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

D. P. SCHWARTZ AND C. R. BREWINGTON

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

Preparation of homologous series of esters of pyruvic acid 2,6-dinitrophenylhydrazone with primary and secondary aliphatic alcohols and a partial series of tertiary aliphatic alcohols was described in Part I of this series (*1*). In Part IV, preparation of a homologous series of amides of *n*-aliphatic primary amines and a partial series of amides of symmetrical secondary amines with pyruvic acid 2,6-dinitrophenylhydrazone was presented (*3*).

The present manuscript is concerned with the preparation of a series of derivatives of *n*-aliphatic mercaptans with the reagent and resolution of the derivatives by thin-layer partition chromatography. Separation of homologous series of the alcohol and amine derivatives has already been described (*2,4*).

APPARATUS AND REAGENTS [1]

The apparatus and materials used for the preparation of the alcohol derivatives (*1*) were employed in this study. The mercaptans were obtained from various sources (Table 1) and were the highest purity obtainable from the specified source.

The thin-layer chromatographic equipment was identical to that described earlier (*2*).

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

TABLE 1

PHYSICAL PROPERTIES OF THIOESTERS OF PYRUVIC ACID 2,6-DINITROPHENYLHYDRAZONE

Mercaptans	Source[a]	mp[b] (C°)	E[c]	C (%) Found	C (%) Calc	H (%) Found	H (%) Calc
C_1	E–K	185–186	6835	40.49	40.4	3.72	3.70
C_2	B	165–166	7132	42.81	42.4	3.89	4.18
C_3	B	147.5–148	7187	44.62	44.4	4.50	4.62
C_4	B	102–103	6908	46.58	46.07	4.93	5.01
C_5	B	95–97	7044	48.80	47.6	4.45	5.38
C_6	M–C–B	98.5–100	7201	48.54	49.0	5.32	5.72
C_7	E–K	102.5–103	7067	50.63	50.38	5.93	6.03
C_8	M–C–B	102–103	7224	51.97	51.7	7.19	6.32
C_9	M–C–B	100.5–101.5	7088	52.36	52.8	6.50	6.60
C_{10}	M–C–B	100–101.5	7203	54.00	53.90	6.74	6.85
C_{11}	K + K	99–100	7315	54.92	54.8	7.16	7.09
C_{12}	M–C–B	104–105	7183	55.86	55.8	7.24	7.31
C_{14}	M–C–B	106–106.5	7271	58.15	57.7	7.82	7.73
C_{16}	M–C–B	107–107.5	7383	59.87	59.3	8.38	8.08
C_{17}	K + K	106.5–107	7195	59.73	59.8	8.37	8.25
C_{18}	B	109.5–110	7450	60.50	60.6	8.67	8.42
		Average	7168				

[a] Legend for sources: E–K = Eastman–Kodak, Rochester, New York; B = Baker Chemical Co., Phillipsburg, New Jersey; M–C–B = Matheson, Coleman, and Bell, East Rutherford, New Jersey; K + K = K + K Labs, Plainview, New York.

[b] Melting points were obtained with the Fisher Johns apparatus and are uncorrected.

[c] Molar Extinction Coefficients were obtained in benzene at 407 mμ with a 1 cm^2 cell.

EXPERIMENTAL METHOD

Thioesters of pyruvic acid 2,6-dinitrophenylhydrazone were prepared in an analagous manner to the alcohol derivatives (*1*). The derivatives were also isolated from the reaction mixture as described in that paper and were recrystallized from absolute ethanol to a constant molar extinction coefficient. The yields were approximately 90%.

Thin-layer partition chromatography of the thioester derivatives was done exactly as described for the amide derivatives (*4*). Exposure of the finished plate to diethylamine vapor gives reddish-violet spots.

