Carbohydrate Research, 38 (1974) 333–338

Note

Reactivity of *N*-acetyl-3-*O*-*p*-tolylsulfonyl-DL-serine methyl ester: nucleophilic displacement by water at C-3 *versus* elimination

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(Received May 6th, 1974; accepted in revised form, June 17th, 1974)

Base-catalyzed degradation of glycopeptides containing 3-glycosyloxy amino acids results in α,β-unsaturated amino acids, which are difficult to quantitate accurately[1,2]. While preparing model compounds to compare the rates of elimination in aqueous buffer for various methyl 3-substituted-2-acetamidopropionates by using polarography[3–5], difficulty was encountered in the synthesis of *N*-acetyl-3-*O*-*p*-tolylsulfonyl-DL-serine methyl ester (**1**). The reaction of *p*-toluenesulfonyl chloride in dry pyridine with *N*-acetyl-DL-serine methyl ester (**2**) at any temperature or reactant concentration failed to produce the desired compound (**1**). Thin-layer chromatographic evidence discussed later has shown that **1** in pyridine at room temperature and atmospheric hymidity undergoes hydrolysis to **2** and *p*-toluenesulfonic acid. On the other hand, the reaction of **2** with *N*-*p*-tolylsulfonylimidazole[6] in benzene–chloroform–tetrahudrofuran containing a catalytic amount of sodium amide resulted only in methyl 2-acetamidoacrylate (**3**). This behavior is in distinct contrast to the stability of other analogs, such as *N*-acetyl-3-chloro-DL-alanine methyl ester[7] (**4**) and *N*-acetyl-3-*O*-acetyl-DL-serine methyl ester[7] (**5**), which were synthesized in high yield according to literature preparations. Even *N*-benzyloxycarbonyl-3-*O*-*p*-tolylsulfonyl-DL-serine methyl ester (**6**) can be synthesized in good yield by the usual method[8], although **6** is reactive both toward elimination[9] and nucleophilic substitution[10] of the 3-*p*-tolylsulfonyloxy leaving group.

In earlier work, Ginsberg and Wilson[11] found that *N*-benzyloxycarbonyl-serine derivatives do not form oxazolines by neighboring-group reaction of the *N*-carbonyl oxygen atom at C-3 to displace the *p*-tolylsulfonyloxy leaving-group; *N*-benzoyl derivatives form either the oxazoline or the elimination product; *N*-acyl serine esters yield both products, whereas the amides favor the oxazoline; the *N*-benzyloxycarbonyl amide of serine gives exclusively the elimination product; strong bases favor elimination, and more-polar solvents favor formation of oxazolines. No previous reference has been found either to synthesis of **1** or of the oxazoline

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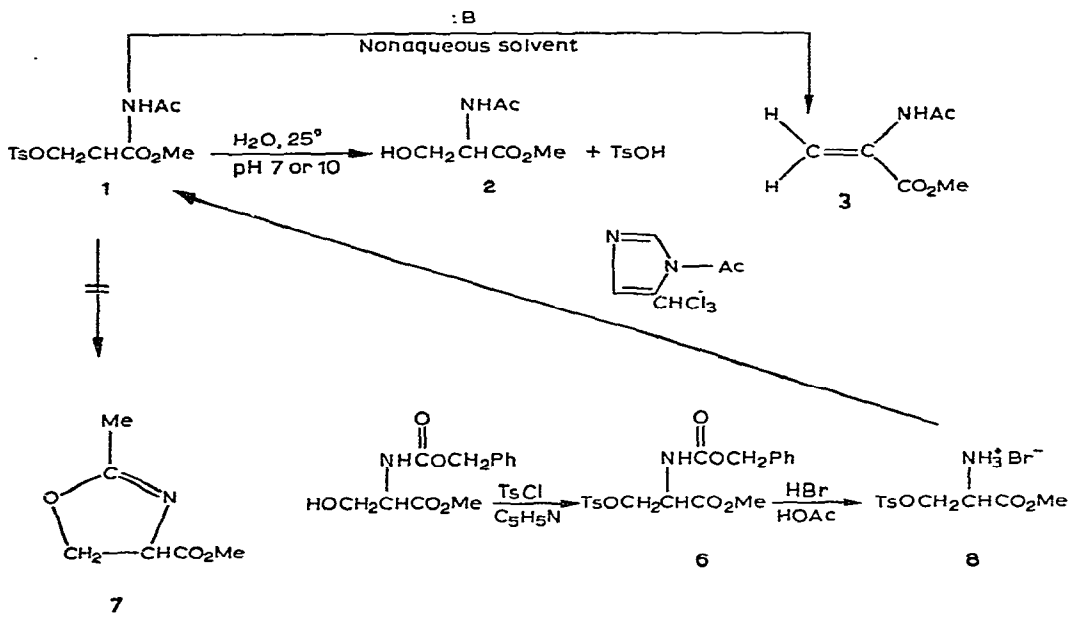

