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Short Communication

Analysis of commercial hesperidin methyl chalcone by high-performance liquid chromatography

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ABSTRACT

A high-performance liquid chromatographic procedure for the determination of the different components present in commercial samples of hesperidin methyl chalcone (HMC) was developed. It employs a C_{18} column and an elution gradient with a mixture of methanol and 0.01 *M* aqueous phosphoric acid and a diode-array detector. Eighteen compounds were detected and their UV spectra characterized. The spectra indicate that HMC is a mixture of flavanones and chalcones with different degrees and/or positions of methylation.

INTRODUCTION

Hesperidin methyl chalcone (HMC) is widely used in the pharmaceutical industry as vasodilatator [1], vascular permeability modifier [2], capillary resistence enhancer [3] and prostaglandin E stabilizer [4]. The use of HMC as a colouring agent in cosmetics has also been reported [5].

HMC is usually synthesized by methylation of hesperidin with dimethyl sulphate in alkaline media [6]. However, the structures of the products formed in the reaction are not well known and some attempts have been made to identify the large number of compounds that are formed in the synthesis of HMC. For example, six methylhesperidin derivatives with different degrees of methylation were found by partition and adsorption chromatography [6].

This paper describes a high-performance liquid chromatographic (HPLC) method for the separation of hesperidin methyl derivatives in order to study the methylation reaction and elucidate all the products formed. Eighteen compounds were separated and their spectra analysed with the aid of a diode-array detector.

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EXPERIMENTAL

Chromatography

HPLC analysis was performed using a Beckman liquid chromatograph with a Model 110B solvent-delivery module and a System Gold Module 168 diode-array detector. An IBM Model 30 286 computer was used for all computations.

A 250 × 4 mm I.D. μBondapak C_{18} (Waters Assoc., Milford, MA, USA) column with an average particle size of 5 μm was used.

Separation was performed by gradient elution with a mixture of solvent A (methanol) and solvent B (0.01 *M* aqueous phosphoric acid). The gradient profile being 30% of A for 20 min, then increasing to 45% of A in 30 min, remaining constant there for 30 min at a flow-rate of 1 ml/min.

Reagents

Commercially available HMC was obtained from Merck (Barcelona, Spain), Takeda (Tokyo, Japan) and Zoster (Murcia, Spain).

Procedure

HMC (40 mg) was dissolved in 10 ml of dimethyl sulphoxide and the solution was filtered through a 0.45-μm nylon membrane and injected into the liquid chromatograph. Peaks were monitored at both 345 and 280 nm.

