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Methods for the Isolation and Characterization of Constituents of Natural Products

IX. Separation of Alcohol, Primary Amine and Thiol Derivatives of Pyruvic Acid 2,6-Dinitrophenylhydrazone into Classes

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Methods for the preparation of alcohol (5), amine (7), and thiol (9) derivatives of pyruvic acid 2,6-dinitrophenylhydrazone¹ were described in previous reports from this Laboratory. We have also presented methods for the separation of the alcohol derivatives into tertiary, secondary, and primary classes (10), and for the separation of primary from secondary amine derivatives (8). The analysis of the individual members of the separated classes by liquid-liquid thin-layer (6,8,9), and by gas-liquid chromatography of these, and also of a series of thiol derivatives (1) has been described.

The present report is concerned with the separation of alcohols (as a class), primary amine, and thiol derivatives of pyruvic acid 2,6-dinitrophenylhydrazone from each other. Both quantitative column adsorption and qualitative thin-layer adsorption systems are described.

APPARATUS AND MATERIALS²

Magnesium oxide (cat. no. 2477) suitable for chromatographic use was obtained from the J. T. Baker Company, Phillipsburg, N. J. The powder had an adsorption index (Food and Drug Yellow No. 4)

¹ Pyruvic acid chloride 2,6-dinitrophenylhydrazone, the reagent employed for preparation of these derivatives, is now available from the J. T. Baker Co., Phillipsburg, N. J.

² Reference to certain products or companies does not imply an endorsement by the Department over others not mentioned.

of 12-13 and was used without further treatment. The original container was subdivided into 6 or 7 lots and these were stored in tightly sealed containers and stored in the absence of atmospheric moisture after it was noted that MgO lost some of its activity as samples were withdrawn from the original container over a period of time.

Celite 545 was obtained from the Johns-Manville Company, Baltimore, Md. Chloroform, methanol, and benzene were ACS grade. Nitromethane was the Fisher Certified grade. Hexane was the high purity grade produced by the Phillips Petroleum Company, Bartlesville, Okla. Diethylamine (Baker) was redistilled. Silica Gel G was obtained from the Brinkmann Instrument Company, Westbury, N. Y.

The thin-layer equipment was the same as previously described (6). A borosilicate glass column, 3.7 cm i.d. \times 28 cm was employed for column chromatography.

EXPERIMENTAL METHOD

Separation of alcohol, primary amine and thiol derivatives into classes by column adsorption chromatography. The MgO (7.5 g) and Celite 545 (30 g) are slurried in about 150 ml of 0.5% methanol in CHCl_3 in a 500-ml Erlenmeyer flask. The flask is stoppered tightly and shaken at high speed on a mechanical shaker (Eberbach Corp., Ann Arbor, Michigan) for 15 minutes. The slurry is poured quickly through a long-stemmed funnel into the chromatography tube and packed immediately under moderate gas pressure until a few ml of solvent remain above the bed. The sides of the tube are washed down with a few ml of solvent and the sample quantitatively transferred to the column. The classes are separated using 200 ml of 0.5% methanol in CHCl_3 to remove the alcohols, 500 ml of 1% methanol in CHCl_3 to remove the primary amines, and finally 200 ml of 25% nitromethane in CHCl_3 to elute the thiol derivatives.

Separation of alcohol, primary amine, and thiol derivatives into classes by thin-layer adsorption chromatography. Plates of Silica Gel G, 250 μ thick are prepared in the usual manner and activated for 1-2 hours at 100°C before use. The derivatives are spotted from benzene solution and the plate is developed in an equilibrated tank using hexane:benzene:diethylamine (3:2:5). Satisfactory separation

of the classes is effected in about 20 minutes. The solvent should be discarded each day and replaced with fresh solvent when needed. The tank should then be equilibrated 1-2 hours before insertion of a plate.

