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SHORT COMMUNICATION

THE ANTHOCYANINS OF RED CABBAGE (*BRASSICA OLERACEA*)

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Abstract—Leaves of red cabbage (*Brassica oleracea*) contain cyanidin 3,5-diglucoside, cyanidin 3-sophoroside-5-glucoside and cyanidin 3-sophoroside-5-glucoside acylated with 1 and 2 moles of sinapic acid.

IT IS well established[1,2] that red cabbage (*Brassica oleracea*) contains acylated forms of cyanidin 3-sophoroside-5-glucoside, but there is less agreement on the nature of the acyl groups. Harborne[1] found ferulic and *p*-coumaric acids as acyl constituents whereas Stroh and Seidel[3] reported finding three anthocyanins which contained sinapic acid. Harborne[4] considered that the latter authors' material may have been contaminated with sinapoyl esters; not without justification, since the ratios of the extinctions at the wavelength peaks of phenolic acid and anthocyanin (E_{322} nm/E_{520} nm) quoted by Stroh and Seidel are much larger than would be anticipated for anthocyanins containing from 1 to 3 moles of phenolic acid.[5] However, we have now extensively purified red cabbage anthocyanins and isolated anthocyanins of the expected spectral characteristics which are acylated with sinapic acid.

RESULTS

The following four anthocyanins were identified—cyanidin 3,5-diglucoside, cyanidin 3-sophoroside-5-glucoside and cyanidin 3-sophoroside-5-glucoside acylated with one and two equivalents of sinapic acid. The spectra of the two acylated anthocyanins exhibited a double peak in the u.v. (Bands 1a and 1b) in addition to the acyl peak (Band II) (see Table 1). Ferulic and *p*-coumaric acids accompanied sinapic acid in four samples but in such small traces that it was not possible to decide whether they were contaminants or true anthocyanin constituents. Sinapic acid occurred alone in the remaining eight samples. The consistency of the spectral characteristics and R_f values of the samples obtained after chromatography in various combinations of solvents precludes the possibility of the sinapic acid being present as a contaminant.

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TABLE 1. PROPERTIES OF ACYLATED ANTHOCYANINS

Mole ratio of sinapic acid	Number of samples	λ_{max} in MeOH–HCl (nm) Bands 1a	1b	II	III	Ratios (%) *E* Band 1a / *E* Band III	*E* Band 1b / *E* Band III	*E* Band II / *E* Band III	E_{440} / *E* Band III
1	7	279	296	331	529	57^{+7}_{-4}	55 ± 6	56^{+10}_{-4}	14–15
2	5	282	297	327	531	75^{+9}_{-5}	81^{+9}_{-6}	94 ± 9	12–15

	R_f values ($\times 100$) in* BuHCl	BuHClW	1% HCl	HOAc–HCl	2% HOAc
Cyanidin 3-sophoroside 5-glucoside	7	—	57	71	68
Cyanidin monosinapoyl derivative	10	34	50	75	43
Cyanidin disinapoyl derivative	28	61	24	64	20

* On Whatman No. 1 paper; abbreviations: BuHCl, butan-1-ol-2 N HCl (1:1, v/v); BuHClW, butan-1-ol–conc. HCl–water (7:2:5, v/v); 1% HCl, water–conc. HCl (97:3, v/v); HOAC–HCl, acetic acid–conc. HCl–water (15:3:82, v/v); 2% HOAc, water–acetic acid (98:2, v/v).

EXPERIMENTAL

Purification of Anthocyanins

A red cabbage was taken at random from a field containing "Red Acre", "Dwarf Red" and "Langendyke Red" (on later investigation, all three cultivars showed the same qualitative anthocyanin pattern). Leaves were macerated in a blender with MeOH–HCl (97:3, v/v), the extracts were filtered, concentrated and adsorbed onto purified[6] polyvinyl pyrrolidone powder ("Polyclar AT"). The powder was washed with dilute aqueous HCl, the anthocyanins were eluted with MeOH–HCl, concentrated to dryness, extracted with anhydrous methanol containing HCl gas and precipitated with ether. The crude anthocyanins were purified (*a*) by cellulose column chromatography using HOAc–HCl followed by BuHCl. The fractions obtained were chromatographed until pure on Whatman No. 3 paper in solvents BAW (butan-1-ol–acetic acid–water, 4:1:5, v/v, top layer), BAW containing conc. HCl (1–5%) and 2% HOAc, (*b*) by solution in 2×10^{-3} N aqueous HCl and adsorption on a column of purified[7] Zeo-Karb 225. The column was washed thoroughly with 2×10^{-3} N aqueous HCl, followed by methanol containing 0·1% conc. HCl which removed a small amount of browned and degraded material. The anthocyanins were eluted with methanol containing increasing amounts of conc. HCl (from 0·2–2%) and concentrated. Further purification was effected on Whatman No. 3 paper using combinations of solvents BAW containing 1% conc. HCl, BuHClW and HOAc–HCl, until the spectra of the anthocyanins remained constant. Recourse to the use of BAW containing conc. HCl was necessary to avoid complications arising from the continual use of solvents free from mineral acid (BAW and 2% HOAc) which could give rise to the formation of multiple and blue anthocyanin bands.

Identification of Acyl Groups

The acylated anthocyanins were hydrolysed with 2 N NaOH for 2 hr under N_2 at room temperature, followed by acidification and ether extraction.[8] Sinapic acid was identified by comparison with authentic samples of phenolic acids by (*a*) paper chromatography using toluene–acetic acid–water (4:1:5 v/v)[9,10] and benzene–acetic acid–water (2:2:1 v/v),[11] followed by observation of the colours of the spots in the u.v. $\pm$ NH_3 vapour, and after spraying with diazotized *p*-nitraniline; (*b*) TLC on Silica gel G, using water saturated

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n-butyl ether–formic acid (10:1),[12] spots were located by spraying with phosphomolybdic acid,[13] (c) absorbance measurements in 0·05 N NaOH.[14,15] Sinapic acid was accompanied on the chromatograms by a derivative of low R_f, which appeared to be similar to that found after air oxidation of sinapic acid in alkaline buffer,[16] as well as traces of other degraded products which formed streaks. Similar chromatograms were obtained when pure sinapic acid underwent the hydrolysis procedure, even with rigorous exclusion of air. In contrast, ferulic, caffeic and *p*-coumaric acids remained unaffected by the hydrolysis procedure.

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