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DERIVATIZATION OF N-METHYL AND CYCLIC AMINO ACIDS WITH DIMETHYLFORMAMIDE DIMETHYL ACETAL

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SUMMARY

Six amino acids containing either an N-methyl or a cyclic secondary amine were converted to volatile derivatives by reaction with dimethylformamide dimethyl acetal. The amine functionalities were formylated by way of an amide acetal intermediate while the carboxylic acid groups were esterified directly. The resulting N-formyl esters were stable to solvent extraction and exhibited gas chromatography–mass spectrometry properties suitable for assay development.

INTRODUCTION

The reaction of primary amines[1], secondary amines[2,3], and primary amino acids[4,5] with dimethylformamide dimethyl acetal (DMF–DMA) has served as a derivatization step in a variety of analytical methods for biologically important substances. The derivatives are more volatile than the parent compounds and are thus more amenable to gas chromatography (GC–MS) analysis.

A number of derivatization methods for cyclic amino acids, such as proline, have been described in the literature. Representative reactions include formation of phenylthiohydantoins[6], N-dinitrophenyl esters[7], N-trifluoroacetyl *n*-butyl esters[8], and other acyl esters[9]. While these derivatives serve well for proline, the reaction conditions required may induce thermal cyclization of N-monosubstituted γ-amino carboxylic acids, yielding a lactam by-product[10].

Since DMF–DMA reacts with both secondary amines and carboxylic acids to form stable derivatives, an evaluation of this reagent for the derivatization of cyclic and N-methyl acyclic secondary amino acids was undertaken.

EXPERIMENTAL

Materials and methods

Dimethylformamide dialkyl acetals, 4-methylaminobutanoic acid hydrochloride, $H_2{}^{18}O$, and NMR solvents were purchased from Aldrich (Milwaukee, WI, U.S.A.). 1-Methyl-2-pyrrolidinone was obtained from J. T. Baker (Phillipsburg, NJ,

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U.S.A.). 3-Piperidinecarboxylic acid (nipecotic acid) and 2-piperidinecarboxylic acid (isonipecotic acid) were provided by Dr. Alex Chang and N-methyl-D-aspartic acid by Dr. J. Edward Moreton. ^{1}H NMR spectra were recorded on a General Electric QE-300 magnetic resonance spectrometer using tetramethylsilane as reference standard. IR analysis was conducted with an Analect Instruments Model fx-6160 Fourier Transform spectrophotometer. Elemental analyses were performed by Galbraith Laboratories, Knoxville, TN, U.S.A.

Typical derivatization procedure

Solid secondary amino acid (10–50 μg) was dissolved in DMF–DMA (100 μl) in a 1-ml reaction vial and the resulting solution heated at 100°C for 15 min. On cooling, 200 μl of water were added and the mixture was then extracted with chloroform (500 μl). The chloroform layer was concentrated prior to GC–MS analysis.

Synthesis of methyl N-formyl-4-methylaminobutanoate ***1b)***

DMF–DMA (2.0 ml, 15 mmol) was added to 4-methylaminobutanoic acid hydrochloride (1.0 g, 6.8 mmol) in an acylating vessel which was then capped with a PTFE-lined screwtop. The mixture was agitated briefly, and heated for 10 min at 110°C. Water (4.0 ml) was added to the mixture when cool, and the resulting mixture was extracted with chloroform (2 × 10 ml). The combined chloroform layers were evaporated and the residue (crude yield 91%) was distilled to give the desired product in 25% yield: b.p. 100°C at 0.45 Torr; ^{1}H NMR (C^2HCl_3), δ 2.89 and 2.90 ppm (3 H, singlet, N-CH_3, reflecting approximately equal populations of the *E* and *Z* isomers), 3.70 (3 H, singlet, ester CH_3), 8.00 (1 H, singlet, formyl); IR (neat), 1739, 1671 cm^{-1}; C H N analysis of the partially hydrated product was consistent with $C_7H_{13}NO_3 \cdot 1/2H_2O$.

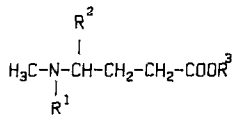
GC–MS

Electron impact (EI) mass spectra were acquired on a Hewlett-Packard mass-selective detector Model 5970 interfaced with a Model 5890 gas chromatograph. An Extrel Simulscan mass spectrometer, interfaced with a Carlo-Erba gas chromatograph, was used to obtain methane positive ion chemical ionization (CI) spectra. Both GC–MS instruments were equipped with a capillary column (HP-1, 12 m × 0.20 mm I.D.) (Hewlett-Packard, Avondale, PA, U.S.A.) and were operated in the splitless injection mode. The column ovens were programmed from 130 to 180°C at 15°C/min following a 1-min solvent delay.

