Presence of Bioactive Glycoconjugates on Different Stages of Saffron Corm

Angela Rubio[1], Pedro Fernández-Nohales[1], Gerrit J. Gerwig[2], Julio Escribano[3], Johannis P. Kamerling[2] and José-Antonio Fernández[1]
[1]Laboratory of Biotechnology, IDR, University of Castilla-La Mancha, Campus Universitario s/n. Albacete E-02071. Spain
[2]Bio-Organic Chemistry, Bijvoet Center for Biomolecular Research, Utrecht, Holland
[3]Faculty of Medicine, University of Castilla-La Mancha, Albacete
Spain

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Abstract

Active glycoconjugates against HeLa cell line were detected in corms of saffron (*Crocus sativus* L.) collected in August. The presence of these compounds in different stages of development have been analysed on a HPLC system. The highly glycosylated fractions appeared from April in the song corm, till March in the mother corm. By contrast, these compounds where not detected in the corms collected during other time periods, suggesting a developmental regulation for the biosynthesis and accumulation of these active glycoconjugates.

INTRODUCTION

In 1999 interesting pharmacological properties were observed in extracts of saffron corms and callus cultures of saffron corms (Escribano et al., 1999a,b), leading to the purification of a HPLC fraction that was active against malignant HeLa cell lines. Microscopy analysis confirmed that cells treated for 5 min exhibited an evident swelling, local plasma membrane evaginations and loss of star-like morphology (Escribano et al., 1999a). To explore the potential therapeutic implication of this cytotoxic fraction (CF), its effects on different human tumoural and non-tumoural cell lines were studied. CF was about eight times more toxic against fibrosarcoma tumoural cells than against non-tumoural fibroblastic cells. Swelling pattern of cells exposed to CF supported the idea of a change in the physical barrier properties of the plasma membrane leading to cell lyses (Escribano et al., 2000). At non-cytotoxic concentrations, CF promoted significant macrophage activation, detected by the release of nitric oxide. Thus, CF may be useful in activating macrophages for defence against tumours (Escribano et al., 1999c).

Latterly, an additional purification step was applied to CF on a C18 column. Several glycoconjugates appeared with similar cytotoxic activity to CF. Due to the importance in the degree of glycosylation for glycoconjugates with pharmacological properties, a preliminary analysis on the presence of highly glycosylated glycoconjugates during different stages of saffron corm development was performed. This analysis is necessary in order to detect when the glycosylation of this compounds occurs.

MATERIALS AND METHODS

Plant Material

Saffron corms were collected from outfields in Tarazona de La Mancha (Albacete, Spain) during twelve months, every 15th of each month. Corms were cleaned and stored at –80ºC or lyophilised until further use.

Reagents

Dichloromethane MeOH, acetone, acetonitrile and TFA were purchase from Merck (Darmstadt, Germany). Ultra-pure water generated by a Milli-Q water-purification system (Millipore, Bedford, MA).

Purification and Detection of Cytotoxic Glycoconjugates from Saffron Corm

0.2 g of lyophilised saffron corms were washed overnight with dichloromethane to remove all the fatty acids. The residue was washed with 0.5 ml of methanol (MeOH), overnight at room temperature, to extract the glycoconjugates that were obtained by precipitation, adding 0.5 ml of acetone to the 0.5 ml of MeOH extract. The pellet, obtained after centrifugation at 3,000 rpm for 5 min, was dissolved in 0.5 ml of water. The sample was then subjected to reversed-phase chromatography using an Intertisil ODS-2, 4.6 X 250 mm, Sugelabor (Madrid, Spain). Column was equilibrated with 36% of acetonitrile with 0.05% of trifluoroacetic acid (TFA) and eluted with the following acetonitrile gradient: 0.05 % of TFA (v/v): from 36-44% in 15 min, from 44-82% in 1 min, 82% 5 min, from 82-36% in 1 min and 5 min to equilibrate the column again. Flow rate was 1.5 ml/min and the wavelength was 208 nm.

RESULTS AND DISCUSSION

Recently, a new purification procedure has been developed to characterise the CF obtained by Escribano et al. (1999a), using a C18 reverse phase column. In the chromatogram three main HPLC fractions were present. These fractions also appeared in lyophilised corms from August after extraction with MeOH and precipitation with acetone (Figure 1). These fractions, named as F1, F2 and F3 were structurally elucidated as glycoconjugates. All these new fractions practically maintain the same IC_{50} against HeLa cells than CF (data not shown).

