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THE ACETYLATION OF 2-METHYL-Δ^2-THIAZOLINE—II

FORMATION AND PROPERTIES OF 2-METHYLENE-3-ACETYLTHIAZOLIDINE*[1,2]

L. V. GROBOVSKY and G. L. SCHMIR

Department of Molecular Biophysics and Biochemistry, Yale University School of Medicine, New Haven, Connecticut 06510

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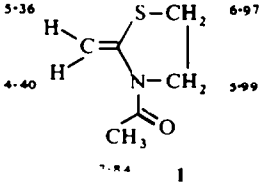
Abstract—The N-acylated ketene-S,N-acetal **1**, postulated to be an intermediate in the acetylation of 2-methyl-Δ^2-thiazoline, has been detected, and characterized by NMR and mass spectra. Evidence has been obtained that the acid-catalyzed hydrolysis of **1** to N,S-diacetylcysteamine involves the transient formation of an intermediate, possibly N,N-diacetylcysteamine.

THE reaction of 2-methyl-Δ^2-thiazoline with acetyl chloride in anhydrous acetonitrile produces dimeric and trimeric compounds; in the presence of small amounts of water, there is rapidly formed a high yield of N,S-diacetylcysteamine.[2] It was supposed that all these products arose from a reactive N-acetylthiazolinium ion, the former *via* selfcondensation reactions, the latter through hydrolytic ring cleavage. No volatile products other than diacetylcysteamine were detected in significant quantities.

In contrast, treatment of 2-methyl-Δ^2-thiazoline with acetic anhydride in 98% acetonitrile-water led to the formation and subsequent disappearance of an unknown volatile material (Fig 1). Diacetylcysteamine, absent after short reaction times, appeared slowly over the course of several hours. The unknown product could be collected in small amounts from the effluent stream of the gas chromatograph, but proved too unstable for isolation and purification on a large scale. Purified samples were generally obtained as dilute solutions in acetonitrile. In what follows, evidence is provided for the assignment of the ketene-S,N-acetal structure **1** to this transient product of the acetylation reaction.



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Spectral properties. The mass spectrum of **1** (Fig 2A) showed an abundant ion at *m/e* 143, presumably the molecular ion of a compound C_6H_9NOS derived from one molecule each of 2-methylthiazoline and acetic anhydride, with loss of acetic acid. Several prominent ions are consistent with the expected fragmentation pattern of **1**. The well-known[3] loss of ketene from acetamido substituents provides the abundant ion at *m/e* 101, a pathway supported by the observation of a metastable ion at *m/e* 71·3

* This work is taken from a dissertation presented by L.V.G. in partial fulfilment of the requirements for the Ph.D. degree, Yale University, 1967.

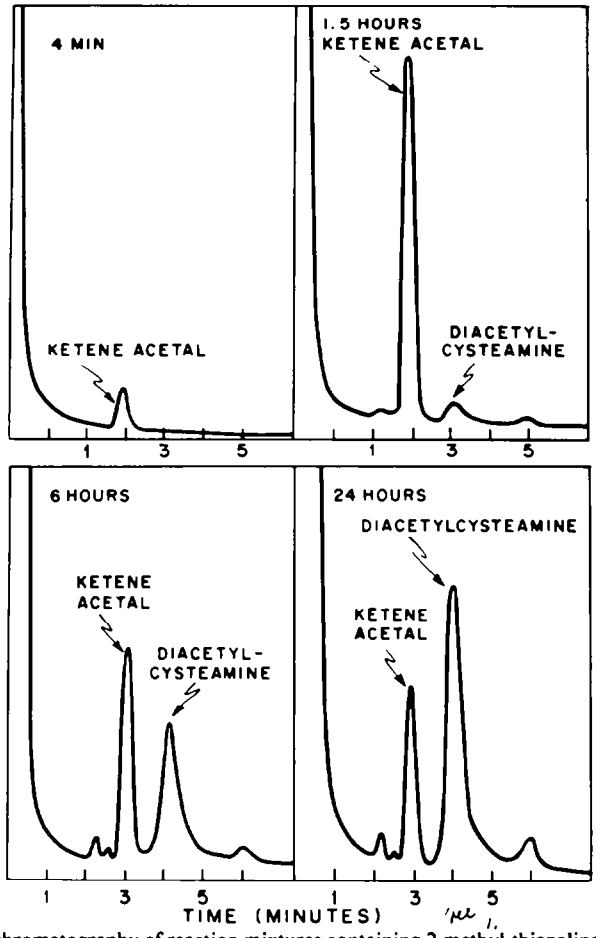
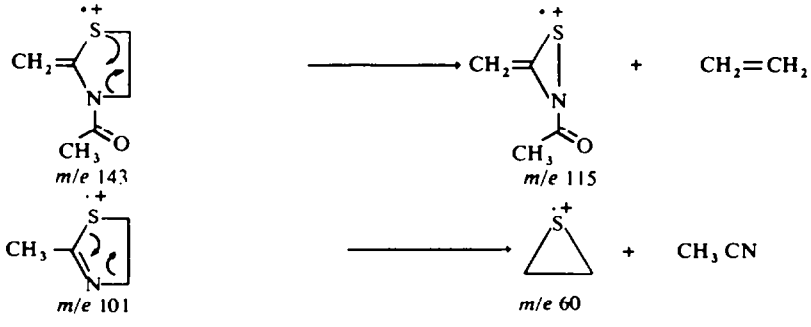


FIG. 1. Gas chromatography of reaction mixtures containing 2-methyl-thiazoline (0·6M) and acetic anhydride (0·3M) in 98% acetonitrile-water, room temperature. Reaction times are shown.

