Gas Chromatography of Barbiturates, Phenolic Alkaloids, and Xanthine Bases: Flash-Heater Methylation by Means of Trimethylanilinium Hydroxide

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Abstract □ Injection of methanol solutions of trimethylanilinium salts of barbiturates, phenolic alkaloids, and dimethylxanthines produced thermal decomposition in the injection port to give methyl derivatives suitable for quantitative gas chromatography. Trimethylanilinium hydroxide appeared to be superior to tetramethylammonium hydroxide for instantaneous methylation of these compounds. Quantitative methylation was achieved under a variety of experimental conditions. The change in retention time resulting from methylation may be used for identification purposes. The 1,3-dimethyl structure of methylated barbiturates was confirmed by NMR spectroscopy.

Keyphrases □ Barbiturates—analysis □ xanthine bases—analysis □ Phenolic alkaloids—analysis □ Trimethylanilium hydroxide—methylating agent □ Methylation—gas chromatograph flash heater □ GLC—analysis □ NMR spectroscopy—structure

During the last few years, gas chromatography has become the method of choice for identifications and quantification of high molecular weight compounds of biological interest (1, 2). The polar nature associated with the acidic functions of barbiturates, phenolic alkaloids, and certain xanthines tends to cause adsorption resulting in loss of material, contamination of the column, and tailing peaks (3). This makes quantitative work difficult or impossible, especially at the submicrogram level. The problem can be partly overcome by careful deactivation of the solid support, and it can often be eliminated completely if the compound to be gas-chromatographed can be converted to a suitable, nonpolar derivative. Trimethylsilyl derivatives are used most extensively for gas chromatography of phenols, alcohols, and amines. The barbiturates do not give stable derivatives with the common silylating reagents; however, they may readily be methylated to dimethylbarbiturates which are more volatile than the unmethylated compounds and can be gas chromatographed with little or no adsorption. The two dimethylxanthines, theophylline and theobromine, have very low volatility, and therefore require a high column temperature for gas chromatography. Furthermore, they have low solubility in most solvents and are difficult to gas chromatograph without considerable adsorption losses. On methylation, both give caffeine which is much more volatile, more soluble in organic solvents, and readily analyzed by gas chromatography. In the same way, the acidic function of phenolic alkaloids, such as morphine, may be methylated to give methyl ethers which are more suitable for quantitative gas chromatography.

Cook *et al.* (4) used diazomethane for methylation of barbiturates for the purpose of gas chromatography. Martin and Driscoll (5) preferred dimethyl sulfate as a methylating agent according to the procedure described by Stuckey (6). Robb and Westbrook (7) showed that various carboxylic acids could be methylated in the flash heater of the gas chromatograph by injecting a methanol solution of their tetramethylammonium salts. This method has also been applied to barbiturates (8) and to purine and pyrimidine bases (9). Stevenson (8) reported quantitative methylation of barbiturates if the molar ratio of tetramethylammonium hydroxide to the barbiturate was at least four and the injection port temperature about 240°. Under these conditions, however, the 5-phenylbarbiturates gave two peaks, one of which appeared to be caused by alkaline decomposition.

Applying the method of Stevenson, the authors found that most barbiturates gave small second peaks due to incomplete methylation, but did not observe any degradation reaction with phenobarbital. With phenolic alkaloids it was even more difficult to achieve complete methylation. It was concluded that a quaternary ammonium base which would produce a better leaving group than trimethylamine, should require shorter reaction time and milder conditions for thermal decomposition of its salts. Such a base is trimethylanilinium hydroxide which is used for commercial methylation of morphine to codeine. Comparative studies showed that trimethylanilinium hydroxide is superior to tetramethylammonium hydroxide for flash-heater methylation of barbiturates, xanthine bases, and phenolic alkaloids.

Based on NMR studies, it was concluded that methylation of barbiturates produced the 1,3-dimethyl derivatives.

EXPERIMENTAL

Apparatus—A gas chromatograph[1] was used with a hydrogen flame detector. Hydrogen was supplied by a hydrogen generator.[2] The column was made from stainless steel tubing, 0.31-cm. (1/8-in.) outside diameter and 152.4 cm. (5 ft.) long, packed with 3% silicone rubber[3] on diatomaceous earth,[4] 100–120 mesh. Helium was used as the carrier gas at a flow rate of 27 ml./min.

Reagents—Trimethylanilinium iodide was prepared by reacting dimethylaniline (reagent grade, free of mono) in ethyl acetate with 1.2 mole equivalents of methyl iodide at room temperature. The crystalline product was filtered and recrystallized from absolute ethanol. Trimethylanilinium hydroxide, about 0.1 *M*, was made by dissolving 263 mg. of the iodide in 10 ml. of reagent grade methanol in a 25-ml. glass-stoppered flask. Finely powdered silver oxide

[1] Aerograph Hy-Fi, model 600B.
[2] Aerograph model 650.
[3] SE-30.
[4] Gas-Chrom Q, Applied Science Laboratories, Inc., State College, Pa.

(175 mg.) was added and the mixture stirred with a magnetic stirrer. After 30 min., a few drops of the supernatant solution was removed, acidified with dilute nitric acid, and tested for iodide with silver nitrate solution. This testing was repeated if necessary, until the reaction was complete. The solution was filtered under nitrogen into an injection vial made of borosilicate glass and closed with a rubber closure previously boiled in methanol. The solution was withdrawn as needed by means of a hypodermic syringe.

