Synthesis of Internal Standards for Analytical Determinations of Primidone and its Metabolite, Phenylethylmalonamide (PEMA)

Kenneth H. Dudley, Daniel L. Bius, and James H. Maguire

Epilepsy and Anticonvulsant Drug Research Laboratory, Department of Pharmacology, University of North Carolina School of Medicine, Chapel Hill, North Carolina 27514

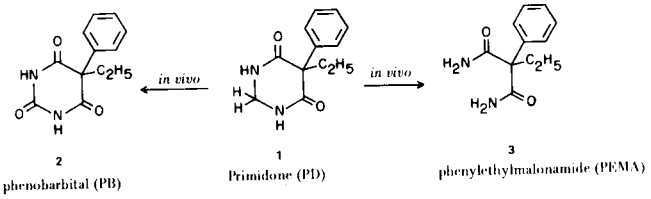
Received March 6, 1978

Synthetic routes are described for the preparation of internal standards for analytical determinations of the antiepileptic agent primidone (**1**) and one of its primary *in vivo* metabolites, phenylethylmalonamide (PEMA, **3**). The internal standards synthesized in the present study are *p*-methylprimidone (**9**) and *p*-methylPEMA (**8**). The internal standards differ in structure from the parent drug and metabolite in that the phenyl substituents of the drug and metabolite have been replaced by *p*-tolyl substituents. The synthetic route for the preparation of *p*-methylPEMA (**8**) entailed conversion of an appropriately substituted malonate to a 4,4-disubstituted pyrazolidine-3,5-dione, and Raney nickel reduction of the latter substance to a malonamide. This same sequence of reactions was useful for the preparation of PEMA (**3**) itself. A fusion of *p*-methylPEMA (**8**) with formic acid and formamidine acetate resulted in the formation of *p*-methylprimidone (**9**).

J. Heterocyclic Chem., 15, 923 (1978)

Introduction.

Primidone (PD, **1**) is one of the major antiepileptic agents, its primary indication being in the treatment and management of complex partial and/or generalized convulsive disorders. In man and in experimental animals, PD (**1**) is converted to phenobarbital (PB, **2**), an antiepileptic agent in itself, and to "phenylethylmalonamide" (PEMA, **3**) (Scheme 1). PD, PB and PEMA accumulate to significant concentrations in body tissues during chronic PD therapy (1).



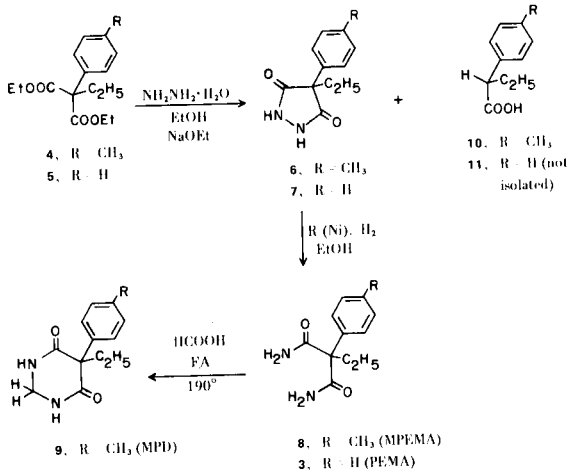
Scheme 1. *In vivo* metabolism of primidone (PD, 1).

The monitoring of plasma concentrations of the antiepileptic agents is now held to be a valuable asset to the therapy of patients with epilepsy (2). Gas-liquid chromatography (glc) is the technology most frequently employed by laboratories for routine quantitative determinations of the antiepileptic drugs (3,4). One of the principal factors contributing to interlaboratory variability in antiepileptic drug determinations has been the unavailability of appropriate internal standards for use in the glc-methods (5).

The present study describes the synthesis of two compounds, "*para*-methylprimidone" (MPD, **9**) and "*para*-methylPEMA" (MPEMA, **8**), candidate internal standards for analytical chromatographic determinations of PD (1) and PEMA (**3**), respectively. The structures of these candidate internal standards differ from those of PD (**1**) and PEMA (**3**) only in that the phenyl substituents of the drug and of the metabolite have been replaced by *para*-tolyl substituents. A convenient laboratory method for the synthesis of PEMA (**3**) is also described.

