

**Methods for the Isolation and  
Characterization of Constituents of Natural Products**  
**VII. Purification of Alcohol Ester, Amide, and Thioester Derivatives of  
Pyruvic Acid 2, 6-Dinitrophenylhydrazone with  
an Anion Exchange Resin**

D. P. SCHWARTZ AND C. R. BREWINGTON

*Dairy Products Laboratory, Eastern Utilization Research and Development  
Division, Agricultural Research, U. S. Department of Agriculture,  
Washington, D. C. 20250*

Preparation, class separation, and chromatographic resolution of derivatives of aliphatic primary, secondary and tertiary alcohol esters, primary and secondary aliphatic amides and aliphatic thioesters with pyruvic acid 2,6-dinitrophenylhydrazone have been described in our previous papers of this series (1-6). We have described also some of the properties and characteristics of the derivatives in these reports and have stated that, among other characteristics the derivatives have the property of being exchanged (or adsorbed) on an anion-exchanger under the proper conditions. This feature makes them particularly valuable in that they can be purified to a high degree for future analyses such as thin-layer chromatography and more particularly for gas-liquid chromatography. The latter technique will be described in the next paper of the series. Furthermore, derivatization can be executed in lipids and the derivatives can be isolated by adsorption chromatography on magnesium oxide and purified further by the ion-exchange technique described below, thus assuring purification when the derivatives are made in practically any type of lipid media.

Schwartz *et al.* (7) have utilized an anion-exchanger in the hydroxyl form to purify dinitrophenylhydrazone derivatives of carbonyl compounds. However, the strongly basic conditions employed were considered unsuitable for the purification of the pyruvic acid ester 2,6-dinitrophenylhydrazones because of the danger of saponification. The procedure described below has been found to be highly satisfactory for all of the compounds tested.

#### APPARATUS AND REAGENTS <sup>1</sup>

Dowex 1 × 2 (100–200 mesh), methanol, glacial acetic acid, and benzene were obtained from the J. T. Baker Co. (Phillipsburg, New Jersey). Diethylamine (Baker) was redistilled before use; acid aluminum oxide, Brockmann Activity Grade 1 for chromatography was deactivated with 8% (w/v) distilled water as described by Schwartz and Brewington (1). Chromatographic columns 1 cm i.d. by about 15 cm were made by cutting old 10-ml graduated pipettes in half.

#### EXPERIMENTAL METHOD

*Preparation of resin.* The alcohol, amine and thiol derivatives of pyruvic acid 2,6-dinitrophenylhydrazone possess acidic properties similar to 2,4-dinitrophenylhydrazones and can therefore be taken up by an anion exchange resin just as 2,4-dinitrophenylhydrazones are.

One gram of wet resin (about 70% moisture) in the chloride form is slurried in distilled water and transferred to a chromatography tube and washed with distilled water until the effluent emerges colorless. The resin is cycled with 2 column volumes of *N* NaOH, distilled water until the effluent emerges neutral, then 2 column volumes of *N* HCl followed by distilled water until a neutral effluent is obtained. The resin is converted to the acetate form by passing 2 column volumes of 15% glacial acetic acid in water over it. The resin is washed with distilled water until the effluent is neutral and then with 3 column volumes of methanol followed by a column volume of 20% diethylamine in benzene:methanol (1:1) (solution A). The latter solution should be prepared fresh each day.

*Purification of derivatives.* Butterfat was chosen as the contaminating lipid for the purification studies because of its complexity and also our interest in it. Approximately 0.5 μmole of the derivative was dissolved in 1 ml of a 1:1 mixture of butterfat and solution A, and transferred quantitatively to the column using a minimum of solution A to effect transfer. The solution was allowed to drain completely and when the column was just dry the sides of it were washed down with solution A and the column then washed with 2 column volumes of solution A to remove the lipid, then with 1 column volume of

<sup>1</sup> Reference to certain products or companies by the Department does not imply an endorsement over others not mentioned.

methanol:benzene (1:1) to remove the diethylamine. The derivatives were then desorbed with 0.1% glacial acetic acid in methanol:benzene (1:1). The acetic acid solution was added until the effluent emerged colorless.

*Recovery and thin-layer chromatographic analyses.* The acetic acid effluent was blown down on the steam bath under a gentle air stream, the residue taken up in the appropriate amount of benzene, and the solution read at the wavelength of maximum absorption (1,4,6). Recoveries were calculated from readings of solutions of the compounds not put through the procedure. Each compound put through the ion-exchange column and the standards were subjected to thin-layer partition chromatography using the procedures of Schwartz and Brewington (2,5,6).

