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UNIVERSITY OF LOUISVILLE

SEPARATION AND IDENTIFICATION
OF THE 2,4-DINITROPHENYLHYDRAZONES
OF THE OXIDATION PRODUCTS OF GLYCEROL

A Dissertation

Submitted to the Faculty

Of the Graduate School of the University of Louisville

In Partial Fulfillment of the

Requirements for the Degree

Of Master of Science

Department of Chemistry

Gwendolyn Parker McClure

1948

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NAME OF STUDENT: Gwendolyn F. McClure

TITLE OF THESIS: Separation and
Identification of
the 2,4-Dinitrophenyl-
hydrazones of the
Oxidation Products
of Glycerol

APPROVED BY READING COMMITTEE COMPOSED OF THE
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DATE: Aug 16, 1948

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PURPOSE

The purpose of this research was to separate chromatographically the 2,4-dinitrophenylhydrazones of the oxidation products of glycerol. The chromatographic method was chosen because no other procedure afforded such adequate separation. Since the compounds formed were so similar in structure that the usual methods of identification other than melting point could not be used, comparative chromatography and comparative spectrophotometry were utilized.

PREPARATION

OXIDATION OF GLYCEROL

The materials with which this paper is concerned, the oxidation products of glycerol, were prepared by the method of C. E. Moore (7). Glycerol was oxidized with hot potassium dichromate-acetic acid solution

with the result that any of the following compounds theoretically might be possible:

FORMATION OF 2,4-DINITROPHENYLHYDRAZONES

Since all of the above compounds are colorless, the advantages of converting them to colored derivatives for the purposes of separation and identification are obvious. All except glycerol, dihydroxy propionic acid and hydroxy malonic acid could theoretically react with 2,4-dinitrophenylhydrazine to yield the following 2,4-dinitrophenylhydrazones:

The formation of many of these compounds was of course unlikely, but the actual problem consisted of determining which ones were present and identifying them. The presence of particular ones of these would ascertain just which of the glycerol oxidation products were present.

CHROMATOGRAPHY

The use of chromatography appeared to offer the best means of separation of the 2,4-dinitrophenylhydrazones of these oxidation products of glycerol because of its unique applicability to a problem of this type. Tswett (11) in 1906 first stumbled upon this simple technique when he poured a petroleum extract from green leaves down a vertical tube of calcium carbonate and found that the apparently homogeneous substance separated out into bands of different color. Becoming aware of many of the innumerable possibilities of this method, he also obtained lecithin crystals from egg yolk. However, these isolated experiments did not convince other investigators of the scope of chromatography, and the method was practically abandoned until 1931 when it was used by Kuhn, Winterstein and Lederer (6) to split carotene into its alpha and beta components and to isolate each of these. Since that time the literature has been filled with various applications, theories and concepts of chromatography. Among the principal applications as cited by Strain (9) are:

1. Resolution of mixtures into their constituents
2. Determination of the homogeneity of chemical substances
3. Comparison of substances suspected of being identical
4. Purification of substances
5. Concentration of materials from dilute solutions
6. Recognition and control of technical products
7. Quantitative separation of one or more constituents from complex mixtures
8. Determination of molecular structure
9. Combination with electrophoretic separations
10. Regeneration of substances from complex addition compounds.

Chromatographic work is usually carried out in vertically placed glass columns which are packed with an adsorbant. The chromatogram is formed upon the addition of the material under investigation and is recognized by the formation of visible bands when work is with colored compounds. With colorless compounds, the use of the streak technique or use of ultra-violet light is necessary. It will be noted that the bands which are formed are adjacent to one another. Development of the column consists of adding large quantities of pure solvent so that the bands will move down the column in such a manner that they will separate from one another leaving spaces of adsorbent between. When the desired amount of development has been completed, it is then necessary to remove the individual bands. This may be accomplished by extruding with gas, cutting the tube between bands or by digging out each part. The individual fractions may be eluted by washing with a solvent which will loosen the combination of material with adsorbent (adsorbate), thus leaving the original mixture separated into its constituents.

The difficulties encountered with this technique are those of finding the suitable initial solvent, adsorbent, developer, eluant and the correct combination of these. The selection of the initial solvent is naturally somewhat dependent upon the solubilities of the specific materials. It must not only dissolve the greatest amount of material possible but must provide the ideal medium for adsorption. The adsorbent must demonstrate different affinities for the various constituents, but yet must

be of such a nature that it will not react chemically or decompose these substances. The developer may sometimes be identical with the initial solvent or contain a small proportion of it. Development may be performed with entirely different solvents or mixtures of solvents. The eluant must gently break down the adsorbate or reverse the adsorption which has occurred. Elution from some adsorbents is very difficult because the material is so tenaciously bound.

