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Gas chromatographic alkylation studies of phenytoin, mephenytoin and primidone: investigation of butylated derivatives

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Abstract: The alkylation of phenytoin, mephenytoin and primidone with *n*-alkyl iodides in *N,N*-dimethylacetamide with tetramethylammonium hydroxide was investigated by gas chromatography. With methyl iodide phenytoin and mephenytoin were each converted into a single derivative; the use of other alkyl iodides yielded more than one product. Primidone was converted with methyl iodide and butyl iodide into a major derivative (> 90%) and a minor one. Butylation of the compounds by this method was compared with butylation in an acetone–butyl iodide mixture with potassium carbonate, caesium carbonate or silver oxide added, and with on-column butylation. All these methods resulted in the production of more than one derivative. The derivatives were identified by mass spectrometry and by ^{1}H NMR and ^{13}C NMR. With the acetone–butyl iodide–silver oxide method the main derivatives were *O*-butylated compounds. The other methods yielded predominantly *N*-butylated derivatives.

Keywords: *Alkylation for gas chromatography; butylation; phenytoin; mephenytoin; primidone.*

Introduction

The gas chromatographic (GLC) analysis of acidic compounds is often hindered by irreversible adsorption of these compounds on to the column packing material, resulting in badly tailed peaks in the chromatograms. This can be remedied by conversion of acidic compounds into less polar derivatives with better chromatographic properties. Many alkylation methods for acidic pharmaceuticals have been described [1]. In a previous article [2] a generally applicable clean-up and derivatization method was described for the GLC-analysis of various types of acidic drugs. The compounds were butylated according to the method of Greeley [3]. Butylation was preferred to methylation, because some compounds are demethylated *in vivo*; upon methylation the parent compound and its metabolite will yield the same derivative. Butylation of the antiepileptic agents

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mephenytoin (MTN), phenytoin (PTN) and primidone (PMD) was found to result in the appearance of more than one peak in the chromatograms, suggesting incomplete derivatization and/or the conversion of these compounds into more than one derivative [2].

The purpose of this work was: (a) the identification of the butylated derivatives of MTN, PTN and PMD; (b) an investigation of the derivatives formed by alkylation according to Greeley [3] with other *n*-alkyl iodides; (c) a comparison of the results of the butylation method of Greeley [3] with those obtained with other butylation techniques.

Experimental

Chemicals and reagents

PTN and tetramethylammonium hydroxide (20% in methanol; TMAH) were obtained from Aldrich-Europe (Beerse, Belgium). MTN was from Brocacef B.V. (Maarssen, The Netherlands). PMD was supplied by ICI Holland N.V. (Rotterdam, The Netherlands). The structural formulae of PMD and of the hydantoins PTN and MTN are shown in Fig. 1. Methyl, ethyl, *n*-propyl, *n*-butyl, *n*-pentyl, *n*-hexyl and *n*-heptyl iodides and tetrabutylammonium hydroxide (25% in methanol; TBAH) were obtained from Fluka (Buchs, Switzerland). Caesium carbonate (reinst), potassium carbonate (zur Analyse) and silver oxide (zur Analyse) were purchased from Merck (Darmstadt, FRG). The solvents were at least of analytical reagent grade.

Gas chromatography

GLC was performed on a Packard–Becker Model 419 gas chromatograph equipped with flame ionization detectors. The glass columns were packed with 10% SE30 on 80–100 mesh Chromosorb WHP, with 3% OV17 or with 3% SP1000, both on 100–120 mesh Chromosorb WHP (Chrompack, Middelburg, The Netherlands). Depending on the analysis the column oven temperature was between 160° and 260°C. The injection port and detector temperatures were 270°C; when the on-column alkylation procedure was studied the injection port temperature was 320°C. The carrier gas (nitrogen) flow rate was 30 ml/min and the air flow rate was 300 ml/min. Retention times and peak areas were measured with the Data Analyser System IVB (Spectra-Physics, Santa Clara, USA).

