THE CHEMISTRY OF THE PENICILLINS

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I. *Degradation and Early Studies in Synthesis*

(i) *Introduction.*—This account outlines structural organic chemical work on the penicillins and related compounds. This was mostly carried out under an Anglo-American agreement,[1] and many references are made to the original reports which have been published in limited form.[2] About 700 reports were submitted during the term of this agreement, and there were several hundred earlier contributions so that many must inevitably receive scant if any notice. Space limitations preclude any but incidental reference to the general production of penicillins, their assay,[3] early work on their concentration, and invaluable physicochemical contributions, *e.g.*, on the titration characteristics, *X*-ray crystallography, and infra-red absorption of penicillins and their derivatives.

(ii) *The Known Penicillins.*—The first degradation product of penicillin concentrates to be recognised was an α-amino-acid, penicillamine (see below), but more characteristic of the penicillins was their behaviour on treatment with cold dilute acid. It had been early observed that concentrates of penicillin, though themselves non-crystalline and containing, as is now known, as little as 20% of pure penicillin,[4] afforded a crystalline *penillic acid*,[5] ultimately assigned the formula $C_{14}H_{20}O_4N_2S$,[6] after the aqueous

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[1] Cmd. 6757.

[2] References appear in the following form: CPS, reports of British origin to the Committee for Penicillin Synthesis of the Medical Research Council; PEN, reports to the Therapeutic Research Corp. of Great Britain; there are a few other British reports of obvious origin. Reports from American Laboratories to the Office of Scientific Research and Development bear the following designations: A, Abbott Labs.; B, W. E. Bachmann, Univ. of Michigan; C, R. D. Coghill, Northern Reg. Res. Lab., U.S. Dept. of Agr.; Cl, G. L. Clark, Univ. of Illinois; Cu, Cutter Labs.; D, V. du Vigneaud, Cornell Univ. Med. Coll.; F, Food and Drug Administration; H, Heydon Chem. Corp.; J, J. R. Johnson, Cornell Univ.; L, Lilly Res. Labs.; M, Merck & Co., Inc.; PD, Parke-Davis & Co.; P, Chas Pfizer & Co., Inc.; R, H. M. Randall, Univ. of Michigan; Sh, Shell Development Co.; S, Squibb Inst. for Med. Res.; U, Upjohn Co.; W, Winthrop Chem. Co.; Wo, R. B. Woodward, Harvard Univ. The American reports were also given CPS numbers which are listed in CPS 673 and Supplement. Where reports were signed the authors have been quoted; for others the laboratory alone is indicated. These principles are being adopted in the forthcoming Penicillin Monograph which presents the original results in more extended form.

[3] League of Nations Bull. Health Organisation, 1945, **12,** No. 2.

[4] These concentrates contained *ca.* 300 units/mg. The present "unit" is the specific pencillin activity in 0·6 microgram of pure sodium benzylpenicillin [3] and is for most practical purposes equivalent to the "unit" of earlier work.

[5] W. M. Duffin and S. Smith, *Nature,* 1943, **151,** 251; PEN 69, 81.

[6] Owing to the crude nature of penicillin concentrates used for early degradative work the presence of sulphur in the antibiotic was not at first appreciated, and some of the early formulæ and structural conclusions, which it would be useless to detail, were erroneous.

solution had been kept for a short time at pH 2. These concentrates had been prepared by using a form of partition chromatography,[7] whereas other concentrates, prepared by orthodox chromatography, treatment with aluminium amalgam, and other steps,[8] appeared to give a " penicillinic acid " (this term is no longer used in this connection, being reserved for " penicillin free acid "—see below) with a C_{15} formula on similar treatment.[9] It soon appeared that the penicillin then known could be hydrogenated, giving rise to a dihydropenicillin, and thence to a *dihydropenillic acid,* $C_{14}H_{22}O_4N_2S$.[10] There was also a growing suspicion that *Penicillium notatum* was capable of producing more than one " penicillin ", a suspicion which was strengthened when the antibiotic produced in submerged culture gave a new *penillic acid,* $C_{16}H_{18}O_4N_2S$.[11] As penicillin preparations were purified so their compositions approximated more closely to that of monobase salts of penillic acids until, with the crystallisation of salts from submerged fermentation, it became clear that penicillin free acid and the corresponding penillic acid were isomeric mono- and di-basic acids respectively,[12] and a similar relationship was accepted for other penicillins. Degradation of various penicillins was thus investigated almost simultaneously, although the distinction between them only later became quite certain.

" Penicillinic acid " was recognised as a complex of the acids $C_{14}H_{20}O_4N_2S$ and $C_{16}H_{18}O_4N_2S$ and not a distinct entity, so that three penicillins remained characterised ; the following formulæ are of the unstable free acids, and the basis for the distinguishing names is apparent : *pent-2-enylpenicillin,* $C_{14}H_{20}O_4N_2S$ (also termed earlier penicillin-I or -F) (surface fermentation) ; n-*amylpenicillin,* $C_{14}H_{22}O_4N_2S$ (a reduction product of pent-2-enylpenicillin and also a natural penicillin—*gigantic acid*—produced by *Aspergillus giganteus* [13]) ; and *benzylpenicillin,* $C_{16}H_{18}O_4N_2S$ (penicillin-II or -G) (submerged fermentation). It should be mentioned that penicillins are produced not exclusively by *P. notatum* but by various *Penicillia* and *Aspergilli* and perhaps by other moulds.[14] p-*Hydroxybenzylpenicillin,* $C_{16}H_{18}O_5N_2S$ (penicillin-III or -X),[15] was soon recognised in *P. notatum* cultures, followed later by n-*heptylpenicillin,* $C_{16}H_{26}O_4N_2S$ (penicillin-K) [16] and by *flavacidin,* $C_{14}H_{20}O_4N_2S$, an isomeride of pent-2-enylpenicillin obtained from *A. flavus.*[17] Other " natural " penicillins have been foreshadowed, but the evidence for their existence is not discussed here as it depends on chromatographic behaviour (which is deceptive when applied to crude penicillin), differential

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[7] J. R. Catch, A. H. Cook, and I. M. Heilbron, *Nature,* 1942, **150,** 633.

[8] E. P. Abraham and E. Chain, *Brit. J. Exp. Path.,* 1942, **23,** 103.

[9] E. P. Abraham, W. Baker, E. Chain, and R. Robinson, PEN 79, 87.

[10] R. Bentley, J. R. Catch, A. H. Cook, J. A. Elvidge, R. H. Hall, and I. M. Heilbron, PEN 99, 102, 105.

[11] Merck & Co., Inc., Rep. on Purification, Sept., 1943.

[12] For first report of crystallisation see S 3 (not generally circulated).

[13] F. J. Philpot, *Nature,* 1943, **152,** 725 ; D. Crowfoot, B. Low, and G. Schmidt, PEN 117.

[14] See M. E. Johns, F. J. Philpot, and A. V. Pollock, *Nature,* 1946, **158,** 446.

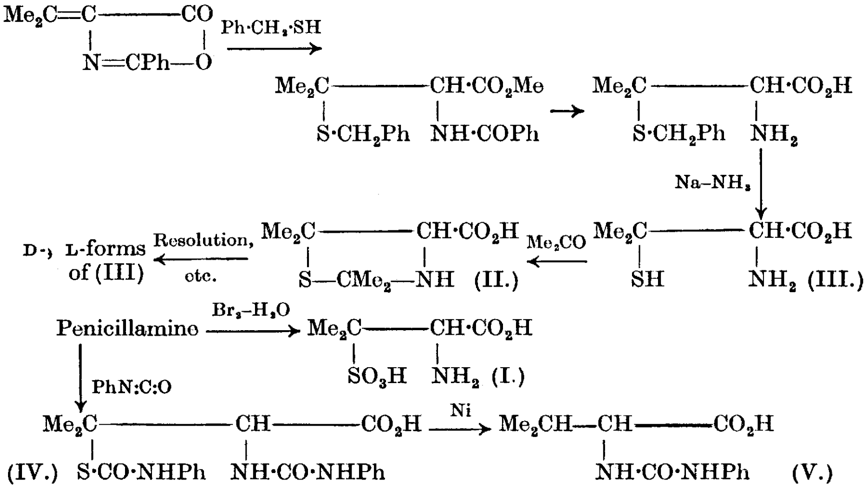
[15] J. R. Catch, A. H. Cook, and I. M. Heilbron, CPS 23.