Results.

The series of reactions employed in the synthesis of PEMA (**3**), MPEMA (**8**), and MPD (**9**) are summarized in Scheme 2.



Scheme 2. Summary of the sequence of reactions for the synthesis of "*p*-methylprimidone" (MPD, **9**) and "*p*-methylPEMA" (MPEMA, **8**).

In 1906, Conrad and Zart (6) described the ethoxide-catalyzed condensations of some malonates with phenylhydrazine, the reactions resulting in pyrazolidine-3,5-dione derivatives. With the reactants employed in the present study, the Conrad and Zart procedure was found to cause extensive formation of an undesirable by-product. By modification of the procedure, the formation of by-product could be reduced considerably, but it could not be eliminated. In the application of the modified procedure to the preparation of 4-ethyl-4-(*p*-tolyl)pyrazolidine-3,5-

dione (**6**), a by-product identified as 2-(*p*-tolyl)butyric acid (**10**) was isolated in 16% yield. The desired pyrazolidine-3,5-dione **6** was obtained in crude form in 64% yield. Degradations of this sort have been noted previously in the chemistry of the barbiturates (7,8). Some other examples include a degradation of 1,3-dimethyl-5,5-dibenzylbarbiturate in aqueous ethanolic borohydride solution (9) and a degradation of PB by trimethylphenylammonium hydroxide (TMPAH) in a gas chromatographic on-column methylation reaction (10).

In the preparation of 4-ethyl-4-phenylpyrazolidine-3,5-dione (**7**), the intermediate required for the synthesis of PEMA (**3**), thin layer chromatography provided evidence for the formation of a similar by-product, which was assumed to be 2-phenylbutyric acid (**11**). Inasmuch as this by-product did not interfere with the purification of **7** by direct recrystallization, it was not further investigated. With the other reaction (*p*-tolyl series, **4** → **6** + **10**), an unorthodox extraction technique had been required in order to accomplish a nearly complete separation of **6** and **10**.

The pyrazolidine-3,5-dione **6** was converted to MPEMA (**8**) by hydrogenation over Raney nickel (Ni). The reductive cleavage proceeded at room temperature, but a short reflux period enabled the reaction to be completed within 25 minutes. The yield of pure MPEMA (**8**) was better than 60%. PEMA (**3**) was formed in about 60% yield by application of the same reductive cleavage reaction to **7**. Applications of Raney Ni for the reductive cleavage of the nitrogen-nitrogen bond have been reported by Ainsworth (11), who studied the conversion of carboxylic acid hydrazides to amides, and by Hinman (12), who studied the reductive cleavages of some aliphatic and alicyclic diacylhydrazine derivatives.

"*p*-Methylprimidone" (MPD, **9**) was prepared in better than 50% yield by heating to fusion a mixture of MPEMA (**8**), formic acid, and formamidine acetate (FA). Essentially the same reaction, the exception being the omission of FA, had been reported by Boon, *et al.*, (13) as a method for the synthesis of PD (**1**). Although Boon, *et al.*, (13) had found that the use of FA in their fusion mixture did not affect the yield of PD (**1**), the addition of FA to the fusion mixture of formic acid and MPEMA (**8**) improved the yield of MPD (**9**) in our study. The sample of MPD (**9**), as prepared by the modified method of Boon, *et al.*, (13), was identical in all respects to another sample prepared by an alternative route in an independent study (*cf.* Experimental).

Discussion.

The use of appropriately tailored synthetic internal standards in glc can be helpful as a safeguard against the pitfalls and variables which affect the reliability of a method (14). An appropriate internal standard may be viewed as a compound which has the same functional groups and basically the same chemical structure as the solute of interest, yet the structural modification in the internal standard does not alter the chemical and chromatographic properties too differently from those exhibited by the solute itself (14). With a structural modification being made at a site sufficiently distant from functional groups of the solute, differences between the solute and internal standard are minimized in extraction properties (partition ratios) and in glc-properties (peak shapes, retention times, response factors, and rates of formation, or of destruction of glc-derivatives). The use of 5-ethyl-5-(*p*-tolyl)barbituric acid ("*p*-methylphenobarbital," MPB) in the glc-determination of PB may be cited as an example to emphasize this point. The *per*-methylated derivatives of the two compounds are formed and degraded to essentially the same extent in the gas chromatographic on-column methylation technique (15,16).