Gas chromatography–mass spectrometry (GLC–MS) and mass spectrometry

GLC–MS analyses of the derivatization mixtures were performed on a Jeol JMS-07 mass spectrometer–gas chromatograph combination (Tokyo, Japan), equipped with a 1.5 m 3% OV1 (Chrompack) glass column. The carrier gas was helium (flow rate, 30 ml/min). The injection port temperature was 250°C; the column oven temperature was between 130° and 190°C. The ion source temperature was 225°C; the ionizing current and electron energy were 100 μA and 70 eV, respectively. The mass spectra of two of the derivatives prepared and isolated on a preparative scale, O^2-butyl MTN and O^2,O^4-dibutyl PTN (Fig. 1), were obtained with a Carlo Erba 4200 gas chromatograph combined with a Kratos MS 80 mass spectrometer (Manchester, UK); column, 3% CP Sil 5; helium flow, 30 ml/min; injection port temperature, 250°C; column oven temperature programmed from 160° to 260°C, 4°C/min. The ionizing current and electron energy were 100 μA and 70 eV, respectively. The mass spectra of the other isolated derivatives were obtained with an AEI MS-902 mass spectrometer (Manchester, UK).

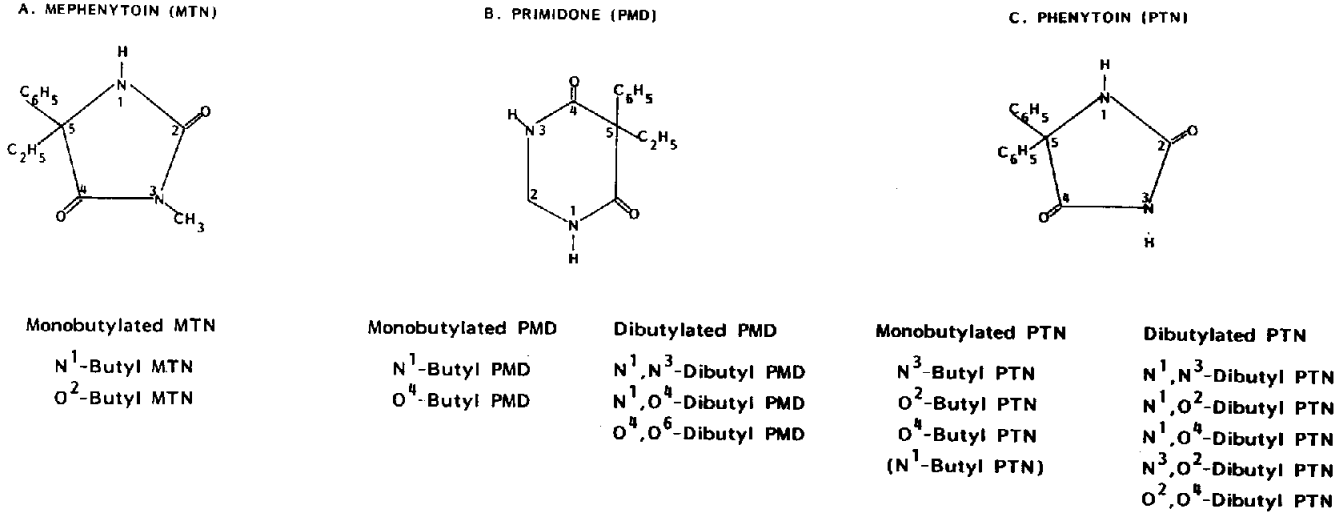

Figure 1
Potential products formed by *N*-butylation or *O*-butylation of mephenytoin, phenytoin and primidone.

The ion source temperature was 150°–180°C; the ionizing current and electron energy were 100 μA and 70 eV, respectively. The samples were introduced by the direct insertion probe technique.

NMR spectrometry

^1H NMR spectra were obtained with a Varian EM-390 spectrometer (Palo Alto, USA). ^{13}C NMR spectra were run on a Varian CFT-20 spectrometer. The compounds were dissolved in $CDCl_3$ or CD_3OD.

Preparative liquid chromatography

Chloroform solutions of the residues, obtained with the preparative derivatization procedures (see below), were placed in the sample reservoir of a Chromatospac Prep 100 preparative liquid chromatograph (Jobin Yvon, Longjumeau, France), equipped with a variable wavelength UV detector. After elution with chloroform through a column with 100 g of silica gel (Kieselgel H nach Stahl, Merck), the appropriate fractions of the eluate containing the pure derivatives were evaporated under reduced pressure. The derivatives were tested for purity by GLC (see above) and by thin-layer chromatography on 0.25 mm silica gel F254 plates (5 × 10 cm, Merck) with chloroform–methanol mixtures as eluents.