(calcd. for *m/e* 143 → *m/e* 101; *m/e* 71·3). Alternatively, the acetyl substituent leaves as the acetylium ion CH_3CO^+ (*m/e* 43). Metastable ions were also found at *m/e* 92·5 (calcd. for *m/e* 143→*m/e* 115; *m/e* 92·6) and *m/e* 35·7 (calcd. for *m/e* 101→*m/e* 60; *m/e* 35·6). Both processes appear to represent examples of the "cross-ring" cleavage previously reported for closely related 5-membered heterocycles.[3c, 4]



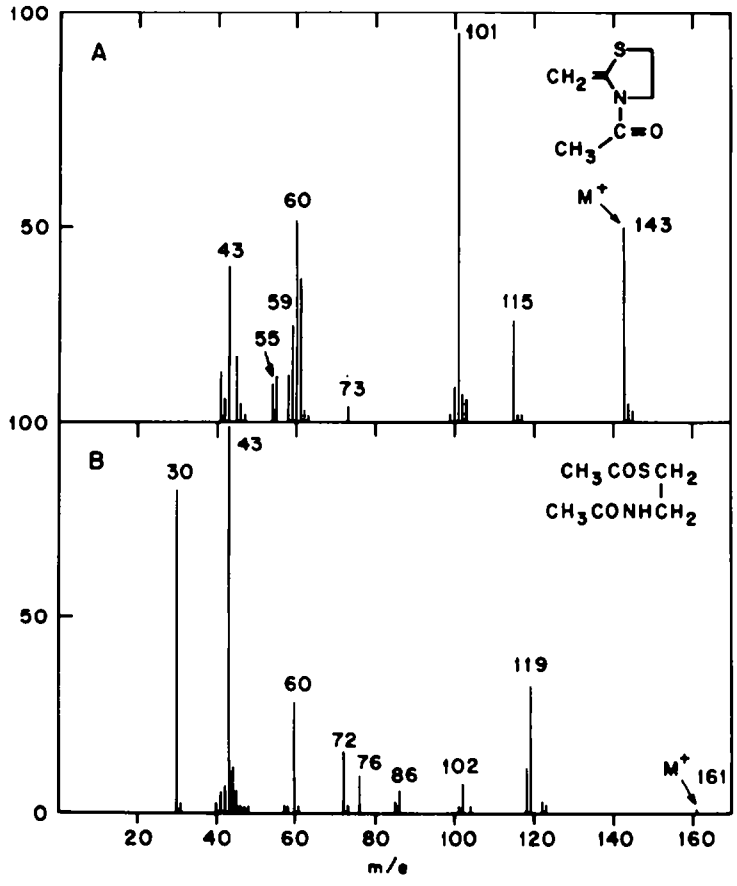
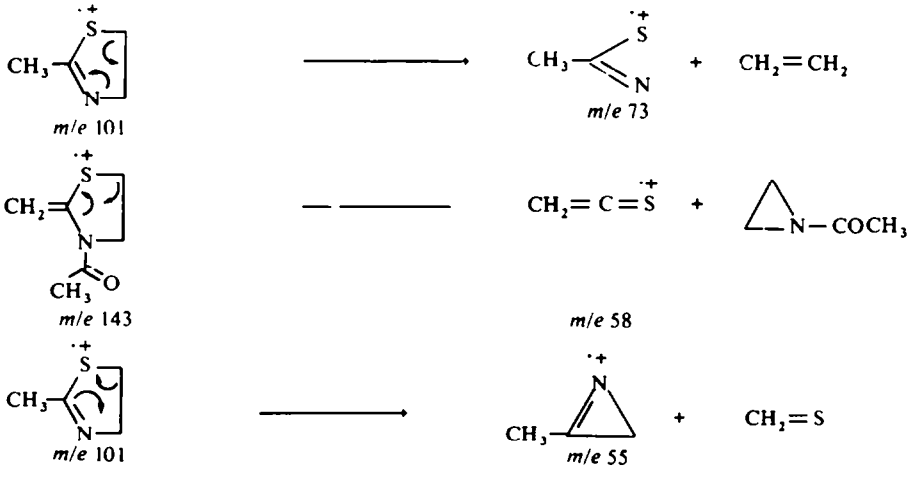


FIG. 2. A. Mass spectrum of 2-methylene-3-acetylthiazolidine.
B. Mass spectrum of N,S-diacetylcysteamine.

Although not supported by corroborative evidence, plausible "cross-ring" cleavages may be suggested for the genesis of the important ions at *m/e* 73. 58 and 55.