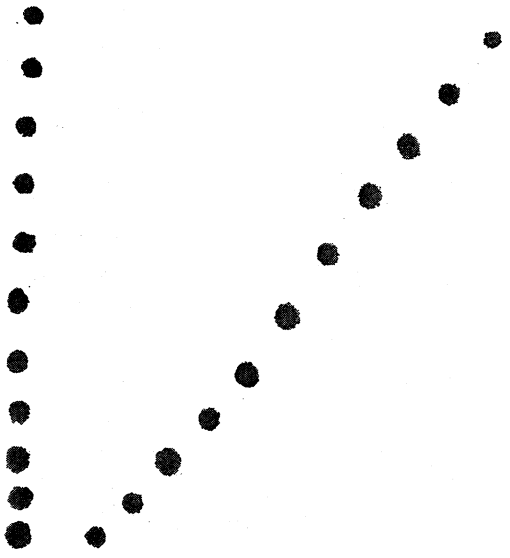

FIG. 1. Separation of thioester derivatives of pyruvic acid 2,6-dinitrophenylhydrazone by normal thin-layer partition chromatography. Support Microcel T-38; stationary phase-polyethylene glycol 400; mobile phase-hexane:benzene (7:3) saturated with stationary phase. Diagonally from top to bottom C_{11} through C_1 thioester derivatives; column on left represents mixture of all 11 derivatives.

RESULTS AND DISCUSSION

Some physical properties of the thioester derivatives are given in Table 1. All of the derivatives crystallize extremely well as shiny orange plates, and except for 3 or 4 members, they proved to be pure after one recrystallization. The molar extinction coefficient, which averaged to 7168, is somewhat higher than that found for the alcohol and amine derivatives (*1,3*).

Figure 1 shows the resolution achieved by thin-layer partition chromatography for the first 11 members of the series. Figure 2 is a reproduction of the reversed-phase chromatogram showing separation of the C_{10} through C_{18} derivatives. As little as 1.2×10^4 μmole of a thioester derivative can be chromatographed and detected after exposure of the plate to diethylamine vapor.

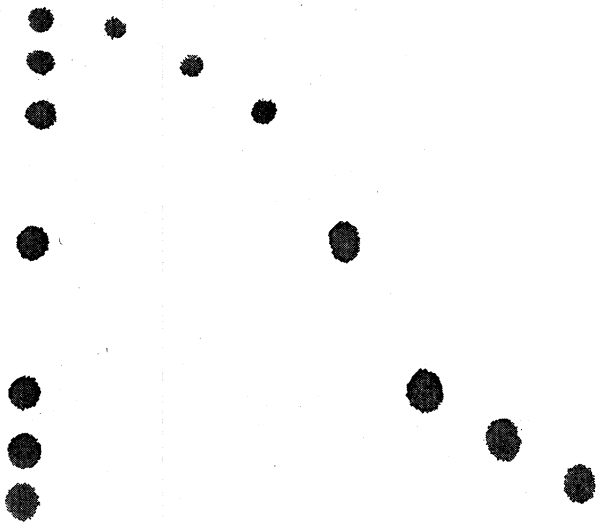

FIG. 2. Separation of thioester derivatives of pyruvic acid 2,6-dinitrophenylhydrazone by reversed-phase thin-layer partition chromatography. Support Microcel T-38; stationary phase-mineral oil; mobile phase-acetonitrile; water (3:1). Diagonally from top to bottom C_{10}, C_{11}, C_{12}, C_{14}, C_{16}, C_{17}, and C_{18} thioester derivatives. Column on left represents mixture of the 7 derivatives.

The thioester derivatives, like the alcohol and amine derivatives, possess properties which should permit their direct isolation from lipids. Thus, they are strongly absorbed on alkaline adsorbents, and are exchanged on an anion exchanger resin under the proper conditions. In both of these procedures a spectral shift is observed.

SUMMARY

Preparation of a homologous series of thioesters of pyruvic acid 2,6-dinitrophenylhydrazone is described. The derivatives are orange, highly crystalline compounds with an absorption maximum at 407 mμ in benzene, and a molar extinction coefficient near 7100. Separation of a series of the derivatives by normal- and reversed-phase thin-layer partition chromatography is presented.

REFERENCES

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