FRACTIONATION OF COMPLEX MIXTURES OF 2,4–DINITRO– PHENYLHYDRAZONES*

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The advantages of partition over adsorption chromatographic columns for the initial fractionation of mixtures of 2,4-dinitrophenylhydrazones have been increasingly appreciated during the last few years. The literature however reveals little awareness that while partition systems will separate the members of an homologous series, they will not effectively separate dinitrophenylhydrazones of different classes of carbonyl compounds of similar chain-length. The compositions of the fractions obtained from such mixtures will depend on the partition system used (e.g. Kramer and van Duin 1954; Monty 1958) but it would be possible to have included in the one fraction the dinitrophenylhydrazones of, for instance, a C_n alkan-2-one, a C_{n+1} *n*-alkanal, a C_{n+2} alk-2-enal, and a C_{n+4} alka-2,4-dienal.

There are few references to systems suitable for further separating such a mixture, though zinc carbonate columns and/or chromatoplates (van Duin 1958; Badings 1959), and the reverse phase chromatographic system of Ellis, Gaddis, and Currie (1958) have been used with success. In the latter technique, sheets of paper are impregnated with vaseline, spotted with the 2,4-dinitrophenyl-

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hydrazones and developed with a mixture of methanol and water. When only small quantities $(1-20 \ \mu g)$ of compounds are available or when limited information on a fraction rather than characterization of its constituents is required, sheet-paper chromatograms are suitable. However, chromatographic columns are more convenient for the separation of larger amounts $(0.02-10 \ mg)$ of compounds and are thus well suited to the characterization of the separated compounds.

This paper describes the use of alumina adsorption columns for the separation of mixtures of 2,4-dinitrophenylhydrazones of alkan-2-ones, *n*-alkanals, alk-2enals, and alka-2,4-dienals. As early as 1935, Strain suggested the use of alumina columns for the separation of 2,4-dinitrophenylhydrazones. While they have

TABLE 1						
BINARY	MIXTURES	OF	2,4 - DINITROPHENYLHYDRAZONES			
SEP.	ARATED ON AI	UMIN	A CHROMATOGRAPHIC COLUMNS			

Mixture	First Compound Eluted	Second Compound Eluted	
1*	Propionaldehyde	Hexa-2,4-dienal	
2*	n-Butanal	Pent-2-enal	
3	n-Butanal	Hepta-2,4-dienal	
4	n-Pentanal	Hex-2-enal	
5^{*+}	n-Hexanal	Hept-2-enal	
6*	n-Octanal	Hendeca-2,4-dienal	
7*	Butanone	Hepta-2,4-dienal	
8	Pentan-2-one	Hept-2-enal	
9*	Heptan-2-one	<i>n</i> -Heptanal	
10	Heptan-2-one	Non-2-enal	
11	Nonan-2-one	n-Nonanal	
12*	Pent-2-enal	Hepta-2,4-dienal	
13*	Hept-2-enal	Nona-2,4-dienal	
14	Oct-2-enal	Deca-2,4-dienal	
15	Non-2-enal	Hendeca-2,4-dienal	

Carbonyl Compounds

* Mixture also separated by paper chromatographic method of Ellis, Gaddis, and Currie (1958).

[†] Mixture only partially separated by both techniques.

been little used for the separation of complex mixtures, the authors have found them most effective when used for secondary separations as described in this paper. In our work, mixtures of 2,4-dinitrophenylhydrazones, prepared from synthetic compounds or obtained from oxidized dairy products and given a primary separation on Celite-nitromethane partition columns, were added to alumina columns packed in light petroleum. The 2,4-dinitrophenylhydrazones were developed with light petroleum containing increasing amounts of diethyl ether (0.5-10%) and the eluted compounds were characterized by their movement on paper chromatograms, light absorption maxima in both ethanol and ethanolsodium hydroxide, and by melting and mixed melting points (Forss, Dunstone, and Stark 1960). Table 1 lists fifteen binary and Table 2 two quaternary mixtures

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separated on alumina columns. Eight of the binary mixtures (marked with an asterisk in Table 1) were also separated by the method of Ellis, Gaddis, and Currie (1958) with both ascending and descending development.

Two chromatographic systems can be successfully combined for the purification of unknown mixtures of 2,4-dinitrophenylhydrazones. The first should be a partition system which will separate within an homologous series of compounds while the second which may be a partition or an adsorption system should be used to resolve the constituents of the fractions obtained from the first system. Under most conditions alumina columns appear to provide the most convenient means of further separating, for characterization purposes, fractions obtained from partition columns.

TABLE 2								
QUATERNARY MIXTURES OF 2.4-DINITROPHENYLHYDRAZONES SEPARATED ON ALUMINA	COLUMNS							

Mixture	First Compound	Second Compound	Third Compound	Fourth Compound
	Eluted	Eluted	Eluted	Eluted
$\frac{1}{2}$	Butanone	n-Pentanal	Hex-2-enal	Octa-2,4-dienal
	Heptan-2-one	n-Octanal	Non-2-enal	Hendeca-2,4-dienal

Carbonyl Compounds

Experimental

Alumina, chromatographic grade ("British Drug Houses") was digested with 2% HNO₃ at 60 °C for 1 hr, collected on a Buchner funnel, and washed thoroughly with distilled water. It was dried at 350 °C for 8 hr, by which time it had an activity of 2 according to the method of Brockmann and Schodder (1941).

The required amount of alumina was added to the chromatographic column containing light petroleum (b.p. 40–60 °C, free of aromatic hydrocarbons) and was preferably allowed to degas overnight. The alumina was then compacted by tapping the column with a wooden rod.

The mixture of 2,4-dinitrophenylhydrazones was dissolved in a small volume of light petroleum and added to the chromatographic column where it formed a band about 1 mm high. A column 15×1 cm would be suitable for the separation of a 2 mg mixture of two 2,4-dinitrophenylhydrazones. After washing the mixture with about 30 ml light petroleum, 100 ml of 0.5%diethyl ether in light petroleum was added. If there was no movement, a further 100 ml 1% ether was added and so on until the 2,4-dinitrophenylhydrazones moved at a rate of 2-3 cm/hr. Separation was usually achieved in a few hours.

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