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Note

High-performance liquid chromatography of chromenes and benzofurans from the genus *Encelia (Asteraceae)*

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Chromenes (benzopyrans) and benzofurans are common natural products of the family *Asteraceae*[1]. These naturally occurring phytochemicals are known to exhibit a variety of biological activities ranging from cytotoxicity[2] to poisoning insects and livestock[2,3]. They have also been used for chemotaxonomic studies in the *Asteraceae*[1,4–7].

Analyses of these compounds has so far been done mainly by thin-layer chromatography (TLC) or column chromatography. This present study describes the application of reversed-phase high-performance liquid chromatography (HPLC) to the separation of chromenes, benzofurans and coumarins in crude plant extracts from species of the genus *Encelia*.

EXPERIMENTAL

Encelia ventorum, *E. palmeri*, *E. laciniata* and *E. halimifolia* were collected in Baja California, Mexico. Voucher specimens are on file in the UCI herbarium. The stems were dried, ground and extracted for 48 h with chloroform. The crude extracts were taken to dryness, redissolved in methanol, filtered and chromatographed on a Sephadex LH-20 column, eluent methanol. Further purification of analyzed compounds was achieved by repeated preparative TLC on silica gel, solvent system light petroleum (b.p. 30–60°C)–chloroform (50:50 or 70:30). ^{1}H nuclear magnetic resonance (NMR) spectra were recorded on a Varian 390 EM NMR spectrometer.

For HPLC analysis a Waters liquid chromatograph was used, equipped with a solvent pump Model 6000 A, a solvent pump Model M 45, a solvent programmer Model 660, a universal injector Model U6K and a UV detector Model 440. Detection was achieved at 254 nm. The HPLC column was LiChrosorb RP-8 (250 × 4 mm), pore size 5 μm (Alltech, Los Altos, CA, U.S.A.). The crude chloroform extract of *Encelia palmeri* was taken to dryness, redissolved in methanol, filtered, injected and separated using a linear gradient of A (water) and B (acetonitrile–acetic acid, 98:2), starting from 40% B in A to 100% B in 30 min, flow-rate 1.5 ml/min. Identification of the peaks was done by simultanous injection of the isolated and identified compounds. Ammonium acetate (NH_4OAc) was added to solvent B to prevent intramolecular hydrogen bonding, B (methanol, $9.0 \cdot 10^{-2}$ *M* ammonium

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acetate. 2% acetic acid). Methanol was chosen because of the better solubility of ammonium acetate in this solvent.

RESULTS AND DISCUSSION

The genus *Encelia* (tribe *Heliantheae*, family *Asteraceae*) consists of at least 15 species with their principal geographical distribution in the arid regions of the south western United States and Mexico. *Encelia californica* and *E. farinosa* have previously shown to contain chromenes and benzofurans[8,9]. During our chemical screening of the genus we isolated one coumarin, four chromenes and two benzofurans (Fig. 1) from *Encelia ventorum*, *E. palmeri*, *E. laciniata* and *E. halimifolia* and identified them by spectroscopical means (Table I).

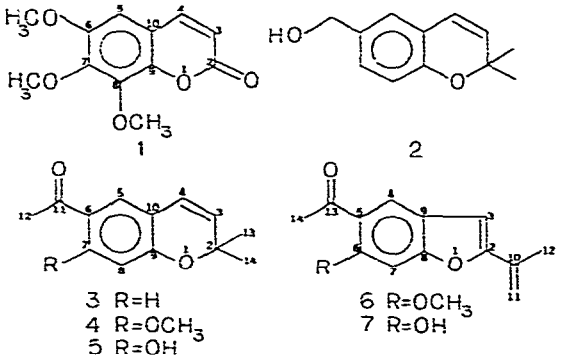

Fig. 1. Structures of coumarin (1), chromenes (2–5) and benzofurans (6, 7) isolated from *Encelia ventorum*, *E. palmeri*, *E. laciniata* and *E. halimifolia*.

