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Note

Determination of Primidone and its metabolites in biological fluids by gas chromatography

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Primidone (Mysoline; 5-ethyl-5-phenylhexahydropyrimidine-4,6-dione)[1], a widely used anti-convulsant, is metabolized in both rats and humans to 2-ethyl-2-phenylmalondiamide (PEMA)[2–4] and phenobarbital (5-ethyl-5-phenylbarbituric acid)[5,6]. These two metabolites are present in substantial amounts in serum and cerebrospinal fluid (CSF) and their quantitative determination is of importance since both compounds exhibit anti-convulsant activity. All three compounds have been analyzed individually by a variety of techniques —Primidone by GC[4,7–9] and TLC[10]; phenobarbital by GC[4,7,9,11] and UV[6,12,13]; and PEMA by GC[4] and TLC[10]. In only one instance, however, all three compounds have been determined by a single instrumental method, this procedure involving two GC injections[4]. We have now developed a method for the simultaneous analysis of Primidone, PEMA and phenobarbital in urine and CSF using a single injection on a gas chromatograph.

EXPERIMENTAL

Materials

Primidone and PEMA were obtained from Ayerst Labs. (New York, N.Y., U.S.A.) and phenobarbital from Mallinckrodt (St. Louis, Mo., U.S.A.). N,N-Dimethylphenobarbital was prepared by reaction of phenobarbital with excess ethereal diazomethane. *n*-Docosane and *n*-octacosane were obtained from Applied Science Labs. (State College, Pa., U.S.A.).

Methods

Analytical gas chromatography was carried out on a F & M Model 400 gas chromatograph (Hewlett-Packard, Palo Alto, Calif., U.S.A.) using 6 ft. × $^1/_8$ in. glass columns with 3% OV-17 on 80–100 Chromosorb G; detector temperature (hydrogen flame), 250°; column temperature, 100–300° at 3°/min. Preparative GC was carried out on a Varian Model 90P-3 (Varian, Palo Alto, Calif., U.S.A.) equipped with a thermal conductivity detector using 5 ft. × $^1/_4$ in. stainless-steel columns with the same packing used on the analytical instrument; detector temperature, 300°; collector temperature, 300°.

Preparation of biological samples

In a typical extraction 20 ml of urine or CSF were acidified to pH 1 with 6 *N* HCl, saturated with NaCl and extracted with three 20-ml portions of ether. The combined extracts were evaporated to dryness under an air jet. Excess ethereal diazomethane was added to the residue and allowed to stand *ca.* 20 min (until nitrogen evolution had ceased) before the solvent was removed under an air jet. Methanol (100 μl) was added to the residue and the resulting solution was subjected to temperature-programmed GC on OV-17.

Identification of compounds in CSF and urine

Three peaks not normally present in CSF were noted at KI (ref. 14) 2165 (I), 2360 (II), and 2850 (III) in the chromatogram (OV-17) of a methylated CSF extract from a patient ingesting 1 g of Primidone daily. A chromatogram of a methylated extract of a 24-h urine specimen from the same patient showed these three peaks in much larger amounts than in CSF. These peaks were unequivocally identified as N,N-dimethylphenobarbital (I), PEMA (II) and Primidone (III) by means of electron impact[15–17] and chemical ionization[18] mass spectroscopy, ^{1}H and ^{13}C nuclear magnetic resonance spectroscopy[19] and by comparison with authentic materials.