EXPERIMENTAL

Solvent

Since the 2,4-dinitrophenylhydrazones are insoluble in such a great many solvents, the search for a suitable one was concerned chiefly with one which would provide the best solubility and one which could be handled easily throughout. The solvents tried were:

- Chloroform
- Ethanol
- Methanol
- Petroleum ether
- Ethyl ether
- Skellysolve
- Acetone
- Ethyl acetate
- Water

Of these chloroform far excelled the others, but even it provided solubility of only approximately 0.6 mg. per 100 cc.

Ethyl ether and ethanol were slightly less satisfactory. The others were very poor.

ABSORBENT

Many of the adsorbents reported by investigators in the field of chromatography were tested, these being:

- Alumina (activated)
- Magnesol
- Magnesol with Celite
- Magnesia
- Fuller's earth
- Cellulose

Silica gel
Starch
Glucose
Silicic acid
Silicic acid with Celite
Bentonite

It was found that upon the basic adsorbents such as magnesia or Magnesol only a wide yellow band similar in color to bands of 2,4-dinitrophenylhydrazine. An explanation of this behavior is given by Strain (1) who recalls that the 2,4-dinitrophenylhydrazones are decomposed by these basic substances. On Fuller's earth one wide orange band was formed, but failed to develop with any of the solvents. Starch, glucose and cellulose exhibited adsorption to only a slight degree. There was no significant development with any of the others except alumina which proved to be the ideal adsorbent for these compounds.

DEVELOPER; ELUANT

In order to accomplish development of the bands formed, the action of some of the previously mentioned solvents was studied. Chloroform performed the task quite well alone, but the addition of ethyl ether hastened the development. In running some of the columns small amounts of ethanol were added to accelerate the division of the lower bands. Since chloroform proved to be such an adequate eluant no further search was made.

As is evident the above searches would have required large quantities of materials if performed in regular columns which are usually 2-3 cm. in diameter and of various lengths. The use of micro columns was thought to be advantageous for conser-

vation of time, space and materials. The micro column was prepared by partially closing one end of a piece of glass tubing (inside diameter 4 mm., length 20-25 cm.), stoppering the small opening with cotton and then filling the tube with alumina, leaving space above the adsorbent for solvent. The tubes were placed vertically in a wire rack ready for use. Only 2-5 cc. of solution of material and 4-5 cc. of developer were required for a chromatogram. When the final decision had been made concerning the most desirable combination of individual components and the use of these forming a good micro chromatogram, then the separations were performed on a larger scale.

PROCEDURE

In order to procure enough of the individual compounds for later identification tubes with a diameter of 3 cm. and length of 35 cm. were packed in the same manner with 300 gm. of alumina. A separatory funnel was used to introduce the solutions and developers at a fairly constant rate. A 2 mg. sample of the 2,4-dinitrophenylhydrazones dissolved in 300 cc. of chloroform was introduced through the funnel after the column had first been wet with solvent. As this solution drained down the column a reddish-orange band appeared near the top.(fig. 1). Upon addition of 300 cc. of chloroform the orange band moved slowly down the column with a wide yellow band forming above it. (fig. 2) At the very top of the column a band of brown was apparent. The addition of more chloroform caused the development of the orange, yellow and brown bands. (fig. 3) The introduction of ethyl ether caused the immediate appearance of

CHROMATOGRAPHIC COLUMNS

FIG 1

FIG 2

FIG 3

FIG 4

FIG 5

a pink band between the brown and yellow ones. (fig. 4) The continued use of ethyl ether at this point interfered with the development of the lowest band into two separate ones, therefore chloroform with a little ethanol was added. The lower band separated into a greenish-yellow band sagging below an orange one. (fig. 5) The completed chromatogram then exhibited a top brown band (I), a delicate pink band (II), a wide, bright yellow band (III), a narrow sharply demarcated bright orange band (IV) and the greenish-yellow bottom band (V).

The individual bands of adsorbate were dug out with a long metal rod and were eluted with chloroform. These liquid portions in turn were evaporated to dryness.

The application of suction to the columns was discarded because of the diffuseness of the bands when it was used. The flow by gravity produced clear, distinct bands.

IDENTIFICATION

BANDS I AND II

Even from the large columns it was impossible to extract sufficient quantities of the compounds of bands I and II for their identification.

