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ALKYLATION STUDIES OF 5,5-DISUBSTITUTED HYDANTOINS

APPLICATION TO THE GAS CHROMATOGRAPHIC ANALYSIS OF PHENYTOIN, MEPHENYTOIN AND NIRVANOL

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SUMMARY

The rate of alkylation of 3-ethyl-5,5-diphenylhydantoin in aqueous acetone at room temperature was found to decrease in the order methyl > ethyl > *n*-propyl, consistent with an S_N2 mechanism. The reactivity of the N_1 nitrogen atom is influenced by the substituent at C_5, and it was observed that 5-ethyl-5-phenylhydantoin (Nirvanol) reacts more slowly than 5,5-diphenylhydantoin (phenytoin). Derivatization conditions were optimized for the perethylation of 5,5-disubstituted hydantoins, as ethylation is sufficient to separate 3-methyl-5-ethyl-5-phenylhydantoin (mephenytoin) from its N-demethylated metabolite (Nirvanol) on an SE-30 column.

INTRODUCTION

Anticonvulsants can be analyzed directly by gas chromatography (GC)[1–4], although better reproducibility and accuracy are obtained after derivatization[5]. Many methods have been used for the derivatization of anticonvulsants, especially on-column alkylation[6–12] and reaction with alkyl iodides[13–17], methylfluorosulfonate[18] or diazoalkanes[19,20].

Barbiturates can be alkylated under mild conditions to give peralkylated products. More drastic conditions are necessary to derivatize the two nitrogen atoms of the hydantoin ring. With diazoalkanes the main product is the 3-methyl or -ethyl derivatives. Recently, Gordos *et al.*[16] prepared the 1,3-dimethyl derivative of diphenylhydantoin by reaction with methyl iodide in acetone in the presence of potassium hydroxide. 3-Methyl-5-ethyl-5-phenylhydantoin (mephenytoin) is demethylated *in vivo* to 5-ethyl-5-phenylhydantoin (Nirvanol) (Table I). In order to separate these two compounds, it is necessary to use higher alkyl groups (such as ethyl or propyl). Kupferberg and Yonekawa[12] reported the separation of mephenytoin and Nirvanol as trimethylsilyl (TMS) derivatives, but N-TMS groups may be labile under GC con-

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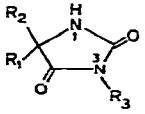
ditions. We have investigated the alkylation of 5,5-disubstituted hydantoins with several alkyl iodides in order to achieve the separation of mephenytoin and Nirvanol by gas–liquid chromatography.

TABLE I

STRUCTURES OF SUBSTITUTED HYDANTOINS



Name	R_1	R_2	R_3
Nirvanol	C_2H_5	C_6H_5	H
Phenytoin (DPH)	C_6H_5	C_6H_5	H
Mephenytoin	C_2H_5	C_6H_5	CH_3
HPPH	C_6H_5	*p*-OH-C_6H_5	H
MPPH	C_6H_5	*p*-CH_3-C_6H_5	H

EXPERIMENTAL

Chemicals and reagents

5,5-Diphenylhydantoin (Mann Labs., New York, N.Y., U.S.A.), 5-ethyl-5-phenylhydantoin, 5-(*p*-methylphenyl)-5-phenylhydantoin, 5-(*p*-hydroxyphenyl)-5-phenylhydantoin (all from Aldrich, Milwaukee, Wisc., U.S.A.) and 3-methyl-5-ethyl-5-phenylhydantoin (Sandoz, Basle, Switzerland) were used as free acids. Methyl, ethyl, propyl and neopentyl iodides were used without purification. All solvents were of analytical-reagent grade.

Preparation of 3-ethyl-5,5-diphenylhydantoin

A 20-ml volume of a diazoethane solution in diethyl ether prepared from N-ethyl-N'-nitro-N-nitrosoguanidine (Aldrich) was added to 100 mg of 5,5-diphenylhydantoin in 10 ml of ethanol. After reaction at room temperature for 5 min, the mixture was dried under a stream of nitrogen. The residue was dissolved in *n*-hexane, in which the diethyl derivatives formed as by-products of the reaction are very soluble. Two crystallizations in *n*-hexane gave 65 and 22 mg of 3-ethyl-5,5-diphenylhydantoin (yield 77%).

