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FORMATION OF A NEW DERIVATIVE OF SECONDARY AMINES DURING TRIMETHYLSILYLATION WITH N,O-BIS(TRIMETHYLSILYL)-FLUOROACETAMIDE

N-(AMINOMETHYLENE)-2,2,2-TRIFLUOROACETAMIDES

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SUMMARY

Trimethylsilylation of secondary amines by the common reagent N,O-bis(trimethylsilyl)trifluoroacetamide (BSTFA) in the presence of dimethylformamide (DMF) produces the previously unknown N-(aminomethylene)-2,2,2-trifluoroacetamide (AMT) derivative, in addition to the trimethylsilyl derivative. This derivative is formed in varying yield by incorporation of CH atoms from DMF, and a CF_3CO group from BSTFA. The structure of the AMT moiety was characterized in the nucleic acid base queuine using stable isotopic labeling, high-resolution mass spectrometry, and ^{13}C and ^{19}F nuclear magnetic resonance spectroscopy. The AMT derivative has unexplored potential for use in gas chromatography and mass spectrometry but its formation may otherwise be undesirable, suggesting that the combined use of BSTFA and DMF be used with caution for silylation of compounds containing secondary amino groups.

INTRODUCTION

Trimethylsilylation is a widely employed derivatization procedure[1,2] used to reduce hydrogen bonding interactions and thus increase volatility for gas chromatography (GC) or mass spectrometry (MS). Of the numerous reagents which are available for this purpose, N,O-bis(trimethylsilyl)trifluoroacetamide[3] (BSTFA) in the presence of 1 % trimethylchlorosilane (TMCS) is one of the most efficient and powerful silyl donors. For silylation reactions, dimethylformamide (DMF) is often a suitable solvent because of its good solubilizing properties and high dielectric constant, which stabilizes the transition state complex formed during transfer of the silyl group. We report here the occurrence of an anomalous derivative formed during silylation of secondary amines. The reaction was first recognized during derivatization of the widely occurring transfer RNA base queuine (1)[4,5]. The unexpected product 2, formed in addition to the persilyl derivative 3, was characterized by MS and

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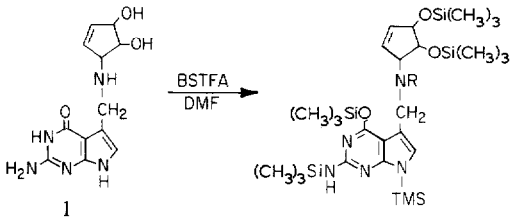


1

2 R = –CH=N–COCF$_3$
3 R = –Si(CH$_3$)$_3$
4 R = –H

nuclear magnetic resonance (NMR) spectroscopy. The side-chain in compound 2 was found to include one atom each of C and H from DMF, and the trifluoroacetyl moiety from BSTFA. A survey of other secondary amines showed the N-(aminomethylene)-2,2,2-trifluoroacetamide to be formed in five out of eight cases, indicating the general nature of the reaction.

Although the number of anomalous reactions reported using silylating reagents and DMF is small with respect to their overall usage, several cases have been documented, including condensation of DMF with primary amines during silylation[6] and reaction of DMF with the reagent hexamethyldisilazane during silylation of tertiary alcohols[7]; however, in the latter case the product was neither isolated nor identified. Markey[8] has reported that silylation of mesoxalic acid using BSTFA or N,O-bis(trimethylsilyl)acetamide (BSA) produces a secondary reaction product in which OH is replaced by CF_3CONH or CH_3CONH, in addition to the principal product resulting from simple silylation. Silylation of 7-methylpurine nucleosides, in which the base has the polar betaine structure, produces the 8-oxo derivative in high yield, by incorporation of oxygen dissolved in the reagent[9]. A silylation-mediated oxidation of dihydropyrimidines has been studied by Kelley *et al.*[10].

EXPERIMENTAL

Materials

BSTFA and BSA containing 1% TMCS were purchased from Pierce (Rockford, IL, U.S.A.). DMF and pyridine solvents were obtained from this and several other sources. [$^2H_7$]N,N-Dimethylformamide (99 atom% ^2H), N,N-dimethyl[^{13}C]formamide (90 atom% ^{13}C), and [^2H]$CHCl_3$ (99.8 atom% ^2H) were from Merck Isotopes (St. Louis, MO, U.S.A.), and N,O-bis([$^2H_9$]trimethylsilyl)trifluoroacetamide was from Regis (Morton Grove, IL, U.S.A.).

Queuine (compound 1, 2-amino-5-{[(4,5-dihydroxy-2-cyclopenten-1-yl)amino]methyl}-3,7-dihydro-4H-pyrrolo[2,3-*d*]pyrimidin-4-one) was isolated from bovine amniotic fluid and was a gift from Dr. J. R. Katze, University of Tennessee Health Sciences Center.

