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Separations of Diastereomeric Organic Acids on Sephadex G-10

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Abstract

The applicability of adsorption chromatography on Sephadex G-10 to the preparative purification of the salts of several mixtures of diastereomeric carboxylic acids as well as the reaction mixture from the photolysis of sorbic acid was investigated. All mixtures were separated, apparently on the basis of relative hydrophobicities of the components, demonstrating the versatility of the method for purifying water-soluble organic compounds.

INTRODUCTION

Sephadex-G is an insoluble hydrophilic gel made by cross-linking water-soluble dextran with epichlorohydrin (*1*). While these gels are widely used for separations based on differences in molecular size (*2–4*), numerous cases in which they have been employed to separate low molecular weight organic compounds of about the same molecular size are known (*5*). Chromatographic separations in these latter cases could well be the result of the adsorption properties of the gels rather than filtration phenomena.

Since separations of structurally similar organic compounds, such as diastereomers, are often difficult in organic chemistry, development of simple and inexpensive chromatographic techniques is of considerable

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interest. Our earlier investigation (*6*) of adsorption chromatography on Sephadex was focused on determining the physical basis for separation of the cis- and trans-isomers of cinnamic acid and defining conditions for maximal resolution by varying the type of Sephadex gel and aqueous eluent. We reported that *cis*- and *trans*-cinnamic acids (**1** and **2**) were separable on Sephadex G-10, and that under all conditions investigated the cis-isomer eluted first. We showed that the difference in elution volumes of the cis- and trans-isomers increases as the degree of cross-linking of the gel is increased, i.e., on changing from G-100 to G-25 to G-10. This result is consistent with previous evidence which indicated that interactions between solutes and cross-linkages in the gel are the major factors influencing separations (*7*). In addition, the elution volumes of both isomers and separations between the two isomers were increased by increasing the "polarity," i.e., ionic strength, of the eluting solvent which is in agreement with earlier conclusions (*8*) that the separation of lipophilic water-soluble compounds on Sephadex was due to hydrophobic interactions (*9*) between the lipophilic portions of solutes and cross-linkages in the gels. These results should be compared to those of Kundu and Maenza (*10*), who found that diastereomeric acids, such as isocrotonic and crotonic, maleic and fumaric, and coumaric and coumarinic, are separable on Sephadex G-10 by elution with 0.5% ammonium bicarbonate. Importantly, their postulate (*10*) that the different spatial configurations of these low-molecular weight isomeric acids, which impart a greater effective size to the trans-isomer, are responsible for the smaller elution volume of the trans-acid and that the gel-filtration phenomenon is responsible for observed separations, seems inconsistant with our observations.

Our continuing studies in this area have a twofold purpose. First, more information is being sought on the nature of the factors responsible for the separations of isomeric, low molecular weight organic acids on Sephadex. Second, the generality and applicability of the method is being developed in order to demonstrate the utility of Sephadex chromatography in separations of a variety of carboxylic acid isomers. Accordingly, we report here our recent investigations of separations of several diastereomeric carboxylic acids, specifically the cis- and trans-isomers of styrylacetic (**3** and **4**), furanacrylic (**5** and **6**), and geranic acids (**7** and **8**). Also investigated were separations of *cis,trans*- and *trans,trans*-2,3-diphenylcyclopropane-1-carboxylic acid (**9** and **10**), *exo*- and *endo*-1-phenyl-5-methyl-bicyclo [3.1.0] hexane-6-carboxylic acid (**11** and **12**), and a mixture of sorbic acid (**13**) and its dimeric photoproducts, **14** and **15** (Fig. 1).

