Sulfate Ester Conjugates: A One-Step Method for Replacing the Sulfate with an Acetyl Group

A simple one-step method is described for replacing the sulfate moiety of a conjugate with an acetyl group. The method is rapid, easy to use, and is especially useful for characterization of compounds which are unstable to conventional hydrolysis conditions.

Sulfate ester conjugates, being nonvolatile, are impossible to characterize by techniques such as gas–liquid chromatography (GLC) and mass spectrometry. In some cases, it is possible to remove the sulfate group by acidic, basic, or enzymatic hydrolysis and then characterize the hydrolysis product. However, in many instances the hydrolysis product is not stable under the conditions necessary to remove the conjugate. Preliminary investigations in this laboratory demonstrated that acidic, basic, and enzymatic hydrolysis all resulted in the degradation of many of the metabolites of carbaryl (1-naphthyl methyl carbamate) isolated from chicken urine (Paulson and Feil, 1969). Subsequent studies were conducted to develop a method to replace the polar conjugating group with a relatively nonpolar protective group. This communication describes how a previously reported acetylation procedure (Sullivan *et al.*, 1967) can be used to remove the sulfate ester conjugate from a variety of compounds and convert them to their acetyl derivatives.

EXPERIMENTAL

Chemicals and Supplies. The source of chemicals was as follows: dehydroisoandrosterone acetate, dehydroisoandrosterone sulfate, estrone, estrone sulfate, *p*-nitrophenyl sulfate, and 1-naphthyl sulfate, Sigma Chemical Co.; 1-naphthyl acetate, K and K Laboratories Inc.; 6-bromo-2-naphthyl acetate, Mann Research Laboratories, Inc.; acetic anhydride and benzene, Fisher Scientific Co.; methane sulfonic acid, Eastman Organic Chemicals, Eastman Kodak Co.; and benzothiophene 4-sulfate and benzothiophene 4-acetate, prepared as previously described (Robbins *et al.*, 1969); Gas Chrom Q, and OV-1, Applied Science Laboratories.

Acetylation Procedure. Ten milligrams of the sulfate ester was mixed with 2 ml. of acetylating reagent (40 part acetic anhydride and 1 part methane sulfonic acid, v. per v.) at 0° C. The mixture was heated at 100° C. for 30 minutes and then cooled to 0° C. A small amount of ice (equivalent to approximately 2 ml. of water) was added, and the mixture was extracted three times with an equal volume of benzene. The combined benzene extracts were concentrated to a small volume and the acetylated compounds were purified using a Barber-Colman 5000 Series gas chromatograph (column, 8 foot, 5-mm. I.D.; support, Gas Chrom Q, 80-100 mesh; liquid phase, 2% OV-1; helium flow rate, 55 ml. per minute; injection port temperature, 350° C.; detector temperature, 350° C.; isothermal, column temperature selected to give 5–10 minute retention time). The efficiency of the acetylation procedure was by comparison of gas chromatographic peak heights with those obtained after GLC of known amounts of authentic samples. Capillary tubes were used for trapping the acetylated compounds from the gas chromatograph. Infrared spectra were taken with a Model 337 Perkin-Elmer Grating Infrared Spectrometer, using the micro KBr technique (1.5-mm. disk with a 4X beam condenser). Ultraviolet spectra were taken with a Bausch and Lomb Spectronic 505 Spectrometer.

RESULTS AND DISCUSSION

Characterization studies, which included GLC retention time and ultraviolet and infrared spectrometry, conclusively demonstrated that the described acetylation procedure removed the sulfate ester from a variety of compounds and converted them to the corresponding acetyl derivatives (Table I). The efficiency of the reaction varied from 23% to 95% of the theoretical yield under the described conditions. The procedure has been used routinely with sample sizes ranging from 50 μg. to 1 gram, using reagents in the same proportions; with few exceptions, the efficiency of the reaction was 50% or greater. In some cases the efficiency of the reaction was improved by increasing the ratio of acetylating reagent to starting material; this approach was especially useful for small sam-

Table I. Reaction of Sulfate Esters with Acetic Anhydride in the Presence of Methane Sulfonic Acid

Starting Material	Acetylation Product	Efficiency of Reaction
1-Naphthyl sulfate	1-Naphthyl acetate[a]	58%
6-Bromo-2-naphthyl sulfate	6-Bromo-2-naphthyl acetate	65%
p-Nitrophenyl sulfate	*p*-Nitrophenyl acetate	58%
Estrone sulfate	Estrone acetate	43%
Dehydroisoandrosterone sulfate	Dehydroisoandrosterone acetate	23%
Benzothiophene 4-sulfate	Benzothiophene 4-acetate	95%

[a] The acetylation product was identified by comparing the infrared and ultraviolet spectra and GLC retention time with an authentic sample.

ples (200 μg. or less) and/or when the conjugated compound was only slightly soluble in the acetylating reagent. Heating the reaction mixture longer (1 to 6 hours) also increased the efficiency of reaction of some compounds with low solubility.

The described acetylation procedure has been especially useful for characterization of compounds which are unstable to conventional hydrolysis conditions. For example, when the sulfate ester of 4-hydroxy carbaryl, isolated from chicken urine (Paulson and Feil, 1969), was hydrolyzed with acid or base, the hydrolysis product was unstable; therefore, the nature of the hydroxylated ring structure could not be determined. However, when the same compound was treated with the acetylation reagent, the stable and relatively nonpolar acetyl derivative of 4-hydroxy carbaryl was produced. The acetylated product was then purified by solvent extraction and gas chromatography, and identified by infrared and mass spectrometry as 4-acetoxy-*N*-acetyl carbaryl (Paulson *et al.*, 1970). Once the nature of the ring structure was known, it was possible to synthesize the sulfate ester of 4-hydroxy carbaryl and thereby determine the structure of the metabolite which had been isolated from chicken urine.

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G. D. Paulson
C. E. Portnoy

U.S. Department of Agricuture
Metabolism and Radiation Research Laboratory
Animal Husbandry Research Division, ARS
Fargo, N.D. 58102

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