RESULTS AND DISCUSSION

Figure 1 is a chromatogram showing separation of the alcohol, primary amine, and thiol derivatives into classes by the column procedure. The following compounds were selected for the attempted separation. Tertiary alcohols: 2-methyl 2-nonanol, *tert*-amyl alcohol, and *tert*-butyl alcohol; secondary alcohols: 2-nonadecanol, 2-dodecanol, 2-heptanol, 2-butanol and 2-propanol; primary alcohols: *n*-octadecanol, *n*-dodecanol, *n*-heptanol, *n*-propanol, ethanol, and methanol; primary amines: *n*-octadecylamine, *n*-dodecylamine, *n*-octylamine, ethylamine, and methylamine; thiols: *n*-octadecylmercaptan, *n*-dodecylmercaptan, *n*-heptylmercaptan, ethylmercaptan, and methylmercaptan. Approximately 0.5 μ mole of each compound was included in the mixture.

Recovery studies were conducted on 0.5 μ mole of the extremes in each class on individual columns. Quantitative recovery was obtained in each case. It was noted, however, that old stock solutions of the derivatives which had been exposed to light at room temperature for several months gave an unadsorbed (yellow) band on the MgO and/or a strongly adsorbed violet band which could not be eluted from the column. In both instances these bands represented only a small percentage of the total color put on the column. Nevertheless, they represent some breakdown product of the derivatives either due to light, heat, or a combination of these. Benzene solutions of the derivatives stored at -18°C for over a year do not show these artifacts, nor do freshly prepared solutions.

Separation of the classes as described takes about 4 hours. The separated classes were checked for the individual members using thin-layer partition chromatography (6,8,9). All of the alcohols were eluted together as shown in Fig. 1 except for the methanol derivative which was found in the primary amine fraction. All of the primary amines were eluted together except methylamine which was found in the thiol class. The anomalous behavior of these two lower members is also manifested in the thin-layer class separation (see below).

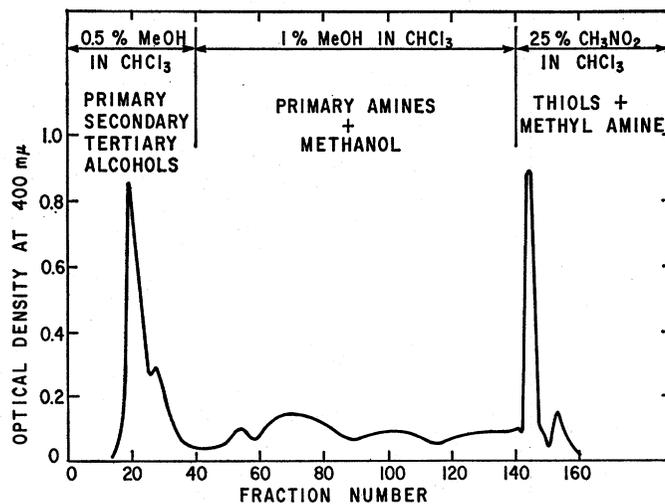


FIG. 1. Separation of alcohol, primary amine, and thiol derivatives of pyruvic acid 2,6-dinitrophenylhydrazone into classes of column chromatography.

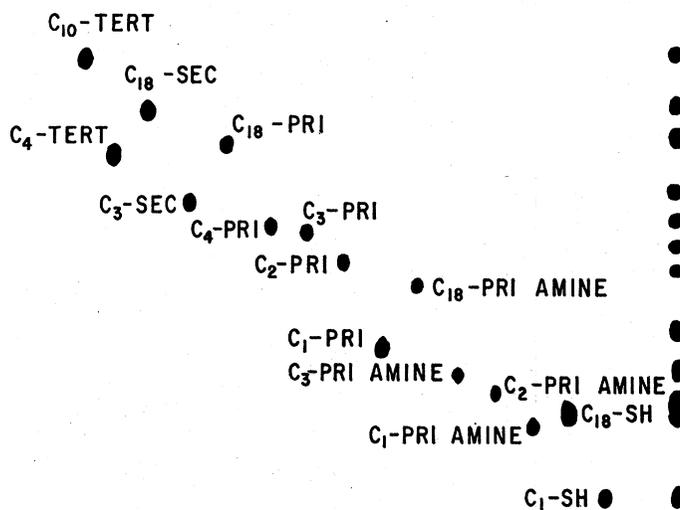


FIG. 2. Separation of alcohol, primary amine, and thiol derivatives of pyruvic acid 2,6-dinitrophenylhydrazone into classes by thin-layer chromatography on Silica Gel G. Solvent: hexane:benzene:diethylamine (3:2.5).