In antiepileptic therapy, two or more drugs are frequently used in combination. Glc-techniques have been developed by investigators so as to permit simultaneous determinations of the antiepileptic drugs in a patient's blood specimen (14). Normally, only one internal standard is employed, the most popular being 5-phenyl-5-(*p*-tolyl)-hydantoin (MPPH), the *p*-methyl derivative of phenytoin (DPH, diphenylhydantoin, Dilantin ®). Recent evidence, however, suggests that such practices can result in unreliable methodology, causing grossly inaccurate determinations of the antiepileptic drugs other than DPH. The use of multiple, appropriately tailored internal standards in methods designed for the simultaneous determinations of antiepileptic drugs has been recommended as a step to improve the reliability of a gas chromatographic method (15,16).

Some encouraging reports have begun to appear on the uses of MPD (**9**) and MPEMA (**8**) as internal standards in glc. MPD has been found to be satisfactory for the determination of PD (**1**), particularly in methods designed for the detection of the native (underivatized) substances (17), or the pentafluorobenzoyl derivatives (18), or the *per*-methylated (on-column methylation technique) derivatives (19). At present, only two laboratories have utilized MPEMA in new methods designed for PEMA (20,21). Both methods involve mild derivatization reactions of PEMA and MPEMA with organic reagents.

EXPERIMENTAL

Thin layer chromatography (tlc) was carried out with microscope slides coated with Silica Gel H. A suitable solvent system was benzene-ethyl acetate-acetic acid (9:1:1). Zones were visualized by spraying the eluted chromatograms with 5% phosphomolybdic acid in ethanol (PMA) and, then, baking the slide on the surface of a hot plate. Color reactions developed within minutes.

Micromelting points were taken on a Kofler hot stage microscope and are uncorrected.

Infrared spectra were measured with a Perkin-Elmer Model 257 instrument; samples were prepared in the form of pressed potassium bromide disks.

Diethyl 2-Ethyl-2-(*p*-tolyl)malonate (**4**).

Ethyl *p*-tolylacetate was prepared by azeotropic distillation of a mixture of *p*-tolylacetic acid (100 g.), benzene (270 ml.), ethanol (130 ml.), and concentrated (sp.g. 1.84) sulfuric acid (1 ml.). The ethyl ester was distilled at 81-83°/0.3 mm, yield, 109 g. (91%).

Diethyl 2-(*p*-tolyl)malonate was prepared by an adaptation of the method of Krapcho, *et al.*, (22). The quantities of materials used in this reaction were as follows: benzene-washed 50% sodium hydride-mineral oil suspension (81 g., 1.69 moles) resuspended in dry benzene (100 ml.), diethyl carbonate (265 g., 2.25 moles), and a solution of ethyl *p*-tolylacetate (100 g., 0.56 mole) in benzene (500 ml.). The product was distilled at 130-132°/0.25 mm, yield, 118 g. (84%).

Anal. Calcd. for $C_{14}H_{18}O_4$: C, 67.16; H, 7.25. Found: C, 67.04; H, 7.25.

Diethyl 2-ethyl-2-(*p*-tolyl)malonate (**4**) was prepared by adaptation of a procedure of Beres, *et al.* (23). The reagents were as follows: diethyl 2-(*p*-tolyl)malonate (50 g., 0.2 mole) in dry *N,N*-dimethylformamide (DMF, 100 ml.); benzene-washed 57% sodium hydride-mineral oil suspension (16.8 g., 0.4 mole) resuspended in dry DMF (100 ml.); and ethyl iodide (32 ml., 0.4 mole). Malonic ester **4** was isolated by vacuum distillation, the fraction with b.p. 135-137°/0.4 mm being recovered, yield, 48 g. (86%).

Anal. Calcd. for $C_{16}H_{22}O_4$: C, 69.02; H, 7.97. Found: C, 68.87; H, 8.20.

4-Ethyl-4-(*p*-tolyl)pyrazolidine-3,5-dione (**6**).