Preparation of 1,3-dimethyl-5-(p-methylphenyl)-5-phenylhydantoin

The internal standard 1,3-dimethyl-5-(*p*-methylphenyl)-5-phenylhydantoin was prepared by reaction of methyl iodide (5 ml) with 5-(*p*-methylphenyl) phenylhydantoin (50 mg) in acetone (100 ml) after addition of 25 ml of 0.5 *N* potassium hydroxide solution.

The solution was refluxed for 3 h, then the derivative was extracted twice with 100 ml of benzene, dried over sodium sulfate and the solvent was evaporated under vacuum. Crystallization from a mixture of methanol and water gave 33 mg (60% yield). No attempt was made to optimize the yield during crystallization.

Study of the rates of reaction

In a standard experiment, 1 mg of the substrate and 1 mg of 1,3-dimethyl-5-

(*p*-methylphenyl)-5-phenylhydantoin (as internal standard) in 2 ml of acetone were mixed with 0.5 ml of ethyl iodide and 0.5 ml of 0.5 *N* potassium hydroxide solution. The reactions were carried out at room temperature or at other temperatures as indicated in the text. At fixed time intervals, 40-μl aliquots were removed, then extracted with benzene (50 μl) after evaporation of the acetone.

Analytical perethylation of 5,5-disubstituted hydantoins

Upon completion of this study, it was found that complete perethylation was obtained under the following conditions: 5 μl of 5 *N* potassium hydroxide solution and 50 μl of ethyl iodide were added to 200 μl of a solution of 5,5-disubstituted hydantoins in acetone (or 2-butanone). After heating the microvial (1 ml) in an aluminum block for 1½ h at 60°, acetone (or 2-butanone) was evaporated under a stream of nitrogen and the perethylated hydantoins were extracted in benzene.

Gas chromatography

For rate studies, the products were separated on a 12 ft. × 2 mm I.D. column packed with 3% OV-225 on Gas-Chrom Q (100–120 mesh) (Applied Science Labs., State College, Pa., U.S.A.) at 245°, and/or on an SE-30 capillary column (37 m × 0.3 mm I.D.) prepared according to the procedure developed in this laboratory[21].

Gas chromatography–mass spectrometry

Structures of derivatives were confirmed by their electron-impact spectra obtained with an LKB 9000 instrument at 70 eV (source temperature 250°) after separation on a 6-ft. 1% SE-30 column.

Quantification was effected with a Finnigan Model 1015 instrument on-line with a System Industries Model 150 data system. The mass spectrometer was operated under chemical ionization conditions with methane as the reagent and carrier gas. GC separations were carried out on a 3-ft. 3% PZ 179 column (Applied Science Labs.).

RESULTS

Preliminary experiments showed that barbiturates could be alkylated in acetone–solid potassium carbonate according to the procedure described by Dünges and Bergheim-Irps[13,14]. The reaction was fast and no decomposition occurred. The dipentyl derivative of phenobarbital could be prepared in 15 min at 80°.

When these conditions were applied to the derivatization of 5,5-diphenylhydantoin, it was not possible to obtain a complete reaction, as shown in Table II. When methanol was added, the yield improved considerably, the reaction being quantitative (99%) with methyl iodide. The 2,3-dimethyl derivative was produced to the extent of 1.5–2% of the 1,3-dimethyl form.

When potassium hydroxide was used instead of potassium carbonate according to the procedure of Gordos *et al.*[16], both methylation and ethylation were complete with phenytoin. Further, the proportion of 2,3-isomer was lower. These preliminary experiments were carried out at 80–100°; it was imperative to have a tightly closed vessel in order to avoid any loss of acetone. A few experiments failed because of this requirement.

