SYNTHESIS AND MOLECULAR AND CRYSTAL STRUCTURE OF 1-(5-BENZYL-2-TETRAZOLYL)-1-CYCLOPENTANECARBOXYLIC ACID. PENICILLIN AND CEPHALOSPORINS BASED ON THIS ACID

A. O. Martirosyan,[1] M. V. Aleksanyan,[1] S. S. Terzyan,[2] Yu. Z. Ter-Zakharyan,[1] R. V. Agababyan,[1] A. A. Karapetyan,[2] Sh. L. Mndzhoyan,[1] and R. A. Tamazyan[2]

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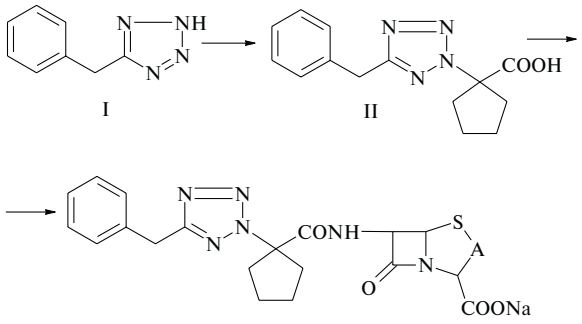
In continuation of our previous investigation devoted to the synthesis and study of the structure – activity relationship for some 7-[1-(tetrazolyl)acetamido]cephalosporanic acids [1], we synthesized 1-(5-benzyl-2-tetrazolyl)-1-cyclopentanecarboxylic acid (II) and related penicillin (IV) and cephalosporins (III, V). The synthesis of compounds III – V was of special interest because many of the known penicillins containing trisubstituted acetic acids in the side chain [2], especially some of the 6-[1-(aryl)-1-cyclopentanecarboxamido]penicillanic acids [3], are resistant to staphylococcal penicillase and active with respect to both sensitive staphylococci and resistant strains.



Acid II was synthesized by alkylating 5-benzyltetrazole (I) with 1-bromo-1-cyclopentanecarboxylic acid ethyl ester in the presence of sodium ethylate in ethanol, followed by hydrolysis of the ester group. The x-ray diffraction measurements showed that the alkylation takes place regioselectively at the second position of the tetrazole ring. The target products III – V were obtained by the chloroanhydride method involving acylation of 7-aminocephalosporanic acid (7-ACA), 6-aminopenicillanic acid (6-APA), and 7-aminodeacetoxycephalosporanic acid (7-ADCA), respectively.

The proposed structures and purity of the reaction products were confirmed by TLC and by the IR and ^{1}H NMR spectroscopic data. The IR spectra of compounds III – V contain characteristic absorption bands due to the carbonyl groups of β-lactam (1760 – 1780 cm^{-1}), acetoxymethyl (1710 – 1740 cm^{-1}), amide (1670 – 1680 cm^{-1}), and carboxy (1610 – 1625 cm^{-1}) fragments. The ^{1}H NMR spectra exhibit signals due to protons of the aromatic ring (6.9 – 7.2 ppm), benzyl CH_2 group (4.3 – 4.4 ppm), 2-CH_2 cephem nucleus (3.6 – 3.8 ppm), acetoxymethyl moiety (2.0 – 2.2 ppm), and methyl groups of penicillanic acid (1.7 and 1.8 ppm).

EXPERIMENTAL CHEMICAL PART

The data of elemental analyses (N, S) agree with the values calculated using the empirical formulas. The IR absorption spectra were recorded on a UR-20 spectrophotometer using samples prepared as nujol mulls. The ^{1}H NMR spectra were measured on a Mercury 300 spectrometer. TLC analyses were performed on Silufol UV-254 plates (Czech Republic).

1-(5-Benzyl-2-tetrazolyl)-1-cyclopentanecarboxylic acid (II). To sodium ethylate prepared from 0.46 g (0.02 mole) of metallic sodium and 20 ml of anhydrous ethyl alcohol were added 3.2 g (0.02 mole) of 5-benzyltetrazole (I) and the reaction mixture was heated to boiling. To this boiling mixture were added with stirring 4.5 g (0.02 mole) of 1-bromo-1-cyclopentanecarboxylic acid ethyl ester and boiling was con-

[1] Mndzhoyan Institute of Fine Organic Chemistry, National Academy of Sciences of the Republic of Armenia, Yerevan, Armenia.
[2] Center for Investigation of the Structure of Molecules, National Academy of Sciences of the Republic of Armenia, Yerevan, Armenia.