Analytical derivatization studies

Method a [4, 5]. A 90 μl volume of a solution of PTN, MTN or PMD in acetone (0.5–1 mg/ml) was transferred into a glass capillary tube (5 cm × 3 mm I.D.) and mixed with 10 μl of *n*-butyl iodide. Potassium carbonate (5–10 mg) was added and the tube was closed. After heating for 1 h at 80°C, 1 μl of the solution was injected into the gas chromatograph.

Method b. The same procedure as described under Method a was followed, but caesium carbonate was used instead of potassium carbonate.

Method c. The same procedure was followed, with silver oxide used instead of potassium carbonate.

Method d [2, 3]. An 80 μl volume of a solution of PTN, MTN or PMD in *N,N*-dimethylacetamide (DMA) (1 mg/ml) was transferred into a 1.5 ml capped polypropylene centrifuge tube and mixed with 10 μl TMAH; 20 μl of *n*-butyl iodide was added. The contents of the tube were mixed and allowed to stand at room temperature for 10 min. After centrifugation (2500 ***g***, 5 min) 1 μl of the supernatant was injected into the gas chromatograph.

Method e; pyrolytic alkylation [6]. A 50 μl volume of a solution of PTN, MTN or PMD in methanol (1 mg/ml) was transferred into a 1.5 ml capped polypropylene centrifuge tube and mixed with 50 μl TBAH; 1 μl of the resulting solution was injected into the gas chromatograph.

The compounds were also methylated following the same procedure with TMAH instead of TBAH.

Preparation and isolation of derivatives

N^3-butyl PTN (Fig. 1) was prepared by refluxing for 6 h a solution of 0.5 g PTN with 5 g potassium carbonate and 10 ml butyl iodide. After evaporation of the solvent under reduced pressure the residue was taken up in 100 ml of diethyl ether; the resulting mixture was filtered and the filtrate was evaporated under reduced pressure. The residue was dissolved in 15 ml of chloroform. N^3-butyl PTN was separated from other components in the mixture by preparative liquid chromatography. N^1-butyl MTN, N^1,N^3-dibutyl PTN and N^1,N^3-dibutyl PMD (Fig. 1) were prepared as described for N^3-butyl PTN, with 5 g caesium carbonate instead of potassium carbonate. O^2-butyl MTN, O^2, O^4-dibutyl PTN and O^4, O^6-dibutyl PMD (Fig. 1) were prepared as described for N^3-butyl PTN with 2 g silver oxide instead of potassium carbonate.

Results

Alkylation studies with various n-alkyl iodides according to method d

PTN and MTN were derivatized according to method d with all the *n*-alkyl iodides from methyl iodide up to *n*-heptyl iodide. GLC analysis of the derivatization mixtures showed that, with the exception of methylation, alkylation of PTN always resulted in the formation of two major derivatives, each with a peak area of at least 10% of the sum of the peak areas in the chromatogram. A small peak of a third derivative was often observed between the two main peaks. After ethylation of PTN only one peak was discernable in the chromatograms obtained with 10% SE30 as the stationary phase. However, analysis of the ethylation reaction mixture with 3% OV17 and 3% SP1000 showed that at least two derivatives were formed whose peaks coincided on 10% SE30. Methylation of PTN and MTN resulted in one derivative peak only with all three stationary phases. Alkylation of MTN with ethyl iodide and the other *n*-alkyl iodides always resulted in a mixture of alkylated MTN (one major and one minor component) and underivatized MTN. Butylation of PMD yielded two derivatives, one major component (peak area 93% ± 1.2% (n = 6) of the sum of peak areas) and one minor component. Similar results were obtained upon methylation of PMD; here the peak area of the major derivative was 95% ± 1.1% (n = 4).

Butylation according to methods a–e

A survey of representative GLC chromatograms (on 10% SE30) obtained using derivatization methods a–e, is shown in Fig. 2. Similar chromatograms were obtained with the GC–MS analysis (with OV1) of the derivatization mixtures.

The peaks of the chromatograms attributed to derivatives of MTN, PMD and PTN are numbered in order of increasing retention times. Two derivatization products were observed for MTN, five for PMD and six for PTN. For peaks of which the mass spectra could be recorded, the *m/e* values of the molecular ions are given.

Pyrolytic methylation/gas chromatography

MTN, PMD and PTN were methylated according to method e. MTN and PTN were completely permethylated with this technique, resulting in the formation of only one derivative of each; the same retention times for the derivatives were found as with method d. Pyrolytic methylation of PMD gave rise to two peaks in the chromatograms, one major peak (peak area > 95% of the sum of areas) and one minor one. The retention times for these peaks were identical to those found using method d.

