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Isolation and Identification of a Novel Impurity of Erythromycin A 9-Oxime Desosaminehydrazinium Salt

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Abstract In the manufacturing process for Biaxin® (clarithromycin), erythromycin-A oxime, an intermediate, is obtained in high yield, when erythromycin-A is treated with hydroxylamine/isopropyl alcohol in the presence of acetic acid. An unusual impurity, the desosamine hydrazinium salt, is generated in this step of the synthetic pathway, and has been isolated and characterized by using one and two-dimensional NMR spectroscopy in conjunction with MS and EDS.

Keywords erythromycin A 9-oxime, hydrazinium salt, NMR

Erythromycin is a well established macrolide antibiotic that is used to treat a wide variety of bacterial infections in man [1, 2]. Erythromycin's instability under the acidic conditions that are present in the stomach can give inactive by-products resulting in low bioavailability [3, 4]. As a result, a number of semi-synthetic erythromycin derivatives have been developed to improve acid stability in the gastrointestinal track [4~8]. Erythromycin-A 9-oxime is an important intermediate in the synthesis of Biaxin® (clarithromycin), a second-generation macrolide antibiotic, which has proven to be a clinical and commercial success due to its improved antibacterial activity and pharmacokinetic profile [7, 9]. Erythromycin-A oxime is obtained in high yield when erythromycin-A is treated with hydroxylamine/isopropyl alcohol in the presence of acetic acid, as shown in Scheme 1 [10]. A new impurity has been identified in this step of the synthetic pathway, and in this report we would like to present information on the isolation and characterization of this compound and discuss the possible mechanism by which it is formed.

Formation and isolation of the impurity

Erythromycin A 9-oxime has been prepared from the reaction of erythromycin-A with hydroxylamine in methanol [11, 12]. Since this reaction was found to produce a number of degradation impurities, an improved procedure was developed to prepare the 9-oxime. In the improved process, erythromycin-A is treated with hydroxylamine and acetic acid in isopropyl alcohol [10]. This modification provides a better impurity profile, however it also leads to a new impurity that was not previously observed in this step of the reaction sequence. The concentration of this new impurity was found to increase with prolonged reaction times and/or with raised reaction temperatures. When a reaction mixture of erythromycin-A, acetic acid and hydroxylamine was heated to 58°C in isopropyl alcohol for 72 hours (or at 70°C for 24 hours), the new impurity was observed by HPLC at a concentration of 4.7 (or 6.0) area-percent, respectively.

Two different methods were used to isolate the impurity from this reaction. In the first method, isopropyl acetate and aqueous sodium hydroxide solution (6 M) were added to the reaction mixture after the reaction was complete. A solid precipitate was collected from the solution by filtration and was found to contain 60% of the unknown impurity by HPLC. The isolated impurity was recrystallized twice from methanol and once from a mixture of methanol and ethyl acetate. The recrystallized material was found to be 93.6% pure by HPLC. NMR, MS and EDS (energy dispersive X-

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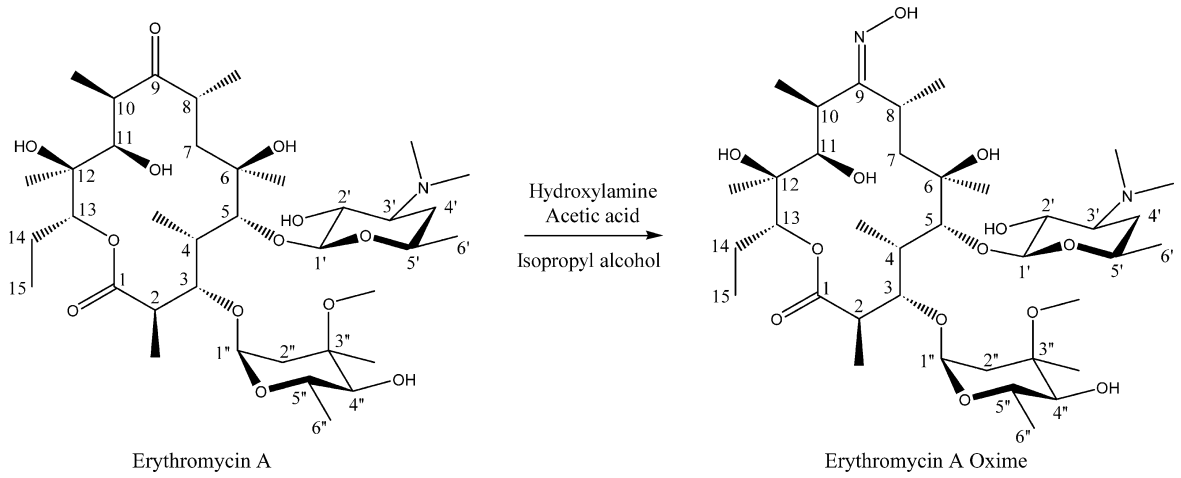


