urinaire afin d'extraire par une faible quantité de solvant, permettant d'injecter directement, après méthylation, le tributylphosphate. La méthylation est complète et rapide dans ce solvant. La deuxième difficulté est la très grande volatilité du méthyloxalate formé. Il faut évaporer avec beaucoup de précaution l'éther et le méthanol.

Le tributylphosphate ne gêne pas l'analyse chromatographique malgré sa faible volatilité, mais il est recommandé d'injecter des volumes de l'ordre de 1 à 2 μl seulement. Au-delà il y a une saturation de la colonne, ce solvant ne sortant que vers 240°.

Malgré ces quelques inconvénients, cette méthode par chromatographie gazeuse permet une analyse quantitative rapide de plusieurs échantillons urinaires. Aucune purification urinaire n'a été nécessaire. Le profil chromatographique d'une solution témoin et urinaire est comparable. Aucun produit urinaire n'interfère sur ces colonnes qui analysent les petites molécules (voir Fig. 1).

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Nitration of aromatic compounds during development of silver nitrate-impregnated silicic acid columns with carbon tetrachloride

Chromatography on silver nitrate-impregnated silicic acid has proved to be a valuable device for olefin separations[1,2]. Relatively few cases of destructive chemical reactions of silver nitrate/silicic acid with the sample are known. One instance of olefin epoxidation has been reported[3]. Alkyl bromides react[4]. Olefins have been destroyed deliberately by silver nitrate/silicic acid at 300° in gas chromatography[5]. WENKERT *et al.*[6] have recently communicated results on nitration of phenols in *ca.* 10% yield by silver nitrate/silicic acid in benzene at 25–80°.

We wish to warn chromatographers of another, more serious nitration reaction occurring on silver nitrate/silicic acid in the presence of carbon tetrachloride. This results from rapid chemical reaction of carbon tetrachloride with silver nitrate to

produce phosgene and a nitrating agent thought to be dinitrogen pentoxide; a more detailed account of the reactions involved is being published elsewhere. In view of the inertness of tetrachloromethane to silver nitrate in homogeneous solution[7], others may also be tempted to develop chromatograms on silver nitrate/silicic acid with this solvent. The following examples give an idea of the results which may be expected from doing so.

When *trans*-stilbene is chromatographed in hexane–carbon tetrachloride on silver nitrate/silicic acid, none of the starting material can be recovered unchanged. A spectrum of products results, from which 4,4'-dinitrostilbene (1.7 %) can be isolated. Attempted elution of N-methylacetanilide with carbon tetrachloride–chloroform results in recovery of about 40 % each of starting material and *p*-nitro-N-methylacetanilide. Benzene in carbon tetrachloride–hexane is nitrated by silver nitrate/silicic acid to the extent of 12 % (static conditions) to 28 % (stirred) in 2.8 h in batch experiments at room temperature. Benzene elution of silver nitrate/silicic acid columns previously contacted with carbon tetrachloride produces copious quantities of nitrobenzene in the effluent.

Our experiments show that neither benzene nor anisole is nitrated by silver nitrate/silicic acid in the absence of carbon tetrachloride. The nitration of phenols observed by WENKERT *et al.*[6] may thus involve the presence of the acidic hydroxyl group, not just a highly reactive aromatic nucleus.

At least one instance of development of chromatograms on silver nitrate/silicic acid with chloroform has been reported[8]. We find that chloroform too reacts rapidly with silver nitrate/silicic acid, although no nitrating agent results. The good results obtained in the TLC separations using chloroform[8] may be due to the speed of TLC development, or it may indicate that silver chloride/silicic acid is also an effective adsorbent. However, one should approach the use of any halogenated solvent with caution.

Experimental

Nitration of trans-stilbene. A solution of 504 mg (2.79 mmole) of *trans*-stilbene in 10 ml of hexane–carbon tetrachloride (2:1) was applied to a 14.4-g column of silver nitrate/silicic acid prepared in the same solvent. Over a period of 8 h the column was eluted with hexane–carbon tetrachloride and carbon tetrachloride–benzene mixtures, benzene, and ether. The combined eluates from two such experiments were evaporated to 2.61 g of a mobile orange oil containing much nitrobenzene. This deposited crystals (45 mg) on treatment with carbon tetrachloride–hexane, and these were recrystallized from acetone to give 25 mg (0.093 mmole, 1.7 %) of 4,4'-dinitrostilbene [m.p. 296–299° (reported[9] m.p. 292–294°); mol. wt.: calc. for dinitrostilbene, 270.064; obs., 270.065 ± 0.002 (mean of five mass spectrometric measurements)].

The remaining oil was rechromatographed on 19 g of silicic acid prepared in benzene–hexane (1:1). With 150 ml of this solvent was eluted 952 mg of material containing much nitrobenzene which was allowed to evaporate on standing. The residue was recrystallized from ethanol to give 47 mg of unidentified nitro compound(s), m.p. 180–212° (gas evolution). Continued elution with various solvents in the above chromatogram produced a long series of fragrant orange oils totalling 1.00 g and possessing IR spectra characteristic of variously nitrated aromatic ketones and hydroxy ketones.

Nitration of N-methylacetanilide. A solution of 500 mg of N-methylacetanilide in 6 ml of carbon tetrachloride was applied to a 15-g column of silver nitrate/silicic acid prepared in carbon tetrachloride. Elution with 315 ml of carbon tetrachloride and 350 ml of chloroform removed 663 mg of green oil and crystals. The NMR spectrum showed this to be a mixture of four substituted N-methylacetanilides, two of which were recovered starting material (204 mg, 1.37 mmole, 41 %) and *p*-nitro-N-methylacetanilide (288 mg, 1.48 mmole, 44 %). The material was rechromatographed on 19 g of silicic acid. After 335 mg of oils was eluted by carbon tetrachloride, methylene chloride and methylene chloride–acetic acid (150:1), 226 mg of yellow crystals was eluted with 600 ml of methylene chloride–acetic acid (100:1). This was recrystallized from water to give 109 mg (0.562 mmole, 17 %) of *p*-nitro-N-methylacetanilide, m.p. 153–154° (reported[10] m.p. 153°), with the required NMR spectrum: τ 2.06 (singlet), 3.37 (singlet), 7.48 (doublet, J 9 Hz), and 8.15 (doublet, J 9 Hz) p.p.m.

Nitration of benzene. To the freshly dried silver nitrate/silicic acid sample was added 10 ml of carbon tetrachloride–hexane (1:2). After 1–15 min, 250 μl (214 mg, 2.74 mmole) of benzene was injected and the mixture was agitated and allowed to stand for the specified time. It was then transferred to a small chromatography column and eluted with ether to provide one or two (depending on the amount of silicic acid used) 25.0-ml product solutions which were analysed by gas chromatography.

Silver nitrate-impregnated silicic acid. Mallinckrodt chromatographic silicic acid (6.1 % water loss at 150°), 56.1 g, was slurried with 160 ml of methanol and added to a solution of 14.00 g of silver nitrate in 60 ml of 50 % aqueous methanol. The resulting slurry was evaporated at 40–55° and 15 Torr in a rotary evaporator to yield a white powder which was lightly ground in a pyrex mortar. The product was dried to constant weight at 150° just before use, and the resulting samples thus contained 1.24 mmole silver nitrate/g. Material dried to constant weight at 120° possessed the same chemical properties.

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