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THIN-LAYER AND GEL CHROMATOGRAPHY OF SOME ALIPHATIC POLYCARBOXYLIC ACIDS

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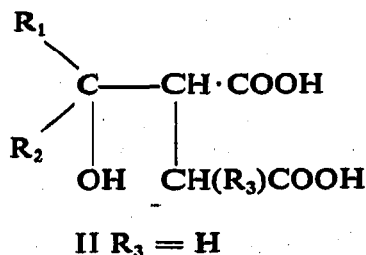
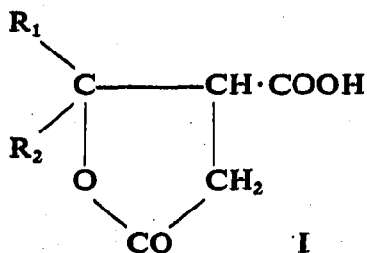
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SUMMARY

Mixtures of telomers obtained by the radiation-induced addition of 2-octanol to maleic acid and containing 2-10 carboxylic groups per molecule were analyzed by thin-layer chromatography on silica gel impregnated with potassium hydrogen sulphate in *n*-hexane-acetone as solvent and by gel chromatography in tetrahydrofuran on styrene-divinylbenzene copolymers. R_M values, relative zone velocities and molecular weights of the individual telomers were calculated. By a combination of thin-layer and gel chromatography, polycarboxylic acids were successfully fractionated and identified.

INTRODUCTION

The addition of secondary alcohols to maleic acid, effected by a photoreaction¹ or ionizing radiation², gives rise predominantly to γ,γ -dialkyl derivatives of paraconic acid (I), which are also known in a linear form (II):



These compounds have been prepared earlier by using common synthetic methods³, and their fractionation by means of paper chromatography has been described⁴. Higher secondary alcohols, by radiation-induced addition to maleic acid, yield some

further adducts besides the already mentioned simple products. As we have shown⁵, these adducts are telomers that contain several (2–5) molecules of maleic acid per molecule of alcohol. Then, for formula II:



The above telomers have not yet been prepared by classical synthetic methods. Polycarboxylic acids formed by radiation-induced addition are difficult to fractionate because of their highly hydrophilic character and considerable chemical similarity. It might be expected that the fractionation of the individual telomers in the reaction mixtures, the determination of their chemical character and their identification may be carried out by using thin-layer chromatography (TLC) on silica gel combined with gel chromatography, if one chooses a suitable gel packing for a particular fractionation⁶. As both the molecular weight and effective volume of the molecules of telomers with the same structural basis differ from each other by a certain constant increment, a logarithmic dependence of the elution volumes of the individual compounds on their molecular weight could be assumed.

EXPERIMENTAL

Chemicals

Maleic acid was prepared by heating redistilled maleic anhydride with an excess of redistilled water and dried *in vacuo* (0.2 mm Hg) at 50°. Octanol-2, analytical-reagent grade (Fluka, Buchs, Switzerland) was redistilled *in vacuo*. Silica gel H for TLC according to Stahl (E. Merck, Darmstadt, G.F.R.) was used for thin-layer chromatography. Column chromatography was carried out on silica gel for column chromatography (E. Merck), 0.05–0.2 mm (70–325 mesh). S-gel 832 (Institute of Macromolecular Chemistry, Czechoslovak Academy of Sciences, Prague), a styrene-divinylbenzene copolymer with an exclusion limit of about 1000 molecular weight units, was used as the packing for the gel chromatography columns. γ,γ -Dimethylparaconic acid (terebic acid) and γ,γ -methylhexylparaconic acid (MHPAc) were used as reference compounds. These compounds were synthesized by Stobb's condensation⁷ of acetone or methyl hexyl ketone with succinic acid diethyl ester catalyzed by sodium alkoxide to give the β,γ - and γ,δ -unsaturated semi-esters of the respective acids, which, by hydrolysis with concentrated hydrochloric acid^{3,8}, yielded the required γ,γ -dialkyl derivatives of paraconic acid. The other chemicals and solvents used were of analytical-reagent grade (Lachema, Brno, Czechoslovakia).

Methods

The preparations of samples of 0.35 M solutions of maleic acid in alcohol and their irradiation have been described in an earlier paper². After irradiation with the absorbed dose of $1.6\text{--}9 \cdot 10^{20}$ eV/ml, the unreacted octanol-2 was removed by distillation *in vacuo* (15 mm Hg); solutions for chromatography were prepared from the dry residue containing the mixture of products.

