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Communication

Acetylation of methyl 5-amino-1*H*-[1,2,4]triazole-3-carboxylate★✪

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Acetylation with acetic anhydride of methyl 5-amino-1*H*-[1,2,4]triazole-3-carboxylate, one of the hetareneamino acids, was studied using HPLC, ^{1}H NMR, FTIR and GC-MS. The compound has a significantly decreased susceptibility to acetylation compared to 5-amino-1*H*-[1,2,4]triazole itself. Two isomeric diacetylated products were found.

Hetareneamino acids have the potential to form hetarene oligopeptides, ones of the most promising small molecules controlling gene expression [1–3], and moreover to be the constituents of natural [4] and artificial [5] peptides useful for biological and non-biological purposes. They owe this to their properties such as a flat, rigid system capable of acting both as a hydrogen bond acceptor and donor, the electrostatic potential near nitrogen atoms, the ability to tautomerism and the amphoteric character. The readily available 5-amino-1*H*-[1,2,4]triazole-3-carboxylic acid (ATC) [6] is such an interesting amino acid. Its chemistry as well as the properties, including structural and spectral ones, of its derivatives are, however, little known. Only the methyl ester obtained through direct esteri-

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Abbreviations: ATC, 5-amino-1*H*-[1,2,4]triazole-3-carboxylic acid; ATC-OMe, methyl 5-amino-1*H*-[1,2,4]triazole-3-carboxylate; ATC(Ac)-OMe, methyl 1-acetyl-5-amino-1*H*-[1,2,4]triazole-3-carboxylate; Ac-ATC-OMe, 5-acetylamino-1*H*-[1,2,4]triazole-3-carboxylate; Ac-ATC(Ac')-OMe, methyl 1-acetyl-3-acetylamino-1*H*-[1,2,4]triazole-5-carboxylate; Ac-ATC(Ac)-OMe, methyl 1-acetyl-5-acetylamino-1*H*-[1,2,4]triazole-3- carboxylate; FTIR, Fourier transform infrared spectroscopy; GC-MS, gas chromatography-mass spectrometry; ^{1}H NMR, proton magnetic resonance.

fication [6] and its 1-acetyl derivative synthesised totally *via* linear substrate cyclisation [7] are described.

Acylation of the hetareneamino acid exo-amino group poses, however problem [e.g. 8]. The ability of ATC and its derivatives to undergo acylation, especially a selective one, which is of practical importance for the potential incorporation into a peptide chain, is unexplored. A model reaction of acylation is acetylation. Based on our previous work on the acetylation of 5-amino-1*H*-[1,2,4]triazole [9] we studied acetylation of methyl 5-amino-1*H*-[1,2,4]triazole-3-carboxylate, which is the simplest protected form of ATC allowing one to avoid undesired side reactions at its free carboxyl group. The reactions with Ac_2O were investigated using HPLC, ^1H NMR spectroscopy, FTIR spectroscopy and GC-MS. The results were compared with those from the acetylation of 5-amino-1*H*-[1,2,4]triazole itself. The compounds isolated were characterized.

MATERIALS AND METHODS

The results of acetylation experiments were followed first of all using a Beckman "System Gold" chromatograph working at 210 nm as a rule and occasionally at 250 nm, with an Alltech Alltima C_{18}, 5 μm, 150 × 4.6 mm reversed-phase column, 0.1% trifluoroacetic acid/acetonitrile (90:10, v/v) as a mobile phase and reference substances. ^1H NMR spectra were taken in a $(CD_3)_2SO$ solution on a Bruker Advance DRX 300 MHz spectrometer with tetramethylsilane internal standard. FTIR spectra were recorded on a Philips Analytical PU9800 FTIR spectrometer at a 2 cm^{-1} nominal resolution in KBr and dimethylsulfoxide. A GC HP 6890 chromatograph with an HP-5 column and an MS 5973 (EI) mass spectrometer as a detector were used.

