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Note

Model compound sorption by the resins XAD-2, XAD-8 and diethylaminoethylcellulose

An useful application to flavonoids isolation

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High recoveries of aromatic acids and phenols from water are possible by adsorption on macroreticular resins such as XAD and diethylaminoethyl (DEAE) cellulose[1]. However, due to the lack of data on the distribution coefficients, K_D, as a function of pH, a more systematic study is desirable.

The sorption of model compounds, phenol (P), cinnamic acid (C), benzoic acid (B), 3,5-dihydroxybenzoic acid (DH), 3,5-dimethoxy-4-hydroxybenzoic acid (DM), coumarin (CM), rutin (RU), isoorientine (IO) and isovitexine (IV) on XAD-2, XAD-8 and DEAE-cellulose was then investigated. Among these compounds, rutin, isoorientine and isovitexine belong to the group of flavonoids, which are found ubiquitously in plants and have many pharmacological properties[2]. Standards are not available for many of the flavonoids and the traditional methods for their isolation are based on thin-layer or column chromatography using different supports (silica, cellulose, polyamide, Sephadex LH-20) and aqueous organic solvents[3,4].

In recent years, semipreparative high-performance liquid chromatography (HPLC) has been applied for the purification of flavonoids, mainly using reversed-phase columns[5]. Nevertheless, the isolation of flavonoids from crude plant extracts through simple column chromatography has potential as confirmed by the results described in this paper.

EXPERIMENTAL

Materials

Coumarin and rutin were obtained from C. Roth (Karlsruhe, F.R.G.). All other test compounds were from Carlo Erba (Milan, Italy). Isoorientine and isovitexine were isolated from *Passiflora incarnata* L. extracts according to the literature[6]. These extracts were obtained from different commercial sources. All chemicals used were of reagent grade.

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Resins

XAD-2 and XAD-8 macroreticular resins (Rohm & Haas, Philadelphia, PA, U.S.A.) were thoroughly purified by sequential solvent extraction with methanol, acetonitrile and diethyl ether in a Soxhlet extractor for 8 h per solvent. The purified resins were stored under methanol in glass stoppered bottles to maintain their purity.

DEAE-cellulose (Bio-Rad, Richmond, CA, U.S.A.) was pretreated by the following procedure: about 50 g of dry cellulose were mixed in 10 ml of 0.5 *M* HCl for 1 h. The cellulose was rinsed with deionized water in a Büchner funnel until the pH was neutral; it was then suspended in 0.5 *M* NaOH for 1 h and rinsed with deionized water until the pH was neutral. Pretreated DEAE-cellulose was stored in the dark at 4°C.

Instrumentation

A Perkin-Elmer scanning spectrophotometer Model 550 SE was used for standard and eluate analysis.

HPLC was performed with a Waters M-501 pump fitted with a μBondapak C_{18} column (25 cm × 4.6 mm I.D.). Peaks were monitored with a Waters M-481 absorbance detector at 340 nm: output was measured with a Shimadzu Model CR3A integrator, using the external standard quantitation method. The eluent was 2-propanol–tetrahydrofuran–water (5:15:80, v/v/v) at a flow-rate of 0.7 ml/min.

Batch experiments

Batch distribution coefficients, K_D

$$K_D = \frac{\text{mg material adsorbed per g of resin}}{\text{mg material in solution per ml solution}}$$

were obtained by shaking overnight in stoppered flasks approximately 100 mg of resin with 25 ml of sample at a fixed pH, adjusted to a value between 2 and 9 by adding 0.1 *M* HCl or 0.1 *M* NaOH. After equilibration for 24 h at 25°C, suitable aliquots were taken from the unknown and standard flasks and the concentration of the solute in each was determined spectrophotometrically.

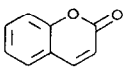
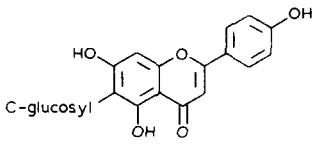
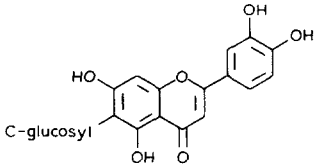
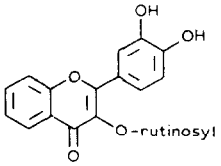
Column experiments

For the experiments on artificial samples glass columns (10 mm I.D.) were packed by using a water–XAD-2, water–XAD-8 or water–DEAE-cellulose slurry rinsed with 500 ml of distilled water to remove methanol. The DEAE-cellulose bed was 50 mm, those of the XADs was 220 mm and the reservoir volume was 250 ml. The flow-rate was maintained and controlled by a peristaltic pump and was set to 1 ml/min for DEAE-cellulose and 5 ml/min for XADs. The eluting agent was passed through the column and the effluent was collected by a fraction collector and monitored with the Perkin-Elmer spectrophotometer.

