phenyl-β-D-glucuronid, 1-Naphthyl-β-D-glucuronid und 2-Naphthyl-β-D-glucuronid, die häufig als Substrate zum Nachweis und zur Aktivitätsbestimmung von Glucuronidase dienen, ergeben mit dem Sprühreagenz keine Blaufärbung. Diese Glucuronide löschen aber die bei 254 nm anregbare Fluoreszenz der DC-Platten und können so auf den Chromatogrammen vor dem Besprühen mit dem Naphthoresorcin-Reagenz einwandfrei erkannt werden.

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Mono- and bidimensional separation of oxyacids by thin-layer chromatography*

In the course of investigations, while trying to identify the degradation products of γ-irradiated D-glucose, a method was required for the analysis of a mixture of mono- and dicarboxylic oxyacids. Acid mixtures are easily separated using thin-layer chromatography. Some reports have been made on this subject: PASTUSKA[1] uses plates of 0.1 *M* boric acid-impregnated Kieselgel for the separation of galacturonic and glucuronic acids; dicarboxylic and carboxylic acids have been separated on silica gel by BRAUN AND GEENEN[2] and by PASTUSKA AND PETROWITZ[3]; a mixture of Kieselgel and Kieselguhr has also been used[4]; better separations have been obtained using highly purified cellulose[5–7].

Experimental

Preparation of plates. A slurry of Cellulose MN 300 HR (Macherey, Nagel & Co.) in water (20 g/100 ml) was mechanically shaken for 2 min and applied to 20 × 20 cm

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TABLE I

COLOR REACTION OF THE ACIDS WITH AMMONIUM VANADATE

No.	*Acid*	*Daylight*	*U.V. light*
1	Glucuronic	Yellow	Blue
2	Gluconic	Yellow	Blue
3	Saccharic	Red-brown	Dark
4	Tartronic	White-gray	Gray
5	Tartaric	Red	Dark
6	Glyceric	White	Gray
7	Oxalic	Yellow	Gray
8	Glycolic	White	Gray

glass plates to a thickness of 350 μ, using a DeSaga adjustable applicator. The plates were dried for 24 h at room temperature, scraped to produce a straight edge and used without other treatment.

Solvents. The following solvents were used in both mono- and bidimensional separations:

Solvent A: isopropanol–ethyl acetate–water (23.5:65:11.5);
Solvent B: formic acid–ethyl acetate–water (1:3:1);
Solvent C: ethyl acetate–acetone–water (4:5:1);
Solvent D: butanol–acetic acid–diethyl ether–water (9:6:3:1).
Each solvent is a single-phase system.

Solvent A has been used for the separation of sugars[8], but never, to our knowledge, for acids. Solvent B was selected because it gives a good separation of gluconic and glucuronic acids[6]. Solvents C and D have (ref. 9) been used mainly in the separation of sugars and sugar derivatives.

Spray reagents. Bromophenol Blue: a 0.04% solution of 95% ethyl alcohol was adjusted to a definite blue color (pH 6.7) with dilute sodium hydroxide. Ammonium vanadate: a saturated solution of ammonium vanadate in water was used[10].

Procedure. The acids, dissolved in water (10 mg/ml), were applied to the plates with a microsyringe (Hamilton Company, Inc., Whittier, Calif.), using 2 μl for each spot, under a stream of cold air. The glass chromatographic tanks, lined with What-

TABLE II

R_F VALUES OF THE ACIDS OBTAINED BY MONODIMENSIONAL SEPARATION ON CELLULOSE PLATES

No.	*Acid*	*Solvent*							
		A		*B*		*C*		*D*	
1	Glucuronic	0.13	0.13[a]	0.28	0.27[a]	0.16	—[a]	0.11	0.11[a]
2	Gluconic	0.13	—	0.34	0.33	0.16	0.16	0.12	—
3	Saccharic	0.59	0.58	0.49	0.48	0.61	0.61	0.36	0.35
4	Tartronic	0.73	0.72	0.60	—	0.73	0.73	0.49	—
5	Tartaric	0.58	—	0.49	—	0.61	—	0.36	—
6	Glyceric	0.55	—	0.62	0.60	0.58	—	0.49	0.48
7	Oxalic	0.88	0.87	0.70	0.69	0.88	0.88	0.67	0.66
8	Glycolic	0.75	—	0.73	0.72	0.73	—	0.65	—

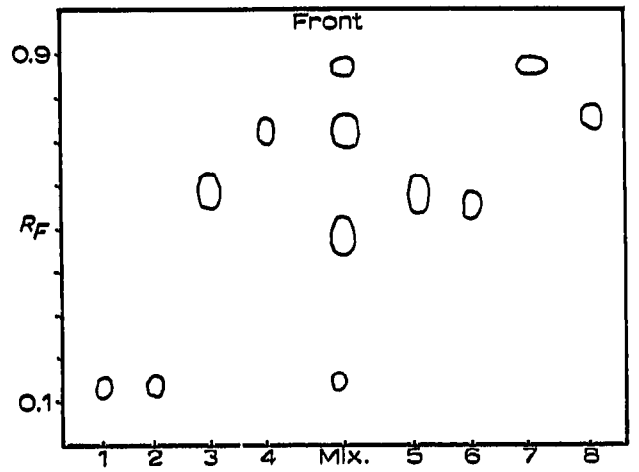
[a] The values reported refer to the mixture.

man No. 1 paper saturated with the developing solvent, were pre-equilibrated for 30 min, then developed ascendingly at room temperature (23°). The front solvent was allowed to reach 14.5 cm. After development the plate was dried under a stream of warm air and sprayed. Bromophenol Blue gives yellow spots; ammonium vanadate gives different colors, which are reported in Table I.

