



## Methods for the Isolation and Characterization of Constituents of Natural Products

### XVI. Quantitative Microdetermination of Diols as *Bis*-Esters of Pyruvic Acid 2,6-Dinitrophenylhydrazone: Separation from Monohydric Alcohol Derivatives and Resolution of an Homologous Series

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#### INTRODUCTION

In a recent report from this laboratory (2), a quantitative micro procedure was described for determining a wide variety of monohydric alcohols as colored esters of pyruvic acid 2,6-dinitrophenylhydrazone. This paper reports an extension of the method for quantitatively determining micro amounts of dihydric alcohols as *bis*-esters of pyruvic acid 2,6-dinitrophenylhydrazone. In addition, a procedure is given for separating the dihydric alcohol derivatives from the monohydric. A thin-layer partition chromatographic system is also described for separating an homologous series of the *bis* derivatives of terminal diols.

Although diols react quantitatively with several reagents (1, 4) there is at present no simple procedure for quantitating nonvicinal diols in the presence of monohydric alcohols.

#### MATERIALS AND APPARATUS

The source of reagents and purification of benzene were the same as previously described (2) except for the following modifications. Pyruvic acid chloride 2,6-dinitrophenylhydrazone was recrystallized three times from pure, dry benzene (2). CaH<sub>2</sub> was pulverized in a mortar and stored in a desiccator. Acidic alumina (Woelm, Activity Grade I, Waters Associates, Framingham, MA) was partially deactivated by addition of 8% distilled water. The dihydric alcohols were obtained from the Aldrich Chemical Co., Milwaukee, WI, except for 1,4-dodecanediol and

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1,5-tetradecanediol which were prepared by  $\text{LiAlH}_4$  reduction of the corresponding lactones. Methyl-*threo*-9,10-dihydroxystearate was kindly supplied by Dr. G. Maerker, ARS, EMN, USDA, Philadelphia, PA.

#### EXPERIMENTAL METHODS

*Preparation of dihydric alcohol solutions.* These were made up to contain between approximately 1.5–2.1  $\mu\text{moles/ml}$ . The solvents used for solution of the alcohols are listed in Table 1. Where mixed solvents were employed, the diol was dissolved first in the more polar solvent and diluted with the less polar solvent.

*Preparation of reagent acid solutions.* A benzene solution of pyruvic acid chloride 2,6-dinitrophenylhydrazone was prepared to contain 23.5  $\mu\text{moles/ml}$  and was stored over 10–20 mg  $\text{CaH}_2/50$  ml solution. A benzene solution of triethylenediamine was made up to contain 37  $\mu\text{moles/ml}$  and was also stored over  $\text{CaH}_2$  in a similar manner.

*General assay procedure.* Assays were conducted in 10-ml dry test tubes fitted with Teflon-lined screw caps. Using a single 0.5 ml graduated pipette, 0.00, 0.25, 0.50, 0.75, and 1.00 ml aliquots of the diol solution were pipetted into the tubes. This was followed by 0.6 ml (14.1  $\mu\text{moles}$ ) of the acid chloride solution, permitting the solution to run down the side while rotating the tube. Benzene was added in the same manner to the appropriate tube to bring the volume to 1.6 ml. A few milligrams (microspatula tip) of  $\text{CaH}_2$  were added, the tubes loosely stoppered and allowed to stand until gas evolution ceased. When dry, 0.6 ml (22.2  $\mu\text{moles}$ ) of the triethylenediamine solution was added while carefully shaking the tube. The solution turned bright red upon addition of the base, became turbid and eventually faded to a lighter color.

*Isolation and quantitation of derivatives.* The reaction mixture can be analyzed immediately following addition of the base or within a few hours. The contents of the tube were transferred to a chromatographic column (approximately 1 cm i.d.  $\times$  17 cm) containing about 5 g of alumina which had been poured dry into the column. There is considerable gassing in the solution above the column bed due to the reaction of  $\text{CaH}_2$  with water in the solvents and in the alumina of the surface of the column, but this does not affect the results. The column was washed with about 20 ml of benzene which will elute most monohydric alcohols.<sup>2</sup> The diol derivatives were then eluted with methylene chloride until all color below a slow-moving impurity band was removed. The solvent was evaporated on the steam bath under  $\text{N}_2$ , the

<sup>2</sup> For the known diols used in this study, no or only traces of monhydric alcohol derivatives were found. For unknowns with monohydric alcohols present the procedure described below should be used.