Chart 1. Synthesis and reactions of *N*-acetyl-3-*O*-*p*-tolylsulfonyl-DL-serine methyl ester (1).

that would result from it, namely, 4-methoxycarbonyl-2-methyloxazoline (7). In contrast to the known synthesis of the phenyl-substituted oxazoline[11,12], we were also unable to synthesize 7.

Synthesis of 1 was effected in 40% overall yield from the readily available 3-*O*-*p*-tolylsulfonyl-DL-serine methyl ester hydrobromide[13] (8) and *N*-acetylimidazole[14,15] (9) reacting in anhydrous medium. The reactivity of 9 has been well studied by Jencks *et al.*[16,17], although reaction with the serine 3-hydroxyl group rather than the amino nitrogen atom was assessed[16]. Other methods for synthesis of 8, by *N*-acetylation of 6 were unsuccessful, presumably because of the reactivity of C-3. Detailed instrumental studies (see experimental section) indicate that, in the presence of water at room temperature, 1 undergoes facile nucleophilic displacement at C-3. This contrasts with the behavior of 1 in nonaqueous solvents containing a catalytic amount of base, where the only product formed from 1 is that of elimination (3). The fact that 1 cannot be stored under atmospheric humidity, without eventual complete conversion into 2 and *p*-toluenesulfonic acid, attests to this reactivity. Fortunately, once 1 is formed, rapid isolation from a cold, aqueous medium nevertheless affords the product in adequate yield.

Polarographic examination of the reaction of 1 in aqueous buffers at pH 9–10 revealed no evidence for β-elimination[5] and the only product identified was 2. This behavior is compared in Table I with that of 4 and 5, which underwent elimination in a normal manner under various conditions of ionic strength with basic, aqueous buffer. The rates of elimination of 4 and 5 were calculated from the increase in

limiting current for reduction of **3**. At pH values from 9 to 10, saponification of the methyl ester of **4** and **5** has been shown not to occur[3–5]. The degree of formation of oxazoline (**7**) during the elimination reactions of **4** and **5** has never been ascertained, either in this study or the earlier reports[3–5], although product isolation, polarography, and n.m.r. spectroscopy have been used in attempts to discern **7** as a possible intermediate[3–5]. However, in terms of limiting currents for **3**, these polarographic data definitely support the marked contrast in reactivity between **1**, **4**, and **5**. Finally, the possibility of elimination from **1** to form **3**, followed by Michael addition of water to **2**, is not substantiated by either n.m.r.-spectral or polarographic data.