TABLE II

PERALKYLATION OF 5,5-DIPHENYLHYDANTOIN AT 80°C

Solvent	*Derivative formed (%)*		
	Methyl	*Ethyl*	*Pentyl*
Acetone–K_2CO_3	85	23	2–3
Acetone–methanol–K_2CO_3	99	60–80	—

The two nitrogen atoms of the hydantoin ring are not equivalent; N_3, which is more acidic than N_1, is similar to the nitrogen atom in the barbiturates. In order to study the rate of alkylation at N_1, 3-ethyl-5,5-diphenylhydantoin was prepared with diazoethane, as described under Experimental.

Alkylation of 3-ethyl-5,5-diphenylhydantoin

The rates of reaction of 3-ethyl-5,5-diphenylhydantoin at room temperature under various conditions are shown in Fig. 1. The reaction is very slow for ethylation in the mixture acetone–methanol–potassium carbonate at room temperature. The rates were compared for the reactions with methyl, ethyl and propyl iodide in the presence of potassium hydroxide. Complete methylation occurred within 45 min. The rate decreased with increasing length of the alkyl chain.

The order of reaction methyl > ethyl > *n*-propyl is expected for a typical S_N2 reaction[22]. It is also possible that steric hindrance may retard the attack of the N_1 nitrogen. Under the same conditions, 3-methyl-5-ethyl-5-phenylhydantoin was ethylated at a much slower rate than 3-ethyl-5,5-diphenylhydantoin.

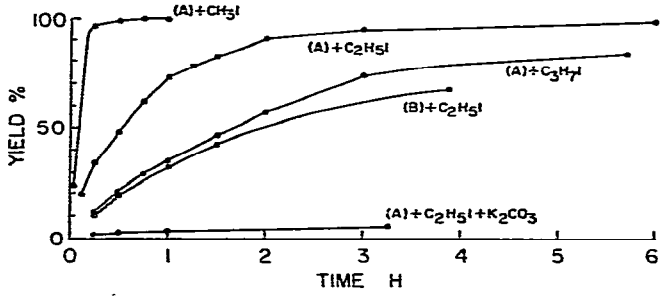


Fig. 1. Reaction rates at room temperature of 3-ethyl-5,5-diphenylhydantoin (A) and 3-methyl-5-ethyl-5-phenylhydantoin (B) in acetone–KOH with alkyl iodides. The lower curve was obtained with K_2CO_3 instead of KOH. Conditions as described under Experimental.

Ethylation of 5,5-diphenylhydantoin and 5-ethyl-5-phenylhydantoin

Phenytoin and Nirvanol were ethylated at room temperature using acetone–potassium hydroxide–water–ethyl iodide. A marked difference was also observed between the reaction of the two compounds, phenytoin being alkylated faster (Fig. 2). After 6 h, phenytoin was almost completely alkylated whereas only 50% of Nirvanol reacted. At 50°, the rate of reaction of the latter was only slightly higher than that for DPH at room temperature. From the differences in rates between

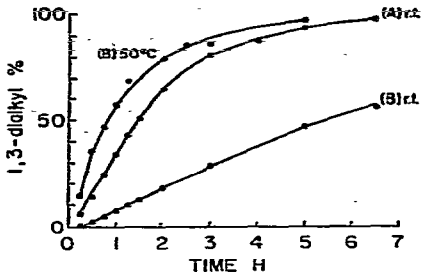


Fig. 2. Perethylation rates of 5,5-diphenylhydantoin (A) and 5-ethyl-5-phenylhydantoin (B) in acetone–KOH at room temperature (r.t.) and at 50° (upper curve).

Nirvanol and phenytoin and between mephenytoin and 3-ethyl-5,5'-diphenylhydantoin, it appears that the replacement of a phenyl by an ethyl group at C_5 decreases the rate of reaction considerably.

The practical application of this difference in rate is that phenytoin is not the best standard for the analysis of mephenytoin or Nirvanol; 5-(*p*-methylphenyl)-5-ethylhydantoin would be more appropriate.

Starting with DPH and Nirvanol, alkylation at N_3 was measured by monitoring the 3-monoethyl derivative. As shown in Fig. 3, a maximal concentration of the 3-ethylated product was obtained within 30–45 min, then it declined. The curve is the sum of the formation of the 3-alkyl derivative from the free hydantoin and of the conversion of the 3-alkyl into the 1,3-diethylated product.