OV-17 (1%) on 100–120 mesh Gas-Chrom Q was purchased from Applied Science Labs. (State College, PA, U.S.A.). Vapor-deposited AgBr photographic plates for MS were a product of Ionomet (Brighton, MA, U.S.A.).

Trimethylsilylation

Queuine (5–10 μg) in methanol was transferred to a melting point capillary and dried *in vacuo*. Dry DMF (1 μl) was added, followed by 9 μl of BSTFA with 1%

TMCS. The tube was sealed, then heated for 1 h at 100°C. An analogous procedure was used in experiments involving other reagents such as BSA, [$^2H_{18}$]BSTFA or pyridine, and on a larger scale for derivatization of other secondary amines (50–100 μg) or queuine used for NMR studies (2 mg).

Gas chromatography

Measurements were made with a Varian 2100 gas chromatograph with 3 ft. × 0.25 in. O.D. × 2 mm I.D. silanized glass columns with 1 % OV-17 on Gas-Chrom Q. The helium flow-rate was 40 ml/min, with injector and flame ionization detector temperatures of 280°C. The column was temperature programmed at 10°/min from 200 to 280°C.

Mass spectrometry

GC–MS studies were carried out using an LKB 9000S instrument interfaced to a PDP-11/40 data system, under the following conditions: ionizing energy 70 eV, ion source and separator temperatures 270°C. High-resolution mass spectra were acquired using a Varian MAT 371 instrument. Samples were introduced by direct probe and the spectra were photographically recorded at resolution 15,000. Photographic plates were processed with a Gaertner M1205PC comparator interfaced to a Varian SS100C data system.

Nuclear magnetic resonance spectroscopy

Derivatized queuine was dried under vacuum and the products dissolved in an appropriate solvent. Fourier transform (FT) NMR spectra were recorded at a probe temperature of 30°C using 5-mm sample tubes.

^{19}F NMR spectra were recorded using a Varian XL-100 instrument operating at 94.1 MHz, using [$^2H_7$]DMF solvent and CCl_3F internal standard. ^{19}F chemical shifts are reported relative to CCl_3F as 0.0 ppm. Positive values indicate upfield shifts (more shielding) with CCl_3F being the low field end. A control solution was also examined, consisting of DMF–BSTFA–TMCS (10:90:1) heated (without queuine) as described above.

^{13}C NMR spectra were acquired at 75.5 MHz using a Varian SC-300 spectrometer, with ^1H decoupling. For ^{13}C NMR measurements, derivatization was carried out as described in the previous section, but using [1-^{13}C]DMF, following solution in [^{2}H]$CHCl_3$. Chemical shifts are reported relative to tetramethylsilane as 0.0 ppm.

RESULTS AND DISCUSSION

Characterization of the derivative from the natural base queuine

Trimethylsilylation of queuine under the conditions employed produced two derivatives, as shown by GC of the products (Fig. 1). The earlier eluting peak is the hexasilyl derivative 3, as determined by its mass spectrum. The later eluting peak is a derivative of queuine as indicated by the *m/z* 379 ion in its mass spectrum (Fig. 2), which characteristically[4,5,11] represents the 7-deazaguanine portion of the molecule (see following discussion). The exact molecular weight was measured by high-resolution MS as 760.3079. The even molecular weight value of 2 compared with the odd

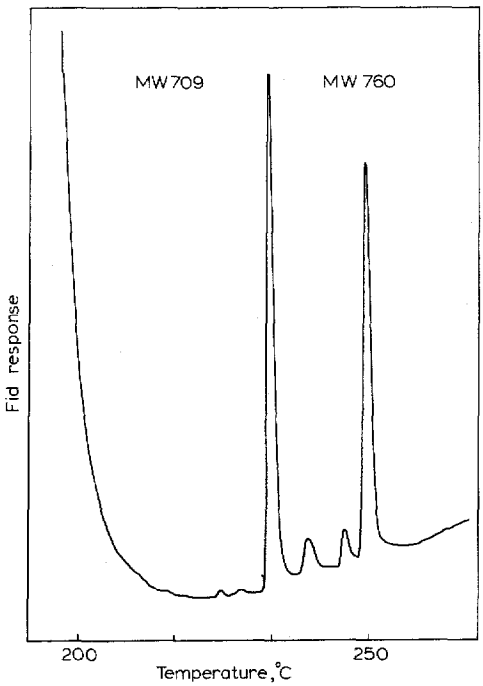
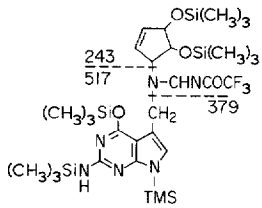