TABLE I

PSEUDO-FIRST-ORDER RATE CONSTANTS FOR THE β-ELIMINATION REACTION OF **4** AND **5**[a]

Compound	*Buffer*	*Ionic strength*	*pH*	$k^b \times 10^4$
1	[c]	0.1–1.0	9.5	[d]
4	[e]	0.2	9.7	40
4	[e]	0.5	10.0	60
5	[e]	0.5	9.1	5
5	[e]	0.5	9.7	50
5	[c]	0.1	9.5	1
5	[c]	0.5	9.5	2
5	[c]	1.0	9.5	[c]3

[a]Basis of calculation was the increase in the limiting current of compound **3** as a function of time. [b]Rate constants are expressed in sec^{-1}. [c]Britton–Robinson buffer. See experimental section for description. [d]No detectable elimination reaction. [e]Carbonate buffer.

In comparing neighboring-group participation of the *N*-acyl carbonyl group and specific anchimeric assistance for solvolytic reactions[18,19], this present evidence suggests that the *N*-acetyl group of **1**, **4**, or **5** cannot form a stable oxazoline (**7**) involving C-3 of serine because **7** is much more reactive to hydroiysis than its 2-phenyl analogs[11]. On the other hand, elimination occurs exclusively in **4** and **5** because neighboring-group participation provides less-effective assistance to solvolysis of their leaving groups than for **1**.

In conclusion, the synthesis of **1** and demonstration of its varied reactivity should permit modification of serine by nucleophilic displacement or the employment of **1** as a convenient intermediate for the synthesis of **3**. Utilization of *N*-acetylimidazole for rapid, mild *N*-acetylation of amino sugars and amino acids containing reactive vicinal substituents may also be recommended as a result of this work.

EXPERIMENTAL

General methods. — T.l.c. was performed by the ascending technique on Eastman Chromagram sheets (Type 6060, silica gel with fluorescent indicator) with 1:1 chloroform–ethyl acetate, utilizing an Eastman developing apparatus 6071

and detection by u.v. light. N.m.r. spectra were recorded with a Varian A-60 spectrometer, with saturated solutions in chloroform-*d* and tetramethylsilane as internal reference. Microanalyses were determined by Galbraith Laboratories, Inc., Knoxville, Tenn. 37921.

N-*Acetyl-3*-O-p-*tolylsulfonyl*-DL-*serine methyl ester* (**1**). — Imidazole (1.4 g) and freshly distilled acetyl chloride (1 ml) in chloroform (10 ml) were stirred for 0.5 h and the precipitated imidazolium hydrochloride was filtered off. To the filtrate containing *N*-acetylimidazole was added **8** (1.8 g) in dry methanol (5 ml) (**8** was prepared from **6** by using hydrogen bromide[13]). After 30 min, the solvent was removed under vacuum. Upon treating the syrupy residue with water (15 ml) followed by cooling in ice, crystals of **1** formed, which were quickly filtered from the aqueous medium. Purification was effected immediately by dissolving the crude **1** in methanol (1 ml), adding water to the point of turbidity, and cooling in an ice bath to effect crystallization; yield, 0.6 g (40%), decomposition point 89–90° (uncorrected); R_F 0.70; n.m.r.: δ 1.98 (3-proton singlet, Ac), 2.40 (3-proton singlet, *p*-tolyl CH_3), 3.72 (3-proton singlet, ester CH_3), 4.35 (2-proton multiplet, $-OCH_2-$, collapsed to a doublet upon shaking the solution with D_2O), 6.7 (1-proton doublet, NH, disappeared upon deuteration), 7.39, and 7.83 (4-proton symmetrical AA'BB' doublets, $J_{A,B}$ 9 Hz, *p*-tolyl ring protons).

Anal. Calc. for $C_{13}H_{17}NO_6S$: C, 49.52; H, 5.44; N, 4.40; S, 10.17. Found: C, 49.34; H, 5.33; N, 4.35; S, 10.20.

