

ACID HYDROLYSIS OF BENZYLPENICILLIN ANILIDES

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In an effort to determine the factors affecting penicillin stability in acid media, benzylpenicillin anilides were synthesized and subsequently hydrolyzed at 30° in a mixture of dimethylformamide and glycocoll buffer at a pH of 2.0. The log of the penicillin anilide β -lactam ring amide bond hydrolysis velocity constant was found to vary linearly with the amine pK. The hydrolysis reaction was found to follow Hammett's equation. The value of the constant ρ (-0.12) indicates insensitivity of the reaction to structural changes in the anilide molecule.

Penicillin and its derivatives deactivate rapidly in acid or alkaline media. This is due to the instability of the amide bond in the β -lactam ring. Much work has been done on the stability of penicillin and of its derivatives. Nevertheless, very little information is available regarding the effects of structural variation in benzylpenicillin derivatives upon the rate of the β -lactam ring opening. Doyle and coworkers [1, 2], while studying the stability of substituted phenylpenicillins and of α -substituted phenylpenicillins, have shown that the introduction of electron acceptor substituents into the side acyl resulted in a reduction of the hydrolysis rate. The same effect was observed when the carboxyl group in penicillin was replaced by an aryl amide group [3]. Studies of polymerized benzylpenicillin amides yielded identical results [4]. It was assumed that the resistance to hydrolysis of the β -lactam ring of penicillin amides depended on the basicity of the amine. This assumption was checked and investigated further in the present work. We felt that benzylpenicillin anilide derivatives would be best suited for such a study, because they differ so markedly in their basicity constants. With this in mind, we synthesized a number of benzylpenicillin anilides, many of them not described previously (see Table 1).

Anilide synthesis was carried out in the usual manner [5] via the mixed anhydride of benzylpenicillin and ethoxyformic acid. The anilides were hydrolyzed at 30°, in a mixture of dimethylformamide and glycocoll buffer (pH 2.0). Under these conditions hydrolysis of the amide bond in the β -lactam ring, proceeds

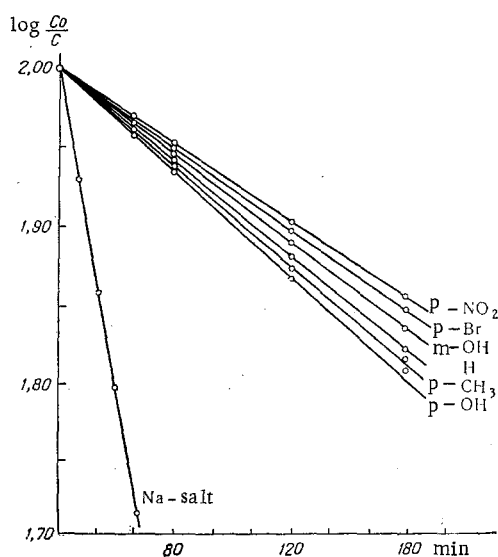


Fig. 1. Hydrolysis of benzylpenicillin and its anilides.

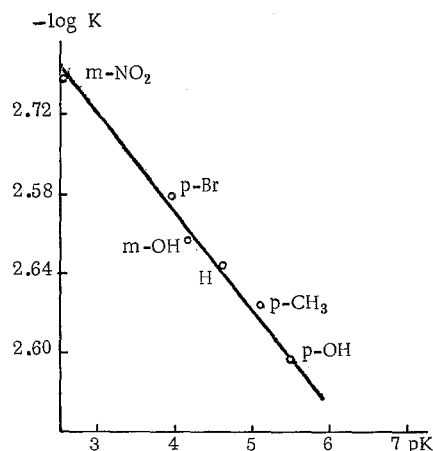
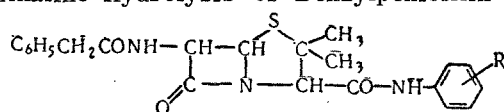


Fig. 2. Velocity constants of hydrolysis of benzylpenicillin anilides as a function of basicity of substituted anilines.

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TABLE 1. Alkaline Hydrolysis of Benzylpenicillin Anilides



R	Melting point (in deg) *	Empirical formula	Found %		Calculated %		Aniline pK	K · 10 ⁻³ · min ⁻¹
			C	N	C	N		
H	129—130a	C ₂₂ H ₂₃ N ₃ O ₂ S	64,50	9,82	64,50	10,20	4,58	2,28
p-CH ₃	190—191b	C ₂₃ H ₂₆ N ₃ O ₂ S	65,41	9,48	65,30	9,91	5,12	2,39
p-OH	128—131c	C ₂₂ H ₂₃ N ₃ O ₄ S	60,90	9,85	61,90	9,89	5,50	2,53
p-Br	169—170b	C ₂₃ H ₂₃ N ₃ O ₂ BrS	53,16	8,76	53,80	8,62	3,91	2,10
p-NO ₂	205—207b	C ₂₂ H ₂₂ N ₄ O ₂ S	59,00	12,90	58,20	12,30	1,02	1,83
m-NO ₂	159—161c	C ₂₂ H ₂₂ N ₄ O ₂ S	58,10	11,87	58,20	12,30	2,50	1,87
m-OH	112—115c	C ₂₂ H ₂₃ N ₃ O ₄ S	60,90	9,49	61,90	9,89	4,17	2,21
m-NO ₂		C ₂₂ H ₂₃ N ₃ O ₄ S	—	—	—	—	—	16,50
-NHC ₆ H ₄ X=OH		C ₁₆ H ₁₈ N ₂ O ₄ S	—	—	—	—	—	—

*Crystallization solvents: a) carbon tetrachloride; b) acetone; c) ethylacetate + petroleum ether.

