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Separation of Aliphatic Alcohols by Chromatographic Adsorption of Their 3,5-Dinitrobenzoates

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Forty pairs of aliphatic 3,5-dinitrobenzoates have been subjected to chromatographic adsorption by Brockmann's fluorescence technique. In this procedure, ultraviolet radiation shows the adsorbed zones as dark bands on a bright fluorescent background. Of these 40 pairs, involving 12 aliphatic alcohols from methyl to hexyl, 23 yielded two zones, 11 gave a single zone of varying composition, and 6 were completely inseparable. The normal primary alkyl dinitrobenzoates from methyl to amyl were easily separated from one another.

CHROMATOGRAPHIC methods have proved of great value in separating small quantities of similar compounds. They have been applied chiefly to naturally occurring colored substances of more or less complex structure. Application to colorless compounds depended on the development of suitable means for observation of the chromatogram. This has been accomplished in several ways: empirically, by examining successive filtrate fractions or by arbitrary sectioning of the extruded chromatogram; by streaking the extruded column with a color-producing reagent; by pretreating the adsorbent or washing the developed column with a reagent that gives a reversible color reaction with the adsorbate; by examining under ultraviolet radiation if the substances fluoresce; by converting the substances to colored or fluorescent derivatives before adsorption; and by introducing a colored substance with adsorptive properties similar to those of the colorless materials.

In a new and elegant procedure for the location of adsorbed bands of colorless and nonfluorescent materials, described by Brockmann and Volpers (*1*), the adsorbents are pretreated with a fluorescent substance so that the entire column becomes fluorescent. Under ultraviolet illumination, bands of adsorbed nonfluorescent material then reveal themselves by a local absence or diminution of fluorescence. By using only radiation absorbed by the substances being separated, such chromatographic procedures can be extended to many colorless substances. Brockmann and Volpers have shown that by using 2537 Å. radiation and quartz adsorption tubes it is possible to locate adsorbed substances that show absorption in this region. They stated that ergosterol and ergosteryl acetate, invisible with 3650 Å. radiation on a fluorescent column, were easily seen when 2537 Å. radiation was used.

The fluorescent material for treating the adsorbent must be chosen with the following essentials in mind: It must not react with the substances being separated, and it must be strongly adsorbed so that it will not be displaced or leached from the column on development or elution. Brockmann and Volpers used morin on alumina, calcium carbonate, and magnesium oxide, berberine on silicic acid, and diphenylfluorindine sulfonic acid on calcium carbonate. Each of these was adsorbed from methanol solution. The following separations on alumina-morin were described: xylene musk, musk ambrette, and musk ketone; ergosteryl and cholesteryl *p*-nitrobenzoates; several aromatic aldehydes; phorone–mesityl oxide; and the *p*-phenylphenacyl esters of acetic and benzoic acids.

Sease (*4*) used fluorescent adsorption columns prepared by mixing fluorescent zinc sulfide with ordinary adsorbents. He separated mixtures of cinnamaldehyde, xanthone, *p*-nitrobenzyl bromide, salicylaldehyde, azoxybenzene, nitrobenzene, and iodoform on silicic acid by this technique.

Table I. Chromatographic Separation of 40 pairs of Dinitrobenzoates Involving 12 Aliphatic Alcohols

	Ethyl	*n*-Propyl	Iso-propyl	*n*-Butyl	Iso-butyl	*sec*-Butyl	*tert*-Butyl	*n*-Amyl	Iso-amyl	2-Pentyl	*n*-Hexyl
Methyl	G										
Ethyl		G	G								
n-Propyl			P	G	G	G	G				
Isopropyl				F	F	F	G				
n-Butyl					O	P	F	G	G	G	G
Isobutyl						P	F	F	F	G	G
sec-Butyl							P	P	P	G	G
tert-butyl								O	O	P	P
n-Amyl									O	P	P
Isoamyl										O	P
2-Pentyl											O

G = good separation. Bands well separated; components recovered with melting point differing by 2° or less from that of original crystalline material.
F = fair separation. Bands close together; components recovered with some contamination, as shown by change in melting point of one or both.
P = poor separation. Single band, but top and bottom halves were distinctly different in composition, as shown by difference in melting points.
O = no separation. Top and bottom halves of band showed no significant difference in melting points.

