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Note

Simple micro-acylating technique with gaseous reagents

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Acylation of a compound containing a derivatizable group (hydroxy, amino, thiol) can be useful for improving its gas chromatographic performance characteristics, facilitating mass spectral identification and enhancing detectability. The more prevalently used acylating reagents and methods have been reviewed by Blau and King[1]. We report a simple, relatively rapid technique for forming the acetate, trifluoroacetate and pentafluoropropionate derivatives of some hydroxyl-containing compounds in which no base or catalyst is used and which is performed with gaseous reagents. Vapor phase acylation has been used[2–4], but we consider these techniques less convenient than the one proposed in this paper.

EXPERIMENTAL

*Materials**

Acetyl chloride (AcCl) (J. T. Baker, Phillipsburg, N.J, U.S.A.), trifluoroacetic anhydride (TFAA) (Aldrich, Milwaukee, WI, U.S.A.) and pentafluoropropionic anhydride (PFPA) (Regis, Mortons Grove, IL, U.S.A.) were used as received. Melting point capillaries open at both ends (1.6–1.8 × 100 mm), glass wool (0.005–0.007 mm diameter), and Celite 545 were from Fisher Scientific (King of Prussia, PA, U.S.A.). Both the glass wool and Celite were purified as described for glass wool[5]. The purified Celite was placed in a 5-ml screw cap vial and the sleeve portion of a rubber septum (A. H. Thomas Co., Philadelphia, PA, U.S.A., cat. No. 8753-D32) was pulled over the threads. The septum was pierced repeatedly with a hypodermic needle until a glass capillary could be inserted smoothly. Chromosorb 102 obtained from Sigma (St. Louis, MO, U.S.A.), was washed with acetone and methanol and dried.

Preparation of micro column

A melting point capillary containing a plug of glass wool** about 3 cm from the end is inserted through the septum on the Celite vial, and, while the index finger is

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

** A convenient way to obtain the plug is to take a piece of glass wool with forceps and push it tightly into a screw cap vial. The capillary is pushed into the wool and rotated until a small plug is retained, then pushed back to 3 cm.

held over the open end, the capillary is pushed up and down into the Celite. The amount of Celite obtained in this way gives a bed about 1–1.5 cm long after the Celite is tamped tightly with wires 1.2–1.3 mm in diameter used as tamping rods. The capillary is tapped several times on the bench top to get rid of any loose Celite. There should be about 1 cm of space from the end of the bed to the end of the capillary to permit facilitation of subsequent manipulations.

Acylation

All compounds (substrates) were studied in submicrogram (0.2–0.8 μg) and microgram (2–8 μg) amounts. A volume of the solution of the substrate dissolved in a suitable solvent (carbon disulfide, toluene, dichloromethane, or ethyl acetate) was applied to the column as follows: the tip of a 10-μl syringe containing the solution was placed onto the bed (glass wool-free end) and the contents were expelled slowly onto the Celite. At least half of the Celite should remain unwetted. The capillary was inserted wetted end down through a septum fitted on a vial containing the acylating reagent and the vapors of the acylating reagent were drawn through the bed by a slight vacuum (15–20 cmHg) applied to the other end (Fig. 1). After 10 min the acylating vial was removed, and air was pulled through the tube for about 30 sec to dissipate most of the acylating reagent. The column was then eluted with CS_2 or CH_2Cl_2 by light air or nitrogen pressure to force the solvent through the bed*. The first 3–4 μl (3–4 mm) of effluent emerging was taken up as thoroughly as possible with a clean syringe for gas–liquid and thin-layer chromatographic analyses.

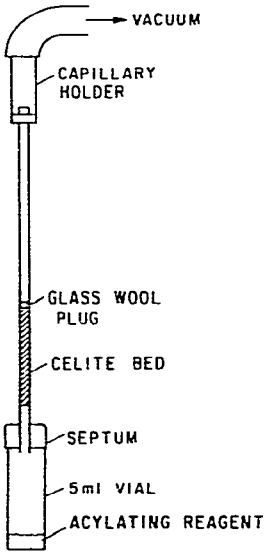

Fig. 1. Apparatus for acylation with gaseous reagents.

* If air pressure is used, a convenient apparatus is a rubber bulb with a netted reservoir and connecting tube (A. H. Thomas Co., cat. No. 1957-K10). The connecting tube can be fitted with a holder such as those supplied with Microcaps or one made from a medicine dropper bulb or septum.

Acylation of very dilute solutions

The procedure for forming acyl derivatives on submicrogram amounts of substrate was also investigated in a situation more likely to occur during the isolation of trace constituents from biological materials, *i.e.*, having a relatively large volume of a very dilute solution of the substrate. For this purpose the bed of Celite was made about 3–4 cm long in the capillary, then 0.2–0.4 μg of the substrate dissolved in 100 μl of CH_2Cl_2 was applied to the bed in 10-μl aliquots while a vacuum was pulled on the opposite end of the capillary. When the wetted area was warmed with the fingers, the solvent evaporated almost instantaneously, so that the entire 100 μl could be applied to the column in a few minutes. Acylation was then effected as above.

Gas–liquid chromatography (GLC)

A Hewlett-Packard 5750 gas chromatograph with flame ionization detector was used. Columns were (a) 183 cm × 2 mm I.D. glass containing 3% SP-2100 on 100–120 mesh Supelcoport and (b) 244 cm × 3.2 mm I.D. stainless steel packed with 7.5% ethylene glycol adipate and 2% phosphoric acid on 80–100 mesh Anakrom

TABLE I
ACYLATION OF VARIOUS COMPOUNDS ON A CELITE COLUMN WITH GASEOUS REAGENTS

AcCl = Acetyl chloride; TFAA = trifuoroacetic anhydride; PFPA = pentafluoropropionic anhydride. a = 183 cm × 2 mm I.D. glass packed with 3% SP-2100 on 100–120 mesh Supelcoport; b = 244 cm × 3.2 mm I.D. stainless steel packed with 7.5% ethylene glycol adipate and 2% phosphoric acid on 80–100 mesh Anakrom ABS.

