Biosynthesis of Penicillins

The Editorial Board of the Monograph on the Chemistry of Penicillin

THIS BRIEF SUMMARY IS A PRELIMinary notice of findings secured up to the end of 1945 in a collaborative effort of a large number of American and British investigators working under the auspices of the Office of Scientific Research and Development and the Medical Research Council. Full details will be published in the forthcoming penicillin monograph, together with an account of experiments not referred to in this report.

In the consideration of methods for increasing the fermentative production of penicillin, it appeared that the yields might be limited by the inability of the mold to produce adequate amounts of essential intermediates. Studies of the biochemistry of penicillin formation were undertaken in several laboratories to determine whether products from the chemical degradation of penicillin, possible metabolic intermediates, or other similar substances might be capable of stimulating the production of penicillin. These studies have been successful; by the addition of certain precursors to the fermentative media the yield of penicillin has been substantially increased. The method has also been found to be applicable to the synthesis of new penicillins, several of which have been obtained in yields representing as much as 60–100 per cent of the total penicillin formed. A number have been isolated in pure crystalline form.

Studies carried out in the Northern Regional Research Laboratories (*5*) indicated stimulation of penicillin production in surface cultures by phenylacetic acid. Attempts to demonstrate an influence on the type of penicillin produced were not successful. The effect on submerged cultures was questionable, and the work was carried no further in the Peoria laboratory.

Independently, investigators in the Glaxo Laboratories (*17*) undertook experiments with the express intention of studying the biosynthesis of penicillin. The stimulus for this effort was provided by a report from Imperial Chemical Industries (*3*) that two different strains of *Penicillium notatum* produced principally benzylpenicillin on a corn-steep medium, but principally 2-pentenylpenicillin on a synthetic medium. It was found that a constituent of corn-steep protein, phenylalanine, in 0.25–1 per cent concentration, or a number of deamination or decarboxylation products of phenylalanine in smaller concentrations, stimulated penicillin production

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, is to be published by the Princeton University Press.

in surface cultures. By means of chromatographic separations, the production of benzylpenicillin in a synthetic medium in the presence of phenylacetamide by *P. notatum* was demonstrated under cultural conditions which, without the adjuvant, had previously given no indication of producing any highly active principle other than 2-pentenylpenicillin (*4*).

TABLE 1

REPRESENTATIVE COMPOUNDS EFFECTIVE AS BENZYLPENICILLIN PRECURSORS*

	Stimulation	Concentration (mg. %)	Reported by:
DL-Phenylalanine	1.82	250	Glaxo (*17*)
Phenylacetic acid	+	10	" (*17*)
	−	11	Lilly (*7*)
Phenylacetamide	2.0	100	Glaxo (*17*)
β-Phenylethylamine	2.0	50	" (*17*)
N-Phenylacetyl-DL-valine	1.64	20	Lilly (*8*)
N-(2-Hydroxyethyl)-phenylacetamide	1.57	14	" (*10*)
N-(2-Aminoethyl)-phenylacetamide	1.56	14	" (*9*)
2-Aminoethyl phenylacetate (hydrochloride)	1.26	17	" (*11*)
N-Allylphenylacetamide	1.48	14	" (*13*)
N-Methylphenylacetamide	1.04–1.73	10	Cutter (*6*)
	1.53–1.83	25	Heyden (*16*)
N-Hydroxymethylphenylacetamide	1.55	10	Abbott (*1*)
N-(2-Hydroxyethyl)-γ-phenylbutyramide	1.35	16	Lilly (*12*)
δ-Phenylbutylamine (sulfate)	1.39–1.54	13–25	Heyden (*16*)

* "−" indicates no stimulation in yield; "+" indicates positive stimulation; the numbers under "Stimulation" represent the ratio: units in test container/units in control container.

Among the American workers, the Upjohn Company (*18*) had reported testing the effects of several compounds, and the Lilly Research Laboratories (*7*) had initiated extensive studies on precursors. The Lilly studies led to the discovery of several groups of phenylacetyl derivatives that were effective in increasing penicillin yields—in this case, with submerged cultures. Table 1 lists a number of representative compounds found effective in the various laboratories. Considerable specificity was evident in the ability of the molds to utilize compounds. Although N-phenylacetyl-L-valine was an effective precursor, N-phenylacetyl-D-valine did not serve as a stimulant. Mixtures of phenylacetic acid and DL-valine or phenaceturic acid and acetyl-DL-valine were ineffective. The group of phenylacetylated amino acids that proved to be stimulatory was found to be limited.

It is of interest that the response of different strains to specific compounds varies. It has already been pointed out that certain submerged-culture strains did not utilize phenylacetic acid. It is now recognized that the more recently discovered strains, Q-176 and X1612, respond to the addition of this compound.