To a warm sodium ethoxide solution, prepared by dissolution of 3.5 g. (0.15 mole) of sodium in 100 ml. of absolute ethanol, were added 20 g. (0.072 mole) of **4** and 5 g. (0.10 mole) of hydrazine hydrate. The mixture was stirred for 1 hour at 25°, after which the temperature was increased to 75° (oil bath) and maintained for 1 hour, and, then, the temperature was increased further to cause gentle refluxing of the solution for 1 hour. This mode of heating decreased the formation of by-product **10**. The progress of the reaction was followed by tlc, and completeness was observed at the end of the reflux period. The oil bath temperature was increased to 110°, and the ethanol was distilled off. The residue, a difficultly soluble sirup, was dissolved in 150 ml. of cold water, the aqueous solution was extracted with 3 x 50 ml. of ether, and the ether extracts were discarded. The aqueous solution was chilled and was acidified to *p*H 3 by the slow addition of concentrated hydrochloric acid. A white semi-solid precipitate formed. Hexane (30 ml.) was added to the cold suspension and, after thorough shaking, the mixture was allowed to stand in an ice-bath for 5 minutes. The white solid was filtered off from the two-phase solvent system, the solid was washed quickly with hexane, and the filtrate was saved for the isolation of by-product **10**. The white solid amounted to 10.1 g. (64% yield) of **6** contaminated slightly by **10** and by a small amount of another unidentified product (tlc). Compound **6** (10.1 g.) was dissolved in hot ethyl acetate (100 ml.) and the solution was filtered to remove a small amount of insoluble material. After the filtrate had cooled hexane (100 ml.) was added slowly and the mixture was allowed to stand at 4°. The crystalline product **6**, homogeneous by tlc, amounted to 5.6 g., m.p. 172-173°. An analytical sample, m.p. 175.5-177°, was prepared by one further recrystallization. The sample was dried at 80° and then again at 100° immediately before analysis; ir: 3250-2900 (NH), 1730 (C=O), 1670 cm^{-1} (C=O).

Anal. Calcd. for $C_{12}H_{14}N_2O_2$: C, 66.04; H, 6.47; N, 12.83. Found: C, 66.20; H, 6.64; N, 12.63.

The procedure of Conrad and Zart (9) was not suitable for the preparation of **6**, because by-product **10** was formed extensively and could not be removed by repeated recrystallization.

Isolation of 2-Tolybutyric Acid (by-product **10**).

The hexane layer of the saved-filtrate from the above experiment was collected. The aqueous layer was washed with an additional portion of hexane (50 ml.) and the hexane fractions were combined, dried over sodium sulfate, and evaporated to an oil that crystallized upon standing overnight at 25°. The homogeneous product (tlc) amounted to 2 g. A sample (1.5 g.) was dissolved in warm methanol (5 ml.) and this solution was diluted slowly with water (15 ml.) over a period of 2 days. The crystalline precipitate (0.5 g.), m.p. 57.5-63°, was filtered off, washed with water, and vacuum-dried (40°).

A sample of 76.3 mg was titrated with 0.1*N* sodium hydroxide in 5% methanol. The estimated molecular weight was 173 (theor. M.W.: 178) and the p*K'*a was 4.52; ir: 2740-3300 (CO*OH*) with peak at 2960, and 1705 cm^{-1} (C=O).

Anal. Calcd. for $C_{11}H_{14}O_2$: C, 74.13; H, 7.92; N, 0.00. Found: C, 74.20; H, 7.99; N, < 0.10.

4-Ethyl-4-phenylpyrazolidine-3,5-dione (**7**).

Compound **7** was prepared from diethyl 2-ethyl-2-phenylmalonate (20 g., 0.076 mole), hydrazine hydrate (5 g., 0.10 mole), and a solution of sodium ethoxide prepared by dissolution of sodium (3.5 g., 0.152 mole) in absolute ethanol (100 ml.). The reaction was carried out by the procedure described by Conrad and Zart (9) in their preparation of 1-phenyl-4,4-diethylpyrazolidine-3,5-dione. The yield of crude **7** ranged from 48-68%, and the material was contaminated heavily with a by-product presumed to be of structure **11**. Compound **7** was obtained in homogeneous form (tlc) by recrystallization of the mixture from ethyl acetate-hexane. An impure sample of 23.3 g. (combined from 4 runs) furnished 18.4 g. of pure **7**, m.p. 201-202°; ir: 2950-3250 (NH), 1728 (C=O) and 1675 (C=O) cm^{-1}; lit. (24) m.p. 198°.

