

Paper chromatography of keto acid 2,4-dinitrophenylhydrazones

Numerous workers interested in the biologically important keto acids have appreciated the potential advantages to be gained by the chromatography of these unstable compounds as their 2,4-dinitrophenylhydrazones. These derivatives are in general relatively stable and by virtue of their colour easily located on chromatograms and estimated after elution. However, attempts at paper chromatography have met with only a fair measure of success. The principal disadvantage encountered has been the tendency of the compounds to travel as double spots, thus making interpretation of one-dimensional chromatograms confusing in many cases and of two-dimensional chromatograms impossible.

The introduction in recent years of modified forms of paper has prompted the reinvestigation described below.

Experimental

Materials. Most 2,4-dinitrophenylhydrazones were prepared by the addition of a solution of 2,4-dinitrophenylhydrazine (0.2 % w/v) in 2N HCl to an aqueous solution of the keto acid or its sodium salt; keto acids were obtained commercially or by synthesis. The products were dissolved in ethyl acetate before use; solutions stored at 4° did not appear to deteriorate in any way.

The derivative from 3,4-dihydroxyphenylpyruvic acid was prepared without isolation of the unstable¹ keto acid.

The derivative from 2,5-dihydroxyphenylpyruvic acid, formed in very poor yield from the sodium salt of the easily lactonised keto acid, was obtained in solution only.

In three cases, those of α -keto adipic acid, α -keto- β -methylvaleric acid and α -keto- γ -methylthiolbutyric acid, the keto acid was prepared by oxygenation of the corresponding L-amino acid in the presence of L-amino acid oxidase and catalase². The derivatives were formed by direct addition of 2,4-dinitrophenylhydrazine reagent to the crude reaction mixture. In these cases and the previous case the derivatives were extracted into ethyl acetate, then into aqueous NaHCO₃ and finally, after acidification, into ethyl acetate. Chromatography indicated that the products were homogeneous.

Chromatography. All chromatograms were carried out by the ascending method at room temperature (20–25°). Using the slower-running direction of the paper, a solvent flow of 20–25 cm required an overnight run for Whatman No. 20 paper but only about 3 h for Whatman No. SG 81 paper.

Results recorded in Table I were taken from single experiments in which all compounds were chromatographed simultaneously in the same tank; absolute R_F values were subject to the usual variation with conditions, particularly when volatile solvents were employed.

Discussion

Application of orthodox methods of paper chromatography to keto acid 2,4-dinitrophenylhydrazones added little to previous knowledge, except that the use of Whatman No. 20 paper rather than Nos. 1 or 4 was found to result in distinctly more compact spots. The most satisfactory solvents appeared to be of the type alcohol-water such as *sec.*-butanol-water (Table I). However, in some runs homologues of

TABLE I

 R_F VALUES ($\times 100$) OF KETO ACID 2,4-DINITROPHENYLHYDRAZONES

Solvents: (A) *sec.*-Butanol-water (4:1);
 (B) 5% w/v aqueous NaHCO₃;
 (C) Benzene-acetic acid (19:1);
 (D) Benzene-formic acid (19:1);
 (E) Chloroform-acetic acid (39:1);
 (F) 2-Nitropropane-formic acid (99:1).

Acid	Solvent A	Solvent B	Solvent C	Solvent D	Solvent E	Solvent F
	Whatman paper No. 20	Whatman paper No. 20	Whatman paper No. SG 81	Whatman paper No. SG 81	Whatman paper No. SG 81	Whatman paper No. SG 81
Glyoxylic	31 ^a	50 ^b	35 ^a	20 ^b	57 ^b	58 ^b
Pyruvic	42	55	47	33	80	73
α -Keto- <i>n</i> -butyric	64	22, 30, 60	52, 57	44	85	79
α -Keto- <i>n</i> -valeric	73	34, 58	56, 60	48	87	81
α -Keto-isovaleric	74	45	62	50	87	82
α -Keto- <i>n</i> -caproic	76	30, 55	61, 65	48	88	81
α -Keto-isocaproic	77	63 ^a	62	48	88	82
α -Keto- β -methyl- <i>n</i> -valeric	77	47	67	51	89	84
Ketomalonic	40	69 ^b	4	2	2	8
Oxalacetic	42	67 ^b	18	6	27	20
α -Ketoglutaric	44 ^a	54 ^b	21	8	42	26
α -Keto adipic	53	57	25	10	47	29
Phenylpyruvic	74	29, 56	63	49	88	80
<i>o</i> -Hydroxyphenylpyruvic	76	23	21	20	30	52
<i>m</i> -Hydroxyphenylpyruvic	69	30 ^b	19	12	35	60
<i>p</i> -Hydroxyphenylpyruvic	68	30	21	11	39	60
4-Hydroxy-3-methoxyphenyl- pyruvic	61	27	33	23	71	64
2,5-Dihydroxyphenylpyruvic	71	dec	5	1	9	28, 34
3,4-Dihydroxyphenylpyruvic	31	15	3	1	3	22
3-Indolylpyruvic	76	streaks	45	36	77	79
3-Indolylglyoxylic	51	1	13	14	28	53
α -Keto- γ -methylthiol- <i>n</i> -butyric	68	32	51	39	83	74
Acetoacetic	88 ^a	67	dec	dec	dec	dec
Laevulinic	84	61	58	43	86	64

^a Signifies that an additional weak second spot or streak was observed; dec indicates decomposition with no well defined spot visible or, in the case of acetoacetate, with the formation of the acetone derivative.

pyruvic acid gave double spots and dicarboxylic acids gave very low values. The same phenomena resulted from addition of ammonia to the solvent and probably indicates hypersensitivity of the procedure to the presence of volatile amines in the atmosphere. The use of aqueous solvents (*e.g.* NaHCO₃, Table I) was not satisfactory in general, but such may be of use in identifying dicarboxylic keto acids because of the high R_F values of their derivatives.

No success attended attempts to chromatograph 2,4-dinitrophenylhydrazones on ion-exchange papers (Whatman Nos. DE 20, AE 30 or ET 20), primarily because of double-spotting although individual compounds often behaved satisfactorily. Use of silicone-treated paper (Whatman No. ST 82) was not successful. Thin-layer chromatography on silica gel or Kieselguhr offered little promise in preliminary experiments in that it was never possible to obtain simultaneously both compact single spots and adequate separations.

Much more promising results were obtained using papers loaded with silica gel (Whatman No. SG 81) or aluminium hydroxide (Whatman No. AH 81). Only the former paper, which appeared much the superior, was investigated in detail.

Some excellent results were observed, small compact single spots being usually obtained. Nevertheless only a few of many solvent systems tried were satisfactory, these being mainly hydrocarbons or chlorinated hydrocarbons in the presence of small amounts of organic acids. Typical examples are recorded in Table I. The relative order of R_F values was much the same in all solvents and absolute values could be controlled by variation of the amount of acid incorporated in the solvent mixture. Other solvents yielded double spots or unduly high R_F values (or both) but 2-nitropropane containing formic acid proved useful particularly since the relatively high R_F values of phenolic derivatives enables its use as a second solvent for two-dimensional work.

It is interesting that of the two β -keto acid 2,4-dinitrophenylhydrazones included in the series examined, that of oxalacetic acid appeared to be quite stable to the conditions of chromatography on Whatman No. SG 81 paper, whilst that of acetoacetic acid decomposed completely—the initial spot immediately separated into two, the slower running of which rapidly disappeared to form a trail behind the faster running (acetone) spot.

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