DICAFFEOLYQUINIC ACIDS FROM Helichrysum italicum AND Achillea cartilaginea

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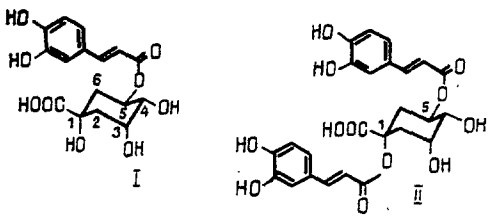
The quinic acid caffeates of the flowers of Helichrysum italicum and the herbage of Achillea cartilaginea have been studied with the use of HPLC, ^{13}H NMR spectroscopy, and chemical transformations.

According to the literature, the caffeoylquinic acids of the artichoke [1] possesses pronounced cholagogic properties, the activity of a dicaffeoylquinic acid (cynarin) being three times greater than that of monocaffeoylquinic acids. In this connection, great interest is presented by such plants as the yarrow Achillea cartilaginea Ledeb. and the everlasting Helichrysum italicum Juss., which, according to our results, contain considerable amounts of dicaffeoylquinic acids [2-4].

It appeared of interest to carry out a comparative investigation of the component compositions of these plants by the HPLC method, which permits the most objective evaluation of the quality of medicinal plant raw material and phytopreparations, especially those containing such labile compounds as dicaffeoylquinic acids. For these purposes we used chromatographic conditions described previously [3]. The assignment of the peaks of the substances on the chromatograms of the extracts was made on the basis of the retention times of the individual substances (Table 1).

The results of a comparative HPLC investigation of extracts of Helichrysum italicum and Achillea cartilaginea (Fig. 1) showed a similarity of the plants with respect to their caffeoylquinic acid contents, the dominating components in both cases being dicaffeoylquinic acids (DCQAs), the amounts of which were from 40 to 75% of the total of phenolic compounds (Table 2). The extracts studied contained three out of the four possible isomers of caffeoylquinic acid (CQA) and five out of the six isomers of dicaffeoylquinic acid, the main ones in each case being chlorogenic acid (I) and 1,5-DCQA (II).



It must be mentioned that a similar HPLC pattern has been observed for extracts of the artichoke (Cynaria scolymus L.) [5], in which the above-mentioned acids were present, although the main component was not 1,5-DCQA (7.3%) but chlorogenic acid (73.0%). It is interesting that the various factors affecting the final composition of artichoke extract have been studied by the HPLC method [6]. It was shown here that the presence of ethanol as solvent in a concentration above 30% suppressed the isomerization reactions of chlorogenic and 1,5-dicaffeoylquinic acids.

On a chromatogram of an extract of H. italicum (Fig. 1A) we also identified a number of flavonoids that we had isolated previously [7-9] (peaks 5, 8, 9, and 10 – Table 1), among which bitaloside, bitalosidin (a 3',4',5,6,7,8-hexahydroxyflavone 7-glycoside), and isoquercitin (quercitin 3-glucoside) predominated; the total amount of flavonoids was ~7%

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TABLE 1. Comparative Investigation of Aqueous Extracts of the Everlasting and the Yarrow by the HPLC Method

Name of the substance (component of the extract)	Hitalicum, flowers		A. cartilaginea, herbage	
	peak	RRT*	peak	RRT
3-Caffeoylquinic acid	1	0,10	1	0,12
4-Caffeoylquinic acid	2	0,13	2	0,15
5-Caffeoylquinic acid	3	0,15	3	0,17
Caffeic acid	4	0,20	4	0,21
Bitaloside	5	0,23	—	—
1,3-Dicaffeoylquinic acid	6	0,28	6	0,27
p-Coumaric acid	7	0,37	—	—
Helichrysin	8	0,44	—	—
Bitalosidin	9	0,55	—	—
Isoquercitrin	10	0,66	—	—
3,5-Dicaffeoylquinic acid	11	0,87	11	0,87
1,5-Dicaffeoylquinic acid	12	1,00	12	1,00
1,4-Dicaffeoylquinic acid	13	1,29	13	1,30
4,5-Dicaffeoylquinic acid	14	1,89	14	1,88

*The retention time of the comparison peak (peak 12) was 12.5 min.