RESULTS AND DISCUSSION

Hydrochloride of amino acid ester was obtained with the $SOCl_2$-methanol method and converted into free ester [10]. As X-ray diffraction reveals, it is methyl 5-amino-1*H*-[1,2,4]triazole-3-carboxylate. As the chemical shift of the amino group protons (Fig. 1) and the regioselective course of the annular monoacetylation reaction show (Fig. 3), the ester in solution, however, mainly occurs in the tautomeric methyl 3-amino-1*H*-[1,2,4]-5-carboxylate form.

The annular acetylation of 5-amino-1*H*-[1,2,4]triazole is very fast and gives two monoacetylated products, the kinetic one:

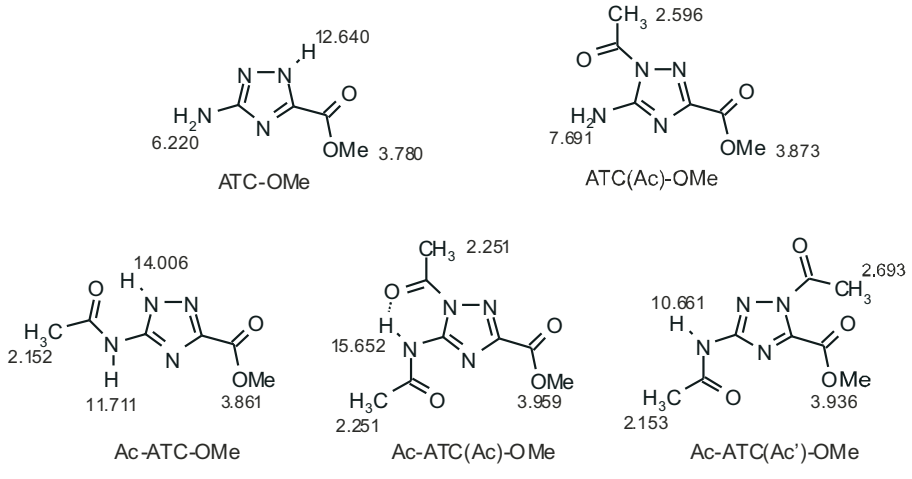

Figure 1. Proton chemical shifts (ppm) of methyl 3-amino-1*H*-[1,2,4]triazole-5-carboxylate and its acetylated derivatives in $(CD_3)_2SO$ solution.

For picture clarity, units have been omitted.

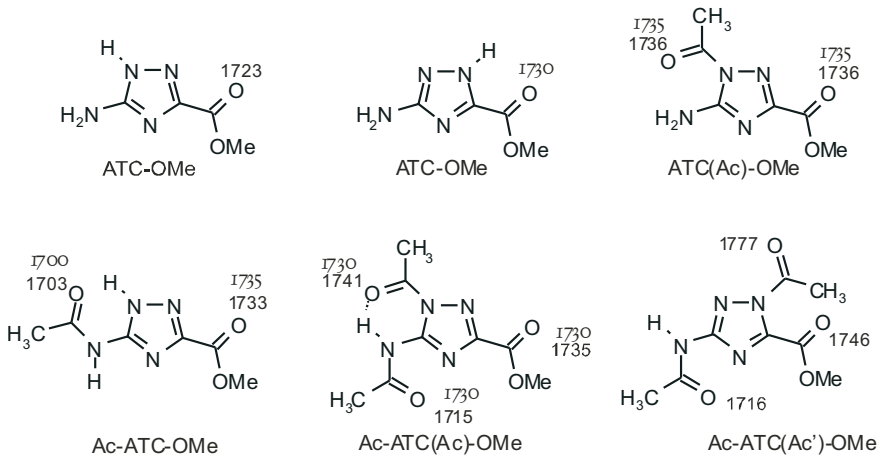

Figure 2. The frequencies in the AI region (cm^{-1}) of methyl-5-amino-1*H*-[1,2,4]triazole-3-carboxylate and its acetylated derivatives in KBr and *dimethylsulfoxide*.

For picture clarity, units have been omitted.