Owing to the lack of suitable model compounds, the UV spectrum (λ_{max} 248mμ, ε_{max} *ca* 5000 in CH_3CN) offered little structural information. The NMR spectrum (Fig 3), however, disclosed the presence of the acetamido methyl group (7·86τ), two adjacent methylene groups (triplets centered at 6·97 and 5·99τ, J_{AB} *ca* 8 c/s)*, and two non-equivalent spin-coupled methine protons (5·36 and 4·40τ, $J < 3$ c/s).[*a] The position of the latter signals may be compared to the chemical shifts in the range of 4·7–6·0τ reported for analogous protons in acyclic ketene-S,N-acetals.[7]

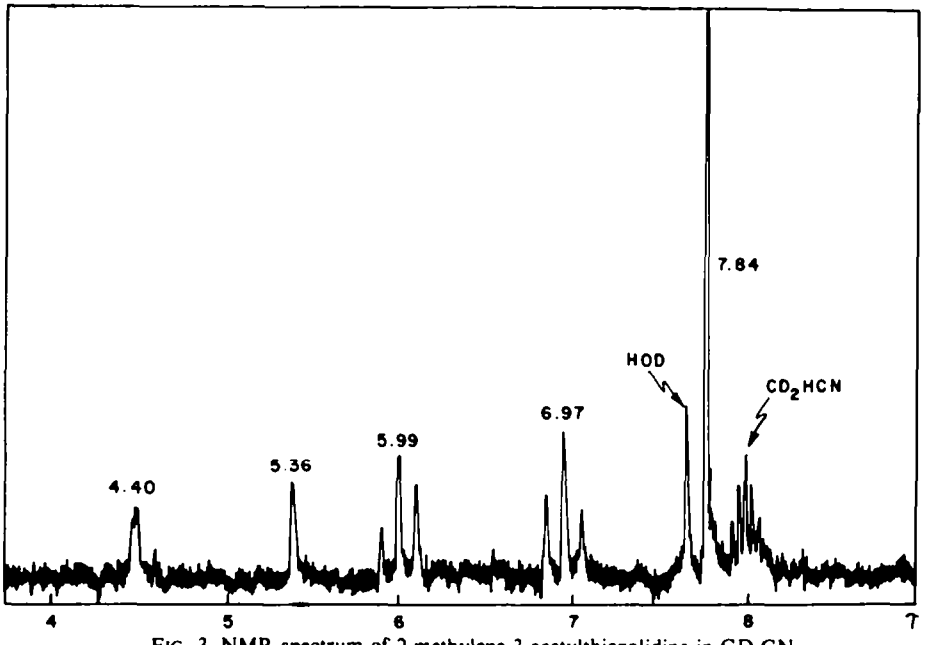


FIG. 3. NMR spectrum of 2-methylene-3-acetylthiazolidine in CD_3CN.

Hydrolysis. The instability of **1** was noted early when attempts at its purification by conventional methods led to its ready transformation to gummy non-volatile materials or to N,S-diacetylcysteamine. On evaporation under reduced pressure of dilute solutions of **1** in acetonitrile, followed immediately by VPC analysis of the oily residue, it was noted that less than 10% of the original compound remained. Exposure to 0·2M acetic acid in acetonitrile resulted in total destruction of the ketene acetal in less than one min. The suspected facile polymerization of **1** recalls the experience of McElvain and Curry[8] with the ketone-O,O-acetal 2-methylene-1,3-dioxolane.

Fragmentary observations were made on the rates of the first-order, acid-catalyzed disappearance of the ketene acetal in 20% acetonitrile-water at 25° (Table 1). At lower pH, hydrolysis was too rapid to be followed. At all values of pH in the range 3·0–7·9, the ketone acetal is *eventually* quantitatively converted to N,S-diacetylcysteamine.[*b] Under some conditions, however, there occurs the accumulation of an intermediate, evidence for which is given next.

* The methylene protons of 2-methylthiazoline resonate at 6·77 and 5·88τ with J_{AB} *ca* 8 c/s.[5]

[*a] The small size of the coupling constant of the methine protons of **1** is characteristic of many geminal protons of the type $CH_2=C$.[6]

[*b] The assumptions on which are based the calculations of the yield of diacetylcysteamine are presented in the Experimental.

TABLE 1. HYDROLYSIS OF THE KETENE ACETAL 1[a]

pH	Buffer	Buffer conc., M	$k \times 10^2$,[b] min^{-1}
5·25	Acetate	0·22	443
6·15	Phosphate	0·20	94·4
7·18	Phosphate	0·20	17·4
7·90	Phosphate	0·17	3·75

[a] In 20% CH_3CN-H_2O, 25°, 1 at 2×10^{-4} M.
[b] First order rate constant for disappearance of 1 calculated from decrease in absorbance at 260 mμ.

Complete UV spectra of solutions of 1 undergoing hydrolysis (Fig 4) showed that, at pH ⩽ 6·2, the rate of disappearance of ketene acetal (decrease in ε_{260}) was significantly faster than the rate of formation of diacetylcysteamine (increase in ε_{235}). At room temperature, the half-life of 1 is about 1 min at 6·15 and 0·1 second* at pH3; half-lives of appearance of diacetylcysteamine are *ca* 6 min and 22 min, respectively. The presence of a clear isosbestic point (*ca* 229mμ) at pH3 is consistent with the rapid formation of an intermediate, slowly converted to N,S-diacetylcysteamine. At pH 6·15, the rates of the two consecutive processes are much less different, and the isosbestic point is seen only in late stages of the reaction. Presumably, at pH ⩾ 8, conversion of the intermediate to final product would occur faster than the relatively slow, acid-catalysed disappearance of 1. At pH 3·0–7·9, UV spectra taken on completion of all spectral change indicated quantitative hydrolysis of 1 to N,S-diacetylcysteamine. In contrast to the slow formation of product in largely aqueous solution, when hydrolysis was carried out in 99·5% CH_3CN—H_2O with $1·6 \times 10^{-4}$M HCl, the first spectrum (1 min) showed an evanescent maximum at 290mμ (N-acetyl-2-methylthiazolinium ion?); formation of N,S-diacetylcysteamine was completed in < 2 min.