[16] A 20, 21, 26.

[17] J. Fried, W. L. Koerber, and O. Wintersteiner, *J. Biol. Chem.,* 1946, **163,** 341.

biological assays, etc., and not on chemical characterisation. *p*-Hydroxybenzylpenicillin has—since its phenolic group permits modification of the molecule without destruction of the antibiotic featues—given rise to a series of *halogeno-* and *azo-*p*-hydroxybenzylpenicillins* [18] of more therapeutic than chemical interest.[18] Certain compounds such as phenylethylamine or *p*-hydroxyphenylacetic acid when added to *P. notatum* fermentations allow a measure of control to be exercised over the type of penicillin produced so that, for example, much increased yields of *p*-hydroxylbenzylpenicillin can be produced at will.[19] By taking advantage of this adaptability many substituted phenylacetic acids were led to produce " biosynthetic " penicillins, otherwise unknown in Nature, several of which were isolated in pure condition.[20] Finally, a few esters of various penicillins were made in the course of chemical work. The chemotherapeutic possibilities are thus very wide, as the penicillins differ in *in vitro* and *in vivo* response to bacteria though, so far the *in vitro* differences are quantitative rather than qualitative. There is no reason to doubt that all these pencillins are similarly constituted though the degradative work has been almost limited to pent-2-enyl-, *n*-amyl-, benzyl-, *p*-hydroxybenzyl-, and *n*-heptyl-penicillins.

(iii) *Early Degradative and Synthetic Studies.*—Pent-2-enylpenicillin concentrates afforded, in yields proportional to their potencies, an α-amino-acid, *penicillamine*, on hydrolysis with hot dilute mineral acid.[21] Despite consistently unsatisfactory analyses this was finally assigned the formula



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[18] C 16—20.

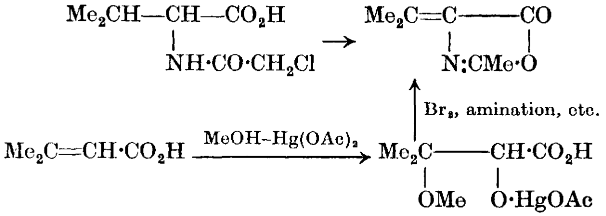
[19] H. R. V. Arnstein, J. R. Catch, A. H. Cook, and I. M. Heilbron, CPS 291, 337, following C 16—20; E. L. Smith, A. E. Bide, and F. A. Robinson, CPS 226. Similar findings have also been briefly reported for *P. chrysogenum* fermentations; see K. Higuchi, F. G. Jarvis, W. H. Peterson, and M. J. Johnson, *J. Amer. Chem. Soc.*, 1946, **68**, 1669.

[20] L 26, 29, 30; U 24, 25; E. L. Smith, A. E. Bide, and W. Graham, CPS 686.

[21] E. P. Abraham, E. Chain, W. Baker, and R. Robinson, *Nature*, 1942, **149**, 356; PEN 67; *Nature*, 1943, **151**, 107; PEN 91.

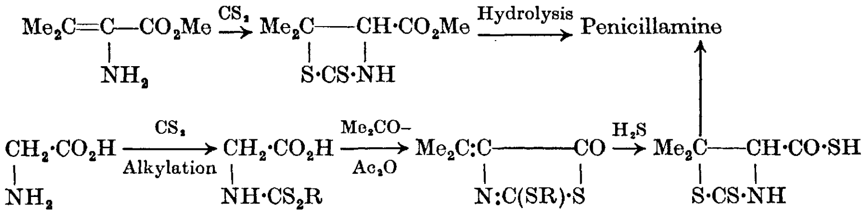
$C_5H_{11}O_2NS$. It was apparently a substituted cysteine as reflected by an indigo colour reaction with ferric chloride, oxidation to a substituted cysteic acid, *penicillaminic acid*, $C_5H_{11}O_5NS$ (I), and condensation with acetone to a thiazolidine, $C_8H_{15}O_2NS$ (II). There was soon general agreement that penicillamine was probably one form of $\beta\beta$-dimethylcysteine (III), and this was confirmed by its identity with the D- (unnatural) modification obtained by modifying an earlier synthesis of methylcysteine.[22] Similar conclusions had meanwhile been reached in America [23] by causing penicillamine from benzylpenicillin to react with phenyl *iso*cyanate to give (IV) which was desulphurised to yield D-valine phenylureide (V). The products from the two penicillins were undoubtedly identical, and the penicillamine from *n*-amylpenicillin and *n*-amylpenillic acid was crystallographically indistinguishable from that obtained from pent-2-enylpenicillin.[10] Other reactions such as attempted acetylation and benzoylation of penicillamine were immediately comprehensible on the above formulation, the products being α-acylamido-$\beta\beta$-dimethylacrylic acids.

Several other syntheses of penicillamine have been accomplished though many are variants of the above, *e.g.*, by using hydrogen sulphide or thioacetic acid instead of benzylthiol, or 2-methyl- instead of 2-phenyl-4-*iso*propylideneoxazolone, the novelty consisting in the mode of preparation of the intermediates, *e.g.*: [24]



$$\begin{tikzcd}
\mathrm{Me_2CH{-}\underset{NH\cdot CO\cdot CH_2Cl}{CH}{-}CO_2H} \arrow[r] & \mathrm{Me_2C{=}\underset{N:CMe\cdot O}{C{-}CO}} \\
\mathrm{Me_2C{=}CH\cdot CO_2H} \arrow[r, "\mathrm{MeOH{-}Hg(OAc)_2}"] & \mathrm{\underset{OMe}{Me_2C}{-}\underset{O\cdot HgOAc}{CH}\cdot CO_2H} \arrow[u, "\mathrm{Br_2,\ amination,\ etc.}"']
\end{tikzcd}$$

A few were unrelated to the foregoing, *e.g.*, the Strecker reaction applied to α-benzylthio*iso*butaldehyde, $Me_2C(S{\cdot}CH_2Ph){\cdot}CHO$ [25] and some were entirely novel as shown by the reactions below, the second method being applicable to the preparation of other amino-acids.[26]



$$\begin{tikzcd}
\mathrm{Me_2C{=}\underset{NH_2}{C}{-}CO_2Me} \arrow[r, "\mathrm{CS_2}"] & \mathrm{\underset{S\cdot CS\cdot NH}{Me_2C{-}CH}\cdot CO_2Me} \arrow[rr, "\mathrm{Hydrolysis}"] & & \mathrm{Penicillamine} \\
\mathrm{\underset{NH_2}{CH_2}\cdot CO_2H} \arrow[r, "\mathrm{CS_2}", "\mathrm{Alkylation}"'] & \mathrm{\underset{NH\cdot CS_2R}{CH_2}\cdot CO_2H} \arrow[r, "\mathrm{Me_2CO{-}}", "\mathrm{Ac_2O}"'] & \mathrm{Me_2C{:}\underset{N:C(SR)\cdot S}{C{-}CO}} \arrow[r, "\mathrm{H_2S}"] & \mathrm{\underset{S\cdot CS\cdot NH}{Me_2C{-}CH}\cdot CO\cdot SH} \arrow[u]
\end{tikzcd}$$

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[22] E. P. Abraham, W. Baker, E. Chain, J. Wand, R. H. Cornforth, and R. Robinson, PEN 100; see also K. Savard, E. M. Richardson, and G. A. Grant, *Canadian J. Res.*, 1946, **24***B*, 28; L. H. Werner, A. Wettstein, and K. Miescher, *Helv. Chim. Acta*, 1947, **30**, 432.

[23] Merck and Co., Inc., Oct., 1943, CPS 14.

[24] *E.g.*, M 10.

[25] J. R. Catch, A. H. Cook, and I. M. Heilbron, CPS 678.

