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Note

Alkylation with dialkylsulfate and diisopropylethylamine

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Alkylation, especially with methyl or ethyl groups, is commonly used to improve the gas chromatographic (GC) characteristics of organic compounds containing acidic hydrogens, as has been reviewed[1,2]. A diversity of alkylation procedures is available, and this diversity appears to be needed due to the different characteristics including limitations of the methods. For example, alkylation with diazomethane is mild and the reagent is volatile, but diazomethane is toxic and product disappearance[3] and also heterogeneity[4] can occur. Here a mild yet powerful alkylation procedure employing dimethylsulfate or diethylsulfate and diisopropylethylamine is presented.

MATERIALS AND METHODS

Chemicals

Phenobarbital and theophylline were acquired from Applied Science (Deerfield, IL, U.S.A.). Thymine and uracil were obtained from Sigma (St. Louis, MO, U.S.A.). Dimethylsulfate and diethylsulfate were purchased from Aldrich (Milwaukee, WI, U.S.A.). Sequanal grade diisopropylethylamine and acetonitrile were obtained from Pierce (Rockford, IL, U.S.A.).

Methylation of theophylline

Theophylline (0.1 mmol) was dissolved in acetonitrile (0.5 ml) in a 5-ml Reacti-vial fitted with a Mininert valve (Pierce). Diisopropylethylamine (0.24 ml, 2.5 mmol) and dimethylsulfate (0.88 ml, 5 mmol) were added and the mixture was stirred at room temperature. High-performance liquid chromatographic (HPLC) analysis after 20 min showed greater than 99% conversion to a single product that co-eluted with caffeine. The same chromatogram was observed after 1 and 2 h of reaction. Only a single product was also evident by thin-layer chromatography (TLC). In order to determine the yield, the reaction mixture after 1 h was diluted with an equal volume of water and aliquots were subjected to HPLC. Caffeine was used as an

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external standard. To confirm the structure of the product, the reaction mixture on a larger scale was diluted with water and extracted with chloroform. The chloroform layer was separated, washed twice with water, and dried over anhydrous sodium sulfate. The residue after solvent evaporation was dried over phosphorus pentoxide *in vacuo* at room temperature and recystallized from toluene. The structure was confirmed by mass spectrometry.

Methylation of the other compounds

The other compounds listed in Table I were methylated similarly. In the case of theophylline, alkylation with diethylsulfate was also done. The structures of the products were confirmed by mass spectrometry except in some cases, as noted, where the product was confirmed by TLC and HPLC comparison with an authentic sample. For ethylation of theophylline, the product had the expected retention time by HPLC.

Chromatography

TLC was done on silica with fluorescence quench detection using hexane–ethyl acetate (75:25, v/v). Preparative HPLC was done on an in-house C_8-bonded silica packing with a gradient of 40–80% acetonitrile in water. Analytical HPLC was performed on a Microsorb C_8 column (15 × 0.46 cm I.D., Rainin, Woburn, MA, U.S.A.) with various gradients of acetonitrile, water and phosphate buffer as required.

RESULTS AND DISCUSSION

The results of the alkylation reaction with dimethylsulfate and diisopropylethylamine are summarized in Table I. As seen, the analytes examined thus far were either converted essentially quantitatively into a single product with complete substitution of active hydrogens by methyl, or remained non-reactive. In the case of theophylline, it was established, as shown, that either methyl or ethyl alkylation could be done. For methylation of uracil, as a test case, the reaction was found to work equally well under either dry or moist conditions.

In some of the reactions a small peak potentially corresponding to unreacted starting material was observed by HPLC, as reflected by a >99% value cited for disappearance of starting material in these reactions. Presumably more severe or prolonged reaction conditions would generally give a 100% conversion. This was demonstrated for the methylation of benzoic acid as seen. However, although >99% of theophylline was methylated after 0.3 h at 35°C, no further methylation took place when the reaction time was extended to 2 h, or when the reaction was conducted for 1 h at 90°C. This is difficult to understand unless the residual peak is an interference. Since this peak was not seen when theophylline was reacted in a later experiment with diethylsulfate, an impurity might have been present in the dimethylsulfate.

A small peak (<1%) corresponding to N-methylaniline was observed by HPLC in the methylation reaction for aniline along with a peak for the major product, N,N-dimethylaniline. This small peak refused to disappear even after a prolonged reaction time (16 h) and the addition of more reagents. Suspecting that this peak was an interference, we obtained its UV spectrum under stop-flow conditions in the HPLC, and surprisingly found that it matched that of N-methylaniline ob-