TABLE 2. Amounts of Phenolic Compounds in Extracts of the Everlasting and the Yarrow (% on the total phenolic compounds)

Compound (number of the HPLC peak)	H. italicum		A. cartilaginea			
	flower		flower		herbage	
	water	70% EtOH	water	70% EtOH	water	70% EtOH
3-CQA (1)	4,3	2,3	1,9	1,5	5,6	2,1
4-CQA (2)	2,6	1,1	1,8	1,9	1,9	1,0
5-CQA (3)	27,7	13,2	26,4	23,9	44,8	35,9
1,3-DCQA (6)	0,5	1,6	—	—	1,0	1,4
Flavonoids (5, 8, 9, 10)	6,8	7,8	12,9	8,5	11,6	16,4
3,5-DCQA (11)	7,0	5,3	1,4	2,0	1,6	—
1,5-DCQA (12)	31,5	52,7	23,4	32,9	14,5	17,7
1,4-DCQA (13)	6,6	7,7	22,6	21,9	15,8	19,9
4,5-DCQA (14)	11,2	6,4	1,5	2,5	1,5	2,3

*The quantitative content was calculated by the method of area normalization.

(see Table 2). The experimental results showed that the aqueous extract of H. italicum also contained ~3% of helichrysoside (6"-p-coumarylisoquercitrin), which was absent from the chromatogram since it is not eluted by the given eluent.

On a chromatogram of an extract of A. cartilaginea (Fig. 1B), of the flavonoids isolated previously [4] rutin and hyperin were identified (RTs 6.9 and 7.7 min, respectively).

A study of the chemical composition of H. italicum flowers and A. cartilaginea herbage showed that questions of the isolation and identification of the caffeoylquinic acids deserve separate discussion.

In a study of the dicaffeoylquinic acids we used the ^{1}H NMR spectra of the substances isolated and of their acetates (Table 3) and also their comparison with literature information [10-15] and our own results on the isolation and identification of 5-caffeoyl- and 4,5-dicaffeoylquinic acids [4, 16].

In the process of isolating the caffeoylquinic acids from the everlasting and the yarrow, on boiling with alcohol not only CQAs and DCAQs but also their ethyl esters were isolated (see Table 3) (their peaks are absent from Fig. 1 – they are eluted by more polar mixtures).

On the whole, it must be stated that the isolation of quinic acid caffeates is an extremely laborious process. The constants of these substances are uninformative, they are