It is obvious that an increase in the yield of a biological product upon addition of a given substance to a medium may be caused through other mechanisms than that of precursor action. For example, a stimulation of production may be due to utilization of a compound either as a vitamin or growth promoter, as a building block to be incorporated into mycelial growth, or to satisfy any one of several other types of metabolic requirements. To ascertain whether the stimulation in penicillin production was due to a direct utilization of the precursor, the Lilly workers suggested (*8*) that deuteriophenylacetyl-DL-N^{15}-valine[1] be added to a culture medium. By means of the deuterium analysis it was shown that 92.5 per cent of the resulting penicillin was derived from the precursor. In sharp contrast, the N^{15} content was only 2.6 per cent of the value expected if the phenylacetylvaline had been utilized intact. Although it is apparent that the phenylacetyl moiety appeared in the penicillin, the role of the amide portion of the molecule remains undefined. N^{15}-Phenylethylamine was also used as a precursor (*2*). There was a significant quantity of N^{15} in the penicillin produced, as judged from the penillic acid isolated from it. The quantity of N^{15} introduced was small, representing only 1.65 per cent of the amount that could have been introduced if the nitrogen of the phenylethylamine were the sole source of one of the nitrogen atoms in the resulting penillic acid.

In contrast to the effective incorporation of benzylpenicillin precursors, no unequivocal utilization of precursors for the natural aliphatic penicillins has been demonstrated.

Arnstein, Catch, Cook, and Heilbron (*2*) used *p*-hydroxyphenylacetic acid in surface cultures. Although there was no increase in absolute yield, they were able to demonstrate that in place of the usual small proportion (about 5 per cent) of *p*-hydroxybenzylpenicillin, approximately 35 per cent of this penicillin was produced in the presence of 0.05 per cent of *p*-hydroxyphenylacetic acid. The Lilly group presented evidence of the production of this penicillin by the use of N-(2-hydroxyethyl)-*p*-hydroxyphenylacetamide in submerged cultures. In lots prepared without precursor, no *p*-hydroxybenzylpenicillin was demonstrable. However, with the use of 0.025 per cent of the compound, an appreciable penicillin fraction was found which had solubility characteristics and a differential assay value comparable to authentic *p*-hydroxybenzylpenicillin. These results clearly offered the opportunity of controlling the production of *p*-hydroxybenzylpenicillin.

The Lilly workers discovered also that derivatives of various substituted acetic acids other than those which occur in natural penicillins could act as precursors, and these led to new penicillins containing the acyl portion of the precursor. In view of the marked specificity exhibited by most enzyme systems, the ability of the mold to utilize a variety of precursors and incorporate portions of these structures into new penicillins could hardly have been predicted. The utilization of N-(*p*-tolylmercapto-acetyl)-DL-valine to form *p*-tolylmercaptomethylpenicillin may be cited as one of numerous examples of the incorporation of a biologically-foreign substance into a penicillin.

In some cases addition of precursors to the culture medium caused an increase in production of penicillin activity. Such stimulation may be interpreted as strong evidence for the utilization of the precursor. In other cases no increase of total yield was experienced, and on occasion a diminution of total yield was observed. Such absence of stimulation cannot be interpreted, however, as constituting proof that the mold fails to utilize the precursor. It has been demonstrated in some cases that

TABLE 2

NEW CRYSTALLINE BIOSYNTHETIC PENICILLINS

Penicillin formed	Precursor used	Activity (U/mg.)	Differential assay*	Reported by:
1. Sodium *p*-methoxybenzylpenicillin	N-(2-Hydroxyethyl)-*p*-methoxyphenylacetamide	1,510	0.82	Lilly (*13*)
2. " *p*-nitrobenzylpenicillin	N-*p*-Nitrophenylacetyl-DL-valine	1,700 (?)	0.86	" (*13*)
3. " *p*-fluorobenzylpenicillin	N-(2-Hydroxyethyl)-*p*-fluorophenylacetamide	1,650	0.89	" (*14*)
4. " *m*-fluorobenzylpenicillin	N-(2-Hydroxyethyl)-*m*-fluorophenylacetamide	2,340	0.76	" (*14*)
5. " *o*-fluorobenzylpenicillin	N-(2-Hydroxyethyl)-*o*-fluorophenylacetamide	1,340	1.08	" (*14*)
6. " *p*-chlorobenzylpenicillin	N-*p*-Chlorophenylacetyl-DL-valine	2,460	0.73	" (*13*)
7. " *p*-bromobenzylpenicillin	N-(γ-*p*-Bromophenylbutyryl)-DL-valine	2,270	0.65	" (*15*)
8. " *p*-iodobenzylpenicillin	N-(2-Hydroxyethyl)-*p*-iodophenylacetamide	2,800 (?)	0.67	" (*15*)
9. " 2-thiophenemethylpenicillin	N-(2-Hydroxyethyl)-2-thiopheneacetamide	1,685	1.13	" (*13*)
10. " phenoxymethylpenicillin	N-(2-Hydroxyethyl)-phenoxyacetamide	1,670	0.87	" (*15*)
11. " *p*-tolylmercaptomethylpenicillin	*p*-Tolylmercaptoacetyl-DL-valine	1,285 (?)	0.83	" (*15*)

