DISODIUM D-6-[α-(1,2,4-TRIAZINE-3,5-DIONE-6-CARBOXAMIDO)-4-HYDROXYPHENYLACETAMIDO]PENICILLANATE, BL-P1908

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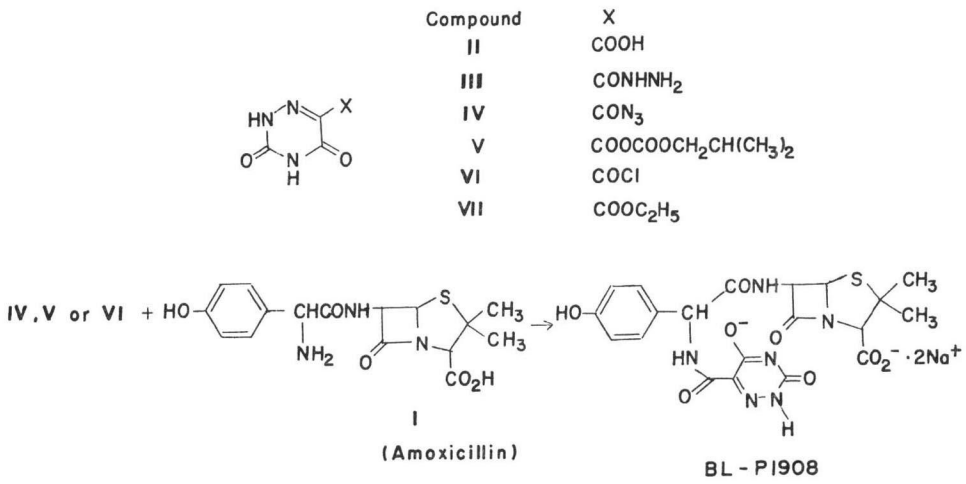
The synthesis of a new penicillin, disodium D-6-[α-(1,2,4-triazine-3,5-dione-6-carboxamido)-4-hydroxyphenylacetamido]penicillanate (BL-P1908), is described. The compound shows superior *in vitro* activity against *Pseudomonas aeruginosa* compared to carbenicillin and ticarcillin and produces higher intramuscular serum levels in mice than does carbenicillin.

The use of antipseudomonal semisynthetic penicillins in modern antimicrobial chemotherapy is becoming increasingly important. The first penicillin agent available for clinical use to combat *Pseudomonas aeruginosa* infections[1] was carbenicillin. This substance demonstrates clinical effectiveness agsinst *Pseudomonas* organisms but has somewhat low potency. A second member of this class of penicillins, ticarcillin, which recently has become available for clinical usage has also been shown to be effective in the treatment of *Pseudomonas* infections[2~4]. Ticarcillin, in *in vitro* studies, appears to be more potent against *Pseudomonas aeruginosa* than carbenicillin[5~9].

We have discovered a new penicillin, disodium D-6-[α-(1,2,4-triazine-3,5-dione-6-carboxamido)-4-hydroxyphenylacetamido]penicillanate (BL-P1908) which exhibits superior *in vitro* activity against *P. aeruginosa* strains compared to carbenicillin and ticarcillin[10].

Fig. 1. Preparation of BL-P1908, disodium D-6-[α-(1,2,4-triazine-3,5-dione-6-carboxamido)-4-hydroxyphenylacetamido]penicillanate.



Chemistry

BL-P1908 was prepared by acylating amoxicillin (**I**) with several activated derivatives of 1,2,4-triazine-3,5-dione-6-carboxylic (**II**)[11~12]: the acid azide (**IV**) (prepared from the hydrazide (**III**)), the mixed anhydride (**V**), and the acid chloride[13] (**VI**). Coupling yields were 6.8%, 24.8%, and 36.4% respectively. In each case, the product was isolated as the water-soluble disodium salt.

Biological Properties

The *in vitro* antimicrobial minimum inhibitory concentrations (MICs) of carbenicillin, ticarcillin and BL-P1908 against 10 *Pseudomonas aeruginosa* strains are compared in Table 1. Intramuscular mouse blood levels of BL-P1908 and carbenicillin are compared in Table 2.

