

THIN-LAYER CHROMATOGRAPHY OF 2,4-DINITROPHENYLHYDRAZINE DERIVATIVES OF HYDROXYCARBONYL COMPOUNDS

E. F. L. J. ANET

C.S.I.R.O., Division of Food Preservation, North Ryde, N.S.W. (Australia)

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In connection with our study of the degradation of carbohydrates it was necessary to separate complex mixtures of mono- and bis-2,4-dinitrophenylhydrazones derived from hydroxycarbonyl compounds. Although the chromatography of 2,4-dinitrophenylhydrazones has been studied extensively, very little has been done with those listed in Table I. WOLFROM AND ARSENAULT^{1,2} reported the separation of some of these compounds on columns of deactivated silica gel, whilst REICH AND SAMUELS³ use an aluminium oxide column to separate the derivatives of pyruvaldehyde and hydroxypyruvaldehyde. Thin-layer chromatography has now been found to be a rapid method of determining the purity of these hydrazones and of examining complex mixtures of them.

METHOD

Adsorbent

Aluminium oxide G* (20 g) mixed with water (40 ml) and silica gel G* (20 g) with water (40 ml) were used as adsorbents. They were applied as slurries, 1½ minutes after mixing, with an applicator⁴ giving a film 0.25 mm thick on glass plates (20 × 5 or 20 × 20 cm). The plates were air dried and could be activated further by drying at 100° for 2 hours. For some separations the plates were deactivated by holding them for a few hours at 20° in an atmosphere of 70% relative humidity. To obtain reproducible R_F values, the water content of the adsorbents must be constant since water lowers their activity. Activated plates were stored in a desiccator, but in hot humid weather some loss of activity took place during handling. However if standard reference substances are used on each plate, rigidly controlled conditions are not necessary.

Solvent

Mixtures of toluene and ethyl acetate were found to be the most useful solvent systems; increasing the proportion of ethyl acetate increased their eluting power. The best mixture depended on the compounds to be separated, the adsorbent, and its activity (see Table I).

Chromatographic procedure

Solutions of the hydrazones (0.01–0.1%) in acetone or tetrahydrofuran were applied to the plates by means of 1 µl capillary pipettes. After a few minutes the plates were

* E. Merck, A.G., Darmstadt.

placed in glass jars of appropriate size containing solvent at the bottom. The sides of the jars were lined with filter paper moistened with the solvent. The adsorbent side of each plate was close to and facing the paper.

The progress of the separation could be seen since all the hydrazones gave yellow spots. Usually when the solvent had travelled 15 cm the plate was removed and allowed to dry for 15 min. The spots were then sprayed with a solution of sodium hydroxide (2%) in ethanol (90%) to give intense characteristic colours (Table I), some of which took a few minutes to develop fully on the silica gel plates. The colours soon faded but a permanent record was obtained by colour photography.

Two-dimensional separations were carried out similarly on the square plates. With aluminium oxide useful results were obtained in our work by running the chromatogram first in solvent (c) on partially deactivated adsorbent, then allowing the plates to dry in air for 20 min and running them at right angles in toluene. Silica gel plates were run first in solvent (b) on activated adsorbent, air dried and run in toluene. The R_F values in the second solvent depended on the humidity of the atmosphere in which the plates were dried, and were higher than those in Table I, but in the same order.

Two-dimensional separations were also carried out with two different adsorbents (Fig. 1). The chromatograms were run as usual on the first adsorbent. After drying for 20 min excess adsorbent was scraped off the plate leaving a strip on one side with the spots of the first separation. The plate was placed in the applicator with the strip of the first adsorbent protruding from the applicator tank. The rest of the plate was coated in the normal way, air dried, and run in the second solvent. Plates precoated with both adsorbents gave unsatisfactory results since the solvents ascended the two adsorbents at different rates, causing distortion of the front and of the spots.

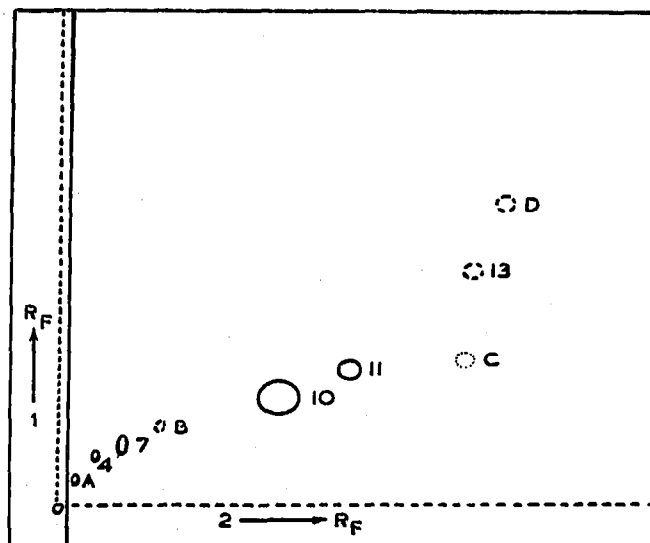


Fig. 1. Two-dimensional chromatogram on two adsorbents. The first adsorbent was silica gel (air dried) and the second aluminium oxide (deactivated). The same solvent system toluene-ethyl acetate 3:1 (v/v) ascended the full length of the plate (20 cm) in the two directions. The sample was the mixture of 2,4-dinitrophenylhydrazine derivatives obtained from the degradation products of fructose heated at 100° in 0.03 N oxalic acid for 2 h. A large proportion of compound 4 had been removed by crystallization from acetone. The colours given by the alkaline spray were: A, 4, B, and C purple; 10, 11, 13, and D brown; and 7 blue. The code for the numbers is as in Table I; A, B, C, and D are unidentified.