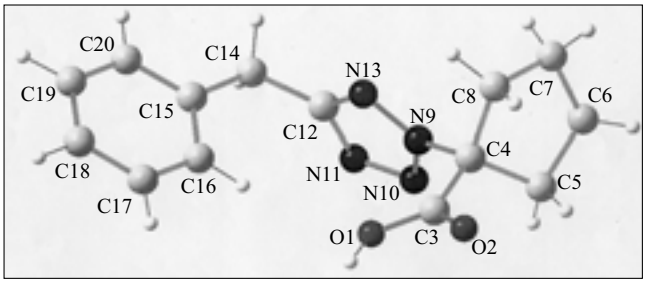


Fig. 1. Molecular structure of 1-(5-benzyl-2-tetrazolyl)-1-cyclopentanecarboxylic acid (II) by x-ray diffraction data.

tinued for 16 h. Then ethanol was evaporated and the residue was dissolved in ether and washed with a 5% $NaHCO_3$ solution. The solvent (ether) was evaporated again and the oily residue was dissolved in 15 ml of methanol. To this solution were added 10 ml of 3 M KOH solution in methanol and the mixture was boiled for 3 h. Then ethanol was evaporated and the residue mixed with water and hydrochloric acid to pH 2. The precipitated crystals were separated by filtration and recrystallized from ethanol to obtain 3.2 g (59%) of acid II; m.p., 124 – 125°C; R_f, 0.5 (acetone – hexane, 1 : 2).

7-[1-(5-Benzyl-2-tetrazolyl)-1-cyclopentylcarbamoyl] cephalosporanic acid (III). A mixture of 2.7 g (0.01 mole) of 7-ACA, 2.5 g (0.03 mole) $NaHCO_3$, 80 ml water, and 60 ml acetone was cooled to 0 – 2°C. To this mixture was added with stirring a solution of 2.7 g (0.01 mole) acid I in 20 ml of acetone. The mixture was stirred for 4 h and allowed to stand overnight in a refrigerator. Then acetone was evaporated in vacuum and the aqueous solution washed with ethyl acetate. To this aqueous solution were added 50 ml of ethyl acetate and 1 N HCl solution to pH 2. The organic layer was separated, washed with glacial acetic acid, and dried over anhydrous sodium sulfate. Finally, ethyl acetate was evaporated and the residue crystallized from hexane or petroleum ether to obtain compound III.

Compounds IV and V were obtained by analogous procedures from 6-APA and 7-ADCA, respectively (Table 1).

Sodium salts of acids III – V. To an ethyl acetate solution of acid III (IV, V) was added an 8% aqueous solution of $NaHCO_3$ to pH 6 – 7. The aqueous layer was separated, washed with ethyl acetate, and lyophilized. Table 1 gives the R_f values of the sodium salts determined in a propanol – water system (7 : 2).

TABLE 1. Yields and Physicochemical Characteristics of Tetrazolyl Penicillanic and Cephalosporanic Acids III – V

Compound	Yield, %	M.p., °C (with decomp.)	Empirical formula	R_f
III	54	88 – 90	$C_{24}H_{26}N_6SO_6$	0.69
IV	81	78 – 80	$C_{22}H_{26}N_6SO_4$	0.66
V	50	103 – 105	$C_{22}H_{24}N_6SO_4$	0.79

Note. R_f values refer to the corresponding sodium salts.

EXPERIMENTAL BIOLOGICAL PART

The antibacterial properties of the sodium salts of acids III – V were studied by the method of double serial dilutions in a meat-infusion broth (pH 7.2 – 7.4) with a load of 2×10^6 microbial cells per ml. The tests were performed with Gram-positive staphylococci (standard strains 209p, Smith, and penicillinase-forming strain 18b) and Gram-negative bacilli (*E.coli*, *B. typhi*, *Sh. dysenteriae*). The activity was characterized by the minimum inhibiting concentration (MIC).

