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Separation of 2,4-dinitrophenylhydrazones into classes by thin-layer chromatography on zinc carbonate

The separation of complex mixtures of carbonyl compounds is a problem which often arises in natural products' chemistry. Conversion of the carbonyl compounds to their 2,4-dinitrophenylhydrazone (2,4-DNPH) derivatives and separation of these by thin-layer chromatography (TLC) is an effective solution. The most frequently employed techniques use adsorption or partition chromatography on silica gel or aluminum oxide, with hydrocarbon or other non-polar solvents[1,2].

In basic solution, 2,4-DNPH derivatives of many carbonyl compounds undergo a distinct change in color. This fact is often made the basis of visualizing small amounts of 2,4-DNPH's on chromatograms. In the presence of base and certain inorganic compounds, such as zinc carbonate or magnesium oxide, a reversible reaction occurs resulting in a change in the adsorptive properties of the 2,4-DNPH's to the inorganic compound and a further change in color. Advantage may be taken of this phenomenon to alter chromatographic behavior of the derivatives[3-6].

In the course of our work, a method was required to separate complex mixtures of carbonyl compounds of widely varying structures. We have developed a procedure which is based on a preliminary separation of the complex mixture of 2,4-DNPH's into classes by TLC on zinc carbonate, using pyridine as developing solvent. This yields separation into three classes of derivatives, based on the structures of the parent carbonyl compounds: aliphatic monocarbonyls, aromatic monocarbonyls, and bis-derivatives of dicarbonyls. These classes are distinguished by R_F and color.

Material and methods

Preliminary class separation. The twenty 2,4-DNPH derivatives used in this study are shown in Table I, along with their color, R_F value and class in the zinc carbonate–pyridine system. All were prepared according to standard procedures[7,8] and recrystallized until a constant melting point in agreement with literature values was obtained. Standard solutions were prepared by dissolving 20 mg of each derivative in 10 ml of reagent-grade pyridine. Mixtures were made by mixing aliquots of the standard solutions. All solutions were brightly colored, ranging from yellow to dark red-brown.

Thin layers of 0.25 mm thickness were prepared on 20 × 20 cm glass plates using an adjustable Desaga spreader. To make sufficient slurry for five plates, 30 g of analytical-grade zinc carbonate was mixed with 60 ml of distilled water and shaken vigorously for several minutes. Prior sieving of the zinc carbonate through a 100-mesh screen was sometimes necessary, depending on the source of the material. After spreading, the layers were dried at room temperature, and activated for 30 min at 130° immediately before use. A layer of satisfactory stability could be prepared in this way without the addition of a binder.

From 1 to 5 μl of the standard solutions were applied to the layers. Most derivatives underwent a distinct color change when applied from pyridine solution. Chromatography was done in glass chambers whose sides were lined with filter paper to facilitate saturation of the atmosphere. The developing solvent, pyridine, was allowed to ascend 10 cm. With some lots of zinc carbonate, best results were obtained if a small amount of water was added to the pyridine (several drops of water in 100 ml of pyridine). The characteristic colors present during development rapidly faded as pyridine evaporated from the finished chromatogram, but reappeared when the layer was lightly sprayed with pyridine.

TABLE I

2,4-DNPH DERIVATIVES AND THEIR PROPERTIES IN THE ZINC CARBONATE–PYRIDINE SYSTEM

2,4-DNPH derivative of	*Color*	*R_F values*[a]	*Class*
Acetone	Yellow-brown	0.82	I
Acetaldehyde	Yellow-brown	0.83	I
n-Butyraldehyde	Yellow-brown	0.63–0.81	I
2-Butanone	Yellow-brown	0.85	I
3-Pentanone	Yellow-brown	0.92	I
Cyclopentanone	Yellow-brown	0.95	I
Cyclohexanone	Yellow-brown	0.88	I
Benzophenone[b]	Yellow-brown	0.84	I
Furfural	Pink-purple	0.07–0.3	II
Benzaldehyde	Pink-purple	0.16–0.44	II
Acetophenone	Pink-purple	0.35–0.66	II
2-Chloroacetophenone	Pink-purple	0.25–0.58	II
Benzoin	Pink-purple	0.14–0.29	II
Formaldehyde[b]	Pink-purple	0.38–0.68	II
Glutaraldehyde[b]	Pink-purple	0.10–0.25	II
p-Benzoquinone, bis-derivative	Dark blue	0	III
Anthraquinone, bis-derivative	Blue	0.02	III
Benzil, bis-derivative	Violet	0–0.13	III
Isatin, bis-derivative	Red	0	III
Glyoxal, bis-derivative	Dark blue	0	III

[a] If the compound moved as a circular or oval zone, the R_F value given is measured from the starting line to the center of the zone. If the compound streaked significantly, the range of R_F values from the bottom to the top of the streak is given.