Anal. Calcd. for $C_{11}H_{12}N_2O_2$: C, 64.68; H, 5.93; N, 13.72. Found: C, 64.54; H, 5.88; N, 13.75.

2-Ethyl-2-(*p*-tolyl)malonamide (*p*-MethylPEMA, MPEMA, **8**).

Compound **6** (3.9 g.) was dissolved in absolute ethanol (200 ml.), and moist W-2 Raney Ni (estimated, 2.4 g.) was added. The mixture was stirred vigorously (magnetically) and heated under reflux for 25 minutes, the completeness of the reaction being indicated by tlc. After the removal of the catalyst, the solution was evaporated to a light yellow oil, which crystallized when triturated under 2-3 ml. of ethyl acetate. MPEMA (**8**) was obtained in homogeneous form (tlc) as white crystals (2.5 g.), m.p. 154.5-155.5°, by recrystallization of the crude crystalline product from ethyl acetate-hexane; ir: 3445 (NH), 3340 (NH), 3190 (NH), 1700-1650 (C=O) cm^{-1}.

Anal. Calcd. for $C_{12}H_{16}N_2O_2$: C, 65.44; H, 7.32; N, 12.71. Found: C, 65.58; H, 7.36; N, 12.68.

2-Ethyl-2-phenylmalonamide (Phenylethylmalonamide, PEMA, **3**).

PEMA (**3**), m.p. 122° with crystallographic changes at 75 and 114°, was prepared in 60% yield by Raney Ni (1.8 g., estimated) reduction of **7** (2 g.) in absolute ethanol (100 ml.); lit. (7) m.p. 121°. The reaction was carried out as described above for MPEMA (**8**).

5-Ethyl-5-(*p*-tolyl)hexahydropyrimidine-4,6-dione (*p*-Methylprimidone, MPD, **9**).

The reaction of MPEMA (**8**, 100 mg., 0.45 mmole) and FA (50 mg., 0.48 mmole) in 90% formic acid (3 ml.) was carried out at 190-195° in a 15 x 125 mm test tube. The reactants were mixed together and the tube was immersed in an oil bath preheated to 90°. The temperature of the bath was raised to 160° over 45 minutes and, then, to 195° over the next hour. With the temperature maintained at 190-195°, successive volumes of 3 x 0.5 ml., 0.3 ml., and 3 x 0.1 ml. of formic acid were added at intervals over the next 5 hours. The progress of the reaction was followed by tlc. The dark brown crystalline residue was recrystallized from a small volume of absolute methanol to provide white needles (53 mg.) of *p*-methylprimidone (MPD, **9**), m.p. 288-292°; ir: 3220 (NH), 1668 (C=O) cm^{-1}.

Anal. Calcd. for $C_{13}H_{16}N_2O_2$: C, 67.20; H, 6.95; N, 12.06. Found: C, 67.13; H, 6.88; N, 12.23.

A reaction of **8** (220 mg.) and 90% formic acid was carried out to completion in the absence of FA. The yield was 50 mg, m.p. 288-291°.

The sample of MPD (**9**) prepared in the present study was compared with a sample furnished to us by Dr. H. Schäfer (25), who synthesized his material, m.p. 305-307°, by desulfurization of an appropriate 2-thiobarbiturate derivative (26). His compound had m.p. 288-292° in our laboratory, and the m.p. of an admixture was not depressed. The two specimens exhibited identical ir, tlc, and gas chromatographic properties.

Acknowledgement.

This work was supported in part by Developmental Disabilities Services Funds as authorized by P.L. 94-103 and awarded by the North Carolina Council on Developmental Disabilities. Other support was by a 1977 Research Grant Award from the Epilepsy Foundation of America and by a National Institute of Environmental Health Sciences Postdoctoral Fellowship Award (E.S. 02281) to J.H.M.

