ELSEVIER

PII: S0308-8146(96)00116-1

Food Chemistry, Vol. 58, No. 3, pp. 215–217, 1997

0308-8146/97 $17.00+0.00

Malonated anthocyanins of garlic *Allium sativum* L.

Torgils Fossen & Øyvind M. Andersen*

Department of Chemistry, University of Bergen, Allégt. 41, N-5007, Bergen, Norway

(Received 27 October 1995; revised version received 14 February 1996; accepted 14 February 1996)

Acidified methanolic extract of inner scale leaves of garlic contain mostly anthocyanins with aliphatic acylation. These are the rare 3″,6″-dimalonylglucoside (13%) and 3″-malonylglucoside (3%) of cyanidin, in addition to cyanidin 3-(6″-malonylglucoside) (71%) and cyanidin 3-glucoside (12%). Copyright © 1996 Elsevier Science Ltd

INTRODUCTION

The colour quality of fresh and processed vegetables, in addition to the documented health benefits of anthocyanins, has led to renewed interest in anthocyanins. The red colour of the inner scales of garlic (*Allium sativum* L.), which has been known from ancient times as an important vegetable, is caused by the presence of anthocyanins. Du and Francis (1975) found the major anthocyanin to be cyanidin 3-glucoside, and suggested the occurrence of mono-, di- and tri-acylated derivatives of cyanidin 3-glucoside in which the acyl moieties were aliphatic acids. Several authors (Borukh & Demkevich, 1976; Hilal *et al.*, 1983) have reported cyanidin 3-glucoside and some unidentified cyanidin derivatives in the bulbs. Mineral acid (HCl) was used in the extraction solvent in all cases; this may account for the finding of cyanidin 3-glucoside as the major anthocyanin (Moore *et al.*, 1982).

This paper presents the anthocyanin content in garlic, which is dominated by anthocyanins with aliphatic acylation. Acylation of the rare 3″-position in the sugar moiety was established for two of the pigments.

MATERIALS AND METHODS

Extraction and separation

Fresh garlic was obtained from the local food market. The inner pigmented scale leaves were collected, cut with a pair of scissors and extracted (twice) with 0.5 M citric acid in methanol. The filtered extracts were combined, concentrated under reduced pressure, purified by partition against ethyl acetate and the use of an Amberlite XAD-7 column (Andersen, 1988). The anthocyanins were separated on a Sephadex LH-20 column (40 cm×1.0 cm; Pharmacia) using methanol–acetic acid–water (10:1:10) as eluent. High-performance liquid chromatography (HPLC) was performed according to a previously published procedure (Andersen & Fossen, 1995). The HPLC chromatograms were recorded as the average values of the absorptions on every second nanometre between 500 and 540 nm. The relative proportions of the anthocyanins are reported as the percentages of the total peak area in the chromatogram without taking into account the different molar absorption coefficients of the pigments.

Authentic cyanidin 3-glucoside, cyanidin 3-(6″-malonylglucoside), cyanidin 3-(3″-malonylglucoside) and cyanidin 3-(3″,6″-dimalonylglucoside) were isolated from red onion and stems of *Allium victorialis* L. (Andersen & Fossen, 1995).

Alkaline hydrolysis

Deacylation with alkali was performed by adding 0.6 ml of 2 M NaOH to 0.4 ml of the sample. The mixture was kept for 2 h in an air-free syringe, acidified with 2 M HCl (0.8 ml) and evaporated to dryness. After washing with diethyl ether (2×2.5 ml), the sample was dissolved in acidified methanol and analysed by HPLC.

Spectral analysis

The ^{1}H- and spin echo ^{13}C-NMR (nuclear magnetic resonance) data were obtained at 400.13 MHz (Bruker AM-400 instrument) and 150.92 MHz (Bruker 600 MHz instrument), respectively. The experiments were performed at 25°C with a 5 mm ^{1}H–^{13}C dual probe using the residual ^{1}H and the ^{13}C signals of the solvent (CF_3COO_2H:$C_2H_3O_2H$; 5:95, v/v) as secondary

*To whom correspondence should be addressed.

references (3.4 and 49.0 ppm from TMS (tetramethylsilane), respectively). UV/Vis absorption spectra were recorded on-line during HPLC analysis using a photodiode array detector (HP 1050), and spectral measurements were made over the wavelength range 210–600 nm in steps of 2 nm.

Cyanidin 3-(6″-malonylglucoside) (4)

^{1}H-NMR: δ9.04 (1H, H-4), δ6.77 (1H, H-6), δ6.99 (1H, H-8), δ8.12 (1H, H-2′), δ7.11 (1H, H-5′), δ8.36 (1H, H-6′), δ5.36 (1H, H-1 glc), δ3.77 (1H, H-2 glc), δ3.63 (1H, H-3 glc), δ3.51 (1H, H-4 glc), δ3.89 (1H, H-5 glc), δ4.64 (1H, H-6A glc), δ4.37 (1H, H-6B glc).

^{13}C-NMR, sugar signals: δ103.64 (C-1″), δ74.68 (C-2″), δ77.91 (C-3″), δ71.31 (C-4″), δ75.93 (C-5 ″), δ65.45 (C-6″).

RESULTS AND DISCUSSION

The HPLC chromatogram of the crude extract of the inner pigmented scale of garlic detected in the visible region showed one major and four minor anthocyanins (Fig. 1). The UV/Vis spectra of pigments 1–5 all showed a λ_{max} at 518–520 nm with $A_{440}/A_{Vis\text{-}max}$ of 29–31% (Table 1), indicating a cyanidin or peonidin nucleus with a sugar unit in the 3-position (Andersen, 1985). In the UV region, 290–340 nm, there was no indication of acylation with aromatic acids for any of the pigments. After alkaline hydrolysis, pigments 2–5 all turned into pigment 1.

