The Chemical Study of Penicillin:

A Brief History

The Editorial Board of the Monograph on the Chemistry of Penicillin

DURING THE DECADE FOLLOWING the discovery of penicillin (*1*) relatively little information was secured as to its chemical nature. Fleming had reported it to be soluble in water and in alcohol, but insoluble in ether or chloroform, and its thermal stability in solution to be maximal at neutrality. Three years later Raistrick and his collaborators (*2*) found that penicillin could be produced in a synthetic medium. They also showed that when solutions of penicillin at pH 7.2 were extracted with ether, some of the antibacterial activity was transferred to the ether, but that when the process was carried out at pH 2, the extraction was almost complete. They likewise noted that the antibiotic extract was sensitive to oxidants and was readily inactivated by evaporation in acid and alkaline solution, but moderately stable at pH 5–6.

After a further interval of three years Raistrick's findings were confirmed, in general, by Reid (*3*), who found, in addition, that the activity was lost on dialysis and that penicillin was adsorbed on charcoal.

In 1940 Chain, Florey, and their collaborators (*4*) prepared penicillin in solid, though inhomogeneous, form and reported on its effectiveness *in vivo* against various pathogenic organisms. In the same year Abraham and Chain (*5*) described the preparation from *B. coli* of an enzyme, penicillinase, which inactivates penicillin. In 1941 the Oxford workers (*6*) published details of a procedure for the concentration of penicillin from culture fluid obtained with the use of Raistrick's synthetic medium. This involved extraction from acid solution with organic liquids and further purification by chromatographic procedures. In this way there was secured (*7*) the barium salt of an acid which proved to be stable when absolutely dry or in organic solvents.

In 1942 Abraham and Chain (*8*), by further refinements of the process, succeeded in producing a barium salt with an activity of 450–500 units/mg. This product was later found (*9*) to contain nitrogen, but the provisional formula suggested, $C_{14}H_{18}O_{10}N_2Ba$, was soon found to be incorrect. One impurity detected was barium furoate. The significance of the nitrogen content was indicated by its linear relation to antibiotic potency. On hydrolysis this substance yielded carbon dioxide, a volatile acid, and the crystalline salt of a base. The authors recognized that their material was not homogeneous, so that these data could not be interpreted with certainty. It was also observed (*10*) that penicillin is inactivated by primary alcohols, by organic bases, and by various metallic salts—for example, those of copper and zinc.

The basic compound, referred to above, received the name penicillamine; it was shown (*11*) to be a primary amine and to contain one strongly and one weakly acidic group. The hydrochloride was at first assigned the formula $C_6H_{11}O_4N \cdot HCl$ and, later, $C_5H_{11}O_2SN \cdot HCl$. In so far as the Oxford work was concerned, the recognition of sulfur in penicillin (see below) followed directly from the study of an oxidation product of penicillamine. The analytical results indicated the uptake of three oxygen atoms and thus suggested conversion of a thiol to a sulfonic acid. The significance of penicillamine as an integral part of penicillin became obvious when it was obtained from penicillin having an activity of 1,000 units/mg. on the Oxford scale of that date.

In the meantime the study of penicillin was taken up in other laboratories. A product showing 750 units/mg. was obtained at the Imperial College of Science, London (*12*), where it was observed that penicillin, on degradation, yielded a product which appeared to be an amino acid. The conversion of penicillin in acid solution into a crystalline product termed penillic acid was recorded early in 1943 by chemists in the Wellcome Research Laboratories (*13*). In the United States, penicillin was obtained in the form of a crude ammonium salt (*14*), which had an activity of 240 units/mg. (*15*), but no evidence was presented as to its chemical nature.

On treatment with diazoalkanes, penicillin concentrates were found to yield esters which proved to be notably more stable than the salts. They showed little activity *in vitro*, but were antibiotically active *in vivo*. The benzhydryl ester was split by catalytic hydrogenation with regeneration of *in vitro* activity (*16*). It was later shown that the methyl and ethyl esters could be hydrolyzed to active penicillins by treatment with sodium hydroxide or sodium bicarbonate solution (*17*, *18*).