The commonly used identification derivatives of the alcohols are neither colored nor fluorescent, and until the procedure just described was reported there was little prospect for application of chromatographic procedures to the separation of aliphatic alcohol derivatives. [The method of "flowing chromatography" described by Claesson (*2*), although applicable to the free alcohols, requires considerable elaborate equipment.] Strain (*6*) suggested that alcohols be separated by chromatographic adsorption of the colored esters of keto acid dinitrophenylhydrazones. The small-scale preparation of these compounds is somewhat difficult, however, and data for more than a few members of the series are not available.

Because the dinitrobenzoates, commonly used for the identification of alcohols, show near-ultraviolet absorption, the authors have studied their separation, using the principle described above. These derivatives are easy to prepare and do not require anhydrous alcohols. Moreover, the melting points of a large number are recorded in the literature.

The adsorbents and fluorescent compounds used in preparing preliminary columns for the separation of the 3,5-dinitrobenzoates were as follows: magnesia-morin, alumina-morin, magnesia-uranine, Magnesol-uranine, Magnesol-oxine, Silene EF–oxine, silicic acid–oxine, silicic acid–rhodamine B (C.I. 749), silicic acid–thioflavine T (C.I. 815), and silicic acid–rhodamine 6G (C.I. 752). Of these ten combinations, the latter two were most satisfactory; the silicic acid–rhodamine 6G afforded better visibility under weak ultraviolet radiation, and was used, therefore, for the work reported below.

The 3,5-dinitrobenzoates of 12 aliphatic alcohols, from methyl to *n*-hexyl, were investigated. Of the 66 possible pairs, 40, including all those close together in the series, were subjected to chromatographic adsorption. Of these, only 6 pairs were completely inseparable; 11 yielded a single zone of varying composition; and the remaining 23 separated with different degrees of sharpness into two bands. The adsorption procedure described below has been successfully applied to the separation and identification of various alcohols in volatile apple concentrate.

EXPERIMENTAL

Preparation of Adsorbent. Silicic acid [(Mallinckrodt AR, precipitated), 610 grams] and a diatomaceous filter aid (305 grams) were mixed dry and then dispersed in methanol (2 liters), and a solution of 40 mg. of rhodamine 6G (Calcozine red 6G extra, Color Index #752) in 50 ml. of methanol was added with stirring. (The filter aid should be white, so as not to interfere with the visibility of the fluorescence of the treated adsorbent.) The slurry was filtered on a Büchner funnel, washed on the funnel with 1 liter of methanol, and left on the funnel until dripping ceased. It was then transferred to a pan and dried in a vacuum oven at about 62.5 cm. (25 inches) of mercury and 100° to 120° C. for 18 hours. The quantity of dye is not critical but should be sufficient to give a colored methanol filtrate.

The dried adsorbent was well shaken and stored in a tightly capped bottle. It was pink in daylight and showed a yellow fluorescence under ultraviolet radiation.

Solvents. The dinitrobenzoates were initially adsorbed from hexane (a petroleum ether fraction boiling at 63° to 70° C.). Development was carried out with a 5.0% (by volume) solution of ether in hexane. Ether was used for elution. (The ether was a C.P. diethyl ether, not further purified. Its label stated that ether of that specification normally contained about 2% alcohol and 0.5% water.)

Preparation of Adsorption Column. A glass column 12 mm. in inside diameter, approximately 300 mm. long, and constricted at the lower end, was used. With a cotton plug in the lower end and suction applied, the dry powder was poured in. To obtain a closely packed column, the side of the tube was tapped while the powder was added. The column was filled to a height of 170 to 200 mm., and the top tamped lightly.

Ultraviolet Sources. A Hanovia Inspectolite was used for the work. Subsequently it was found that a 45-cm. (18-inch), 15-watt Sylvania Blacklite fluorescent-type tube was well adapted, as it provided an even radiation over the entire length of the adsorption column.