TABLE I

^{1}H NMR SIGNALS OF COUMARIN (1), CHROMENES (2–5) AND BENZOFURANS (6,7)

Numbering follows Fig. 1. Chemical shifts are given in δ. All spectra were recorded at 90 MHz in C^2HCl_3 with Tetramethylsilane (TMS) as internal standard.

	1	*2*	*3*	*4*	*5*	*6*	*7*
H-3	d 6.30*	d 5.62*	d 5.60*	d 5.32*	d 5.52*	s 6.98	s 6.90
H-4	d 7.55*	d 6.30*	d 6.30*	d 6.25*	d 6.25*	s 7.88	s 7.86
H-5	s 6.60	d 7.52**	d 7.57**	s 7.46	s 7.25		
H-7		dd 7.65***	dd 7.69***			s 6.53	s 6.48
H-8		d 6.76§	d 6.72§	s 6.29	s 6.28		
H-11		s 4.72				br s 5.68, 5.10	br s 5.70, 5.10
H-12			s 2.50	s 2.50	s 2.50	br s 2.10	br s 2.10
H-13		s 1.47	s 1.47	s 1.47	s 1.45		
H-14		s 1.47	s 1.47	s 1.47	s 1.45	s 2.65	s 2.76
OCH_3	s 3.88, 3.97, 4.05			s 3.88		s 3.92	
OH		s 3.65			s 12.59		s 12.38

* J = 10 Hz.
** J = 3 Hz.
*** J = 9 Hz, 3 Hz.
§ J = 9 Hz.

These seven structurally related compounds were separated by reversed-phase HPLC within 17 min, using an RP-8 column and a linear gradient of water and acetonitrile with 2% acetic acid (Fig. 2A). Addition of acetic acid to the acetonitrile resulted in remarkable improvement of the peak symmetry of compounds 2, 5 and 7, exhibiting hydroxyl groups. The elution sequence of compounds 5 and 7 is affected by intramolecular hydrogen bonding of the hydroxyl groups to the ketone moieties. Addition of ammonium acetate, as previously described with the HPLC separation of phenolic acids[10], partially prevented the hydrogen bonding and caused compounds 5 and 7 to coelute with 4 and 6, respectively. Increasing the amount of ammonium acetate above $9.0 \cdot 10^{-2}$ *M* showed no further effect on the separation.

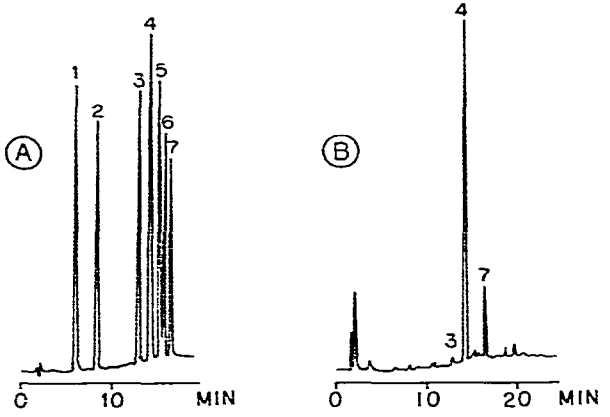

Fig. 2. (a) Separation of compounds 1–7 by reversed-phase HPLC.

Compound No.	t_R *(min)*
1	6.35
2	8.40
3	12.98
4	14.20
5	15.20
6	15.84
7	16.50

(b) HPLC separation of a crude stem extract of *Encelia palmeri*. Only main components are marked. Attenuation 1.0 a.u.f.s., chart speed 0.5 cm/min. For chromatographic conditions see Experimental.

The application of the described method is shown in Fig. 2B. Compounds 3, 4 and 7, the main components of *Encelia palmeri*, were determined in the crude stem extract of the species without any previous purification.

The use of HPLC, as described in this study, offers a quick and sensitive method to screen crude plant extracts for the presence of chromenes, benzofurans and structurally related compounds.

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