in the subsequent fluorometry step. We employ a 0.2 ml RGI micrometer syringe (Kontes Glass Co., Vineland, N.J.) equipped with a blunted 24 gauge needle for spotting. Evaporation of solvent during spotting is promoted by a gentle stream of cool air.

Chromatograms are developed for 40 min in benzene–methanol–98 % formic acid (4:1:0.1, v/v/v) in chromatography chambers which are lined with filter paper to improve saturation. The developed chromatograms are dried thoroughly in a current of moving air for 1 h to insure removal of the last traces of formic acid. The blue-fluorescing scopolin and scopoletin (R_F values 0.75 and 0.85, respectively) are then located under long wavelength (3660 Å) ultraviolet light, and are outlined by dotting the surface of the adsorbent with a dissecting needle. A blank zone of approximately the same size is also outlined. The outlined polyamide zones are then removed quanti-

tatively from the thin layers by means of microvacuum cleaners[5], and the adsorbed scopolin or scopoletin in each individual vacuum cleaner apparatus is eluted directly into a 5 ml volumetric flask by washing the adsorbent with approximately 5 ml of warm methanol added in small increments. The resulting scopolin, scopoletin, and blank solutions are allowed to equilibrate to room temperature, and are then adjusted to exactly 5 ml volume with methanol and thoroughly mixed.

Determination of the fluorescence of each scopolin and of each scopoletin solution is made with a Turner Fluorometer, Model 110, using Pyrex cuvets and a high sensitivity attachment. The fluorometer is equipped with a general purpose ultraviolet lamp (General Electric No. F4T4/BL). A Corning No. 7-60 narrow pass filter, with peak transmission at 360 mμ is used as the primary filter. The secondary filter consists of a combination of a Wratten No. 2A sharp cut filter, which passes all wavelengths longer than 415 mμ and a Wratten No. 48 narrow pass filter, with peak transmission at 460 mμ. The instrument is zeroed with the blank solution obtained from each individual thin-layer chromatogram before determination of fluorescence of scopolin and scopoletin solutions from that chromatogram.

Under the conditions employed, fluorescence intensities of scopolin and scopoletin solutions are linear functions of the concentrations of these compounds in the range 0–1 μg/ml for scopolin and 0–0.1 μg/ml for scopoletin. Hence, the quantities of scopolin and scopoletin in the sample solutions are determined by comparing fluorescence intensities of the sample and standard solutions.

Scopoletin has a fluorescence intensity which is approximately nine times greater than that of scopolin, and therefore much smaller amounts of scopoletin can be readily determined. This is an advantage since the amount of scopoletin present in properly handled tobacco tissues is usually much smaller than the amount of scopolin present.

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