A preliminary analysis by HPLC was performed with different developmental stages of saffron corms, in order to detect the presence of these glycoconjugates obtained after MeOH extraction and acetone precipitation. These corms were roughly classified as song (S) or mother (M). S were corms growing by the support of M corms. M state also includes dormancy and sprouting corms, as shown in Figure 2. During corm development, from S of November till S of February, F3 is the only fraction present in the chromatogram (Figure 3). S corms from March also present F3 together with an HPLC fraction (called A) that is exclusively for this month (Figure 4). F1, F2 and F3 appeared from S April till M March. In the senescent M from April, F1 and F2 appeared, but in lower amount than the observed from S April till M March (Figure 5).

The presence of F3 is remarkable in all the stages, suggesting the presence of a constitutive compound during the whole corm developmental process. HPLC fraction A appeared exclusively in March, suggesting that this fraction could be a precursor of the highly glycosylated fractions F1 and F2. These fractions are present from April in the developing S corm till the developed or M corms.

It should be interesting to study the expression of glycosyl transferases enzymes in corm tissues since March, due to the importance of glycosyl moiety for the biological activity of a great number of bioactive glycoconjugates (Haralampidis, et al., 2002).

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Figures

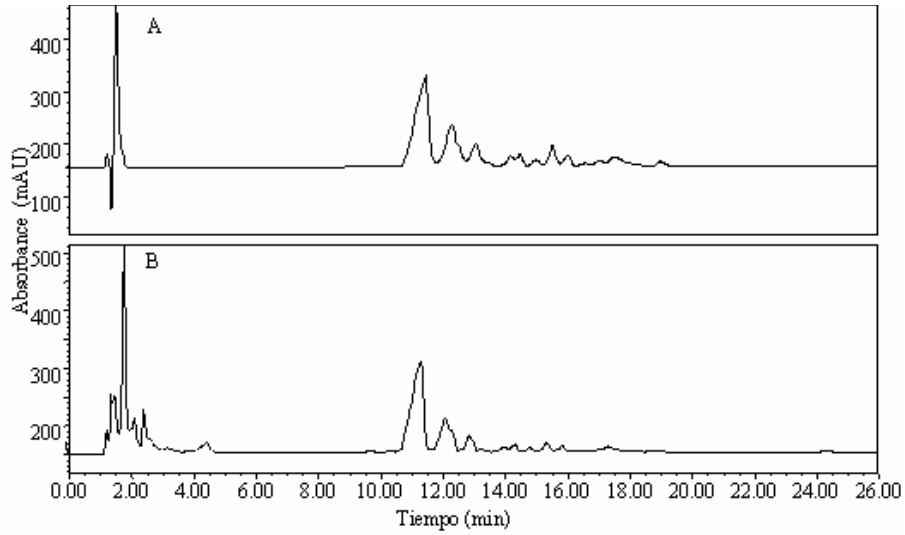

Fig. 1. Comparison between the chromatograms of the cytotoxic glycoconjugates from saffron corm obtained by different methods. (A) CF. (B) Extraction with MeOH and precipitation with acetone.

