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Synthesis and Characterization of Cephalosporin Antibiotics

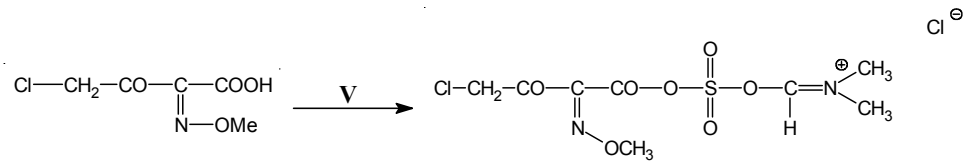
SARITA SHRIVASTAVA and DANTU MURALIKRISHNA*†
Department of Chemistry, Government Motilal Vigyan Mahavidyalaya, Bhopal-462 016, India
E-mail: dmuralikrishna@lupinpharma.com

In present studies, the synthesis, characterzation and stability of cephalosporin antibiotics are described.

Key Words: Synthesis, Cephalosporin antibiotics.

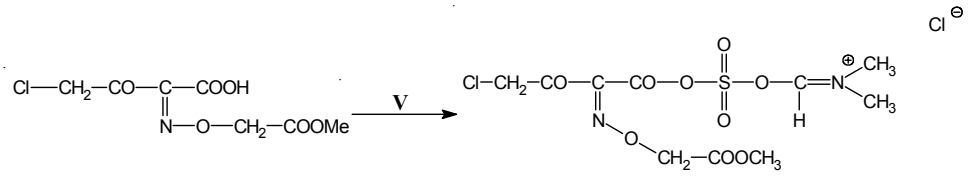
INTRODUCTION

Cephalosporins are considered as highly effective antibiotics with low toxicity and can be used for treatment of variety of bacterial infections[1]. Many of these compounds have been synthesized and reported in the literature[2-8]. The compounds, 4-chloro-2-methoxyimino-3-oxobutyric acid-N,N-dimethyl formiminium chloride chlorosulfate (**I**) and 4-chloro-2-methoxy carbonyl methoxyimino-3-oxobutyric acid-N,N-dimethyl formiminium chloride chlorosulfate (**II**). However the experimental details, stability and the spectral data were not reported therein for the compounds **I** and **II**. Present work deals with the preparation, stability of the title compounds of cephalosporin antibiotics and subsequent study of their use in the synthesis of cephalosporin antibiotics. Therefore, it is reported herein the preparative methods, stability and the spectral data of these compounds. Also the stability of these compounds is confirmed by converting to corresponding cephalosporin antibiotics after prolonged storage. The processes for preparation of the antibiotics, ceftriaxone sodium and cefixime are also described herein utilizing these compounds **I** and **II**. The compound, 4-chloro-2-methoxyimino-3-oxobutyric acid N,N-dimethyl formiminium chloride chloro sulfate (**I**) is an useful intermediate for the preparation of antibiotic, ceftriaxone sodium and is prepared from 4-chloro-2-methoxyimino-3-oxobutyric acid (**III**) and the compound, 4-chloro-2-methoxy carbonyl methoxyimino-3-oxobuyyric acid-N,N-dimethyl formiminium chloride chlorosulfate (**II**) is an useful intermediate for the preparation of antibiotic, cefixime and is prepared from 4-chloro-2-methoxy



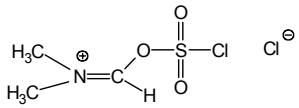
III **I**

†**Present address:** General Manager (R&D), Lupin Ltd., Mandideep-462 046, India.



IV **II**

carbonyl methoxyimino-3-oxo-butyric acid (**IV**), by activation with the compound (**V**), that obtained from the action of sulfuryl chloride and dimethyl formamide.



V

The compounds have been kept at -10 ºC and studied the purity on storage up to 120 h at this temperature. Also studied the stability of these compounds on reaction with water. Further the compounds have been analyzed by ^{1}H NMR, mass, IR and the data have been included herein.