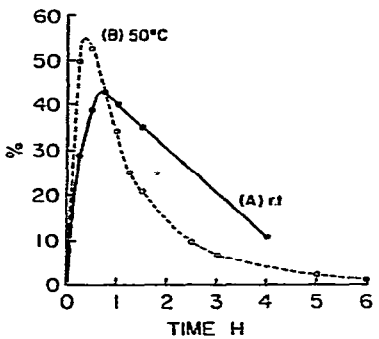


Fig. 3. Concentration of the 3-ethyl derivative during the perethylation of 5,5-diphenylhydantoin (A) and 5-ethyl-5-phenylhydantoin (B) (Fig. 2). The yield is expressed as a percentage of the total hydantoin.

Influence of the concentrations of potassium hydroxide and water

For analytical purposes the above experiments were carried out on a smaller scale: 50 μl of 0.5 *N* potassium hydroxide solution were added to the sample in acetone (200 μl), then the reaction was started by addition of ethyl iodide (50 μl).

The ethylation of 5-ethyl-5-phenylhydantoin at N_1 increased with an increase in the potassium hydroxide concentration. Starting with 50 μl of 0.01 *N* potassium hydroxide solution, only the 3-monoethyl derivative was formed. These results are

similar to those of Gordos *et al.*[16], who found that at pH 13 only the dimethyl derivative of diphenylhydantoin was formed whereas two products were observed at pH 11.5.

It was also found that water slowed the reaction rate, a better perethylation yield being achieved with 5 μl of 5 *N* than with 50 μl of 0.5 *N* potassium hydroxide solution. However, it was observed that with the former conditions permethylation was not complete, only 40–60% of the dimethyl derivative being formed. This result will be discussed later.

Application to the analysis of Nirvanol and mephenytoin

Mephenytoin is metabolized to Nirvanol; these two compounds cannot be differentiated after methylation with either methyl iodide or diazomethane unless the deuterated analogs are used and quantitation based upon selected ion monitoring methods.

1-Ethylmephenytoin and 1,3-diethyl-Nirvanol were well separated on an SE-30 capillary column (Fig. 4).

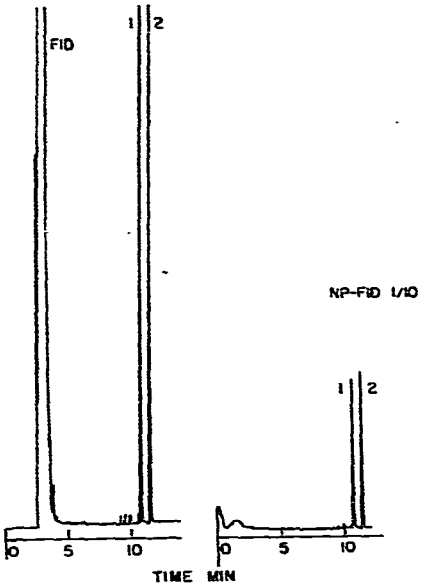


Fig. 4. Separation on a 35-m SE-30 capillary column of perethylated mephenytoin (1) and Nirvanol (2). The chromatogram on the left was obtained with a flame-ionization detector. Injection of one tenth of the sample at the same attenuation with a nitrogen detector gave the chromatogram on the right. Derivatives were prepared by heating mephenytoin and Nirvanol in acetone (200 μl)–0.5 *N* KOH (50 μl) with ethyl iodide (50 μl) for 1 h at 65°.

Small amounts of N,O- and O,O'-isomers were eluted before the main derivatives.

The percentage of these isomers was further decreased if tetraethylammonium hydroxide in methanol was used instead of 0.5 *N* potassium hydroxide solution.