*Removal of fatty acids from derivatives.* Fatty acids are the only class of compounds from butterfat which might be expected to contaminate the derivatives obtained from the ion-exchange column. The fatty acids are easily removed from the derivatives by taking the residue from the ion-exchange column up in  $\text{CHCl}_3$  and passing it over a 5-g column of acidic alumina. The fatty acids are retained on the alumina and the derivatives pass through quantitatively. The column is washed with  $\text{CHCl}_3$  until all color is removed.

## RESULTS AND DISCUSSION

Table 1 lists the derivatives put through the ion-exchange procedure and their recoveries. The derivatives chromatographed as single spots with the same mobility as the standards. As can be noted in Table 1, a long- and a short-chain compound in each class were studied. On the basis of the results it is likely that all derivatives between the extremes in these classes will hold on the resin and be recovered to a similar degree.

The entire procedure from the time the derivative is put on the exchanger until the last of it has been removed takes about 10 minutes.

The capacity of the resin for derivatives was determined to be about 7-8  $\mu\text{moles}$  per dry gram, but the long-chain secondary amines appear to be held less strongly than the other derivatives and the capacity of the resin for these derivatives is accordingly less. However, the capacity of the resin for all derivatives is increased as the benzene con-

**TABLE 1**  
**RECOVERY OF ALCOHOL ESTER, AMIDE AND THIOESTER DERIVATIVES OF PYRUVIC ACID 2,6-DINITROPHENYLHYDRAZONE FROM ANION-EXCHANGER**

Derivative	Recovery <sup>a</sup> (%)
<b>Alcohols</b>	
methanol	99
<i>n</i> -octadecanol	99
isopropyl	99
2-octadecanol	99
<i>tert</i> -butyl	97
3-methyl-3-heptyl	96
<b>Amines</b>	
methyl	99
<i>n</i> -octadecyl	99
dimethyl	99
diamyl	95
<b>Thiols</b>	
methyl	99
<i>n</i> -octadecyl	99

<sup>a</sup> On approximately 0.5  $\mu$ moles of each derivative. concentration in solvent A is decreased. This manipulation will, of course, depend on the solubility of the contaminating lipid in methanol.

We have utilized the ion-exchange procedure for purifying derivatives from non-volatile contaminants in the preparation of semimicro amounts of derivatives and for the purification of derivatives made in butterfat, both with gratifying results.

#### SUMMARY

A quantitative ion-exchange procedure is described for purification of alcohol ester, amide and thioester derivatives of pyruvic acid 2,6-dinitrophenylhydrazone. The derivatives are exchanged (or absorbed) on an anion-exchanger in the acetate form and desorbed with acetic acid solution after removal of contaminating lipid (butterfat). Fatty acids which may contaminate the derivatives are removed on an acidic alumina column.

#### REFERENCES

1. SCHWARTZ, D. P., AND BREWINGTON, C. R. Methods for the isolation and characterization of constituents of natural products. I. Derivatives of

- alcohols with pyruvyl chloride 2,6-dinitrophenylhydrazine. *Microchem. J.* **11**, 430-436 (1966).
2. SCHWARTZ, D. P., AND BREWINGTON, C. R. Methods for the isolation and characterization of constituents of natural products. II. Separation of homologous series of esters of pyruvic acid, 2,6-dinitrophenylhydrazine by thin-layer chromatography. *Microchem. J.* **12**, 1-6 (1967).
  3. SCHWARTZ, D. P., BREWINGTON, C. R., AND SHAMEY, JENNIE. Methods for the isolation and characterization of constituents of natural products. III. Separation of alcohol esters of pyruvic acid 2,6-dinitrophenylhydrazine into classes by column and thin-layer chromatography.
  4. SCHWARTZ, D. P., AND BREWINGTON, C. R. Methods for the isolation and characterization of constituents of natural products. IV. Amide derivatives of amines with pyruvyl chloride 2,6-dinitrophenylhydrazine. *Microchem. J.* **12**, 192-195 (1967).
  5. SCHWARTZ, D. P., AND BREWINGTON, C. R. Methods for the isolation and characterization of constituents of natural products. V. Separation of 2,6-dinitrophenylhydrazine pyruvamides into classes and resolution of the individual members. *Microchem. J.* **12**, 547-554 (1967).
  6. SCHWARTZ, D. P., AND BREWINGTON, C. R. Methods for the isolation and characterization of constituents of natural products. VI. Preparation of thioesters of pyruvic acid 2,6-dinitrophenylhydrazine and resolution of a homologous series by thin-layer partition chromatography. *Microchem. J.* **13**, 000 (1968).
  7. SCHWARTZ, D. P., JOHNSON, A. R., AND PARKS, O. W. Use of ion-exchange resins in the micro analysis of 2,4-dinitrophenylhydrazones. *Microchem. J.* **6**, 37-44 (1962).