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SHORT COMMUNICATION

XANTHYLIUM DERIVATIVES IN GRAPE EXTRACTS

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Abstract—A yellow compound, which developed during storage of grape juice, was isolated and characterized as the glucose derivative of a 1,3,6,8-tetrahydroxyxanthylium salt.

MONOMERIC and consensed leucoanthocyanidins[1] and model leucoanthocyanin-polyphenol condensation products[2] on acid treatment yield xanthylium salts and varying amounts of anthocyanidins.[3] Plant extracts containing anthocyanins, grape juices and wines, on the other hand, change their original anthocyanin profiles on prolonged storage even at relatively low temperatures and form polymeric pigment fractions.[4] The formation of these condensation and degradation products is simultaneous with the increase in the absorption in the 420–460 nm region of the spectra,[5] the λ_{max} region of the hydroxylated xanthylium salts. Since the formation of the xanthylium salts requires acidic conditions[6,7] and the pH of most plant extracts is known to be acidic, xanthylium salts could be formed upon prolonged storage as a result of condensation from leucoanthocyanidin and/or reactive structural transformation products of anthocyanins, such as flav-2-ene radicals.

RESULTS AND DISCUSSION

The chromatographic behavior of the isolated yellow compound was similar to that of the monoglucosides of flavonoids. It migrated as a well defined single yellow spot in all solvent systems used. On exposure to NH_3 it developed an orange color, similar to the hydroxylated xanthylium salts reported by Jurd and Somers.[2] The spectrum in 1% aqueous HCl showed sharp, well defined absorption maxima at 439 and 269 nm and a minor peak at 302 nm (Fig. 1). The absorption ratios of the two wavelengths at 269 and 439 nm were 1·11, well within the range for the reported data for the 1,3,6,8-tetrahydroxyxanthylium chloride.[2] On extraction of the yellow compound into BuOH its λ_{max} shifted to 454, 305, and 272 nm. Addition of NaOH to the aqueous acidic solution of the compound resulted in a pronounced bathochromic shift to 486, ~465, and 273 nm. The development of the double λ_{max} after addition of NaOH is characteristic for the trihydroxyxanthylium structure; however, the position of the shoulder at ~465 nm is rather high for this compound (λ_{max} for 1,3,6-trihydroxyxanthylium chloride after addition of NaOH: 483,433 nm).

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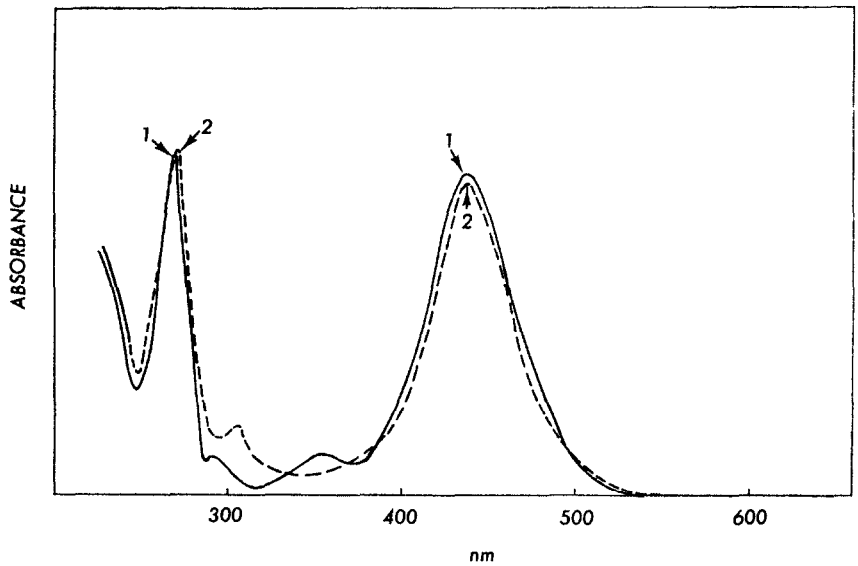

FIG. 1. SPECTRUM IN 1% AQUEOUS HCl OF (1) 1,3,6,8-TETRAHYDROXYXANTHYLIUM CHLORIDE AND (2) YELLOW COMPOUND ISOLATED FROM GRAPE JUICE

Hydrolysis of the yellow compound showed the presence of glucose and two well defined yellow spots which had similar R_f values to the tetrahydroxyxanthylium compounds obtained by heating the leucocyanidin–phloroglucinol condensation product[2] (R_f 0·41; 0·66; 0·60 and 0·29, 0·47; 0·46 in solvent systems A, B and C, respectively).

