SYNTHESIS AND BIOLOGICAL ACTIVITY OF (2-HYDROXY-1-NAPHTHYL)-METHYLAMINO ACETAMIDO-EPICILLIN AND CEPHRADINE, AND (2-HYDROXY-1-NAPHTHYL)-METHYLACETAMIDO 6-APA†

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(Received for publication August 26, 1976)

Two new penicillins and a new cephalosporin have been synthesized by condensing 2-hydroxy-1-naphthaldehyde with epicillin, 6-aminopenicillanic acid and cephradine, and subsequently reducing the SCHIFF bases with $NaBH_4$. The antimicrobial activities of these compounds are also described.

In an attempt to prepare antibiotics with broad spectra of activity, especially active against resistant organisms, we have synthesized new penicillin and cephalosporin derivatives. From the readily available epicillin (**I**), cephradine (**II**) or 6-aminopenicillanic acid (**III**), SCHIFF bases were prepared by reacting these β-lactam antibiotics with 2-hydroxyl-1-naphthaldehyde[1]. The resulting SCHIFF bases were reduced with $NaBH_4$ at pH 6.0[2] to yield the respective derivatives **VIII**, **IX**, and **X**.

The *in vitro* activities of compounds **VIII**, **IV** and **X** were compared with those of **I**, **II** and **III** respectively. All of the compounds had comparable biological activities, except that the semisynthetic naphthyl derivatives were more active against the penicillin-resistant *Staphylococcus aureus* SC 2400 (Table 1).

The *in vivo* activities of compounds **II** and **IX** were tested in mice infected with *S. aureus* SC 2400. In these experiments, 1.5×10^8 cells were injected intraperitoneally and the antibiotics administered subcutaneously or orally in divided doses at 1 and 5 hours after

Fig. 1. Preparation of penicillin and cephalosporin derivatives (**VIII**, **IX**, **X**) from epicillin (**I**), cephradine (**II**) and 6-aminopenicillanic acid (**III**) via SCHIFF bases (**V**, **VI**, **VII**).

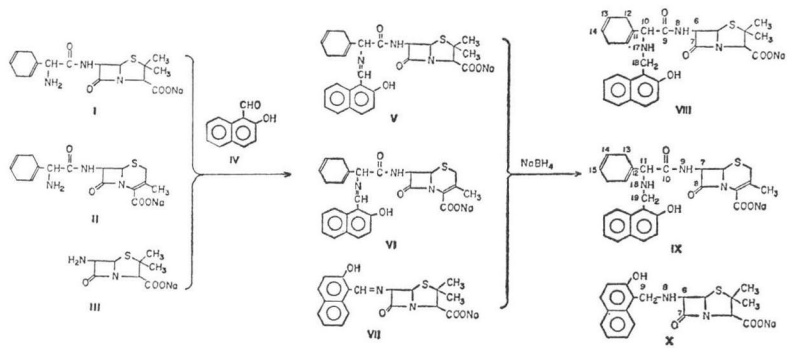

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Table 1.

Organism	MIC values in μg/ml					
	I	VIII	II	IX	III	X
Staphylococcus aureus, SC 2399	0.6	0.19	2.9	6.3	37.5	18.7
Staphylococcus aureus, SC 2400	>100	12.5	18.7	12.5	>100	18.7
Escherichia coli, SC 8294	2.4	3.6	10.0	6.3	12.5	37.5
Escherichia coli, SC 8194	NT	NT	>50	25.0	NT	NT
Pseudomonas aeruginosa, SC 8329	25	>100	>50	50.0	>100	>100
Streptococcus pyogenes, SC 3862	0.12	0.19	NT	NT	12.5	25.0

SC=Squibb Culture Collection: NT=Not tested.

Table 2.*

Organism	Route	ED_{50} mg/kg	
		II	IX
S. aureus, SC 2400	s.c.	11.3 (2.6~48.1)	22.4 (20.5~24.5)
	p.o.	19.8 (12.4~31.5)	30.3 (15.6~60.7)

* Each value represents the geometric mean of at least two experiments, and statistical formula for estimation of 95% confidence limits (in parentheses) was taken from methods reported by MILLER and coworkers[4].

infection. At least three different doses of the test antibiotic were used in twofold increments; each dose group consisted of 10 mice. All animals were observed for a period of 6 days, and the ED_{50} was determined by the method of REED and MUENCH[3]. Tests were repeated at least twice. The results showed that compound **II** was more active than compound **IX** in *in vivo* (Table 2).

For determinations of the serum concentrations, compounds **II** and **IX** were each administered as a single subcutaneous dose (100 mg/kg) to groups of three mice each. At specified intervals, mice were bled from the retroorbital sinus, the serum was separated immediately by centrifugation and stored at 4°C. Sera were assayed on the day of collection by a disc-diffusion assay, with *Sarcina lutea* SC 2495 as the test organism. The results (Table 3) showed that compound **IX** had a higher and more prolonged blood level than did compound **II**.