Scheme 1.

ray spectroscopy) were used to determine the structure of this impurity, isolated as the acetate salt (compound **1**, see Fig. 1).

A second method was used to isolate the chloride salt of the impurity. In this procedure, isopropyl acetate and aqueous sodium hydroxide were added to the solution after the reaction was complete and the aqueous layer was separated and treated with 20% aqueous sodium chloride. After this mixture was agitated for a few minutes, a white solid was filtered from the solution, and this material exhibited the same retention time as compound **1**, by HPLC (with 80% purity). The isolated material was then recrystallized from methanol, yielding compound **2** in 90% purity. This compound was identified as the chloride salt (**2**) of the impurity (see Fig. 1) by NMR, MS and EDS.

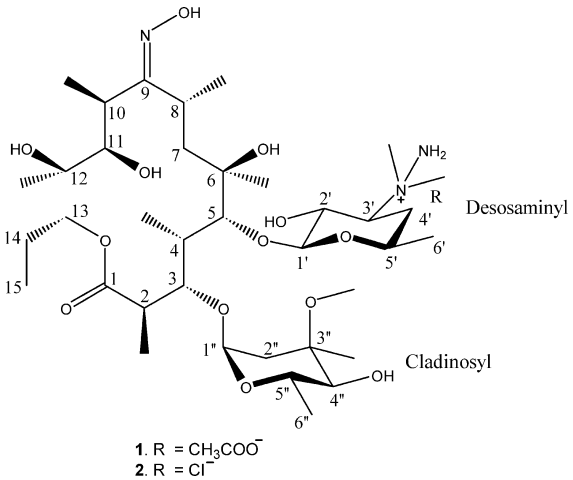


Fig. 1 The structure of the impurity that was isolated as two different salt forms.

Identification of the impurity

Both compounds **1** and **2** were freely soluble in DMSO-d_6 and therefore NMR spectra were acquired in this solvent. The ^1H and ^{13}C chemical shifts for both compounds were assigned with the aid of standard g-DQCOSY, g-HSQC, and g-HMBC experiments.

The proton and carbon chemical shifts for compound **1** and **2** could be assigned from these studies, as shown in the assigned g-HSQC spectra (Fig. 2B and E) and Table 1 for compound **2**. Cross-peaks for the methine protons at positions 8 and 10 did not show reasonable intensity in the g-HSQC spectra for both compounds. However, these signals were clearly visible when the NMR data was acquired in pyridine-d_5 (see Table 1). Both compounds showed the similar correlations between the $-NH_2$, 3′-$N(CH_3)_2$ and 3′ CH groups in the g-HMBC spectra (Fig. 2C and F). The carbon chemical shifts for the 3′-$N(CH_3)_2$ group in the desosamine sugar are normally at about 40 ppm in erythromycin. In contrast, the carbon chemical shifts for the 3′-$N(CH_3)_2$ group in the impurity are in the 50∼60 ppm range, which is even further downfield than the -OCH_3 (∼49 ppm) group at position 3″. This fact suggests that the nitrogen on the desosamine ring is charged, as expected in the case of a hydrazinium salt of a tertiary amine. Exact-mass MS measurements for both compounds indicate a MW of 764.4918 (*m*/*z*), which is consistent with the proposed molecular formula $C_{37}H_{70}N_3O_{13}^+$.