By 1943 recognition of the potential military importance of penicillin had led to restriction of chemical information on the subject. Investigation was continued with increasing intensity, in both academic and industrial research laboratories, but, in general, the results were privately communicated to other recognized workers in the field rather than to the scientific press. This exchange

From Chapter I of the monograph entitled *The chemistry of penicillin,* now in preparation under the supervision of the National Academy of Sciences and the Office of Scientific Research and Development, to be published by the Princeton University Press.

of information on the chemistry of penicillin was effected in Britain at first through unofficial conferences of interested workers and later by those sponsored by the Ministry of Supply and the Medical Research Council. There were also special agreements between certain pharmaceutical manufacturers in England (especially May and Baker, Ltd., one of the firms which entered the Therapeutic Research Corporation of Great Britain, Ltd.) and in the United States (Merck & Co., Inc., E. R. Squibb & Sons, and subsequently Chas. Pfizer & Co., Inc.). Information secured by the American firms was subsequently communicated to the Committee on Medical Research and disseminated through it; a similar procedure was followed in England, where the five firms participating in the Therapeutic Research Corporation, as well as Imperial Chemical Industries, Ltd., and various academic groups, reported to the Medical Research Council.

During the first half of 1943, progress in the chemical studies was made principally in Britain, where, in addition to penicillamine, 2-pentenylpenillic acid and 2-pentenylpenillamine were isolated as conversion products of the impure preparations then available. Experimental work in the United States was at first primarily directed toward problems of production and purification. It was found in the Northern Regional Research Laboratory and in the Merck and Squibb laboratories that chromatographic procedures, the efficacy of which had been demonstrated in Britain for the concentration of penicillins in the form of their free acids, could be applied advantageously to the more stable sodium salts. The partition chromatography of Martin and Synge was adapted for the purification and separation of the penicillins by chemists of Imperial Chemical Industries, Ltd. In the summer of 1943, MacPhillamy, Wintersteiner, and Alicino, of the Squibb group, succeeded (*19*) in crystallizing the sodium salt of benzylpenicillin. This important achievement, which made possible the accurate chemical study of the pure compound, immediately led to the recognition by the same investigators of sulfur as a constituent of the molecule. Coincidentally, the presence of sulfur in (impure) barium penicillin, as well as in penicillamine, penicillaminic acid, penillic acid, and other well-defined derivatives of penicillin, was discovered independently by the chemists in Oxford (*20*). Soon afterward the Oxford workers reported the crystallization of alkali metal salts of their penicillin.

At about the same time it became clear that the penicillin which had been obtained in crystalline form in the United States was not identical with the penicillin with which the British investigators had been working. Among other differences between the two was the far greater reluctance of the latter to crystallize. The chemical distinction between them was clearly brought to light during the middle of 1943 by observations in several quarters. One was the demonstration by Stodola, Wachtel, and Coghill, of the Northern Regional Research Laboratory, that the two varieties of penicillin give different, though analogous, crystalline derivatives when the free acids of the respective penicillins are treated with benzylamine. The second observation was the demonstration in the Merck laboratories, by analytical data on crystalline penillic acids, that there were at least two penicillins.[1] The American preparations had been found (*21*) to yield phenylacetic acid on hydrolysis, whereas the antibiotic studied in Britain yielded 2-hexenoic acid under similar conditions (*22*). Very convincing evidence was also obtained by the chemists of Imperial Chemical Industries, Ltd., at an early stage of the development. The main constituents of their own penicillin and of Merck penicillin were chromatographically separated (*23*). This was later confirmed by direct comparison of the derived penillic acids (*24*). It appears that these reports had a limited circulation.