Serum samples were withdrawn half an hour after dosing mice with compounds **II** and **IX**, and electrophoretic comparison of the two compounds was made. At pH 4.0, both compounds **II** and **IX** were zwitterions with an Eh* value of zero. At pH 8.3 in the presence of formamide compound **II** migrated essentially as a monoanion with Eh value of -37, whereas compound

* Eh value is the relative mobility of the compound with respect to picric acid as 100.

Table 3.

Compound	Serum concentration μg/ml at various intervals after dosing			
	0.5 hr	1.0 hr	3.0 hrs	6.0 hrs
II	42 (31~58)	14 (10~21)	<1	<1
IX	57 (53~61)	39 (30~49)	1.9 (1.0~3.5)	<1

IX moved as a dianion with Eh value of −73. The results also revealed that only one bioactive component was present in the serum sample from compound **IX**. It can therefore be concluded that the *in vivo* activity of **IX** is attributable to its intact molecule, and not to enzymatically produced cephradine (**II**).

Experimental Section*

Preparation of 6-[2-(1, 4-cyclohexadien-1-yl)-2-[[(2-hydroxy-1-naphthyl)methylene]amido] acetamido]-3, 3-dimethyl-7-oxo-4-thia-1-azabicyclo [3.2.0]-heptane-2-carboxylic acid, sodium salt (**V**).

A sodium salt of epicillin (**I**) was formed by dissolving 1,404 mg (4 millimoles) of epicillin and 336 mg (4 millimoles) of $NaHCO_3$ in a mixture of 40 ml of H_2O and 360 ml of methanol. A total of 860 mg (5 millimoles) of 2-hydroxy-1-naphthaldehyde (**IV**) was added to the sodium salt solution, the reaction mixture was stirred for 20 hours at 5°C, methanol was removed *in vacuo* at 10°C, and the remaining aqueous portion was lyophilized. The resultant solid was washed with ether until the washings became clear, and the remaining yellowish solid was dried. The solid (compound **V**) weighed 2,210 mg.

Preparation of 6-[2-(1, 4-cyclohexadien-1-yl)-2-[[(2-hydroxy-1-naphthyl)methyl]amido]acetamido]-3, 3-dimethyl-7-oxo-4-thia-1-azabicyclo[3.2.0]-heptane-2-carboxylic acid, sodium salt (**VIII**)

Compound **V**, 2,000 mg (3.6 millimoles), was dissolved in 150 ml of potassium phosphate buffer (0.05 M, pH 6.0). A solution of 455 mg (12 millimoles) of $NaBH_4$ dissolved in 12 ml of H_2O was added dropwise for 1 hour, with vigorous stirring on an ice-bath. The mixture was acidified to pH 3.0 with 1 N HCl, and the resultant solid was isolated by filtration and washed with cold H_2O. The product obtained after drying was a white solid and weighed 502 mg. A total of 409 mg (0.79 millimoles) of the solid (free acid) and 75.6 mg (0.9 millimoles) of $NaHCO_3$ were dissolved in a mixture of 10 ml of H_2O and 100 ml of methanol. Methanol was removed *in vacuo* at 10°C, then the aqueous portion was lyophilized, and 403 mg of a light brownish solid (VIII) was obtained. mp 117~120°C. NMR (DMSO d_6): δ 1.4 (6H, $2CH_3$ at C_2), δ 5.36 (2H, H at C_5 and C_6), δ 5.6 (2H, H at C_{13} and C_{14}), and δ 7.1~7.8 (6H, naphthyl H).

Anal. Calcd for $C_{27}H_{27}N_3SO_5Na \cdot 4H_2O$: C, 53.99; H, 5.83; N, 7.00; S, 5.34; Na, 3.83.
Found: C, 59.68; H, 5.98; N, 7.31; S, 5.53; Na, 3.32.

Preparation of 7-[2-(1, 4-cyclohexadien-1-yl)-2-[[(2-hydroxy-1-naphthyl)methyl]amido]acetamido]-3-methyl-8-oxo-5-thia-1-azabicyclo[4.2.0]oct-2-ene-2-carboxylic acid, sodium salt (**IX**).

A sodium salt solution of cephradine (**II**) was formed by dissolving 1,396 mg (4 millimoles) of **II** and 336 mg (4 millimoles) of $NaHCO_3$ in a mixture of 30 ml of H_2O and 350 ml of methanol. To the solution was added 712 mg (4.2 millimoles) of **IV**, and the reaction mixture was stirred for 18 hours at 5°C. Methanol was removed *in vacuo* at 10°C and the remaining aqueous solution was lyophilized to yield 2,125 mg of a greenish-yellow solid (**VI**). A total of

* All mps were determined on a Kofler hot-stage microscope and are uncorrected. The nmr spectra were measured on a Varian A-60-A spectrometer in DMSO (Me_4Si). Analyses were determined by Mr. J. ALICINO of the Analytical Chemistry Department of the Squibb Institute. Where analyses are indicated by symbols of the elements, analytical results obtained for those elements were within ±0.4% of the theoretical values.

