

Semisynthetic Penicillins

III.* Aminopenicillins *via* Azidopenicillins

BERTIL EKSTRÖM, ANTONIO GOMÉZ-REVILLA,
RENÉ MOLLBERG, HUGO THELIN and BERNDT SJÖBERG

Research Laboratories, AB Astra, Södertälje, Sweden

Azido substituted penicillins have been prepared by acylation of 6-aminopenicillanic acid with chlorides, azides and ethoxyformic anhydrides of azido acids. The azido groups can be smoothly converted into amino groups by catalytic hydrogenation over palladium or, preferably, over nickel catalyst. Good results were obtained with penicillins derived from carboxylic acids having the azido group in the α -position or in a benzene ring, whilst compounds with the azido group in the β - or γ -position were not satisfactorily reduced and gave inconsistent results.

The properties of the prepared penicillins are briefly described.

One of the main objects of the development of new, semisynthetic penicillins by acylation of the penicillin nucleus, 6-aminopenicillanic acid (6-APA, I), which was instituted when the latter was made easily available by the work of Batchelor *et al.*¹, is to find compounds which are active against a wider range of micro-organisms than the conventional penicillins, obtainable by fermentation processes. The first result of this search for broad-spectrum penicillins was α -aminobenzylpenicillin (Ampicillin)² which is as effective as benzylpenicillin (Penicillin G) against Gram-positive bacteria and has also very good activity against many Gram-negative organisms.

Against *Salmonella*, *p*-aminobenzylpenicillin³ is more active than the unsubstituted benzylpenicillin and the relative activity of D-4-amino-4-carboxybutylpenicillin (Synnematin B, Cephalosporin N, Penicillin N)⁴ against *Salmonella typhi* and *Staphylococcus aureus* is greater than that of its N-acylated derivatives.⁵ Further, introduction of an alkyl or acyl substituent on the amino group of α -aminobenzylpenicillin resulted in compounds which are less active against Gram-negative bacteria than the parent compound,⁶ hence it seems

* Paper II, B. Sjöberg, B. Ekström, P. Moses and S. Gronowitz. *Acta Pharmaceutica Suecica*. In press.

that the activity against this type of micro-organisms is connected with the presence of a free amino group in the molecule. It was therefore deemed worthwhile to prepare series of penicillins containing this group in the side chain.

During the acylation of 6-APA with an amino acid, the amino group of the latter must be masked with a suitable protecting group, to prevent it from taking part in the reaction.

In their synthesis of α -aminobenzylpenicillin and of a number of other aminopenicillins, Doyle *et al.*^{7,8} used the benzyloxycarbonyl group and first prepared N-benzyloxycarbonyl-aminopenicillins, from which the protecting groups were removed by catalytic hydrogenation to give the corresponding aminopenicillins. Application of this procedure, however, met with certain obstacles⁸ which limited the general use of the method. For instance, when this group was used to protect an α -substituted α -amino acid, *e.g.* α -amino- α -phenylpropionic acid, acylation by the mixed anhydride method could be performed only with difficulty, probably due to steric hindrance;* owing to poisoning of the catalyst by the sulphur atom in the penicillin molecule, unusually large amounts of the palladium catalyst were needed for the hydrogenation; finally, in attempts to prepare analogues of α -aminobenzylpenicillin containing chlorine in the benzene ring, extensive dechlorination occurred during the hydrogenation. The limitations of the benzyloxycarbonyl procedure thus made it desirable to have available alternative routes to the aminopenicillins. In this paper, the first result of our investigations in this direction are presented.

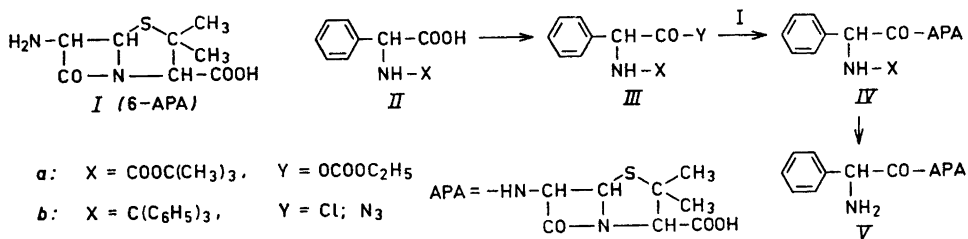
It is well known from peptide chemistry that it is very difficult to split off an N-benzyloxycarbonyl group by catalytic hydrogenation if the molecule also contains a sulphur atom.⁹ In such cases the protecting group can be removed by chemical reduction with sodium in ammonia, according to the method of du Vigneaud.¹⁰ Early experiments on the inactivation of benzylpenicillin with gaseous ammonia,¹¹ as well as our own experiences from reactions of penicillins in liquid ammonia show, however, that the β -lactam ring of the penicillin nucleus would be split very rapidly under these reduction conditions, making it unpractical to use this modification. The same would also apply to the N-*p*-toluenesulphonyl and other protecting groups which are removed in an analogous way. The lability of the β -lactam ring of the penicillin nucleus towards nucleophilic reagents and alkalis also makes protecting groups such as the N-phthalyl and N-trifluoroacetyl group unattractive, as their removal, with hydrazine and dilute alkali resp., would also lead to rapid inactivation of the penicillin.**¹¹

The N-benzyloxycarbonyl group and other N-protecting groups of the carbamic acid type can also be split off by treatment with anhydrous hydro-

* By modification of the conditions for the acylation it has since been possible to achieve better yields of the penicillins in question (personal communication from Beecham Research Laboratories).

** The results obtained by Schallenberg *et al.*¹² indicate that the ease with which the N-trifluoroacetyl group is hydrolyzed highly depends on the structure of the protected derivative; under favourable circumstances, 50 % hydrolysis was obtained after 40 min at room temperature and pH 12. We found that α -aminobenzylpenicillin under these conditions is almost completely inactivated within 10 min.

chloric or hydrobromic acid in organic solvents,¹³ *e.g.* acetic acid or nitromethane. Although it has been shown¹⁴ that the β -lactam ring in the methyl ester of benzylpenicillin is very rapidly opened up under such conditions, we found it worthwhile to try this method in the case of the aminopenicillins, since the introduction of a hetero substituent in the α -position of the side chain has been found to greatly improve the acid-stability of the penicillin.¹⁵ As the *N-t*-butoxycarbonyl group is split off with special ease, the *N-t*-butoxycarbonyl- α -aminobenzylpenicillin (IV a), prepared from *N-t*-butoxycarbonyl- α -aminophenylacetic acid (II a), was chosen for these experiments. Treatment of the penicillin with anhydrous hydrochloric or hydrobromic acid in nitromethane solution at room temperature or at 0–5° led to its immediate inactivation. The same result was obtained when the *t*-butoxycarbonyl group was removed by dissolution of the penicillin in trifluoroacetic acid according to the procedure of Schwyzer.¹⁶ Keeping IV a in water-dioxane solution at