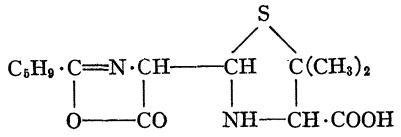
On the other hand, penicillamine having the same configuration was obtained from both types. After some uncertainty as to its constitution (*20*), penicillamine was recognized by the Oxford workers to be β,β-dimethylcysteine. This was demonstrated by chemical (*25*) and crystallographic comparison with a synthetic sample (*26*). Penicillamine had in the meantime been shown to yield thiazolidines on condensation with carbonyl compounds, and it was suspected that the same ring system was present in the penicillin molecule (*27*). Grounds for this suspicion had been furnished by the observation (*28*) that when the Oxford penicillin was decomposed by mercuric chloride, it yielded a carbonyl compound, probably an aldehyde, as well as penicillamine. This aldehyde was characterized in the form of a crystalline 2,4-dinitrophenylhydrazone and a condensation product with dimedone, and was shown to have the composition $C_8H_{13}O_2N$ (*27*). It was recognized as hexenoylaminoacetaldehyde and as the source of the glyoxal-osazones which were obtained early from mother liquors resulting after hydrolysis and separation of penicillamine. Meanwhile the Imperial College of Science group (*29*) had shown that the antibiotic studied in Britain could be reduced to a dihydro derivative which was biologically active and was recognized as a "natural" penicillin. It afforded n-caproic acid on hydrolysis. The position of the double bond in the hexenoic acid obtained from the unreduced antibiotic was determined at Oxford by permanganate oxidation to propionaldehyde. The Imperial College workers found that the penilloaldehyde from their dihydropenicillin afforded a dinitrophenylhydrazone which could be further changed to the glyoxal osazone. They therefore suggested that the aldehyde was n-caproylaminoacetaldehyde. In both series the identity of the penilloaldehydes was confirmed by synthesis.

[1] Systems of nomenclature using letters (in the United States) and Roman numerals (in Britain) were initiated late in 1943. However, in the interests of clarity and uniformity, workers in the field agreed early in 1946 on the nomenclature now in use, which involves a designatory prefix such as "benzyl."

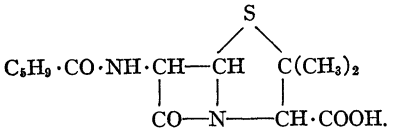
Analogous conclusions and hypotheses were attained independently and simultaneously by American workers. The chemists in the Squibb Institute (*30*) and in the Merck laboratories (*31*) produced evidence that the carbonyl compound secured by treatment of the American crystalline penicillin with mercuric chloride was phenylacetylaminoacetaldehyde. It was, for example, found to be oxidized to phenaceturic acid. At the same time the Merck group demonstrated that when the product formed by the action of benzylamine upon benzylpenicillin was treated with mercuric chloride and penicillamine was liberated, the benzylamine group remained in amide linkage in the residual portion of the molecule. A strictly analogous result was obtained with the methyl ester which resulted from the action of methanol upon benzylpenicillin. It was also shown by members of the Imperial Chemical Industries group (*32*) that the methyl ester of pentenylpenicillin, on treatment with mercuric chloride, yielded the methyl ester of penicillamine. The identity of the acid group in penicillin with the carboxyl group of penicillamine was thus definitely established by two independent methods.

In the recognition of degradation products of penicillin, invaluable aid was rendered by X-ray crystallographic measurements. For instance, the 2,4-dinitrophenylhydrazone of the penilloaldehyde secured in Oxford was shown by this method (*33*) to be identical with the hydrazone prepared from synthetic 2-hexenoylaminoacetaldehyde (*34*).

On the basis of these and many other findings, the Oxford workers proposed (*35*) the thiazolidine-oxazolone formula



as the simplest expression for their penicillin. An exactly corresponding formula was independently proposed for benzylpenicillin by the Merck, Squibb, and Abbott groups. However, the chemists in both the Oxford and the Merck laboratories drew attention to the fact that the presence of a basic group, indicated by this structure, could not be detected in penicillin, and both proposed, as a possible alternative, the β-lactam structure



At this point it was hoped that, as the penicillins were relatively simple compounds, synthetic methods for their production could be developed without much difficulty. The urgency of the need for large quantities of penicillin by the military forces made imperative the intensive exploration of this field on a wider front and on a basis of international collaboration. At the instance of the director of the Office of Scientific Research and Development and the chairman of the Committee on Medical Research in Washington, D. C., and the secretary of the Medical Research Council in London, the necessary diplomatic agreements were reached. The American group of collaborators was enlarged by the inclusion of 8 more industrial research laboratories and 10 academic laboratories.[2] Contracts between these organizations and the governmental agencies were entered into, according to the terms of which each contractor undertook to conduct experimental investigations in connection with the chemical structure of penicillin and the synthesis of penicillin or a therapeutic equivalent. All contractors undertook to report to their Government all pertinent information available to them at that time and thereafter to furnish monthly progress reports.[3] All of this information was transmitted as expeditiously as possible to each contractor in Britain and in the United States. In the United States the contracts, which began during December and January 1943–44, remained in force until November 1, 1945 in the case of the industrial organizations[4] and December 31, 1945 in the case of the academic institutions. In Britain, as already stated, the early collaboration was an informal by-product of a Penicillin Production Committee, sponsored by the Ministry of Supply. On January 1, 1944, the Medical Research Council set up a Committee on Penicillin Synthesis (CPS) under the