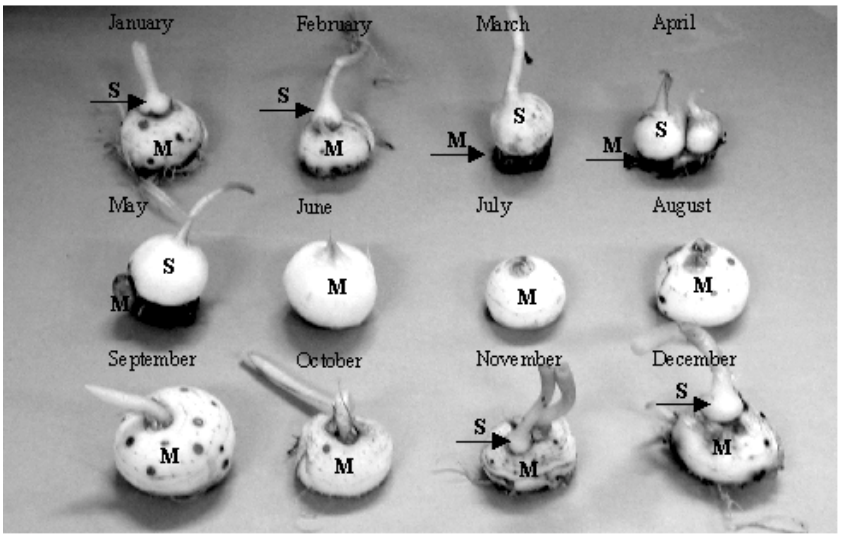

Fig. 2. Corms were collected during one year, the 15th of each month. These corms were classified as song (S) or mother (M). S were the corms that were growing thanks to the support of other corm, called M. M represent the dormancy and sprouting corms.

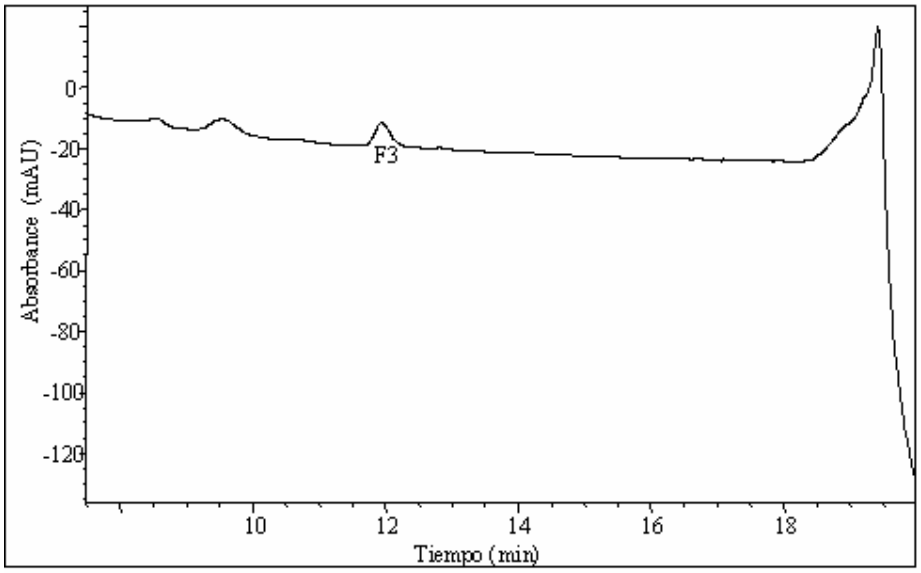

Fig. 3. Chromatograms observed in S corms extracts from November till February. F3 is the only fraction that appear during this period.

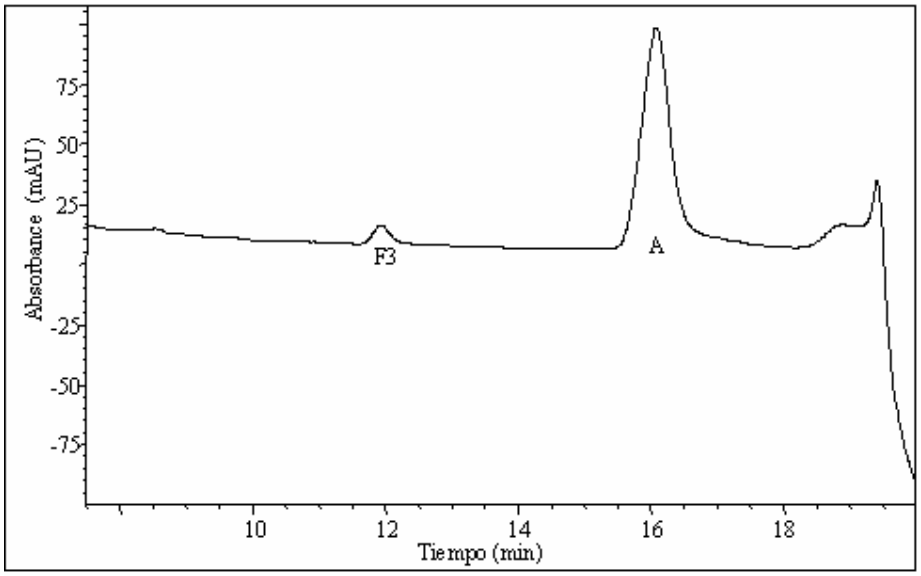

Fig. 4. Chromatograms observed in S corms from March. In addition to F3, a new fraction appeared. This fraction called A is exclusive of this month and stage of development.