Quantitation of Primidone and its metabolites in urine and CSF

Quantitation of Primidone, phenobarbital and PEMA by GC methods was carried out using the procedure described above for the extraction, derivatization and GC of urine and CSF. Docosane was employed either as an external or internal standard for phenobarbital and PEMA; however, octacosane, due to its low solubility in methanol, was only employed as an external standard. Straight-line correlations were observed for the amounts of N,N-dimethylphenobarbital (in the range of 1 to 4 μg) and PEMA (2 to 6 μg) *vs.* the peak ratio (compound/docosane). Similarly a straight-line correlation was noted for the amount of Primidone (2 to 5 μg) *vs.* area Primidone/area octacosane. Quantities as small as 40 ng can be detected in a standard solution containing these three compounds. In a typical urine or CSF extract, however, the limit of detection depends on the proximity of other peaks to those of Primidone and its metabolites. Thus, quantities less than *ca.* 250 ng are difficult to detect. Recoveries from urine by our procedure, as determined from four samples (in duplicate) containing different amounts of the three compounds, are 75 $\pm$ 5%, 96 $\pm$ 4%, and 96 $\pm$ 5%, respectively, for phenobarbital, PEMA, and Primidone.

DISCUSSION

Our method for the quantitation of Primidone and its metabolites by GC means has several advantages over that recently reported by Baumel and co-workers[4]. Their technique involves extraction from acid solution into chloroform followed by trimethylsilylation of Primidone and PEMA. Because phenobarbital does not form a stable silylated derivative a second injection of underivatized sample is necessary for the analysis of phenobarbital. In our procedure extraction with ether gives recoveries comparable to (phenobarbital) or better (Primidone and PEMA) than the 80% to 90% recoveries reported by Baumel. Diazomethane produces the N,N-dimethyl derivative of phenobarbital but does not react with Primidone or PEMA. All three

compounds are stable whereas the trimethylsilyl derivatives of Primidone and PEMA are easily hydrolyzed and, in general, unstable. Furthermore, the method reported here gives quantitative information on all three compounds from a single analytical run.

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REFERENCES

1 J. Y. Bogue and H. C. Carrington, *Brit. J. Pharmacol.*, 8 (1953) 230.
2 J. Y. Bogue, H. C. Carrington and S. Bentley, *Acta Neurol. Belg.*, 56 (1956) 640.
3 R. L. Foltz, M. W. Couch, M. Greer, K. N. Scott and C. M. Williams, *Biochem. Med.*, 6 (1972) 294.
4 I. P. Baumel, B. B. Gallagher and R. H. Mattson, *Arch. Neurol.*, 27 (1972) 34.
5 J. M. Thorp, *Congr. Int. Biochim. Resumés Commun., 3me, Bruxelles, 1955*, Vol. 1, Société Belge de Biochemie, Liège, p. 132; *C.A.*, 50 (1956) 14127a.
6 T. C. Butler and W. J. Waddell, *Proc. Soc. Exp. Biol. Med.*, 93 (1956) 544.
7 E. M. Baylis, D. E. Fry and V. Marks, *Clin. Chim. Acta*, 30 (1970) 93.
8 M. A. Evenson, P. Jones and B. Darcey, *Clin. Chem.*, 16 (1970) 107.
9 H. J. Kupferberg, *Clin. Chim. Acta*, 29 (1970) 283.
10 J. M. Fujimoto, W. H. Mason and M. Murphy, *J. Pharmacol. Exp. Ther.*, 159 (1968) 379.
11 J. C. Van Meter, H. S. Buckmaster and L. L. Shelley, *Clin. Chem.*, 16 (1970) 135.
12 G. L. Plaa and C. H. Hine, *J. Lab. Clin. Med.*, 47 (1956) 649.
13 M. T. Bush and E. Helman, *Life Sci.*, 4 (1965) 1403.
14 E. Kováts, *Adv. Chromatogr.*, 1 (1965) 229.
15 M. W. Couch and C. M. Williams, *Arch. Mass Spectral Data*, 3 (1972) 468.
16 J. N. T. Gilbert, B. J. Millard and J. W. Powell, *J. Pharm. Pharmacol.*, 22 (1970) 897.
17 R. A. Locock and R. T. Coutts, *Org. Mass Spectr.*, 3 (1970) 735.
18 R. L. Foltz, M. W. Couch, M. Greer, K. N. Scott and C. M. Williams, *Biochem. Med.*, 6 (1972) 294.
19 K. N. Scott, in preparation.