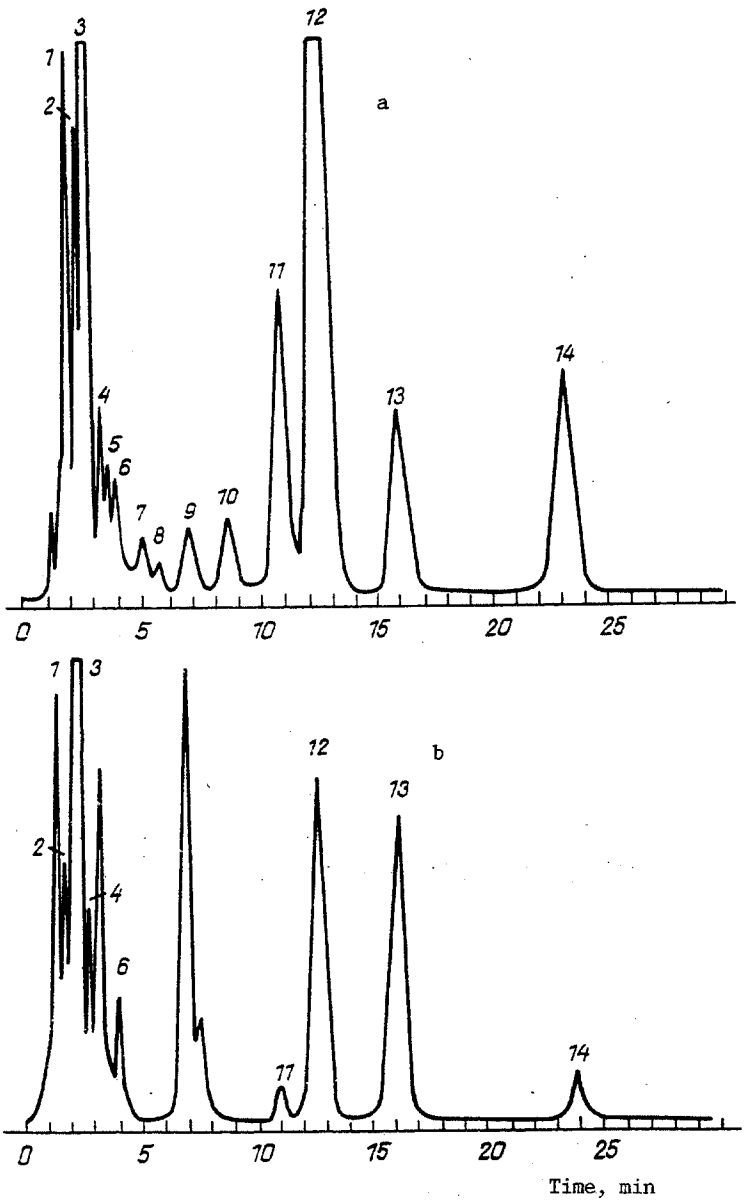


Fig. 1. HPLC of aqueous extracts of H. italicum flowers (A) and A. cartilaginea herbage (B); the numbers of the peaks correspond to those given in Table 1.

isolated in the amorphous state, and the melting points and angles of rotation given for them by different authors differ extremely considerably [10-12]. An individual element of confusion is also introduced by two different systems of nomenclature for quinic acid used by authors without appropriate explanations even in publications of recent years (sometimes both nomenclatures are used simultaneously [5]). The extremely laborious separation of the CQA isomers and, particularly, the DCQA isomers is due primarily to their lability and migration [12, 13] not only during extraction but also during chromatographic separation.

In view of this, we carried out the identification of the caffeates isolated not only by studying their NMR spectra (Table 3) but also by checking their individuality by the HPLC method with assignments to definite peaks on the chromatogram.

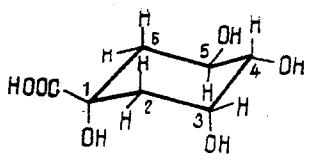
In the NMR spectra of all the caffeoylquinic acids and their acetates, the caffeic acid residues were clearly identified from the presence of doublets (J = 16 Hz) of a trans double bond (each DCQA has two doublets: of 2H-α, ~6.4 ppm, and of 2H-β, ~7.8 ppm). The aromatic protons give overlapping signals in the 7.0-7.4 ppm region with intensities of 3H (for the CQAs) or 6H (for the DCQAs). In addition, in the case of the acetates, one or two caffeic acid residues were confirmed by the signals of aromatic acetoxy groups at 2.3 ppm (singlets

TABLE 3. Details of the ^1H NMR Spectra of Quinic Acid Caffeates

Compound	Solvent (deuterated)	Chem. shifts of the quinic acid protons, δ		
		H-3 q 4 and 3 Hz	H-4 dd 9 and 3 Hz	H-5 td 9.4 and 10 Hz
Quinic acid	Pyridine	4,56	3,93	4,76
5-Dicaffeoylquinic acid	Pyridine	4,67	4,21	6,08
Acetate of 5-CQA	Chlf.	5,75	5,14	5.58
Ethyl ester of 5-CQA	Acetone	4,00	3,62	5,10
1,3-Dicaffeoylquinic acid	Pyridine	6,22	4,36	5,08
1,4-Dicaffeoylquinic acid	Pyridine	4,80	5,48	5,01
1,5-Dicaffeoylquinic acid	Pyridine	4,63	4,28	6,00
Acetate of 1,5-DCQA	Chlf.	5,75	5,15	5,45
Ethyl ester of 1,5-DCQA	Acetone	4,09	3,73	5,07
Acetate of the ethyl ester of 1,5-DCQA	Chlf.	5,65	5,18	5,65
3,5-Dicaffeoylquinic acid	Acetone	5,44	4 08	5,49
4,5-Dicaffeoylquinic acid	Pyridine	4,73	5,53	6,16
Acetate of 4,5-DCQA	Chlf.	5.66	5,24	5.66
Ethyl ester of 4,5-DCQA	Acetone	4,17	5,27	5,16
Acetate of the ethyl ester of 4,5-DCQA	Chlf.	5,65	5,24	5,65

with an intensity of 6H or 12H, respectively). The numbers of aromatic and aliphatic acetoxy groups were retained in the ethyl esters, which confirmed the esterification by ethanol of the COOH group of the quinic acid. The ether esters showed additional signals of the CH_3CH_2O group (quartet at 4.2 ppm and triplet at 1.2 ppm, J = 7 Hz).