The isolation of isovitexine and isoorientine from the *Passiflora incarnata* extract was performed by loading 2 g of the extract on a DEAE-cellulose column (bed height 270 mm, 16 mm I.D.) and eluting with 0.01 *M* HCl at a flow-rate of 1.2 ml/min.

Final purification was effected by passing the eluates through a column of XAD-2, same size as that of the DEAE-cellulose, and desorbing the flavonoids with methanol.

TABLE I
COMPOUNDS EXAMINED

Compound	*Abbreviation*	*Compound*	*Abbreviation*	*Formula*
Phenol	P	Coumarin	CM	
Benzoic acid	B			
		Isovitexine	IV	
Cinnamic acid (*trans*)	C			
3,5-Dihydroxybenzoic acid	DH	Isoorientine	IO	
3,5-Dimethoxy-4-hydroxybenzoic acid	DM			
		Rutin	RU	

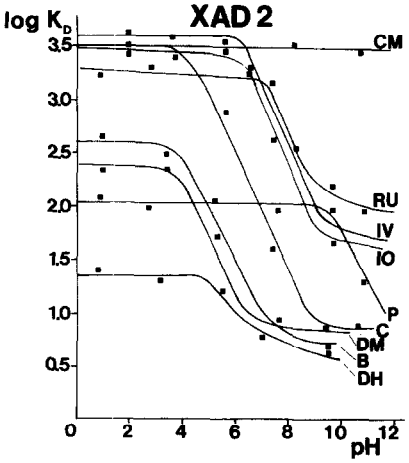


Fig. 1. Effect of pH on K_D for the uptake by XAD-2. Symbols as in Table I.

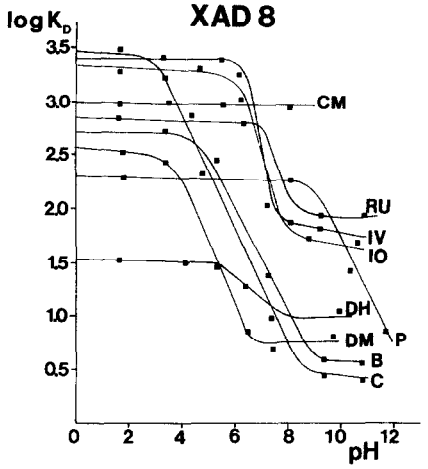


Fig. 2. Effect of pH on K_D for the uptake by XAD-8.

Solutions

Test solutions of aromatic acids and phenol (30–100 mg/l) in water were prepared. Samples of 200 μl of *Passiflora incarnata* L. aqueous extract (total flavones approximately 1%) were used without any previous treatment.

RESULTS AND DISCUSSION

The adsorptive forces involved when using XAD-2 and XAD-8 resins are mainly van der Waal interactions[7]. However, since the compounds examined (Table I) contain phenolic or/and carboxylic groups, their sorption on XAD-2 and XAD-8 is influenced by the pH as shown in Figs. 1 and 2. The K_D values of phenol, benzoic acid and cinnamic acid decrease sharply in a pH range which is close to the pK_a of each compound. On the other hand, 3,5-dimethoxy-4-hydroxybenzoic and 3,5-dihydroxy-

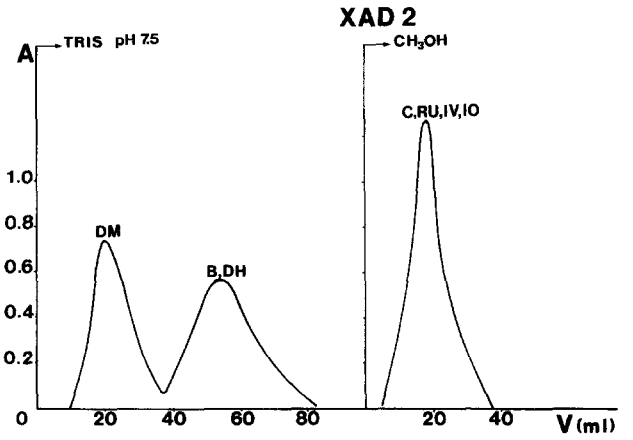


Fig. 3. Desorption from XAD-2.