Table 1. Minimum inhibitory concentrations (MICs)* of BL-P1908, carbenicillin and ticarcillin against clinical isolates of *Pseudomonas aeruginosa*.

Bristol Culture No.	Carbenicillin	Ticarcillin	BL-P1908
A-9827	63	32	2
A-9843a	63	32	2
A-9910	63	32	2
A-9925	63	63	2
A-9926	63	32	2
A-20126	63	63	2
A-20127	125	63	4
A-20227	63	32	2
A-20574	125	63	2
A-20641	125	63	2

* The MIC values are in μg/ml and were determined by the two-fold tube dilution method. Nutrient broth was used as the test medium in these experiments, with the number of organisms/ml being 10^4.

Clearly, BL-P1908 shows superior *in vitro* potency against these organisms, with a 32~64-fold activity increase compared with carbenicillin and a 18~32-fold increase in activity compared with ticarcillin. Peak mouse blood levels at 15 minutes were about 1.5 times those of carbenicllin.

Table 2. Mouse blood levels*.

Minutes after administration	Carbenicillin	BL-P1908
15	18.1	28.4
30	8.1	15.1
60	<4.6	3
90	<4.6	<1.8

* A dose of 40 mg/kg was administered intramuscularly. The results are in μg/ml and are the average of 8 mice per experiment.

Experimental Section

Melting points were determined in capillaries on a Mel-Temp apparatus and are uncorrected. IR spectra were determined on a Beckman Spectrophotometer IR4240. NMR spectra were determined on a Perkin-Elmer R12B Nuclear Magnetic Resonance Spectrometer.

1,2,4-Triazine-3,5-dione-6-carboxylic acid hydrazide (**III**)

To a solution of 4.2 g (0.023 moles) of ethyl 1,2,4-triazine-3,5-dione-6-carboxylate (**VII**)[11] in 50 ml of ethyl alcohol, 6 ml of hydrazine (64% in H_2O) was added causing a precipitate to form. The reaction mixture was refluxed for 17 hours. The mixture was cooled in an ice-bath. The solid was filtered, washed with alcohol and air-dried giving 4.35 g (91.3%) of **III** as the hydrazine salt, mp 230~260°C (dec.), IR spectrum consistent for **III**.

Anal. Calcd for $C_4H_9N_7O_3$: C, 23.65; H, 4.47; N, 48.26.
Found: C, 24.06; H, 4.51; N, 48.43.

Sodium D-6-[α-(1,2,4-Triazine-3,5-dione-6-carboxamido)-4-hydroxyphenylacetamido]penicillanate (BL-P1908).

(a) Acid Azide Coupling

A solution of 1.01 g (0.005 moles) of **III** in 50 ml of H_2O, 20 ml 1 N HCl and 70 ml of N,N-dimethylformamide (DMF) was cooled to $-3°C$. A solution of 0.83 g (0.012 moles) of sodium nitrite in 4 ml of H_2O was added to the hydrazide solution and the mixture was stirred at $-3°$ to $-5°C$ for 30 minutes.

A solution of 2.09 g (0.005 moles) of **I** in 25 ml of H_2O and 10 ml of THF containing 3.36 g (9.04 moles) of $NaHCO_3$ was cooled to 4°C. The acid azide solution above was added all at once to the penicillanic acid solution, the cooling bath was removed and the reaction mixture stirred at room temperature for 19 hours. The mixture was filtered and the filtrate concentrated to near dryness *in vacuo*. The residue was dissolved in 50 ml of H_2O and the solution was acidified to pH 2.5 with 42% H_3PO_4. The aqueous phase was extracted three times with 100-ml portions of EtOAc. The combined organic extracts were washed (H_2O) and dried (Na_2SO_4). After bubbling dry nitrogen through the EtOAc solution for 30 minutes (to purge HN_3), a solution of Na 2-ethylhexanoate in *n*-BuOH (2.17 ml, 0.005 moles) was added, causing a precipitate to separate. The solid was collected by filtration and air-dried. The product was reprecipitated from 20 ml of methanol with 30 ml of EtOAc. The solid was filtered, washed with Me_2CO and air-dried, giving 0.188 g (6.8%) of BL-P1908, mp 250~260°C (dec.); IR (KBr) 1773 (β-lactam), 1670, 1540 (CONH), 1610 (COO^-) and 820 cm^{-1} (*p*-phenyl); NMR δ 1.50, 1.54 (2s, 6H, C_2-$(CH_3)_2$), 4.25 (s, 1H, C_3-H), 5.55 (s, 2H, C_5-H and C_6-H), 5.66 (s, 1H, PhCHN) and 7.47, 7.00 (q, 4H, J=9, *p*-phenyl).

