PENICILLINS FROM 3,3-DISUBSTITUTED 2-ARYLACRYLIC ACIDS AN APPROACH TO BROAD-SPECTRUM ACTIVITY

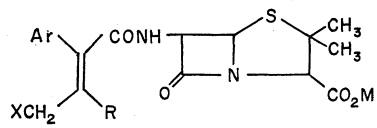
R. RAAP*, C. G. CHIN and R. G. MICETICH

R & L Molecular Research Ltd.,
8045 Argyll Road, Edmonton 82, Alberta, Canada

(Received for publication April 24, 1971)

The syntheses and antibacterial activities of various sterically hindered penicillins from 2-aryl-3-methylcrotonic acids (**1a**), 2-aryl-3-azidomethylisocrotonic acid (**1b**) and 2-aryl-3-aminomethylisocrotonic acids (**1c**) are reported. Although the penicillins **1a** and **1b** show good activity against Gram-positive microorganisms, they are only effective against penicillinase-producing strains of *Staphylococcus aureus* at small inocula. The aminopenicillins **1c** exhibit broad-spectrum activity and are not appreciably serum-bound. The presence of the amino group has resulted in increased penicillinase resistance and a 4~50 fold increase in Gram-negative activity.

Of the penicillins[1~4] presently used in antibacterial therapy none is active against all three general types of bacteria: Gram-positive and Gram-negative bacteria and penicillinase-producing staphylococci. This feature is present in cephalosporins such as cephalothin and cephaloridine and has greatly contributed to their success. In a search for truly broad-spectrum penicillins we synthesized several penicillins of the type **1**, derived from 3,3-disubstituted 2-arylacrylic acids[5]. In **1a** the β-lactam ring was expected to be sterically protected against attack by the enzyme penicillinase to approximately the same extent as in oxacillin (cf. reference **6**) and as the penicillins were of the arylacetic acid type some Gram-negative activity also seemed to be possible, particularly in the heteroaromatic series. Furthermore, the presence of allylic aminomethyl groups such as in **1c** could conceivably enhance the Gram-negative activity considerablv without jeopardizing the penicillinase resistance.



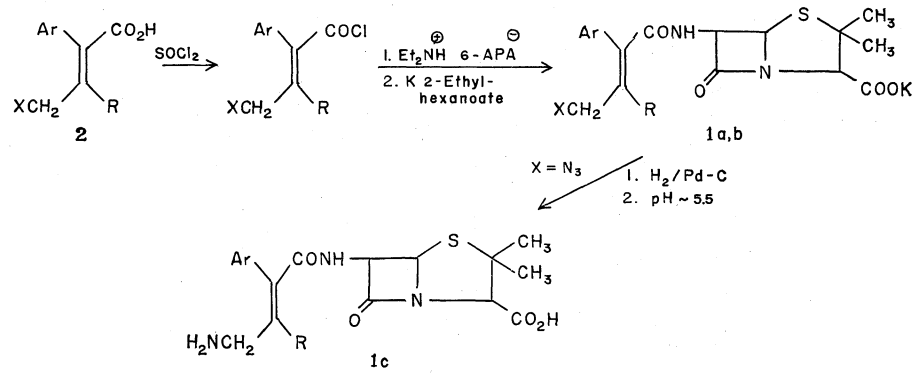
1a. X=H; R=CH_3; M=K
1b. X=N_3; R=CH_3, C_2H_5; M=K
1c. X=NH_2; R=CH_3, C_2H_5; M=H

Synthesis

The syntheses of the penicillins **1** are outlined in Scheme 1. The acids **2** (X=H, N_3) reacted with thionyl chloride at or below room temperature to give the acid chlorides, which upon reaction with the triethylammonium salt of 6-aminopenicillanic acid (6-APA) in methylene chloride gave the triethylammonium salts of the penicillins. The potassium salts **1a** and **1b** were obtained by treatment of methanolic solutions of the triethylammonium salts with potassium 2-ethylhexanoate. The azidopenicillins

* Present address: Camosun College, P. O. Box 490, Victoria, B. C., Canada.

Scheme 1.



1b were converted to the aminopenicillins **1c** by catalytic hydrogenation, using 10% palladium on charcoal as catalyst. The preparation of aminopenicillins from azidopenicillins has been reported by EKSTRÖM *et al.*[7]

Antibacterial Activity

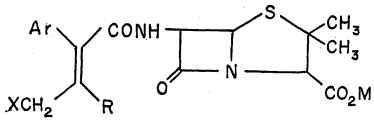
The penicillins were tested by Drs. M. H. PINDELL and K. E. PRICE and their associates of the Microbiology Department of Bristol Laboratories, Syracuse, N. Y., using published techniques[8]. Minimum inhibitory concentrations (MIC) were determined using a twofold serial dilution technique in heart infusion broth in the absence of serum and in the presence of pooled human serum.