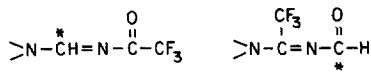
Fig. 1. Gas chromatogram of the reaction products from trimethylsilylation of queuine by BSTFA in DMF.

value of 3 (709) requires that an odd number of nitrogen atoms be gained or lost from compound 1 during silylation. That the second component is not an impurity was further suggested by its failure to form when either pyridine or BSA was used. In the latter experiments only 3 and the lower derivative 4 (mol.wt. 637) were observed. Silylation using $[^2H_{18}]$BSTFA and $[^2H_9]$TMCS in DMF resulted in a molecular ion shift of 45 mass units, indicating the presence of five silyl groups in 2. After application of the nitrogen rule and other constraints such as numbers of rings and double bonds[12], no satisfactory computer-derived elemental compositions were obtained from the exact molecular mass using the elements C, H, N, O, S, P, and Si. Based on recognition of the requirements for BSTFA, fluorine was added to the element list. A single possible composition resulted: $C_{30}H_{55}N_6O_4Si_5F_3$ (calc. 760.3081). After subtraction of compositional elements of queuine and $[Si(CH_3)_3]_5$, the additional elements added during derivatization were determined to be C_3HNOF_3.

The site of modification in 2 was readily established by comparison of its mass spectrum with the previously reported spectrum[4] of the persilyl derivative 3. Presence of the characteristic *m/z* 379 ion[5] indicates no modification in the 7-deazaguanine portion of the molecule while *m/z* 243 likewise indicates that no structural change has occurred in the cyclopentenediol moiety (structure 5). These assignments point to substitution of C_3HNOF_3 at the secondary amino group, a conclusion which is corroborated by the major ion 517.1847 ($C_{19}H_{32}N_6O_2Si_3F_3$) due to loss of the cyclopentenediol ring. A detailed analysis of the mass spectrum of 2 is presented elsewhere[13].



The role of DMF was investigated by use of the isotopically labeled solvents [$^2H_7$]N,N-dimethylformamide and N,N-dimethyl[^{13}C]formamide. In both cases the molecular mass shifted from 760 to 761, and all side-chain containing ions shifted one mass unit, indicating the incorporation of a hydrogen atom and a carbonyl carbon of DMF during silylation. As a result of these experiments, and with the knowledge that a portion of BSTFA is incorporated, side-chain structures 6 and 7 were considered possible.



6 7

*carbon from DMF

The ^{13}C NMR spectrum of the ^{13}C-enriched product showed two peaks at 163.8 ppm (*s*, 75%) and 161.7 ppm (*s*, 25%). The ^{19}F NMR spectrum exhibited resonances at 75.06 ppm (*s*, 75%) and 75.17 ppm (*d*, 25%, J_{HF} = 2.6 Hz), which are distinguished from peaks which could result from residual BSTFA, measured at 73.37 (*s*) and 77.77 (*s*) ppm in the BSTFA blank. The major peak at 75.06 ppm is as expected for a CF_3CO group attached to nitrogen as in 6 (refs. 14, 15). The CF_3 group in 7 is more deshielded and would appear downfield from the found values[16,17]. The data therefore support structure 6, and therefore structure 2*.

The presence of two resonances in both ^{19}F- and ^{13}C-NMR spectra are attributed to *syn*- and *anti*-isomerism about the carbon–nitrogen double bond, which has been extensively studied by NMR techniques[18,19]. In the ^{13}C NMR spectrum the minor isomer a steric compression shift of 2.1 ppm upfield; because steric compression of this carbon could occur only in the *syn*-isomer, we accordingly assign the *syn* structure to the minor isomer. This is supported by the ^{19}F data, in which the minor resonance is shifted upfield (0.11 ppm). Due to close spatial proximity of the side-chain hydrogen to the CF_3 group in this isomer, they interact with J_{HF} = 2.6 Hz. This is an example of long-range "through space" coupling[20,21].

anti (75%)
^{13}C 163.8 ppm
^{19}F 75.06 ppm

syn (25%)
^{13}C 161.7 ppm
^{19}F 75.17 ppm

* N-{[[4,5-Bis(trimethylsiloxy)-2-cyclopenten-1-yl][[4-(trimethylsiloxy)-7-(trimethylsilyl)-2-[(trimethylsilyl)amino]-7H-pyrrolo[2,3-*d*]pyrimidin-5-yl]methyl]amino]methylene}-2,2,2-trifluoroacetamide.