[26] A. H. Cook, G. Harris, and I. M. Heilbron, CPS 583.

Clearly the penicillins differed among themselves in the detail of the non-penicillamine moiety. Acid hydrolysis of (pent-2-enyl) penicillin concentrates and, more significantly at the time, of pent-2-enylpenillic acid gave ultimately a dinitrophenylhydrazone,[27] an increased yield resulting from mild alkaline hydrolysis of pent-2-enylpenicillin followed by treatment with mercuric chloride. Of various formulæ, the evidence of *X*-ray crystallography favourėd $C_8H_{13}O_2N$ for the parent carbonyl compound,[28] and direct analysis suggested the formula, $C_8H_{15}O_2N$, for the carbonyl compound from *n*-amylpenicillin, the derivatives of which were crystallographically distinct from those of pent-2-enylpenicillin. The dinitrophenylhydrazone from *n*-amylpenicillin was degraded to a glyoxalosazone by excess of 2 : 4-dinitrophenylhydrazine, and having regard to the composition and non-basicity of the carbonyl compound and formation of *n*-hexoic acid on acid hydrolysis, the formula $n\text{-}C_5H_{11}\cdot CO\cdot NH\cdot CH_2\cdot CHO$ was suggested.[29] *n*-Amylpenilloaldehyde, as the product from *n*-amylpenicillin was·termed, was synthesised from bromoacetal and *n*-hexoamide[30] as well as from *n*-hexoyl chloride and aminoacetal,[31] and identified with compounds of natural origin. Meanwhile pent-2-enylpenilloaldehyde[32] had been oxidised

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[27] E. P. Abraham, W. Baker, E. Chain, and R. Robinson, PEN 79, 87, 97.

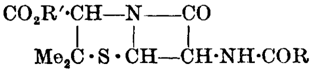
[28] D. Crowfoot and B. Low, PEN 98.

[29] R. Bentley, J. R. Catch, A. H. Cook, J. A. Elvidge, R. H. Hall, and I. M. Heilbron, PEN 102, 106.

[30] *Idem,* PEN 110.

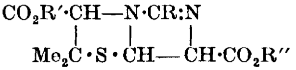
[31] E. P. Abraham, W. Baker, E. Chain, and R. Robinson, PEN 109.

[32] The rather confusing general nomenclature of further penicillin degradation products is summarised for convenient reference here :



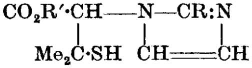
$$\begin{array}{rcccl} \mathrm{CO_2R'{\cdot}CH} & — & \mathrm{N} & — & \mathrm{CO} \\ | & & | & & | \\ \mathrm{Me_2C} & \cdot\mathrm{S}\cdot & \mathrm{CH} & — & \mathrm{CH{\cdot}NH{\cdot}COR} \end{array}$$

(or alternative formulations)
Penicillins (penicillinic acids).



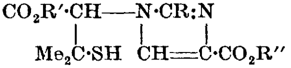
$$\begin{array}{rcccl} \mathrm{CO_2R'{\cdot}CH} & — & \mathrm{N} & \mathrm{{\cdot}CR{:}} & \mathrm{N} \\ | & & | & & | \\ \mathrm{Me_2C} & \cdot\mathrm{S}\cdot & \mathrm{CH} & — & \mathrm{CH{\cdot}CO_2R''} \end{array}$$

(or tautomerides)
Penill-ates (-ic acids).



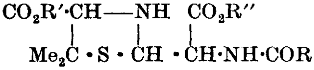
$$\begin{array}{rcccl} \mathrm{CO_2R'{\cdot}CH} & — & \mathrm{N} & \mathrm{—CR{:}} & \mathrm{N} \\ | & & | & & | \\ \mathrm{Me_2C{\cdot}SH} & & \mathrm{CH} & = & \mathrm{CH} \end{array}$$

Penillamines.



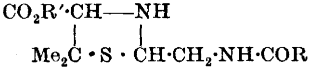
$$\begin{array}{rcccl} \mathrm{CO_2R'{\cdot}CH} & — & \mathrm{N} & \mathrm{{\cdot}CR{:}} & \mathrm{N} \\ | & & | & & | \\ \mathrm{Me_2C{\cdot}SH} & & \mathrm{CH} & = & \mathrm{C{\cdot}CO_2R''} \end{array}$$

iso*Penill-ates (-ic acids).*



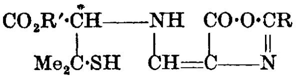
$$\begin{array}{rcccl} \mathrm{CO_2R'{\cdot}CH} & — & \mathrm{NH} & & \mathrm{CO_2R''} \\ | & & | & & | \\ \mathrm{Me_2C} & \cdot\mathrm{S}\cdot & \mathrm{CH} & \cdot & \mathrm{CH{\cdot}NH{\cdot}COR} \end{array}$$

Penicillo-ates (-ic acids).



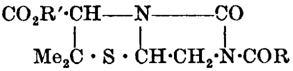
$$\begin{array}{rcl} \mathrm{CO_2R'{\cdot}CH} & — & \mathrm{NH} \\ | & & | \\ \mathrm{Me_2C} & \cdot\mathrm{S}\cdot & \mathrm{CH{\cdot}CH_2{\cdot}NH{\cdot}COR} \end{array}$$

Penillo-ates (-ic acids).



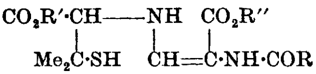
$$\begin{array}{rcccccl} \mathrm{CO_2R'{\cdot}CH} & — & \mathrm{NH} & & \mathrm{CO} & \mathrm{{\cdot}O{\cdot}} & \mathrm{CR} \\ | & & | & & | & & \| \\ \mathrm{Me_2C{\cdot}SH} & & \mathrm{CH} & = & \mathrm{C} & — & \mathrm{N} \end{array}$$

Penicillen-ates (-ic acids).



$$\begin{array}{rcccl} \mathrm{CO_2R'{\cdot}CH} & — & \mathrm{N} & — & \mathrm{CO} \\ | & & | & & | \\ \mathrm{Me_2C} & \cdot\mathrm{S}\cdot & \mathrm{CH} & \mathrm{{\cdot}CH_2{\cdot}} & \mathrm{N{\cdot}COR} \end{array}$$

Penillon-ates (-ic acids).



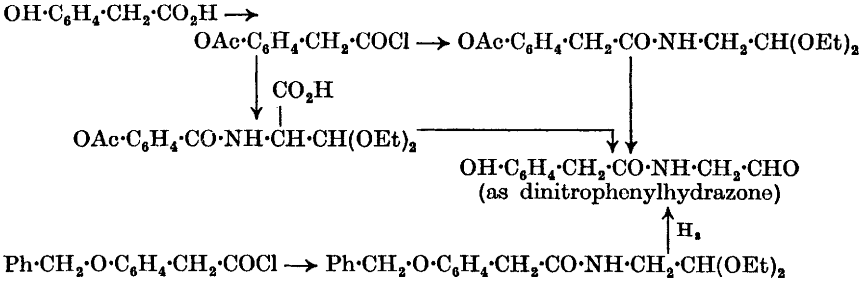
$$\begin{array}{rcccl} \mathrm{CO_2R'{\cdot}CH} & — & \mathrm{NH} & & \mathrm{CO_2R''} \\ | & & | & & | \\ \mathrm{Me_2C{\cdot}SH} & & \mathrm{CH} & = & \mathrm{C{\cdot}NH{\cdot}COR} \end{array}$$

Penamaldates (-ic acids).

$CHO\cdot CH(CO_2R'')\cdot NH\cdot COR$
Penald-ates (-ic acids).

$CHO\cdot CH_2\cdot NH\cdot COR$
Penilloaldehydes.

