

Methods for the Isolation and Characterization
of Constituents of Natural Products XX. Rapid
Column Procedures for Forming Isopropylidene
Derivatives of Submicrogram to Micromole
Amounts of Diols

DANIEL P. SCHWARTZ¹

*Eastern Regional Research Center, Agricultural Research Service, U. S.
Department of Agriculture, Philadelphia, Pennsylvania 19118*

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A simple, essentially instantaneous and complete reaction for the formation of isopropylidene (acetonide) derivatives of submicro- and microgram quantities of diols is described. The diol dissolved in acetone:hexane (1:4) is brought in contact with a column of Celite charged with a H_3PO_4 - P_2O_5 mixture and the resulting acetonide eluted for analysis. The procedure was modified so that micromole amounts of diols can be quantitatively derivatized in a continuously flowing column.

INTRODUCTION

This paper describes a simple, rapid procedure for preparing isopropylidene (acetonide) derivatives of diols at the submicro- and microgram level. The 1,2- or 1,3-diol dissolved in an acetone-hexane solution is brought in contact with a micro column of Celite charged with a P_2O_5 - H_3PO_4 mixture. Complete derivatization occurs essentially instantaneously and the product is conveniently eluted for analysis or other manipulation. The method was also extended so that micromole amounts of derivative can be formed continuously and quantitatively in a flowing system.

Isopropylidene derivatives are utilized frequently in structural studies, as an alkali stable protective group, and in synthesis (4). In lipid analysis, they have found their main utility in studies of glyceryl ethers (1,2,6). They also have been employed in the location of double bond position of osmium tetroxide hydroxylated fatty acid methyl esters (3) and long-chain olefins (5).

EXPERIMENTAL PROCEDURES

Materials

Celite 545 (Fisher Sci. Co.,² Pittsburgh, Pa.); phosphorus pentoxide

¹ Author to whom correspondence should be sent.

² Reference to brand or firm names does not constitute endorsement by the U. S. Department of Agriculture over others of a similar nature not mentioned.

and acetone (Reagent grade, J. T. Baker, Chem. Co., Phillipsburg, N. J.); n-hexane (Nanograde, Mallinckrodt Chem. Co., St. Louis, Mo.): Melting point capillaries open at both ends, 100 mm long, 1.2-1.4 mm id \times 1.6-1.8 mm od (Kimble #34,500) were cut approximately in half and used as columns in the microgram procedure; disposable Pasteur Pipettes (145 \times 7 mm OD) were used for columns in the micromole procedure. They were cut just below the crimp to facilitate insertion of column materials and were plugged with a small wad of glass wool.

Preparation of Catalyst Powder

Celite was dried at 160° for 16 hr. Water (0.25 ml) was ground with 10 g of the Celite in a mortar and 2.5 g P₂O₅ was added and the mixture ground intimately for a few minutes. The powder was stored in a desiccator over P₂O₅ when not in use.

Derivatization at the Submicro- and Microgram Level

A cut capillary was dabbed into the catalyst powder until approximately 2 cm was retained. The powder was pushed into the capillary with a tamper made from the straight portion of a paper clip and held there while being pressed into a compact column about 1 cm long with another tamper. From 0.3 to 30 μ g of the diol dissolved in up to 5 μ l of hexane:acetone (4:1) was applied to the column by placing the tip of a 10 μ l syringe on the bed and expelling the solution slowly. The column was then eluted with the same solvent until 5-8 μ l (about 5-8 mm) emerged. This was withdrawn as completely as possible with a clean syringe. Light pressure could be used to force the solvent through the column. If pressure was used, the entire procedure could be accomplished in about 30 sec. The effluent was analyzed by both gas-liquid and thin-layer chromatography as described below.

The procedure for forming isopropylidene derivatives on submicrogram amounts of substrates was also investigated in a situation more likely to be encountered in practice. The diol (0.7 μ g of 1-octadecyloxy-2,3-propanediol) dissolved in 100 μ l of 20% acetone in hexane was applied to the column as follows. A light vacuum was exerted on one end of the capillary and the solution applied in 5 μ l aliquots to the bed. This volume wetted only about half of the column. Evaporation of the solvent occurred practically instantaneously and the next 5 μ l aliquot was applied, etc. The entire 100 μ l could thus be applied in this manner in only a few minutes. After the last aliquot had been evaporated, the column was eluted as above.

Derivatization at the Micromole Level

A tared Pasteur pipette was dabbed into the catalyst powder until about 3 cm was retained. This represented from 200-250 mg. The variation was not critical for the concentrations of diols studied. The powder was

tamped lightly to eliminate air pockets. The subsequent flow rates varied from approximately 2.70 to 3.25 min/ml and from the results (Table 1), the variation in flow rate was also not critical for the concentrations studied. Seven ml of a 10% acetone in hexane solution containing from 20 to approximately 44 μ moles of the diol/ml was pipetted on the column, one ml at a time, and the effluent collected immediately in a tared vial. After the last aliquot had drained, one column volume of solvent was added to complete the elution of the acetone. Solvent in the tared vial was evaporated under a stream of N₂ until the residue was constant in weight.

