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Note

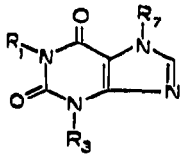
Specific gas chromatographic determination of xanthines and barbiturates by flash-heater *N*-butylation

Many of the polar ureide compounds give unsymmetrical gas chromatographic peaks, which are unsatisfactory in quantitative work. MACGEE[1] described flash-heater methylation of xanthines and some related compounds, and STEVENSON[2] reported on a similar method for barbiturate drugs. In these procedures, the ureide drugs are dissolved in ethanolic or methanolic solutions of tetramethylammonium hydroxide. Pyrolysis in the hot injection block of the instrument results in practically instantaneous conversion of ureide N–H groups to N-methyl derivatives, reducing their polarity and thereby improving their chromatographic characteristics. BROCHMANN-HANSSEN AND OKE[3] improved the method by use of trimethylanilinium hydroxide, which affords more efficient on-column methylation.

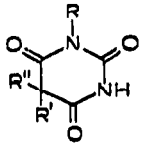
A major disadvantage of flash-heater N-methylation in some applications is formation of the same derivative from different compounds. Thus, KUPFERBERG[4] noted that phenobarbital and its 1-methyl derivative, mephobarbital, cannot be distinguished from each other using this technique. Similarly, 1,3-dimethylxanthine (theophylline), 3,7-dimethylxanthine (theobromine) and the monomethylxanthine metabolites of these alkaloids are all converted to 1,3,7-trimethylxanthine (caffeine), and they cannot be distinguished from that compound or from one another. It was considered that alkylation with a group other than methyl should produce unique derivatives, which could be chromatographically resolved. Ethylation, using triethylanilinium hydroxide as the reagent and theophylline as the test compound, was successful; however, the resolution of the presumed 1,3-dimethyl-7-ethylxanthine from caffeine was poor. Trials with tetra-*n*-butylammonium hydroxide, using the commercially available titrant, a 25% methanol solution (about 1 *M*), afforded good

TABLE I

STRUCTURAL RELATIONSHIPS AMONG UREIDES STUDIED



Xanthine	R_1	R_3	R_7
Caffeine	CH_3	CH_3	CH_3
Theobromine	H	CH_3	CH_3
Theophylline	CH_3	CH_3	H
1-Methylxanthine	CH_3	H	H
Xanthine	H	H	H



Barbiturate	*R'*	*R''*	*R*
Alphenal	C_6H_5	Allyl	H
Butabarbital	1-MePr	C_2H_5	H
Hexobarbital	C_6H_9	CH_3	CH_3
Mephobarbital	C_6H_5	C_2H_5	CH_3
Phenobarbital	C_6H_5	C_2H_5	H
Secobarbital	1-MeBu	Allyl	H

separations of closely related xanthines and barbiturates without detriment to the simplicity of the method or peak symmetry. A synopsis of the structural relationships of the test compounds is presented in Table I.

Experimental

Add 7 μl of methanolic 25% tetra-*n*-butylammonium hydroxide (about 7 μmol) to 1 ml of methanolic ureide solution containing not more than about 1.5 μequiv. of N–H groups, at least a 4:1 molar ratio of alkylating agent to test compound. Inject 1 μl of the solution into the gas chromatograph, using a minimum injector block temperature of 270° and adjusting other chromatographic parameters, according to the compound tested, to obtain satisfactory retention times and recorder response. Using theophylline as the test compound and a Perkin-Elmer Model 900 instrument equipped with flame ionization detector, suitable parameters were: Column, 6 ft. × $^1/_4$ in. O.D. glass packed with 3% OV-17 on 100–200 mesh Gas Chrom Q; injector and manifold temperature, 270°, oven temperature, 220°, helium flow-rate, 60 ml/min.

Results and discussion

A chromatogram illustrating the resolution of several xanthines from each other and their separation from caffeine, using flash-heater butylation, is presented

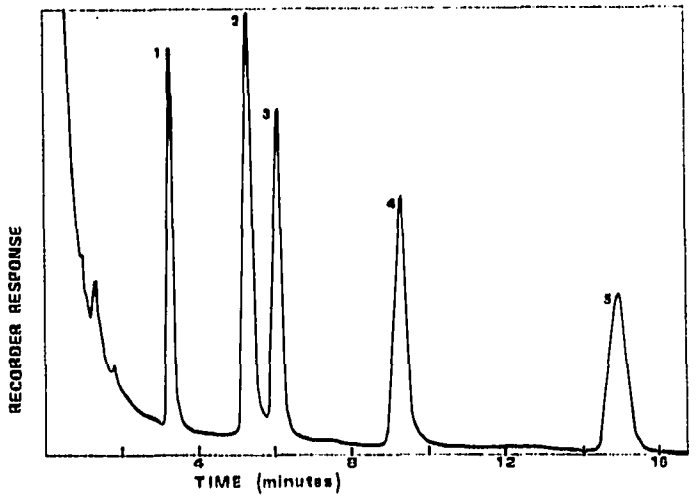


Fig. 1. Chromatogram of xanthines following flash-heater N-butylation. 1 = Caffeine; 2 = theophylline; 3 = theobromine; 4 = 1-methylxanthine; and 5 = xanthine.