[2] The following groups collaborated in the general program: In America, the industrial participants were Abbott Laboratories; Cutter Laboratories; Heyden Chemical Corporation; Eli Lilly and Company; Merck & Co., Inc.; Parke, Davis and Company; Chas. Pfizer & Co., Inc.; Shell Development Company; Squibb Institute for Medical Research; The Upjohn Company; Winthrop Chemical Company, Inc. The academic and governmental participants were U. S. Department of Agriculture, Northern Regional Research Laboratory; Cornell University Medical College, Department of Biochemistry, and Russell Sage Institute; Federal Security Agency, Food and Drug Administration; Harvard University, Department of Chemistry; University of Illinois, Department of Chemistry; University of Michigan, Departments of Chemistry and Physics; National Bureau of Standards; Naval Medical Research Institute; The Rockefeller Institute for Medical Research.

In Britain the industrial participants were Boots Pure Drug Company, Ltd.; British Drug Houses, Ltd.; Glaxo Laboratories, Ltd.; Imperial Chemical Industries, Ltd. (Alkali Division); Imperial Chemical Pharmaceuticals, Ltd.; May and Baker, Ltd.; Wellcome Foundation, Ltd. The academic and governmental participants were Cambridge University, Departments of Chemistry and Colloid Science; Imperial College of Science, London, Department of Organic Chemistry; The London Hospital Medical Unit; Manchester University, Department of Chemistry; National Institute for Medical Research, Hampstead, London; Oxford University, Department of Crystallography, Dyson Perrins Laboratory, Sir William Dunn School of Pathology, and the Department of Physical Chemistry.

[3] Arrangements have been made for the deposition of a complete file of these reports with the U. S. Department of Commerce, Office of Technical Services, from which reproductions of desired portions can be obtained on request. Copies of these reports are also being filed in scientific libraries in Britain.

[4] The industrial participants in the chemical projects performed the subject work of their contracts without financial aid from their Governments. In both countries, however, grants were made to the academic groups in support of their work.

chairmanship of Sir R. Robinson and including representatives of the industrial groups, academic centers, and the Ministry of Supply. The regular exchange of British and American reports then began, but because of delay in the first months much work was duplicated. Attention is drawn elsewhere to the more important consequences of this situation.

The unrestricted exchange of current information at frequent intervals resulted in as close a collaboration as is possible among a widely distributed group of laboratory teams, but no attempt was made to avoid duplication of effort in the various laboratories. In consequence it is difficult or impossible, except in a relatively few specialized phases of the joint effort, to assign sole scientific credit for individual findings secured during the period covered by the contracts.[5] In the outline that follows no attempt is made to do more than touch upon some of the more significant results.

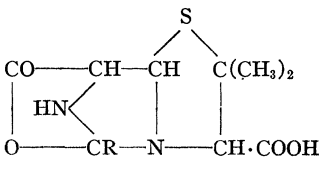
In the winter of 1943–44, an important problem was the confirmation and clarification of the structure assigned to penicilloic acids, the primary product of the hydrolysis of penicillins. The major part of the light thrown on this problem was supplied by the Merck laboratories. The D (or "unnatural") configuration of penicillamine was established by successive treatment with phenylisocyanate and with Raney nickel catalyst, whereby the sulfur atom was replaced by hydrogen; the product was identical with the phenylureide derivative of D-valine (*31*).

As stated in *Science* (*36*), the constitution of the penaldic acid, with which penicillamine is combined in benzylpenicilloic acid, was established (*31*) by its conversion on catalytic hydrogenation into cyclohexylacetylalanine. Under less drastic conditions of hydrogenation corresponding derivatives of serine were obtained. As these amino acid derivatives were optically inactive, they yielded no information as to the configuration of that part of the penicilloic acid molecule. This information was secured only later, when it was shown in the Merck laboratories (*37*) that under suitable conditions of hydrogenation derivatives of L-alanine, which has the "natural" configuration possessed by the amino acids of proteins, were formed.