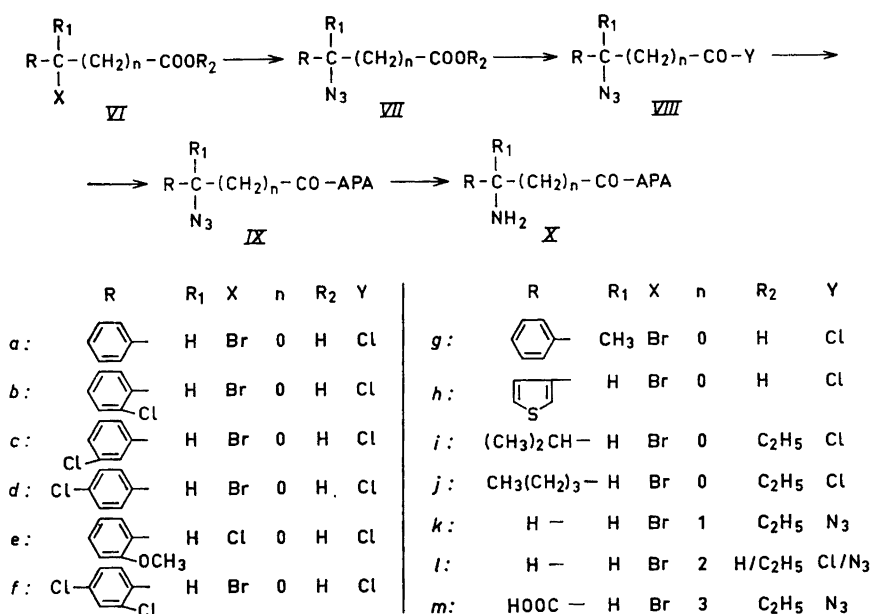


pH 2 for some hours at room temperature did lead to the formation of α -aminobenzylpenicillin (V) but only in trace amounts. A further protecting group which can be split off under mild acidic conditions or by catalytic hydrogenation is the triphenylmethyl (trityl) group. Its use, however, was precluded by the fact that we were not able to acylate 6-APA with the acid chloride or azide (III b) of *N*-triphenylmethyl- α -aminophenylacetic acid, probably due to steric hindrance, which has been found to greatly influence the rate of alkaline hydrolysis of *N*-triphenylmethylamino acid esters.¹⁷ The azide was also found to decompose in chloroform solution at room temperature.

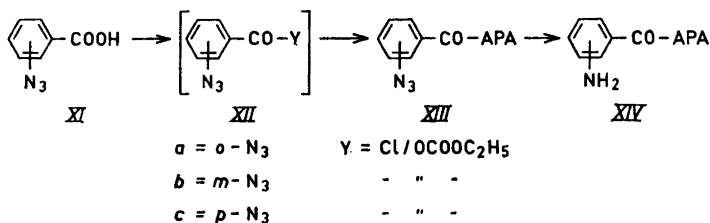
It thus seemed that none of the protecting groups commonly used in peptide syntheses which could be split off with nucleophilic or electrophilic agents were suitable for the preparation of aminopenicillins, because the conditions required for their removal led to destruction of the penicillin molecule; only a process involving catalytic hydrogenation for the release of the amino group would be mild enough. It was therefore of interest to investigate the possibility to use, *e.g.*, oximino-, alkoximino-, phenylhydrazono- or azido acids, instead of amino acids, for the coupling reaction, as their nitrogen functions can be reduced by catalytic hydrogenation to the amino group. They have been used in the preparation of amino acids and in peptide syntheses.^{18–21} We found it impossible to reduce catalytically the α -oximino-, α -alkoximino- or α -phenylhydrazonobenzylpenicillins to α -aminobenzylpenicillin.²² Similarly, reduction of the α -oximino group in other sulphur-containing peptides took place only with great difficulties.²³ In the case of the α -azido derivative, however,

the reduction could be carried out very readily. From further experiments with other azidopenicillins, it soon became clear, that in this reaction we had found a good method for the synthesis of aminopenicillins.*

The use of α -azido acid intermediates for the synthesis of peptides was introduced in 1932 by Bertho and Maier²⁷ and by Freudenberg *et al.*²⁸ There have been only scanty reports of their use, probably because it is not so easily applicable as the benzyloxycarbonyl method introduced in the same year, which utilizes readily available amino acids as starting materials. The preparative routes to the azido- and aminopenicillins, described in this paper, are illustrated in flowsheets 1 and 2.



Flow-sheet 1.



Flow-sheet 2.

* The results described in this paper form the basis for patent applications.²⁴ After completion of the greater part of the present work, some of the compounds (IX a; IX j, X j) were mentioned in the literature²⁵ though without any experimental details. A closely related patent application, describing the preparation of them as well as of compounds IX d and X d, has also appeared.²⁶

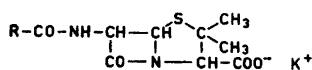
Azido acids. With the exception of the three azido benzoic acids, which were prepared by treatment of the corresponding diazonium perbromides with ammonia, the azido acid derivatives used for the acylation of 6-APA were prepared by reacting the corresponding halogen-substituted acids or esters with sodium azide in aqueous acetone or in dimethylformamide. The α -azido-phenylacetic acid (VII a) has previously been prepared by treatment of an aqueous solution of the sodium salt of α -bromo or α -chloro phenylacetic acid with sodium azide, whereby considerable amounts of mandelic acid were formed as by-product.^{29,30} We found that the formation of the latter can be suppressed to a large extent by performing the reaction in a suspension of the halogeno acid, sodium azide and sodium carbonate in acetone containing 5–20 % of water.* After the reaction, the acetone was removed *in vacuo* at room temperature, and the residue was dissolved in water and acidified to liberate the azido acid, which was isolated by extraction with ether. The method works equally well with the substituted phenyl acetic acids (VII b-f) and also with the α -azido-3-thienylacetic acid (VII h). In the case of the α -azido- α -phenylpropionic acid (VII g) the sodium carbonate was omitted in order to diminish the risk of formation of atropic acid by hydrogen bromide elimination. The reaction was performed in dimethylformamide at 60° and the azido acid, which was obtained in low yield, was isolated as its cyclohexylamine salt. Its structure was proved by its reduction to the known α -amino- α -phenylpropionic acid. The aliphatic azido acids or acid derivatives used have already been described in the literature with the exception of ethyl 5-azido-5-carboxyvalerate (VII m), which was prepared by treatment of ethyl 5-bromo-5-carboxyvalerate with sodium azide and potassium carbonate in an acetone slurry.




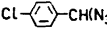

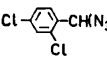


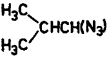
Azidopenicillins. As reactive derivatives for the acylation of 6-APA, the acid chlorides were used in most cases. They were prepared by treating the acids with an excess of thionyl chloride on the water bath, or by adding to the acid the calculated amount of thionyl chloride together with one equivalent of pyridine in dry ether. Due to some risk of violent decomposition, we generally preferred not to purify the acid chlorides, but to use them directly as the crude products, obtained after removing excess thionyl chloride *in vacuo*, or filtering off the pyridine hydrochloride. In three cases (VIII k, l, m) the acid azides were used for the coupling reaction. By treating them with hydrazine, the appropriate esters (VII k, l, m) were transformed into their corresponding hydrazides, which in the first two cases were isolated as the acetone derivatives, which could be nitrosated with sodium nitrite at pH 3 in a mixture of ether and water. The ether phases, containing the acid azides formed, were separated and used directly for the acylation. The hydrazide of 5-azido-5-carboxyvaleric acid was isolated as its hydrazine salt and was nitrosated in the same way. The three azidobenzoic acids (XI a, b, c) were coupled as the acid chlorides as well as *via* mixed carbonic acid anhydrides (XII, Y = COOC₂H₅), prepared from the acids and ethyl chloroformate.

The acylation reaction (VIII–IX, XII–XIII) was performed essentially as already described for preparation of various penicillins.^{31,32} The acid

* This method has been worked out by Mr. T. Rydh in our Development Laboratories.

Table 1. Azidopenicillins.