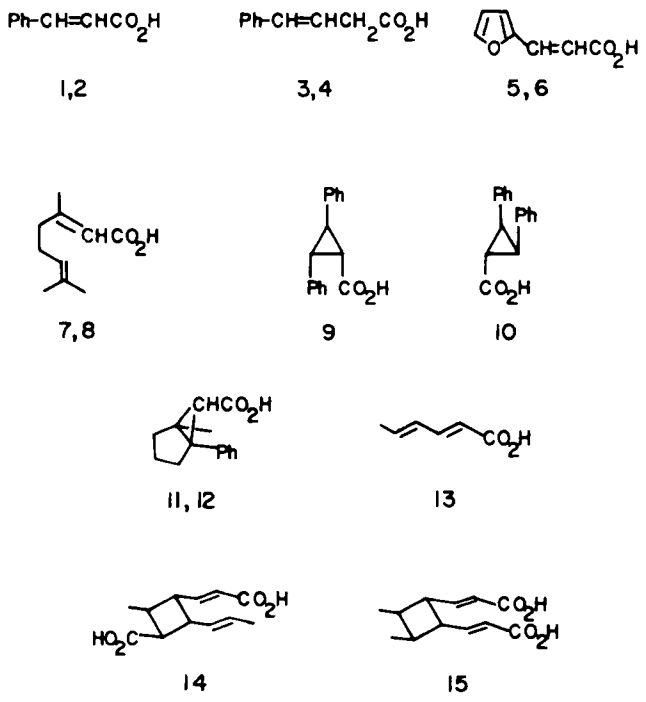

FIGURE 1.

EXPERIMENTAL

General

Nuclear magnetic resonance spectra were determined with a Varian T-60 spectrometer. Ultraviolet spectra were recorded on a Beckman Acta-III ultraviolet-visible spectrophotometer. Melting points were determined in capillary tubes with a Mel-Temp melting point apparatus and are recorded uncorrected.

Citral and styrylacetic acid were purchased from Aldrich Chemical Co., *trans*-cinnamic and sorbic acids from Sigma Chemical Co., 2-furanacrylic acid from Eastman Organic Chemicals, and Sephadex G-10 and Blue Dextran 2000 from Pharmacia Fine Chemicals. Prepared as referenced were *cis*-, *trans*-, and *trans,trans*-2,3-diphenylcyclopropane-1-carboxylic

TABLE 1

Photolysis Conditions for Photoisomerization

Acid[a]	Solvent	Filter[b]	Irradiation time (hr)	Ratio of cis to trans acid[c]
Cinnamic	Methanol	Pyrex	20	0.7
Styrylacetic	10% Acetone in acetonitrile	Quartz	5	0.6
2-Furanacrylic	Methanol	Quartz	5	0.4
Sorbic	Methanol	Pyrex	5	—

[a]The trans-isomers (5 g) were dissolved in 75 ml of the solvents indicated.

[b]Each photolysis mixture was contained in a 25 × 200 mm quartz or Pyrex tube, as indicated. The tubes were tied to the quartz immersion well with flexible latex tubing.

[c]Approximated using NMR spectroscopy.

acids (*11*), *endo*- and *exo*-1-phenyl-5-methylbicyclo[3.1.0]hexane-6-carboxylic acids (*12*), and *cis*- and *trans*-geranic acids (*13*).

Photolyses

Irradiations were conducted using a 450-W Hanovia medium-pressure lamp contained in a quartz water-cooled immersion well. The conditions employed for each photoisomerization are summarized in Table 1. Following irradiation for the times indicated, the solvent was evaporated *in vacuo* yielding mixtures of cis- and trans-isomers and, in the case of sorbic acid, of the photodimers and sorbic acid.

Chromatographic Separations

These were performed on a 2.7 × 200 cm column of Sephadex G-10 eluting with 0.05 *M* potassium phosphate (monobasic) adjusted to pH 8.0 with potassium hydroxide and containing 0.1 *M* potassium chloride. The mixtures of diastereomers (*19*) were dissolved in a minimum of the pH 8.0 buffer, adding sodium hydroxide as required. Fractions (4 ml) were collected with a Gilson Mini-Escargot Fractionator at a flow rate of 90 to 120 ml/hr. All separations were carried out at room temperature. The eluted compounds (Table 2) were detected by measuring the absorbance of each fraction at an appropriate wavelength (Table 3) against a blank of the eluting buffer. Fractions containing one component were pooled, acidified to pH 3.0 with phosphoric acid, and extracted with ether. The

