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CHEMISTRY OF PENICILLIN

By the Committee on Medical Research, O.S.R.D., Washington, and the Medical Research Council, London

THIS brief summary of results obtained by British and American chemists, issued under the joint auspices of the Committee on Medical Research (O.S.R.D., Washington) and the Medical Research Council (London), is a preliminary notice of the principal findings secured up to the end of 1944 in a collaborative effort of a large number of investigators, unnamed at present. It implies some corrections of published data; authors of early publications are among those who have cleared up these points. For the sake of clearness, the account is not given in chronological order of development. The primary object of this communication is to disclose significant facts which have been confirmed by unequivocal synthesis and to record a few essential points which are still matter for conjecture. Full details will be published at a later stage, together with an account of experiments not referred to in this report.

Several antibiotics of the penicillin class are known and all have the empirical formula $C_9H_{11}O_4SN_2 \cdot R$. In F-penicillin (known in Britain as penicillin-I), R is Δ^2-pentenyl, $-CH_2 \cdot CH{=}CH \cdot CH_2 \cdot CH_3$; in dihydro-F-penicillin, R is *n*-amyl; in G-penicillin (known in Britain as penicillin-II), R is benzyl; in X-penicillin (also known as penicillin-III), R is *p*-hydroxybenzyl; in K-penicillin (a recent addition to the series), R is *n*-heptyl. The best elementary analyses are of pure crystalline sodium salts. Determinations of the molecular weights of the sodium salt and of the methyl ester of G-penicillin indicate that the empirical formulae truly represent the molecular weights.

The penicillins are strong monobasic acids of pK about 2.8; electrometric titration does not disclose the presence of a basic group. Slow titration with perchloric acid in acetic acid solution indicates such a group, but the penicillin is biologically inactivated by this treatment; rapid titration gives a negative result.

The ultraviolet and infrared absorptions, crystal

structure (by x-ray methods, including full electron distribution of the rubidium salt of G-penicillin) and polarimetric and polarographic behavior of the penicillins and their derivatives have been studied.

Sodium G-penicillin contains one hydrogen atom replaceable by deuterium on equilibration with heavy water.

On treatment with hot dilute mineral acids the penicillins afford one molecule of carbon dioxide, an amino acid termed penicillamine and other products. Penicillamine, obtainable by several other degradation processes, has been identified by analytic and synthetic methods as *d*-β,β-dimethylcysteine. Penicillamine with the same steric configuration is derived from F- and G-penicillins; it belongs to the *d*- or "unnatural" series of α-amino acids.

Synthetic penicillamine has been resolved, and numerous derivatives of the optically active enantiomorphs and the racemic form have been prepared. These include penicillamine disulfide (tetramethylcystine) and penicillaminic acid (dimethylcysteic acid) as well as a long series of thiazolidines and S- and N-substituted derivatives.

After removal of penicillamine from the acid hydrolysates of F-penicillin, careful treatment allowed of the isolation of an aldehyde, $C_8H_{13}O_2N$, in the form of its 2,4-dinitrophenylhydrazone and its condensation product with dimedone. Similarly, dihydro-F-penicillin gave rise to derivatives of an aldehyde, $C_8H_{15}O_2N$. G-penicillin afforded phenaceturic acid, phenylacetamide and an aldehyde, $C_{10}H_{11}O_2N$. Phenylacetic acid had previously been recognized as a hydrolytic product of G-penicillin.

These penilloaldehydes have been identified by analysis and synthesis as follows: F-penilloaldehyde, Δ^2-hexenoylaminoacetaldehyde; dihydro-F-penilloaldehyde, *n*-hexoylaminoacetaldehyde; G-penilloaldehyde, phenylacetylaminoacetaldehyde.

It was inherently probable that the carbon dioxide liberated when penicillin is hydrolyzed in hot acid solution was derived from an unstable carboxyl group and, taking into consideration the nature of penicillamine and the penilloaldehydes, a probable precursor was penilloaldehyde-carboxylic acid,

$$R \cdot CO \cdot NH \cdot CH(CO_2H) \cdot CHO$$

now termed a *penaldic acid.* This was conclusively demonstrated to be correct.

G-penicillin and benzylamine afford a crystalline compound, $C_{30}H_{36}O_4N_4S \cdot H_2O$, which has the composition of a hydrated addition compound of one molecule of G-penicillin and two molecules of benzylamine and is the mono-benzylamine salt of the mono-benzylamide of a dicarboxylic acid. Degradation of this substance by means of mercuric chloride afforded penicillamine and G-penaldic acid benzylamide which was catalytically reduced to hexahydrophenylacetylserine hexahydrobenzylamide,

$$C_6H_{11} \cdot CH_2 \cdot CO \cdot NH \cdot CH(CH_2OH) \cdot CO \cdot NH \cdot C_7H_{13},$$

identified with a synthetic specimen.