No.	R	Method	Yield %	Purity %	Stability ^a		Activity ^b (μg/ml)	
					pH 2.1	penicillinase	<i>S. aureus</i> , Oxford	<i>E. coli</i>
IXa		A	84	83	67	0.6	0.03	25
IXb		A	81	74	22	1.0	0.03	250
IXc		A	81	81	14	1.0	0.01	250
IXd		A	83	86	28	0.8	0.01	125
IXe		A	92	88	17	0.9	0.01	250
IXf		A	82	73	8.5	1.2	0.03	> 250
IXg		A	52	41	—	—	—	—
IXh		A	68	88	42	0.6	<0.006	25
IXi		A	86	82	24	1.6	0.06	> 250
IXj	CH ₃ (CH ₂) ₃ CH(N ₃)—	A	85	81	30	0.8	0.03	> 250
IXk	N ₃ —CH ₂ CH ₂ —	B	31	79	7.4	1.2	0.25	—
IXl	N ₃ —(CH ₂) ₃ —	A	49	38	—	—	0.25	62.5
		B	20	26	—	—	—	—
IXm	HOOC—CH(CH ₂) ₃ — N ₃	B	36	53	1.6	2.5	1.25	> 250

^a Stability at pH 2.1. Time for 50 % inactivation of the penicillin relative to that of benzylpenicillin (K-salt) at pH 2.1 and 37°, determined in 0.1 M phosphoric acid in 50 % ethanol.

Stability against penicillinase. Rate of inactivation of the penicillin with *B. cereus* penicillinase (Neutrapen[®], Schenlabs Pharmaceuticals, Inc., New York) relative to that of benzylpenicillin (K-salt) at pH 7.0 and 25° in 0.1 M phosphate buffer solution.

^b Activity as minimum inhibitory concentration (MIC) in μg/ml, determined in a serial dilution test on agar plates.

Table 1. Continued.

No.	R	Method	Yield %	Purity %	Stability ^a		Activity ^b (μg/ml)	
					pH 2.1	penicil- linase	<i>S.aureus</i> , Oxford	<i>E.coli</i>
XIIIa		A	68	56	1.0	1.8	0.13	> 250
		C	64	84				
XIIIb		A	48	42	2.9	1.4	0.25	> 250
		C	89	55				
XIIIc		A	43	48	0.9	1.3	0.25	> 250
		C	76	83				

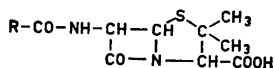
chlorides, dissolved in dry acetone, were added to a stirred solution or suspension of 6-APA in aqueous acetone, which was maintained neutral by the addition of sodium bicarbonate. The reactions with the acid azides were made in a stirred mixture of water and ether, using sodium bicarbonate as the acid binding component, while the acylations with the mixed carbonic acid anhydrides were performed in a homogeneous solution in aqueous dimethylformamide with triethylamine as base. These three procedures are referred to in Table 1 as method A, B and C, respectively. The penicillins formed were isolated from the reaction mixture by extraction with ether, after acidification to pH 2 with hydrochloric acid. Under these conditions any unreacted 6-APA remains in the aqueous phase as the hydrochloride. From the ether solution, the penicillins were extracted back into water with potassium bicarbonate solution and finally the potassium salt of the penicillins were isolated by freeze-drying. The yields and purities of the azido penicillins (IX a–f, h–j) obtained from the disubstituted acetic acids *via* the acid chlorides and of the penicillins (XIII a–c) obtained from the azido benzoic acids *via* the mixed ethoxyformic anhydrides were generally satisfactory. In the latter case, however, the products were contaminated with minor amounts of ethoxy-penicillin, formed by reaction of 6-APA with the carbonic carbonyl group of the anhydride or with excess ethyl chloroformate remaining from the preparation of the anhydride. When compounds XIII a–c were prepared *via* the acid chlorides, the azido penicillins were the only antibacterially active compounds. In case of the penicillin IX g, with the side chain derived from a trisubstituted acetic acid, *viz.* α -azido- α -phenylpropionic acid, the yield was lower than normally but still satisfactory, presumably due to steric hindrance in the acylation reaction. Low yields were also noted for the three compounds (IX k–m) prepared from monosubstituted acetic acids *via* the acid azides and also in the case where IX l was prepared *via* the acid chloride.














The products obtained were generally pure enough to be used directly for biological and stability tests. To verify the assumed structures, some of the azido penicillins were purified for elemental analysis. The results are given in Table 4. All of the penicillins showed in their IR-spectra, in addition to the

sharp band around 1770 cm^{-1} characteristic for the β -lactam moiety of the molecule, also a strong absorption at $2100\text{--}2120\text{ cm}^{-1}$, indicating the presence of an azido group. The compounds which were prepared *via* the acid azides also showed an additional weaker absorption band at 2040 cm^{-1} , probably due to potassium azide present in the product. (An authentic sample of potassium azide was found to have a strong absorption at 2040 cm^{-1}).

Aminopenicillins. An azido group can be reduced to an amino group either by chemical reduction or by catalytic hydrogenation.³³ We came to the conclusion that the conditions generally used in the first case — aluminium amalgam, hydrogen bromide in acetic acid, or warming with triphenylphosphine — are so destructive that they would lead to opening of the sensitive β -lactam ring of the penicillin molecule, and that only the catalytic hydrogenation method was of interest. Bertho and Maier²⁷ investigated the conditions for the hydrogenation of azido groups, using precious metal catalysts, and found that a PtO_2 -catalyst was suitable. With this catalyst the hydrogenation was complete within $\frac{1}{2}$ —2 h at room temperature and atmospheric pressure, using pure methanol, 70 % methanol, ethyl acetate, or 0.1 N hydrochloric acid as solvents. We found that palladium on calcium carbonate was a good catalyst, but also that a Raney-nickel catalyst was quite excellent for this purpose, which from a technical viewpoint was of considerable importance. The hydrogenation was preferably performed in water as it allowed the direct use of the potassium salt of the azidopenicillins. The catalyst was always prehydrogenated in water. It was possible to perform the hydrogenation at atmospheric pressure, but as short reaction times were essential a slight overpressure of hydrogen, 3—4 atm., was used. Hydrogenation times were 30—60 min. Afterwards any unreacted azidopenicillin was removed from the reaction solution by extraction with ether after acidification to pH 2. The aminopenicillin was then precipitated by adjusting the pH of the solution to the isoelectric point (about 5), and concentrating *in vacuo* at room temperature. Depending on the solubilities of the aminopenicillins and on the extent of concentration, the products obtained with this procedure (method A, Table 2) were contaminated to various degrees with inorganic salts and by-products. Since the purity of the products was more essential than the yields, we did not try to get maximal isolation of the penicillins. The yields in Table 2 refer to the specific fractions with the noted purities. In many instances, further crops with lower purities were also obtained. The three aminophenylpenicillins (XIV a, b, c) were isolated as the potassium salts by freeze-drying of the filtered and neutralized hydrogenation solutions. Alternatively, the aminopenicillins could be extracted at pH 2, after removal of unreacted azidopenicillins, with a solution of the sodium salt of 1,2-bis-(2-ethylhexyloxycarbonyl)-ethanesulphonic acid (Aerosol OT) in ether or methylisobutylketone. The aminopenicillin was precipitated from this solution by the addition of triethylamine. This method has been used for the isolation of 6-APA,³⁴ and its application for the isolation of α -aminobenzylpenicillin³⁵ and its sulfone³⁶ has also been recently described. The products obtained in this way (method B, Table 2) were also impure, the chief contaminations probably being the amino acids corresponding to the side chains of the penicillins and decomposition products thereof.

Table 2. Aminopenicillins.