The kinetics of the hydrolysis of 1 were also followed by VPC assay for 1 and diacetylcysteamine. At pH 7·9, disappearance of ketene acetal paralleled appearance of diacetylcysteamine, both processes occurring with $t_{\frac{1}{2}}$ 24 min, in reasonable agreement with the rate of decrease of absorbance at 260mμ (Table 1). The rate of reactions at pH 2·0–5·25 was too rapid for VPC assay, all ketene acetal having disappeared at the time of the first sample injection (45 sec). For all reactions at pH 2·0–7·9, the yield of diacetylcysteamine was nearly quantitative, varying randomly in the range 85–99%. Interestingly, no significant time lag in product formation was seen, even at pH ⩽ 3.

That N,S-diacetylcysteamine was the final product of hydrolysis of 1 was established in several ways: (a) by comparison of VPC retention times to that of authentic material; (b) final UV spectra of reaction mixtures; (c) hydrolysis of 1 in 17% CH_3CN—0·001N HCl (2 hr) followed by combined VPC-mass spectrometry of the reaction product,[*a] (d) the NMR spectrum of the product of hydrolysis of 1 in 17% CD_3CN—0·001N DCl (1

* Too fast to measure: extrapolated from data of Table 1.
[*a] The mass spectrum of authentic N,S-diacetylcysteamine (Fig 2B) exhibits a weak molecular ion at *m/e* 161 (relative intensity 0·3). The abundant fragments at *m/e* 119 and *m/e* 102 may have arisen from the molecular ion by loss of ketene and acetamide, respectively. Ions at *m/e* 43 and *m/e* 30 probably consist of CH_3CO^+ and $CH_2{=}NH_2^+$, respectively. [3a]

hr) was identical to that of authentic material; integration of the singlets corresponding to the Me groups of N,S-diacetylcysteamine showed equal areas ($2{\cdot}8 \pm 0{\cdot}2$ protons), a fact discussed in the sequel.

The reaction of 2-methylthiazoline with acetic anhydride

Some aspects of the overall acetylation reaction are recorded here.[*b] The time course of the reaction in 99·5% and 98% MeCN—H_2O (e.g. Fig 1) suggests a precursor-product relationship between the ketene acetal and N,S-diacetylcysteamine, although detailed kinetic studies were not carried out. In 98% MeCN—H_2O, with the reactants at $0{\cdot}3M$, the maximum accumulation of **1** (30% yield) occurs after 2 hr. VPC coupled directly with mass spectrometry was employed to identify the intermediate and final products of reactions carried out in 99·5 (2 hr), 98 (6 hr) and 50% (15 min) MeCN—H_2O. The mass spectra of chromatographic fractions corresponding to **1** and N,S-diacetylcysteamine were identical to those of isolated ketene acetal and diacetylcysteamine, respectively. Diacetylcysteamine was also isolated by VPC from a reaction in 95% MeCN and identified by IR[2] and UV spectra(λ_{max} 231mμ, ε_{max} 4750 in 95% ethanol-water).

Some kinetic data on the rates of reaction of acetic anhydride with 2-methylthiazoline and water are provided in Table 2. The marked rate increase of the aminolysis reaction in 50% MeCN—H_2O is consistent with the expected effect of increasing dielectric constant on a reaction leading to a charged transition state. Similarly, the effect of increasing water content on the rates of hydrolysis of acetic anhydride parallels the observations of Bunton, *et al.*,[9] for the hydrolysis of acid anhydrides in dioxane-water mixtures.

The final yield of N,S-diacetylcysteamine is not particularly dependent on water concentration, except in solvents containing less than 5% water (Table 3). Low yields in predominantly non-aqueous solvent may reflect competition between hydration and polymerization of the ketene acetal. It is noteworthy that high yields of products are obtained in pure water; in contrast, when acetyl chloride was used, the yield of diacetylcysteamine fell dramatically once water content reached 10% or more.[2] Presumably, the highly reactive acid chloride discriminates much less between the competing nucleophiles (water and 2-methylthiazoline) than does the less reactive, more selective, acid anhydride.

DISCUSSION

The reaction mechanism (Scheme 1) previously advanced[2] to account for the products of the reaction of 2-methylthiazoline with acetyl chloride is strengthened by the isolation and partial characterization of the proposed intermediate **1** in the present study. Although simple ketene-S,N-acetals have become available in recent years,[7] few examples of their N-acyl derivatives have been reported,[10] and little is known of their chemistry. Equally few instances of N-acylated ketene-O,N-acetals seem to exist in the literature.[11]

[*b] Additional details, particularly progress curves describing the changes in relative concentration of reactants and products with time may be found in the Ph.D. dissertation of L.V.G., available from University Microfilms, Ann Arbor, Michigan, Order No. 68-4873.