In Table 1 the MIC values of the penicillins **3**~**31** against various types of bacteria are compared with the values for oxacillin and ampicillin, obtained under the same conditions. Indications of the extent of serum binding and the effect of the inoculum size (10^8 and 10^2 culture dilutions) were obtained for *Staphylococcus aureus* (Smith) and a penicillinase-producing strain of *S. aureus* respectively. The Gram-positive inhibitory activities of **3**~**23** and the extent of serum binding are approximately the same as for oxacillin. These compounds generally also exhibit good activity against the penicillinase-producing strain of *S. aureus* at small inocula, however the MIC values drastically increase at higher inocula, in contrast to oxacillin whose values remain the same. This indicates that compounds **3**~**23**, with the possible exception of **8** and **11**, are considerably more susceptible to staphylococcal penicillinase than oxacillin. The Gram-negative activities of **3**~**23** are not substantially improved over oxacillin.

The presence of an amino group in **24**~**31** has resulted in a substantial improvement in activity. These aminopenicillins are all highly active against Gram-positive microorganisms and are not appreciably serum-bound; they maintain their resistance to penicillinase at higher inocula and the Gram-negative inhibitory activity has improved **4**~**50** fold. Against Gram-negative microorganisms, with the exception of *Proteus morganii*, ampicillin is however still considerably more active. Phenyl ring substitution causes a slight loss in the Gram-negative activity but has a beneficial effect on the penicillinase resistance.

Against *S. aureus*, rather surprising, *ortho* aryl ring substitution frequently

Table 1. Minimum inhibitory concentrations (μg/ml) of penicillins from 3,3-disubstituted 2-arylacrylic acids



No.	Ar	R	X	M	*Diplococcus pneumoniae* +5 % serum	*Staphylococcus aureus*			
						Penicillin G sensitive (Smith)		Penicillin G resistant (BX 1633-2)	
						−serum	+50 % serum	10^3 dilution	10^2 dilution
3	phenyl	CH_3	H	K	0.08	0.08	1.7	2.5	>10
4	*o*-chlorophenyl	〃	〃	〃	0.08	0.16	2.5	—	—
5	*m*-chlorophenyl	〃	〃	〃	0.04	0.06	2.5	1.3	>10
6	*p*-chlorophenyl	〃	〃	〃	0.6	0.08	>5	0.3	>10
7	2,4-dichlorophenyl	〃	〃	〃	0.04	0.07	2.5	0.6	>10
8	2,6-dichlorophenyl	〃	〃	〃	0.08	0.11	2.5	0.6	1.3
9	3,4-dichlorophenyl	〃	〃	〃	0.016	0.04	5.0	0.3	>10
10	*p*-methylphenyl	〃	〃	〃	0.3	0.06	>5	0.4	10
11	*p*-methoxyphenyl	〃	〃	〃	0.16	0.08	2.5	0.9	2.5
12	*o*-fluorophenyl	〃	〃	〃	0.04	0.11	2.5	5	>10
13	*o*-methylphenyl	〃	〃	〃	0.08	0.16	3.3	1.3	>10
14	2-thienyl	〃	〃	〃	0.008	0.06	1.0	1.3	>10
15	5-chloro-2-thienyl	〃	〃	〃	0.6	0.04	5.0	0.3	>10
16	2,5-dichloro-3-thienyl	〃	〃	〃	0.08	0.08	3.3	0.9	>10
17	3-methyl-2-thienyl	〃	〃	〃	0.16	0.23	2.5	1.3	10
18	2-thiazolyl	〃	〃	〃	0.08	0.3	0.6	>10	>10
19	N-methyl-2-pyrrolyl	〃	〃	〃	0.16	0.3	2.5	8	125
20	phenyl	〃	N_3	〃	0.16	0.23	>5	1.3	>10
21	*p*-chlorophenyl	〃	〃	〃	0.16	0.11	3.3	0.45	7
22	2,4-dichlorophenyl	〃	〃	〃	0.3	0.08	5.0	0.6	7
23	*p*-methylphenyl	〃	〃	〃	0.16	0.16	5.0	0.6	5
24	phenyl	〃	NH_2	H	0.08	1.0	1.3	5	10
25	phenyl	C_2H_5	〃	〃	0.08	1.3	2.5	2.5	2.5
26	*o*-chlorophenyl	CH_3	〃	〃	0.08	1.3	1.3	2.5	5.0
27	*p*-chlorophenyl	〃	〃	〃	0.08	0.6	1.3	0.8	1.8
28	2,4-dichlorophenyl	〃	〃	〃	0.04	0.3	0.8	1.3	1.3
29	3,4-dichlorophenyl	〃	〃	〃	0.04	0.3	0.8	0.45	1.3
30	*p*-methylphenyl	〃	〃	〃	0.02	0.3	1.3	0.6	1.3
31	*p*-methoxyphenyl	〃	〃	〃	0.08	1.3	2.5	2.5	2.5
	oxacillin				0.08	0.08	2.5	0.3	0.3
	ampicillin				0.008	0.06	0.06	125	>500