No.	R	Catalyst	Work-up method	Isolat. product		Stability ^a		Activity ^b (μg/ml)	
				Yield %	Purity %	pH 2.1	penicillinase	<i>S.aur.</i> , Oxford	<i>E.coli</i>
<i>Xa</i>	 -CH(NH ₂)-	Ni	A	27	83	80	0.7	0.06	6.25
				86	61				
<i>Xb</i>	 -CH(NH ₂)-	Ni	B	62	66	37	0.8	0.03	25
<i>Xc</i>	 -CH(NH ₂)-	Ni	A	28 ^c	82	67	0.6	<0.006	6.25
<i>Xd</i>	Cl-  -CH(NH ₂)-	Ni	B	67	84	>100	—	0.13	25
<i>Xe</i>	 -CH(NH ₂)-	Ni	B	35	69	61	1.1	0.25	25
<i>Xf</i>	Cl-  -CH(NH ₂)-	Ni	B	72	56	—	1.0	0.13	62.5
<i>Xg</i>	 -CH(NH ₂)-	Ni	A	78	21	—	—	—	250
<i>Xh</i>	 -CH(NH ₂)-	Ni	B	68	80	58	0.8	0.13	2.5
<i>Xi</i>	 -CH(NH ₂)-	Ni	A	115	42	52	1.5	1.25	>250
<i>Xj</i>	CH ₃ (CH ₂) ₂ CH(NH ₂)-	Ni	A	98	40	56	1.1	0.25	>250
<i>Xk</i>	H ₂ N-CH ₂ CH ₂ -	Pd		40 in solution					
<i>Xl</i>	H ₂ N-(CH ₂) ₃ -	Pd		20 in solution					
<i>Xm</i>	HOOC-  -	Ni	A		<10				
<i>XIVa</i>	 -	Ni	A	99 ^d	52	0.3	2.2	0.13	>250
<i>XIVb</i>	 -	Ni	A	119 ^d	39	1.8	1.6	0.63	250
<i>XIVc</i>	H ₂ N-  -	Ni	A	95 ^d	45	0.9	2.0	0.25	>250








^{a, b} See corresponding footnotes in Table 1.

^c After recrystallization.

^d Isolated as potassium salt.

Table 3.

$$\begin{array}{c} \text{R}-\text{CH}-\text{COOH} \\ | \\ \text{N}_3 \end{array}$$

No.	R	Yield ^a %	M.p. ^b °C	Cryst. ^c solvent	Formula	Calculated %						Found %						
						C	H	Cl	N	S	C	H	Cl	N	S			
VIIa		85	103-4 ^d	B														
VIIb		64	62-3	B-PE	$\text{C}_6\text{H}_4\text{ClN}_3\text{O}_2$	45.40	2.86	16.76	19.86		45.2	2.70	16.9	20.1				
VIIc		69	50-1	C-PE	»	»	»	»	»		45.4	2.93	16.84	19.28				
VIIe		66	107-8	B-PE	»	»	»	»	»		45.7	2.96	16.6	20.0				
VIIe		37	95-6	B-L	$\text{C}_6\text{H}_4\text{N}_3\text{O}_3$	52.17	4.38		20.28		52.38	4.41		20.11				
VIIi		85	60-1	B	$\text{C}_6\text{H}_3\text{Cl}_2\text{N}_3\text{O}_2$	39.05	2.05	28.82	17.08		39.3	2.00	29.1	17.0				
VIIh		68	71-2	B	$\text{C}_6\text{H}_3\text{N}_3\text{O}_2\text{S}$	39.34	2.75		22.94	17.50	39.6	2.77		23.0	17.5			

^a after one crystallization. ^b analytical samples. ^c B = benzene, C = cyclohexane, L = ligroin, PE = petroleum ether. ^d literature^{29,30} gives m.p. 98.5°C and 98-101°C. ^e from the α -chloro acid. ^f α -bromo-3-thienylacetic acid was prepared according to Gronowitz *et al.*³⁰

The penicillins having the azido group in α -position on the side chain or in a benzene ring were smoothly hydrogenated over Raney nickel under the conditions used. Extractions, analysis or chromatograms showed in general that very little if any azido penicillins remained after the hydrogenations. It was found, however, that the age of the catalyst was of importance; thus in some cases incomplete hydrogenation was noted with older preparations, whilst after changing to fresh catalyst, the reduction could easily be performed. It is noteworthy that even α -azido-3-thenylpenicillin (IX h) containing two sulphur atoms, could readily be hydrogenated over Raney nickel to the corresponding aminopenicillin (X h). Though thiophene compounds are often readily desulphurized with Raney nickel,³⁷ the reduction of the azido group appeared to be completely selective, and chromatographic analysis revealed no by-products derived from an opening of the thiophene ring.

In the reduction of the chlorosubstituted α -azidobenzylpenicillins (IX b, c, d, f), some dehalogenation occurred, as the chromatograms of the hydrogenated solutions showed the presence of minor amounts of a second penicillin which in the first three cases had the same R_F value as that of α -aminobenzylpenicillin. These contaminants were readily eliminated during the work-up procedure. Unsatisfactory and inconsistent results were obtained with the penicillins having the azido group in the β - or γ -position of the side chain (IX k, l). With Raney nickel, no reduction took place in one experiment, whilst in other ones reduction was accompanied by complete loss of penicillin activity. As the inactivation could be caused by the alkaline conditions obtained during the hydrogenation of the azido group, some experiments were performed in a 1 M borate buffer at pH 7, but even under these conditions, extensive decomposition occurred. With a 5 % Pd/CaCO₃ catalyst, the amino penicillins (X k, l) were obtained in solution in yields of 40 % and 20 %, respectively, together with a little unreacted azido penicillin, though no amino penicillin could be isolated from such solutions. Poor results were also obtained with 4-azido-4-carboxybutylpenicillin (IX m), where a product with very low purity was obtained.

Some of the amino penicillins were purified for elemental analysis by reprecipitation from aqueous solutions (Table 4).

Properties. The acid stability of the prepared penicillins, as measured by their half life in 50 % ethanol at pH 2.1, relative to that of benzylpenicillin, was determined and the results are collected in Tables 1 and 2. It is seen that the α -azidopenicillins follow the general trend of α -heterosubstituted penicillins, namely, they show an enhanced acid stability,¹⁵ although in general they are not as stable as the corresponding aminopenicillins. The stability against *Bacillus cereus* penicillinase was also determined and compared with that of benzylpenicillin. It was found that all of the penicillins investigated show a relatively low stability. This is the expected result, since none of the compounds show the steric hindrance around the carbonyl group of the side chain found in penicillins such as 2,6-dimethoxyphenylpenicillin³⁸ or 3-*o*-chlorophenyl-5-methylisoxazolylpenicillin³⁹ which are highly stable against penicillinase.

The penicillins were also tested for their activity against a number of Gram-positive and negative bacteria. In Tables 1 and 2, MIC values against *Staphylococcus aureus*, Oxford and an *Escherichia coli* strain (non penicillinase

Table 4. Purified penicillins.

No.	M.p. ^a °C	Formula	Calculated %					Found %				
			C	H	Cl	N	S	C	H	Cl	N	S
IXa	194	C ₁₆ H ₁₆ N ₅ O ₄ SK	46.47	3.90		16.94	7.76	46.2	4.11		16.6	7.17
IXb	201	C ₄₆ H ₃₂ Cl ₂ N ₁₂ O ₈ S ₂ ·H ₂ O ^b	53.47	5.05		15.59	5.95	53.5	5.27		15.1	6.08
IXc	195	C ₁₆ H ₁₅ ClN ₅ O ₄ SK·H ₂ O	41.24	3.68		15.03	6.88	41.3	3.67		14.8	6.65
IXd	205	»	»	»	»	»	»	42.1	3.61		14.8	6.67
IXf	192	C ₁₆ H ₁₄ Cl ₂ N ₅ O ₄ SK	39.83	2.93	14.70		6.65	39.8	3.31	14.5		6.29
IXi	206	C ₁₃ H ₁₈ N ₅ O ₄ SK	41.14	4.78		18.46	8.45	40.8	5.08		18.6	7.97
Xa	202	C ₁₆ H ₁₆ N ₅ O ₄ SK·H ₂ O	52.30	5.76		11.44		52.2	5.79		11.4	
Xc	196	C ₁₆ H ₁₈ ClN ₃ O ₄ S	50.06	4.73	9.24	10.95		50.31	4.70	8.87	10.27	
Xd	203	C ₁₆ H ₁₈ ClN ₃ O ₄ S·H ₂ O	47.82	5.02	8.82	10.46	7.98	48.7	5.16	8.83	10.5	7.95
Xh	218	C ₁₄ H ₁₇ N ₃ O ₄ S ₂ ·H ₂ O	45.02	5.13		11.25	17.17	45.24	5.29		10.87	17.66
XIVa	200	C ₁₅ H ₁₆ N ₃ O ₄ SK·H ₂ O	46.02	4.63		10.73		46.62	4.54		10.24	

^a with decomposition. ^b dibenzylethylenediamine salt.

producer) are shown. Most of the penicillins show good activity against the former and some of them — IX c, d, e, and h and X c — are as active as benzylpenicillin (MIC 0.01 $\mu\text{g}/\text{ml}$). The MIC-values against *E. coli* are of a different order, but again, using benzylpenicillin (MIC 25 $\mu\text{g}/\text{ml}$) as reference, it is seen that of the azidopenicillins only the benzyl derivative (IX a), the thenyl derivative (IX h), and possibly also the γ -azidopropylpenicillin (IX l) can compete with it, the other compounds being virtually inactive.