Anal. Calcd for $C_{20}H_{18}N_6O_8SNa_2 \cdot 4H_2O$: C, 38.71; H, 4.22; N, 13.55.
Found: C, 39.05; H, 4.00; N, 13.17.

(b) Mixed Anhydride Coupling

A mixture of 0.78 g (0.005 moles) of **II**, 0.7 ml (0.005 moles) of triethylamine (TEA) and 1.5 g of Linde 4A molecular sieves (powdered) in 50 ml of DMF was stirred at room temperature for 20 minutes. The sieves were removed by filtration and the filtrate cooled to $-15°C$ in an apparatus protected from moisture. Isobutyl chloroformate, 0.63 ml (0.005 moles) was then added and the mixture stirred at $-15°$ to $-20°C$ for 20 minutes.

A solution of 2.09 g (0.005 moles) of **I** and 0.7 ml (0.005 moles) of TEA in 25 ml of H_2O was prepared and cooled to 4°C. The penicillanic acid solution was added quickly to the mixed anhydride solution. The reaction mixture was stirred for a few minutes in the cold, then for 1½ hours without cooling. The solvent was removed *in vacuo* and the residue dissolved in 50 ml of H_2O. The aqueous phase was layered with 100 ml of EtOAc and it was acidified to pH 2.0 with 42% H_3PO_4. The phases were separated and the aqueous phase extracted twice more with 100-ml portions of EtOAc. The combined organic extracts were washed (H_2O), dried (Na_2SO_4) and treated with a solution of Na 2-ethylhexanoate in *n*-BuOH (2.17 ml, 0.005 moles) causing the product to separate. The product was collected by filtration, washed with EtOAc, then anhydrous diethyl ether and air-dried. A solution of this solid in 15 ml of methanol was treated with activated carbon, then diluted slowly with 40 ml of EtOAc. The product which precipitated was filtered, washed with Me_2CO and air-dried giving 0.68 g (24.8%) BL-P1908. The IR and NMR spectra and elemental analysis were consistent.

(c) Acid Chloride Coupling

A suspension of 1.56 g (0.010 moles) of **II** in 100 ml of CH_2Cl_2 in an apparatus protected from moisture, was gassed with dry hydrogen chloride for 5 minutes. Phosphorus pentachloride, 4.16 g (0.020 moles), was added to the suspension and the mixture stirred at room temperature for 23 hours. The insoluble material was removed by filtration, washed with CH_2Cl_2 and air-dried, giving 1.4 g (79.0%) of crude **VI**, mp 189~190°C (gas evolution).

A solution of 2.09 g (0.005 moles) of **I** in 50 ml of H_2O was obtained by the addition of 1.4 ml (0.010 moles) of TEA. The penicillanic acid solution was cooled to 4°C, 0.87 g (0.005 moles) **VI** was added rapidly and the reaction mixture stirred at 4°C for 30 minutes, then without cooling for 1½ hours. The reaction mixture was filtered and the filtrate adjusted to pH 2.2 with 42% H_3PO_4. The aqueous phase was extracted three times with 100-ml portions of EtOAc. The combined organic extracts were washed (H_2O), dried (Na_2SO_4) and treated with a solution of Na 2-ethylhexanoate in *n*-

BuOH (2.17 ml, 0.005 moles) causing a precipitate to separate. The solid was removed by filtration, washed with EtOAc, then anhydrous diethyl ether and air-dried. The solid was then stirred in 40 ml of anhydrous diethyl ether for 2 hours. The material was filtered, washed with anhydrous diethyl ether and air-dried giving 1.0 g (36.4%) BL-P1908. The IR and NMR spectra and elemental analysis were consistent.

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