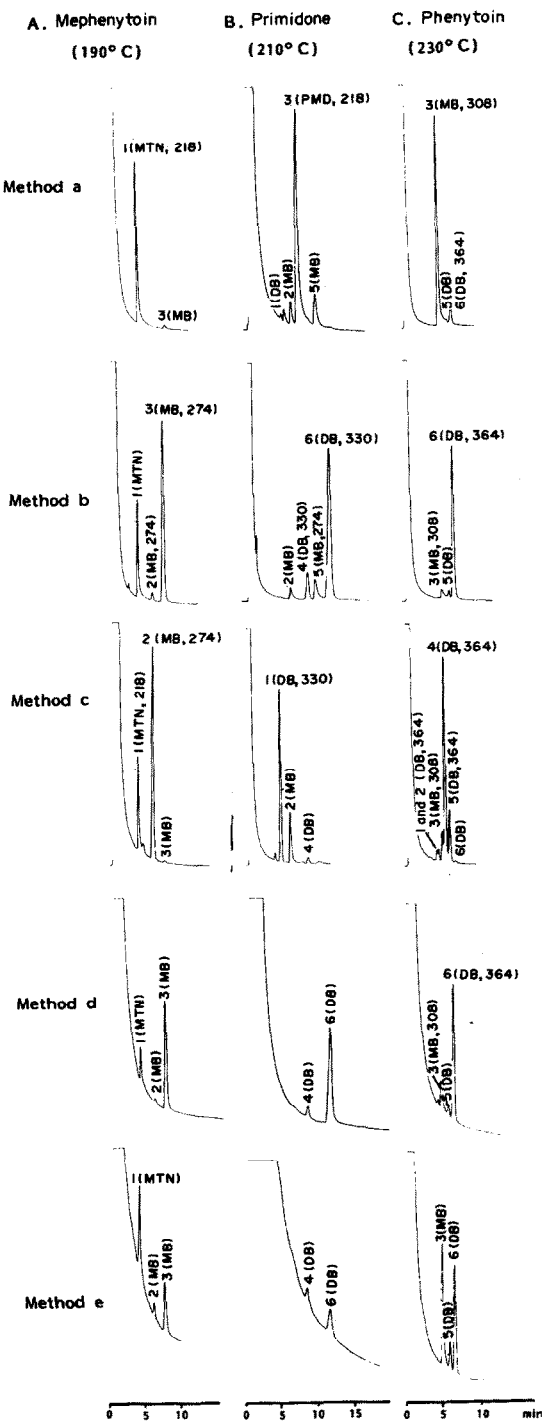

Figure 2
Gas chromatograms obtained after butylation of mephenytoin, phenytoin and primidone by methods a–e. The peaks are numbered according to increasing retention times; *m/e* values of the molecular ions are given together with the indications MB (monobutyl) or DB (dibutyl). The peaks correspond to A_1, MTN; A_2, O^2-butyl MTN; A_3, N^1-butyl MTN; B_1, O^4,O^6-dibutyl PMD; B_2, O^4-butyl PMD; B_3, PMD; B_4, N^1,O^4-dibutyl PMD; B_5, N^1-butyl PMD; B_6, N^1,N^3-dibutyl PMD; C_1, dibutyl PTN; C_2, dibutyl PTN; C_3, N^3-butyl PTN; C_4, O^2,O^4-dibutyl PTN; C_5, dibutyl PTN; C_6, N^1,N^3-dibutyl PTN.

Mass spectra and NMR spectra of purified derivatization products

Values of *m/e* and relative abundances of the principal mass fragments from the electron impact mass spectra of N^1-butyl MTN, O^2-butyl MTN, N^1,N^3-dibutyl PMD, O^4,O^6-dibutyl PMD, N^3-butyl PTN, N^1,N^3-dibutyl PTN and O^2,O^4-dibutyl PTN are shown in Table 1. ^{1}H NMR chemical shifts for these compounds were measured and will be discussed below. ^{13}C-NMR chemical shifts (except O^4,O^6-dibutyl PMD) are reported in Table 2.