Among the amino compounds, good activity is found only for α -amino-benzylpenicillin (X a) and its analogues, of which the *m*-chloroderivative (X c) and the α -amino-3-thenylpenicillin (X h) are comparable with the first mentioned compound. It is noteworthy that the introduction of a methyl group in the α -position in α -aminobenzylpenicillin to form X g reduces the activity.*

It has previously been shown^{8,40} that in the case of α -aminobenzylpenicillin the product prepared from D- α -aminophenylacetic acid is more active than the corresponding L-epimer. Our product (X a) as well as the other penicillins with an asymmetric center in the acyl group are mixtures of the D- and L-epimers, as racemic starting materials were used for the preparation of the side chains. We have found that the *in vitro*-activity of X a against the two above-mentioned microorganisms and some others is a little lower (1–2 dilution steps) than that of the pure D-epimer.

EXPERIMENTAL

The following azido acids and acid derivatives, used for the acylation of 6-APA, were prepared as described in the literature: *o*-, *m*-, and *p*-azidobenzoic acid,^{41,42,43} α -azido-isovaleroyl chloride,⁴⁴ α -azidocaproic acid,⁴⁵ γ -azidobutyric acid,⁴⁶ and the acetone derivatives of the hydrazides of β -azidopropionic,⁴⁷ and γ -azidobutyric acids.⁴⁸

α -Bromo-*m*-chlorophenylacetic acid (VI c) was prepared by the addition of bromine (56 g, 0.35 mole) during 5 h to a mixture of *m*-chlorophenylacetic acid⁴⁸ (45 g, 0.26 mole) and red phosphorus (1 g) at 100°C. The reaction mixture was allowed to cool and was then treated with 200 ml of water and extracted with ether. The combined ether extracts were washed with a bisulfite solution and with water, dried and concentrated *in vacuo* to give the bromo acid as an oil. Distillation gave the pure acid (34.5 g, 53%), b.p. 132–136°/0.5 mm; n_D^{25} 1.586. (Found: Br 31.8. Calc. for $\text{C}_6\text{H}_5\text{BrClO}_2$: Br 32.03).

α -Bromo-*p*-chlorophenylacetic acid (VI d) was prepared in an analogous way. Yield: 45%, m.p. 95–96° (from benzene). (Found: C 39.0; H 2.83; O 12.6. Calc. for $\text{C}_6\text{H}_5\text{BrClO}_2$: C 38.51; H 2.42; O 12.83).

α -Chloro-*o*-methoxyphenylacetic acid (VI e). *o*-Methoxymandelic acid (52 g, 0.23 mole), obtained by hydrolyzing its methyl ester⁴⁹ (oil. Found: equiv. wt. 185. Calc. for $\text{C}_9\text{H}_{10}\text{O}_4$: 182.2), was treated for one hour at room temperature with thionyl chloride (82 g, 0.69 mole) and a few drops of dimethylformamide, then warmed on a water-bath for 30 min. Excess thionyl chloride was removed *in vacuo* and the residue was treated with icewater and extracted with ether. After washing with brine and drying over anhydrous sodium sulphate, the ether solution was concentrated *in vacuo* to give VI e as a semi-solid. Crystallization from cyclohexane gave the pure product (33 g, 72%), m.p. 105–106°. (Found: C 53.93; H 4.66; Cl 17.49. Calc. for $\text{C}_9\text{H}_9\text{ClO}_3$: C 53.88; H 4.52; Cl 17.68).

α -Bromo-2,4-dichlorophenylacetic acid (VI f). 2,4-Dichlorophenylacetic acid⁵⁰ (6.7 g, 0.033 mole) and thionyl chloride (12 g, 0.1 mole) were refluxed for 30 min. Heating was discontinued and bromine (5.7 g, 0.036 mole) was added, whereafter the mixture was kept

* The low activity of this compound could partly be attributed to the low purity of the preparation used for the biological assay, but even a 73% pure sample prepared in another way gave a MIC of 125 $\mu\text{g}/\text{ml}$.

under slow reflux for several days until the bromine colour disappeared. The excess of thionyl chloride was removed *in vacuo* and the residue was poured into ice water, taken up in ether and washed with water. After drying, the ether solution was evaporated to give an oil, which slowly crystallized. Recrystallization from benzene gave the pure acid (4.4 g, 47 %), m.p. 97–97.5°. (Found: C 34.2; H 1.82; Br 28.4; Cl 25.4; O 11.0. Calc. for $C_8H_5BrClO_2$: C 33.84; H 1.77; Br 28.14; Cl 24.97; O 11.27).

α-Azido-o-chlorophenylacetic acid (VII b). A mixture of *α*-bromo-*o*-chlorophenylacetic acid⁵¹ (82 g, 0.33 mole), sodium azide (22 g, 0.33 mole) and sodium carbonate (19 g, 0.18 mole) was stirred overnight in 500 ml of 95 % acetone. The acetone was evaporated *in vacuo* and the residue was dissolved in water, acidified with dilute sulphuric acid and extracted with ether. The combined ether extracts were washed with water, dried over anhydrous sodium sulphate, and finally concentrated *in vacuo* at room temperature to give VII b as an oil, which crystallized from benzene-petroleum ether. Yields, physical constants, and analytical data for this and the other azido acids, which were prepared in the same manner, are given in Table 3.

α-Azido-α-phenylpropionic acid (VII g) was prepared by stirring a mixture of *α*-bromo-*α*-phenylpropionic acid⁵² (11.5 g, 0.05 mole) and sodium azide (3.6 g, 0.055 mole) in 50 ml of dimethylformamide at 60° for 4.5 h. The reaction mixture was left overnight and was then poured into 600 ml of water and extracted with chloroform. The combined organic extracts were washed with water, dried over anhydrous sodium sulphate and concentrated *in vacuo*, leaving an oil (7.8 g), which was dissolved in dry ether (50 ml) and treated with cyclohexylamine (5 g, 0.05 mole), whereby the cyclohexylamine salt of VII g (3.5 g, m.p. 151–153°) precipitated. Repeated crystallizations from ethanol finally gave 1.9 g (13 %) of the pure salt, m.p. 177–179° (decomp.) (Found: C 62.47; H 7.68; N 18.73. Calc. for $C_9H_{13}N_3O_2$: C 62.04; H 7.64; N 19.30).