Derivatives of penaldic acid were synthesized in several laboratories by the condensation of ethyl formate or orthoformate with esters of phenylacetyl glycine, and when the products were condensed with synthetic D-penicillamine, esters of penicilloic acids were obtained. In a very complete study, in the Merck laboratories (*38*), methyl esters of three of the four theoretically possible diastereoisomers of the penicilloic acids derived from D-penicillamine were synthesized, and it was shown that at least two of these were formed by the action of methanol upon benzylpenicillin. Somewhat later, esters of the fourth isomeric form of penicilloic acid were synthesized in the Squibb Institute (*39*).

It has already been mentioned that the conversion of penicillins into penillic acids contributed largely to the recognition of the existence of more than one variety of penicillin. The constitution of benzylpenillic acid was confirmed by synthesis in two laboratories during 1944. An optically inactive monomethyl ester was prepared by Cook, Elvidge, and Heilbron (*40*) by condensing the methyl ester of phenylthioacetyldiethoxyalanine with DL-penicillamine. The dimethyl ester of optically active penillic acid, identical with the product from benzylpenicillin, was synthesized by the Merck group (*41*) by condensing the Schiff base from N-formyl-α-formylglycine methyl ester and benzylamine with the methyl ester of D-penicillamine.

Attempts, made in many laboratories, to produce penicillins by anhydrization of penicilloates met with almost universal failure. It is worthy of note, however, that they did provide the first synthetic material possessing antibiotic activity, even though the potencies were small and not proved to be of penicillin type (*42*). In the early stages of the research, the oxazolone, or azlactone, structure indicated above was that to which most attention was paid, but, as the work progressed, increasing difficulty was experienced in applying it to the penicillins. This was especially the case in connection with the physicochemical investigations such as the electrometric studies by Neuberger (*43*) and many others. A number of structures which had been proposed at the outset were excluded, during the first half of 1944, by the observation that the penicillin molecule contains only one labile hydrogen atom other than that of the carboxyl group. This was indicated in the Abbott laboratories by the action of Grignard reagent upon methyl benzylpenicillinate (*44*) and at Cornell University by equilibration of sodium benzylpenicillinate with deuterium oxide (*45*). The structural limitations thereby imposed were met by the formulas set forth above, as well as by a tricyclic formula



[5] Shortly after the contracts between OSRD and the industrial participants had expired, a brief summary announcement of the principal findings was published in *Science* (*36*) and in *Nature* (1945, **156**, 766). At a conference, attended by scientific representatives of all the cooperating groups, held on January 9, 1946, it was decided that detailed reports of the results secured under the collaborative program should, in general, be published in a monograph rather than in individual papers in the scientific press. However, provision was made for the publication, in advance of the monograph, of papers which, in the opinion of the Editorial Board, would not conflict with the plan. Several such articles have appeared.

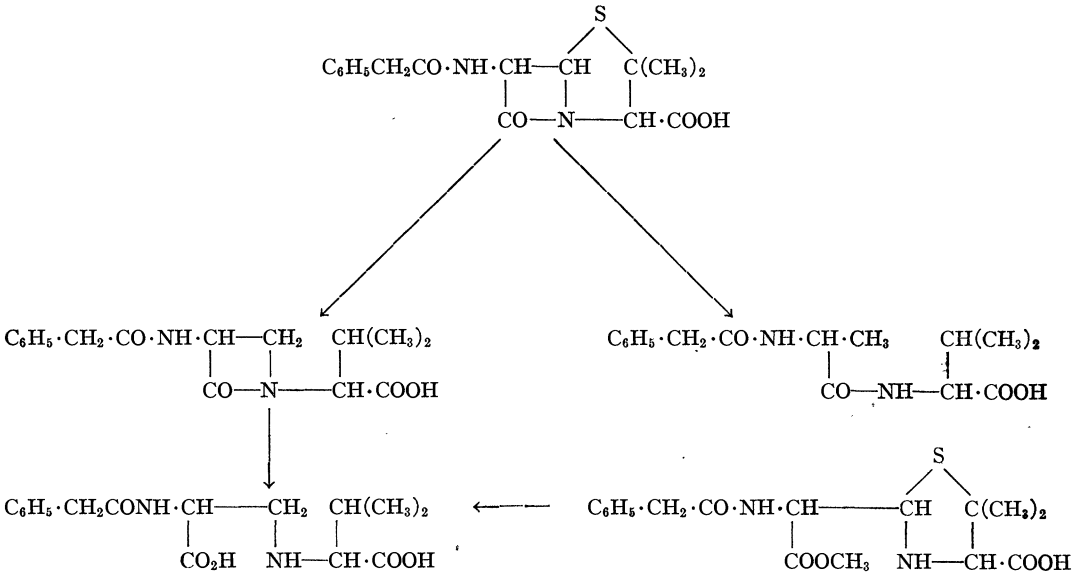
When the monograph was planned, it was hoped that its writing could be completed in six months; unfortunately, this estimate proved unduly optimistic.