It was found that compound IV exhibits activity with respect to staphylococci (MIC = 0.1, 0.05, and 31.2 μg/ml for the strains 209p, Smith, and 18b, respectively) and inhibits the growth of *Sh. dysenteriae* with a MIC = 100 μg/ml, while being inactive toward *E.coli* and *B. typhi*. Compounds III and V were significantly less active than IV.

X-Ray diffraction study of 1-(5-Benzyl-2-tetrazolyl)-1-cyclopentanecarboxylic acid (II). The monoclinic unit cell parameters of the first modification, measured at room temperature on an Enraf-Nonius CAD-4 automated diffractometer and refined using 24 reflections with $14 < \vartheta < 18°$, are as follows: $a = 6.008(1)$ Å, $b = 10.900(2)$ Å, $c = 20.898(4)$ Å; $\beta = 96.99(3)°$; $V = 1358.3(5)$ Å^3. A diffraction experiment performed on the same instrument included the measurement of 3211 reflections with $1 > 2\sigma(I)$ for $-7 \le h \le 7$, $0 \le k \le 12$,

TABLE 2. The Coordinates of Nonhydrogen Atoms in the First Structural Modification of 1-(5-Benzyl-2-tetrazolyl)-1-cyclopentanecarboxylic Acid (II) (see Figure 1)

Atom	x/a	y/b	z/c	$U_{eq.}$, Å^2
O1	0.5280(3)	0.45258(14)	0.20846(7)	0.0612(4)
O2	0.5895 (4)	0.5560(2)	0.12191(9)	0.1100(8)
C3	0.5124 (4)	0.4702 (2)	0.14663(10)	0.0537(5)
C4	0.3890(3)	0.3687(2)	0.10629(8)	0.0418(4)
C5	0.5410(4)	0.3026(2)	0.06402(11)	0.0528(5)
C6	0.3799(5)	0.2445(2)	0.01036(12)	0.0687(7)
C7	0.1624 (5)	0.3155(3)	0.00729(13)	0.0700(7)
C8	0.2001(4)	0.4165(2)	0.05715(10)	0.0532(5)
N9	0.2966(2)	0.28061(13)	0.14996(6)	0.0371(4)
N10	0.4122 (3)	0.18629(14)	0.17534(7)	0.0437(4)
N11	0.2977(3)	0.14415(14)	0.22051(7)	0.0458(4)
C12	0.1164(3)	0.2161(2)	0.22107(9)	0.0422(4)
N13	0.1111(2)	0.30320(14)	0.17694 (7)	0.0433(4)
C14	– 0.0479(4)	0.2041(2)	0.26857(11)	0.0542(5)
C15	– 0.0287(3)	0.3052 (2)	0.31878(9)	0.0456(5)
C16	0.1529(4)	0.3835(2)	0.32814(11)	0.0570(6)
C17	0.1660 (4)	0.4744(2)	0.37437(11)	0.0641(6)
CIS	– 0.0027(5)	0.4885(2)	0.41207(11)	0.0671(7)
C19	– 0.1847(5)	0.4116(3)	0.40353 (12)	0.0725(7)
C20	– 0.1986(4)	0.3206(2)	0.35737(11)	0.0594(6)

$-24 \le I \le 24$, $\vartheta_{max} = 25°$ using graphite-monochromatized Mo K_α radiation.

The systematic extinctions unambiguously determined the space group $P2_{1/c}$ ($Z = 4$). All calculations were performed using the SHELXTL program package. After averaging of the symmetrically equivalent reflections, the array contained 1666 inequivalent reflections ($R_{av} = 0.015$). The structure was solved by direct methods, with the coordinates of hydrogen atoms determined by difference Fourier syntheses. The structure was refined by full-matrix least squares in an anisotropic approximation for nonhydrogen atoms; the divergence factor $R = 0.0354$, $S = 0.944$. The atomic coordinates and equivalent thermal parameters are listed in Table 2. The molecular structure is presented in Fig. 1.

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