Table 1
Partial electron-impact mass spectra of the butylated derivatives of mephenytoin, primidone and phenytoin

Compound*	*m/e* and (relative abundance)
O^2-butyl MTN	274(3); 245(33); 219(7); 189(100); 160(4); 132(6); 104(38)
N^1-butyl MTN	274(3); 246(16); 245(100); 231(6); 189(39); 174(9); 117(11); 104(25)
O^4,O^6-dibutyl PMD	330(4); 302(20); 301(43); 246(16); 245(15); 231(14); 175(24); 174(27); 147(20); 146(100); 117(45); 103(9)
N^1,N^3-dibutyl PMD	330(19); 302(35); 301(39); 245(9); 146(100); 117(16); 104(2); 103(7)
N^3-butyl PTN	308(24); 279(9); 231(9); 208(41); 207(24); 181(29); 180(100); 165(26); 104(59)
O^2,O^4-dibutyl PTN	364(50); 309(14); 308(27); 307(36); 252(68); 251(100); 208(71); 180(78); 165(23); 105(77); 104(32)
N^1,N^3-dibutyl PTN	364(67); 322(24); 321(100); 287(18); 265(24); 222(12); 208(14); 195(14); 194(31); 180(5); 165(17); 103(17)

* See Fig. 1.

Discussion

Identification of the derivatives

The mass spectra of the derivatives (Table 1) show the characteristic features of fragmentations previously described for the underivatized products [7] and for the methyl derivatives [8, 9]. The intensity ratios of some clusters of peaks, e.g. *m/e* 104 and 103 for hydantoins, are different in the spectra of some of the derivatives. Although there is some evidence that (at least in hydantoins which are *N*-butylated) the expulsion of a propyl radical leads to a significant M-43 fragment, the mass spectra cannot be used for establishing the exact location of the substituted butyl groups.

The location of the substituted *n*-butyl group(s) in the derivatives could be established with NMR spectroscopy. The assignment of the ^{13}C NMR chemical shifts of Table 2 have been made with correlation tables and calculations using chemical shift incremental theories [10, 11]: results for both the *N*-butyl and the *O*-butyl derivatives are shown. These results were used for the establishment of the location of the butyl group(s). In the ^{1}H NMR spectra, the chemical shifts of the 1:2:1 triplet resonances of the X–CH_2 group were found near $\delta = 3.3$ for X = N (N^1-butyl MTN, 3.16; N^1,N^3-dibutyl PMD, 3.50 and 3.51; N^3-butyl PTN, 3.55; N^1,N^3-dibutyl PTN, 3.30 and 3.57) and near $\delta = 4.3$ for X = O (O^2-butyl MTN, 4.50; O^4,O^6-dibutyl PMD, 4.01; O^2,O^4-dibutyl PTN, 4.43 and 4.48). This is in agreement with the expected relative values for the shifts [11]. The resonances of the other H atoms in the ^{1}H NMR spectra of the derivatives were also found to be in agreement with the expected values.

Knowing the identity of a number of the main derivatization products, the identity of most of the other derivatives could be deduced with the help of the available GLC and GC–MS data (Fig. 2). The *m/e* values of the molecular ions in the mass spectra of the

Table 2
^{13}C NMR chemical shifts of the butylated derivatives of mephenytoin, primidone and phenytoin (δ_c, ppm)

	Hydantoins						Primidone derivatives	
Compound*	O^2-butyl MTN	N^1-butyl MTN	N^3-butyl PTN	O^2,O^4-dibutyl PTN	N^1,N^3-dibutyl PTN	Calculated†	N^1,N^3-dibutyl PMD	Calculated†
Solvent	$CDCl_3$	$CDCl_3$	CD_3OD	$CDCl_3$	$CDCl_3$		CD_3OD	
Carbon atom‡								
C-2	158.0	157.1	158.0	169.7	155.9		65.1	
C-4	181.5	174.3	175.5	193.1	173.7		172.5	
C-5	75.0	71.5	71.2	82.9	77.4		61.9	
C-e	25.4	25.4	—	—	—		—	
C-f	30.6	24.7	—	—	—		33.9	
C-g	8.3	7.7	—	—	—		13.2	
C-1′	140.2	137.2	140.9	139.9	137.6		139.9	
C-*o*	128.0	128.9	129.6	128.1	128.7		129.4	
C-*m*	125.8	126.4	128.3	127.3	128.4		132.1	
C-*p*	127.2	128.7	129.4	127.6	128.7		131.1	
$C\text{-}N_\alpha$	—	41.0	39.4	—	39.0 + 41.9	41	48.8	47
$C\text{-}N_\beta$	—	31.1	31.1	—	30.0 + 30.2	~30	32.7	31
$C\text{-}N_\gamma$	—	20.3	20.8	—	19.9 + 19.9	20	23.0	20
$C\text{-}N_\delta$	—	13.6	13.9	—	13.4 + 13.5	14	16.1	14
$C\text{-}O_\alpha$	68.4	—	—	68.7 + 72.1	—	62 + 65	—	62
$C\text{-}O_\beta$	33.5	—	—	30.2 + 30.7	—	34	—	36
$C\text{-}O_\gamma$	18.9	—	—	18.8 + 19.0	—	20	—	20
$C\text{-}O_\delta$	13.1	—	—	13.4 + 13.7	—	14	—	14