EXPERIMENTAL

Preparation of 4-chloro-2-methoxyimino-3-oxobutyric acid- N,N-dimethyl formiminium chloride chlorosulfate (I): 10.47 g (0.143 mol) of dimethyl formamide were added to the mixture of 19.3 g (0.143 mol) of sulfuryl chloride and 16 mL methylene chloride slowly in 0.5 h at -20 to -10 °C. The temperature of the mixture was slowly raised to 20 °C and was stirred for 2 h at 20-22 °C. Further 63 mL of dichloro methane were added and settled for the separation of reagent N,N-dimethyl formiminium chloride chlorosulfite (**V**) as bottom layer. The compound **V** was added to the mixture of 20 g (0.111 mol) of 4-chloro-2-methoxyimino-3-oxobutyric acid (**III**) and 160 mL of dichloromethane in 0.5 h at -25 to -15 °C. Then the temperature of the reaction mixture was raised slowly and stirred for 2.5 h at 5 to 10 °C to obtain the compound **I** with 97.15 % purity by HPLC.

^{1}H NMR in DMSO-d_6 (400 MHz): δ (ppm), 2.71 (3H, s, =N-CH_3); 2.87 (3H, s, =N-CH_3); 4.05 (3H, s, =N-O-CH_3); 4.87 (2H, s, $ClCH_2$); 7.93 (1H, s, CH=N); (IR ν_{max}, cm^{-1}): 1715 (CH_2C=O); 1782 (C=O-O); 1584 (C=N); 2951 (N-$(CH_3)_2$; Mass (ESI mode) m/z: 352.1 amu.

Preparation of 4-chloro-2-methoxy carbonyl methoxyimino-3-oxobutyric acid- N,N-dimethyl formiminium chloride chlorosulfate (II): 7.91 g (0.108 mol) of dimethyl formamide were added slowly in 0.5 h at -20 to -10 °C to the mixture of 14.57 g (0.108 mol) of sulfuryl chloride and 16 mL dichloromethane. The temperature of the mixture was slowly raised to 20° and was stirred for 2 h at 20-22 °C. Further, 63 mL of dichloro methane were added and settled for the separation of reagent N,N-dimethyl formiminium chloride chlorosulfite (**V**) as bottom layer. The compound

(**V**) was added to the mixture of 20 g (0.084 mol) of 4-chloro-2-methoxy carbonyl methoxyimino-3-oxobutyric acid (**IV**) and 200 mL of dichloromethane in 0.5 h at -25 to -15 °C. Then the temperature of the reaction mixture was slowly raised and stirred for 3 h at 5 to 10 °C to obtain the compound **II** with 98.27 % purity by HPLC.

^{1}H NMR in DMSO-d_6 (400 MHz): δ (ppm), 2.71 (3H, s, =N-CH_3); 2.87 (3H, s, =N-CH_3); 3.68 (3H, s, -CO-O-CH_3); 4.82 (2H, s, $ClCH_2$); 7.93 (1H, s, CH=N); 4.96 (2H, s, N-O-CH_2). (IR ν_{max}, cm^{-1}): 1714 (CH_2C=O); 1759 (C=O-O); 1595 (C=N); 2954 (N-$(CH_3)_2$; Mass (ESI mode) m/z: 409.1 amu.

Preparation of ceftriaxone sodium: 29.0 g (0.179 mol) of hexamethyl disilazane and 4.3 g (0.038 mol) of trimethyl chloro silane were added to the suspension of 39.2 g (0.105 mol) of **VIII** and 600 mL of dichloromethane and were refluxed for 8 h for completion of silylation. The silylated (**VIII**) was added to the compound that obtained from (i), above in 0.5 h at -80 to -50 °C. Then 16.62 g (0.137 mol) of dimethyl aniline was added as base in this reaction. Reaction was monitored by HPLC. After completion of reaction, 315 mL of water and 157 mL of THF were added at room temperature and stirred, separated the organic layer which contains the intermediate product. 9.65 g (0.126 mol) of thiourea and 8.86 g (0.105 mol) of sodium bicarbonate were dissolved in 80 mL of water and added in 0.5 h to mixture of the above organic layer and 235 mL water and stirred for 1 h at 5 to 10 °C and at pH around 5.5 adjusting with sodium bicarbonate solution. Reaction was stirred for 2 to 3 h monitoring by HPLC. After completion of the reaction, the aqueous layer was separated and treated with activated carbon and was filtered. To the filtrate 140 mL of ethyl acetate and 31 mL of isopropyl alcohol were added and adjusted the pH to 2.8 gradually by formic acid to obtain white solids of ceftriaxone acid. The product was filtered and dried at 45 °C for 4 h under reduced pressure. 31.5 g of ceftriaxone acid was obtained (51.25 % molar yield).