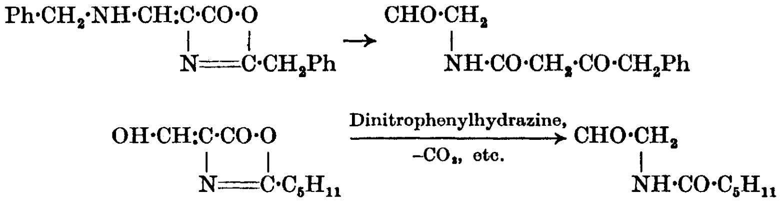
to an acid, $C_8H_{13}O_3N$,[33] and then hydrolysed to glycine [31] so that it was constitutionally similar to *n*-amylpenilloaldehyde. As also it gave propaldehyde on oxidation it was formulated as *n*-hex-β-enamidoacetaldehyde, $Me{\cdot}CH_2{\cdot}CH{:}CH{\cdot}CH_2{\cdot}CO{\cdot}NH{\cdot}CH_2{\cdot}CHO$, and this was confirmed by synthesis.[34] Almost simultaneously benzylpenicillin was similarly being degraded to *benzylpenilloaldehyde*, $C_{10}H_{11}O_2N$, and as it afforded phenylacetic acid on hydrolysis (this acid had been obtained from benzylpenicillin much earlier, when its significance could not be established) it was formulated as $Ph{\cdot}CH_2{\cdot}CO{\cdot}NH{\cdot}CH_2{\cdot}CHO$,[35] and almost immediately synthesised. p-*Hydroxybenzylpenilloaldehyde*, affording *p*-hydroxyphenylacetic acid on hydrolysis, was formulated as $p\text{-}OH{\cdot}C_6H_4{\cdot}CH_2{\cdot}CO{\cdot}NH{\cdot}CH_2{\cdot}CHO$ [36] and synthesised as follows : [37]



$$\begin{tikzcd}[column sep=small]
OH{\cdot}C_6H_4{\cdot}CH_2{\cdot}CO_2H \arrow[r] & OAc{\cdot}C_6H_4{\cdot}CH_2{\cdot}COCl \arrow[r] \arrow[d] & OAc{\cdot}C_6H_4{\cdot}CH_2{\cdot}CO{\cdot}NH{\cdot}CH_2{\cdot}CH(OEt)_2 \arrow[d] \\
 & OAc{\cdot}C_6H_4{\cdot}CO{\cdot}NH{\cdot}\overset{CO_2H}{\overset{|}{C}H}{\cdot}CH(OEt)_2 \arrow[r] & \begin{array}{c} OH{\cdot}C_6H_4{\cdot}CH_2{\cdot}CO{\cdot}NH{\cdot}CH_2{\cdot}CHO \\ \text{(as dinitrophenylhydrazone)} \end{array} \\
Ph{\cdot}CH_2{\cdot}O{\cdot}C_6H_4{\cdot}CH_2{\cdot}COCl \arrow[rr] & & Ph{\cdot}CH_2{\cdot}O{\cdot}C_6H_4{\cdot}CH_2{\cdot}CO{\cdot}NH{\cdot}CH_2{\cdot}CH(OEt)_2 \arrow[u, "H_2"']
\end{tikzcd}$$

n-*Heptylpenilloaldehyde*, $n\text{-}C_7H_{15}{\cdot}CO{\cdot}NH{\cdot}CH_2{\cdot}CHO$, was obtained much later,[38] and the penilloaldehyde from flavacidin appears to be n-*hex-γ-enamidoacetaldehyde*,[17] $Me{\cdot}CH{:}CH{\cdot}CH_2{\cdot}CH_2{\cdot}CO{\cdot}NH{\cdot}CH_2{\cdot}CHO$. In addition to these rational syntheses, some penilloaldehydes have been obtained in less direct ways,[39] *e.g.* :

$$n\text{-}C_5H_{11}{\cdot}CO{\cdot}NH{\cdot}CH(CHO){\cdot}CO_2Et \xrightarrow[\text{etc.}]{\text{Hydrolysis,}} n\text{-}C_5H_{11}{\cdot}CO{\cdot}NH{\cdot}CH_2{\cdot}CHO$$



$$\begin{array}{l} Ph{\cdot}CH_2{\cdot}NH{\cdot}CH{:}C{\cdot}CO{\cdot}O \\ \qquad\quad\; | \qquad\quad | \\ \qquad\quad N{=\!=}C{\cdot}CH_2Ph \end{array} \longrightarrow \begin{array}{l} CHO{\cdot}CH_2 \\ \qquad | \\ NH{\cdot}CO{\cdot}CH_2{\cdot}CO{\cdot}CH_2Ph \end{array}$$

$$\begin{array}{l} OH{\cdot}CH{:}C{\cdot}CO{\cdot}O \\ \qquad\quad | \qquad | \\ \qquad\quad N{=\!=}C{\cdot}C_5H_{11} \end{array} \xrightarrow[-CO_2,\ \text{etc.}]{\text{Dinitrophenylhydrazine,}} \begin{array}{l} CHO{\cdot}CH_2 \\ \qquad | \\ NH{\cdot}CO{\cdot}C_5H_{11} \end{array}$$

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[33] E. P. Abraham, W. Baker, E. Chain, and R. Robinson, PEN 103.

[34] *Idem*, PEN 111 ; D. Crowfoot and B. Low, PEN 112.

[35] S 3 ; Merck & Co. Inc., CMR Report, Oct., 1943.

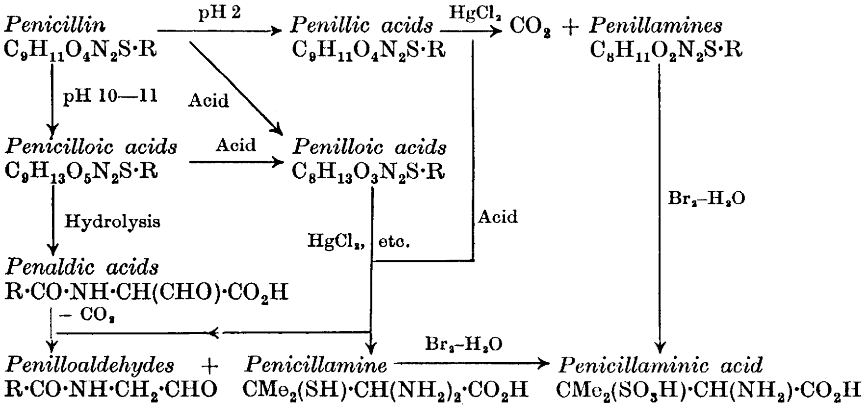
[36] J. R. Catch, A. H. Cook, and I. M. Heilbron, CPS 23.

[37] A. H. Cook, A. R. Graham, and I. M. Heilbron, CPS 314 ; U 13b ; E. P. Abraham, W. Baker, E. Chain, D. G. I. Felton, F. E. King, and R. Robinson, CPS 685.

[38] A 20, 21, 26.

[39] R. Bentley, J. R. Catch, A. H. Cook, J. A. Elvidge, R. H. Hall, and I. M. Heilbron, PEN 114 ; U 11 ; J. Attenburrow, D. F. Elliott, B. A. Hems, and F. A. Robinson, CPS 383.

When penilloaldehydes were obtained by mild alkali inactivation of penicillins followed by treatment with mercuric chloride the last step was accompanied by elimination of 1 mol. of carbon dioxide,[40] and this was obtained also from penillic acids by treatment with mercuric chloride or by acid hydrolysis.[40, 41] Under the former conditions the penillic acids yielded the various *penillamines* (*e.g.*, *pent-2-enylpenillamine*, $C_{13}H_{20}O_2N_2S$), whereas acid hydrolysis ultimately afforded penicillamine and a penilloaldehyde. These changes, including some intermediates which await discussion, are represented schematically below :



The salient features of the more complex of these products may be summarised as follows :

	Acid groups.	Basic group.	Thiol group.	Ultra-violet absorption.
Penicillin	1	0	0	—
Penillic acid	2	0	0	Characteristic absorption.
Penillamine	1	1	1	
Penicilloic acid	2	1	0	—
Penilloic acid	1	1	0	—

The penillamines could not be hydrolysed to penicillamine [41] and, as they contained a thiol grouping with all their oxygen in the form of the penicillamine carboxyl, the potential though not actual penilloaldehyde grouping must have been bound wholly through the penicillamine N atom. These facts were well represented by (VI). Penillic acids contained all their oxygen in the form of 2 carboxyl groups of which one was labile, and having regard to their potential thiol and labile carboxyl groups, difficult hydrolysability, and an absorption spectrum quite distinct from that of the penillamines, it was suggested that they were represented by one of the (probably

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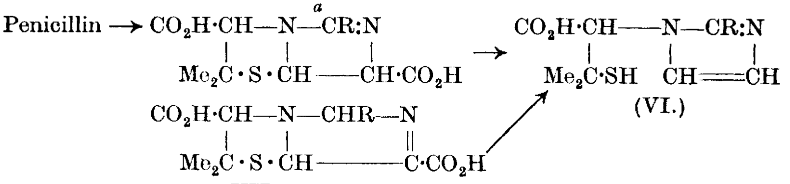
[40] E. P. Abraham, W. Baker, E. Chain, and R. Robinson, PEN 91.