TABLE 1  
THE DETERMINATION OF DIHYDRIC ALCOHOLS AS *Bis*-ESTERS OF PYRUVIC ACID 2,6-DINITROPHENYLHYDRAZONE

Compound	Solvent	Range investigated ( $\mu$ moles)	No. of assays within range	Average yield %	Average deviation %
1,2-Cyclohexanediol <sup>a</sup>	20% methylene chloride in benzene	0.48-1.44	4	101.3	$\pm 2.0$
2,5-Dimethyl-2,5-hexanediol		0.61-2.40	4	96.2	$\pm 1.6$
Methyl- <i>threo</i> -9,10-dihydroxystearate		0.39-1.57	3	102.3	$\pm 0.2$
1,4-Dodecanediol	methylene chloride	0.51-2.07	4	100.5	$\pm 0.6$
2-Ethyl-1,3-hexanediol		0.49-1.95	4	97.5	$\pm 2.0$
2,5-Hexanediol		0.45-1.80	4	97.6	$\pm 2.5$
1,9-Nonanediol		0.49-1.97	4	100.0	$\pm 1.5$
1,8-Octanediol		0.47-1.88	4	98.2	$\pm 1.3$
1,5-Tetradecanediol		0.41-1.65	4	107.5	$\pm 0.2$
1,6-Hexanediol		0.54-2.16	3	100.6	$\pm 0.4$
1,4-Butanediol	5% 2-butanone in methylene chloride	0.61-1.84	3	101.8	$\pm 2.0$

<sup>a</sup> Two spots on thin-layer plate.

derivative taken up in benzene (or in the case of short-chain diols, in methylene chloride) and read at 400 m $\mu$  against the blank. The concentration of derivative was calculated using  $E = 11,850$ . This value was experimentally determined on pure *bis* derivatives of the diols and is very close to twice the molar absorptivity determined for monohydric alcohol derivatives. (2).

*Purity of derivatives.* Each diol derivative prepared in the assay procedure was checked by thin-layer chromatography using the system described below. Authentic crystalline derivatives were spotted with the derivatives obtained in the assay.

*Separation of an homologous series of terminal dihydric alcohol bis-derivatives by thin-layer partition.* This was achieved on plates of Microcel T-38 coated with polyethylene glycol 400. The plates (8  $\times$  10 in.) were prepared as described by Schwartz *et al.* (3). The derivatives were spotted from benzene solution (except 1,4-butanediol which was spotted from ethyl acetate) and the chromatogram developed with hexane: benzene (65:35) saturated with stationary phase.

*Separation of diols from monohydric alcohol derivatives.* A complex mixture of 26 monohydric derivatives was prepared in order to determine whether a mixture of dihydric alcohol derivatives could be separated from them. The following monohydric alcohol derivatives were used in a concentration of approximately 0.15  $\mu$ mole each/ml of benzene: 2-methyl-2-nonanol, 4-methyl-1-penten-3-ol, dihydrolanosterol, 4-methyl-4-penten-2-ol,  $\beta$ -phenylethanol, lanosterol, 3-ethyl-3-hepten-1-ol, *cis*-9,10-epoxyoctadecan-1-ol, 2-methyl-1-penten-3-ol, 3-methyl-5-hexen-3-ol, 3-phenyl-1-propanol, L-menthol, 2-undecanol, farnesol, 3-methyl-2-hexanol, 4-methyl-3-pentanol, L-isopulegol, 3-decanol, cinnamyl alcohol, 2-ethyl-5-hexen-3-ol, citronellol, 2,2-dimethyl-3-octanol, isobutyl alcohol, 2,2-dimethyl-1-propanol, and methanol. The dihydric alcohol mixture consisted of 1,8-octanediol, 1,5-tetradecanediol, methyl-*threo*-9,10-dihydroxystearate, 2-ethyl-1,3-hexanediol and 1,4-dodecanediol, each in a concentration of approximately 0.3  $\mu$ mole/ml of solvent. Following derivatization in the manner described, the solution was quantitatively transferred to a chromatographic column (1.2 cm i.d.  $\times$  31 cm) containing 15 g of alumina which had been poured into the column about two-thirds filled with benzene. All monohydric alcohol derivatives were eluted within 50 ml of benzene; the dihydric alcohol derivatives were then eluted with methylene chloride.

## RESULTS AND DISCUSSION

Table 1 lists 12 dihydric alcohols representing primary, nonvicinal secondary, tertiary, mixed primary and secondary, and vicinal sec-

ondary alcohols. Also given in Table 1 are the solvent systems used for dissolving them, the range (in micromoles) over which the compound was assayed, the number of determinations made within the range and the yield. The yield (based on 100% purity of the starting material) is the average of the yields obtained at the various concentrations assayed within the range.