20 g (0.036 mol) of above acid was dissolved in 120 mL water using triethyl amine (till clarity) at pH 5.5-6.0. The solution was treated with 2 g of activated carbon at 0-5 °C, stirred, filtered and washed the carbon bed with 10 mL of water. To the filtrate, 12.85 g (0.0774 mol) of 2-ethyl sodium hexanoate solution in 800 mL of acetone were added to obtain white solids of ceftriaxone disodium at 30 ºC. The slurry was cooled to 10 ºC, stirred for 1 h at same temperature. The product was filtered, washed with acetone (50 mL) and dried under reduced pressure at 25 °C. 18.5 g (77 % molar) of ceftriaxone disodium was obtained. This material was dissolved in 45 mL of water and was treated with 1.5 g of activated charcoal, stirred at 20 ºC for 0.5 h, filtered and washed the carbon bed with 20 mL of mixture of water and acetone (1:1) and the filtrates were combined. 270 mL of acetone was added to this solution slowly in 0.5 h to precipitate out the material. The mixture was cooled to < 10 ºC, stirred for 1 h and was filtered, washed with 60 mL of acetone. The product was dried at 30 ºC to obtain 16.7 g (92 % molar) of pure ceftriaxone disodium.

Characteristics: Off white crystalline powder; Purity: 98.6 %; Specific rotation: -158º; (IR ν_{max}, cm^{-1}): 3276 (-NH); 1741 (-C=O of lactum); 1604 (-C=N oxime);

1650 (CONH); Mass (m/z)-amu: 554.8 $(M+H)^+$ and 576.8 $(M+Na)^+$; ^{1}H NMR in DMSO-d_6: δ, 9.53-9.46 (1H, d, CO-NH); 7.22 (2H, s, NH_2); 6.72 (1H, s, -CH-of thiazole); 5.55-5.49 (1H, m, -CH-C of lactum); 4.98-4.96 (1H, d, -CH-S of lactum); 4.43-4.37 (1H, d, -CH_2-S); 4.13-4.07 (1H, d, -CH_2-S);); 3.81 (3H, s, N-O-CH_3); 3.47-3.43 (2H, ABq, -S-CH_2); 3.53 (3H,s,-N-CH_3).

Preparation of cefixime: 10.27 g (0.0637 mol) of hexamethyl disilazane and 7.1 g (0.0653 mol) of trimethyl chlorosilane were added to the suspension of 18 g (0.079 mol) of **IX** and 600 mL of dichloromethane in a 1 L round bottom glass vessel equipped with a condenser and were refluxed for 3 h for completion of silylation. The silylated compound was added slowly to the compound **II**, that was obtained above from (ii) in 15-30 min at -70 to -45 °C. Further 0.58 g (0.006 mol) of N-methyl morpholine was added as base in this reaction. Reaction was stirred at -20 to -15 ºC and monitored by HPLC. After completion of reaction, 180 mL of water was added and stirred for 20 min at 10-15 ºC. Further the mixture was cooled to 5 ºC and the intermediate product was filtered, washed with dichloromethane (90 mL) followed by washing with water (90 mL). The product was slurried in water (90 mL) and filtered to obtain 33.3 g of wet material. The wet material was suspended in water (185 mL) and cooled to 10-15 ºC and adjusted the pH to ~5.5 by 10 % sodium bicarbonate solution. 9.5 g (0.125 mol) of thiourea was added and raised the temperature to 30-35 ºC. The reaction mixture was stirred for 2 h and simultaneously maintained the pH around 5.5 with 10 % aqueous bicarbonate solution. After completion of the reaction, the mixture was treated with activated carbon (10 g) with a pinch of EDTA and stirred for 0.5 h at room temperature and filtered, washed the carbon bed with 40 mL of water. The temperature of the filtrate (solution) was raised to 55 ºC and adjusted the pH to 2.3 by 15 % solution to precipitate out the product. The slurry was cooled to 5 ºC, stirred and filtered, washed with 300 mL of water. This product was carried forward to subsequent reaction without drying. The wet cake is suspended in 300 mL water and cooled to < 5 ºC and added chilled solution of sodium hydroxide (*ca.* 8.5 g in 175 mL water) to pH 13 and agitated for 10 min at < 5 ºC. Further the mixture was acidified to 4.8-5.2 pH by dilute HCl. Then to the aqueous solution acetone (180 mL) was added and gradually pH was brought down to 2.5 at *ca.* 30 ºC to precipitate out the material slowly in 15-20 h. Further mixture was cooled to 5 ºC and the slurry was filtered, washed with water (20 mL) and dried the pure cefixime at 40 ºC under reduced pressure. 9 g of cefixime were obtained (22.5 % molar) which was further purified. The above material was suspended in 75 mL of water and dissolved at pH 5.8-6.0 by dilute ammonia at < 5 ºC. The solution was treated with 1 g of activated charcoal and a pinch of EDTA, stirred for 0.5 h and was filtered. The carbon bed was washed with 10 mL of water and was mixed with the main filtrate. The solution was added to the mixture of 180 mL of ethanol and 150 mL of water at < 5 ºC. Then pH of the mixture was adjusted to 2.5 by dilute HCl to isolate white solid material. The product was filtered and washed with 15 mL of water. The product was dried at 40 ºC till the water content was between 10-12 ºC to obtain 7.65 g (85 % molar) of pure cefixime trihydrate (**VII**).