TABLE 2

Chromatographic Data for the Separation of Diastereomers on Sephadex

Acids separated	Component	Elution volume (ml)	K_{av}	Crude weight (g)	Pure weight (g)	Recrystallization solvent
Cinnamic	cis (1)	921	0.72	0.45	0.35	Water/ethanol
	trans (2)	1904	2.11	0.52	0.43	Water/ethanol
Styrylacetic	cis (3)	1307	1.27	0.38	0.20	Petroleum ether
	trans (4)	1497	1.54	0.60	0.43	Petroleum ether
Furanacrylic	cis (5)	820	0.58	0.20	0.14	Water
	trans (6)	1173	1.08	0.70	0.45	Water
Geranic	cis (7)	982	0.81	0.32[a]	—	—
	trans (8)	1116	1.00	0.13[a]	—	—
2, 3-Diphenylcyclopropane-1-carboxylic	cis, trans (9)	3667	4.61	0.38	0.28	Water/methanol
	trans, trans (10)	4500	5.79	0.45	0.35	Water/methanol
1-Phenyl-5-methylbicyclo[3.1.0]-hexane-6-carboxylic	exo (11)	1136	1.06	0.20	0.17	Hexane
	endo (12)	1243	1.22	0.17	0.10	Hexane
Sorbic	dimer 14	616	0.29	0.35[a]	—	—
	dimer 15	700	0.41	0.10[a]	—	—
	trans, trans (13)	854	0.63	0.45	0.25	Water/ethanol

[a] Oil

TABLE 3

Physical Constants and Ultraviolet Spectral Data of Separated Components

Acid	Isomer	Melting point (°C)		Ultraviolet spectra			
				λ_{max}		log ε	
		Observed	Reported	Observed	Reported	Observed	Reported
Cinnamic	cis (**1**)	55–56	58	259[a]	264 (*14*)	3.93	3.97
	trans (**2**)	132	133	270[a]	273	4.30	4.30
Styrylacetic	cis (**3**)	58–59.5	—	241[a]	—	4.15	—
	trans (**4**)	86–87	87 (*31*)	250[a]	—	4.27	—
Furanacrylic	cis (**5**)	102	106–108 (*15*)	300[a]	299 (*15*)	4.05	4.14
	trans (**6**)	140–141	141	297[a]	296	4.33	4.37
Geranic	cis (**7**)	Oil	—	220, 275[b]	220, 275 (*32*)	3.87, 2.46	4.12, 1.65
	trans (**8**)	Oil	—	220, 275[b]	220, 275	3.76, 2.31	4.08, 1.86
2, 3-Diphenylcyclopropane-1-carboxylic	cis, trans (**9**)	154–155	157–158 (*11*)	219[a]	—	3.99	—
	trans, trans (**10**)	150–152	154.4–155.5	223[a]	—	4.00	—
1-Phenyl-5-methyl bicyclo [3.1.0] hexane-6-carboxylic	exo (**11**)	197–199	198–200 (*12*)	217[a]	—	3.76	—
	endo (**12**)	108–111	—	226[a]	—	3.54	—
Sorbic	dimer (**14**)	Oil	—	206[c]	206 (*16*)	3.82	—
	dimer (**15**)	Oil	—	206[c]	206	3.98	—
	trans, trans (**13**)	132–133	134.5	254	254	4.39	4.41

[a] Determined in water/ethanol (20/80).
[b] Determined in methanol.
[c] Determined in water.

combined ether extracts were dried over anhydrous sodium sulfate, and the solvent was removed *in vacuo* giving the crude product. Data for these separations, the solvents used for recrystallization and the yield of pure products are given in Table 2.

Each component was characterized by comparison of its melting point, (if it was crystallizable), and its UV spectrum and NMR spectrum with those reported, with the exception of *cis*-styrylacetic acid.

