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ENZYMATIC SYNTHESIS OF AMOXICILLOIC ACIDS

M. Vorona[1], G. Veinberg[1]*, E. Liepinsh[1], H. Kažoka[1], G. Andrejeva[1], and E. Lukevics[1]

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(α*R*,2*R*,4*S*)-2-[2(*R*)-Amino-2-(4-hydroxyphenyl)acetylamino]-4-carboxy-5,5-dimethyl-2-thiazolidinyl-acetic acid (amoxicilloic acid) (**2**) is the first product of the hydrolytic clevage of the semisynthetic penicillin amoxicillin **1**, used for the treatment of bacterial infections. This explains the large number of analytical investigations on the identification of these compounds in biological solutions and industrial samples.

Analysis of literature data showed that samples of amoxicilloic acid (α*R,2R,4S*)-**2** and (α*R,4S*)-**2** for analytical and chemical investigations were obtained by alkaline hydrolysis of amoxicillin **1**, acidification or neutralization of the reaction mixture with subsequent dehidration [1, 2].

Preparative hydrolytic clevage of the β-lactam ring in natural and semisynthetic penicillins, carried out to obtain the valuable penicilloic acid **2**, is a routine problem, which is complicated in the case of amoxicillin:

a) the amphoteric properties amoxicilloic acids, caused by the presence of amino and carboxylic groups, excludes the extraction of the initial or final reaction products from aqueous solutions by organic solvents;

b) the configurational instability in acid and basic media of the 2C chiral center in (α*R,2R,4S*)-**2** which leads to the formation of the corresponding racemate (α*R,4S*)-**2** [3, 4];

c) the formation in quantity of the similar substance 2',5'-dioxopiperazine isomer of amoxicillin **3** and amoxilloic acid **4** and also other products, makes it difficult to isolate and purify the valuable product (α*R,2R,4S*)-**2**.

In this connection we have developed an effective method for obtaining analytically pure amoxicilloic acid (α*R,2R,4S*)-**2** by hydrolytic clevage of the β-lactam ring of amoxicillin **1** in the presence of the immobilized penicillinase of *Bacillus cereus* at pH 8. The constant value of the pH of the solution, decreased as the result of formation of additional carboxyl groups, is maintained by the addition of 10-15% ammonia solution. Chromatographic monitoring of the end of the reaction is indicated by the decrease in the pH of the medium and the disappearance of the amoxicillin spot. After removal of the immobilized penicillinase by filtration, the filtrate was acidified to pH 5.5 with dilute hydrochloric acid, partially evaporated and maintained at 5°C for several hours. The finely crystalline precipitate was filtered and dried in the air. According to

* To whom correspondence should be addressed, e-mail: gveinberg@osi.lv.

[1]Latvian Institute of Organic Synthesis, Riga LV-1006, Latvia.

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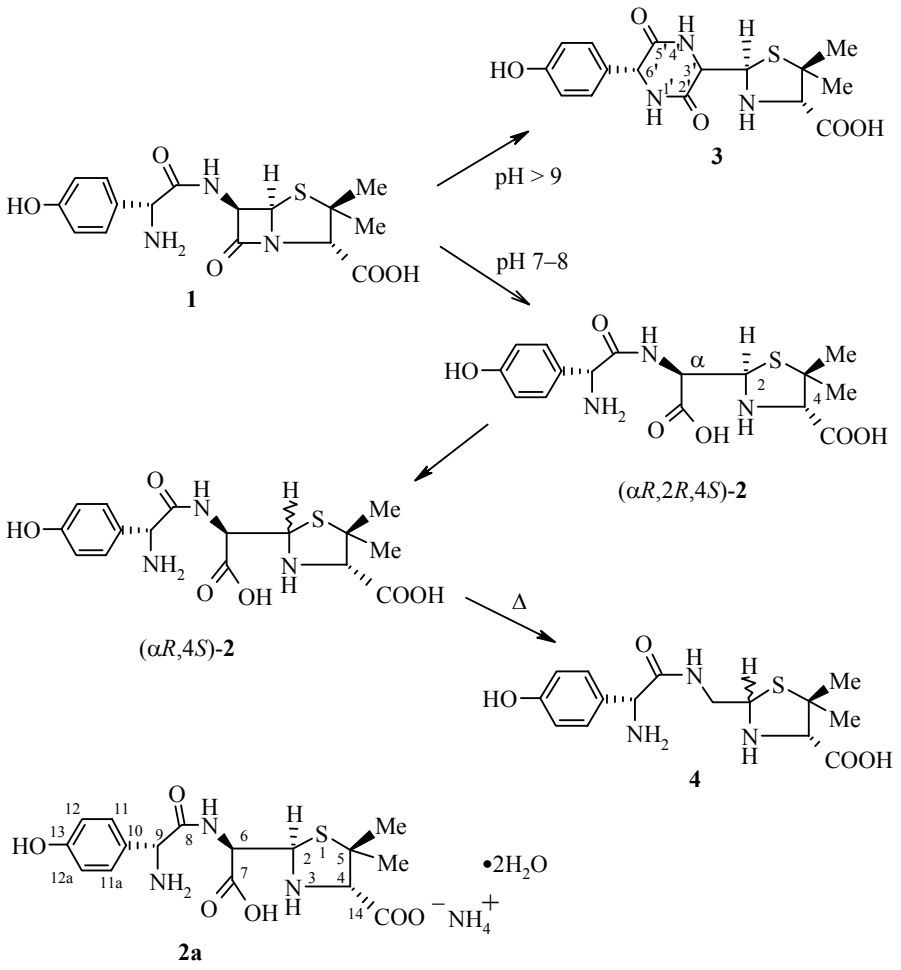
elemental analysis and derivatographic analysis, the material obtained was the ammonium salt of amoxicilloic acid **2a** with two molecules of water of crystallization, and according to HPLC data, it was >99.5% pure with no amoxicillin within the limits of the method sensitivity.