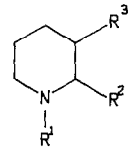
RESULTS AND DISCUSSION

Six secondary amino acids were converted to their N-formyl methyl ester derivatives by reaction with DMF–DMA (Fig. 1 and Table I). Since lactam by-products were not observed for compounds **1** and **2**, it was concluded that thermal cyclization of these γ-amino acids was avoided under the reaction conditions employed.

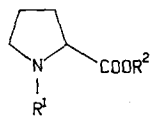
4-Methylaminobutanoic acid (**1**) was studied as a model N-substituted γ-amino acid. Esterification of the carboxyl group with DMF–DMA provided the methyl ester. This reaction involves an electrophilic alkoxyliminium intermediate derived from



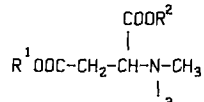
	R^1	R^2	R^3
1	H	H	H
1a	$CH(OCH_3)_2$	H	CH_3
1b	CHO	H	CH_3
2	H	3-pyridyl	H
2a	$CH(OCH_3)_2$	3-pyridyl	CH_3
2b	CHO	3-pyridyl	CH_3



	R^1	R^2	R^3
4	H	COOH	H
4a	$CH(OCH_3)_2$	$COOCH_3$	H
4b	CHO	$COOCH_3$	H
5	H	H	COOH
5a	$CH(OCH_3)_2$	H	$COOCH_3$
5b	CHO	H	$COOCH_3$



	R^1	R^2
3	H	H
3a	$CH(OCH_3)_2$	CH_3
3b	CHO	CH_3



	R^1	R^2	R^3
6	H	H	H
6a	CH_3	CH_3	$CH(OCH_3)_2$
6b	CH_3	CH_3	CHO

Fig. 1. Structures of secondary amino acids and the corresponding amide acetal and N-formyl methyl ester derivatives.

dissociation of an alkoxyl unit from the reagent[11,12]. The weakly nucleophilic carboxylate moiety displaces an alkyl group from this reactive intermediate by an S_N2 mechanism producing the ester along with methanol and dimethylformamide. In contrast, esterification methods based on acid catalysis[13] or mixed anhydride formation[14] activate the carboxyl function of the analyte, promoting susceptibility to

TABLE I

GC–MS CHARACTERISTICS OF N-FORMYL METHYL ESTER DERIVATIVES OF SOME N-SUBSTITUTED AMINO ACIDS

Derivative	*Retention time (min)*	*Ions (relative intensity, %)*[a]					
		CI	*EI*				
		MH^+	M^+	$[M-28]^+$	$[M-59]^+$	$[M-87]^+$	*Other ions*
1b	2.2	160 (100)	159 (10)	131 (26)	100 (68)	72 (100)	130 (18), 86 (68)
2b	7.1	237 (100)	236 (29)	n.d.	177 (8)	149 (53)	207 (45), 175 (37), 163 (100), 121 (100)
3b	2.5	158 (100)	157 (10)	129 (13)	98 (100)	70 (75)	
4b	3.5	172 (100)	171 (100)	143 (6)	112 (79)	84 (32)	142 (66, 82 (84), 56 (94)
5b	3.4	172 (100)	171 (27)	143 (5)	112 (100)	84 (25)	56 (43)
6b	4.3	204 (83)	n.d.	n.d.	n.d.	n.d.	102 (100), 58 (32)

[a] n.d. = not detected.