[41] R. Bentley, J. R. Catch, A. H. Cook, J. A. Elvidge, R. H. Hall, and I. M. Heilbron, PEN 106, 113.

tautomeric) structures (VII).[29], [33] In this case the penillic acid–penillamine reaction would consist in fission of the thiazolidine ring to liberate a thiol grouping and simultaneous conversion of a carboxyiminazoline into an iminazole ring. Although several postulates remained to be supported these formulations credibly explained the hydrolysis of penillic acids by

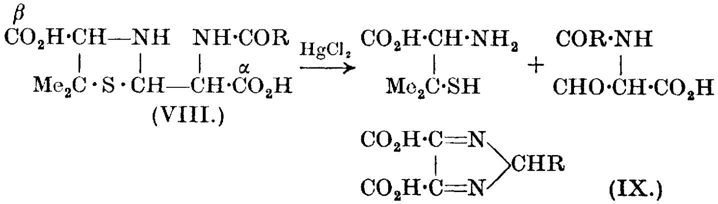


(VI.)

(VII.)

(R = C_5H_9, C_5H_{11}, CH_2Ph. etc.)

fission of bond *a* to give thiazolidines (VIII) (*penicilloic acids*) which would undergo disruption by mercuric chloride to α-formyl acids (*penaldic acids*) and thence to the penilloaldehydes :



(VIII.)

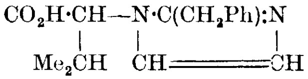
(IX.)

Paucity of material precluded more detailed studies of this kind in the pent-2-enyl, *n*-amyl, and *p*-hydroxybenzyl series though the behaviour of synthetic compounds was closely examined. Study of derivatives of benzylpenicilloic acid was more precise, being assisted by the quantities of material available, the formation of α-penicilloic esters (see VIII) by inactivation of benzylpenicillin by alcohols, and the fortunate circumstance that benzylpenicillin reacted with bases such as benzylamine to give α-monoamides of benzylpenicilloic acid. That these derivatives are in fact derivatives with respect to the labile (α) carboxyl group was only established later.

The penillamines and penillic acids were thus represented as substituted iminazoles and iminazolines respectively, the latter owing their light absorption essentially to the system $N:C{\cdot}CO_2H$. A comparison of the absorption spectra of penillamines and penillic acids with iminazole, 2-*n*-amyl- and 2-benzyl-iminazole, and the corresponding 4 : 5-iminazoledicarboxylic acids respectively showed satisfactory compatibility with the proposed formulations [41] ; the dicarboxylic acids (IX) seemed to provide a fair comparison as they could be regarded as containing a duplicated absorbing system of the above kind.

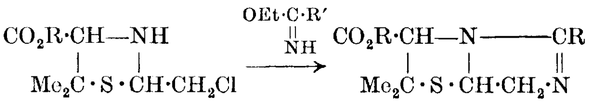
It is suitable, despite some divergence from chronological order, to discuss immediately the further chemistry of the degradations already outlined, as they suggested directly and indirectly a major part of the subsequent synthetic work. Later degradations and their influence on attempted syntheses can thus be viewed in the light of growing experience of unfamiliar ring-systems and appreciation of initially unsuspected potentialities of the penicillin molecule.

Penillamines. Verification of the structures indicated above was first sought, though unsuccessfully, by attempted synthesis of complex iminazoles by interaction of 2-amyl- or -benzyl-iminazole as a sodium or silver derivative with α-halogeno-$\beta\beta$-dimethyl-acrylates or -propionates. The nearest approach of this kind was the ultimate synthesis of a dethiodihydrobenzylpenillamine (X) which, however, could not be dehydrogenated. Another approach consisted in the interaction of suitable 2-chloromethyl-thiazolidines (XI) with imino-ethers,[42] but, despite the resemblance to the successful synthesis of dimethyl benzylpenillate (see below), the thiazolidines (XI) did not react in the desired manner. However, despite initial unsuccessful attempts,[43] the DL-penilloic acids could be dehydrated by phosphorus oxychloride to give " decarboxypenillic acids " (XII) together with complex diketopiperazines. When the former were treated with mercuric chloride under the conditions used for preparing the penillamines, cleavage of the thiazolidine ring took place and DL-penillamines were produced. The products were chemically indistinguishable from the penillamines of natural origin ; moreover the similarity extended to their microscopic crystallography, and *X*-ray examination suggested that the above representation of the penillamines was correct, the synthetic material behaving as a DL-mixture rather than a racemic compound.[44] Repetition of the syntheses with D- and L-penicillamine *via* the optically active penilloic acids gave D(—)- and L(+)-*n*-amyl and -benzyl-penillamines of which the D(—)-compounds were identical with those of natural origin. DL-*p*-Hydroxybenzylpenillamine was obtained similarly from penicillamine and *p*-acetoxyphenylacetamidodiethylacetal mentioned above. An analogous synthesis of (XII) ($R = H$, $R' = CH_2Ph$) by loss of hydrogen sulphide from the phenylthioacetamidomethylthiazolidine (XIII) was also successful.[45]



$$\begin{array}{l} CO_2H\cdot CH—N\cdot C(CH_2Ph){:}N \\ \quad |\qquad\quad |\qquad\qquad\quad | \\ Me_2CH\quad CH{=\!=\!=\!=}CH \end{array}$$

(X.)



$$\begin{array}{l} CO_2R\cdot CH—NH \\ \quad |\qquad\quad | \\ Me_2C\cdot S\cdot CH\cdot CH_2Cl \end{array} \xrightarrow{OEt\cdot C(:NH)\cdot R'} \begin{array}{l} CO_2R\cdot CH—N——CR \\ \quad |\qquad\quad |\qquad\quad \| \\ Me_2C\cdot S\cdot CH\cdot CH_2\cdot N \end{array}$$

(XI.) (XII.)

Attempts to apply the principle of the latter synthesis to *n*-amylpenillamine unexpectedly failed. Dithio-*n*-hexoic acid was only inconveniently accessible by reaction of *n*-amylmagnesium bromide with carbon disulphide,

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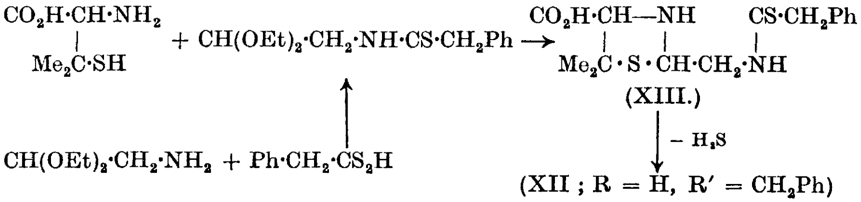
[42] F. C. Copp, W. M. Duffin, S. Smith, and S. Wilkinson, CPS 3, 72.

[43] L 4 ; U 11.

[44] A. H. Cook, J. A. Elvidge, I. M. Heilbron, and A. L. Levy, CPS 67, 313 ; cf. D. Crowfoot, B. Low, and G. Schmidt, CPS 53.

