1 This research was assisted by a grant from the National Science Foundation.
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0014-4754/83/101084-02$1.50+0.20/0

Stability of ceftizoxime in aqueous solution

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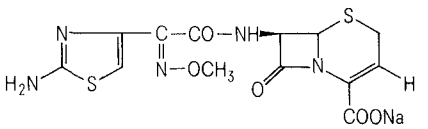
Summary. Ceftizoxime, when assayed using the disc agar-diffusion method with *Bacillus subtilis* ATCC 6633 as the indicator microorganism in buffered (pH 6) medium, retains its potency in aqueous solution at 4 °C for about 3 months. Even after 4 months, some residual activity can be found. However, when this solution was assayed on the non-buffered medium, a seemingly quicker but misleading loss of potency was observed. This resulted from inadequate sensitivity of the assay. The sensitivity of the ceftizoxime assay was increased by lowering the incubation temperature from 37 °C to 30 °C. The pH- and incubation temperature-dependent potency must be kept in mind when assaying ceftizoxime.

Ceftizoxime is a member of the third generation parenteral 2-amino-4-thiazolyl-α-methoxyimino-acetyl cephalosporins. It is unique since it contains only 1 H-atom at the 3-position of the β-lactam-dihydro-thiazine nucleus as shown in the figure 1.



Chemical structure of ceftizoxime.

Ceftizoxime possesses a wide spectrum of antibacterial activity with an unusually great potency and a high degree of β-lactamase stability[1]. The compound has an excellent therapeutic application and safety record. Therefore, it is important to make its bio-assay at optimal conditions. In this report, the results of the study of its stability in aqueous solutions employing such optimal conditions is communicated.

Materials and methods. A solution of ceftizoxime containing 10 µg/ml was prepared by dissolving 2.5 mg in 250 ml of deionized water. This stock-solution was kept at 4 °C throughout the study. Initially and later at selected times between August 15 and December 12, 2-fold dilutions were made from the stock-solution to obtain concentrations of 5, 2.5, 1.25, 0.6 and 0.3 µg/ml. These were assayed using the disc (6.35 mm diameter, Schleicher-Schuell Inc.) agar-diffusion method. Both commercial 'Penseed' agar and 'Penseed' agar buffered to pH 6.0 with phosphate buffer were seeded with the appropriate dilutions of *B. subtilis* ATCC 6633 spores. Prior to assay, discs saturated with the appropriate dilutions of ceftizoxime were placed on the surface of the seeded agar plates as described earlier[2]. The plates were incubated overnight at 37 °C, but on 2 occasions were additionally incubated at 30 °C. The diameters of the inhibition zones were measured and recorded. In all assays and for each dilution, 3 discs were used and the data given in the tables represent the average values of the inhibition zone diameters of the 3 discs.

Results and conclusion. The table represents the average diameters (mm) of the inhibition zones for each concentration used (5, 2.5, 1.25, 0.6, and 0.3 µg/ml). Over the study period of 2 months, the inhibition zone sizes (a reflection of activity) did not change markedly, thus reflecting maintained stability. After 2 months, inhibition zone sizes diminished slowly, and after 4 months, diminished at a faster rate. For assay purposes (*B. subtilis* ATCC 6633), the buf-

Time related stability of ceftizoxime (µg/ml) in aqueous solution under various assay conditions

Date of assay	Diameter of inhibition zones in mm																			
	pH 6, 37 °C					Non-buffered 37 °C					pH 6, 30 °C					Non-buffered, 30 °C				
	5*	2.5*	1.25*	0.6*	0.3*	5*	2.5*	1.25*	0.6*	0.3*	5*	2.5*	1.25*	0.6*	0.3*	5*	2.5*	1.75*	0.6*	0.3*
8/15	23	20	16.5	13.4	10	21.5	15.5	11.5	9	0										
8/16	23	19.5	16.5	14	9	20.5	16.5	13	9	0	28.5	25	20	17.5	14	29.5	25.5	21.5	17.5	11.5
8/27	23.4	22	18.5	15.5	9	20.5	18	14.5	10.5	0										
9/4	24.5	22	18.5	15.5	11	22	18.5	14	11	0										
9/13	24.5	22	18	14	10.5	19	16	12.5	9	0										
9/17	24.5	21.5	18	14.5	11.5	19	16.5	11.5	7.5	0										
10/15						20	16.5	13.5	7.5	0										
10/18	22.5	20	16.5	12.5	7.5	21	18	12.5	7.5	0										
10/30	24.5	21.5	18	12.5	7	20.5	17	12.5	6.5	0										
11/8	22.5	20	18	13	9	15.5	12	9	0	0	27	23	21	18	12	18	13	9	0	0
12/12	19	15.5	11	0	0	18	13	10	0	0										

* Ceftizoxime, µg/ml.

fered (pH 6) medium is preferred over the non-buffered one. The data also indicate that 30 °C is also preferred for incubation than 37 °C. If the assay is performed at optimal conditions (slightly acidic medium and at 30 °C incubation) diluted solutions of ceftizoxime retain their original potency at 4 °C for 3 months. In keeping with this premise, ceftizoxime was found to be a very stable cephalosporin in aqueous solution for a long period of time.