The electron-impact mass spectra of the perethylated derivatives of mepheny-

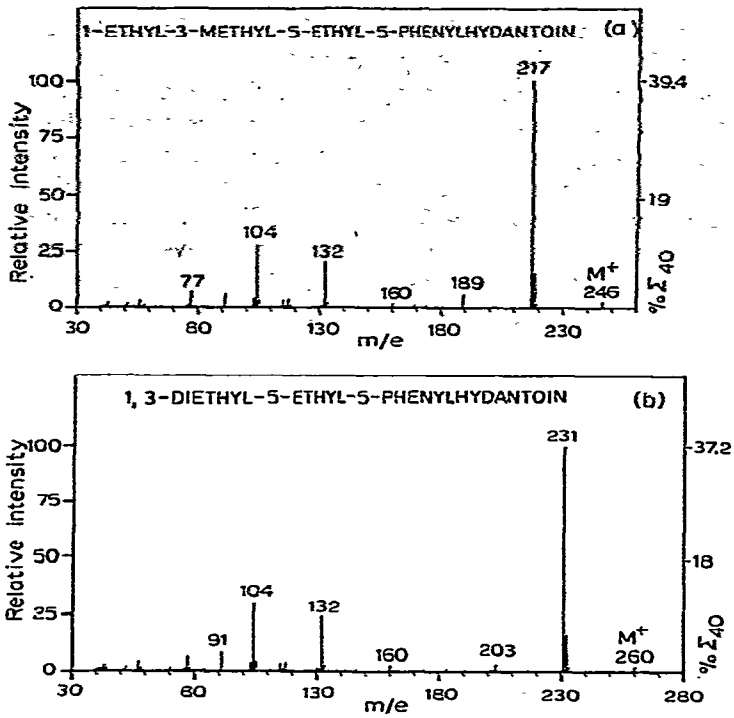


Fig. 5. Electron-impact mass spectra of perethylated mephenytoin (a) and Nirvanol (b) at 70 eV.

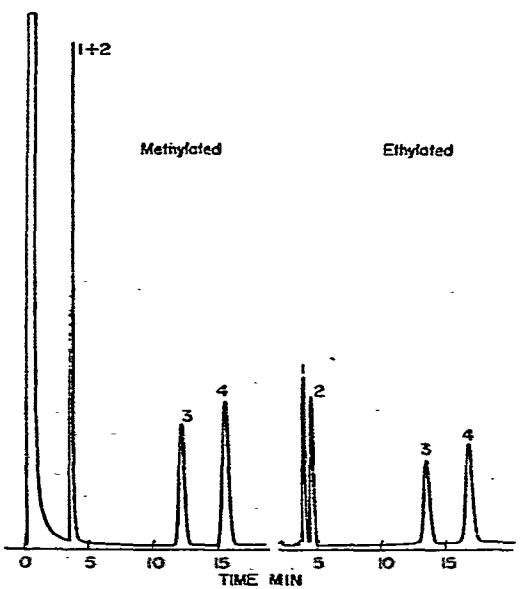


Fig. 6. Separation on a 3-ft. 5% SE-30 column of mephenytoin (1), Nirvanol (2), phenytoin (3) and *p*-methylphenytoin (4) as their permethylated derivatives (left) and as their perethylated derivatives (right). The perethylated products were prepared by heating the hydantoin mixture with ethyl iodide (50 μl) in acetone (200 μl) and 20% tetraethylammonium hydroxide in methanol (50 μl) for 1 h at 65°.

toin and Nirvanol are shown in Fig. 5. The molecular ion is of low intensity; the main fragmentation arises from the loss of the ethyl group at C_5.

The separation of mephenytoin, Nirvanol, phenytoin and 5-(*p*-methylphenyl)-5-phenylhydantoin as their permethylated and perethylated derivatives on an SE-30 column is shown in Fig. 6. As expected, a single peak was obtained for mephenytoin and Nirvanol after methylation. Perethylation was sufficient to achieve a baseline resolution between the two compounds even on a 3-ft. column. The methylene unit (MU) values of alkylated derivatives of 5,5-disubstituted hydantoins were recorded (Table III), and indicate that the 3-methyl is separated from the 3-ethyl derivative of 5-ethyl-5-phenylhydantoin. Nirvanol and mephenytoin could be resolved after ethylation at N_3.

TABLE III

MU VALUES

5% SE-30 column.