* See Fig. 1.
† Calculated on basis of the formula $\delta = -2.3 + \Sigma_{A_i}$ + correction [10].
‡ C-e, C-f and C-g are the carbon atoms of the $N\text{-}CH_3$, C_2H_5 (α) and C_2H_5 (β), respectively. The aromatic carbon atoms are indicated with 1′, *o* (ortho), *m* (meta) and *p* (para), respectively.

separate peaks indicate the number of butyl groups incorporated in the parent compounds. The *O*-butylated products had shorter retention times than the *N*-butylated products; this was shown by comparing the retention times of O^2-butyl MTN and N^1-butyl MTN (peaks A_2 and A_3, Fig. 2), of O^4, O^6-dibutyl PMD, N^1, O^4-dibutyl PMD and N^1,N^3-dibutyl PMD (peaks B_1, B_4 and B_6, Fig. 2) and of O^2,O^4-dibutyl PTN and N^1,N^3-dibutyl PTN (peaks C_4 and C_6, Fig. 2). The identity of the derivatives corresponding with the peaks in the chromatograms is given in Fig. 2. All the theoretically possible butylated derivatives of MTN and PMD (Fig. 1) were observed, whereas five dibutyl derivatives and one monobutyl derivative of PTN could be traced.

Mechanism of the alkylation reactions

The anions of acidic compounds such as the hydantoins MTN and PTN act as nucleophiles in a substitution reaction with alkyl iodides (methods a–d) and with tetraalkylammonium ions (method e) as the substrates. In dipolar aprotic solvents like acetone and DMA and with unbranched *n*-alkyl iodides the reactions usually take place according to a bimolecular (S_{N2}) mechanism [12]. MTN, PMD and PTN are ambidentate nucleophiles; they have two atoms, N and O, in each deprotonated acidic group with which they can attack the alkyl iodide.

S_{N2} reactions proceed preferentially through the more polarizable atom in the nucleophile; in the case of MTN, PMD and PTN this is the N-atom. Indeed with methods a, b, d and e, *N*-butylated products were predominantly formed with only minor amounts of *O*-butylated derivatives. Butylation of the compounds in acetone with silver oxide (method c) resulted in the formation of mainly *O*-butylated products. The silver ions promote the ionization of butyl iodide by precipitation of silver iodide, resulting in the shift from an S_{N2} to a S_{N1} attack by the nucleophiles on butyl iodide [12]. In S_{N1} type reactions the alkylation of ambidentate nucleophiles proceeds preferentially through the atom in the nucleophile on which electron density is higher, i.e. the O atom. Ijdenberg [5] reported that alkylation in acetone with silver oxide resulted in more than one PTN derivative, and one derivative for primidone. We observed under the same derivatization conditions the conversion of both compounds into more than one derivative, while MTN was only partially derivatized.

Alkylation studies of PTN, PMD and MTN

PTN is a dibasic acid; the pK_a of the imide group at N^3 is 8.3 [13]. This group is smoothly butylated with each of the methods a–e. The amide group at N^1 has extremely weak acidic properties; a considerable concentration of a strong base is therefore needed to deprotonate this group and make it accessible to an alkylation reaction. Butylation of PTN in acetone with potassium carbonate (method a) yielded mainly N^3-butyl PTN with only a minor amount of N^1,N^3-dibutyl PTN. The amide group at N^1 in MTN is apparently even less reactive than the corresponding group in PTN [14]. With method a virtually no MTN was butylated and indeed MTN was only partially derivatized with any of the other methods. The rate of butylation of the amide function was considerably higher when caesium carbonate was used (method b) instead of potassium carbonate. This may be attributed at least partly to the better charge separation — and therefore increased reactivity — of the nucleophile and the counter-ion in the case of the larger (softer) caesium ions [15, 16]. PMD, also possessing two very weakly acidic amide groups, remained largely underivatized by method a, but was converted by method b, with N^1,N^3-dibutyl primidone as the main derivative.