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0014-4754/83/101085-02$1.50+0.20/0

Occurrence of 3-methoxy octadecanoic acid in yeast lipid[1]

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Summary. The fatty acid composition of a new strain of the yeast *Rhodotorula glutinis,* grown in molasses, has been studied and found to contain palmitic, stearic, oleic, linoleic and linolenic acids, and small amounts of other constituents. In addition, 3-methoxy octadecanoic acid has been shown to be present in the glycolipid fraction.

The fatty acid compositions of numerous fungal species have been described since the introduction of gas chromatography. Besides the typical fatty acids such as palmitic, stearic, oleic, linoleic and linolenic acids, unusual fatty acids with hydroxy, keto and epoxy functions have also been reported[5].

Several yeasts and yeast-like fungi produce extracellular hydroxy or acetylated long chain fatty acids. For example, 3-D-hydroxy-palmitic acid has been identified as a product of *Saccharomyces malanga*[6]. Acetylated fatty acids such as 8,9,13-triacetoxydocosanoic and 13-oxo-8,9-diacetoxydocosanoic acids have been isolated as products of a yeast-like fungus closely related to *Torulopsis fujisanesis.* Partially acetylated and esterified 8,9,13-trihydroxydocosanoic acid has been isolated from a *Rhodotorula* species[7]. The presence of 17-L-hydroxyoctadecanoic and 17-L-hydroxyoctadecenoic acids have been confirmed in the extracellular glycolipids from *Torulopsis magnaliae*[8] and 13-hydroxydocosanoic was found in the extracellular glycolipid of *Candida bogoriensis*[9]. Recently, 7-methoxytetradec-4(E)-enoic acid[10] and 7-methoxy-9-methylhexadeca-4(E), 8(E)-dienoic acid[11] have been isolated from the marine blue gree alga *Lyngbya majuscula.*

A preliminary study in a search for a yeast producing high levels of oil led to a new isolate of *Rhodotorula glutinis* from soil[12]. Besides the typical fatty acids, this oleaginous yeast strain contained a novel methoxy fatty acid in the glycolipid fraction. The present report describes the production, isolation and characterization of this hitherto unreported acid.

Materials and methods. The primary criterion for selecting the yeast isolate used in this study was its ability to survive and metabolize under nitrogen-deficient conditions, producing fat in high yield[13]. Characterization of the genus and species of this red yeast isolate was carried out according to the procedure of Van der Walt[14]. Finally, the strain was

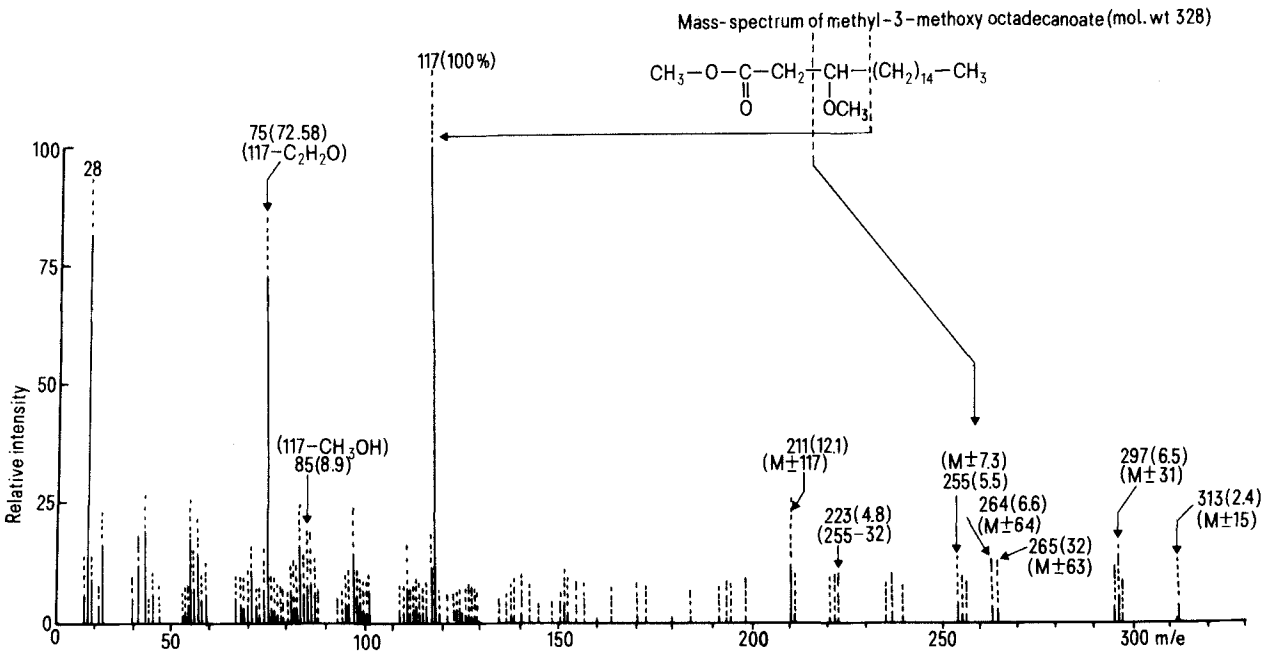

Figure 1. Gas chromatograms of the methyl esters of the fatty acids of the glycolipid fraction of *R. glutinis* lipid, on 10% DEGS column. Oven temperature, 180 °C; detector and injection port temperatures, 250 °C. Nitrogen flow rate, 60 ml/min.