Characteristics: Light yellowish white crystalline powder; Purity: 99.5 %; Specific rotation: - 83.8°; (IR ν_{max}, cm^{-1}): 3562 (-OH); 3296 (-NH); 1770 (-C=O of lactum); 1737 (-C=O of carbamate); 1670 (CONH); 1591 (oxime C=N); Mass (m/z)-amu: 454 $(M+H)^+$ and 476 $(M+Na)^+$; ^1H NMR in DMSO-d_6: δ, 9.61-9.57 (1H, d, CO-NH); 7.29 (2H, s, NH_2) 6.98-6.89 (1H, dd, -CH=); 6.81 (1H, s, -CH-of thiazole); 5.84-5.78 (1H, dd, -CH-C of lactum); 5.63-5.54 (1H, d, -CH of vinyl); 5.34-5.28 (1H, d, CH of vinyl); 5.21-5.15 (1H, d, -CH-S of lactum); 4.60 (2H, s, -N-O-CH_2); 3.88-3.45 (2H, ABq, -S-CH_2).

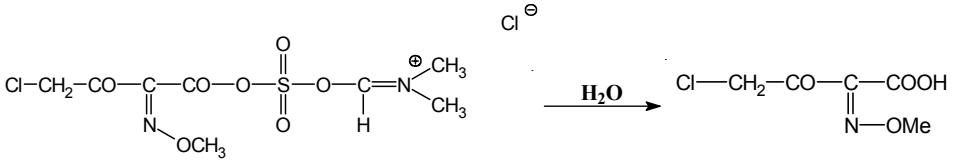
RESULTS AND DISCUSSION

The present work reports the study of stability and spectral analysis of title compounds. In brief, the study reveals that these compounds are stable at -10 ºC. No significant drop in purity was observed on storage up to 120 h at -10 ºC under dry atmospheric conditions (Table-1). The samples are analyzed by high pressure liquid chromatography (HPLC) using C18, 4.6 mm × 150 mm, 5 µ (Inertsil ODS) column. However these compounds are sensitive to moisture and can get hydrolyzed on treatment of water yielding to corresponding acid. This has been confirmed by reaction of compound (**I**) with water. Compound **I** was treated with water (Fig. 1) and the hydrolyzed compound was extracted in dichloro methane. The solution was concentrated to strip out the solvent. Further on addition of xylene and cooling to very low temperature (-10 ºC) the corresponding acid was isolated. The isolated acid has been tested by IR, NMR and Mass. The PMR spectra of hydrolyzed compound shows singlet due to 2H proton of $ClCH_2$ at 4.61 ppm, singlet due to $NOCH_3$ at 4.18 ppm and a singlet at 10.26 ppm due to proton of carboxylic acid group and confirms as acid. Appearance of signal at 3398 cm^{-1} due to hydroxyl functionality in FTIR and the mass (m/z) of 179.1 further confirms the compound as acid.

TABLE-1
STABILITY ON STORAGE

Compound	At 0 h (%)	After 120 h (%)
I	96.79	96.41
II	98.33	98.09

Reaction with water:



Compound (**I**) Acid

Fig. 1.

Conversion to cephalosporin antibiotics: The compound **I** was stored for 120 h at -10 ºC and converted to the antibiotic, ceftriaxone sodium (**VI**). The synthetic scheme (Fig. 2) is outlined hereunder.