TABLE 2. REACTION OF ACETIC ANHYDRIDE WITH 2-METHYLTHIAZOLINE AND WATER[a]

Solvent MeCN-H_2O, %	Acetic anhydride M	Thiazoline M	k, M^{-1} min^{-1} [b]
100	0·3	0·3	$10·7 \times 10^{-3}$
98	0·3	0·3	$13·1 \times 10^{-3}$
98	0·3	·6	$12·6 \times 10^{-3}$
50	0·3	0·3	*ca* 1·5[c]

Solvent MeCN-H_2O, %	Acetic anhydride M	$k \times 10^3$, min^{-1} UV[d]	VPC[b]
90	0·24	*ca* 0·1[e]	
70	0·32	1·5	1·5
50	0·32	8·9	8·9
30	0·32	21·4	21·4
1	0·32	120	
1	0·004	120	

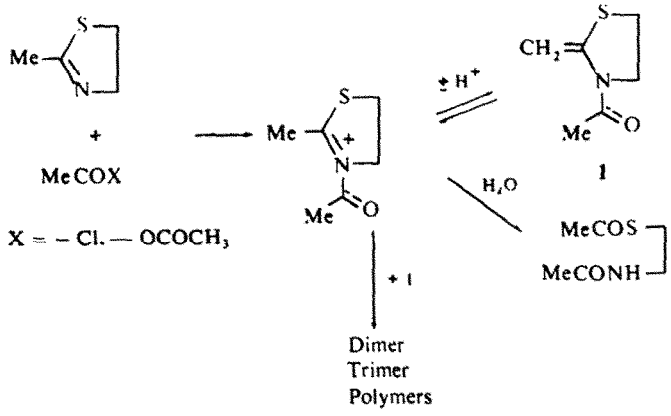
[a] At 25°
[b] Rate of disappearance of acetic anhydride measured by VPC.
[c] Reaction too rapid for accurate determination of rate constant
[d] Rate of decrease of absorbance measured at 270 mμ, except for ·004M, where 230 mμ was used.
[e] First order plot linear for first half-life only.

TABLE 3. SOLVENT EFFECTS ON YIELD OF N,S-DIACETYLCYSTEAMINE[a]

Solvent MeCN-H_2O, %	Time (hr)	Yield[c]	Time[b] (hr)	Yield[c]
98	5	17	30	34
95	8	25	48	50
90	4	38	48	75
80	6	40	36	81
70	2	45	24	91
60	1	46	7	92
50	0·3	43	5	85
40	0·4	46	6	92
30	0·75	40	6	79
20	0·15	38	2·3	75
10	0·15	40	2·3	79
0	0·10	38	1·3	75

[a] Room temperature; 2-methylthiazoline at 0·6 M; acetic anhydride at 0·3 M.
[b] No increase in yield noted after this time.
[c] By VPC assay, and based on acetic anhydride.

SCHEME 1



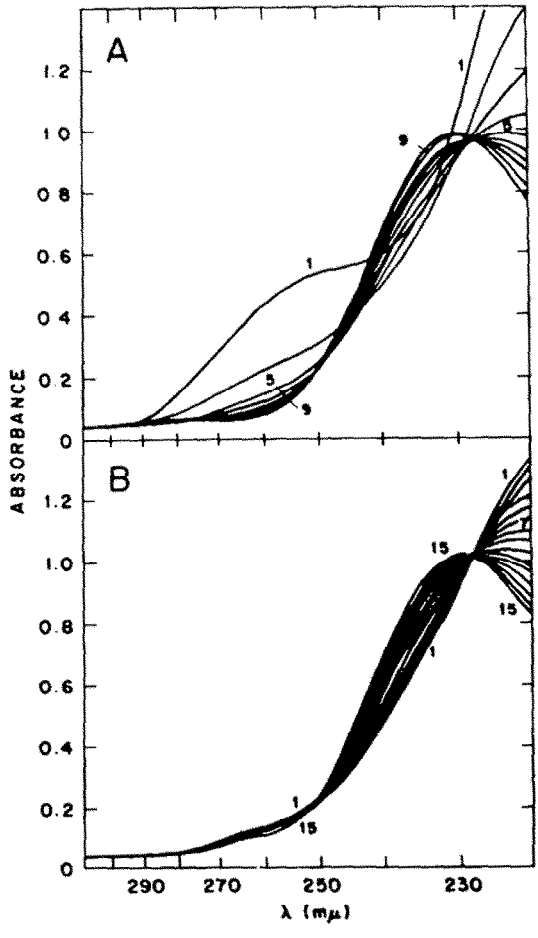
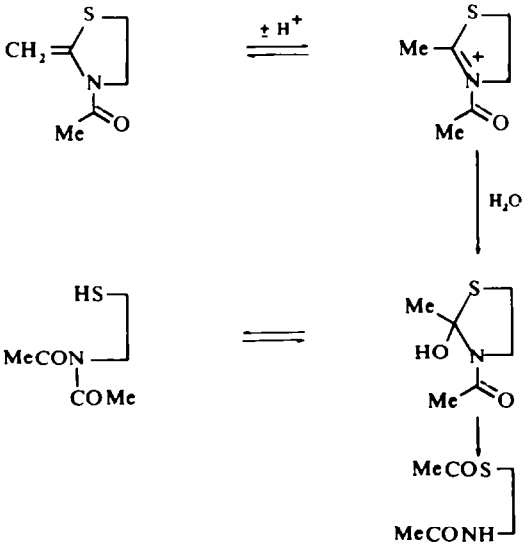


FIG. 4. Hydrolysis of 1 in 20% CH_3CN—H_2O, room temperature; 1 at 2×10^{-4}M. A, pH 6·15 (0·2M phosphate buffer): (1) after 1 min; (5) after 6 min; (9) after 18 min. B, pH 3 (HCl): (1) after 1 min; (7) after 20 min; (15) after 80 min.