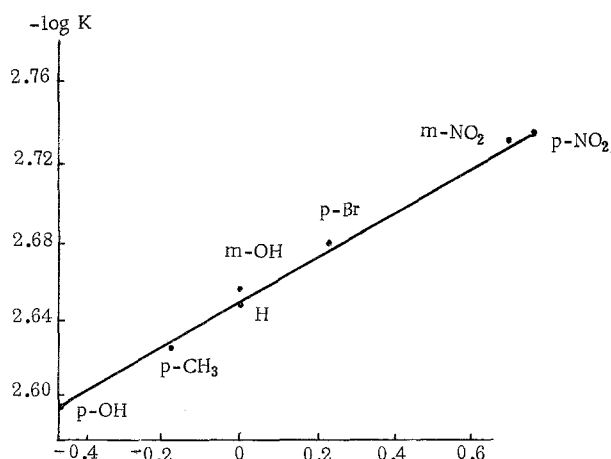


Fig. 3. Velocity constants of hydrolysis of benzylpenicillin anilides as a function of Hammett's σ constants for the respective substituents in aniline; $\rho = -0.12$.

was, nonetheless, possible to study its effect upon electron density on the nitrogen atom in the β -lactam ring. It was found that electron donor substituents increased, and electron acceptor substituents decreased the velocity constant of hydrolysis. A linear relationship was established between the logs of the reaction velocity constants, the pK of the amine (Fig. 2), and Hammett's constant (Fig. 3), thereby confirming applicability of the Hammett equation to the given reactions.

The reaction constant ρ has the value -0.12 . The negative sign of ρ bespeaks a favorable effect of electron donor substituents upon the reaction velocity constants, while its low absolute value indicates slight sensitivity of the reaction to structural variation in the anilide molecule.

EXPERIMENTAL

Anilide Synthesis. 0.00268 mole of potassium benzylpenicillinate was dissolved in 10 ml water, and the solution was acidified with 0.5 N HCl to a pH of ca 2.0. The aqueous phase was saturated with KCl, and the liberated benzylpenicillanic acid was extracted several times with 3 ml portions of chloroform. The extracts, dried with anhydrous Na₂SO₄ and cooled to -5° , were mixed with 0.00268 mole of triethylamine in chloroform and allowed to stand for 30 min. After addition of 0.00268 mole of ethoxyformic acid chloroanhydride in chloroform to the formed triethylamine benzylpenicillinate, the solution was stirred for 30 min at -5° , and then treated with 0.00268 mole of the respective amine in chloroform (in the case of nitroanilines

as a pseudounimolecular reaction. The reaction velocity constant was computed, and was also determined graphically, as for a first order equation. Velocity constants obtained for hydrolysis of the anilides were found to be about one order of magnitude lower than that of benzylpenicillin ($16.5 \cdot 10^{-3}$), but they differed little from each other (see Table 1 and Fig. 1).

The substantial difference between the velocities of hydrolysis of benzylpenicillin and of the anilides is due to a sharp decrease in the electron density on the nitrogen atom of the penicillin β -lactam ring, when COOH is replaced by the arylamide group CONHC₆H₄R. The introduction of electron donor or electron acceptor substituents into the benzylpenicillin anilide molecule leads to no significant change in the velocity constant of hydrolysis. This is caused by a considerable damping of the inductive effect in the intermediate thiazolidine ring. Even though the position of the substituent was far removed from the reaction site, it

and aminophenols acetone was used as the solvent). The reaction went to completion in 3 h at a temperature of 0-5°. The reaction product was first washed with distilled water, then with 20 ml of 0.2 M citric acid, 25 ml 0.2 M Na₂HPO₄, again with distilled water, and finally dried with anhydrous Na₂SO₄. The chloroform was removed under vacuum without the addition of heat, and the residue was crystallized from a suitable solvent to yield the anilide.

Kinetic Studies. A weighed sample of the benzylpenicillin-anilide (0.05-0.07 g) was dissolved in a 50 ml volumetric flask, in 15 ml of dimethylformamide (purified by vacuum distillation), and diluted to the 50 ml mark with a pH 2.0 glycocoll buffer. The resultant solution was kept at 30 ± 0.2° in a thermostat, and samples were taken from the reaction vessels at given time intervals and analyzed iodometrically [7] for residual, non-hydrolyzed, benzylpenicillin.

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