Tetramethylammonium hydroxide solution, about 0.1 *M*, was prepared in the same way from tetramethylammonium bromide.

Gas Chromatography—To a screw-cap containing the substance or substances to be gas chromatographed was added the methylating agent in approximately 100% excess. The solution was diluted with methanol if necessary, and 1 μl., containing 0.2 to 1.0 mcg. of each compound was injected into the flash heater with a microsyringe.[5] The injection port temperature was usually 250° for the barbiturates and 275° for the xanthines and the phenolic alkaloids. The column temperatures were 130°, 137°, and 210° for barbiturates, xanthines, and phenolic alkaloids, respectively. Single-component solutions were gas chromatographed in order to determine the completeness of the methylation and to detect possible decomposition products. Mixtures of barbiturates were also chromatographed for the purpose of determining relative retention times and resolution of closely related compounds.

NMR Spectroscopy—The barbiturates were methylated with dimethyl sulfate as described by Martin and Driscoll (5) and their NMR spectra determined in hexadeuterio acetone and hexadeuterio dimethylsulfoxide with a NMR spectrometer[6] and internal tetramethylsilane standard. The dimethylbarbiturates prepared in this way had the same retention times as the products obtained by flash-heater methylation.

RESULTS AND DISCUSSIONS

Flash-heater methylation of barbiturates with trimethylanilinium hydroxide appeared to give complete reaction under widely different conditions. Excess of reagent ranging from 50 to 500% gave the same results. The injection port temperature was varied from 200° to 350° with no apparent effect. Packing of the stainless steel flash heater with glass wool, or inserting a glass sleeve packed with glass wool in the flash heater as recommended by Stevenson (8), did not seem to affect the results. A plot of peak height against the amount of barbiturate injected (0.1 to 1.0 mcg.) gave a straight line passing through the origin, indicating that the method is suitable for quantitative analysis. When stored for 48 hr. in a refrigerator, the barbiturate salt solutions gave the same gas chromatograms, qualitatively and quantitatively, as when they were first prepared. Most barbiturates gave single, symmetrical peaks when the methylation was carried out with trimethylanilinium hydroxide. Minor second peaks representing unmethylated compounds occurred with tetramethylammonium hydroxide. Barbiturates containing brominated susbstituents as well as the 2-thio analogs gave two or more peaks with both reagents due to decomposition. A large number of barbiturates can be separated as their dimethyl derivatives, including amobarbital and pentobarbital which do not separate on silicone rubber as the unmethylated compounds (10, 11). Probarbital could be separated from diallylbarbituric acid and phenobarbital from cyclobarbital. These two pairs did not separate with Stevenson's system. A barbiturate having a methyl substituent in the 1-position cannot be separated from the parent compound with no *N*-substituent because they give the same dimethyl derivatives. However, such substances can readily be differentiated on the basis of the peak shifts produced by complete methylation. A gas chromatogram of a mixture of twelve barbiturates is illustrated in Fig. 1.

It is generally assumed that the dimethylbarbiturates have the methyl substituents in Positions 1 and 3. This is based on the observation that the wavelength of maximum absorption at 228 mμ is unaffected by changes in pH (6). The authors have confirmed the 1,3-structure by NMR spectroscopy which showed that the two

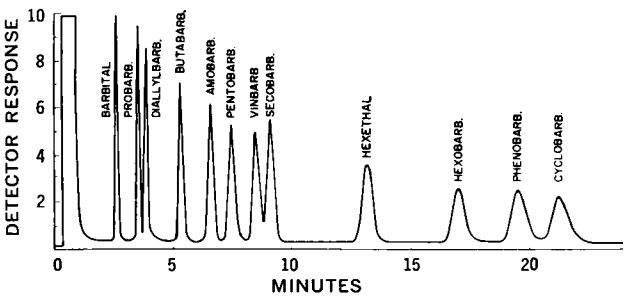

Figure 1—*Gas chromatogram of 12 barbiturates after flash-heater methylation with trimethylanilinium hydroxide.*

methyl groups of dimethylamobarbital, dimethylbarbital, and dimethylphenobarbital are equivalent. In hexadeuterio acetone the methyl substituents produced a singlet at τ 6.75, equivalent to six protons for the dimethyl derivatives and three protons for the monomethyl derivatives (metharbital and mephobarbital). In hexadeuterio dimethylsulfoxide the *N*-methyl substituents resonated at τ 6.80 for dimethylbarbital and at τ 6.78 for dimethylphenobarbital. A two-proton signal corresponding to the acidic hydrogens of unmethylated barbiturates (687 c.p.s. for barbital and 700 c.p.s. for phenobarbital in DMSO) disappeared on methylation.

The phenolic opium alkaloids, morphine, codamine, laudanine, and reticuline, gave complete methylation of the phenolic hydroxyl groups with trimethylanilinium hydroxide, but not with tetramethylammonium hydroxide. Thus, morphine gave rise to codeine without methylation of the secondary alcohol group in the 6-position. The monophenolic benzylisoquinolines, codamine and laudanine, and diphenolic reticuline gave the same fully methylated alkaloid laudanosine. The peak shifts resulting from the methylation of these alkaloids are useful for identification purposes.

The xanthine alkaloids, theobromine and theophylline, were quantitatively converted to caffeine by flash-heater methylation with trimethylanilinium hydroxide.

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