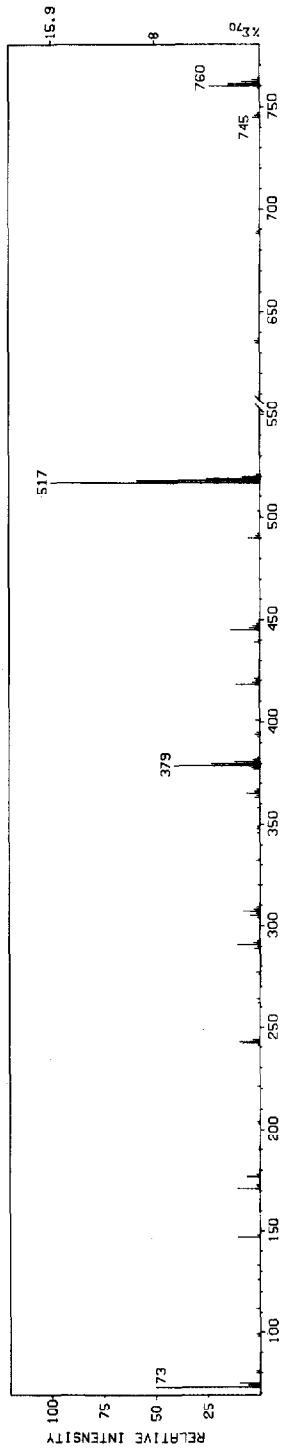
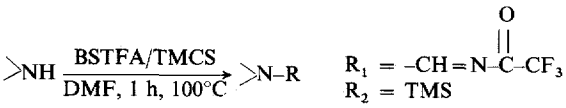


Fig. 2. Mass spectrum of the later eluting product (mol.wt. 760) shown in Fig. 1.

TABLE I

REACTION OF SECONDARY AMINES WITH BSTFA AND DMF



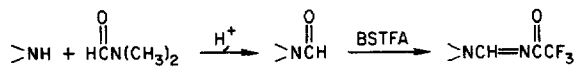
Compound	R_1			R_2			*Relative yield* R_1/R_2
	m/z		*Relative intensity*	*m/z*		*Relative intensity*	
	M	*M − 15*	*M/M − 15*	*M*	*M − 15*	*M/M − 15*	
N,N-Diisopropylamine	224	*	–	*	158	–	1.5
N,N-Diphenylamine	292	*	–	241	226	0.5	0.3
N-Methylaniline	230	*	–	179	164	0.75	0.1
N^6-Methyladenine	344**	329	4.0	293	278	0.15	0.5
N^6-Isopentenyladenosine	674***	659	3.4	623	608	3.5	4.0
Adenine	*	*	–	279	264	0.3	–
Imidazole	*	*	–	140	125	1.3	–
N^6-*p*-Toluidineadenine	*	*	–	297	282	5.0	–

* Not observed.
** One TMS and one AMT group.
*** Three TMS and one AMT group.

Formation of the AMT derivative from other secondary amines

The generality of the reaction between BSTFA, DMF and secondary amines was qualitatively examined, using the eight compounds listed in Table I. No attempt was made in the present study to optimize reaction conditions or quantitatively measure the reaction yields. The ratios of AMT and TMS derivatives were estimated from GC peak heights. No reaction with primary amines was observed. Identities of reaction products from secondary amines were confirmed by GC–MS. Molecular ions (M) and M − 15 ions were characteristically[22] observed for all derivatives containing TMS groups, while M of the appropriate mass values were formed by AMT derivatives. Five of the eight amines studied formed AMT derivatives, while all formed TMS derivatives. The cyclic amines adenine and imidazole, and N^6-*p*-toluidineadenine, produced no AMT product, but significant amounts were produced from the remaining compounds, showing the general nature of the reaction.

The initial observation of AMT formation with the polyfunctional base queuine led to consideration that the *cis*-diol function was required, perhaps by reaction with DMF to form an intermediate cyclic acetal[23]. This interpretation is excluded by the findings shown in Table I, and also because the AMT derivative was formed after blocking the *cis*-diol by an isopropylidene group. It is worthwhile to speculate that DMF is involved in the initial reaction step as suggested by the arrangement of atoms in compound 2. In general, the carbonyl carbon of DMF is insufficiently electrophilic for attack by secondary amines. However, protonation of DMF during silylation may render DMF sufficiently electrophilic for reaction:



Previously it has been shown that dimethylformamide dialkylacetal reacts with secondary amines to produce an N-formyl derivative[24], which is the postulated intermediate in the reaction above.

The analytical utility of the AMT derivative remains largely unexplored, but it may be useful for GC–MS or mass spectrometry alone. We are currently developing a GC–MS assay for queuine, based on the AMT derivative, which affords a molecular ion approximately ten times more abundant than that of the persilyl derivative[4].

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