Thin-layer chromatography on silica gel

Glass plates, 9 × 15 cm, were coated with a 0.5-mm layer of silica gel H containing 1% (w/w) of sodium hydrogen sulphate and dried at 90° for 1 h, and 300–500 μg of the mixture of products in the form of *ca.* 2% solutions in acetone and 10% in water were placed on the start. The reference compounds (maleic acid, terebic acid and MHPAc) were applied in the same solution in amounts of 20–50 μg. An *n*-hexane–acetone mixture in the proportions 1:1 (system S1) or 1:3 (system S2) was used for developing. The chromatograms were developed at room temperature and detected on drying by spraying with the following solutions: detection D1, solution of 0.1% of dimethyl yellow and 0.1% of pentamethylene red in ethanol (red spots on a yellow background); detection D2, solution of 0.5% of potassium permanganate in 2.5% (v/v) sulphuric acid (white spots on a pink background); detection D3, solution of 5% of potassium bichromate in 40% (v/v) sulphuric acid (brown to black spots on a yellow-green background); after spraying, the chromatograms were heated at 150–200° for 3–5 min.

Gel chromatography

Gel chromatography was carried out on a system of five columns, 1200 mm long and 8 mm in diameter, packed with S-gel 832. The samples were diluted with tetrahydrofuran to *ca.* 10% solutions and injected into the column in 0.3–0.4-ml amounts. Tetrahydrofuran was used as the eluent and the analyses were performed at room temperature at a flow-rate 35 ml/h. A series-connected R-4 differential refractometer (Waters Ass.) with a UV detector (Czechoslovak Academy of Sciences, Prague) operating at 254 nm were used as detectors; 1 count volume corresponded to 2.72 ml and the void volume of the system (V_0) was 47.0 counts.

RESULTS AND DISCUSSION

Preliminary experiments have shown that the system *n*-hexane–acetone is suitable for the fractionation of compounds such as dicarboxylic and polycarboxylic acids on a thin layer of silica gel. Impregnation of this sorbent with sodium hydrogen sulphate prevents spreading of the spots of the compounds being analyzed, which, during chromatography on unimpregnated silica gel or paper, form very elongated and therefore inseparable spots. In the system used here, the properties of carboxylic groups prevail and consequently the compounds are separated according to the number of carboxyl groups in the molecule. An example of the TLC separation of a mixture of telomers obtained by radiation-induced addition during the irradiation of a solution of maleic acid in 2-octanol is shown in Fig. 1.

A survey of R_F values of the chromatographed acids is given in Table I. It is remarkable that the differences ΔR_M in system S1 observed for the pairs 6 and 8, 9 and 11, 11 and 14, and 14 and 15 vary within a comparatively narrow range (0.40–0.54) with an average value of 0.48, *i.e.* for one COOH group $\Delta R_M = 0.24$ if the change involved is not that between *cis*- or *trans*-configurations (*cf.*, maleic and fumaric acids). Hence, from the magnitude of the difference ΔR_M , it is possible to draw some conclusions about the number of carboxyl groups in a molecule, as has been shown for paper chromatography⁴. Further resolution was obtained by using different methods of detection. Detection D1 revealed compounds with at least one

TABLE I
CHROMATOGRAPHY OF POLYCARBOXYLIC ACIDS

Solvent systems and detection methods are explained in the text.