In comparing the NMR spectra of compound **1** and **2**, some chemical shift differences can be found in the region of the desosamine sugar at the 2′, 3′ and 3′-$N(CH_3)_2$ positions. In compound **1**, an acetate signal is also observed in the proton spectrum at a level that corresponds to a 1 : 1 mole-ratio of compound **1** to acetate, and EDS confirms that no other heteroatoms are present except oxygen. In the

Table 1 ^{1}H and ^{13}C assignments of compound **2** in DMSO-d_6 and pyridine-d_5

Position	Group	DMSO-d_6		Pyridine-d_5	
		^{1}H (ppm)[a]	^{13}C (ppm)[b]	^{1}H (ppm)[c]	^{13}C (ppm)[d]
1	C=O	—	174.6	—	175.7
2	CH	2.79	44.2	3.18	45.1
2-Me	CH_3	1.10	16.0	1.33	16.3
3	CH	3.83	78.1	4.49	79.5
4	CH	1.94	38.4	2.51	39.3
4-Me	CH_3	1.00	9.3	1.55	9.8
5	CH	3.47	82.9	4.07	84.5
6	C=O	—	74.1	—	74.7
6-Me	CH_3	1.32	26.5	1.96	27.1
7	CH_2	1.49	37.3	1.87, 1.75	38.2
8	CH	3.58*	25.0	4.28	25.7
8-Me	CH_3	0.99	18.7	1.10	19.0
9	C=NOH	—	168.9	—	170.1
10	CH	2.64	32.7*	2.94	33.2*
10-Me	CH_3	1.08	14.6	1.48	15.0
11	CH	3.53	70.3	4.32	71.2
12	C=O	—	74.1	—	74.6
12-Me	CH_3	1.01	17.0	1.36	17.0
13	CH	5.13	76.0	5.70	77.0
14	CH_2	1.82, 1.37	20.9	2.17, 1.64	21.5
15	CH_3	0.74	10.6	0.87	10.7
1′	CH	4.50	100.7	5.15	101.9
2′	CH	3.58	70.5	4.24	72.2
3′	+NCH	3.58	75.4	4.56	75.7
3′-NMe	$+N(CH_3)_2$	3.35, 3.22	55.2, 53.2	3.94, 3.67	54.8, 53.1
4′	CH_2	2.14, 1.49	32.7	2.44, 1.68	34.0
5′	CH	3.75	65.5	4.30	66.4
6′	CH_3	1.13	20.9	1.29	21.2
1″	CH	4.75	95.4	5.09	96.4
2″	CH_2	2.27, 1.53	34.8	2.38, 1.46	35.3
3″	C=O	—	72.6	—	73.3
3″-Me	CH_3	1.14	21.1	1.36	21.3
3″-OMe	CH_3	3.23	48.9	3.60	49.5
4″	CH	2.92	77.2	3.24	78.5
5″	CH	3.97	64.9	4.49	66.0
6″	CH_3	1.16	18.5	1.50	18.9
9	N-OH	10.70	—	12.86	—
11	OH	4.12	—	4.34	—
2′	OH	6.32	—	8.33	—
3′	$N-NH_2$	6.36	—	7.88	—
4″	OH	4.43	—	5.60	—

[a] The ^{1}H chemical shifts are referenced relative to the residual signal from DMSO-d_6 assigned as 2.50 ppm.

[b] The ^{13}C chemical shifts are referenced to DMSO-d_6 assigned as 39.5 ppm.

[c] The ^{1}H chemical shifts are referenced relative to the middle residual signal from pyridine-d_5 assigned as 7.55 ppm.

[d] The ^{13}C chemical shifts are referenced to the middle signal from pyridine-d_5 assigned as 135.5 ppm.

*Not accurate due to broad or overlapping signals.