The major difference in the course of the reaction of 2-methylthiazoline with acetyl chloride as compared to that with acetic anhydride consists of the accumulation of **1** when acetylation is performed with acetic anhydride in solvents of low water content (0·5–2% H_2O). It seems reasonable to suggest that, under the latter conditions, the kinetically favored product is **1**, arising in a reversible reaction from the initially formed N-acetylthiazolinium ion by proton abstraction. The accumulation of the ketene acetal may be favored by the relatively strongly basic acetate ion, absent when acetylation is performed with acetyl chloride. According to this hypothesis, the thermodynamically stable product is N,S-diacetylcysteamine, to which the ketene acetal is eventually largely transformed despite the competition of polymerization reactions under some conditions. The relatively high acidity of the exocyclic 2-methyl group of the intermediate cation is suggested by the readiness with which analogous protons are exchanged for deuterium in cationic thioimidate and N-acylimidate esters.[12] With the closely related 2,3-dimethylbenzthiazolium ion, evidence has been adduced that alkaline treatment results in the reversible formation of "methylene bases" (and derived dimers) analogous to **1**, which are slowly transformed to the product of hydrolytic ring cleavage.[13]

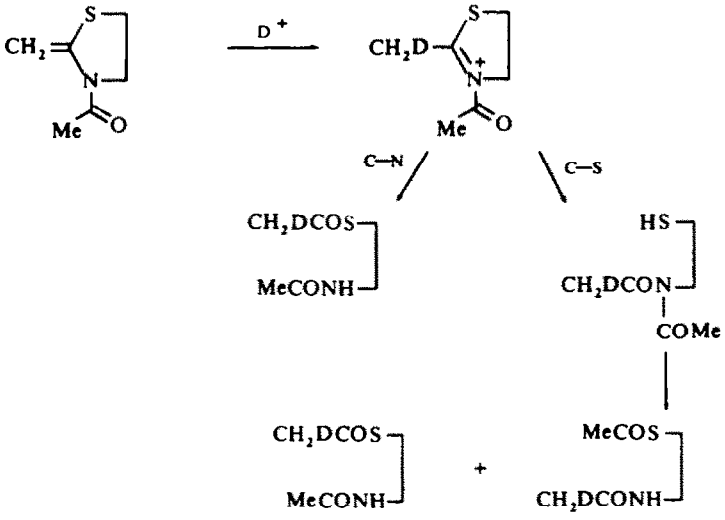
The pH-dependence of the hydrolysis of **1** (Table 1) indicates that this reaction is susceptible to acid catalysis, an observation consistent with the well-established specific and general acid catalysis of the hydrolyses of enamines[14] and ketene-O,O-acetals.[15] Despite the preliminary nature of the present study of the hydrolysis of **1**, the available data suggest some tentative conclusions concerning the detailed mechanism of the ring-opening reaction leading from the N-acetylthiazolinium cation to N,S-diacetylcysteamine. It is clear (Fig 4) that at least one intermediate accumulates in the course of the hydrolytic process. A plausible structure for this intermediate is that of N,N-diacetylcysteamine, the product of C—S bond cleavage in an initially formed carbinolamine (Scheme 2).

SCHEME 2



Rearrangement (possibly base-catalysed) of N,N- to N,S-diacetylcysteamine may then take place to yield the observed product. The following lines of evidence support this hypothesis; (a) the non-specific end absorption in the UV spectrum of the intermediate (Fig 4) is in accord with its proposed structure; (b) hydrolysis in the presence of D_2O yield N,S-diacetylcysteamine possessing equal amounts of hydrogen in its two Me groups. The stoichiometry of the hydrolysis of **1** requires the introduction of at least one deuterium atom in the product, even if no rapid exchange reactions occur with the Me protons of the N-acetylthiazolinium ion. The consequences of two possible ring-opening mechanisms are summarized in Scheme 3; clearly, product formation *via* direct C—N bond cleavage should result in methyl signals in the NMR apectrum with relative areas

SCHEME 3



of 2 : 3. The experimental result is entirely consistent with the randomization of isotope predicted (if secondary isotope effects are small) by the pathway involving initial C—S bond cleavage followed by the equally probable migration of the acetyl or deuterioacetyl group. Finally, the apparent absence of a lag in the formation of N,S-diacetylcysteamine when the hydrolysis is followed by VPC could result either from lack of resolution of the isomeric diacetylcysteamines or from thermally induced N→S acetyl transfer.

The usual products of acylation of Δ^2-oxazolines are the N,O-diacyl-ethanolamines derived (formally) from C—N bond cleavage.[16] Some instances of the isolation of N,N-diacylethanolamines have been reported, however, as in the acylation of 2-methyl-oxazoline with phthaloylglycyl chloride,[17] and in the related reaction with O,O-diisopropylphosphoryl chloride.[18]* Rearrangements similar to that of Scheme 2 have been suggested to take place in the reaction of 2-amino-Δ^2-thiazoline with acylating agents.[19]

The pathways of breakdown of carbinolamines derived from imidate and thioimidate esters are known to be profoundly affected by pH and by the presence of general acid-base catalysts, both C—N and C—O (or C—S) bond cleavage occurring depending on

* The proposed[18] mechanism, involving nucleophilic attack by water on C-5 of an intermediate N-phosphoryloxazolinium cation seems less likely than ring opening proceeding by addition of water to C-2 of the cation.

reaction conditions.[20] It would hardly be surprising if the mode of decomposition of the putative 2-hydroxy-2-methyl-3-acetyl-thiazolidine (Scheme 2) were found to be subject to similar influences.