1 g of the salt, in 60 ml of 30 % ethanol, was hydrogenated for 2 h at 4 atm. hydrogen pressure, over a 5 % Pd/CaCO₃ catalyst (1 g). After filtering off the catalyst and washing it with water, the combined filtrates were washed with ether, acidified with hydrochloric acid and evaporated to dryness. The residue was dissolved in 25 ml of ethanol and treated with pyridine till a sample, diluted with water, showed pH 3–4. Upon concentration, the solution deposited colourless crystals (0.2 g), m.p. 261–263°. Reprecipitation from water-methanol raised the melting point to 265–267° (decomp.), which is the reported melting point for *α*-amino-*α*-phenylpropionic acid.⁵³

A second portion of the salt was hydrogenated as above. After filtration, the solution was evaporated to dryness and the residue was treated with 98–100 % formic acid on the water bath for 4 h. The formic acid was removed *in vacuo* and the residue was washed with small amounts of cold N hydrochloric acid and ice water, dried and crystallized from ethanol to give *α*-formamido-*α*-phenylpropionic acid, m.p. 179–180° (decomp.). An authentic sample⁵⁴ had m.p. 181–182° (decomp.), and a mixed melting point determination between the two products gave 179.5–180.5° (decomp.). The two products also showed the same IR-spectrum.

The free *α*-azido-*α*-phenylpropionic acid, which was obtained as an oil after acidification of the cyclohexylamine salt and extraction with ether, was directly converted into its acid chloride (see below).

5-Azido-5-carboxyvalerohydrazide. Ethyl 5-bromo-5-carboxyvalerate⁵⁵ (75.0 g, 0.3 mole) was stirred together with potassium carbonate (20.8 g, 0.15 mole) and sodium azide (21.5 g, 0.33 mole) in a mixture of water (60 ml) and acetone (250 ml) for 6 h at room temperature and was then allowed to stand for further 72 h in the dark. The acetone was distilled off *in vacuo*, water was added and the aqueous solution acidified with conc. hydrochloric acid and extracted with ether. After washing with water and drying over anhydrous sodium sulphate, the ether solution was evaporated *in vacuo* to give the ethyl 5-azido-5-carboxyvalerate as an oil (58 g, 91 %), which was not characterized. The acidic ester (10.8 g, 0.05 mole) was treated with hydrazine hydrate (7.5 g, 0.15 mole) in a crystallization dish, left overnight at room temperature and then kept for 24 h in an evacuated desiccator over conc. sulphuric acid. The hydrazine salt of 5-azido-5-carboxyvalerohydrazide thus obtained was filtered off and washed well with ethanol (50 ml), giving the desired product, (8.6 g, 73 %) m.p. 85–87°. Two recrystallizations from ethanol gave an analytically pure sample, m.p. 86–87°. (Found: C 31.01; H 6.72; N 42.00. Calc. for $C_6H_{11}N_3O_3$: C 30.90; H 6.48; N 42.04).

The hydrazine salt (7 g), suspended in dry dioxane (150 ml), was treated with excess hydrogen chloride under stirring and cooling in an ice bath. The mixture was left overnight and the crystalline hydrochloride of 5-azido-5-carboxyvalerohydrazide filtered off and washed with warm acetonitrile. After drying, it weighed 7.1 g, m.p. 100–110° (decomp.). (Found: Cl 17.1. Calc. for $C_9H_{11}N_5O_3 \cdot HCl$: Cl 14.92). The product probably contained some hydrazine hydrochloride.

Preparation of azidopenicillins

Method A: via acid chlorides. Most of the acid chlorides of the azido acids were prepared by treatment of the latter with 2 equiv. of thionyl chloride on the water bath for 30–60 min, until the evolution of hydrogen chloride ceased. α -Azido- α -phenylpropionic acid was treated with 4 equiv. for 2 h at 40°C. α -Azidocaproyl chloride (VIII j), b.p. 70–75°/7 mm, like other aliphatic α -azido acid chlorides, could be purified by distillation. Similarly, α -azidophenylacetyl chloride (VIII a) could be distilled, b.p. 112°/8 mm, though decomposition was noted on one occasion, and after distillation of *m*-azidobenzoyl chloride, a violent explosion occurred when air was admitted into the distillation residue. We therefore preferred not to distill the acid chlorides but to use directly the crude products obtained after removing excess of thionyl chloride *in vacuo* at 50–60°C. To ensure that all of the latter was removed, the residues were treated three times with their own volume of dry benzene, which was also distilled off *in vacuo*. The γ -azido butyryl chloride (VIII l, Y = Cl) and the acid chlorides of the azidobenzoic acids (XII a-c, Y = Cl) were prepared by treating the acids, dissolved in dry ether together with the calculated amount of pyridine, with one equivalent of thionyl chloride under ice cooling. After 1 h, the precipitated pyridine hydrochloride was filtered off and the ethereal solution concentrated *in vacuo* at room temperature and used directly for the acylation.

α -Azidobenzylpenicillin (IX a) 6-APA (18.5 g, 0.085 mole) was dissolved in 300 ml of 30 % acetone by addition of sodium bicarbonate, while stirring and cooling in an ice bath. Phenylazidoacetyl chloride, (VIII a) (16.6 g, 0.085 mole), dissolved in 10 ml of dry acetone, was added dropwise to the solution, sodium bicarbonate (a total of 21 g 0.25 mole) being added to keep the mixture neutral. Stirring and cooling were continued for 2 h, the reaction mixture was then washed twice with ether, acidified to pH 2 with dilute hydrochloric acid and extracted twice with ether. The ether solution, containing the free azidopenicillin, was washed twice with water and then extracted with 85 ml of N potassium bicarbonate solution. The aqueous phase was washed with ether and then freeze-dried to give the potassium salt of IX a (29.4 g, 84 %) with a purity of 83 % (hydroxylamine assay). The penicillin was purified for elemental analysis (Table 4).

Method B: via acid azides. *α -Azidoethylpenicillin (IX k).* The acetone derivative of β -azidopropionyl hydrazide⁴⁷ (5.1 g, 0.03 mole) was dissolved, with stirring and cooling in an ice bath, in a mixture of water (15 ml), 2 N hydrochloric acid (15 ml), and ether (30 ml) and treated with a solution of sodium nitrite (2.8 g, 0.04 mole) in 10 ml of water till a drop of the reaction mixture gave a blue colour with starch-iodide paper.

The aqueous phase was separated and extracted with ether (3 × 10 ml). The combined ether solutions were washed twice with small volumes of water and then added to a rapidly stirred solution of 6-APA (6.5 g, 0.03 mole) and sodium bicarbonate in 125 ml of 40 % acetone. Stirring was continued for 3 h at room temperature and more sodium bicarbonate (a total of 7.6 g, 0.09 mole) was added to keep the mixture neutral. The reaction mixture was worked up as in method A. By freeze-drying, the potassium salt of IX k (3.3 g, 31 %) with a purity of 79.2 % (hydroxylamine assay) was obtained.

Method C: via mixed anhydrides. *o-Azidophenylpenicillin (XIII a).* To *o*-azidobenzoic acid⁴¹ (6.5 g, 0.04 mole) dissolved in dry dimethylformamide (50 ml) containing dry triethylamine (4.0 g, 0.04 mole), ethyl chloroformate (4.4 g, 0.04 mole) was added dropwise while stirring and cooling to –10° in an ice-salt mixture. After all the chloroformate had been added, the mixture was stirred for 20 min and then dry, ice-cold acetone (50 ml) was added in one portion, immediately followed by a chilled solution of 6-APA (8.6 g, 0.04 mole) and triethylamine (4.0 g, 0.04 mole) in 50 ml of water. The cooling bath was removed and stirring continued for 90 min, when 50 ml of water was added and the pH of the mixture was adjusted to 7. The reaction mixture was worked up as in method A. By freeze-drying, the potassium salt of XIII a (10.3 g, 64 %) with a purity of 83.8 %

(hydroxylamine assay) was obtained. Paper chromatography revealed that the product contained, besides the azido penicillin ($R_F = 0.58$), also a small amount of a second component with the same R_F -value (0.43) as ethoxyphenicillin.

Preparation of aminopenicillins

The catalysts used for the hydrogenation of the azidopenicillins were palladium on calcium carbonate (5 %) and Raney nickel, W 5.⁵⁶ The hydrogen pressure varied from 1–4 atm. and the solvent was always water. A list of the prepared penicillins appears in Table 2. The hydrogenation techniques used are illustrated by the following examples.