As Table 1 clearly shows, the reagent acylates all of the diols investigated in quantitative or near-quantitative yield. The *bis* derivatives of all of the diols studied were exclusively formed. This was substantiated by the absence of any colored bands following the diol band on the alumina column and also by TLC. There was always a very small impurity band well above the diol band which moved very slowly with methylene chloride on the column and did not offer any problem in the collection of the diol band.

The separation of the mixture of 28 monohydric alcohol derivatives from the 5 dihydric alcohol derivatives was readily accomplished on the 15-g alumina column. Separation was clean-cut with about 5 cm separating the tail end of the monohydric alcohol band and the leading edge of the diol fraction as the former was leaving the column. A near quantitative yield of both the monohydric and dihydric alcohol fractions was obtained in two trials; with 0.46 and 0.92  $\mu$ moles of dihydric alcohol mixture and with 0.40 and 0.80  $\mu$ moles of monohydric alcohol mixture. The yields were 106 and 104% for the dihydric alcohols and 96 and 100% for the monohydric alcohols, respectively. Thin-layer partition chromatography of the diol derivatives showed it to be entirely free of spots other than the diol spots.

It should be pointed out that vicinal diols do not react quantitatively with the reagent under conditions established earlier (2) for acylation of monohydric alcohols. Increasing the concentration of triethylenediamine to the level specified in this report effectively remedies this situation. The increase in the concentration of base still gives a quantitative yield of monohydric alcohol derivatives and no adverse effect of the increase has been noted.

Figure 1 is a reproduction of a thin-layer partition plate showing the separation of an homologous series of terminal diol *bis* derivatives. Exposure of the plate to diethylamine vapor gives greyish-blue spots.

Interferences in the acylation of diols by the reagent are the same as for the acylation of monohydric alcohols. Interference by water, thiols, amines, and fatty acids has been discussed (2). Methods are being perfected in this laboratory to effectively remove amines, thiols and fatty acids from mixtures containing alcohols.

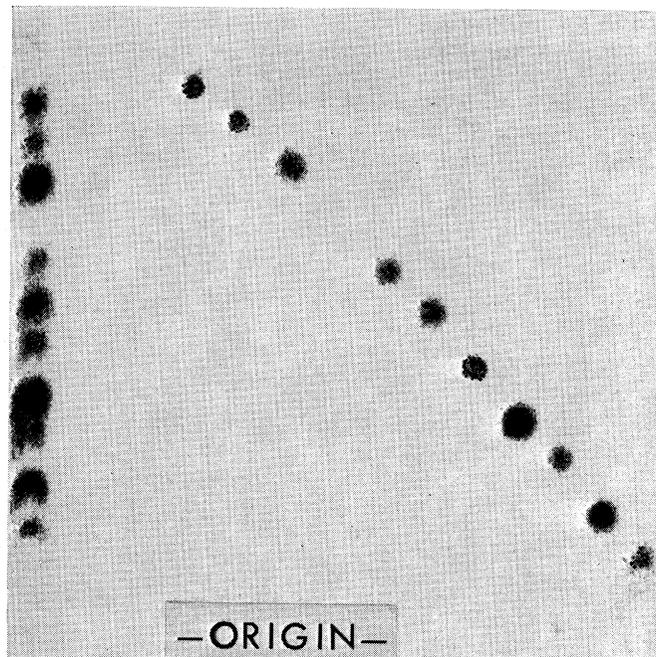


FIG. 1. Thin-layer partition chromatography of an homologous series of terminal diols as *bis*-esters of pyruvic acid 2,6-dinitrophenyl-hydrazone. Diagonally from top to bottom: C<sub>14</sub>, C<sub>13</sub>, C<sub>12</sub>, C<sub>10</sub>, C<sub>4</sub>. Column on left is mixture; stationary phase is polyethylene glycol 400 on Microcel T-38; mobile phase is hexane:benzene (65:35) saturated with polyethylene glycol 400.

#### SUMMARY

A quantitative colorimetric method is described for the acylation of micro- and submicromole amounts of dihydric alcohols as *bis*-esters of pyruvic acid 2,6-dinitrophenylhydrazone. The *bis*-esters which are obtained exclusively, can be cleanly and quantitatively separated from a complex mixture of monohydric alcohol derivatives on an alumina column. Separation of an homologous series of terminal diol derivatives by thin-layer partition chromatography is also described.

#### REFERENCES

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