Compound	*GLC column*	*AcCl*	*TFAA*	*PFPA*	*Comments*
Cholesterol	a (230)	+	+	+	Complete* (shoulder on peak)
Diethyl stilbestrol	a (180)	+	+	+	Complete (2 peaks)
Epicoprostanol (5β-cholestan-3α-ol)	a (250)	+	+	+	Complete
β-Estradiol	a (215)	+	+	+	Complete, (bis derivative)
Estradiol-3-benzoate	a (265)	+	+	+	Complete
Furfuryl alcohol	b (95)	−	−	−	Decomposed
p-Hydroxypropiophenone	b (220)	+	+	+	90%
Lanosterol + dihydrolanosterol (*ca.* 65 : 35)	a (250)	+	+	+	Both complete
Methyl 9,10-dihydroxystearate	a (208)	+	+	+	Complete (bis derivative)
Methyl α-hydroxypalmitate	a (160)	+	+	+	Complete
Methyl 12-hydroxystearate	a (180)	+	+	+	Complete
o-Methoxyphenol	b (110)	+	+	+	Complete
1-Naphthol	b (200)	+	+	+	Complete
10-Nonadecanol	a (175)	+	+	+	Complete
1-Octadecanol	a (160)	+	+	+	Complete
Testosterone	a (212)	+	+	+	Complete (2 peaks)
3,4,5-Trimethyoxybenzyl alcohol	b (220)	+	+	+	Complete
Triphenylmethanol		−	−	−	No reaction, TLC

* Denotes complete disappearance of parent compound.

ABS. Helium was the carrier gas exiting from the columns at 37 ml/min. The oven was operated isothermally, with temperatures selected to give adequate separation between the acylated compound and the substrate (Table I).

Thin-layer chromatography (TLC)

Microscope slides (2.5 × 10 cm) coated with 250-μm thick layers of silica gel G (Analtech, Newark, DEL, U.S.A.) were used. The plates, after being spotted with the reaction mixture and substrate, were developed in a suitable solvent (benzene, methylene chloride or ethyl acetate), and dried. The spots were revealed when charred with 50% aq. sulphuric acid.

RESULTS AND DISCUSSION

The compounds investigated and their ability to acylate with the reagents studied are given in Table I. With the exception of the highly hindered triphenylmethanol and the acid-labile furfuryl alcohol, all of the compounds were acylated satisfactorily at both the submicrogram and microgram levels. Complete disappearance of the parent compound was noted in all instances in the positive reactors except for *p*-hydroxypropiophenone of which about 10% remained unacylated after 10 min; with a 20 min exposure to the acylating reagents, only a trace of unacylated *p*-hydroxypropiophenone could be detected.

Although 10 min exposure to the acylating reagents was chosen as the standard reaction time based on initial observations with acetyl chloride, we checked several compounds and found that they could be acylated completely in much shorter exposure times. Cholesterol, lanosterol and dihydrolanosterol were completely acylated with TFAA and PFPA in 3 min, and testosterone in 0.25 min. Diethyl stilbestrol, however, required 10 min. The other compounds in Table I were not investigated in this regard.

Dihydroxy compounds (methyl 9,10-dihydroxystearate and β-estradiol) gave the bis derivative exclusively as determined by GLC and GLC–mass spectrometry (MS). Testosterone gave two peaks with all reagents; a minor peak with a shorter retention time was identified as the bis acyl derivative and the other peak proved to be the mono (C_{17}) acyl derivative. The ratio of the enol di-acyl derivative to the mono-acyl derivative was dependent on exposure time to the acylating vapor. The bis derivative as percent of total area (bis + mono) with time was: 19% at 20 min; 19% at 10 min; 10% at 5 min; 7% at 2.5 min; 6.8% at 1 min; 5.2% at 0.5 min; and 1.1% at 0.25 min. Thus, it is possible to form the mono-trifluoroacetate and mono-pentafluoropropionate practically exclusively and still get complete acylation of the 17-hydroxyl group in only 15 sec.

Diethyl stilbestrol gave two peaks with identical mass spectra, both being the bis derivative. We assume that these are the *cis* and *trans* isomers, although we did not determine which peak was which.

The acylating procedure was also tried with Chromosorb 102 (a styrene–divinylbenzene polymeric adsorbent) as a support for the deposition of the substrate. The three compounds investigated, 10-nonadecanol, cholesterol, and 1-octadecanol, were completely acylated with TFAA and PFPA in 10 min. AcCl was not studied. It would, therefore, seem feasible to concentrate adsorbable compounds from aqueous

solution onto a micro column of Chromosorb 102 (constructed, for example, in the tip portion of a disposable Pasteur pipette) and after drying, directly acylate the adsorbed susceptible structures.

Although we did not investigate detection of the fluorinated derivatives by electron capture, there is no obvious reason why nanogram or even picogram amounts of a substrate should not be satisfactorily acylated and recovered from the bed in a suitable solvent for electron capture capture detection. The technique of depositing multiple aliquots of a very dilute solution onto the support would then facilitate derivatization and detection of extremely small amounts of substrate.

ACKNOWLEDGEMENTS

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