[45] E. P. Abraham, W. Baker, E. Chain, and R. Robinson, CPS 71 ; A. H. Cook, J. A. Elvidge, and I. M .Heilbron, CPS 77.



$$\begin{array}{l}CO_2H\cdot CH\cdot NH_2\\ \quad |\\ Me_2C\cdot SH\end{array} + CH(OEt)_2\cdot CH_2\cdot NH\cdot CS\cdot CH_2Ph \rightarrow \begin{array}{l}CO_2H\cdot CH - NH \quad CS\cdot CH_2Ph\\ \qquad | \qquad\quad | \qquad\qquad |\\ Me_2C\cdot S\cdot CH\cdot CH_2\cdot NH\end{array}$$

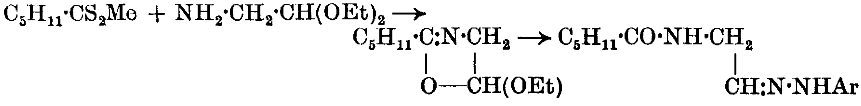
$$CH(OEt)_2\cdot CH_2\cdot NH_2 + Ph\cdot CH_2\cdot CS_2H \uparrow$$

(XIII.)

$$\downarrow -H_2S$$

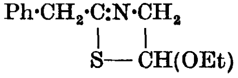
(XII ; R = H, R′ = CH_2Ph)

but was converted into its methyl ester. Reaction of the latter with aminodiethylacetal led not to a thiohexamido-compound but by loss of ethylthiol to 5-ethoxy-2-*n*-amyloxazoline (XIV), as was confirmed by the ready



$$C_5H_{11}\cdot CS_2Me + NH_2\cdot CH_2\cdot CH(OEt)_2 \rightarrow \begin{array}{l}C_5H_{11}\cdot C{:}N\cdot CH_2\\ \qquad | \quad |\\ \qquad O - CH(OEt)\end{array} \rightarrow \begin{array}{l}C_5H_{11}\cdot CO\cdot NH\cdot CH_2\\ \qquad\qquad\qquad |\\ \qquad\qquad CH{:}N\cdot NHAr\end{array}$$

(XIV.)

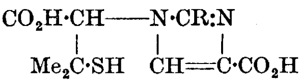


$$\begin{array}{l}Ph\cdot CH_2\cdot C{:}N\cdot CH_2\\ \qquad\quad | \quad |\\ \qquad\quad S - CH(OEt)\end{array}$$

(XV.)

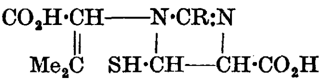
conversion of the product into the dinitrophenylhydrazone of *n*-amylpenilloaldehyde. By contrast, phenylthioacetamidodiethylacetal could also be cyclised (by loss of ethanol), to give 5-ethoxy-2-benzylthiazoline (XV).[46]

iso*Penillic acids.* Pent-2-enylpenillic acid exhibits an irreversible change in ultra-violet absorption at pH 10, the product being a mercapto-dicarboxylic acid isomeric with the original penillic acid but undergoing no further change on treatment with mercuric chloride ; in particular the lability of one of the carboxyl groups of penicillins and the penillic acids is no longer evident in the new iso*penillic acid.* The precise isomerisation conditions remain somewhat obscure as the presence of oxygen appeared to be important and indeed in some circumstances seemed of more moment than the alkalinity. On the basis of formula (VII) for the penillic acids, and having regard to what must have been a rearrangement in the sense $-S\cdot\overset{|}{\underset{|}{C}}-\overset{|}{\underset{|}{C}}H \rightarrow \cdot SH + \overset{|}{\underset{|}{C}}=\overset{|}{\underset{|}{C}}$, two formulations for pent-2-eny*liso*penillic acid, (XVI) and (XVII) ($R = C_5H_9$), came under consideration. Of these, (XVII) represented the *iso*penillic acid with at least thioacetal-like reactivity, and the alternative formulation (XVI) as a penillaminecarboxylic acid seemed the better repre-



$$\begin{array}{l}CO_2H\cdot CH - N\cdot CR{:}N\\ \qquad | \qquad\quad | \qquad |\\ Me_2C\cdot SH \quad CH{=}C\cdot CO_2H\end{array}$$

(XVI.)



$$\begin{array}{l}CO_2H\cdot CH - N\cdot CR{:}N\\ \qquad \| \qquad\quad | \qquad |\\ Me_2C \quad SH\cdot CH - CH\cdot CO_2H\end{array}$$

(XVII.)

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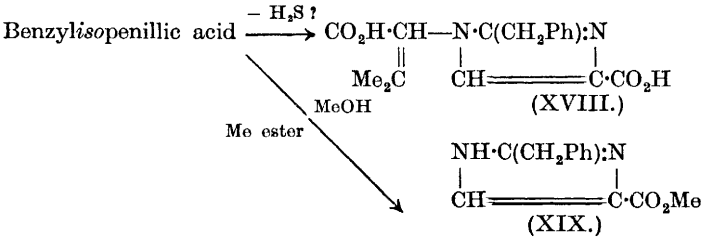
[46] A. H. Cook, J. A. Elvidge, and I. M. Heilbron, CPS 273 ; E. P. Abraham, E. Chain, W. Baker, and R. Robinson, CPS 43.

sentation.[47] *n*-Amylpenillic acid has not been observed to undergo any similar change, but benzylpenillic acid and its near derivatives undergo a number of changes in presence of alkali. Under ordinary conditions at pH 11·6 a fall in ultra-violet absorption is associated with fission of the iminazoline ring with formation of benzylpenicilloic acid and probably its decarboxylation product, benzylpenilloic acid; reaction in this sense probably occurs also when dimethyl benzylpenillate is kept with butanolic alkali.[48] With aqueous or warm methanolic barium hydroxide, however, the product is a *benzyl*iso*penillic acid* comparable with the pentenyl compound above. Dimethyl benzyl*iso*penillate is formed unexpectedly from dimethyl benzylpenillate in acetic acid or by thermal rearrangement in boiling xylene.[49]

The reactions of benzyl*iso*penillic acid still offer some perplexities. Thus loss of hydrogen sulphide did not appear to lead to dimethylacrylic acid derivatives such as (XVIII), whereas methanolysis resulted unexpectedly in fission of the penicillamine C–N bond with formation of the iminazole (XIX);[50] the formation of (XIX) is paralleled by the formation of the corresponding acid by methanolysis of benzylpenillic acid itself, a reaction which may perhaps be compared with the fission of α-acylamidoacrylic acids to pyruvic acids.



Penillic acids. Although the light-absorbing centre of the penillic acids had been considered earlier in relation to iminazolecarboxylic acids, the question continued to excite interest and several compounds containing the system $C{\cdot}C(CO_2H){:}N$ were examined. This confirmed the origin of the light absorption of penillic acids relative to that of the penillamines,[51] and the entire carbon–nitrogen skeleton (of benzylpenillic acid) was revealed by two further pieces of evidence. The relation of the labile carboxyl group to the remainder of the potential penilloaldehyde moiety was deduced from the product of inactivation of benzylpenicillin with benzylamine,[52] and this relation was confirmed by degradation of benzylpenillic acid to known iminazole derivatives.

Treatment of sodium benzylpenicillin with benzylamine led to inactivation and formation of a benzylamide derivative of a thiazolidine. In confirmation of this it was immediately cleaved by mercuric chloride whereas

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[47] E. P. Abraham, W. Baker, E. Chain, and R. Robinson, CPS 11; E. R. Holiday, CPS 47.

[48] M 6, 7, 48, 53.

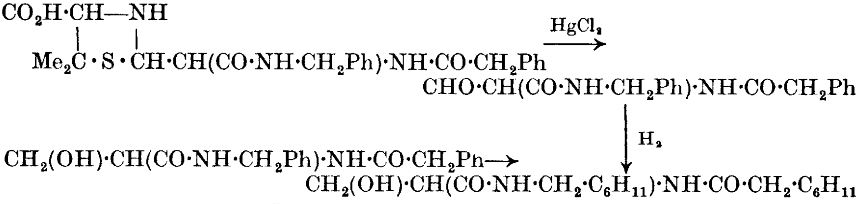
[49] S 24; M 36, 58.

[50] M 41, 61.

[51] See, for example, M 15, 25, 34, 41, 45; S. 21; PD 10.