In all the compounds investigated, the quinic acid has the COOH conformation shown below, (e) (IUPAC numbering):



The choice among the isomers of CQA and DCQA followed clearly from an analysis of the H-3, H-4, and H-5 signals of the quinic acid residue which, on acetylation of the geminal hydroxy groups, shifted downfield by approximately 1.5 ppm in comparison with the corresponding signals in quinic acid itself (see Table 3). It must be mentioned that the C-4 signal (the most readily determined, a characteristic dd with J = 9 and 3 Hz) witnessed the retention of the quinic acid conformation in all the conformations studied (see Table 3) (previously, inversion of the quinic acid ring on the silylation of chlorogenic acid has been reported [16]).

Table 3 gives no signals of the quinic acid protons at C-2 and C-6. The fact is that in the majority of compounds described in the literature [14, 15] and in those isolated by us, these protons exhibit magnetic equivalence and form overlapping signals in a narrow region (2.4-2.8 ppm). Only in cynarin (1,3-DCQA) is it easy to distinguish the signals of all four protons: δ 2.90 (H-2ax, J = 16 and 4 Hz), 3.50 (H-2eq, J = 16 and 4 Hz), 2.68 (H-6ax, J = 13 and 10 Hz), 3.30 (H-6eq, J = 13 and 4 Hz).

Thus, the parameters of the ^1H NMR spectra of the substances investigated, in combination with the results of HPLC analysis, have permitted an identification of the CQAs and DCQAs present in the flowers of Helichrysum italicum and in the herbage of Achillea cartilaginea. The investigations performed permit HPLC to be recommended as the most promising method for the qualitative and quantitative study of dicaffeoylquinic acids.

EXPERIMENTAL

The ^1H NMR spectra were taken on a Varian HA-100D instrument at 100 MHz (internal standard TMS).

HPLC was conducted on a SP 8700 liquid chromatograph (Spectral Physics, USA) with SP 4100 computer-integrator, a variable-wavelength SP 8400 UV detector, and a Rheodyne 7125 injector with a loop volume of 10 μl. Separation was achieved on a Brownlee Labs analytical column (4.6 × 100 mm) with the sorbent Spheri 5 C_8 fitted with a Pellicular C_{18} (30-40 μm,

4.6 × 70 mm) precolumn in the isocratic regime. The mobile phase was water–acetonitrile–tetrahydrofuran–acetic acid (85:10:2:3), the rate of flow of eluent 1.5 ml/min, the analytical wavelength 325 nm, the sensitivity 0.04 absorption units full scale, and the volume injected 10 μl.

Preparation of the Extracts for HPLC. A weighed sample of the air-dry raw material was extracted in a ratio of 1:20 with heating in the boiling water bath for 30 min (water) or 15 min (70% ethanol).

For TLC we used Silufol$_{254}$ in the chloroform–methanol–water (26:14:3) and chloroform–methanol (in various ratios) systems with detection in UV light at wavelengths of 254 and 360 nm and by spraying with diazotized sulfanilic acid and with dilute sulfuric acid (110°C).

Isolation of the Substances. We had isolated hydroxycynamic and chlorgenic acids in the course of earlier investigations of the chemical composition of Helichrysum italicum flowers [2].

In order to isolate the dicaffeoylquinic acids, the flowers of H. italicum (Crimean Zonal Exploration Station of the All-Union Scientific-Research Institute of Medicinal Plants, 1989 harvest) were extracted with 80% ethanol at the boil, and the combined extracts were evaporated to the viscous state and were chromatographed on silica gel L 40/100 [chlf–MeOH (100:0 → 60:40)]. The fractions obtained were repeatedly rechromatographed with an alternation of sorbents (polyamide, silica gel, Sephadex LH-20). The course of separation of the DCQAs was monitored by the HPLC method and by ^{1}H NMR spectroscopy. The substances isolated are listed in Table 3.

The acetylation of the substances was carried out with acetic anhydride in pyridine at 20°C. The acetates were purified by chromatography on silica gel in hexane–chloroform.

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