1,019 mg (1.84 millimoles) of **VI** was dissolved in 400 ml of potassium phosphate buffer (0.05 M, pH 6.0), and to the solution was added dropwise for 3 hours a solution of 325 mg (8.55 millimoles) of $NaBH_4$ in 15 ml of H_2O, with vigorous stirring at 5°C. The reaction mixture was acidified to pH 3.0, and centrifuged to separate a light brownish solid. The solid was washed twice with 5.0-ml portions of cold H_2O and dried *in vacuo* to yield 670 mg of product (free acid). A total of 432 mg (0.838 millimoles) of the free acid and 73 mg (0.872 millimoles) of $NaHCO_3$ were dissolved in a mixture of 10 ml of H_2O and 30 ml of methanol. Methanol was removed *in vacuo* at 10°C, and the remaining aqueous solution was lyophilized to yield 380 mg of a light brownish-yellow solid (**IX**). mp 110~113°C. NMR (DMSO d_6): δ 2.02 (3H, CH_3 at C_3), δ 2.68 (1H, H at C_{10}), δ 3.93 (1H, H at C_{11}), δ 4.16 (1H, H at C_{13}), δ 5.06 (2H, H at C_6 and C_7), and δ 8.83 (1H, H at C_9).

Anal. Calcd for $C_{27}H_{26}N_3SO_5Na \cdot H_2O$: C, 59.44; H, 5.17; N, 7.70; S, 5.82; Na, 4.22.
Found: C, 59.14; H, 5.32; N, 8.01; S, 6.03; Na, 3.69.

Preparation of 6-[[(2-hydroxy-1-naphthyl)-methyl]amido]-3, 3-dimethyl-7-oxo-4-thia-1-azabicyclo[3.2.0]-heptane-2-carboxylic acid, sodium salt (**X**)

The sodium salt of 6-aminopenicillanic acid (**III**) was formed by dissolving 1,130 mg (6.0 millimoles) of **III** and 504 mg (6.0 millimoles) of $NaHCO_3$ in a mixture of 40 ml of H_2O and 16 ml of methanol. To the solution was added 1,238 mg (7.2 millimoles) of **IV**, and the reaction mixture was stirred for 18 hours at 5°C. Methanol was removed from the reaction mixture *in vacuo* at 10°C, and the remaining aqueous solution was lyophilized. Excess aldehyde was removed by washing the resultant solid with ether, and 2,650 mg of a yellowish solid (**VII**) was obtained. A total of 2,584 mg (4.85 millimoles) of **VII** was dissolved in 120 ml of potassium phosphate buffer (0.05 M, pH 6.0), and to this solution was added a solution of 252 mg (6.65 millimoles) of $NaHB_4$ dissolved in 5.5 ml of H_2O. The $NaBH_4$ solution was added dropwise during 0.5 hour, with vigorous stirring on an ice-bath. The reaction mixture was acidified to pH 3.0 with 1 N HCl, and 1,568 mg of a white solid product (free acid) was isolated after the resulting solid had been washed with cold H_2O and cyclohexane. A total of 1,440 mg (3.88 millimoles) of the free acid and 352 mg (4.52 millimoles) of $NaHCO_3$ were dissolved in a mixture of 25 ml of H_2O and 125 ml of methanol, and methanol was removed *in vacuo* at 10°C. The remaining aqueous solution was lyophilized to yield 1,350 mg of a greenish brown solid (**X**). mp 113~117°C. NMR (DMSO d_6): δ 1.42 (6H, $2CH_3$ at C_2), δ 3.83 (1H, H at C_3), δ 4.15 (1H, H at C_9), δ 5.25 (1H, H at C_5), and δ 7.1~8.0 (6H, naphthyl H).

Anal. Calcd for $C_{19}H_{19}N_2SO_4Na \cdot 2\frac{1}{2}H_2O$: C, 51.99; H, 5.48; N, 6.39; S, 7.30; Na, 5.24.
Found: C, 51.50; H, 5.07; N, 6.24; S, 7.90, Na, 5.09.

Electrophoresis

Separation of the antibiotics by paper electrophoresis was carried out in a Buchler analytical electrophoresis apparatus, using 0.05 M pyridine acetate, pH 4.0, and 0.1 M N-ethylmorpholinium acetate in 30% formamide, pH 8.3. A total of 250 μl of mouse serum was applied to Whatman 3 mm paper, alongside 25 μg each of compounds **II** and **IX**. A total of 1 μl of a saturated solution of picric acid and caffeine was spotted in an adjacent lane to serve as mobility and electroosmotic indicator respectively. Electrophoresis was performed at 10 volts/cm for 90 minutes. Electropherograms were dried, and either bioautographed on *S. aureus* 209P (S.C. 1276) or sprayed with β-lactam[5], ferric chloride[6], ATFIELD's reagent[7] or diazotized *p*-bromoaniline respectively.

Acknowledgement

We are grateful to Dr. G. J. MIRAGLIA, Mr. H. BASCH and Mr. F. E. PANSY of the Squibb Institute for the biological testings.

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