RESULTS

Diastereomeric Acids Used

Mixtures of the cis- and trans-isomers of cinnamic (*14*) (**1** and **2**), styrylacetic (**3** and **4**), and 2-furanacrylic (*15*) (**5** and **6**) acids were individually obtained by photoisomerization of the corresponding trans-acids. Pertinent data for the photochemical reactions are included in Table 1. *cis*-Styrylacetic acid was thoroughly characterized since it has not been previously reported. The cis-isomer was separated from the trans-isomer by Sephadex chromatography, as described below, and was recrystallized from petroleum ether. Its spectral and analytical properties are well in accord with its assigned structure. Especially characteristic is its UV spectrum, which contains an absorbance maximum at lower wavelength than that of the isomeric trans-acid. In addition, the NMR chemical shift for the α-vinyl proton at C-3 of *cis*-styrylacetic acid is located further upfield than that of the corresponding proton in the trans-isomer. Both observations pattern the behavior for the cis-isomers of cinnamic and 2-furanacrylic acids (see Table 4).

A mixture of *cis*- and *trans*-geranic acids (**7** and **8**) was prepared by silver oxide oxidation of citral (*13*). The pure diastereomeric *cis,trans*- and *trans,trans*-2,3-diphenylcyclopropane-1-carboxylic acids (**9** and **10**) were independently prepared by the method of Blatchford and Orchin (*11*), involving the copper-catalyzed addition of ethyl diazoacetate to *cis*- and *trans*-stilbene followed by saponification of the intermediate ester. A mixture of the *exo*- and *endo*-1-phenyl-5-methylbicyclo[3.1.0]hexane-6-carboxylic acids (**11** and **12**) was prepared by the published route (*12*) from ethyl diazoacetate and 1-methyl-2-phenylcyclopentene.

We had anticipated that photolysis of sorbic acid (**13**), which has the trans,trans-stereochemistry, would lead to production of the other three diastereomers. However, the only photoproducts isolated after irradiation and chromatography on Sephadex were the known cyclobutane photodimers, **14** and **15** (*16*).

TABLE 4

Nuclear Magnetic Resonance Spectral Data

$RC(H_\beta)=C(H_\alpha)COOH$

Acid	Isomer	Chemical shift (δ)	
		H_α	H_β
Cinnamic	cis (1)	5.86	6.98
	trans (2)	6.21	7.78
Furanacrylic	cis (5)	5.81	6.91
	trans (6)	6.30	7.55

$RC(H_a)=C(H_b)C(H_c)_2COOH$

Acid	Isomer	Chemical shift (δ)		
		H_a	H_b	H_c
Styrylacetic	cis (3)	6.66	5.86	3.36
	trans (4)	6.55	6.10	3.25

Chromatographic Separations

Each mixture of acids was separated using the same column and conditions described above. Data for each separation including elution volumes, K_{av}, and weights of materials recovered along with solvents used for recrystallization are given in Table 2. The physical and spectral properties of the pure acids obtained by chromatographic separations are collected in Tables 3 and 4.

The fraction of the volume of the gel that is available to the solute, K_{av}, is defined by Laurent and Killander (*17*) as: $K_{av} = V_e - V_0/V_t - V_0$, where V_e = the elution volume of the solute, V_0 = void volume, V_t = total volume of the gel bed. V_0 is determined from the elution volume of Blue Dextran 2000.

Ziska (*18–20*) has used K_{av} as an adsorption parameter in order to compare the adsorption of peptides to Sephadex. In our work, K_{av} was used to determine the relative adsorption of the acids to the gel, i.e., the higher the K_{av} value, the stronger the solute-gel interaction.

DISCUSSION

In an earlier study we presented evidence to support the conclusion that separations of water-soluble lipophilic stereoisomers, such as cis- and trans-isomers of carboxylic acids, on Sephadex are due to adsorption

phenomena resulting from hydrophobic interactions between the hydrocarbon portions of solutes and the cross-linkage portion of the gels (*6*). The results of our current investigation, which provides additional examples of the utilization of Sephadex chromatography on a variety of structural types, are consistent with this earlier postulate. In all cases studied, the more compact or smaller stereoisomer possessed a smaller elution volume on Sephadex G-10 than did the extended isomer. Thus, on the basis of these findings, it appears that the earlier postulate of Kundu and Maenza (*10*), that separations of isomeric unsaturated carboxylic acids resulted from their size differences, requires revision. Sephadex G-10, the gel used in our study, is designed to exclude molecules of molecular weight on the order of 700, i.e., sites within the gel can be occupied by molecules smaller than this. It is difficult to imagine how small (molecular weight 200) isomeric acids could possess that great a size differential even when solution spheres are taken into account. (Furthermore, one would expect the trans-isomer in each case to be more highly solvated since it has the polar carboxyl group in a less hydrophobic environment.)