1 **3** (α*R*,2*R*,4*S*)-**2** (α*R*,4*S*)-**2** **4** **2a**

^{1}H and ^{13}C NMR spectra in D_2O solution with TMS as internal standard were recorded with a Varian 600 instrument (600 and 150 MHz respectively). Elemental analyses were carried out with a Carlo Erba 1108 analyzer. ESI-MS Mass spectra (mass spectrometer with inductively linked plasma) were recorded on a Micromass Quatro Micro^{TM} API (MeCN). The course of the reaction was monitored by TLC on Merck Kieselgel plates with a 4:1:1 *n*-butanol–water–acetic acid system, with ninhydrin development. HPLC data were obtained with a DuPont Model 8800 apparatus fitted with a UV detector (λ = 210 nm) and a (4.6×150 mm) column filled with Prevail-C18 in the acetonitrile + 0.02M KH_2PO_4 (pH 7), rate of flow 10 μl/min.

Ammonium amoxicilloate dihydrate (2a). Penicillinase of *Bacillus cereus* (Sigma) (1.0 g), immobilized on polyacrylate carrier, was added to a solution of amoxicillin trihydrate (20 g, 47 mmol) in distilled water (250 ml) in a three-necked flask, fitted with a stirrer, pH-meter, and an autotitrator, in a water bath. The mixture was stirred for 1 h at 30°C while maintaining the pH at 7.8-8.2 with 10% ammonia, monitoring the course of the reaction by TLC. The solution was filtered, the filtrate was acidified to pH 5.5 with dilute hydrochloric acid, partially evaporated, and kept in a refrigerator. The white precipitate was filtered off, washed with water and acetone, and dried in the air. Yield 11.2 g (55%); mp 187-188°C. ^{1}H NMR spectrum, δ, ppm (*J*, Hz): 0.96 (3H, s, 5-CH_3); 1.01 (3H, s, 5-CH_3); 2.93 (1H, d, ^3J = 0.6, H-4); 4.10 (1H, dd, ^3J = 1.3,

^3J = 4.7, H-6); 4.90 (1H, dd, ^3J = 1.3, ^3J = 4.7, H-2); 4.97 (1H, s, H-9); 6.80 and 7.26 (4H, two d, ^3J = 7.2, C_6H_4). ^{13}C NMR spectrum, δ, ppm: 23.54 (5-CH_3); 23.96 (5-CH_3); 54.2 (C-9); 56.4 (C-5); 57.5 (C-6); 63.5 (C-2); 73.2 (C-4); 114.4 (C-12); 122.0 (C-10); 127.9 (C-11); 155.0 (C-13); 166.8 (C-8); 173.13 (C-14); 173.54 (C-7). Mass spectrum, *m/z*: 384.1296 [MH^+]. Found, %: C 44.65; H 6.30; N 12.92. $C_{16}H_{28}N_4O_8S$. Calculated, %: C 44.03; H 6.47; N 12.84.

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