1-acetyl-3- amino-1*H*-[1,2,4]triazole, and the thermodynamic one: 1-acetyl-5-amino-1*H*-[1,2,4]triazole. The latter is also unstable, and at room temperature both in solution and in the solid state undergoes isomerisation into the acetylamino derivative [9]. In contrast, monoacetylation of the sample ester, both in dimethylformamide and dimethylsulfoxide solution with 1 eqiv. Ac_2O is slower. Thus, in a dilute dimethylsulfoxide solution, at room temperature after 2 min, Ac_2O undergoes reaction with 5-amino-1*H*-[1,2,4]triazole in 74%, whereas with ATC-OMe merely in 15%. The reaction produces one compound only: methyl 1-acetyl-5-amino-1*H*-[1,2,4]triazole-3-carboxylate, whose structure has been confirmed by X-ray diffraction as well as ^{1}H NMR (Fig. 1) and FTIR spectroscopy (Fig. 2). No intermediate, isomeric annular monoacetylated compound could be detected. Furthermore, methyl 1-acetyl-5-amino-1*H*-[1,2,4]triazole-3-carboxylate, unlike 1-acetyl-5-amino-1*H*-[1,2,4]triazole, is stable. After one year of storage at room temperature, we did observe neither the formation of the acetylamino derivative nor any other change. The ester is, however, isomerised completely into the acetylamino derivative during melting (215°C) and slowly in solutions at heating.

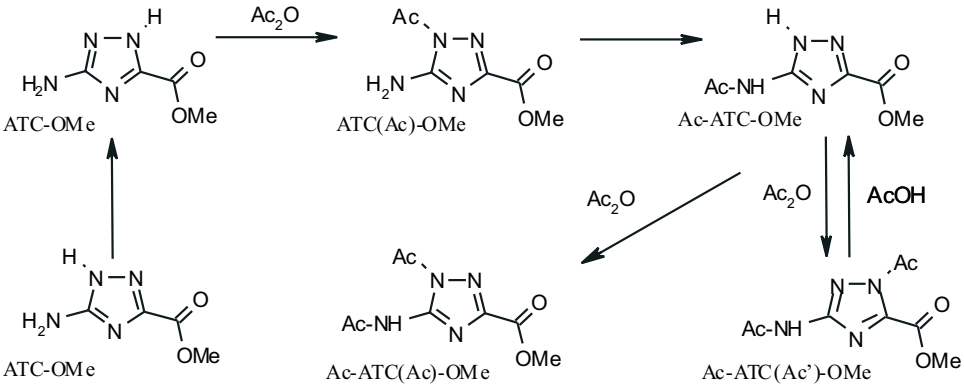

Figure 3. Hypothetical main pathways of the acetylation of ATC-OMe with neat Ac_2O at boiling.

The reaction of 5-amino-1*H*-[1,2,4]triazole with acetic anhydride in excess at room temperature gives the diacetyl derivative in quantitative yield [9]. Under the same conditions, the investigated ester exoamino group does not react at all. Upon 10 days, merely minute quantities of the diacetyl compound methyl

Table 1. The results of reactions of methyl 5-amino-1*H*-[1,2,4]triazole-3-carboxylate and its monoacetylated derivatives with neat Ac_2O at boiling.