In contrast, the orders of elution for each of the stereoisomeric acids investigated seem to correlate well with their predicted hydrophobicity. In the cis-isomers of the unsaturated acids, the polar carboxyl groups are spatially nearer to the hydrophobic side chain. Thus interactions of the hydrophobic groups with the more hydrocarbon-like cross-linkages of the gels would be greater for the trans-diastereomers as a result of less steric interference from the carboxyl group or of greater hydrophobic surface areas. An alternate explanation, consistent with the observed elution orders, is that hydrogen bonding interactions between the carboxyl groups of the acids and hydroxyl functionalities on the gel are responsible for separation. Consistent with this rationale is the prediction that the less crowded trans-isomers would be better able to come into closer proximity to the gel surface. However, our earlier observation that increasing solvent polarity by increasing the ionic strength of the aqueous eluent has no effect on the elution order of this cis- and trans-isomeric acids and increases the elution volumes of both (*6*) seems to eliminate hydrogen bonding interactions as the correct explanation.

Additional data related to our postulate can be derived from Table 2. For example, adsorption to Sephadex, as judged by the K_{av} values, seems to be dependent on the number and hydrophobicity of side chains in the organic acids investigated. The K_{av} values increase in the order (data for extended structures compared): two aromatic rings (**10**, 5.79) > one aromatic ring (**2**, 2.11; **4**, 1.54; **12**, 1.22; **6**, 1.08) > aliphatic chain (**8**, 1.00;

7, 0.81; 13, 0.63; 14, 0.29; 15, 0.41). Furthermore, 2-furanacrylic acid, which contains a more polar aromatic moiety, is adsorbed the least strongly among those compounds containing one aromatic ring.

It is important also to note that the general phenomena discussed above concerning isomeric unsaturated carboxylic acids also hold for other isomeric carboxylic acids having the acid functional group in the vicinity of, or remote from, regions of hydrophobicity. Excellent examples are the exo- and endo-bicyclic acids, **11** and **12**, and diastereomeric diphenylcyclopropane carboxylic acids **9** and **10**. In both cases the isomer having the carboxyl moiety close to the aromatic ring possesses the smaller elution volume.

Our observations, when combined with those which indicate that aromatic and heteroaromatic compounds are more strongly adsorbed to Sephadex than are their aliphatic analogs (*5e*) and that primary alcohols display increasingly stronger adsorptions to Sephadex with increasing chain length (*22*), appear to clearly demonstrate that hydrophobic interactions between solute and gel are the dominant physical forces responsible for separations of small water-soluble molecules on Sephadex. In addition, we have shown by a number of examples that Sephadex is a very versatile adsorbent for chromatographic separations of isomeric acids. Other literature reports have emphasized this same point. Brook and Housely (*24*) found that organic acids could be separated using eluents buffered at low pH. This method, however, lacks attractiveness due to the sparing solubility of most organic acids in acidic media. Sephadex LH-20 has also been employed to separate isomeric and structurally similar organic compounds using both organic (*25–28*) and aqueous eluents (*29*, *30*). Some advantages offered by chromatography on G-10 are: (a) the ionic strength of the aqueous eluent can be manipulated to increase elution volumes and enhance separations, (b) the gel is relatively inexpensive and columns can be reused, (c) Sephadex G-10 in our hands has been more effective than LH-20 in separating *cis*- and *trans*-cinnamic acid, and (d) the physical properties of the column, such as ease of pouring and flow rates, appear superior for G-10.

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