EXPERIMENTAL

UV spectra were obtained with a Perkin-Elmer Model 350 recording spectrophotometer. Mass spectra were determined with an A.E.I. MS-9 Mass Spectrometer operating at an ionizing current of 100μ amp, ionizing potential of 70ev, ion-source temp 225–250°, which was coupled to a Perkin–Elmer Model 800 gas chromatograph. NMR spectra were recorded using Varian Model A—60 spectrometer operating at 60Mc, with TMS as internal standard. Ac_2O was purified according to Lewis.[21] Acetonitrile and N,S-diacetylcysteamine were materials previously used.[1]

2 *Methylene-3-acetylthiazolidine* (1)

a. *Preparation.* A sol of Ac_2O (0·61 g; 6 mmole) in MeCN (10 ml) was added to a sol of 2-methylthiazoline: (0·61 g; 6·0 mmole; distilled from powdered BaO) in 99·0% MeCN—H_2O (10 ml). After 1 hr at room temp. Et_3N (0·84 ml) was added, and the reaction vessel placed in an EtOH-dry ice bath. Solvent was removed *in vacuo* after allowing the reaction mixture to reach room temp, but without application of heat, and the residual oil placed in a sealed vial in EtOH-dry ice. The frozen oil could be stored at −70° for one to two weeks without extensive breakdown of 1. When desired, the ketene acetal was isolated in purified form after VPC on 6ft, ¼in o.d., stainless steel columns, packed with Silicone Gum Rubber SE-30 (Perkin–Elmer), 17% by weight on Anakrom ABS (90/100 mesh). N_2 was the carrier gas, the column temp was 150°, with the injector block at 260°, and the N_2 flow rate was 76 ml/min. Under these conditions, the retention time of 1 was 3 min. Aliquots (20μ l) of the crude oil were injected, and the eluted ketene acetal was collected into a glass capillary tube,[2] which was rinsed once with 50μ l of acetonitrile. The MeCN soln of 1 was immediately placed in an EtOH-dry ice bath. The procedure was repeated with 20μ l aliquots of crude oil, the capillary being rinsed with the same soln of MeCN until the solution of 1 had reached the desired concentration. Typically, four injections of 20μ l samples yielded about 1 mg of ketene acetal in 25μ l of acetonitrile. It is probable that the yield could be considerably increased by the use of a more efficient collecting system. Prior to further use for spectral or kinetic studies, the concentration and purity of acetonitrile solutions of 1 were routinely determined by VPC. The stock solns could be stored in a sealed vial kept in EtOH-dry ice for up to 2 weeks. After 1 hr at room temp, solns of 1 in MeCN showed a decrease in concentration of about 10%.

b. *Assay.* Two methods were used to determine the approximate concentration of solns of 1. In the first, the areas of VPC peaks corresponding to 1 were compared to the peaks produced by known concentrations of N,S-diacetylcysteamine based on the assumption that the response of the flame ionization detector to closely related compounds is roughly proportional to their number of C atoms.[22] With the VPC conditions cited in the preparation of 1, retention times for 1 and N,S-diacetylcysteamine were 3·0 and 4·2 min respectively. In the second method, aliquots of 1 were allowed to undergo hydrolysis in 20% MeCN–0·001N HCl, and the concentration of the resulting N,S-diacetylcysteamine determined from its absorbance at 231mμ. Assuming quantitative conversion of 1 to diacetylcysteamine, typical assays gave 0·21M (VPC) and 0·23M (hydrolysis).

c. *Mass spectrum.* Coupled VPC-mass spectrometry[23] was employed, with the columns described under the preparation of 1, but using a column temp of 190° and He as the carrier gas (20ml/min). The mass spectrum of the ketene acetal was obtained either from acetylation mixtures (*cf* Fig 1) without prior isolation of 1, or from MeCN solutions of 1. In the first case, a one μ l aliquot of a reaction mixture containing Ac_2O and 2-methylthiazoline (each at 0·3M) in 99·5% MeCN—H_2O was removed after 2 hr of reaction and injected for analysis. The mass spectra of the ascending and descending limbs, as well as of the middle and tailing portions of the peak corresponding to 1 were determined. The spectrum of the middle fraction is shown in Fig 2A; the spectra of the other fractions of the peak as well as that of a purified solution of 1 in CD_3CN (*ca* 0·25M) were identical.

d. *The UV absorption spectrum* of 1 was determined on a freshly purified sample of the ketene acetal in MeCN. VPC analysis showed two lower-boiling contaminants, amounting to 2·7% of the peak area of 1, and no diacetylcysteamine; λ_{max} 248mμ, ε_{max} *ca* 5000 in MeCN, ε_{max} *ca* 3600* in 20% MeCN—H_2O, pH 7·9.