which had been proposed by several of the participant groups.

Among these alternatives, the β-lactam structure gradually gained popularity. This formula received support from physicochemical evidence. Infrared absorption spectra were studied principally by the Shell Development Company; Thompson, of Oxford University; Sutherland, of Cambridge University; Randall and collaborators, of the University of Michigan; Merck & Co., Inc.; and the Russell Sage Institute, Cornell University Medical College. An important result was the demonstration by the Shell Development Company of the presence in penicillins of a distinctive absorption band which also appeared in three models containing a fused β-lactam-thiazolidine structure, prepared in its laboratories. Also, other absorption bands found in the spectrum of penicillin were considered to indicate the presence of a monosubstituted amide group. The models here show that the bands extend over a certain range and that the penicillin band is on the margin of this range. Thus, the amide group is perhaps present in a modified form.

all three salts in February and March 1945; it was later fully confirmed by the evaluation of the electron density in three dimensions for the sodium and potassium salts alone. The detailed arrangement of the atoms found in these salts determined not only the essential chemical formula but also the stereochemical relations between the different groups within the penicillin molecule. X-ray spectrum determinations, made by Clark and his group at the University of Illinois, contributed greatly to our knowledge of the various penicillins and their derivatives.

From the standpoint of organic chemistry, the most convincing evidence was secured by a study, carried out in the Merck laboratories (*46*), of the action of Raney nickel catalyst upon sodium benzylpenicillinate. Two products resulted; one was benzyldesthiopenicillin, a monocarboxylic acid $C_{16}H_{20}O_4N_2$, which on hydrolysis yielded D-α-benzyldesthiopenicilloic acid, identical with the product of the action of Raney nickel catalyst on α-methyl D-α-benzylpenicilloate; the other was a monocarboxylic acid $C_{15}H_{22}O_4N_2$, identified with phenylacetyl-L-alanyl-D-valine (see formula given directly below).



Thermochemical data, secured by the National Bureau of Standards and interpreted by Woodward, of Harvard University, suggested the presence of a strained system in penicillin, comparable with that of authentic β-lactams. More cogent still, the CNOS skeleton of the penicillin molecule was conclusively demonstrated through the X-ray crystallographic analysis of sodium, potassium, and rubidium benzylpenicillins by Crowfoot and Low (Oxford University) and by Bunn and Turner-Jones (Alkali Division, Imperial Chemical Industries, Ltd., Northwich). The existence of the β-lactam ring was first shown clearly in electron density projections derived from

As sodium benzylpenicillinate, when similarly treated in the absence of Raney nickel catalyst, underwent no loss of antibiotic activity or change in optical rotation, it may be assumed that these products were formed without intramolecular rearrangement.

In studies of the interaction of thiocyanate and benzylpenicillin, begun in October 1944 and carried on throughout 1945 in the laboratories of Cornell University Medical College and the Squibb Institute, it was found that whereas azlactones in general yield 2-thiohydantoins, penicillin does not do so, but resembles an authentic β-lactam, studied by the Merck group (*47*), in yielding a

thiohydrouracil. Although the oxazolone and β-lactam structures would give a common intermediate with thiocyanate, the results of this intricate investigation may be considered to support the lactam structure. The properties of the sulfur atom in penicillin were also found to accord with this hypothesis. Thus, methyl benzylpenicillinate affords a sulfone (*48*) and a sulfoxide (*49*). Hence, penicillin behaves like an acylated thiazolidine (*50*), in contrast to unacylated thiazolidines, in which the ring is opened on oxidation.