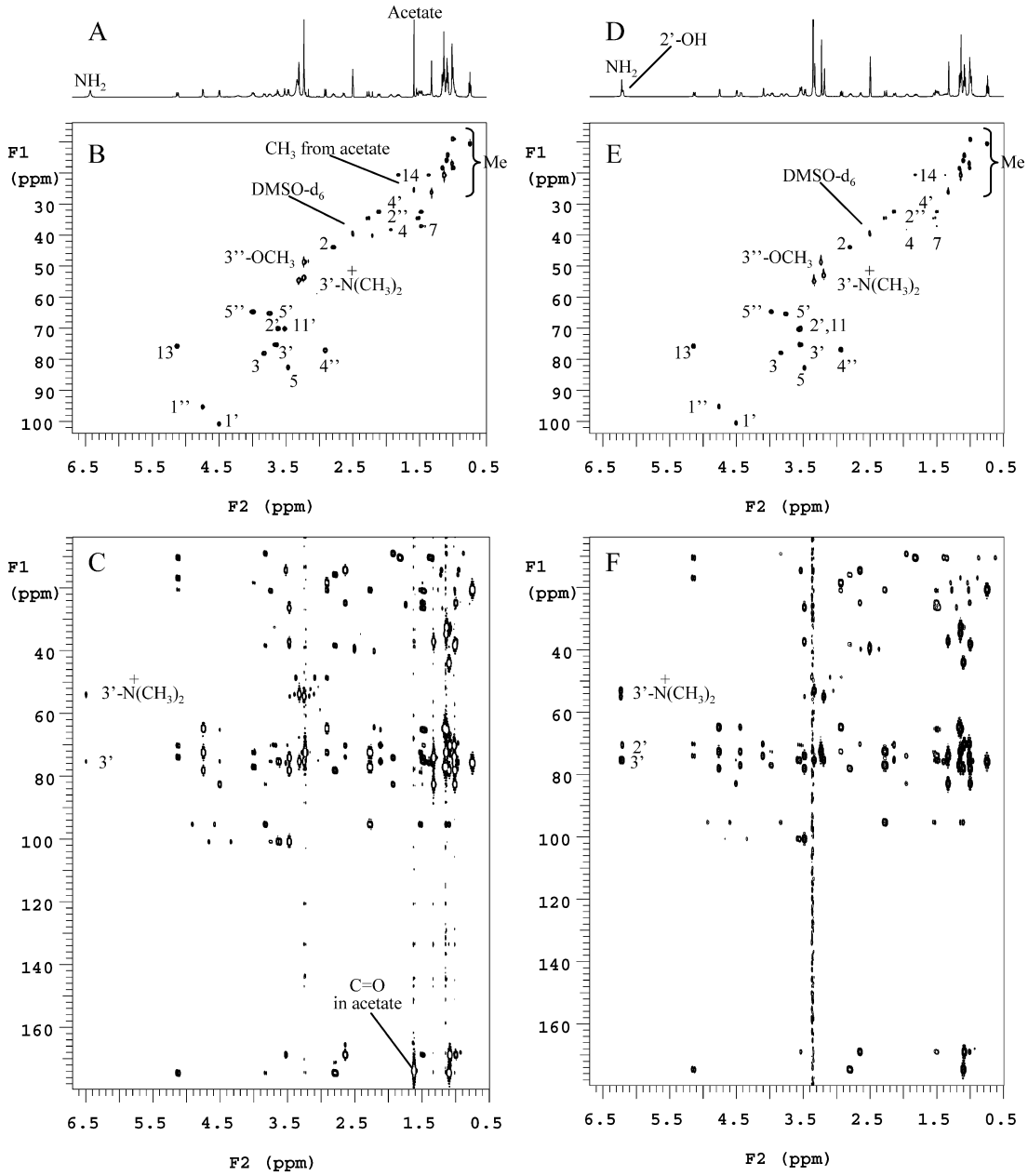
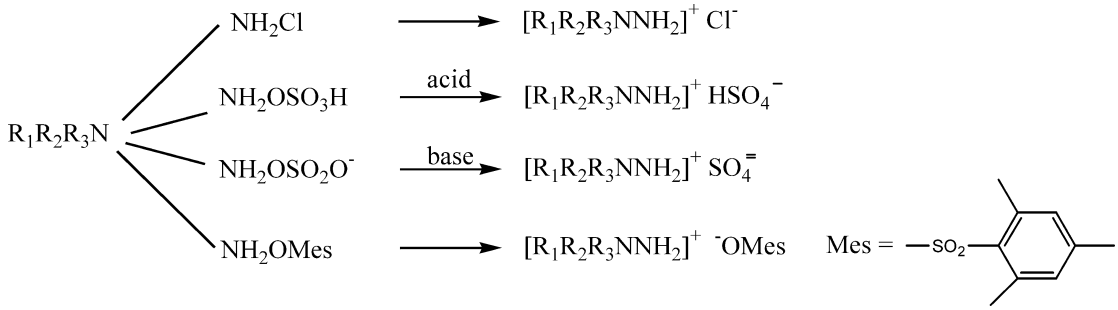


