AN IMPROVED PREPARATION OF DESACETYLCEPHALOGLYCIN

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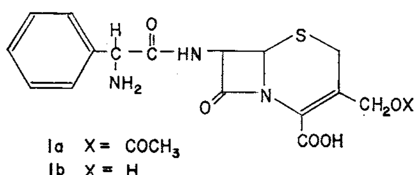
It has been shown by WICK and coworkers[1] that cephaloglycin, 7[(D-α-amino-α-phenyl) acetamido]-3-acetoxymethyl-3-cephem-4-carboxylic acid, **Ia**, is converted, in part, in man to a new biologically active compound, desacetylcephaloglycin, 7[(D-α-amino-α-phenyl)acetamido]-3-hydroxymethyl-3-cephem-4-carboxylic acid, **Ib**. It was also claimed[1] that **Ib** is in fact the predominant therapeutic agent in treating urinary tract infections with oral cephaloglycin. Earlier, KUKOLJA[2] reported the preparation of **Ib** by enzymatic hydrolysis of **Ia**. The compound was then isolated by a lengthy and meticulous chromatography over a cellulose column at low temperature. The product was then crystallized from acetonitrile-water, the yield of which was not reported. This method gave only limited amounts of **Ib**.[1]



We wish to report in this communication some improvements in the preparation and isolation of compound **Ib**. These modifications involve the enzymatic hydrolysis as well as the isolation procedure. Thus, the pH of the hydrolysis reaction was maintained constant at 7.0 by adding triethylamine rather than dilute sodium hydroxide. This enabled us to isolate **Ib** by methanol extraction, possibly as its triethylamine salt. This material was then chromatographed on a cellulose thick layer plate (1,000 microns). The correct zone for **Ib** on the plate was determined by isolating the material in the various zones, and examining them by tlc, bioautographs, ir and nmr. Once the appropriate zone was established, the isolation became a routine procedure since only **Ib** had to be eluted from the plate. The product was finally crystallized from water-acetonitrile in 30~40 % yield. The ir and nmr spectra were consistent with structure, and the biological activity agreed with that reported.[1,2]

Experimental Section

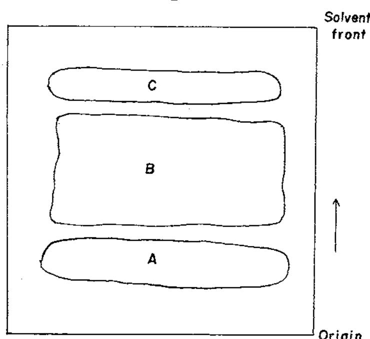
Solvents were evaporated under reduced pressure, below 26°C, in a rotary evaporator. ir spectra were obtained on a Beckman IR-5 Spectrophotometer in KBr. Nmr spectra were taken on a Perkin Elmer R12B Spectrometer. For tlc, Avicel F (250 microns), prescored, Uniplate® plates, (Analtech, Inc.) were used. For thick layer chromatography Avicel F (1,000 microns, 20×20 cm) Uniplate® plates (Analtech, Inc.) were used. In both cases the spots or zones were detected by uv.

Citrus acetylesterase was prepared according to the method described by JANSEN and coworkers[3] as modified by JEFFERY and coworkers.[4] Paper strip chromatography was done on Whatman #1 1/2″ paper strips, and developed descendingly in a solvent system of *n*-BuOH-EtOH-H_2O (8:2:10, upper phase). The chromatograms were bioautographed on agar plates seeded with *Bacillus subtilis* ATCC 6633.

Enzymatic hydrolysis of cephaloglycin: Cephaloglycin, one gram, was dissolved in 100 ml of deionized H_2O and the pH of the solution was adjusted to 7 with triethylamine. To this was added 30 ml of enzyme solution and the volume made up to 1,000 ml with deionized H_2O. The reaction mixture was placed in a water bath at 37°C and stirred constantly for 13 hours. The pH was maintained at 7.0 throughout by adding triethylamine. The reaction was also monitored by paper strip chromatograms. At the end of this period the solution was lyophilized.

Isolation of desacetylcephaloglycin: The freeze-dried material, 1.5 g, was suspended in 200 ml of methanol and the mixture was stirred for 20 minutes. The solution was filtered, and the solvent was evaporated. The residue was triturated with ether to yield

Fig. 1.



950 mg of a brown glass. The ir of the material showed a strong absorption at 1760 cm^{-1}, indicating the presence of a β-lactam. A solution of 75~100 mg of this material in 0.4 ml of 0.1 N acetate buffer, pH 5.25, was applied to a thick layer plate. The plate was then developed at room temperature with a freshly prepared solution of $CH_3CN - H_2O$, 75 : 25. This was complete after approximately 1 1/2 hour. The respective zones were located on the plate by uv (Fig. 1). In preliminary experiments zone A was identified as that of desacetylcephaloglycin. Zone B was shown by ir to contain material with a degraded β-lactam while the material in zone C originated probably from the enzyme solution. Zone A was scraped off and was eluted with 70 ml of the same solvent twice by stirring for 20 minutes. The acetonitrile was evaporated and the residual aqueous solution was freeze-dried to yield 26~30 mg of amorphous **Ib**. Crystalline material was obtained by dissolving 60 mg of crude **Ib** in 0.5 ml of H_2O. Acetonitrile was added until the solution became cloudy. The solution was chilled in ice and then triturated with a glass rod. Small amounts of CH_3CN were further added until no further crystalline material formed. After cooling for an additional hour, the solid was collected by filtration, 22 mg (36.8 %). The material had the following spectroscopical data ; ir, 1770 (β-lactam), 1695 (amide), 1595 (carboxylate) and 705 cm^{-1} (phenyl) ; nmr (D_2O-$NaHCO_3$) 7.45 (s, C_6H_5), 5.64, 5.05, (d, J=4.5, β-lactam), 5.21 (s, $CHNH_2$), 4.23 (s, CH_2OH) and 3.45 ppm (broad, S-CH_2). For analysis the sample was dried at room temperature over P_2O_5 under high vacuum. *Anal.* Calcd. for $C_{16}H_{23}N_3O_8S \cdot 3H_2O$: C 46.04, H 5.55, N 10.07. Found : C 45.69, H 5.17, N 9.64.

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