During the period in which the penicillin molecule was considered to contain the oxazolone (azlactone) ring, many attempts were made to synthesize penicillin-like compounds by condensing appropriate alkoxymethylene oxazolones with penicillamine. Experiments conducted in the Merck laboratories (*51*) showed that a trace of antibiotic activity corresponding to benzylpenicillin could be produced by this general method. It was independently found in Oxford (*52*) that a similar small degree of antibiotic activity was produced in a reaction designed to yield an artificial "styrylpenicillin." The work was extended to other cases including propyl-, *n*-amyl-, benzyl-, and phenylpenicillins, and similar antibiotic activity was obtained. The chemists of May and Baker, Ltd., also condensed 2-phenyl-4-ethoxymethylene oxazolone with DL-penicillamine in pyridine solution and obtained an antibiotic product (*53*). It was later shown by the Oxford workers (*54*) that this activity was destroyed by penicillinase, and it was therefore attributed to a compound of the penicillin type. Support for this interpretation was supplied by the Cornell group, who showed (*55*) that the mixture of the reaction products formed in the condensation of 2-benzyl-4-methoxymethylene-5(4)-oxazolone with penicillamine exhibited the same quantitative relationships in its antibiotic activities toward a series of 7 microorganisms as did pure benzylpenicillin. Evidence strongly suggestive of identity was also secured by the Cornell workers, using the isotope dilution method (*56*).

Attempts to concentrate the antibiotically active product synthesized by the method described a year earlier by the Merck group met with only partial success; chromatographic procedures used by the Upjohn chemists (*57*) and countercurrent distribution technique used at Cornell (*58*) led at best to preparations containing less than 3 per cent and 17 per cent of penicillin, respectively.[6] Although this method of synthesis was based on the thiazolidine-oxazolone structural theory, it must be pointed out that a rational synthesis of the β-lactam structure by way of the oxazolone is conceivable as the result of intramolecular acylation.

[6] At this point the contracts expired; the investigation, continued by the Cornell group as an independent project, led to the isolation, early in 1946, of synthetic benzylpenicillin in crystalline form. An account of the background and results of this work, together with full references, was, at the request of the Editorial Board of the monograph on the chemistry of penicillin, published in November, 1946 (*Science*, 1946, **104**, 431).

Concurrently with the studies of structure, the search for other penicillins was carried on in several laboratories. The principal accomplishments in this line of endeavor were proof of the existence of p-hydroxybenzylpenicillin by the isolation of the corresponding penillic acid and related compounds in the Imperial College of Science (*59*), followed by the isolation of the antibiotic itself in the Northern Regional Research Laboratory (*60*), of n-heptylpenicillin in the Abbott Laboratories (*61*), of flavacidin (probably 3-pentenylpenicillin) from *Aspergillus flavus* in the Squibb Institute for Medical Research (*62*), and the production at the Northern Regional Research Laboratory of halogenated penicillins and aryl azopenicillins by the action of halogens and of diazo compounds upon p-hydroxypenicillin (*63*).

A development of great technical importance resulted from the observation, first recorded by the Northern Regional Research Laboratory (*64*), that the addition of phenylacetic acid and related compounds to media in which penicillin is elaborated in surface culture gives rise to increased yields of antibiotic products. Attempts to induce a similar effect with submerged cultures having failed, the chemists of the Lilly Research Laboratories, in an extensive survey, discovered (*65*) that the yields of penicillin could be raised by the addition of phenylacetyl derivatives of various L-amino acids. It was shown that a strain of *P. notatum*, which had hitherto not been observed to produce any antibiotic but 2-pentenylpenicillin actually produced benzylpenicillin when grown in presence of phenylacetamide (*66*); in presence of p-hydroxyphenylacetic acid a yield of p-hydroxybenzylpenicillin much higher than any isolated in previous fermentations was obtained. This was a clear indication of biosynthesis (*67*). Extension of this principle to analogous compounds, in which the benzyl group of the phenylacetamino acids was replaced by a wide variety of other groups, led to the production and isolation in pure, crystalline form of many novel penicillins (*68*).

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