Method A: α -Aminobenzylpenicillin (X a). A suspension of Raney nickel (1 g) in 30 ml of water was saturated with hydrogen at room temperature and a pressure of 3.5 atm. A solution of the potassium salt of IX a (4.1 g, with a purity of 83 %) in 30 ml of water was added and the mixture shaken for 30 min under hydrogen at a pressure of 3.5 atm. The catalyst was filtered off and thoroughly washed with water.

The filtrate was adjusted to pH 2 with dilute hydrochloric acid and extracted twice with ether. The pH of the aqueous solution was then adjusted to 5 with dilute sodium hydroxide. On concentrating the solution *in vacuo* below 20°, α -aminobenzylpenicillin was obtained (3.0 g, 86 % yield) in the form of a white powder (purity 61 %).

In a duplicate experiment, the aqueous solution of the aminopenicillin was adjusted to pH 4.7 and concentrated *in vacuo* at room temperature to give 0.7 g (27 %) of α -aminobenzylpenicillin with a purity of 83 % (hydroxylamine assay). After adding butanol to the filtrate, it was evaporated *in vacuo* to give a second crop of the penicillin, (2.1 g) with a purity of 39 % (hydroxylamine assay).

Method B: α -Amino-p-chlorobenzylpenicillin (X d). Raney nickel catalyst (6 g) was suspended in water (50 ml) and hydrogenated for 30 min at 4 atm. hydrogen pressure. The potassium salt of IX d (8.2 g with a purity of 84 %) dissolved in water (100 ml) was added and the hydrogenation was continued for 30 min at the same pressure. The catalyst was removed by filtration and washed well with water. The filtrate was acidified to pH 2 with 2.5 N sulphuric acid and washed twice with methylisobutylketone. The aqueous solution was then extracted with 200 ml of an 8 % solution of the sodium salt of 1,2-bis-(2-ethylhexyloxy-carbonyl)-ethanesulphonic acid in methylisobutylketone. The organic phase was filtered through a celite pad and then slowly treated with triethylamine till the pH of the solution as measured by pH-meter was 6.2. The white crystalline precipitate thus obtained was filtered and washed with ether. After drying, the product weighed 4.0 g (67 %) and had a purity of 83.6 % (alkalimetric assay).

o-, m-, and p-Aminophenylpenicillin (XIV a–c). The three aminophenylpenicillins were isolated as their potassium salts by freeze-drying of the hydrogenation solutions. Compound XIV a was purified for elemental analysis and its identity was also proven by a comparison of its IR-spectrum with that of an authentic sample, prepared from *o*-nitrophenylpenicillin by hydrogenation.²⁴

Characterization of the penicillins

The purities of the obtained penicillins were determined according to the hydroxylamine method,⁵⁷ using the potassium salt of benzylpenicillin as standard. Alternatively, an alkaline titrimetric method⁵⁸ was used. For elemental analysis, the azidopenicillins, in the form of their potassium salts, were purified by precipitating them from their methanolic solutions by the addition of ether. This method did not work with *o*-chloro- α -azidobenzylpenicillin (IX b) which was purified by recrystallization of its N,N'-dibenzylethylenediamine salt, prepared by adding the calculated amount of the amine to a solution of the free penicillin in ether. The salt was purified by dissolving it in methylformamide and precipitating with water.

With the aid of dilute alkali, 10–20 % aqueous solutions of the aminopenicillins at pH 7.5–8.0 were prepared. On acidification to pH 4.5–5.5 the penicillins crystallized out. The potassium salt of XIV a was recrystallized from isopropanol-ether. The results of the analysis are given in Table 4.

Some of the purified penicillins contained crystal water and they all decomposed on heating, showing no definite melting points. Paper chromatograms were run in a butanol-ethanol-water (4:1:5, top layer) system and were developed microbiologically.¹

N-t-Butoxycarbonyl- α -aminophenylacetic acid (II a). A mixture of α -aminophenylacetic acid (7.5 g, 0.05 mole), *t*-butoxy-*p*-nitrophenylcarbonate⁵⁹ (17.2 g, 0.075 mole), sodium carbonate (13.3 g, 0.125 mole), *t*-butanol (75 ml), and water (50 ml) was heated on a water bath for 30 min. The butanol layer was evaporated by passing a stream of air through the reaction flask and the remaining aqueous phase was cooled and filtered. The filtrate was acidified to pH 6, washed three times with ether and then further acidified to about pH 1 and extracted with ether. After washing with water and drying, the ether solution was concentrated *in vacuo* to give an oil, which was crystallized from ligroin. Yield: 5.0 g (41 %), m.p. 111–112°. (Found: C 62.18; H 7.07; N 5.65. Calc. for C₁₃H₁₇NO₄: C 62.14; H 6.82; N 5.57).

N-t-Butoxycarbonyl- α -aminobenzylpenicillin (IV a) was prepared from II a (6.0 g, 0.024 mole) according to method C above. Yield: 8.5 g (73 %) of the potassium salt, having a purity of 71 % (hydroxylamine assay).

A dry solution of the free penicillin in nitromethane was treated at 0–5° with one or two equivalents of dry hydrogen chloride or bromide, which in all cases resulted in an immediate precipitation of an oil. On working up, no product with antibiotic activity could be obtained.

IV a (0.5 g) was added with shaking to 5 ml of trifluoroacetic acid. The substance dissolved with evolution of gas and as soon as the solution was clear (45 sec) the trifluoroacetic acid was removed *in vacuo* at 30° leaving a colourless oily residue. This was dissolved in water and neutralized. The solution thus obtained contained no substance with antibiotic activity.

If a solution of IV a in 50 % dioxan was kept at room temperature for 2 h at pH 2, formation of small amounts α -aminobenzylpenicillin could be detected by paper chromatography.

N-Triphenylmethyl- α -aminophenylacetic acid (II b). α -Aminophenylacetic acid, methyl ester hydrochloride (77.5 g, 0.385 mole) was shaken overnight with triphenylchloromethane (108 g, 0.385 mole) and triethylamine (95 g, 0.94 mole) in chloroform (600 ml). The reaction mixture was washed with water, dried and concentrated *in vacuo* to give the methyl ester of II b (163 g), which was crystallized from ethanol, m.p. 100–102°. The ester (26 g, 0.064 mole) was triturated 1 h on a water-bath with potassium hydroxide (4.2 g, 0.075 mole) in 100 ml of water and filtered. The cooled filtrate was cautiously acidified with acetic acid and extracted with ether. The acid was extracted with 25 ml of 2 N sodium hydroxide, reprecipitated with acetic acid and taken up in ether. The ether was removed *in vacuo* to give the acid (5 g, 20 %), m.p. 195–197°. An analytical sample had m.p. 197–198.5° (from benzene). (Found: C 82.13; H 5.92; N 3.21. Calc. for C₂₇H₂₃NO₂: C 82.41; H 5.89; N 3.56). The acid could be converted into its chloride by treatment with phosphorus pentachloride according to Zervas,¹⁷ but all attempts to acylate 6-APA in aqueous solution or in chloroform failed.

N-Triphenylmethyl- α -aminophenylacetohydrazide was prepared by boiling the methyl ester of II b (50 g, 0.123 mole) for 7 h with hydrazine hydrate (50 ml) in a mixture of pentanol (80 ml) and propanol (40 ml). On cooling, the hydrazide crystallized and was collected by filtration. Recrystallization from ethanol gave 34 g (68 %), m.p. 196–197°. (Found: C 79.5; H 6.33; N 10.15. Calc. for C₂₇H₂₅N₂O: C 79.58; H 6.18; N 10.31).