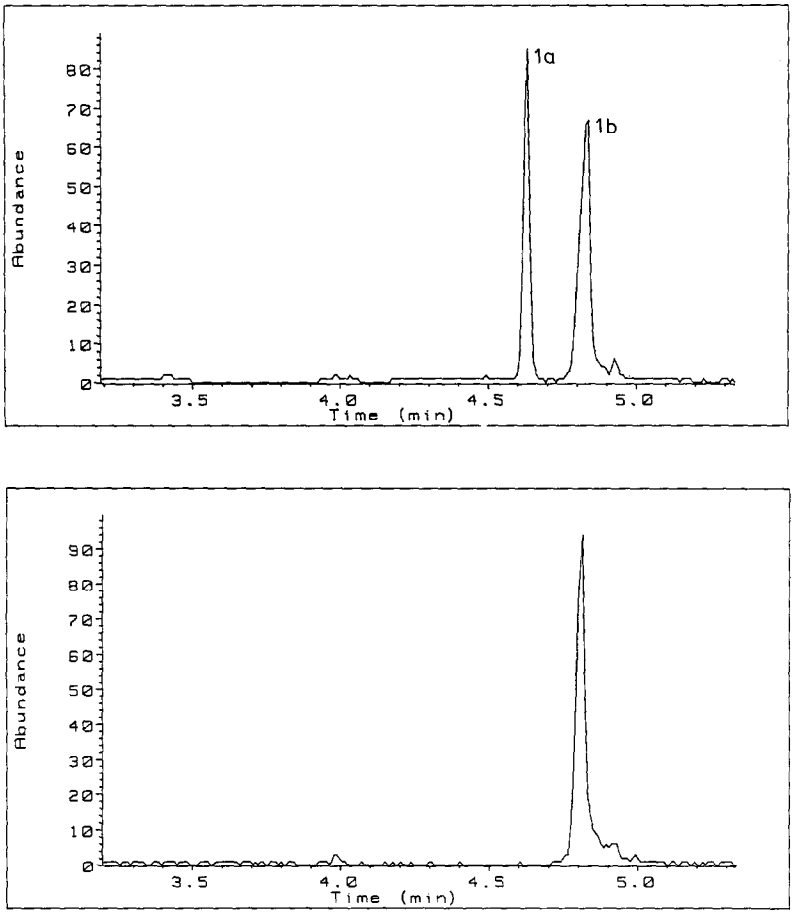
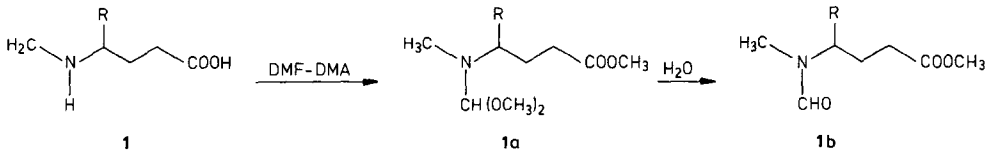


Fig. 2. Total ion current mass chromatograms of the products of the reaction of 4-methylaminobutanoic acid (10 mg) with DMF–DMA (100 μl) before (top) and after (bottom) addition of water (20 μl).

reaction with a wide variety of nucleophiles and thus leading to the formation of multiple products.

Derivatization of the secondary amine moiety with DMF–DMA was less straightforward. Dissolution of 4-methylaminobutanoic acid in DMF–DMA yielded two products on GC–MS analysis (Fig. 2), neither of which was the cyclized product, N-methyl-2-pyrrolidinone. The mass spectrum of the later eluting substance provided evidence for the assignment of an N-formyl methyl ester structure (**1b**) consistent with the properties of an N-formyl derivative previously identified for another secondary amine, desipramine[2,3]. The identity of the earlier eluting peak was assigned as the transamination product, methyl N-dimethoxymethyl-N-methyl-4-aminobutanoate (**1a**). Following addition of water to the reaction mixture, the first peak disappeared while the second peak increased in area (Fig. 2, bottom). This suggested that the N-formyl product was the result of the hydrolysis of the transaminated intermediate.

Consistent with the reactivity of DMF–DMA[11,12,15], Fig. 3 presents a proposed



1 1a 1b

Fig. 3. Formation of the N-formyl methyl ester derivative of 4-methylaminobutanoic acid.

reaction pathway for the derivatization of 4-methylaminobutanoic acid with DMF–DMA. In a study of the reaction of dimethylformamide dialkyl acetals with secondary amines, Wawer and Osek found that the predominant product was an amide acetal resulting from nucleophilic attack of the amine on the acetal carbon with elimination of dimethylamine[15]. The reaction between substituted anilines and various dimethylformamide dialkyl acetals followed second order kinetics[16].

The hydrolysis step in the formation of the N-formyl derivative from the amide acetal intermediate was further verified by an experiment in which ^{18}O-labeled water was used in the hydrolysis step. The observed molecular ion in the mass spectrum of the N-formyl methyl ester indicated the presence of one ^{18}O atom (Fig. 4). Since the formyl and ester oxygens do not exchange readily with water, and in that addition of $H_2{}^{18}O$ to DMF–DMA afforded [^{18}O]dimethylformamide, the most probable source of oxygen in the N-formyl grouping is water and not the reagent.