REFERENCES AND NOTES

(1) B. B. Gallagher and I. P. Baumal, in "Antiepileptic Drugs," D. M. Woodbury, J. K. Penry, and R. P. Schmidt, Eds., Raven Press, New York, N. Y., 1972 p. 361.

(2) H. Kutt and J. K. Penry, *Arch. Neurol.*, **31**, 283 (1974).

(3) A. Richens, in "Clinical Pharmacology of Anti-Epileptic Drugs," H. Schneider, D. Janz, C. Gardner-Thorpe, H. Meinardi, and A. L. Sherwin, Eds., Springer-Verlag, New York, N. Y., 1975, p. 293.

(4) C. E. Pippenger, J. K. Penry, B. G. White, D. D. Daly, and R. Buddington, *Arch. Neurol.*, **33**, 351 (1976).

(5) C. E. Pippenger, H. Paris-Kutt, J. K. Penry, and D. Daly, *J. Anal. Toxicol.*, **1**, 118 (1977).

(6) M. Conrad and A. Zart, *Chem. Ber.*, **39**, 2282 (1906).

(7) H. Aspelund, *Acta Acad. Abo. Ser. B*, **20**, No. 3, 16 pp (1955); *Chem. Abstr.*, **50**, 11351e (1956).

(8) H. Aspelund and S. Stolt, *Acta Acad. Abo. Ser. B*, **20**, No. 4, 26 pp (1955); *Chem. Abstr.*, **50**, 11352b (1956).

(9) K. H. Dudley, I. J. Davis, D. K. Kim, and F. T. Ross, *J. Org. Chem.*, **35**, 147 (1970).

(10) R. Osiewicz, V. Aggarwal, R. M. Young, and I. Sunshine, *J. Chromatogr.*, **88**, 157 (1974).

(11) C. Ainsworth, *J. Am. Chem. Soc.*, **76**, 5774 (1954).

(12) R. L. Hinman, *J. Org. Chem.*, **22**, 148 (1957).

(13) W. R. Boon, H. C. Carrington, N. Greenhalgh, and C. H. Vasey, *J. Chem. Soc.*, 3263 (1954).

(14) K. H. Dudley, in "Antiepileptic Drugs: Quantitative Analysis and Interpretation," C. E. Pippenger, J. K. Penry, and H. Kutt, Eds. Raven Press, New York, N. Y., 1978, p. 19.

(15) E. B. Solow, J. M. Metaxas, and T. R. Summers, *J. Chromatogr. Sci.*, **12**, 256 (1974).

(16) K. H. Dudley, D. L. Bius, B. L. Kraus, and L. W. Boyles, in "Antiepileptic Drugs: Quantitative Analysis and Interpretation," C. E. Pippenger, J. K. Penry, and H. Kutt, Eds., Raven Press, New York, N. Y., 1978, p. 35.

(17) R. N. Gupta, K. Dobson, and P. M. Keane, *J. Chromatogr.*, **132**, 140 (1977).

(18) J. E. Wallace, H. E. Hamilton, E. L. Shimek, Jr., H. A. Schwertner, and K. Blum, *Anal. Chem.*, **49**, 903 (1977).

(19) K. H. Dudley, D. L. Bius, B. L. Kraus, and L. W. Boyles, *Epilepsia*, **18**, 259 (1977).

(20) J. E. Wallace, H. E. Hamilton, E. L. Shimek, Jr., H. A. Schwertner, and K. D. Haegele, *Anal. Chem.*, **49**, 1969 (1977).

(21) R. J. Perchalski and B. J. Wilder, *J. Chromatogr.*, 1978, in press.

(22) A. P. Krapcho, J. Diamanti, C. Cayen, and R. Bingham, *Org. Synth., Coll. Vol. V*, 198 (1973).

(23) J. A. Beres, D. E. Pearson, and M. T. Bush, *J. Med. Chem.*, **10**, 1078 (1967).

(24) H. Ruhkopf, *Chem. Ber.*, **73B**, 820 (1940).

(25) H. R. Schäfer, in "Clinical Pharmacology of Anti-Epileptic Drugs," H. Schneider, D. Janz, C. Gardner-Thorpe, H. Meinardi, and A. L. Sherwin, Eds., Springer-Verlag, New York, N. Y., 1975, p. 124.

(26) H. R. Schäfer, personal communication.