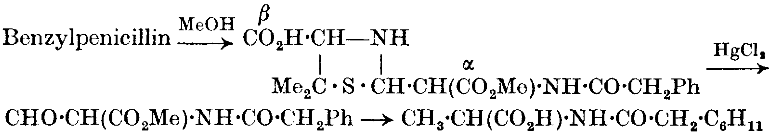
[52] C 4, 5.

after acetylation of the cyclic :NH group it gave no precipitate with that reagent. One of the products of the above cleavage was an aldehyde, catalytic reduction of which gave (XX), identical with the product obtained by hydrogenating the synthetic serine derivative (XXI).[53] The position of the entering benzylamido-grouping, marking that of the "labile" carboxyl group, was thus clear. A similar series of reactions with the product formed from methanol and benzylpenicillin ("α-methyl benzylpenicilloate") led to the same conclusion; treatment with mercuric chloride cleaved it to an aldehyde which, on hydrogenation and saponification, gave the alanine derivative (XXII), which, bearing in mind the penilloaldehyde structure, could only have arisen from a penaldic ester of constitution (XXIII), *i.e.*, an α-carboxyaldehyde. It is obvious that these degradations also reveal the "labile" (α) carboxyl group as that which is originally only latent in penicillin itself. This fact was evident also from the degradation of methyl pent-2-enylpenicillin, the products being carbon dioxide, pent-2-enylpenilloaldehyde, and penicillamine methyl ester, *i.e.*, the "penicillamine" (or β) carboxyl group is free in penicillin.[54] The location of the α-carboxyl group in an iminazoline ring in benzylpenillic acid was moreover indicated by pyrolysis of the dimethyl ester which afforded 4-carbo-



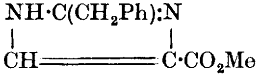
$$\begin{array}{l} CO_2H\cdot CH—NH \\ \quad | \qquad | \\ Me_2C\cdot S\cdot CH\cdot CH(CO\cdot NH\cdot CH_2Ph)\cdot NH\cdot CO\cdot CH_2Ph \xrightarrow{HgCl_2} CHO\cdot CH(CO\cdot NH\cdot CH_2Ph)\cdot NH\cdot CO\cdot CH_2Ph \\ CHO\cdot CH(CO\cdot NH\cdot CH_2Ph)\cdot NH\cdot CO\cdot CH_2Ph \xrightarrow{H_2} CH_2(OH)\cdot CH(CO\cdot NH\cdot CH_2\cdot C_6H_{11})\cdot NH\cdot CO\cdot CH_2\cdot C_6H_{11} \\ CH_2(OH)\cdot CH(CO\cdot NH\cdot CH_2Ph)\cdot NH\cdot CO\cdot CH_2Ph \rightarrow CH_2(OH)\cdot CH(CO\cdot NH\cdot CH_2\cdot C_6H_{11})\cdot NH\cdot CO\cdot CH_2\cdot C_6H_{11} \end{array}$$

(XXI.) (XX.)



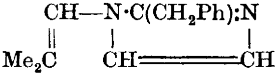
$$\begin{array}{l} \text{Benzylpenicillin} \xrightarrow{MeOH} \overset{\beta}{CO_2H}\cdot CH—NH \\ \qquad\qquad\qquad | \qquad | \qquad \alpha \\ \qquad\qquad Me_2C\cdot S\cdot CH\cdot CH(CO_2Me)\cdot NH\cdot CO\cdot CH_2Ph \xrightarrow{HgCl_2} \\ CHO\cdot CH(CO_2Me)\cdot NH\cdot CO\cdot CH_2Ph \rightarrow CH_3\cdot CH(CO_2H)\cdot NH\cdot CO\cdot CH_2\cdot C_6H_{11} \end{array}$$

(XXIII.) (XXII.)



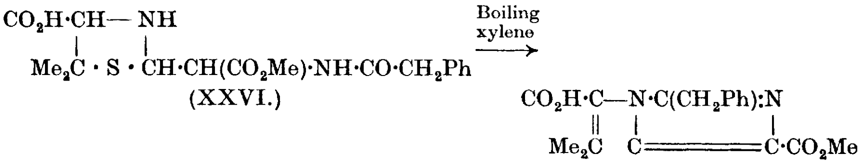
$$\begin{array}{l} NH\cdot C(CH_2Ph){:}N \\ | \qquad\qquad\quad | \\ CH{=\!=\!=}C\cdot CO_2Me \end{array}$$

(XXIV.)



$$\begin{array}{l} \quad CH—N\cdot C(CH_2Ph){:}N \\ \quad \| \qquad | \qquad\qquad | \\ Me_2C \quad CH{=\!=\!=}CH \end{array}$$

'XXV.)



$$\begin{array}{l} CO_2H\cdot CH—NH \\ \quad | \qquad | \\ Me_2C\cdot S\cdot CH\cdot CH(CO_2Me)\cdot NH\cdot CO\cdot CH_2Ph \xrightarrow{\text{Boiling xylene}} \begin{array}{l} CO_2H\cdot C—N\cdot C(CH_2Ph){:}N \\ \quad \| \qquad | \qquad\qquad | \\ Me_2C \quad C{=\!=\!=}C\cdot CO_2Me \end{array} \end{array}$$

(XXVI.) (XXVII.)

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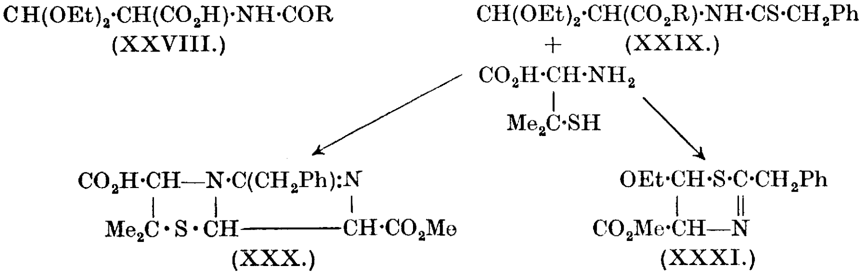
[53] M 13. [54] W. R. Boon, H. C. Carrington, and G. G. Freeman, CPS 28.

methoxy-2-benzyliminazole (XXIV). Benzylpenillic acid itself lost both carboxyl groups on pyrolysis. The resulting base, $C_{14}H_{16}N_2$, could be written almost with certainty as (XXV), for it proved to be identical with that obtained from the synthetic thiazolidine (XXVI) *via* the ester (XXVII).[55]

The structure of the penillic acids thus only required final confirmation by synthesis. Many attempts to modify the synthesis of "decarboxypenillic acids" described above, *i.e.*, to dehydrate appropriate α-penicilloic esters rather than penilloate derivatives, proved unsuccessful [56] though the isolation of what was believed to be (XXV) suggests that the failure was due not to lack of cyclisation but to subsequent reactions. On the other hand experiments to cyclise penicilloic acids led to decarboxylation as did also condensations between penicillamine and the diethylacetal of phenylacetamido- and *n*-hexoamido-formylacetic acid (XXVIII) (R = CH_2Ph or *n*-C_5H_{11}). Partial success however followed adaption of the second of the above penillamine syntheses. Dithiophenylacetic acid and ββ-diethoxyalanine afforded the acid (XXIX) (R = H) which, suffering decarboxylation on attempted condensation with penicillamine, was esterified to give (XXIV ; R = Me). The ester condensed with DL-penicillamine to give a small yield of an acid which had the anticipated light absorption of a penillic acid and was believed to be (XXX). The yield was small and irregular and the reaction complicated by formation of the thiazoline (XXXI).[57]



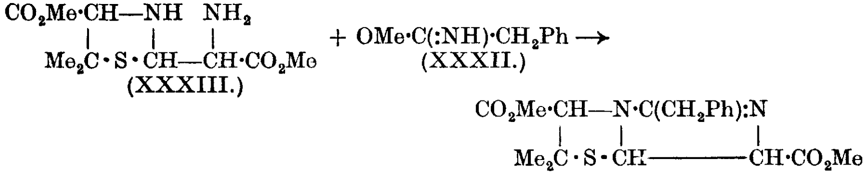
$CH(OEt)_2{\cdot}CH(CO_2H){\cdot}NH{\cdot}COR$
(XXVIII.)