The concentrations of the base catalyst are much higher in methods d and e and large counterions (TMA^+, TBA^+) are present. The pyrolytic butylation (method e) of the anions of MTN and PTN is accompanied by nucleophilic attack of OH^- ions upon the TBA^+ ions, resulting in the formation of methanol and tributylamine. This results in a decrease of the TBA^+ concentration. The pH of the medium is also lowered, which may lead to protonation of the strongly basic anions of MTN, PMD and PTN. The result is that these compounds are not completely perbutylated by this method.

In method d the added OH^- ions deprotonate the compounds to be derivatized, but the OH^- ions react also with *n*-butyl iodide, which is added in excess, to form *n*-butanol (S_{N2} mechanism); the liberated I^- ions are precipitated as TMA^+I^-. The pH of the derivatization medium is therefore continuously lowered until the solution is neutralized. The butylation reaction of PTN and MTN is apparently too slow to allow complete perbutylation before the pH falls below the value needed for the deprotonation of amide groups. PMD was found to be completely perbutylated with methods d and e; two of the three possible dibutyl derivatives of PMD were formed with N^1,N^3-dibutyl PMD as the predominant one, particularly with method d. MTN and PTN were completely permethylated by method d or method e, with virtually 100% yield of N^1-methyl MTN and N^1,N^3-dimethyl PTN, respectively. S_{N2}-type reactions proceed faster when the substrate is less bulky. Apparently the rates of the reactions between the OH^- ions and the substrates are not increased to the same extent as the rates of the alkylation reactions of MTN and PTN, when changing from *n*-butyl iodide to methyl iodide (method d) or from TBAH to TMAH (method e). PMD was also found to be completely permethylated; only a minor amount of N^1,O^4-dimethyl PMD was formed with both methylation procedures (d and e).

Practical consequences for the determination of MTN, PMD and PTN

With methods a–e none of the compounds MTN, PTN and PMD could be completely converted into a single perbutylated derivative. The formation of two or more derivatives from one compound yielding a particular pattern in chromatograms can occasionally be of value for identification purposes, but for quantitative determinations the conversion of a compound into a single derivative or the reproducible formation of a major derivative is preferable. Considerable variation of the relative amounts of the derivatives formed with methods a–e was observed with the exception of the amounts of butylated and methylated derivatives of PMD formed with method d. As the amount of the major derivative was very high in both cases, either methylation or butylation of PMD by this method is suitable for the quantitative GLC assay of this compound. In an effort to find reaction conditions for method d which would lead to the complete conversion of PTN into a single perbutylated product, the relative amounts of DMA, butyl iodide and TMAH in the reaction medium were varied, as well as the reaction time and the temperature; but the results were unsatisfactory.

PTN can be quantitatively permethylated. As PTN is not metabolized *in vivo* into a desmethyl derivative, permethylation of PTN is acceptable for the determination of the drug in biological fluids. Complete methylation of MTN is also possible. However, MTN is partly metabolized *in vivo* to form 5-ethyl, 5-phenyl hydantoin (desmethyl MTN), which upon methylation forms the same derivative as MTN. In this case use of deuterium labelled methylating agents and MS detection would distinguish natural methylated compounds from demethylated metabolites. The pentylation of MTN and desmethyl MTN by a modification of Greeley's method (3) (method d) has been described, but the

possible formation of more than one derivative was apparently not investigated [17].

De Sagher *et al.* [14] developed an ethylation procedure for MTN and desmethyl MTN; they concluded that each of the compounds could be completely derivatized into a single perethylated product by heating in a mixture of acetone (or 2-butanone), ethyl iodide and a concentrated aqueous solution of potassium hydroxide. This procedure was applied by Yonekawa *et al.* [18] in the GC–MS determination of MTN and its metabolite in plasma. However, these authors observed small peaks in the chromatograms eluting before the main derivatives, which they ascribed to minor amounts of other derivatives. As the shape of the MTN peak is fairly good in gas chromatograms obtained with more polar stationary phases such as SP1000, derivatization of this compound can more easily be avoided than in the cases of PTN and of PMD.

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