TABLE I

ALKYLATION WITH DIALKYLSULFATE AND DIISOPROPYLETHYLAMINE IN ACETONITRILE

Solute	*Reaction conditions*[*]	*Number of active hydrogens*	*Number of added alkyls*[**]	*Disappearance of starting material*[***] *(and product yield ± relative standard deviation*[§]*) (%)*
Theophylline	RT, 0.3–2 h	1	1	> 99 (111 ± 15)
	90°C, 1 h	1	1	> 99
	RT, 24 h	1	1 (ethyl)[§§]	100[§§]
Phenobarbital	90°C, 1 h	2	2	> 99
N^4-Pentafluorobenzoylcytosine	35°C, 24 h[§§§]	2	2	100 (87 ± 3.1)
Uracil	90°C, 1 h[†]	2	2	100
Thymine	90°C, 1 h	2	2	100
Benzoic acid	RT, 0.3 h	1	1	> 99
	90°C, 1 h	1	1	100
2,4-Dichloro-3,5-dinitrobenzoic acid	RT, 0.3 h	1	1	100
Succinic acid	90°C, 1 h	2	2	100
Phenol	RT, 90°C, 140°C	1	0	0
Aniline	RT, 1 h[††]	2	2	> 99

[*] In all cases except for alkylation of aniline and N^4-pentafluorobenzoylcytosine, a 25-fold molar excess of dialkylsulfate and a 50-fold molar excess of diisopropylethylamine were used. RT = room temperature.

[**] Alkyl is methyl in all cases and is also ethyl for theophylline as shown.

[***] The disappearance of starting material is based on analysis by HPLC. A single product peak was observed by HPLC and TLC in all cases except for N^4-pentafluorobenzoylcytosine and aniline as described below.

[§] In two cases, as shown, the product was quantitated using an HPLC standard curve obtained with an independent sample of purified product.

[§§] With diethylsulfate as the alkylating reagent, HPLC analysis showed complete disappearance of starting material, along with the formation of a major new peak, assumed to be the desired product. A minor unknown peak (2% of the peak area of the major peak) eluting between the major peak and theophylline was also seen.

[§§§] A 250-fold molar excess of dimethylsulfate and a 500-fold molar excess of diisopropylethylamine were used. Unidentified side products were observed by HPLC in addition to a peak for the major product, N^4-dipentafluorobenzoyl-1,3-dimethylcytosine[5,15].

[†] The product was purified by recrystallization from ethanol. The presence of 200 μl of added water in the reaction had no effect.

[††] A 50-fold molar excess of dimethylsulfate and a 100-fold molar excess of diisopropylethylamine were employed.

tained with an authentic sample. Why a small amount of N-methylaniline resisted further methylation was not clear.

The yield of the product in addition to the disappearance of starting material was determined in two cases. For theophylline, these yield and disappearance values agreed within the precision of the analysis. This was consistent with our observing only a single HPLC peak for product when theophylline is methylated. All of the other reactions behaved similarly by HPLC analysis except the one conducted on

N^4-pentafluorobenzoylcytosine, where side products were observed by HPLC. This motivated us to determine the yield of the major product (87%) in this case as well. The latter yield was obtained only after a thorough optimization of the reaction conditions[5]. In this other work the mixture of products did not change with a longer reaction time, so that the reactant, or an intermediate product, is labile under these reaction conditions. Perhaps this is related to the presence of an electrophoric pentafluorobenzoyl group on this compound.

In a related methylation procedure, Brookes and Lawley methylated cytosine with dimethylsulfate in dimethylformamide, but without any base, both at 37°C and 100°C[6]. Some cytosine remained unchanged and both 1-methylcytosine and 1,3-dimethylcytosine were obtained. Thus it is clear that the diisopropylethylamine base in our procedure makes an important contribution. This base was chosen because of its high pK_a of 10.7 (a value determined in 50% aqueous ethanol[7]) and also because of its anticipated resistance to alkylation based on its sterically hindered amine group[8].

No methylation of phenol was observed even when the reaction temperature was increased to 140°C as seen in Table I. This contrasts with the ability of dimethylsulfate to methylate phenol in the presence of methanolic potassium hydroxide and sodium carbonate along with heating[9]. Phenols are also methylated by dimethyl carbonate and 4-dimethylaminopyridine when a reaction temperature above 150°C is used[10,11].

A related methylation reaction is the use of a dialkylsulfate to convert an anhydride in the presence of the appropriate alcohol to the corresponding ester[12]. Esterification of a carboxylic acid with triethyloxonium fluoroborate in the presence of diisopropylethylamine[8] or another amine base[13] also has been reported.

This methylation technique has some attractive features, aside from the aspects already cited. First of all, the diisopropylethylamine base and the acetonitrile solvent are commercially available in a highly purified form, directly applicable to this reaction. Second, dimethylsulfate and diethylsulfate are similarly available, including a perdeutero form for dimethylsulfate. Such alkylating reagents have been noted to yield clean GC chromatograms[14], and can be removed at the end of a reaction, along with the diisopropylethylamine, by water extraction. Finally, the reaction provides homogeneous reaction conditions. A disadvantage is the toxicity of dialkylsulfates, although this aspect is difficult to avoid with alkylation reagents.

Alkylaton is expected to play an increasing role in the derivatization of compounds containing active hydrogens prior to their determination by GC. This is due not only to the development, including the work here, of improved alkylation reactions, but also is needed because of the limited performance of silyl derivatives for trace GC analysis[16].

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