Compound	*MU value*	*Compound*	*MU value*
5-Ethyl-5-phenylhydantoin:	19.06*	*5-(p-Methylphenyl)-5-phenylhydantoin:*	
3-Methyl-	17.81	3-Methyl-	23.06
3-Ethyl-	18.10	1,3-Dimethyl-	22.75
1,3-Dimethyl-	17.70	1,3-Diethyl-	23.12
1,3-Diethyl-	18.58	*5-(4'-Hydroxyphenyl)-5-phenylhydantoin:*	
1-Ethyl-3-methyl-	18.30	1,3,4'-Trimethyl-	24.4
3-Propyl-	18.66	1,3,4'-Triethyl-	25.68
3-Pentyl-	20.78		
5,5-Diphenylhydantoin:			
3-Methyl-	22.03		
3-Ethyl-	22.31		
1,3-Dimethyl-	21.82		
1,3-Diethyl-	22.36		

* Tailing.

The 3-ethyl-Nirvanol can be prepared by reaction with diazoethane but diethylated side-products are usually present. The reaction is cleaner with ethyl iodide; selective 3-alkylation was achieved by decreasing the concentration of potassium hydroxide solution added.

The conditions used for the derivatization of mephenytoin and Nirvanol are also applicable to the determination of phenytoin, its *p*-hydroxylated metabolite and 5-(*p*-methylphenyl)-5-phenylhydantoin. The electron-impact mass spectrum of perethylated 5-(4'-hydroxyphenyl)-5-phenylhydantoin (HPPH) is shown in Fig. 7. The molecular ion is the base peak and the most intense fragment at *m/e* 275 results from the loss of the phenyl group at C_5.

For analytical work, it should be added that commercially available ethyl iodide is sometimes contaminated with methyl iodide. As methylation is much faster than ethylation, a high yield of dimethyl derivative can be obtained with a reagent containing only 1% of methyl iodide.

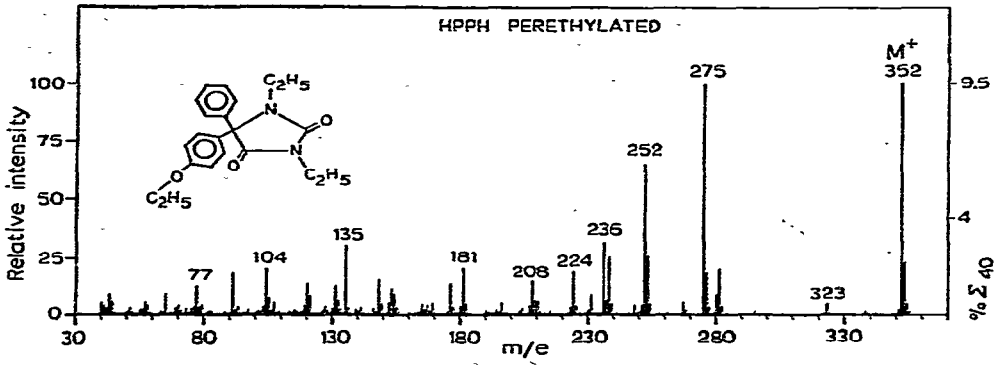


Fig. 7. Electron-impact mass spectrum of 1,3,4′-triethyl-5-(4′-hydroxyphenyl)-5-phenylhydantoin (HPPH) at 70 eV.

DISCUSSION

Hydantoins react with alkyl iodides according to a bimolecular nucleophilic substitution mechanism (S_N2):

$$Y^- + RI \rightarrow YR + I^- \quad (1)$$

where Y^- is the anion corresponding to the hydantoin and R is an alkyl group.

S_N2 reactions as a class have been investigated extensively in solution[23]. More recently, Olmstead and Brauman have studied the rate constants of this type of reaction in the gas phase[24].

As shown in eqn. 1, the hydantoins react as an anion, and therefore the pH of the medium must be such that the nitrogen to be alkylated is present as an anion. The pK value of N_3 is 8.3 (ref. 25); the pK value of N_1 is similar to that of an amide, and therefore a strong base is necessary to ionize N_1. The difference in pK values between N_1 and N_3 is the basis for the possibility of selective alkylation of the hydantoin ring, although it is not possible to alkylate N_1 preferentially unless N_3 is protected[26]. Gordos *et al.*[16] found that the reaction was strongly dependent on pH. At pH > 13, the 1,3-dimethylphenytoin could be prepared, whereas at pH 11.5–13 both the mono- and dimethyl derivatives were formed.