[b] These derivatives are exceptions to the classification scheme discussed in the text.

Preparative separation. We have devised a procedure for separating complex mixtures of 2,4-DNPH's which utilizes class separation with the zinc carbonate–pyridine system as the first step. The mixture is applied as a streak to several preparative zinc carbonate layers (0.50 or 0.75 mm thick) and developed with pyridine. The finished chromatograms are divided into three areas, each containing one class of compounds (see *Results* and Fig. 1), which are scraped from the plates and extracted with ethyl acetate. Each class is then resolved into its individual components by one- or two-dimensional TLC on silica gel. For instance, the derivatives in each class listed in Table I were separated on silica gel by development in the first dimension with ethyl acetate–hexane (1:4), and in the second dimension with *p*-xylene for class I, di-*n*-butyl ether for class II, and lutidine–hexane (1:4) for class III.

Results

The separation of a mixture of all the 2,4-DNPH's listed in Table I using the zinc carbonate–pyridine system is shown in Fig. 1. The compounds separated into three distinct classes characterized by R_F and color. The first class, located near the solvent front, was yellow-brown and contained derivatives of aliphatic monocarbonyl compounds. Class II occupied a large central region of the finished chromatogram, with R_F values ranging from 0.1 to about 0.65. This class was composed of derivatives of aromatic monocarbonyl compounds, and was pink-purple. The third class contained bis-derivatives of both aliphatic and aromatic dicarbonyl compounds. These derivatives had low R_F values and possessed very vivid red, blue, and violet colors.

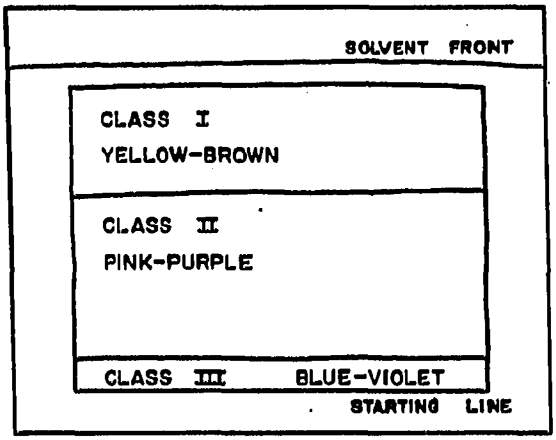

Fig. 1. Separation of the twenty derivatives listed in Table I into classes using the zinc carbonate–pyridine system. The starting mixture was applied as a streak across the bottom of the plate, and solvent was allowed to ascend 10 cm beyond starting line.

Out of the twenty compounds tested, there were three which did not fit into this classification. These were benzophenone which showed characteristic class I behavior, and formaldehyde and glutaraldehyde, which were found in class II.

The derivatives were also distinguished by the shape of the zone, when chromatographed individually. Class I compounds moved as fairly small, circular or oval spots; class II compounds showed extensive streaking; and class III compounds formed very small, compact zones, sometimes exhibiting slight streaking.

Thin layers of magnesium oxide were also used to effect class separation of 2,4-DNPH's, again with pyridine as developing solvent. However, generally better and faster separation of the classes was achieved on zinc carbonate.

Discussion

Zinc carbonate was first used in the chromatography of 2,4-DNPH's by Van Duin[3]. Using solvents containing small amounts of pyridine, Van Duin and others[9] were able to separate the derivatives of aliphatic monocarbonyl compounds into groups containing alkan-2-ones, alkanals, alk-2-enals, and alka-2,4-dienals. Group separations similar to this were also achieved by Urbach[5] on silver nitrate-impregnated aluminum oxide and by others[6,10,11] using magnesium oxide. Our procedure differs from all these in that the classes obtained are composed of derivatives of aliphatic monocarbonyls, aromatic monocarbonyls and bis-derivatives of dicarbonyl compounds, rather than subclasses of aliphatic monocarbonyls. For mixtures of carbonyl compounds containing widely varying structures, a class separation of the kind described here might be a useful first step in the complete resolution of the mixture.

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