Hydrolytic stability of **1**. — In the presence of small quantities of water, **1** decomposed slowly at room temperature, and rapidly at higher temperatures, to *p*-toluenesulfonic acid and **2**. Product identification was made by comparing n.m.r. spectra of freshly prepared **1**, aged samples of **1**, *p*-toluenesulfonic acid and **2** together, and mixtures of all three. Identification of the degree of hydrolysis of **1** was made by comparing the AA'BB' patterns of the *p*-tolyl aromatic protons in **1** with those in *p*-toluenesulfonic acid in conjunction with the acetyl and methyl ester proton signals in **1** and **2**. The chemical shifts for the A and B protons in *p*-toluenesulfonic acid are δ 7.75 and 7.16, respectively, whereas those in **1** are δ 7.83 and 7.39, respectively. The chemical shifts of the acetyl and methyl ester signals for **2** are δ 2.05 and 3.78, respectively, whereas those for **1** are δ 1.98 and 3.72, respectively. The methyl group of *p*-toluenesulfonic acid itself possesses a chemical shift δ 2.33 versus 2.43 for the *p*-toluenesulfonyl group in **1**.

The n.m.r. spectrum for a sample of **1**, stored at room temperature and humidity for 3 weeks, typically showed: δ 1.9 (singlet, integral 29 mm, acetyl group of **2**); 2.0 (singlet, integral 50 mm, acetyl group of **1**); 2.35 (singlet, integral 30 mm, CH_3- of *p*-toluenesulfonic acid); 2.45 (singlet, integral 50 mm, aryl CH_3- of **1**); 3.6–3.7 (2 unresolved singlets, integral 80 mm, methyl esters of **1** and **2**); 3.75–5.00 (multiplet, integral 78 mm, $-CHCH_2-$ of **1** and **2**); 7.15 (doublet, integral 22 mm, B' of *p*-toluenesulfonic acid); 7.2 to 7.8 (multiplet, remainder of aromatic protons, integral 85 mm). Artificial mixtures of **1**, **2**, and *p*-toluenesulfonic acid gave a similar n.m.r. spectrum.

When solutions of **1** in chloroform-*d*, as used for n.m.r., were extracted with D_2O, **2** and *p*-toluenesulfonic acid were removed, and the n.m.r. spectrum of the resultant solution revealed the presence of **1** only. Heating accelerated the decomposition; even when freshly prepared **1** was dried under vacuum at the temperature of refluxing acetone there resulted resolved n.m.r. signals for the proton resonances of the mixture already given, including the acidic proton of *p*-toluenesulfonic acid resonating at δ 13.8, which disappeared in the presence of D_2O.

T.l.c. evidence supports the n.m.r. data on the hydrolytic lability of **1**. Twenty lanes were scribed on Eastman 6060 Chromagram sheets, appropriate markers were spotted, and the chromatograms were eluted in Eastman S-chambers (6071). Aged samples of **1**, samples of **1** kept for >12 h in pyridine, and pyrolyzed samples of **1** (pyrolyzed by heating either on glass cover slips on a Fisher–Johns melting-point apparatus or by heating in open capillary-tubes) showed only two spots on the chromatograms (*p*-toluenesulfonic acid, R_F, <0.1; and **2**, R_F, 0.36). A second elution with methanol showed that the spot at R_F <0.1 was homogeneous. The pyridine solutions of **1** did not reveal the presence of any of the elimination product **3** (R_F, 0.85). Compound **3** was stable in pyridine solution under similar conditions.

Base-catalyzed elimination reaction of **1** *in nonaqueous solvents.* — The procedure of Rothstein[7] was used to study these reactions. In a typical reaction in 1:1 ethyl acetate–ethyl ether with diethylamine as catalyst, **1** was converted into **3** in 35% yield. Characterization of **3** was performed as in the earlier reports[4,5].

Electrochemical studies on **1**, **4**, *and* **5**. — The polarographic methodology used in this study has been discussed in previous papers[3,4]. Reaction of **1**, **4**, and **5**, and determination *in situ* of **3**, was effected in buffers having pH $\leq$ 10 to avoid saponification of the ester group.

