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RESEARCH NOTE

An improved technique for the extraction of precarthamin under mild conditions

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The floret paste of dyer's saffron was suspended separately in methanol, ethanol, acetone and ethyl acetate, and precarthamin in solutions was quantified to assess the capacity of the pigment extractability by the test solvents. On the basis of the experimental data, acetone was found to be most promising solvent for extracting the pH-sensitive, O_2-labile and photo-oxidizable precarthamin under mild conditions. The other three solvents do not appear to be of practical use for the dye preparation, judging from their lower capacities for pigment recovery.

ABBREVIATIONS

AW	Acetone/water (6:4, by vol.)
BAW	*n*-Butanol/acetic acid/water (4:1:2, by vol.)
BEMW	*n*-Butanol/ethyl acetate/methanol/water(4:4:1:2, by vol.)
HOAc	Acetic acid/water (15:85, by vol.)
HPLC	High-performance liquid chromatography
TLC	Thin-layer chromatography
UV	Ultraviolet light
VIS	Visible light

INTRODUCTION

Precarthamin is a poly-oxy chalcoquinoid glycoside, whose structure still remains undetermined. It accumulates in orange–yellow tubular parts of dyer's saffron (*Carthamus tinctorius*) flowers and is readily convertible to carthamin by oxidation through enzymic and/or non-enzymic processes (Saito *et al.*, 1983; Saito & Takahashi, 1985). Because of their lower releasability, methanol/HCl (Saito, 1991, unpublished) and methanol/formic acid (Takahashi *et al.*, 1984) have generally been used as extraction media for the dye. However, these drastic acids seem to be unfavourable, because yield is always indeterminate and, moreover, little or no precarthamin is ultimately acquired from the starting materials, even after being applied in 10 kg loads. The dye has many attractive tinctorial properties from the chemical and biochemical points of view and, furthermore, it can be transformed safely to carthamin on textile fabrics and in processed foods if appropriate techniques are applied (Saito, 1991; Saito, 1991, unpublished). Thus, a more ample supply of the dye would be helpful for experimental purposes as well as for applications. To ensure this, simple and mild techniques are necessary in the extraction process.

Recently, we have shown that precarthamin yield can be multiplied by triturating the floral tissues by mechanical treatments (Saito & Katsukura, 1992). The new findings prompted us to utilize precarthamin for extraction by which less deterioration of the dye is induced. The present work will therefore be centred on the extraction efficiency of precarthamin by organic solvents. Solvent systems and their compositions are the main subject of this introductory test.

MATERIALS AND METHODS

Materials

Organic solvents obtained from Wako Pure Chemical (Osaka, Japan) were all glass-distilled prior to experimental use. Precarthamin and carthamin used as standard specimens were prepared following the reported methods (Takahashi *et al.*, 1985; Saito *et al.*, 1992). Silica-gel TLC plates were purchased from E. Merck (Darmstadt, Germany). Avicel cellulose was supplied by Asahi Kasei Kogyo (Tokyo, Japan). Other chemicals and reagents were all of analytical-grade purity, from several commercial sources.

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Orange-yellow florets of dyer's saffron (283·6 g) were harvested from the freshly opened flowering buds in our experimental field on 27 August 1992.

Extraction of precarthamin

Fresh florets (0·5 g portions) were quickly ground with a pestle and mortar, to which 5 ml of 10–70% (v/v) test solvent has been added. After 5 min, the paste was transferred onto a Büchner funnel, then filtered with suction. The residue was washed with 95 ml of the same solvent. The pooled filtrates (100 ml in total) were evaporated at less than 35°C to remove the solvent.

Detection of precarthamin

Precarthamin was detected by its chromatographic behaviour and elution pattern from an HPLC column. From the chromatography, the extract and a marker specimen were spotted on the same silica-gel TLC plate, which was developed separately in BAW, in HOAc or in BEMW. The R_f values were compared with those of an authentic marker on the dried plate. The colour was observed in daylight or under UV light (365 nm), occasionally by spraying chromogenic reagents (Saito, 1991). Carthamin thus produced was checked tentatively by a spot test and comparison with an authentic marker compound. To carry out HPLC, a Jasco 880 system component series with a Wakosil $5C_{18}$ column (5 μm, 4 mm × 250 mm i.d.) was used. Aliquots (5–10 μl) of the extract from macerated florets were charged on the column, and developed with AW at a flow rate of 0·3 ml/min. Column effluents were monitored at 410 nm, which is a representative peak of precarthamin.

Determination of precarthamin content

Precarthamin content was determined indirectly following our previously reported method (Takahashi *et al.*, 1984; Fukushima *et al.*, 1990), which was slightly modified in this study. In brief, to the solvent-free solution, 5 ml of 0·5 mM $KMnO_4$ and 1 g of Avicel cellulose were added, stirred for several minutes and then filtered. After washing with distilled water (200 ml in total), the residual Avicel and filter paper were dipped together into 60% (v/v) acetone in a beaker. The Avicel in the acetone layer was centrifuged for 5 min at 3500 rpm and the supernatant pipetted out. The acetone extraction was repeated twice or more often; on each occasion fresh 60% aqueous acetone was used as the solvent. The reddish solution thus pooled (100 ml in total) was subjected to spectrophotometric assay. The absorbance measured at 521 nm was used for the indirect estimation of precarthamin. The precarthamin content is expressed in terms of carthamin (MW = 910).

Table 1. The release of precarthamin after trituration of flower florets from dyer's saffron in organic solvents

Solvent[a]	Precarthamin released (μg/ml)			
	Concentration (%)			
	10	30	50	70
Methanol	0·44	0·50	0·43	0·49
Ethanol	0·31	0·33	0·32	0·18
Acetone	0·47	1·12	0·68	0·48
Ethyl acetate	0·37	—	—	—

[a] Solvents were used as aqueous mixtures.

RESULTS AND DISCUSSION

Precarthamin liberated from the fresh floret paste of dyer's saffron into organic solvents can be detected by preparative chromatography methods. In this study, it was tentatively identified on TLC plates with or without chromogenic spray reagents (Saito, 1991) and by examining the HPLC elution pattern (Saito & Kawasaki, 1992; Saito & Katsukura, 1992), on each occasion; the colour, R_f value or the elution times compared closely with those of authentic specimens of precarthamin and carthamin.

Table 1 shows the solvent extractability of precarthamin from the floret pastes, which were prepared after a mechanical treatment of the fresh materials. Aqueous 30% (v/v) acetone is seen to be the most promising solvent for precarthamin. Methanol and ethyl acetate are not as good as previously expected. Ethanol acts rather as an inhibitor. It is not clear whether these differences in the pigment solubilization are characteristic of the organic solvents tested.

Precarthamin is one of the most unstable chalcoquinoid glycosides among *Carthamus* dyes. Therefore, especial circumspection should always be exercised during extraction, isolation and subsequent purification of the dye, when speedy, simple and mild conditions are used. The currently established technique is superior to the old methods using drastic acids in at least the following three respects: (1) the extraction process is simple, (2) H^+ or Cl^- offers no problems and (3) solvent recovery is speedy and easy. Keeping these points in mind, we can now safely obtain the unstable precarthamin from the fresh florets of dyer's saffron.

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