Fig. 2 NMR spectra in DMSO-d_6 of compound **1** (A) ^{1}H, (B) g-HSQC, (C) g-HMBC and compound **2** (D) ^{1}H, (E) g-HSQC, and (F) g-HMBC. Most of peaks in g-HSQC are labeled except for the methyl groups, which are too crowded to display the labels (see Table 1 for the assignments) and some selected peaks of interest are labeled in the ^{1}H and g-HMBC spectra.

case of compound **2**, the proton and carbon signals of the acetate anion were not observed in the spectra, and EDS indicated that chloride was present in the sample. Therefore, we have identified compound **2** as the chloride salt of the parent compound shown in Fig. 1. Further experiments proved that acetate can be replaced by the chloride anion when the compound is placed in a chloride solution during the purification process.

In DMSO-d_6 solutions, the oxime group in both compound **1** and **2** was determined to be in the *E* configuration (where the oxime's hydroxyl group is *syn* to C8). The identifcation of the *E* configuration was supported by the downfield proton shift of H8 (~3.6 ppm), the downfield carbon shift of 10-CH_3 (~15 ppm), the upfield proton shift of 8-CH_3 (~1.0 ppm), and the upfield carbon shift of C8 (~25 ppm), resulting from shielding/deshielding effects based on steric interactions with the *cis*-hydroxyl group of the oxime. The assignments



Scheme 2.

are consistent with the previous reports on oxime conformations [5, 13, 14].

Possible Reaction Mechanism

Several methods have been reported for the preparation of 1,1,1-trisubstituted hydrazinium salts of tertiary amines [15~17]. One well known method is the reaction of chloramine with tertiary amines which provides the corresponding 1,1,1-trisubstituted hydrazinium chlorides in good yield [15]. It is believed that the mechanism of this reaction involves an acid-base interaction, in the Lewis sense, with chloramine being the electrophilic reagent. This reaction can be further modified with other reagents like hydroxylamine-*O*-sulfonic acid [16] and NH_2OMes [17], as shown in Scheme 2. All the NH_2X compounds in this scheme, where X represents an electronegative group, are efficient amination reagents. In these cases, X is also a very good leaving group. The reaction of NH_2OMes with tropinone, which contains both a tertiary amine and a ketone group, gives rise to the *N*-amine salt only. It appears that, in this case, *N*-amination proceeds faster than oxime formation.

In our case, where NH_2OH was used as an amination reagent, the hydroxyl group is not a good leaving group and therefore the formation of 1,1,1-trisubstituted hydrazinium is not as efficient as it would be with the other reagents mentioned above. In fact, in this reaction, the oximation proceeds much faster than the *N*-amination reaction. That explains why the impurity is observed only in the presence of excess amounts of NH_2OH, or with prolonged reaction times and high reaction temperatures. Also trace amounts of NH_2OAc could be formed under the reaction conditions and this might be the active reagent for the formation of this hydrazinium.

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