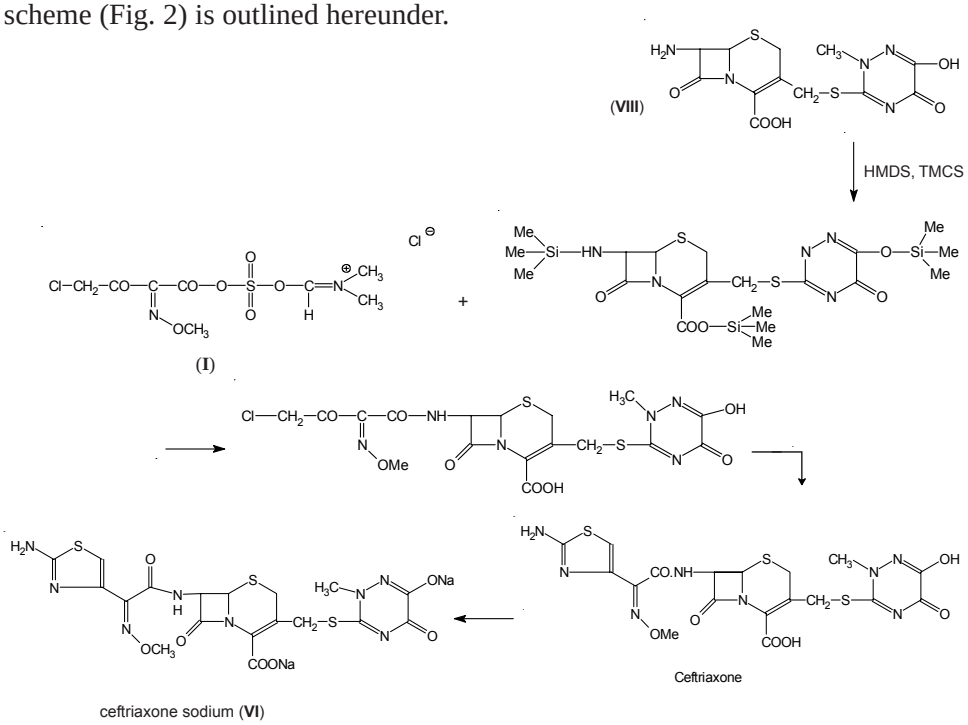


Fig. 2. Synthesis of ceftriaxone

In similar way the compound, **II** was stored for 120 h at -10 ºC and converted to the antibiotic, cefixime (**VII**). The synthetic scheme for cefixime is outlined in Fig. 3.

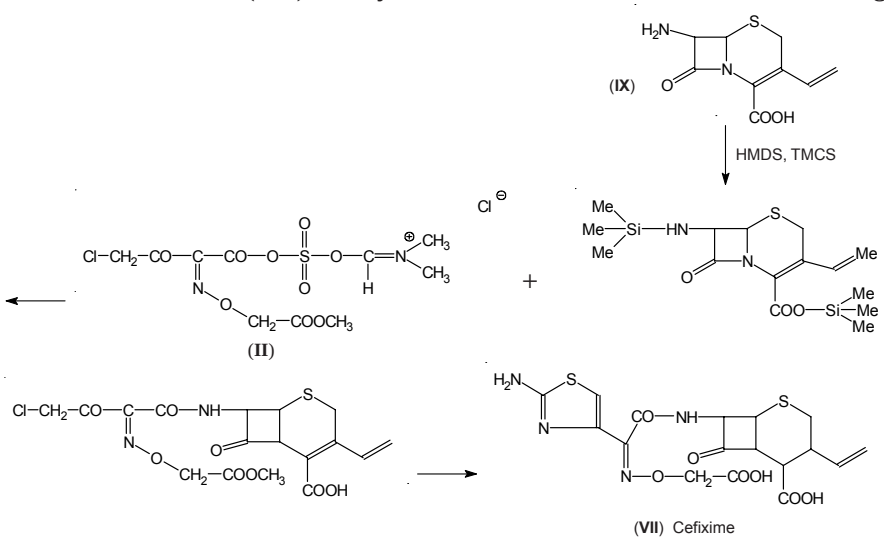


Fig. 3. Synthesis of cefixime

Conclusion

This study concludes that the compounds **I** and **II** are stable at -10 ºC even up to 120 h and further supported by spectral analysis and conversion to the antibiotics, ceftriaxone sodium and cefixime trihydrate, respectively as evidenced by experimental results.

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