in Fig. 1. REISCH AND WALKER[6] needed three column conditions for qualitative separation of some natural and synthetic xanthines, and their chromatograms show a considerable amount of tailing for those compounds with free N–H groups.

A comparison of the separation of some representative barbiturate drugs following on-column methylation using trimethylanilinium hydroxide[3] and butylation by the method described here is shown in Fig. 2. One may note the 2-min difference in retention time of the phenobarbital and mephobarbital butyl derivatives

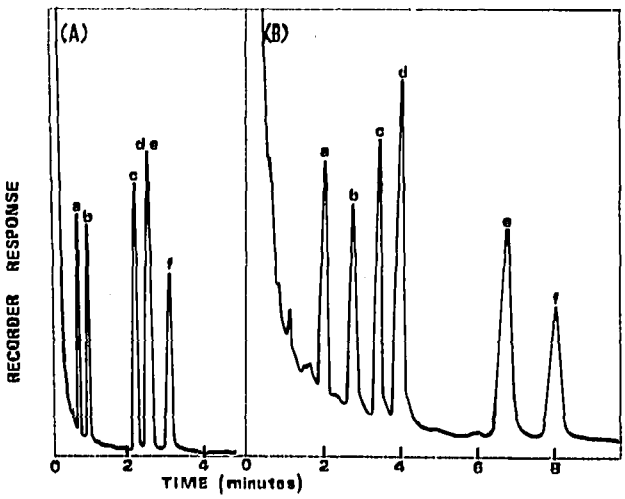


Fig. 2. Chromatogram of barbiturates following flash-heater N-methylation (A) and N-butylation (B). a = Butabarbital; b = secobarbital; c = hexobarbital; d = mephobarbital; e = phenobarbital; and f = alphenal.

in Fig. 2B in comparison with the identity of the methyl derivatives peaks shown in Fig. 2A.

Fig. 3 shows a plot of the molar ratio of butylating agent to test compound, constructed by using a constant amount of reagent (7 μl) added to a serially diluted solution of theophylline. The rectilinear plot obtained using excess reagent illustrates the quantitative capability of the method.

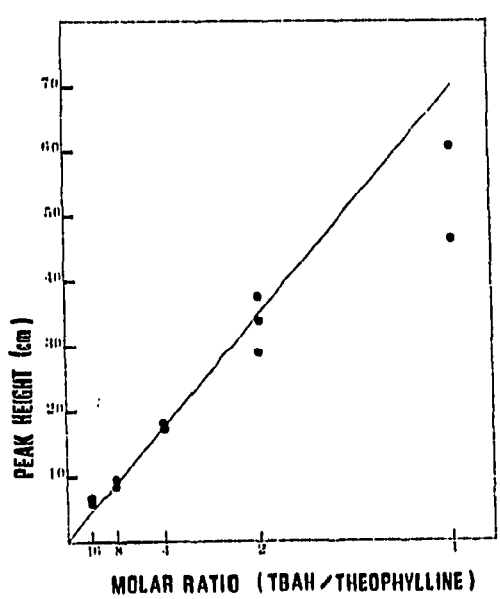


Fig. 3. Relationship of molar excess of tetra-*n*-butylammonium hydroxide (TBAH) to response of alkylated theophylline. Each data point represents a separate injection.

Conclusions

The operationally elegant flash-heater N-methylation method for ureides has been extended to use of on-column butylation, affording a considerable increase in selectivity. Structural isomers, such as theophylline and theobromine, which form the same derivative on methylation, form unique and resolvable derivatives by butylation. Similarly, N-methyl derivatives of synthetic compounds, related as mephobarbital is to phenobarbital, can be distinguished from each other by on-column butylation. Since N-methyl compounds are ubiquitous in nature and are easily accessible in synthesis, the use of N-butylation instead of N-methylation offers a considerable advantage in selectivity in a number of applications.

Applications of the N-butylation procedure and studies using other reagents will be reported in further communications from these laboratories.

Note added in proof

After acceptance of this paper our attention was drawn to the work of MACGEE[6] who used on-column ethylation to separate phenobarbital and mephobarbital.

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