The ^{18}O-labeling experiment was also useful in assigning the identity of the EI mass spectral fragments. As was the case with the mass spectra of ester derivatives of primary amino acids[5], a characteristic $[M-59]^+$ fragment in the spectra of most of the secondary amino acid derivatives was observed (Table I). At first, this fragment was assigned as loss of a carbomethoxy radical from the methyl ester functionality. However, the mass spectrum of ^{18}O-labeled **1b** (Fig. 4) yielded $[M-30]^+$ and $[M-61]^+$ ions rather than the expected $[M-28]^+$ and $[M-59]^+$ ions, indicating that these fragments arise from cleavage at the ^{18}O-containing formyl terminus.

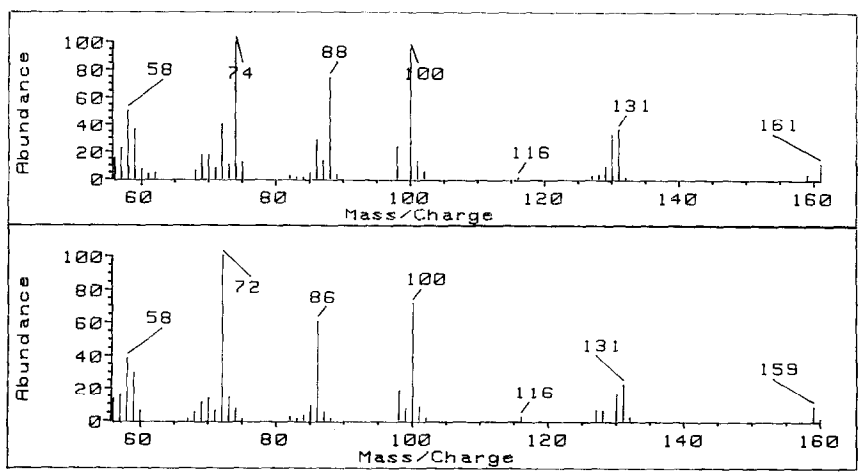


Fig. 4. Mass spectra of 4-methylaminobutanoic acid N-formyl methyl ester derivatives formed in the presence of $H_2{}^{18}O$ (top) and $H_2{}^{16}O$ (bottom).

TABLE II

GC–MS CHARACTERISTICS OF N-DIMETHOXYMETHYL METHYL ESTER INTERMEDIATES IN THE DERIVATIZATION OF SOME N-SUBSTITUTED AMINO ACIDS

Derivative	*Retention time (min)*	*Ions (relative intensity, %)*[a]			
		CI		*EI*	
		$[M-1]^+$	$[M-31]^+$	$[M-31]^+$	*Other ions*
1a	2.1	204 (7)	174 (100)	174 (34)	101 (36), 75 (100), 59 (38)
2a	6.9	–	–	n.d.	87 (61), 74 (100)
3a	2.0	202 (6)	172 (100)	172 (30)	75 (100)
4a	3.2	216 (6)	186 (100)	186 (76)	75 (100)
5a	3.0	216 (5)	186 (100)	186 (71)	75 (100)
6a	3.3	–	–	218 (27)	75 (80)

[a] n.d. = not detected; – = not determined.

Molecular ions were not recorded in EI mass spectra of the amide acetals, although there were strong $[M-31]^+$ peaks, accounted for by loss of a methoxy radical.

Methane positive ion CI mass spectra of the N-formyl methyl ester derivatives were characterized by abundant protonated molecular species (MH^+ ions). Ions of $[MH-32]^+$ appeared in the CI mass spectra of the amide acetals, consistent with loss of methanol[17] (Table II). Present also were $[M-1]^+$ ions of low abundance, accounted for as the resonance-stabilized acetal carbocations.

In summary, reaction between DMF–DMA and N-substituted amino acids yields, in the first step, methyl esters of unstable amide acetal intermediates. Addition of water to these intermediates results in the hydrolysis of the amide acetals yielding stable N-formyl metyl ester derivatives. The derivatization is easy to perform since it is a one-pot reaction with a single reagent. The product is stable to isolation procedures, such as solvent extraction, and GC analysis.

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