# Chemistry of Penicillin

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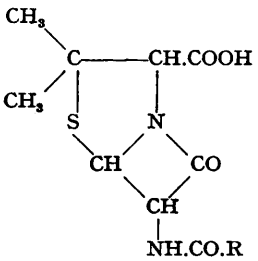
THE constitution of penicillin was elucidated as the result of collaborative research carried out in many laboratories in Great Britain and the U.S.A. during 1943–45.[1] In the course of this work, it was discovered that several different penicillins exist. These vary according to the strain of *Penicillium notatum* or *P. chrysogenum* used and with the cultural conditions. Indeed, the proportion of certain penicillins in a mixture can be increased by addition to the medium of certain "precursors" which the mould is apparently able to assimilate and incorporate into the penicillin molecule.

All the penicillins have the common basic structure




in which the group R varies from one penicillin to another. In the original penicillin produced by Sir Howard Florey and Dr. E. Chain and their colleagues at Oxford, R for the major constituent is a $\Delta^2$-pentenyl group. This species of penicillin was originally termed penicillin F, in order to identify it with the penicillin discovered by Fleming and to distinguish it from another antibiotic produced by *P. notatum*, which was originally called penicillin A and later notatin; it has a structure entirely different from that of the penicillins. The term penicillin F is still used in the U.S.A., but in this country this form is now generally known as penicillin I. Mild methods of reduction convert penicillin I into dihydro penicillin I, in which R is an amyl group, and the activity is apparently undiminished or even slightly increased by the hydrogenation. In America the penicillin originally isolated was not penicillin F, but a different penicillin with approximately the same activity. It was considerably easier to isolate and more stable than penicillin F and was called penicillin G by the Americans; in this country it is known as penicillin II. In this instance the group R is a benzyl group, and the presence of this form in the original American penicillin was associated with the use of a different strain of *P. notatum* and with addition to the medium of corn steep liquor, which contains benzyl derivatives that favour the production of penicillin II in preference to penicillin I. Since the adoption of corn steep liquor for routine production in this country, penicillin II is also the predominant form in British penicillin. Another form of penicillin produced by some strains of *P. notatum* and *P. chrysogenum*, especially in presence of the appropriate precursor, is penicillin III or penicillin X, in which R is a *p*-hydroxy benzyl group. Even under the best conditions, however, it is produced in limited amounts only, but, as it has rather unusual biological properties, interest in this form of penicillin may be expected to increase.

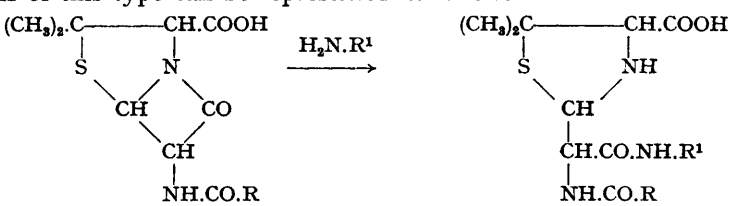
In 1945 a new strain of *P. chrysogenum*, known as Q.176, was generally adopted for submerged culture, because it produced much larger quantities of total penicillin than the strain X.1612 previously employed. It was not immediately realised, however, that, in absence of precursors, the predominant form of penicillin so produced is not the therapeutically valuable penicillin I or penicillin II, but another penicillin termed penicillin K, in which R is a *n*-heptyl group. This form of penicillin is rapidly inactivated in the animal or human organism and is therefore clinically less effective than penicillin I or II. Even in presence of precursors, substantial amounts of penicillin K are produced. The relative effectiveness of the various forms of pencillin is still a matter of controversy, but the balance of evidence is in favour of reducing to a minimum the proportion of penicillin K in material to be used for clinical purposes.

A substance isomeric with penicillin I and with similar biological properties is produced

by *Aspergillus flavus* and has been given the names flavicin and flavicidin.[2] In this substance R is a $\Delta^3$-pentenyl group.

The discovery that different forms of penicillin do not have the same therapeutic value has made it imperative to find methods of estimating the individual penicillins in commercial penicillin, and this is proving to be an extremely difficult task. For a long time the only method of estimating the amount of total penicillins in a mixture was the bacteriological method of assay, and although this has been improved so that the errors of the method now in use are surprisingly small, such a method must always suffer from certain practical disadvantages: for example, that the result can only be known several hours after submission of the sample for test, and that occasional highly erroneous results are obtained. Within recent months several chemical methods of estimating total penicillin have been described. Scudi,[3] for example, published details of a colorimetric method and of a fluorimetric method of assay based on the fact that the $\beta$-lactam ring of all the penicillins is opened by organic bases to form substituted amides and, by using an amine containing a chromophoric group (N-(1-naphthyl-4-azobenzene)-ethylene diamine) or an amine containing a fluorophoric group (7-methoxy-2-chloro-5-$\beta$-aminoethylamino acridine), products which are either coloured or fluorescent are produced and can be estimated by conventional procedures. These methods have very serious limitations, due mainly to the difficulty of completely removing excess of the reagent from the coloured product; moreover, they are tedious and the reagents are difficult to prepare.