results in a slight loss of activity, in contrast to *meta* and *para* substitution which invariably improves the activity.

The *in vivo* activities were consistent with the *in vitro* results. When administered subcutaneously, the CD_{50} values for **11**, **26**, **27**, **29**, **30** and oxacillin in mice infected with the penicillinase-producing strain of *S. aureus* were found to be 14, 22.5, 3.4, 13, 10 and 2.5~9.0 mg/kg respectively.

Gram-negative microorganism				
Salmonella enteritidis	*Escherichia coli*	*Klebsiella pneumoniae*	*Proteus mirabilis*	*Proteus morganii*
63	250	500	—	125
250	500	250	—	500
16	250	63	250	125
125	500	358	250	250
16	250	100	—	125
250	500	250	—	>500
63	250	125	125	125
63	>500	250	500	250
250	500	250	500	500
63	250	125	125	125
32	>250	358	>250	250
16	250	79	32	63
63	500	125	125	125
63	250	125	250	250
63	>250	250	>250	>250
32	125	250	32	63
125	250	250	250	250
32	250	250	125	125
32	500	358	125	125
125	>500	358	500	250
125	>500	>500	250	500
1	16	10	1	4
2	32	32	4	16
2	32	32	4	4
2	32	32	8	8
4	63	90	32	16
2	32	32	8	8
4	32	16	8	8
4	32	16	8	8
63	500	125	250	250
0.13	4	0.5	0.16	63

Experimental

Potassium 6-(2-Aryl-3-methylcrotonamido)penicillanates (**3**～**19**) and Potassium 6-(2-Aryl-3-azidomethylisocrotonamido)penicillanates (**20**～**23**). General Procedure: 2-Aryl-3-methylcrotonic acid[5] or 2-aryl-3-azidomethylisocrotonic acid[5] (0.010 mole) was allowed to react with thionyl chloride (10～20 ml) at 0～25°C until the reaction was completed (as indicated by IR), then the excess of thionyl chloride was removed under reduced pressure. A solution of the crude acid chloride in 15 ml of methylene chloride was added dropwise in 5 minutes to a stirred solution of triethylammonium 6-aminopenicillanate, prepared from triethylamine (0.020 mole) and 6-APA (0.010 mole), in 25 ml of methylene chloride at −20～−40°C. The mixture was allowed to reach ambient temperature in 1～2 hours, then the solvent was removed. A solution of the residue in 50 ml of water was layered with 50 ml of ether, cooled in ice and with stirring brought to pH 2.0～2.5 with 1 N hydrochloric acid. The layers were separated (sometimes a filtration through Celite was required to break the emulsion) and the aqueous layer extracted again with ether. The combined ether solutions were dried ($MgSO_4$) and treated with 4 ml of a 2.4 M solution of potassium 2-ethylhexanoate in *n*-butyl alcohol. Depending on the solubility of the penicillin, additional ether or some *n*-hexane was added. The solvent was decanted from the precipitate which was then triturated with ether until it solidified. The potassium penicillinate was dissolved in the minimum amount of methanol and reprecipitated with ether. The product was collected by filtration and dried *in vacuo* over P_2O_5; yields: 40～70 %. The purities were 80～95 % as estimated from TLC on silica gel (a system containing 3:1:1 of *n*-butyl alcohol, acetic acid and water was used) and the IR and NMR spectra.

IR (Nujol): *e. g.* for **3**: maxima at 1780 (β-lactam carbonyl), 1675 (amide carbonyl) and 1605 cm^{-1} (carboxylate); for **20**: maxima at 2090 (N_3), 1760, 1660 and 1600 cm^{-1}.