Procedure. Approximately 20 mg. of each of two alkyl dinitrobenzoates were weighed to the nearest milligram, dissolved in the minimum volume of warm hexane, and poured into the tube with suction applied. [The 3,5-dinitrobenzoates were prepared by the pyridine procedure outlined by Shriner and Fuson (*5*).] After the solution had passed into the column, the developing solvent (5.0% ether in hexane) was added, the vacuum removed, and development carried out under air pressure of about 30 cm. of mercury. The dinitrobenzoates were visible under ultraviolet radiation as dark brown bands on a yellow fluorescent background. Development was continued until the bands were completely separated, whereupon they were either washed through and collected separately or dug out of the column by a long narrow spatula under ultraviolet inspection, and then eluted with ether. When only a single band was obtained, the band was divided in half, and the halves were eluted separately. The filtrates or eluates were evaporated to dryness on a steam bath, and the melting points of the evaporated residues determined. No recrystallization was ordinarily done, as this might have resulted in some fractionation of mixtures.

RESULTS AND DISCUSSION

Table I shows the results obtained by the adsorption of 40 pairs of dinitrobenzoates involving six normal primary alcohols, two branded-chain primary alcohols, three secondary, and one tertiary alcohol. The pairs marked G and F were separated sufficiently to make two zones visible, with a yellow fluorescent band between. In the pairs marked F, there was so little space between the zones that some admixture probably resulted during removal from the column. In practice, sacrifice of yield or a second adsorption would give good separation in these cases. The pairs marked P gave only a single zone on development, but when the band was arbitrarily divided the two parts gave materials of different melting points, showing that a mixture was initially present. Finally, in the pairs marked O, no separation was obtained, as there was no significant difference in the melting points of the material from the upper and lower halves of the zone.

The blank spaces in the upper right part of Table I represent combinations not investigated, but as the derivatives are listed in the table in order of decreasing strength of adsorption, each of these 26 pairs should be completely separable without difficulty.

Table II shows typical examples of each of the four degrees of separation obtained, with a description of the developed column in each case and the weight, melting point, and identity of the fractions obtained.

When mixtures of the dinitrobenzoates of alcohols of a homologous series are separated by this procedure, they wash through in

the order of molecular weight, the heaviest passing through first. The derivatives of the first five normal primary alcohols can be completely separated from their mixture, five bands appearing. A mixture of *n*-amyl and *n*-hexyl derivatives gives a single band, which may be resolved by arbitrary division and readsorption.

When mixtures of dinitrobenzoates with the same number of carbon atoms are separated, the secondary alcohols wash through below the primary, and the tertiary (judging from *tert*-butyl, the only tertiary alcohol studied) below the secondary. Such separations are poor, however; no actual separation into bands was obtained on the column.

Table II. Description of Chromatograms of Typical Mixtures of Alkyl Dinitrobenzoates Showing Different Degrees of Separation

Degree of Separation (Table I)	Original Mixture	Column after Development	Material from Upper Zone[a]	Material from Lower Zone[a]
Good (G)	26 mg. methyl, m.p., 106° 21 mg. ethyl, m.p., 91–3°	94 mm. yellow 29 mm. brown 30 mm. yellow 19 mm. brown	23 mg. methyl, m.p. 107°	18 mg. ethyl, m.p. 92°
Fair (F)	22 mg. isobutyl, 86–9° 24 mg. isoamyl, 61–3°	162 mm. yellow 23 mm. brown 2 mm. yellow 23 mm. brown	24 mg. isobutyl, m.p. 79–80°	20 mg. isoamyl, m.p. 56–7°
Poor (P)	22 mg. *n*-propyl, 74–8° 22 mg. isopropyl, 121–3°	166 mm. yellow 42 mm. brown[b]	19 mg.[c], m.p. 83–7°	21 mg.[d], m.p. 100–2°
None (O)	21 mg. *n*-amyl, 44° 21 mg. isoamyl, 61–3°	159 mm. yellow 32 mm. brown[b]	18 mg., m.p. 39–40°	22 mg., m.p. 40–3°

[a] Melting points of recovered materials are in all cases those of evaporated residues, not recrystallized.
[b] Single zones divided in half before elution.
[c] By mixed melting point found to be mostly *n*-propyl derivative.
[d] By mixed melting point found to be mostly isopropyl derivative.