Results and discussion

Table II shows the R_F values of the acids, for each solvent, separated monodimensionally; the R_F values referred to the mixture are also given. With solvent A, only four of the eight substances tested could be separated: gluconic and glucuronic acids have the same R_F value as do saccharic and tartaric acids; glyceric acid is too close to tartaric acid (see Fig. 1).

With solvent B five substances could be well separated: glycolic acid was too close to oxalic acid; tartaric and saccharic acids have practically the same R_F values as do tartronic and glyceric acids (see Fig. 2).



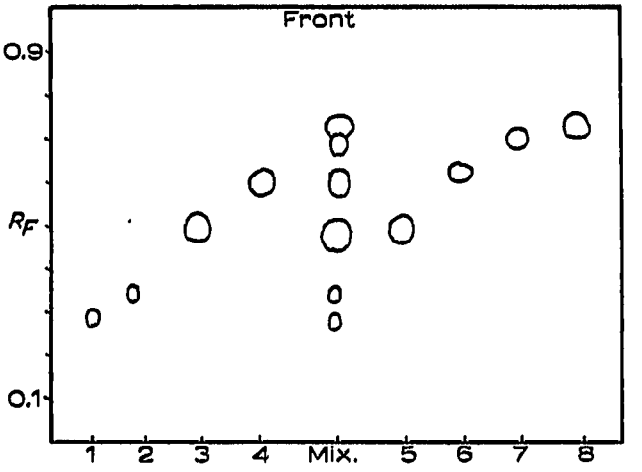


Fig. 1. Oxyacids separated monodimensionally on a cellulose plate using solvent A (isopropanol–ethyl acetate–water, 23.5:65:11.5). The mixture contained (from bottom to top) glucuronic, saccharic, tartronic and oxalic acids.

Fig. 2. Oxyacids separated monodimensionally on a cellulose plate using solvent B (formic acid–ethyl acetate–water, 1:3:1). The mixture contained (from bottom to top) glucuronic, gluconic, saccharic, glyceric, oxalic and glycolic acids.

Solvents C and D gave no better separation (as shown in Table II), and only four substances could be separated; in some cases solvent D gave a tailing effect.

In order to obtain a better separation, two-dimensional chromatograms were run using the combinations AB (first direction, solvent A; second direction, solvent B) and CD (first direction, solvent C; second direction, solvent D). With the combination AB, a good separation was achieved. Fig. 3 shows the position of each acid: tartaric acid is not present because it could not be separated from saccharic acid. With the combination CD, both tartaric and gluconic acid could not be separated from glucuronic acid (see Fig. 4).

In conclusion, using four different solvent systems monodimensionally, a rather poor separation was achieved. With solvent B we obtained satisfactory results: only

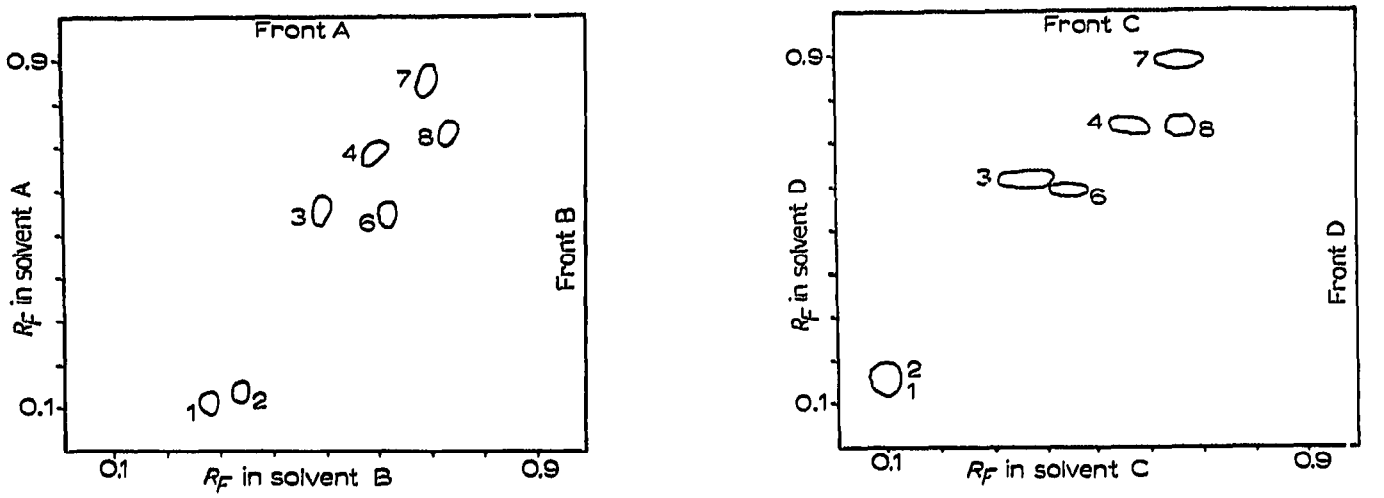


Fig. 3. Oxyacids separated two-dimensionally on a cellulose plate using solvent A (first direction) and solvent B (second direction). 1 = Glucuronic, 2 = gluconic, 3 = saccharic, 4 = tartronic, 6 = glyceric, 7 = oxalic, and 8 = glycolic acid.

Fig. 4. Two-dimensional separation of oxyacids on a cellulose plate using solvent C (first direction) and solvent D (second direction). 1 = Glucuronic, 2 = gluconic, 3 = saccharic, 4 = tartronic, 6 = glyceric, 7 = oxalic, and 8 = glycolic acid.

two of the eight test substances could not be separated. Using two-dimensional chromatograms, we obtained good results in both cases.

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