Preparation of the hydrazones

Most of the hydrazones (mono and bis) could be prepared from the parent carbonyl compounds with 2,4-dinitrophenylhydrazine (2%) in perchloric acid (30%) by the general procedure of NEUBERG, GRAUER, AND PISHA⁵. To prevent osazone formation, derivatives 1, 5, 6 and 9 (Table I) were prepared in the absence of acid, by refluxing a solution of the carbonyl compound and the hydrazine in ethanol^{3,6}. Refluxing solutions of α -hydroxymonocarbonyl compounds with 2,4-dinitrophenylhydrazine in 2 *N* hydrochloric acid yielded the osazones^{1,3,7,8}. This reagent gave with glycerose and dihydroxyacetone a mixture³ of two bishydrazones, 12 and 15, which were separated on an aluminium oxide column.

All the derivatives were obtained pure by recrystallization. Their melting points agreed with those in the literature, except for derivatives 7, 11, and 13 which are new and will be described elsewhere.

RESULTS AND DISCUSSION

Thin-layer chromatography of 2,4-dinitrophenylhydrazine derivatives of hydroxycarbonyl compounds achieved good separation as indicated in Table I. The method is quick, taking about 30 min, and requires extremely small samples. Spots of 0.1 μ g are

TABLE I
THIN-LAYER CHROMATOGRAPHY OF 2,4-DINITROPHENYLHYDRAZONES

2,4-Dinitrophenylhydrazone Parent carbonyl compound	Colour with NaOH	R_F value $\times 100$						
		Al_2O_3 plates				SiO_2 plates		
		a	b	c	d	a	b	c
1 D-Glucose	brown	0	0	0	0	0	0	0
2 D-Glucosone*	purple	0	0	0	3	0	0	10
3 3-Deoxy-D-threo-hexosone*	purple	0	0	0	15	0	2.5	23
4 3-Deoxy-D-erythro-hexosone (3-deoxyglucosone)*	purple	0	0	0	20	0	2.5	26
5 DL-Glycerose	brown	0	0	0	8	0	1.8	12
6 Dihydroxyacetone	brown	0	0	3	25	0	4	17
7 3,4-Dideoxy-trans- Δ^3 -D-hexosone*	blue	0	0	3	46	0	9	43
8 3,4-Dideoxy-D-hexosone*	purple	0	0	7	49	0	9	41
9 Glycolaldehyde	brown	0	13	38	67	0	16	39
10 5-(Hydroxymethyl)-furfural	brown	0	20	50	63	0	21	48
11 2-(Hydroxyacetyl)-furan	brown	1.5	32	60	70	2	26	50
12 Hydroxypyruvaldehyde*	purple	0	25	65	85	0	48	74
13 Bis-(5-methylenefurfural) ether	brown	1	59	85	85	1.5	45	66
14 Glyoxal*	purple	2	73	87	86	9	67	80
15 Pyruvaldehyde*	purple	5	77	89	87	13	70	80
16 Furfural	brown	42	83	89	88	27	62	74

(a) Toluene; (b) toluene-ethyl acetate 3:1 (v/v); (c) and (d) toluene-ethyl acetate 1:1 (v/v);
(a) (b) and (c) adsorbent activated at 100°, (d) adsorbent deactivated, see text.

* These are all $\alpha\beta$ -dicarbonyl compounds, their bishydrazones could also be derived by osazone formation from any of the corresponding α -hydroxy-monocarbonyl compounds. Number 2 was also prepared from D-glucose, D-mannose, and D-fructose, 4 from 3-deoxy-D-glucose, 14 from glycolaldehyde, and both 12 and 15 from either glycerose or dihydroxyacetone.

easily seen and their intensity is increased by the alkaline spray, specially for those giving blue or purple colours. The blue or purple colours seem to be specific for the 1,2-bis-derivatives.

Hydroxyl groups account for most of the adsorption and largely determine the R_F value of these hydrazones as can be seen from Table I, where they are listed in order of decreasing number of hydroxyl groups.

The R_F values also depend on the adsorbent, its activity, and on the solvent system as shown in Table I. It should be noted that reversal of order frequently takes place on changing either the adsorbent or the solvent system. To increase R_F values of polyhydroxy derivatives it is better to deactivate the adsorbent by increasing its water content than to increase the proportion of ethyl acetate beyond 50% since the spots of low R_F values become increasingly elongated. In the solvent systems in Table I all the compounds shown gave single round spots.*

Two-dimensional separations with one or two adsorbents (Fig. 1) were found most useful for the examination of complex mixtures, and this is undoubtedly due to the flexibility in the choice of experimental conditions as the adsorbent, its activity, or the solvent system may be varied for each direction.

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SUMMARY

A simple and rapid method is described for chromatographing mono- and bis-2,4-dinitrophenylhydrazones derived from hydroxycarbonyl compounds. The derivatives were separated by the thin-layer technique on aluminium oxide or silica gel plates with toluene-ethyl acetate mixtures as solvents. Spraying with sodium hydroxide intensified the spots and gave blue or purple colours only with the 1,2-bis-hydrazones. Complex mixtures were resolved by two-dimensional methods using the same or a different adsorbent for each direction.

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* The present work confirms the preparation of pure glycerose hydrazone by the method of REICH AND SAMUELS³. This hydrazone gave a single spot in all the systems in Table I and also on deactivated silica gel using benzene-ether as solvent (*cf.* WOLFROM AND ARSENAULT)^{1,2}.