The spectrum of the aglucone in BuOH–HCl showed absorption maxima at 454, ~375, 305, and 273 nm and absorbance ratio of 1·17 at 273/454 nm.

The chromatographic behaviour and spectral characteristics of both the isolated yellow compound and its aglucone suggest the 1,3,6,8-tetrahydroxyxanthylium structure for the compound, with glucosylation most likely at the 8-OH, possibly formed by 4–8 condensation of leucoanthocyanins and/or flav-2-ene radicals with anthocyanins. The glucosylation of the 8-OH and the site of cleavage of the B-ring of the original anthocyanin molecule at the 7-position are possibly responsible for the spectral differences from the 1,3,6,8-tetrahydroxyxanthylium salt.

Because of the small amount formed, the difficulties encountered in isolation, and the instability of the isolated compound, further attempts are required for the determination of its exact structure.

EXPERIMENTAL

The following TLC solvent systems were used: (A) H_2O–HOAc–conc. HCl (80:20:5, by vol); (B) HCO_2H–3 N HCl (1:1, vol); (C) H_2O–HOAc–conc. HCl (80:40:5, by vol.); (D) *n*-BuOH/1% HCl, organic phase; (E) H_2O–HOAc–conc. HCl (5:5:1, by vol).

Isolation of the Yellow Compound

Seibel 9549 grape juice (40 l.), stored for 2 yr at 4°, was chromatographed on a PVP column (100 × 10 cm) as described previously.[8] The yellow compound eluted between petunin and cyanin, was rechromatographed three times on freshly prepared PVP columns (45 × 2·5 cm). Fractions containing the yellow compound were concentrated to *ca.* 25 ml and extracted with *n*-BuOH satured with 1% aqueous HCl. Counter

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current distribution of this extract on the RONOR-column (400 chambers, 20 rev/min, pump speed, 3·0 ml/min) using *n*-BuOH and 1% aq. HCL acid as mobile and stationary phases, respectively, separated 3 compounds. Compound 1 (not identified) which was eluted with the mobile phase had λ_{max} at 362 and 259 nm in *n*-BuOH, R_f 0·85 in solvent system (E) (colorless, yellow with NH_3).

The yellow compound, followed by a mixture of anthocyanins, was eluted by washing the column with the stationary phase. The fractions containing the yellow compound were concentrated to give a bright yellow solution (10 ml) with λ_{max} at 439 nm (o.d. 0·65) and 269 nm (o.d. 0·72). Chromatography of the solution showed only one compound (yellow, orange with ammonia) with R_fs 0·68; 0·76; 0·76 and 0·14 in solvent systems (A), (B), (C) and (D), respectively.

Hydrolysis of the Yellow Compound

HCl (1 ml of 2 N) and the concentrated solution containing the yellow compound (1 ml) was refluxed for 2 hr, cooled, and the hydrolysate percolated through a PVP column (2 × 1 cm). The column was washed with water until neutral and the effluent evaporated to dryness, silylated,[9] and the sugar component determined using G.C. (Aerograph 200; 3% OV-1 on Gas Chrom. Q., 60–80 mesh, column length: 6, column diameter $\frac{1}{8}$ in.). The aglucone was eluted from the column with MeOH–AcOH (1:1, v/v), concentrated to dryness, and dissolved in the organic phase of *n*-BuOH/1% aq. HCl for determination of the spectra.

The 1,3,6-Trihydroxyxanthylium salt was synthesized according to Jurd.[2]

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