No.	Compound	Thin-layer chromatography					Gel chromatography						
		Detection		System S1		System S2		Elution volume, V_e (counts)	Relative zone velocity, V_0/V_e	Molecular weight			
		D1	D2	D3	N*	R _F	R _M			R _F	R _M	Calculated**	Theoretical
1	Maleic acid	+	+	-	2	0.25	+0.48	0.56	-0.10	78.2	0.601	(141)	116
2	Monoisopropyl ester of 1	+	+	-	1	0.56	-0.10						
3	Mono-2-octyl ester of 1	+	+	-	1	0.68	-0.33			69.9	0.672	(235)	228
4	Fumaric acid	+	+	-	2	0.46	+0.07	0.60	-0.18	74.0	0.635	(185)	116
5	Monoisopropyl ester of 4	+	+	-	1	0.58	-0.14						
6	Terebic acid (DM-telomer***, 1:1)	+	-	+	1	0.48	+0.04	0.62	-0.21	76.6	0.614	158 ^{§§}	158
7	Monoisopropyl ester of 6	-	-	+	0	0.53	-0.05						
8	DM-telomer***, 1:2	+	-	+	3	0.27	+0.43			68.2	0.689	280	274
9	Methylhexylparaconic acid (MH-telomer [§] , 1:1)	+	-	+	1	0.60	-0.18	0.72	-0.42	71.1	0.661	228 ^{§§}	228
10	Mono-2-octyl ester of 9	-	-	+	0	0.71	-0.39			65.5	0.718	340	340
11	MH-telomer [§] , 1:2	+	-	+	3	0.30	+0.37	0.63	-0.23	65.5	0.718	340	344
12	Mono-2-octyl ester of 11	+	-	+	2	0.39	+0.19			61.0	0.770	460	456
13	Di-2-octyl ester of 11	+	-	+	1	0.53	-0.05			57.2	0.822	580	568
14	MH-telomer [§] , 1:3	+	-	+	5	0.11	+0.91	0.47	+0.05	61.1	0.769	460	460
15	MH-telomer [§] , 1:4	+	-	+	7	0.04	+1.38	0.40	+0.18	57.0	0.825	590	576
16	MH-telomer [§] , 1:5	+	-	+	9			0.34	+0.29	55.0	0.855	680	692

* The number of free carboxylic groups.

** Read off from the plot in Fig. 4.

*** Telomers formed by the addition of propanol-2 to maleic acid.

§ Telomers formed by the addition of octanol-2 to maleic acid.

§§ Molecular weights of acids 6 and 9 used as reference compounds.

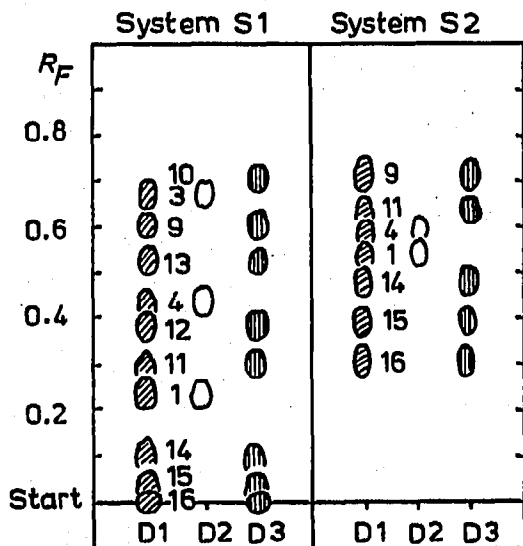


Fig. 1. Thin-layer chromatogram of polycarboxylic acids obtained by radiation-induced addition during the irradiation of a solution of maleic acid in 2-octanol. Numbers of the individual spots correspond to those in Table I.