Reactions of this type can be represented as follows:

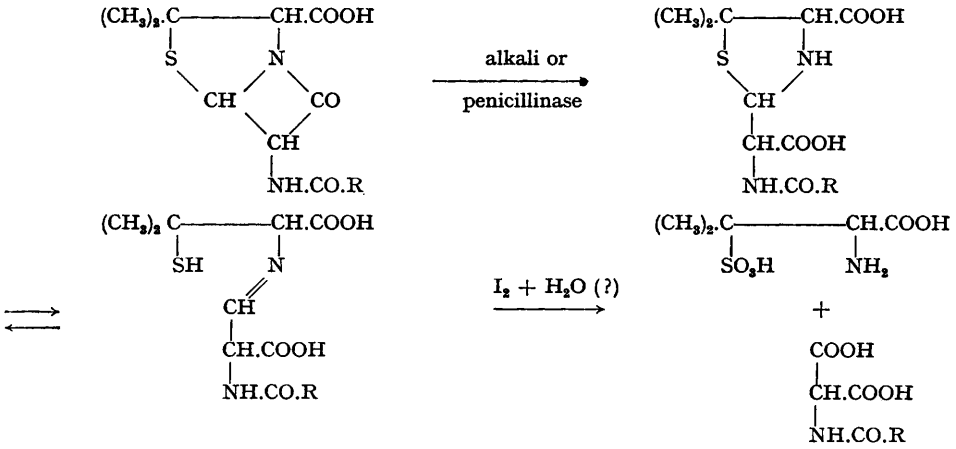



A similar reaction occurs with hydrazines and hydroxylamines and can likewise be utilised for the quantitative estimation of total penicillins. Such methods, however, cannot be used for the differentiation of individual penicillins, as these all behave in the same manner towards these reagents.

A simpler method of estimating total penicillins is based on the fact that, on treatment with alkali or with a specific enzyme known as penicillinase, the $\beta$-lactam ring of penicillin is opened, with the formation of an additional carboxyl group, giving penicilloic acid; the amount of alkali required to neutralise the acidity thus produced is proportional to the penicillin content. These two methods are not very accurate, though the penicillinase modification is, as might be expected, highly specific. An improved procedure, which is likely to prove more useful than either of Scudi's methods, is the iodimetric method of Alicino,[4] which is based on the observation that the difference in the amount of iodine consumed under standard conditions before and after inactivation by alkali is proportional to the amount of penicillin present. The alkali converts penicillin into penicilloic acid, which adds on six to nine equivalents of iodine, depending on the conditions used. The mechanism of the reaction is obscure but, provided the conditions are rigidly standardised, the amount of iodine reacting is always constant. The reaction undoubtedly depends on the fact that the thiazolidine ring in penicilloic acid is readily opened to form a free thiol group, whereas ring-cleavage does not occur with compounds, such as penicillin, in which the thiazolidine nitrogen atom is acylated. The free thiol group is then oxidised by the iodine, presumably to a sulphonic acid group, whilst an aldehyde group is probably liberated and then oxidised as in the scheme on p. 276.

The bacteriological method of assay, in addition to its purely practical disadvantages, also suffers from a very serious theoretical objection, for not only do the individual penicillins differ in therapeutic value, but they also differ in the extent to which they inhibit the growth of test-organisms. The degree of inhibition produced by a mixture of penicillins is therefore dependent on the proportions in which the constituents occur, and the biological assay may give an entirely different result from the chemical method of assay and probably neither will give a satisfactory estimate of the therapeutic efficacy. The proportion of two penicillins in a mixture can be determined from the ratio of the response of two different organisms, such as *Staphylococcus aureus* and *Bacillus subtilis*, but the method is not very accurate

and is invalid if a third penicillin is present; with commercial preparations, this is at present the rule rather than the exception.




It is important, therefore, that satisfactory methods of estimating the individual penicillins should be made available as soon as possible. The only chemical method that has so far given satisfactory results is that of Sheehan *et al.*[5] for the estimation of penicillin II. This method depends on the observation that penicillin II forms with N-ethyl-piperidine a sparingly soluble salt which can be filtered off and weighed. The results are said to be quantitative with material containing not less than 50 per cent. of penicillin II and with a potency not less than 800 units per mg., but in our experience the results may not be reliable with material containing less than 90 per cent. of penicillin II. The method has recently been improved in our laboratories by combining the method of Sheehan *et al.* with that of Alicino. A colorimetric method of estimating penicillin II, actually a modification of the Kapeller-Adler method for the estimation of tyrosine, was described by Page and Robinson[6] but is less specific than the method of Sheehan *et al.*, since impurities containing aromatic rings also react, giving rise to coloured products and thus leading to high results. The same disadvantage applies to spectrophotometric methods of estimating penicillin II that depend on the absorption of the phenyl ring, although a method recently elaborated by Philpotts, Thain, and Twigg[7] has given valuable results with material of high potency.

The only chemical method available for the estimation of penicillin K is one recommended by the Food and Drug Administration of the U.S.A., but it does not give reliable results. In this method the penicillin is distributed between a buffer solution of *p*H 6 and chloroform; most of the penicillin K goes into the chloroform phase, whilst most of the penicillin G remains in the aqueous phase. The amount of penicillin in the chloroform solution is estimated by iodimetric titration. Unfortunately the separation is not sharp and other penicillins present may be distributed more equally between the two phases and so increase the error of the method.

The method to be described by Dr. Goodall and Dr. Levi is, like the foregoing, a distribution method, but is of far greater value, since all the penicillins in a mixture can be estimated simultaneously. Morevoer, it is capable of indicating what penicillins are present, so that a qualitative and quantitative analysis of a mixture may be made at the same time. It is without doubt the most useful method yet described for the examination of mixtures of penicillins.

## References

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