* The differential assay represents the ratio of activity *B. subtilis/Staph. aureus*, with that for benzylpenicillin defined as being 1.00.

[1] The deuteriophenylacetic acid was furnished by David Rittenberg, of the College of Physicians and Surgeons, Columbia University. N^{15}-DL-Valine was prepared and transformed into deuteriophenylacetyl-N^{15}-valine in the Lilly laboratories (*11*). The cultural work and preliminary purification were done at the Abbott Laboratories. Final purification and crystallization were effected by The Upjohn Company (*19*). The isotopic determinations were performed by Dr. Rittenberg.

the precursor is utilized and the corresponding penicillin formed. For example, addition of N-(2-hydroxyethyl)-phenoxyacetamide to the culture medium did not appreciably increase the total units of penicillin activity produced, but a high percentage of the penicillin was isolated in a single fraction which was shown to be the new phenoxymethylpenicillin.

A large number of compounds were prepared and tested as possible precursors of new penicillins, and in several cases the new penicillins were isolated in analytically pure, crystalline form (Table 2). Each of the new penicillins listed, except numbers 2, 8, and 11, gave satisfactory analytical values and was further identified by the ultraviolet absorption curve in comparison with that of the precursor used. The three exceptions, *p*-nitrobenzylpenicillin, *p*-iodobenzylpenicillin, and *p*-tolylmercaptomethylpenicillin, were obtained in a somewhat impure state, but the presence of these new penicillins was demonstrated by examination of their ultraviolet absorption spectra. It will be noted that *p*-bromobenzylpenicillin was isolated after the use of N-(γ-*p*-bromophenylbutyryl)-DL-valine. Examples of terminally phenyl-substituted butyl and butyryl compounds that have been used as benzylpenicillin precursors are given in Table 1. It is apparent that substituted butyric acid derivatives are effective in a manner similar to the corresponding substituted acetic acid derivatives.

Attempts to find precursors for the three-carbon moiety or the penicillamine portion of the penicillin molecule have been unsuccessful.

The following groups have participated in the joint program for the chemical study of penicillin and have contributed to various aspects of the precursor work: In Britain, British Drug Houses, Ltd.; Glaxo Laboratories, Ltd.; Imperial Chemical Industries, Ltd.; Imperial College of Science, London, Department of Organic Chemistry; and Oxford University, Department of Crystallography; in the United States, Abbott Laboratories; U. S. Department of Agriculture, Northern Regional Research Laboratory; Cutter Laboratories; Heyden Chemical Corporation; Eli Lilly and Company; Merck and Co., Inc.; and The Upjohn Company.

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The National Academy of Sciences:

Abstracts of Papers Presented at 1947 Autumn Meeting

Growth of Particles in Smokes and Clouds and the Production of Snow From Supercooled Clouds

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V. J. Schaefer introduced pellets of dry ice into clouds of supercooled water droplets and produced enormous numbers of minute ice crystals which rapidly grew into snowflakes. B. Vonnegut found that silver iodide vaporized into air can give nuclei which induce the formation of snowflakes in supercooled clouds below −5° C. Account is given of the development of these ideas from studies made during the war of fundamental aspects of: (1) gas mask filters; (2) smokes needed for screening purposes; (3) the radio interference caused by the electric charging of aircraft flying through snow; and (4) the icing of aircraft when flying through supercooled clouds. A theory of the growth of particles in aerosols has served as a guide in these various researches. The experiments in progress during the last year on the artificial production of rain and snow, now Project Cirrus with the Signal Corps and the Navy, have led to many interesting results which are described and explained. The generation and wartime use of screening smokes and the effects produced by the seeding of stratus and cumulus clouds are illustrated. A preliminary account of the flight tests over the recent hurricane of October 10–15 is given.

Physical Families of Curves

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In any field of force the most important curves are the trajectories (paths of possible motion). Thus, for the Newtonian field we have the planetary orbits, the conic sections of Kepler. Another important physical family is formed by the generalized catenaries, forms of equilibrium of a suspended chain. Other physical families in the field are brachistochrones and velocity curves.