TABLE II
SORPTION OF SELECTED AROMATIC ACIDS AND FLAVONOIDS ON DEAE-CELLULOSE RESIN (BATCH EXPERIMENTS)

H_nA	pH_a	K_D	
		H_nA–NaOH pH 7.5	10^{-2} M Tris–HCl pH 7.5
Phenol	9.89	–	–
Benzoic acid	4.19	800	80
Cinnaic acid (*trans*)	4.44	1480	420
3,5-Dihydroxybenzoic acid	4.04	5050	580
3,5-Dimethoxy-4-hydroxybenzoic acid	–	4200	500
Coumarin	–	100	8
Isovitexine	–	57000	15600
Isoorientine	–	81000	24100
Rutin	–	11800	2800

benzoic acid exhibit a slope change at higher pH values, while the behaviour of the neutral coumarin is represented by a "flat" curve. As shown in Fig. 3, only three compounds are removed from XAD-2 resin using a pH 7.5 buffer and the others can be eluted as a single large fraction with methanol. This means that an effective separation is not possible and the purification of the flavonoids rutin, isoorientin and isovitexin cannot be attained by means of XAD resins. Unlike the XAD resins, the mechanism of retention by DEAE-cellulose is based on charge rather than on hydrophobic interactions. To measure k_D values the intermediate pH of 7.5 was chosen, so that most of the model compound were in their anionic forms. The data in Table II indicate that the molecular complexity plays an important role in determining the extent of sorption as confirmed by the uptakes of rutin, isoorientin and isovitexin. An efficient separation of these flavonoids from the other aromatic compounds was then achieved (Fig. 4). As

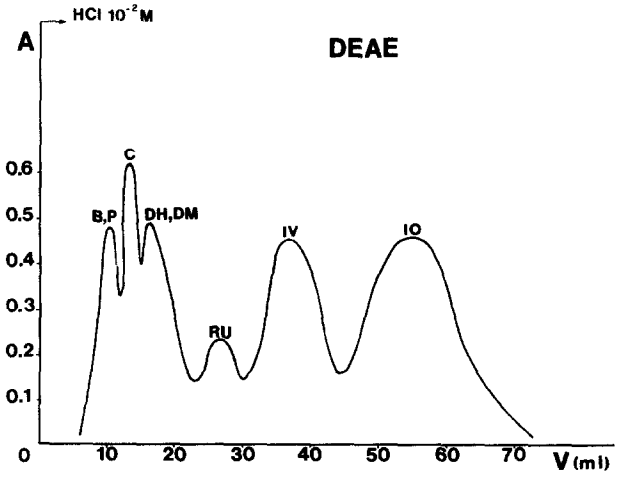


Fig. 4. Desorption from DEAE-cellulose.

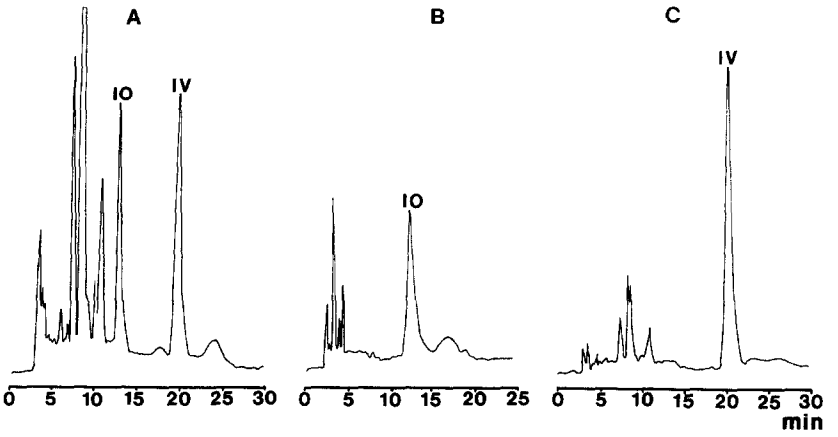


Fig. 5. HPLC analysis before and after the isolation of isoorientin and isovitexin from *Passiflora incarnata* L. extracts. (A) *Passiflora incarnata* L. extract; (B) and (C) components eluted from DEAE-cellulose with 0.01 *M* HCl.

far as the ionic strength is concerned, a small increase produces a sharp decrease in K_D values, as shown in Table II.

In conclusion, the sorption of aromatic acids and phenols on XAD resins is favoured by pH values in the range of the pK_a of the adsorbed compound, but desorption is aspecific. On the other hand the interactions between the same compounds and DEAE-cellulose at pH 7.5 are so enhanced by the molecular complexity that a selective elution from the resin can be performed using 0.01 *M* HCl. By this approach, isoorientiion and isovitexin have been isolated from the other carboxylic and phenolic components in a 2-g *Passiflora incarnata* L. extract (Fig. 5). The amounts recovered, after removal of HCl by adsorption chromatography on XAD-2, were 37 and 25 mg of isovitexine and isoorientine, respectively. The extension of this approach to other plant extracts is under investigation.

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