BAND III

It was noted that Band III was similar in color to the band exhibited by pure 2,4-dinitrophenylhydrazine in a previously run micro column. This pointed to the possibility of their being identical substances. In order to ascertain this fact melting points and mixed three tube chromatograms (8) were run. Melting points performed simultaneously on a melting point block were 203° C. for both pure 2,4-dinitrophenylhydrazine and band III. The mixed micro chromatogram failed to show any separation of the one yellow band formed. This mixed chromatographic technique is acclaimed by Zeebaster (12) who says that "the strength of adsorption affinity generally depends to a far greater extent on molecular structure than do the indications usually concerned with proof of homogeneity, such as melting point, boiling point and spectrum." Thus the assumption is that band III was identical with 2,4-dinitrophenylhydrazine

BAND IV

The extracted material from band IV melted at 283° C. Of the possible derivatives the melting point of the osazone was closest, being listed as 277-8° C. (decomposition) (5). It was then decided to compare band IV with the osazone on mixed melting points and mixed chromatograms. Since there was no osazone available, it was prepared according to the directions of Fuson and Schriner (2).

This freshly prepared compound demonstrated an identical melting point with that of band IV. In individual columns as well as in the mixed column a single orange band appeared. This substantiated the fact that the osazone and the material of band IV were the same.

BAND V

Since the third band was 2,4-dinitrophenylhydrazine and the fourth the osazone, it was thought that the lowest band might be a higher molecular weight compound, perhaps the tris 2,4-dinitrophenylhydrazone of 1,3-propanone-dial. A literature search failed to find any mention of this compound, however a similar one, tris phenylhydrazone of 1,3-propanone-dial had been prepared by Chargoff and Magasnik. (1)

It was decided to prepare the tris 2,4-dinitrophenylhydrazone of 1,3-propanone-dial from acetone dicarboxylic acid converted

to the oxime. The acetone dicarboxylic acid was made according to Ingold and Nichols (4) and converted to oxime according to Geissman, Schlatter and Webb. (3) The oxime was then treated with 2,4-dinitrophenylhydrazine in hydrochloric acid and refluxed for two hours to form the tris 2,4-dinitrophenylhydrazones.

This newly prepared compound had a melting point of 243° C. and the substance of band V one of 244° C. A mixed chromatogram did not separate the wide greenish yellow band, but a faint pink band, similar to the pink band in the large column, appeared very high in the tube. Since the individual samples showed the characteristic band on mixed chromatograms the pink was assumed to be an impurity. As an additional test of the homogeneity of the two substances they were compared spectrophotometrically, the results of which are represented in fig. 6. All of these comparison tests seemed to indicate that band V was identical with the new compound.

A Kjeldahl analysis showed the new compound to contain 25.98% nitrogen (theoretical 26.84%), thus reaffirming its identity as the tris 2,4-dinitrophenylhydrazene of 1,3-propanone-dial.

SPECTROPHOTOMETRIC COMPARISON

T = transmission read on Coleman Universal Spectrophotometer, slit width 20 μ .

CONCLUSION

Since the two top bands obtained by the chromatographic adsorption of the 2,4-dinitrophenylhydrazones of the oxidation products of glycerol failed to yield any solid, it is supposed that these were impurities. The brown band (I) of strongly adsorbed material appears on almost every chromatogram. The pink band is interesting in that it not only was in the large columns but a very similar one was in the mixed micro chromatogram of the tris and the material of band V. It has been suggested by Dr. H. I. Bowman that this pink band might be traced to a metallic impurity.

Band III was 2,4-dinitrophenylhydrazine which was undoubtedly was retained from the conversion to the hydrazones.

Band IV, which was identified as the 2,4-dinitrophenyl-osazone of dihydroxy acetone, seemed to be present in the greatest concentration. This particular derivative could have been formed from any one of these oxidation products of glycerol: glyceraldehyde, 1,3-dihydroxy propanone or hydroxy propanone-al.

Band V, tris 2,4-dinitrophenylhydrazone of 1,3-propanone-dial, was evidently obtained from one of the following: 1,3-dihydroxy acetone, hydroxy propanone-dial or propanone dial.

SUMMARY

The 2,4-dinitrophenylhydrazones of the oxidation products of glycerol were separated chromatographically and two of them identified, one being 2,4-dinitrophenyllosazone of glyceraldehyde and the other tris 2,4-dinitrophenylhydrazone of 1,3-propanone-dial.

A preparation for the tris 2,4-dinitrophenylhydrazone of 1,3-propanone-dial was cited.

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