Primary derivatives containing the same number of carbon atoms—e.g., *n*- and isobutyl, *n*- and isoamyl—cannot be separated by this method.

Secondary or tertiary derivatives of one group may overlap with the primary derivatives of the next higher group and thus prevent separation—for example, *tert*-butyl cannot be separated from *n*-amyl or isoamyl; 2-pentyl cannot be separated from *n*-hexyl.

In the work reported above, individual dinitrobenzoates were prepared and then mixed. As a check on the applicability of the procedure to the separation of alcohols for identification purposes, the following experiment was done.

Methyl, ethyl, *n*-propyl, *n*-butyl, and *n*-amyl alcohols were mixed, and the mixture was reacted with dinitrobenzoyl chloride in the presence of a small amount of pyridine. About 100 mg. of the liquid product were washed with petroleum ether, which was then adsorbed on the column. The reaction product was adsorbed without preliminary crystallization in order to be certain of the presence of all five alcohol derivatives in the adsorbed solution. After development, five well defined zones were obtained; the melting points of the materials from the zones confirmed the separation.

Any dinitrobenzoic acid in the samples is very strongly adsorbed at the top of the column and easily separable from any of the derivatives examined.

The visibility of the adsorbed bands under ultraviolet radiation is excellent. On columns of the size used above, bands containing about 1 mg. of derivative can be discerned. Visibility of faint bands is considerably increased if the column is examined from above with the line of sight making an angle of about 30° with the side of the tube. The stronger bands are also visible in daylight, showing as reddish pink bands on the salmon-pink background, but the visibility in daylight is not good enough to allow elimination of ultraviolet examination. This visible color varies with the particular batch of adsorbent used.

VARIATION IN ADSORBENTS

The work reported above was done entirely with one lot of silicic acid (Mallinckrodt AR, control RXN1). In order to survey the effect of source of adsorbent, four other silicic acids were tested. In each case a solution containing methyl, ethyl, *n*-propyl, *n*-butyl, and *n*-amyl dinitrobenzoates was adsorbed and developed. Such a mixture was separated into five bands by the Mallinckrodt preparation (control RXN1).

Mallinckrodt AR (control REY) separated the test mixture into four bands, the lowest containing butyl and amyl derivatives, which were poorly separated. (Control RXN1 had been shipped and stored in a fiberboard drum; control REY had been shipped and stored in a glass jar. When the latter material was exposed in a thin layer to the laboratory atmosphere for 24 hours before treatment with the rhodamine 6G, it gave five bands in this test.)

Merck reagent grade (control 357) separated the test mixture into five bands.

Baker C.P. powdered (control 31,546) separated the test mixture into five bands, but the separation between the butyl and amyl bands was faint. It was necessary to grind this absorption to 150-mesh before use. Silicic acid was prepared from sodium silicate solution by the procedure described by Ramsey and Patterson (*3*). A column made up of 150-mesh material separated the test mixture into four bands.

REGENERATION OF ADSORBENT

When the adsorbed zones had been washed through the column, it could be prepared for re-use by washing with hexane. When the zones were mechanically removed from the column before elution, the adsorbent after elution was saved and regenerated by washing with methanol and drying as in the original preparation of the adsorbent. The activity of regenerated adsorbent was fully equal to that of the original material.

There was no tendency for the fluorescent dye to leach from the column when washed with hexane, the developing solution, or the ether eluant. More active eluants might cause some dye to appear in the filtrate. It was not necessary to use more active eluants to remove the dinitrobenzoates. In one case, ether eluted 97.3% of the adsorbed methyl dinitrobenzoate (the most strongly adsorbed dinitrobenzoate studied) from the column.

No evidence of decomposition of the adsorbed dinitrobenzoates by the ultraviolet radiation was obtained, but as a precaution the columns were shielded from the source except when observations were being made.

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