As expected for an S_N2 reaction in solution, the relative rate of alkylation decreased in the order methyl > ethyl > *n*-propyl. The decrease in reactivity from methyl to *n*-propyl is due to the increasing steric hindrance during the near approach of the alkyl iodide[22].

The effect of solvent on the rates of S_N2 reactions has been extensively studied. It is well known that for reactions described by eqn. 1, the rate increases from a protic solvent (water, methanol) to an aprotic solvent (acetone, dimethylformamide). In the method described by Greeley[15] for the mild alkylation of barbiturates, dimethylformamide is used as the solvent, which explains the rapid derivatization. The solvent effect is important, as shown by the fact that the well known order of nucleophilicity in water ($I^- > Br^- > Cl^- > F^-$) is reversed in acetone[23] and in the gas phase[24].

Protic solvents are more sensitive than aprotic solvents to charge localization, so it is expected that the rate should be faster in acetone or dimethylformamide than in an aqueous acetone solution. This effect was observed in the present study; the addition of 25 μmole of potassium hydroxide in 5 instead of 50 μl of water resulted in a faster reaction. Under these conditions, although the perethylation was complete, it was not possible to obtain complete methylation. The hydantoin anion Y^- and OH^- (in excess) are competing for the alkylating agent. After reaction with OH^-, the corresponding alcohol is formed. If the rate of hydrolysis is much faster than the rate of reaction with Y^-, methyl iodide is hydrolysed and its concentration becomes the limiting factor for the methylation of Y^-. It was observed that after addition of 5 *N* potassium hydroxide solution (5 μl), the rates of alkylation were increased but the maximal yield of permethylation was only 60% and could not be improved with time.

In his extensive review of the chemistry of hydantoins, Ware[27] stated that "N_1 substituents cannot be introduced, through action of alkyl halides in alkaline solution, into a hydantoin which does not have a double bond or a phenyl group attached to the C_5 carbon atom". This activation of C_5 substituents was first pointed out by Johnson and Bates[28]. The replacement of a phenyl by an ethyl group at C_5 may decrease the reactivity at N_1, which would explain the difference in rate observed between phenytoin and Nirvanol.

The separation of mephenytoin and Nirvanol after perethylation was easily achieved with a packed or a capillary column coated with SE-30. The alkylated derivatives are stable and give symmetrical peaks. Although N-TMS derivatives are easily prepared[12], they hydrolyze rapidly and may decompose on the GC column. Acetyl- and TMS-hydantoins are not stable, losing preferentially the group at N_3[29]. 3-Acetyl-1,5,5-trimethylhydantoin is a mild acetylating agent which can react selectively with phenols in the presence of alcohols. It is probable that 3-TMS-hydantoins are mild silylating agents.

In this work acetone was used as the solvent; owing to its high volatility (b.p. = 56°), evaporation may occur during the perethylation reaction as heating is required. This loss of solvent can be avoided by using 2-butanone (b.p. = 80°) instead of acetone. Complete perethylation was achieved under these conditions (2-butanone, 5 *N* potassium hydroxide solution, ethyl iodide)[30].

An alternative method to the preparation of peralkylated derivatives is to alkylate the acidic N_3 nitrogen selectively. Diazoalkanes give as the main product the 3-alkylhydantoins, together with a variable amount of dialkylated products. It is thus preferable to alkylate with dilute potassium hydroxide solution or in the presence of potassium hydrogen carbonate in order to avoid alkylation at N_1. The selective alkylation at N_3, discovered by Pinner[31] in 1888, cannot be used as it requires the exact addition of an equivalent of potassium hydroxide to the hydantoin.

3-Monoalkyl derivatives have good GC properties on polar phases such as the PZ 179. On that phase they give symmetrical peaks. Columns coated with SE-30 tend to produce tailing peaks.

The procedures described here are applicable to the determination of 5,5-disubstituted hydantoins used for anti-epileptic therapy. The generality of the method for the analysis of other anticonvulsants is under investigation.

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