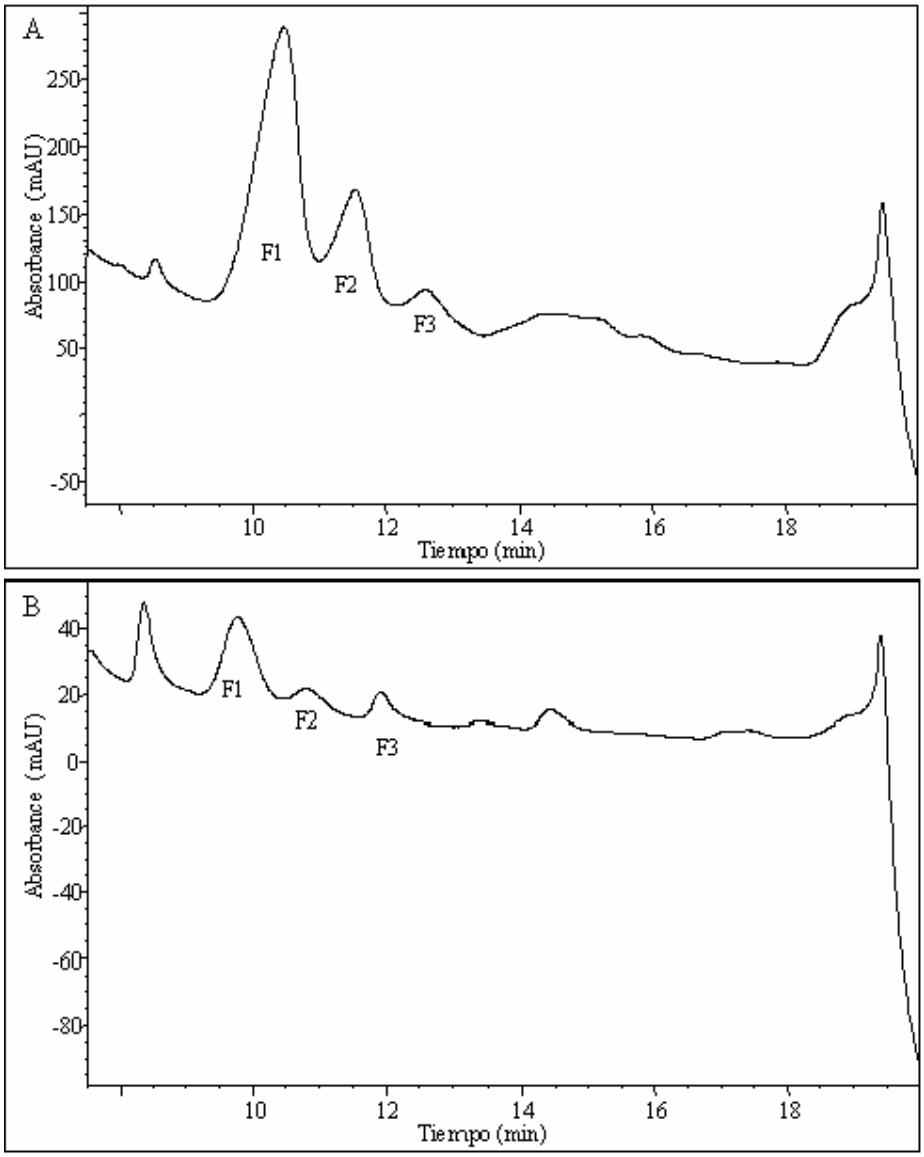

Fig. 5. Chromatograms of highly glycosilated glygoconjugates. (A) Chromatogram observed from the extracts of S corm from April and May and M corms from June till March. In addition to F3, two new more polar fractions appear in the chromatogram. F1 and F2 correspond to different glycosilated forms. (B) In the senescent corm from April (M), F1 and F2 appeared but in lower amount than the observed in the extracts of S corm from April and May and M corms from June till March.