Chromatography

The effluents from all procedures were checked by gas-liquid (GLC) and thin-layer chromatography (TLC). For GLC, a Hewlett-Packard Model 5750A, equipped with a flame-ionization detector, was employed. A 4' \times 1/8" silanized stainless steel column packed with 3% OV-225 on 100-200 mesh Gas Chrome Q was used. Helium was the carrier gas supplied at a pressure of 30 ml/min. The column was programmed from 110° to 250° at 10°/min. The identity of the isopropylidene derivatives was substantiated by comparison of the retention time with the derivative made by a classical literature procedure (2) and by mass spectrometry using the LKB 9000 GC-MS. TLC was carried out on precoated silica gel G plates using chloroform as solvent. Spots were revealed by spraying with a 20% solution of conc. H₂SO₄ in 95% ethanol and heating at 200°C.

RESULTS AND DISCUSSION.

The following compounds were examined with the submicro- and microgram derivatization procedure: 1-hexadecyloxy-2,3-propanediol (chimy alcohol), 1-octadecyloxy-2,3-propanediol (batyl alcohol),

TABLE I
QUANTITATIVE ASPECTS OF ISOPROPYLIDENE DERIVATIZATION OF
DIOLS ON A CELITE-H₃PO₄-P₂O₅ COLUMN

Compound	Amount over column* (μ moles)	Yield of derivative (% of theory)
1-Hexadecyloxy-2,3-propanediol	140	99
1-Octadecyloxy-2,3-propanediol	145	99
Methyl <i>erythro</i> -9,10-dihydroxystearate	140	100
Methyl <i>threo</i> -9,10-dihydroxystearate	150	100
1-Monopalmitin	144	98
1-Phenyl-1,2-ethanediol	310	96
<i>meso</i> -1,2-diphenyl-1,2-ethanediol	175	100

* Flow rates were from 2.70-3.25 min/ml.

1-hexadecylthio-2,3-propane-diol, 1,2-hexadecanediol, methyl *threo*-9,10-dihydroxystearate, methyl *erythro*-9,10-dihydroxystearate, a mixture of *cis*- and *trans*-1,2-cyclohexanediol, *trans*-1,2-cycloheptanediol, *meso*-1,2-diphenyl-1,2-ethanediol, 1-phenyl-1,2-ethanediol, 1-monopalmitin, 2-hexadecyloxy-1,3-propanediol, and 1,1,2,2-tetraphenyl-1,2-ethanediol (benzopinacole). With the exception of the last compound, which did not react, all of the vicinal diols were converted completely to the corresponding acetonides as shown by GLC and TLC. The derivatives gave the characteristic, intense P-15 ion in the mass spectrum. The 2-hexadecyloxy-1,3-propanediol was estimated to be derivatized to the extent of 95% by peak height measurement of the acetonide.

It was possible to derivatize as little as 0.3 μg of the glyceryl ethers and readily detect the acetonides by GLC (range 10, attenuation $\times 4$). In the more practical experiment in which 0.7 μg was taken up in 100 μl of solvent and applied to the micro column in aliquots, the derivative was easily detected under the same GLC conditions.

The results obtained in the micromole procedure are summarized in Table 1. Derivatization was quantitative or nearly so in all cases when based upon weight of the expected derivative.

No attempt was made to establish the upper capacity of the micromole column for quantitative derivatization. This would ostensibly depend upon the water content of the acetone-hexane solution which would affect the amount of P_2O_5 remaining on the Celite. However, the system appears to be capable of being expanded to derivatize millimole and even mole quantities of diols, although at this scale the procedure would not offer a marked advantage over the classical techniques in which yields are generally also very good.

Using the prescribed solvent (20% acetone in hexane), there is no elution of phosphoric acid and/or P_2O_5 . This solvent system appears suitable for dissolving most of the commonly occurring diol-containing lipids. Attempts were made to use more polar solvent systems (e.g., 20% acetone in dichloromethane and 20% acetone in benzene), but some acid appeared in the residue after a few ml had gone over the column. However, the use of 20% acetone solutions was arbitrarily chosen and no attempts at using lower concentrations of acetone were made.

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Mr. E. J. Saggese of this laboratory supplied the methyl-*threo*- and *erythro*-9,10-dihydroxystearates and Dr. Wm. Ferrell, University of Detroit donated the 1-hexadecylthio-2,3-propanediol.

SUMMARY

A simple, essentially instantaneous and complete reaction for the formation of isopropylidene (acetonide) derivatives of submicro- and microgram quantities of diols is de-

scribed. The diol dissolved in acetone:hexane (1:4) is brought in contact with a column of Celite charged with a H_3PO_4 - P_2O_5 mixture and the resulting acetonide eluted for analysis. The procedure was modified so that micromole amounts of diols can be quantitatively derivatized in a continuously flowing column.

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