Identification of compounds by ^{1}H NMR, FTIR and GC-MS

Substrate	The composition of reaction mixture by HPLC [%][b]	36 equiv. Ac_2O Reaction time (min)				53 equiv. Ac_2O Reaction time (min)				
		5	10	30	60	5	10	30	60	180
ATC-OMe	ATC-(Ac)-OMe [c]	41.0	6.8	1.1	1.0	55.2	0.9	-		
	Ac-ATC-OMe [d]	12.9	9.8	11.6	11.2	13.0	11.2	11.9		
	Ac-ATC(Ac')-OMe [e]	40.4	68.0	60.8	60.4	39.9	66.0	56.5		
	Ac-ATC(Ac)-OMe [f]	5.7	15.0	25.7	25.7	1.5	21.0	30.6		
Ac-ATC-OMe	ATC-(Ac)-OMe	-	-	-		-	-	-	-	-
	Ac-ATC-OMe	21.5	11.8	12.7		10.2	7.6	9.2	10.6	9.0
	Ac-ATC(Ac')-OMe	55.8	59.0	49.5		78.0	75.2	57.0	57.3	47.5
	Ac-ATC(Ac)-OMe	21.8	27.8	36.6		11.8	16.7	32.8	31.8	42.7
ATC(Ac)-OMe	ATC-(Ac)-OMe	34.8	8.6	-	-	43.0	25.9	0.9	-	-
	Ac-ATC-OMe	5.9	8.3	9.4	11.9	5.2	5.4	6.7	7.8	8.3
	Ac-ATC(Ac')-OMe	54.5	70.1	65.9	53.4	48.8	61.3	68.5	64.9	63.0
	Ac-ATC(Ac)-OMe	4.5	12.1	23.8	34.0	3.0	7.0	23.8	27.3	27.0

[a] 0.1% Trifluoroacetic acid/acetonitrile (90:10, v/v) ; [b] in addition to the enumerated compounds, others unidentified constitute 0–1.8% (a complement to 100.0%); [c] t_R = 8.07; [d] t_R = 4.18; [e] t_R = 9.18; [f] t_R = 7.67.

1-acetyl-3-acetylamino-1*H*-[1,2,4]triazole-5-carboxylate was detected. The reaction requires an elevated temperature. Table 1 collects the results of reactions of methyl 5-amino-1*H*-[1,2,4]triazole-3-carboxylate and both its monoacetyl derivatives with neat acetic anhydride at boiling. These results from Table 1 and additional ones allow the following conclusions to be drawn.

ATC(Ac)-OMe is the first product of acetylation. Of the two monoacetylated derivatives of methyl 5-amino-1*H*-[1,2,4]triazole-3-carboxylate, Ac-ATC-OMe reacts faster with acetic anhydride and it is this compound that is the main substrate for the second acetylation. This is also shown by the results of acetylation of each of the monoacetylated substrate separately at room temperature. Ac-ATC-OMe can be assumed to be mostly formed from ATC(Ac)-OMe by intermolecular transacetylation, although direct acetylation of the exoamino group of ATC-OMe cannot be ruled out. Each experiment from Table 1 shows that Ac-ATC(Ac')-OMe is formed as the first diacetylated product and subsequently converted into its isomeric compound Ac-ATC(Ac)-OMe. The system aims at equilibrium with approximately equimolar quantities of both isomeric diacetyl derivatives, 45% each, and with 10% of Ac-ATC-OMe. This shows that the isomerisation can be considered as a process with the participation of the latter compound, but not as an intramolecular phenomenon. Both diacetylated compounds were characterised by ^{1}H NMR (Fig. 1) and FTIR spectroscopy (Fig. 2). An outstanding feature of methyl 1-acetyl-*1H*-[1,2,4]triazole-5-acetylamino-3-carboxylate is a very strong intramolecular hydrogen bond. Triacetylation that takes place in the case of 5-amino-1*H*[1,2,4]triazole [9] could not been observed for methyl 5-amino-1*H*-[1,2,4]triazole-3-carboxylate.

CONCLUSION

The acetylation of methyl 5-amino-1*H*-[1,2,4]triazole-3-carboxylate as compared to the parent 5-amino-1*H*-[1,2,4]triazole is influenced by the presence of the methoxycarbonyl group. Monoacetylation of the ring nitrogen atom is slower, no isomeric products are observed and the resulting only compound is stable at room temperature. In contrast to 5-amino-1*H*-[1,2,4]triazole, introduction of the second acetyl group requires elevated temperature and, similar to 5-amino-1*H*-[1,2,4]triazole, two isomeric diacetyl derivatives are formed. Figure 3 presents hypothetical main pathways of the acetylation of ATC-OMe with acetic anhydride at boiling.

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