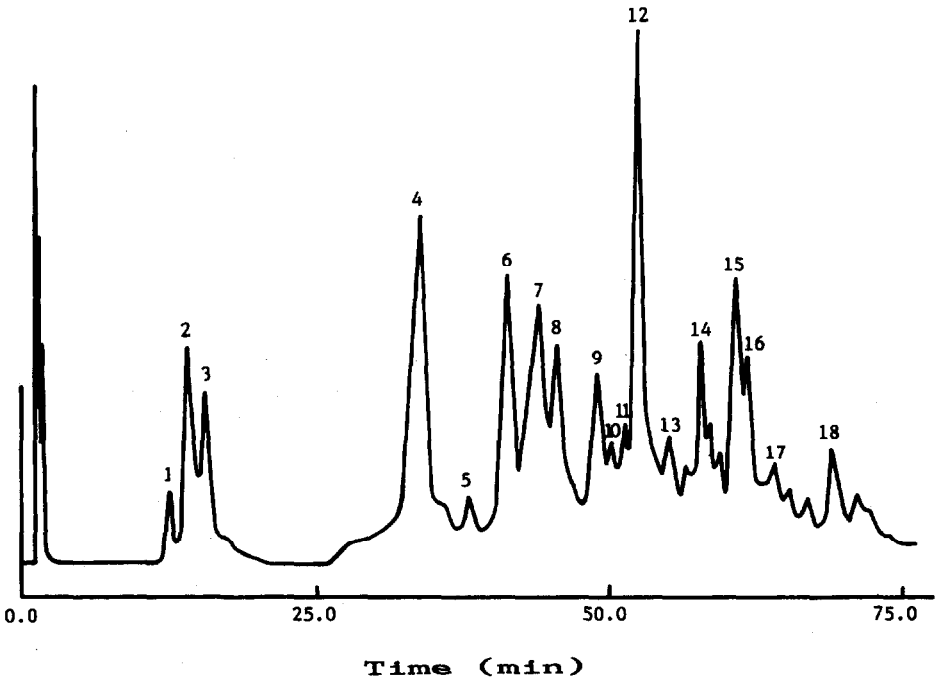

Fig. 1. High-performance liquid chromatogram of commercial HMC from Zoster. See text for eluents and elution profile. Column, μBondapak C_{18} (5 μm) (250 × 4 mm I.D.). Detection: 280 nm. Peak identification as in Table I.

RESULTS AND DISCUSSION

Owing to the complex composition of commercial HMC, the different chromatographic system mentioned in the literature [7–16] were not able to attain the complete resolution of the different compounds present. Chromatographic systems that use direct stationary phases, used for the resolution of flavonoids of high molecular weight, generally polymethoxylated and polybenzoylated flavones, and which employ mobile phases of low polarity [7–9] such as heptane–isopropanol or chloroform–methanol–water, did not permit a good resolution of the samples of commercial HMC analysed, since peaks 1–3 and 4–12 in Fig. 1 overlapped. C_8 and C_{18} reversed-phase columns were tried [10], the latter showing greater peak resolution, for which reason it was used in the following assays. Acetonitrile–water mixtures [11–14] presented problems of resolution, producing grouping of peaks 1–3, 4–5, 6–8, 9–12 and 14–16. Methanol–water combinations (acedified with acetic or phosphoric acid) [10,15], although resolving a greater numbers of peaks than acetonitrile–water combinations, were not capable of resolving all the species later found in HMC because peaks 1–3, 6–8 and 13–16 overlapped. The use of mixtures of water and other alchols such as *tert.*-butanol [16] did not improve the resolutions obtained with methanol–water mixtures (acidified with acetic and phosphoric acid).

Because methanol–water mixtures (acidified with acetic and phosphoric acid) showed a greater capacity to resolve the different species present in commercial HMC, different gradients of methanol–0.01 *M* aqueous phosphoric acid were tried, complete resolution of all peaks being obtained when the gradient profile was 30% methanol–70% 0.01 *M* phosphoric acid for 20 min, rising to 45% methanol in 30 min and remaining constant there for 30 min, at a flow-rate of 1 ml/min. Fig. 1 shows the results for a commercial sample of HMC from Zoster. Other samples from different manufacturers, showed the same distribution of peaks, although their relative proportions differed slightly (data not shown).

Spectral analysis of the peaks in Fig. 1 by means of a diode-array detector showed three UV patterns for methyl derivatives of hesperidin (Fig. 2). The first (Fig. 2a) was a typical flavanone spectrum with a maximum at 280 nm and a low-intensity maximum at 320 nm. The peaks designated F in Table I corresponded to this pattern. The second group of peaks, designated C, had a chalcone spectrum with a maximum at 345 nm (Fig. 2b). The remaining peaks, designated O, had an unidentified spectrum, with a maximum at 285 and others between 330 and 345 nm (Fig. 2c). All the compounds listed in Table I showed spectra similar although unidentified, to one of the three spectra shown in Fig. 2.

Bearing in mind the spectral characteristics of structures F and C, the absorption ratios between 280 and 345 nm were used to compare the different compounds resolved in the chromatogram in Fig. 1. This ratio was higher than 1 for the F compounds, owing to their characteristic maximum at 280 nm. However, this ratio was not constant, varying between a maximum of 7.85 for compounds 2 and 3 and 1.60 for compound 9. Identical 280/345 nm ratios and different retention times suggest an identical degree of methylation but distinct positions [9]. On the other hand, the different ratios suggest different degrees of methylation. The chalcone-type structures, C, with a maximum at 345 nm, showed 280/345 nm ratios lower than 1 (Table I), varying between a maximum of 0.7 (compound 16) and 0.32 (compound 4).