Buffers were of ionic strengths and pH values given in Table I. A standard carbonate buffer was prepared in the usual way. A Britton–Robinson buffer is a general buffer system prepared by titrating a stock solution of 0.04M acetic acid, 0.04M phosphoric acid, and 0.04M boric acid with 0.2M sodium hydroxide to the desired pH value[20,21].

Typical reaction conditions were as follows: 20 μmoles of the substrate (**1**, **4**, or **5**) was added to 10 ml of deoxygenated and thermostatted buffer in the polarographic cell. Polarograms were then taken at various time intervals and the current–time parameters were analyzed. Rate constants were calculated from the values of half lives obtained from the plot of $\log (i_\alpha - i)$ *vs.* t, where i_α is the current due to reduction of **3** present at the end of reaction (usually 4 h) and i is the current due to reduction of **3** at any time. Results are presented in Table I. In these aqueous solutions, **1** was only converted into **2**, and no detectable quantity of **3** was formed by elimination.

ACKNOWLEDGMENT

This study was supported in part by the Agricultural Research Service, U.S. Department of Agriculture, Grant 12-14-100-9208(71), administered by the Northern Marketing and Nutrition Research Division, Peoria, Illinois 61604.

REFERENCES

1 W. PIGMAN AND J. MOSCHERA, *Advan. Chem. Ser.*, 117 (1973) 220–241, and references cited.
2 A. GOTTSCHALK, in A. GOTTSCHALK (Ed.), *Glycoproteins*, 2nd ed., Elsevier, Amsterdam, 1972, p. 470–476.
3 D. N. SCHLUTER, G. MAMANTOV, AND J. R. VERCELLOTTI, *J. Chem. Soc., Perkin Trans. II*, (1973) 1663.
4 D. N. SCHLUTER, G. MAMANTOV, AND J. R. VERCELLOTTI, *J. Chem. Soc., Perkin Trans. II*, (1973) 1670.
5 D. N. SCHLUTER, Ph.D. Dissertation, University of Tennessee, 1972.
6 H. A. STAAB, *Angew. Chem., Int. Ed., Engl.*, 1 (1962) 351.
7 E. ROTHSTEIN, *J. Chem. Soc.*, (1949) 1968.
8 J. P. GREENSTEIN AND M. WINITZ, *Chemistry of the Amino Acids*, Vol. 3, J. Wiley and Sons, New York, 1961, p. 2225.
9 I. PHOTAKI, *J. Amer. Chem. Soc.*, 85 (1963) 1123.
10 D. THEODOROPOULOS, I. L. SCHWARTZ, AND R. WALTER, *Biochemistry*, 6 (1967) 3927.
11 S. GINSBERG AND I. B. WILSON, *J. Amer. Chem. Soc.*, 86 (1964) 4716.
12 E. M. FRY, *J. Org. Chem.*, 14 (1949) 887.
13 J. P. GREENSTEIN AND M. WINITZ, *Chemistry of the Amino Acids*, Vol. 2, J. Wiley and Sons, New York, 1961, p. 1249.
14 T. WIELAND AND G. SCHNEIDER, *Ann. Chem.*, 580 (1953) 159.
15 L. F. FIESER AND M. FIESER, *Reagents for Organic Synthesis*, Vol. 1, J. Wiley and Sons, New York, 1967, p. 13.
16 B. M. ANDERSON, E. H. CORDES, AND W. P. JENCKS, *J. Biol. Chem.*, 236 (1961) 455.
17 J. GERSTEIN AND W. P. JENCKS, *J. Amer. Chem. Soc.*, 86 (1964) 4655.
18 L. GOODMAN, *Advan. Carbohyd. Chem.*, 22 (1967) 109–175.
19 B. CAPON, *Chem. Rev.*, 69 (1969) 407.
20 J. A. C. FRUGONI, *Gazz. Chim. Ital.*, 87 (1957) 403.
21 J. HEYROVSKÝ AND P. ZUMAN, *Practical Polarography*, Academic Press, London, 1968, p. 179.