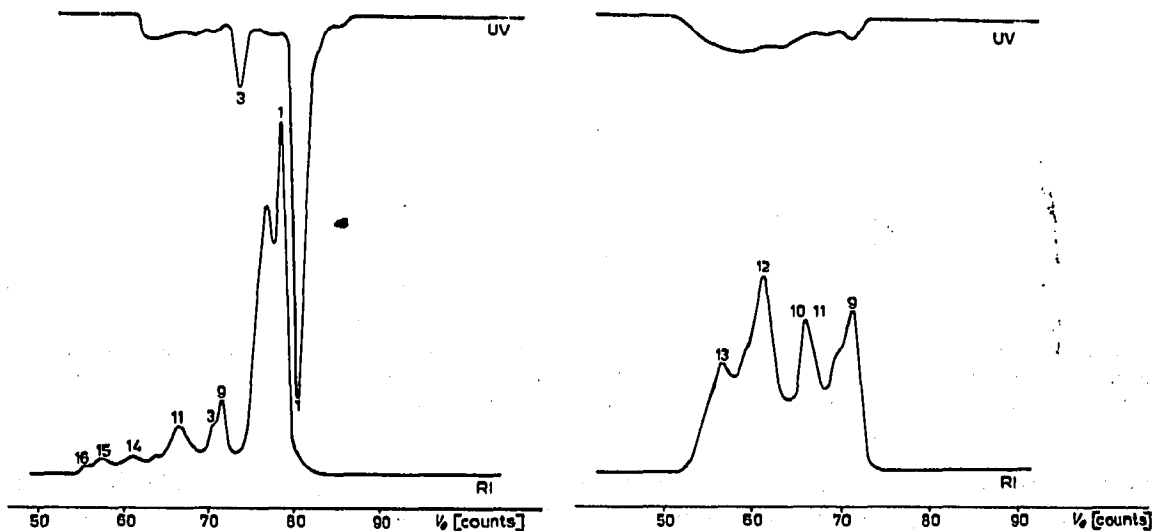


Fig. 2. Gel chromatogram of a mixture of polycarboxylic acids obtained by irradiation of a solution of maleic acid in 2-octanol with a dose of $1.6 \cdot 10^{20}$ eV/ml. S-gel 832; tetrahydrofuran flow-rate, 35 ml/h. RI, recorded with differential refractometer; UV, recorded with UV detector at 254 nm; numbers of the individual peaks correspond to those in Table I.

Fig. 3. Gel chromatogram of a fraction prepared from a raw mixture of polycarboxylic acids by separation on a silica gel column by elution with *n*-hexane-acetone (1:1). Experimental conditions of gel chromatography as in Fig. 2.

free carboxyl group in the mixture, detection D2 demonstrated the presence of compounds with a C=C double bond, and detection D3 also demonstrated the presence of neutral compounds besides those already mentioned. Neither maleic nor fumaric acid, nor the esters of these acids, give spots if detection D3 is used, which permits the determination of the products of telomerization even when an excess of the acids is present in the initial mixture.

Gel chromatography, which makes possible the separation of individual compounds on the basis of the shape and size of molecules, enabled the telomer molecules under investigation to be determined in a mixture from their elution volumes without isolation. As the telomers investigated here differ from MHPAc (mol. wt. 228) by a contribution of 1–4 molecules of maleic acid (mol. wt. 116), their molecular weights could be determined by comparing their elution volumes with that of MHPAc. Figs. 2 and 3 show examples of chromatograms of a mixture of telomers that were prepared by radiation-induced telomerization during the irradiation of a solution of maleic acid in 2-octanol and separated by gel chromatography. The corresponding elution volumes read off from the maxima of these curves are given in Table I. These values were compared with the graph representing the dependence of the partition coefficients on the molecular weight of telomers (Fig. 4), which was constructed by

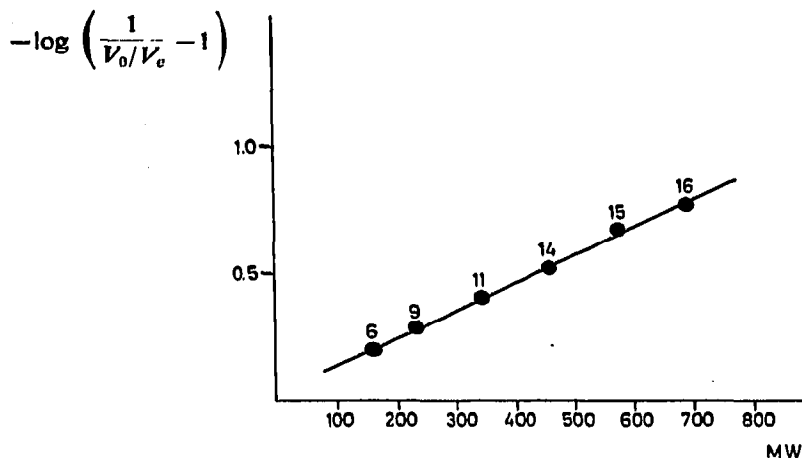


Fig. 4. Dependence of the partition coefficients of polycarboxylic acids (obtained by gel chromatography) on their molecular weights. The straight-line dependence confirms an oligomer-homologous series. Numbers of the individual points correspond to those in Table I.

plotting the elution volumes of synthetically prepared reference compounds (MHPAc and terebic acid*) against the logarithm of their molecular weights. In this way, approximate molecular weights were obtained for the individual telomers present in the mixture, which are given in the last but one column in Table I. It can be seen that the agreement between the calculated and theoretical molecular weights of the individual compounds is very good.

It has been the objective of this paper to demonstrate that by combining

* MHPAc is a homologue of terebic acid (mol. wt 158) differing by C_5H_{12} in the γ -alkyl group.

suitable chromatographic methods one can analyze successfully and rapidly even very complicated mixtures of unknown polycarboxylic acids and identify the constituents. The method was used in practice for analyzing and evaluating radiation-chemical reactions, which will be published elsewhere.

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