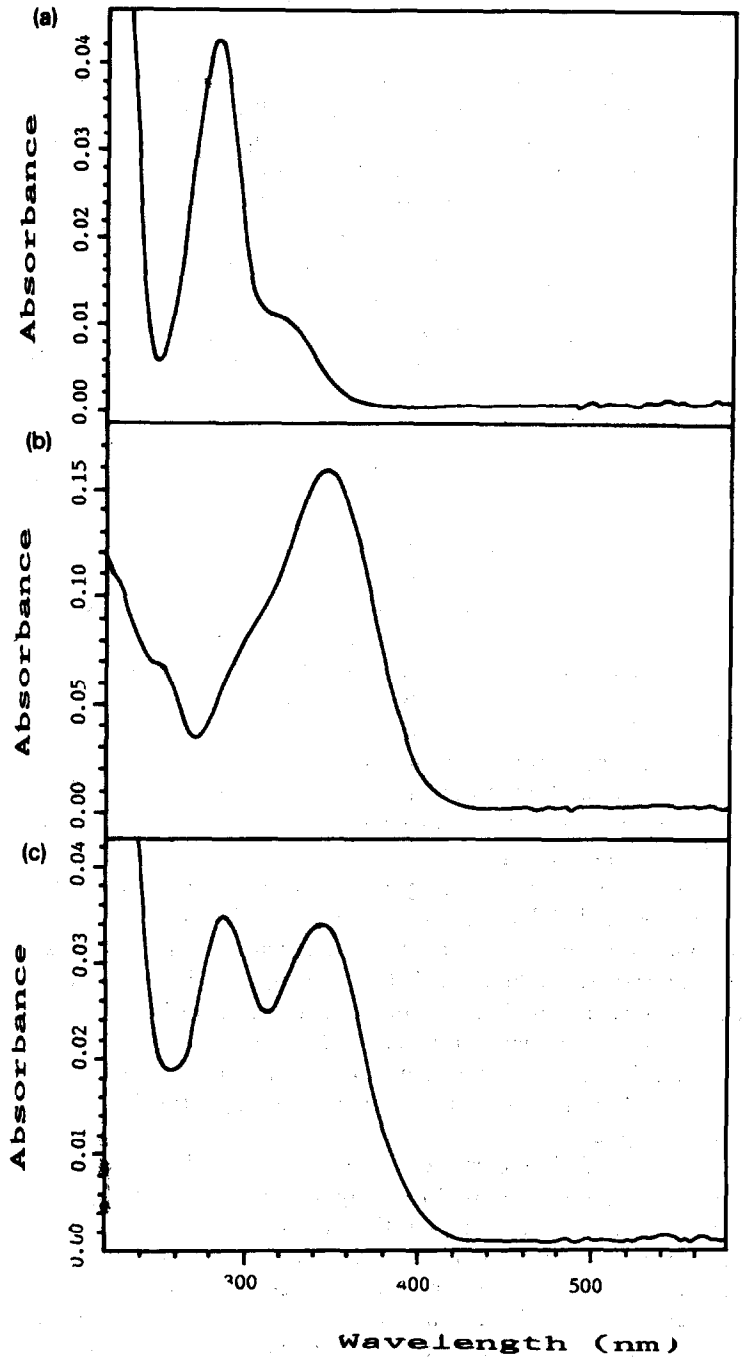

Fig. 2. UV spectrum of different methyl derivatives of hesperidin. (a) Flavanone species (F); (b) chalcone species (C); (c) others species (O).