* Not corrected for some hydrolysis.

c. *NMR spectrum.* A sample of **1** purified by VPC was collected directly into CD_3CN, yielding a solution (*ca* 0·25M) contaminated by *ca* 1% diacetylcysteamine, 1·5% 2-methylthiazoline and an unidentified substance (retention time 2·5 min) in amount of 1% of the peak area of **1**. The NMR spectrum, measured in 25μl microcells, showed: for CD_3CN (Fig 3), 7·84τ, singlet, 3H; 6·97τ, triplet, 2H; 5·99τ, triplet, 2H; 5·36τ, doublet, 1H; 4·40τ, doublet, 1H; the sharp signal at 7·71τ and the quintet centered at 8·08τ are due to HOD and CD_2HCN, respectively. For $CDCl_3$, corresponding resonances were at 7·73, 6·98, 5·95, 5·23 and 4·64τ.

Hydrolysis of **1**

a. *Kinetics.* the rate of hydrolysis of the ketene acetal (*ca* 2×10^{-4}M) was determined spectrophotometrically by the decrease of absorbance at 260mμ of buffered solns of **1** in 20% MeCN—H_2O at 25°. Reactions were followed either with the use of a Zeiss PMQ II spectrophotometer equipped with a water-jacketed cell holder, or with a Beckman model DU spectrophotometer converted to a linear direct reading instrument by a Gilford Model 220 optical density converter. Absorbance was recorded continuously by means of a Honeywell–Brown electronic recorder. The reaction mixtures were kept at 25° with Beckman thermospacers connected to a circulating bath. Reactions were initiated by adding 1·5μl of the soln of **1** in CH_3CN to the buffer solution, previously equilibrated at 25° in the cell holder, and mixing was carried out with a stream of air bubbles.

Final optical densities were obtained after 7–10 half lives of reaction and first-order rate constants were calculated using the integrated form of the first-order rate equation. Complete spectra of reactions at pH 3·0–6·2 were determined at selected time intervals to obtain an estimate of the rate of formation of N,S-diacetylcysteamine (from the increase in absorbance at 235mμ). Final spectra indicated complete conversion of **1** to diacetylcysteamine.

b. *Gas chromatography.* Hydrolysis was carried out at pH 2–7·9 using buffered solutions in 20% MeCN—H_2O at 25°. Buffers used were phosphate or formate at pH 2–4 (both at 0·2M), and HCl at pH3. For pH > 5, the buffers are given in Table 1. Reactions were initiated by the addition of 10μl of a solution of **1** in MeCN (*ca* 0·25M) to 40μl of the buffer, both solns having been previously equilibrated at 25°. The disappearance of **1** and the appearance of diacetylcysteamine were simultaneously determined by VPC, using the conditions described above. On completion of hydrolysis, there remained, in all cases, an unidentified volatile residue with the same retention time as **1**, amounting to 15% of the initial peak area of **1**. Yields of diacetylcysteamine were calculated by comparison of peak areas to those of standard solutions.

c. *Products of hydrolysis.* The ketene acetal, freshly purified by VPC, was collected directly into a soln of 17% CD_3CN—0·001 N DCl, and kept at room temp for 1 hr. At that time, VPC analysis showed that the soln contained diacetylcysteamine (0·35M), contaminated by about 1% 2-methylthiazoline, 9% of a substance with retention time 2·5 min, and 6% of a high boiling component (retention time 6 min). The NMR spectrum was identical, in all major aspects, to that of N,S-diacetylcysteamine.

Hydrolysis of **1** was also performed in 20% MeCN—0·2M phosphate buffer (pH 7·2), and 17% MeCN—0·001 N HCl, by adding 1μl of the stock soln of **1** in MeCN to 4μl of buffer. VPC analysis of the product soln showed the same composition as that of the soln used for the NMR spectrum. The mass spectrum of the major product peak, determined using the coupled VPC-mass spectrometer system, was identical to that of N,S—diacetylcysteamine.

Reaction of acetic anhydride with 2-methylthiazoline

a. *Kinetics.* The rate of disappearance of Ac_2O was followed by VPC, using 1μl aliquots of reactions carried out as given in Table II. With the column temperature at 90° and a flow rate for carrier N_2 of 50 ml/min., the retention time for acetic anhydride was 1 min. Second-order rate constants were calculated using the appropriate integrated rate equations for reactants at unequal or nearly equal concentrations,[24] with the assumption that the diasppearance of acetic anhydride is due mainly to acetylation of thiazoline and not to competing hydrolysis.

b. *Products.* The time course of the acetylation reactions was followed by VPC (e.g. Fig 1), using columns and conditions as described in the preparation of **1**. Retention times are given above under *Assay* for **1**. The coupled VPC-mass spectral method, as described above, was used to identify the fractions corresponding to **1** and diacetylcysteamine for reactions performed in 99·5, 98 and 50% MeCN—H_2O with reactants at 0·3M.

Hydrolysis of acetic anhydride. Slow reactions were carried out in sealed ampoules immersed in a constant temp bath at 25°. With half-lives of < 24 hr, reaction mixtures were kept in volumetric flasks, from

which aliquots were periodically removed, or followed directly in the cell compartment of the spectrophotometer, using stoppered 4cm cuvettes. Reactions were initiated by the addition of a stock soln of the anhydride in MeCN to the appropriate MeCN—H_2O mixture, both equilibrated previously at 25°.

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