A difference in color exists for the three classes which aids in their classification. The alcohols are yellowish-violet, the amines are violet and the thiols are rust-red. The thiols are easily distinguishable from the amines.

Separation of the classes by thin-layer adsorption chromatography on Silica Gel G in the alkaline solvent system is shown in Fig. 2. The classes separate nicely, but the methanol derivative moves into the primary amine class and methylamine into the thiol class as with the column separation. The colors displayed on the column are also evident on the plate. Very small amounts (about 5×10^{-4} μ mole) of a derivative can be seen on the plate.

The complexity of natural products, especially food products, is manifested by the large number of peaks that can be obtained by capillary gas chromatography (e.g., 2,3,4,12). Although sophisticated instrumental techniques such as capillary gas chromatography coupled with mass spectrometry afford a great deal of information, it is doubtful whether this technique, as presently used, is capable of separating and characterizing all of the volatile constituents in a food product, for example. Evidence gathered in this Laboratory indicates that a fairly large number of constituents which we can identify in the volatiles by techniques which we have described in this series and in another report regarding the identification of carbonyl compounds (11) go undetected or unidentified by capillary gas chromatography-mass spectrometry. In view of this, it would seem that instrumental techniques, in their present state, could beneficially be supplemented by methods such as are described here and in our previous publications, in order to gain a more complete picture of the spectra of compounds present in natural products.

SUMMARY

Quantitative column and qualitative thin-layer chromatographic procedures are described for separating a mixture of alcohol, primary amine, and thiol derivatives of pyruvic acid 2,6-dinitrophenylhydrazide into classes. Magnesium oxide is used as the adsorbent in the column procedure and Silica Gel G in the thin-layer technique. The alcohols, amines, and thiol derivatives which are eluted in that order, show differences in color during chromatography which aid in their proper classification.

REFERENCES

1. BASSETTE, R., BREWINGTON, C. R., AND SCHWARTZ, D. P., Methods for the isolation and characterization of constituents of natural products. VIII. Gas-liquid chromatographic resolution of alcohol ester, amide, and thioester derivatives of pyruvic acid 2,6-dinitrophenylhydrazone. *Microchem. J.* **13**, 000 (1968).
2. BONDAROVICH, H. A., GIAMMARINO, A. S., RENNER, J. A., SHEPHARD, F. W., SHUGLER, A. J., AND GIANTURCO, M. A., Some aspects of the chemistry of tea. A contribution to the knowledge of the volatile constituents. *J. Agr. Food Chem.* **15**, 24-28 (1967).
3. DAY, E. A. AND ANDERSON, D. F., Gas chromatographic and mass spectral identification of natural components of the aroma fraction of Blue cheese. *J. Agr. Food Chem.* **13**, 1-4 (1965).
4. DAY, E. A. AND LIBBEY, L. M., Cheddar cheese flavor: Gas chromatographic and mass spectral analyses of the neutral components of the aroma fraction. *J. Food Sci.* **29**, 583-589 (1964).
5. 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-dinitrophenylhydrazone. *Microchem. J.* **11**, 430-436 (1966).
6. 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-dinitrophenylhydrazone by thin-layer chromatography. *Microchem. J.* **12**, 1-6 (1967).
7. 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-dinitrophenylhydrazone. *Microchem. J.* **12**, 192-195 (1967).
8. SCHWARTZ, D. P. AND BREWINGTON, C. R., Methods for the isolation and characterization of constituents of natural products. V. Separation of 2,6-dinitrophenylhydrazone pyruvamides into classes and resolution of the individual members. *Microchem. J.* **12**, 547-554 (1967).
9. 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-dinitrophenylhydrazone and resolution of a homologous series by thin-layer partition chromatography. *Microchem. J.* **13**, 000 (1968).
10. 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-dinitrophenylhydrazone into classes by column and thin-layer chromatography. *Microchem. J.* **12**, 186-191 (1967).
11. SCHWARTZ, D. P., HALLER, H. S., AND KEENEY, M., Direct quantitative isolation of monocarbonyl compounds from fats and oils. *Anal. Chem.* **35**, 2191-2194 (1963).
12. TANG, C. S. AND JENNINGS, W. G., Volatile components of apricot. *J. Agr. Food Chem.* **15**, 24-28 (1967).