TABLE I

RETENTION TIMES, SPECTRAL CHARACTERISTICS AND 280/345 nm ABSORPTION RATIOS OF METHYLHESPERIDIN DERIVATIVES IN COMMERCIAL HMC ON μBONDAPAK C_{18} USING A DIODE-ARRAY DETECTOR

No.	t_R (min)	Spectrum	Absorbance ratio (280/345 nm)
1	13.21	F	7.84
2	15.47	F	7.85
3	17.82	F	7.85
4	33.83	C	0.32
5	38.24	C	0.63
6	41.43	F	4.10
7	43.85	O	1.23
8	45.53	F	1.61
9	49.05	F	1.60
10	50.07	F	2.70
11	51.24	F	1.83
12	52.20	C	0.38
13	54.86	O	1.04
14	57.56	C	0.54
15	60.50	F	5.18
16	61.38	C	0.70
17	63.71	O	1.00
18	68.54	O	1.04

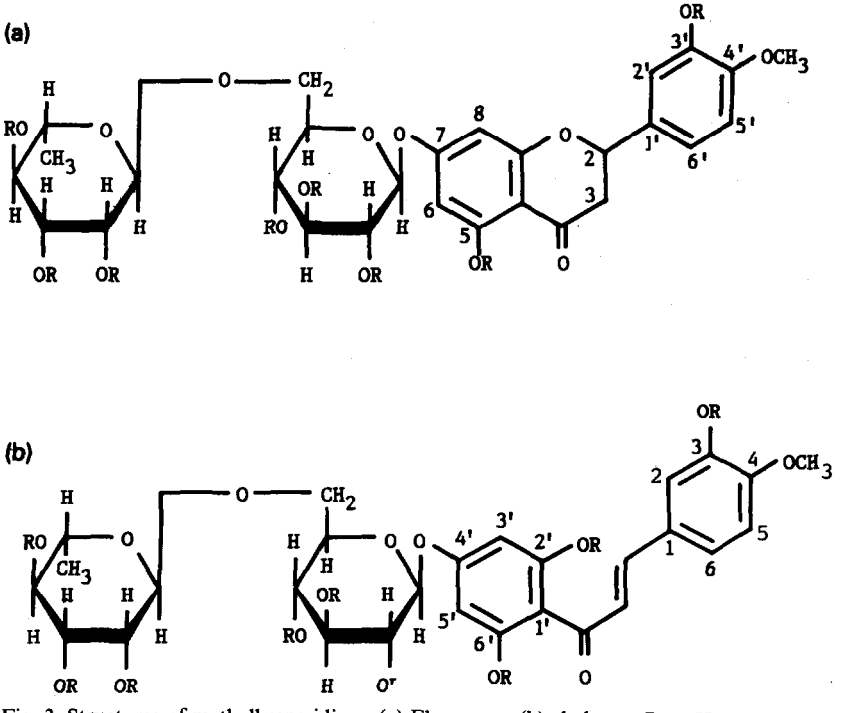

Fig. 3. Structures of methylhesperidines. (a) Flavanone; (b) chalcone. R = H or CH_3.

No differences were observed in the peak distribution at either wavelength. All peaks included in Tabl I were detected was pure compounds by the diode-array detector. The purity criteria employed, included in the spectrum-treatment program, were based on the comparison of the spectra at the half-height of each peak. When these and their derivatives coincided, the peak was considered to be pure. The peaks not considered did not fulfil this criterion.

The different chalcone–flavanone spectra were caused by methylation of the free hydroxyl groups at the 2′-position of chalcone compounds (Fig. 3). This conclusion is based on the equilibrium between the flavanone and chalcone structures, which depends on the pH, and with the eluent employed (pH 3.3) the chalcone structure cannot be maintained if the hydroxyl group at C-2′ is not methylated.

This work demonstrates that commercial HMC is a mixture of chalcone and flavanone species with an unspecified pattern of methylation. Further, no compound predominantes. HPLC with diode-array detection provides an excellent method of detecting not only the presence of distinct methylhesperidines in commercial HMC but also their chalcone–flavanone structure.

Details of the isolation and identification of the different peaks in Fig. 1 will be reported elsewhere.

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