NMR: *e. g.* for **3** (in D_2O): singlets at τ 2.77 (5H, phenyl H's), 4.48 (2H, β-lactam H's), 5.82 (1H, C^3-H), 8.06 (3H, CH_3 *trans* to phenyl), 8.46 (3H, CH_3 *cis* to phenyl) and 8.56 (6H, *gem* dimethyl).

Anal. Calcd. for **3**, $C_{19}H_{21}N_2O_4SK$: C 55.32, H 5.13, N 6.79, S 7.77.
Found: C 54.71, H 5.13, N 6.65, S 7.80.

6-(3-Aminomethyl-2-arylisocrotonamido)penicillanic Acids (**24**~**31**). General Procedure: A mixture of potassium 6-(2-aryl-3-azidomethylisocrotonamido)penicillanate (0.010 mole), 10 % palladium on charcoal (1~2 g) and water (50~100 ml) was hydrogenated at 15~20 psi for 16 hours. The mixture was filtered through Celite, the filtrate cooled in ice and brought to pH 2.0 with dilute hydrochloric acid. A voluminous precipitate was removed by filtration through Celite. The clear and almost colorless filtrate was adjusted to pH 5.5~6.0 with dilute aqueous sodium hydroxide and then concentrated to dryness under reduced pressure (below 30°C). The solid residue was twice extracted with 5~10 ml of methanol. The methanol-soluble material was reprecipitated with ether and subjected to chromatography on Sephadex-G 10 (column: 60 cm in length and 2 cm in diameter). The penicillin was eluted with water and the eluate collected in 10 ml fractions. On the basis of TLC the proper fractions were chosen, combined and freeze-dried to give the aminopenicillins as white amorphous solids in 80~90 % purity (as estimated from the NMR spectra). The yields of purified **24**~**31** were low and amounted to 18, 26, 11, 5, 4, 2.3, 20 and 15 % respectively. None of these aminopenicillins were obtained in analytical purity and the characterization is based on IR and NMR spectra.

IR (Nujol): *e. g.* for **24**: maxima at 1775, 1650 and 1605 cm^{-1}.

NMR: *e. g.* for **24** (in D_2O): singlets at τ 2.57 (5H, phenyl H's), 5.78 (1H, C^3-H), 6.36 (2H, $H_2N\underline{CH_2}$), 7.95 (3H, CH_3 *trans* to phenyl), 8.49 (3H) and 8.55 (3H) (*gem.* dimethyl), and an AB quartet (J=4.5 Hz) at τ 4.50 (2H, β-lactam H's).

Acknowledgements

The authors are grateful to Professor R. U. LEMIEUX, Director of Research, for advice and to Messrs. V. R. BAKER, S. CHERWONKA, R. A. FORTIER, R. R. THOMAS, A. VANSON and P. K. WOLFERT for technical assistance.

References

1) DOYLE, F. P. & J.H.C. NAYLER: Penicillins and related structures. Advan. Drug Res. 1 : 1~69, 1964
2) STEWART, G. T.: The penicillin group of drugs. Elsevier Publishing Co., Amsterdam, 1965
3) ABRAHAM, E. P.: The cephalosporin C group. Quart. Rev. (London) 21 : 231~248, 1967
4) ABRAHAM, E. P.: Penicillins and cephalosporins. Top. Pharm. Sci. 1 : 1~31, 1969
5) RAAP, R.; C. G. CHIN & R. G. MICETICH: Syntheses of 3,3-disubstituted 2-arylacrylic acids. Can. J. Chem. 49 : 2143~2151, 1971
6) Loevens Kemiske Fabrik Produktionsaktieselskab: (1-Phenylbuten-1-yl)penicillins. Belgian Patent 633, 397 (Nov. 4, 1963); Chem. Abstr. 60 : 15878, 1964
7) EKSTRÖM, B.; A. GOMÉZ-REVILLA, R. MOLLBERG, H. THELIN & B. SJÖBERG: Semisynthetic penicillins. III. Aminopenicillins *via* azidopenicillins. Acta Chem. Scand. 19 : 281~299, 1965
8) GOUREVITCH, A.; G. A. HUNT, J. R. LUTTINGER, C. C. CARMACK & J. LEIN: Activity of α-phenoxyalkyl penicillins against sensitive and resistant staphylococci. Proc. Soc. Exptl. Biol. & Med. 107 : 455~458, 1961