The hydrazide (8 g, 0.02 mole), in a mixture of chloroform (20 ml), water (50 ml), and conc. hydrochloric acid (2 ml), was nitrosated at 0° by the slow addition of sodium nitrite (1.38 g, 0.02 mole) dissolved in water (30 ml). The chloroform layer was separated, washed with water and dried, whereby an evolution of gas started after a short time. The IR-spectrum of the solution showed a strong band at 2240 cm⁻¹, indicating the presence of an isocyanate group, while the expected azide band at 2140 cm⁻¹ was much weaker. Upon attempted acylation, the chloroform solution was used directly and stirred with an aqueous solution of 6-APA as in method B; no acylation product, however, could be detected.

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REFERENCES

1. Batchelor, F. R., Doyle, F. P., Nayler, J. H. C. and Rolinson, G. N. *Nature* **183** (1959) 257.
2. Rolinson, G. N. and Stevens, S. *Brit. Med. J.* **2** (1961) 191.
3. Tosoni, A. L., Glass, D. G. and Goldsmith, L. *Biochem. J.* **69** (1958) 476.
4. Abraham, E. P., Newton, G. G. F., Schenck, J. R., Hargie, M. P., Olson, B. H., Schuurmans, D. M., Fischer, M. W. and Fusari, S. A. *Nature* **176** (1955) 551.
5. Newton, G. G. F. and Abraham, E. P. *Biochem. J.* **58** (1954) 103.
6. Unpublished results of Beecham Research Laboratories and of this laboratory.
7. Doyle, F. P., Nayler, J. H. C. and Smith, H. *Brit. Pat. Spec.* 873, 049/1961.
8. Doyle, F. P., Fosker, G. R., Nayler, J. H. C. and Smith, H. *J. Chem. Soc.* **1962** 1440.
9. Greenstein, J. P. and Winitz, M. *Chemistry of the Amino Acids*, John Wiley & Sons, Inc., New York 1961, p. 1238.
10. Sifferd, R. H. and du Vigneaud, V. *J. Biol. Chem.* **108** (1935) 753.
11. Peck, R. L. and Folkers, K. In Clarke, H. T., Johnson, J. R. and Robinson, R. (Eds.) *The Chemistry of Penicillin*, Princeton University Press, Princeton 1949, p. 148.
12. Schallenberg, E. F. and Calvin, M. *J. Am. Chem. Soc.* **77** (1955) 2779.
13. *Ref. 9*, p. 1243.
14. Hunter, J. H., Hinman, J. W. and Carter, H. E. *Ref. 11*, p. 909.
15. Doyle, F. P., Nayler, J. H. C., Smith, H. and Stove, E. R. *Nature* **191** (1961) 1091.
16. Schwyzer, R., Surbeck-Wegmann, E. and Dietrich, H. *Chimia* **14** (1960) 366.
17. Zervas, L. and Theodoropoulos, D. M. *J. Am. Chem. Soc.* **78** (1956) 1359.
18. Wieland, Th., Müller, R., Niemann, E., Birkhofer, L., Schöberl, A., Wagner, A. and Söll, H. In Müller, E. (Ed.) *Methoden der Organischen Chemie (Houben-Weyl)*, 4th Ed. Vol. XI/2, Georg Thieme Verlag, Stuttgart 1958, p. 310.
19. Ferris, A. F. *J. Org. Chem.* **24** (1959) 1726.
20. Hartung, W. H., Kramer, D. N. and Hager, G. P. *J. Am. Chem. Soc.* **76** (1954) 2261.
21. *Ref. 9*, p. 816.
22. Unpublished results of this laboratory.
23. Moses, P., Dahlbom, R. and Sjöberg, B. *Arkiv Kemi.* **22** (1964) 451.
24. Sjöberg, B. and Ekström, B. *Brit. Prov. Pat. Appl.* 26488/1961 and 26489/1961 *Brit. Pat. Spec.* 940,488/1963 and 940,489/1963.
25. Claesen, M., Van Dijck, P. J., Vanderhaeghe, H. and De Somer, P. *Antibiot. Chemotherapy* **12** (1962) 187.
26. Vanderhaeghe, Claesen, Van Dijck, and Parmentier, *South African Pat. Appl. No.* R61/1961.
27. Bertho, A. and Maier, J. *Ann.* **498** (1932) 50.
28. Freudenberg, K., Eichel, H. and Leutert, F. *Ber.* **65** (1932) 1183.
29. Forster, M. O. and Müller, R. *J. Chem. Soc.* **97** (1910) 126.
30. Darapsky, A. *J. prakt. Chem.* **99** (1919) 179.
31. Doyle, F. P., Nayler, J. H. C. and Rolinson, G. N. *Brit. Pat. Spec.* 870,395/1961.
32. Perron, Y. G., Minor, W. F., Holdrege, C. T., Gottstein, W. J., Godfrey, J. C., Crast, L. B., Babel, R. B. and Cheney, L. C. *J. Am. Chem. Soc.* **82** (1960) 3934.
33. *Ref. 18*, p. 353.
34. *Brit. Pat. Spec.* 882,277/1961 to Beecham Research Laboratories, Ltd.
35. *Fr. Pat.* 1,332,557/1963 to Bristol-Myers Co.
36. Johnson, D. A., Panetta, C. A. and Cooper, D. E. *J. Org. Chem.* **28** (1963) 1927.
37. Goldfarb, Ya., Fabrichnyi, B. P. and Skalavina, I. F. *Tetrahedron* **18** (1962) 21.
38. Rolinson, G. N., Stevens, S., Batchelor, F. R., Cameron Wood, J. and Chain, E. B. *Lancet* **1960 II** 564.
39. Nayler, J. H. C., Long, A. A. W., Brown, D. M., Acred, P., Rolinson, G. N., Batchelor, F. R., Stevens, S. and Sutherland, R. *Nature* **195** (1962) 1264.
40. Sjöberg, B., Ekström, B., Forsgren, U. and Örtengren, B. *Elfte Nordiska Kemist-mötet, Åbo, Finland* 1962, p. 273.

41. Bamberger, E. and Demuth, E. *Ber.* **34** (1901) 1309.
42. Bamberger, E. and de Werra, J. *Ber.* **35** (1902) 3711.
43. Griess, P. *Ann.* **135** (1865) 121.
44. Freudenberg, K. and Keller, R. *Ber.* **71** (1938) 329.
45. Darapsky, A. *J. prakt. Chem.* **146** (1936) 219.
46. Curtius, Th. and Giulini, W. *Ber.* **45** (1912) 1045.
47. Curtius, Th. and Franzen, H. *Ber.* **45** (1912) 1037.
48. Kenner, J. and Morton, F. *J. Chem. Soc.* **1934** 679.
49. Barthel, W. E., Leon, J. and Hall, S. H. *J. Org. Chem.* **19** (1954) 485.
50. Schlesinger, A. H. and Frill, E. J. *J. Am. Chem. Soc.* **78** (1956) 6123.
51. G. Pat. 279,198/1914 to Farbwerke vorm. Meister Lucius & Brüning (*Chem. Zentr.* **1914:II** 1136).
52. Fittig, R. and Kast, H. *Ann.* **206** (1881) 24.
53. Steiger, R. E. In Horning E. C. (Ed.) *Org. Syn. Coll. Vol. III* (1955) 88.
54. McKenzie, A. and Clough, G. W. *J. Chem. Soc.* **101** (1912) 390.
55. Schwenck, E. and Papa, D. *J. Am. Chem. Soc.* **70** (1948) 3626.
56. Adkins, H. and Billica, H. R. *J. Am. Chem. Soc.* **70** (1948) 695.
57. Boxer, G. E. and Everett, P. H. *Anal. Chem.* **21** (1949) 670.
58. Örtengren, B. *Private communication.*
59. Andersson, G. W. and McGregor, A. C. *J. Am. Chem. Soc.* **79** (1957) 6180.
60. Gronowitz, S., Sjögren, I., Wernstedt, L. and Sjöberg, B. *Arkiv Kemi* **23** (1964) 129.

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