The penicillins are readily inactivated by methanol and the products are methyl esters. Methanol-inactivated G-penicillin was degraded to methyl G-penaldate,

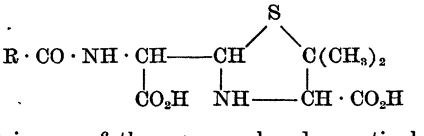
$$C_6H_5 \cdot CH_2 \cdot CO \cdot NH \cdot CH(CO_2CH_3) \cdot CHO$$

the constitution of which was proved by its catalytic reduction to N-hexahydrophenylacetylalanine. The latter was identical with a specimen prepared by similar reduction of phenylacetylalanine. F- and G-penicillins are converted by the action of diazomethane into mono-methyl esters and these are degraded by mercuric chloride in aqueous solution with formation of the methyl ester of penicillamine.

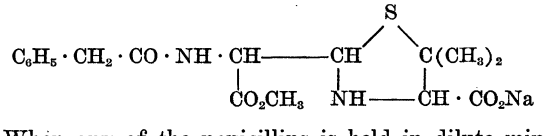
These observations serve to show (1) that the acidic group in penicillin is identical with the carboxyl group in penicillamine, (2) that by the addition of the elements of water to penicillin a second carboxyl is produced, (3) that it is this new carboxyl which breaks down to carbon dioxide by the action of hot dilute mineral acids.

The dicarboxylic acid obtained by hydrolysis of penicillin at the site of the potential carboxyl is termed *penicilloic acid.* This acid is produced in the form of salts by treatment of penicillin with alkalies and is presumably the product of the action of the enzyme penicillinase on penicillin.

Derivatives of penicilloic acid have been synthesized and the outcome of much work that can not here be described in detail is that penicilloic acids are undoubtedly thiazolidines of the formula:



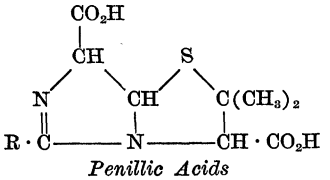
where R is one of the groups already particularized. Thus, "methanol inactivated" sodium G-penicillin is one of the stereoisomeric forms of the structure:



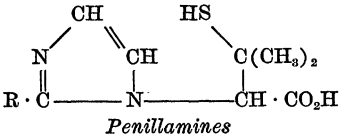
When any of the penicillins is held in dilute mineral acid solution at about 30° a change occurs which may be followed polarimetrically. Crystalline isomerides of the penicillins, termed *penillic acids,* may then be readily isolated. These substances are shown by electrometric titrations to be dibasic acids containing a basic group; thiol groups are absent.

Treatment of the penillic acids with cold aqueous mercuric chloride involves loss of a molecule of car-

bon dioxide and formation of substances termed *penillamines*. These compounds are mono-basic, mono-acidic and contain a thiol group. Analysis and synthesis have shown that the annexed expressions represent the respective structures:

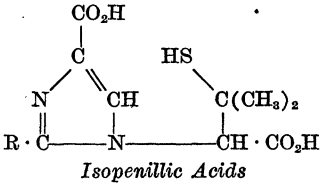


Penillic Acids



Penillamines

Hydrolysis of the penillic acids by hot dilute acids affords penicillamine, the penilloaldehydes and carbon dioxide but the penillamines are resistant to hydrolysis. F- and G-penillic acids are convertible by baryta into the isomeric isopenillic acids.

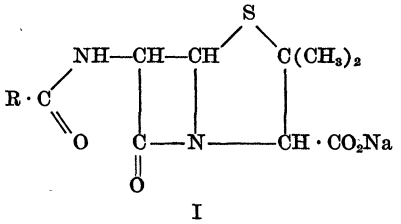


Isopenillic Acids

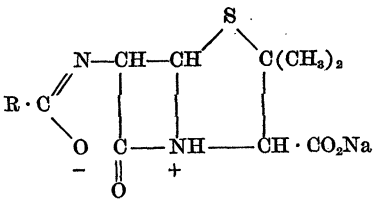
Methyl G-penicillin is changed by mercuric chloride in neutral media into the isomeric methyl G-penicillenate which, on hydrolysis by means of aqueous sodium hydroxide, affords the sodium salt of 4-hydroxymethylene-2-benzyloxazolone. The addition of the elements of thiocyanic acid to methyl penicillin and the transformations of the primary product have been investigated in detail with important results.

Finally, the action of Raney nickel catalyst on sodium G-penicillin in aqueous solution affords desthio-G-penicillin, $C_{16}H_{20}O_4N_2$, together with phenylacetyl-*l*-alanyl-*d*-valine, $C_{16}H_{22}O_4N_2$.

Naturally the workers in this field have formed views as to the full constitution of the penicillins. At present it can be stated that the formulae which are now receiving the most active attention contain respectively a β-lactam structure (I) and an incipient azlactone grouping (II):



I



II

The following groups have participated in the joint program for the chemical study of penicillin:

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*British Drug Houses, Ltd.
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The London Hospital Medical Unit
Manchester University, Department of Chemistry
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