The relatively long HPLC retention of the major pigment, 4, and the similarity between the UV/Vis spectra of pigments 1 and 4 (Table 1) indicated that pigment 4 was a derivative of pigment 1 acylated with aliphatic acid(s). The ^{1}H- and ^{13}C-NMR spectra of pigment 4

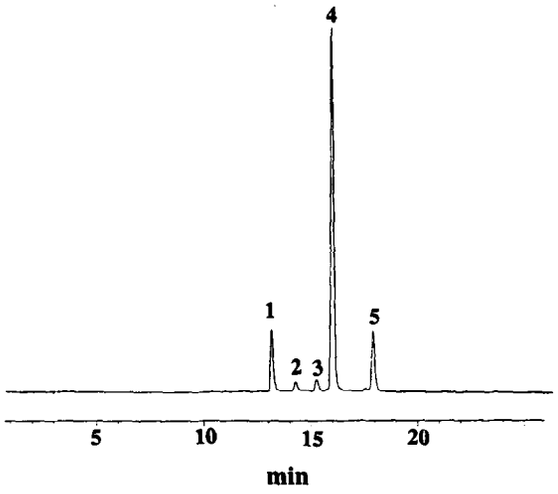
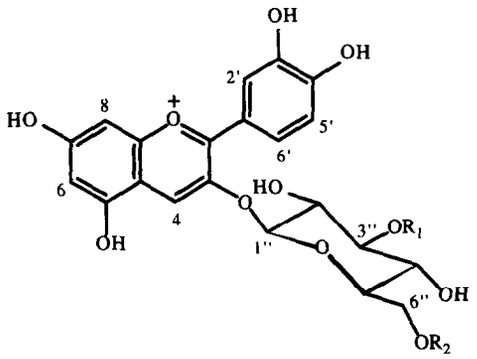


Fig. 1. The HPLC separation of anthocyanins in garlic detected at 520 ± 20 nm. Identification of peaks: 1, cyanidin 3-glucoside; 2, cyanidin derivative; 3, cyanidin 3-(3″-malonylglucoside); 4, cyanidin 3-(6″-malonylglucoside); 5, cyanidin 3-(3″,6″-dimalonylglucoside).

Table 1. Spectral and chromatographic data and relative proportions of the anthocyanins in the inner pigmented scales of garlic

Compound number	On-line HPLC			
	Vis-max (nm)	A_{440}/A_{max} (%)	R_t (min)	Area (%)
1	518	29.8	13.02	12
2	518	31.4	14.12	1
3	518	29.2	14.89	3
4	520	29.1	15.95	71
5	520	28.8	17.73	13



1: R_1 = H, R_2 = H
3: R_1 = malonyl, R_2 = H
4: R_1 = H, R_2 = malonyl
5: R_1 = malonyl, R_2 = malonyl

Fig. 2. Structure of the anthocyanins in garlic.

were in accordance with cyanidin, glucose and one malonic acid moiety (Andersen & Fossen, 1995). Of particular value for the identification of the malonyl group was the proton signal at 3.48 ppm integrating for two protons. The downfield chemical shifts of C-6″ (65.4 ppm) and H-6″A/H-6″B (4.64 and 4.37 ppm, respectively) showed that the malonyl group was connected at the glucosyl 6″-position.

Pigments 1, 3, 4 and 5 co-chromatographed (HPLC) with authentic cyanidin 3-glucoside, cyanidin 3-(3″-malonylglucoside), cyanidin 3-(6″-malonylglucoside) and cyanidin 3-(3″,6″-dimalonylglucoside), respectively, while pigment 2 was tentatively identified as cyanidin 3-acylglucoside. (Fig. 2)

Malonated anthocyanins constitute 87% of the total anthocyanin content of garlic (Table 1). The finding of acylation of the 3″-position in the sugar moieties of anthocyanins has so far been restricted to plants belonging to the genus *Allium* (Andersen & Fossen, 1995).

REFERENCES

Andersen, Ø. M. (1985). Chromatographic separation of anthocyanins in cowberry (lingonberry) *Vaccinium vites-idea* L. *J. Food Sci.*, **50**, 1230–1232.

Andersen, Ø. M. (1988). Semipreparative isolation and structure determination of pelargonidin 3-*O*-α-L-rhamnopyranosyl

(1→2)-β-D-glucopyranoside and other anthocyanins from the tree *Dacrycarpus dacrydioides*. *Acta Chem. Scand.*, **42**, 462–468.

Andersen, Ø. M. & Fossen, T. (1995). Anthocyanins with unusual acylation pattern from stem of *Allium victorialis*. *Phytochemistry*, **40**, 1809–1812.

Borukh, I. F. & Demkevich, L. I. (1976). The pigments in some varieties of West Ukraine garlic. *Izv. Vyssh. Uchebn. Zaved. Pishch. Tekhnol.*, **3**, 47–49.

Du, C. T. & Francis, F. J. (1975). Anthocyanins of garlic (*Allium sativum* L.). *J. Food Sci.*, **40**, 1101–1102.

Hilal, S. H., Shabana, M. M. & Ibrahim, A. S. (1983). Flavonoids and anthocyanins of the flowers of *Allium aschersonianum* Barb and *Muscari comosum* Mill. *Egypt. J. Pharm. Sci.*, **24**, 67–74.

Moore, A. B., Francis, F. J. & Clydesdale, F. M. (1982). Changes in chromatographic profile of anthocyanins of red onion during extraction. *J. Food Protect.*, **45**, 738–743.