$CH(OEt)_2{\cdot}CH(CO_2R){\cdot}NH{\cdot}CS{\cdot}CH_2Ph$
(XXIX.)

(XXX.)

(XXXI.)

Dimethyl benzylpenillate was ultimately synthesised from phenacetiminomethyl ether (XXXII) and 4-carbomethoxy-5 : 5-dimethyl-2-aminocarbomethoxymethylthiazolidine (XXXIII). From the optically pure thiazolidine from pure D-penicillamine the product was identical with the penillate of natural origin, dimethyl benzyl*iso*penillate (see above) being



(XXXIII.)

$OMe{\cdot}C(:NH){\cdot}CH_2Ph$
(XXXII.)

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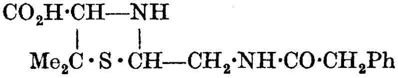
[55] M 36, 44 ; S 19. [56] CPS 5 ; M 23, 32, 36 ; B 4 ; PD 15 ; W 8.
[57] A. H. Cook, J. A. Elvidge, and I. M. Heilbron, CPS 199 ; S 29 ; L 14.

formed as a by-product.[58] So far analogous experiments to obtain other penillic acids have not met with success.[59]

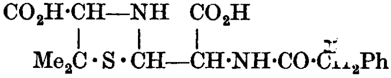
When a " tricyclic " formulation (see below) was considered reasonable for penicillin, attention was given to the possibility of reversing the penillic acid change. Despite attempts involving irradiation of the penillic acids, treatment with boron trifluoride, aluminium chloride, aluminium *tert.*-butoxide, ascaridol, etc., no sign of reactivation was observed, nor, in view of the now recognised remoteness of the penicillin from penillic acid structures, is this surprising.[60]

Early views on the structure of the penicillins. With the above structures in mind it became permissible to consider possible penicillin formulations. It was at once probable that penicillin contained a thiazolidine ring, for in addition to the penillic acid other products were soon isolated which appeared to possess this feature. Thus when aqueous benzylpenillic acid was heated it lost carbon dioxide and was converted into a *benzylpenilloic acid*, $C_{15}H_{20}O_3N_2S$, also formed on boiling sodium benzylpenicillin with dilute sulphuric acid.[61] This compound itself contained neither thiol nor amino-group, but behaved like thiazolidines derived from cysteine and various aldehydes in undergoing fission by aqueous mercuric chloride. Benzylpenilloic acid furnished penicillamine and phenylacetamidoacetaldehyde and was therefore regarded as the thiazolidine (XXXIV).[62]



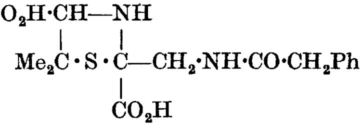
$$\begin{array}{l} \mathrm{CO_2H{\cdot}CH{-}NH} \\ \qquad | \qquad\ | \\ \mathrm{Me_2C\cdot S\cdot CH{-}CH_2{\cdot}NH{\cdot}CO{\cdot}CH_2Ph} \end{array}$$

(XXXIV.)



$$\begin{array}{l} \mathrm{CO_2H{\cdot}CH{-}NH\quad CO_2H} \\ \qquad | \qquad\ | \qquad\ | \\ \mathrm{Me_2C\cdot S\cdot CH{-}CH{\cdot}NH{\cdot}CO{\cdot}CH_2Ph} \end{array}$$

(XXXV.)



$$\begin{array}{l} \mathrm{CO_2H{\cdot}CH{-}NH} \\ \qquad | \qquad\ | \\ \mathrm{Me_2C\cdot S\cdot C{-}CH_2{\cdot}NH{\cdot}CO{\cdot}CH_2Ph} \\ \qquad\qquad\ | \\ \qquad\qquad \mathrm{CO_2H} \end{array}$$

(XXXVI.)

Again, benzylpenicillin was inactivated by very dilute alkali, adding 1 mol. of water to give a labile dibasic acid, *benzylpenicilloic acid*, which in turn broke down slowly in acid solution to give benzylpenilloic acid.[63] In view of the liberation of carbon dioxide without a thiol group appearing, only structure (XXXV) for benzylpenicilloic acid came into consideration (disregarding possible stereochemical variations). The possibility represented by (XXXVI) was more remote because benzylpenicilloic acid was degraded to benzylpenilloaldehyde supposedly *via benzylpenaldic acid* (XXXVII); the corresponding intermediate from (XXXVI) would be the pyruvic acid (XXXVIII) which presumably would have been stable; the

$\mathrm{Ph{\cdot}CH_2{\cdot}CO{\cdot}NH{\cdot}CH(CO_2H){\cdot}CHO}$ (XXXVII.)

$\mathrm{Ph{\cdot}CH_2{\cdot}CO{\cdot}NH{\cdot}CH_2{\cdot}CO{\cdot}CO_2H}$ (XXXVIII.)

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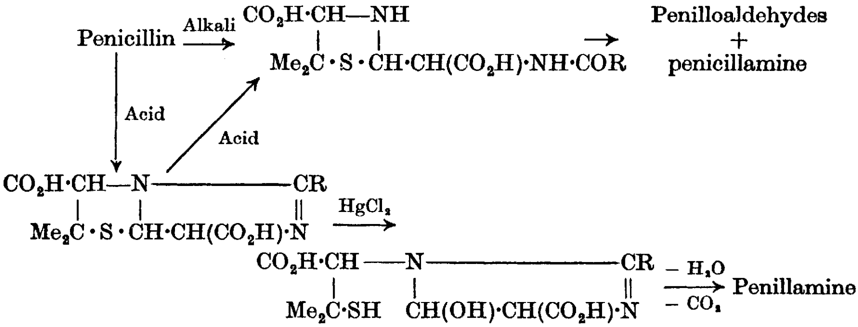
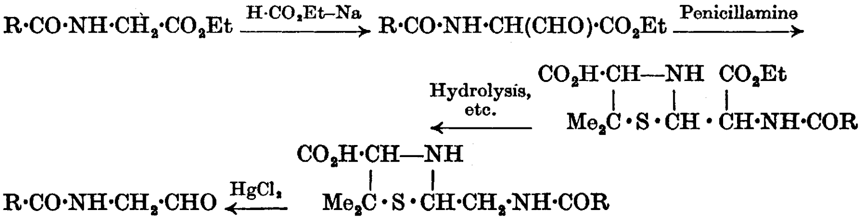
[58] M 50.
[59] P 26, 28.
[60] M 53; L 9; U 25.
[61] Merck & Co. Rep., April—July, 1943; S 1.
[62] *Ibid.*, Oct., 1943; Squibb Inst. Rep., Sept.—Oct., 1943.
[63] M 1, 2.; cf. 64.

stability of 2-carboxythiazolidines and the instability of 2-carboxymethylthiazolidines has since been amply established experimentally. Further, structure (XXXV) seemed biogenetically more acceptable as the central C_3 grouping thus became an α-amino-acid rather than the aminopyruvic acid demanded by (XXXVI). The penilloate and penicilloate structures were rigidly proved some time later (see later).

Although the products were not isolated in pure condition the similar breakdown of pent-2-enylpenicillin in dilute barium hydroxide solution was also believed to proceed *via* a thiazolidine,[64] which in turn afforded pent-2-enylpenilloaldehyde. Evidence pointed here to the various penilloaldehydes arising from carboxylated precursors, and placing the second carboxyl group as in (XXXV) above satisfactorily explained all the transformations known at the time.[65] Compounds of the *n*-amyl-penicilloate, -penilloate, and -penaldate series were soon synthesised[66] and found to behave exactly as did the corresponding natural compounds. Syntheses of this type were later intensively studied in other series[67] (R = Ph, CH_2Ph) because of the possibility of removing water or alcohol from the penicilloic acids or esters to give penicillins. Meanwhile, as described above, location of the labile carboxyl group had been finally established by fixation as a benzylamide and hydrogenation of the degradation product to a serine derivative, and in other ways.





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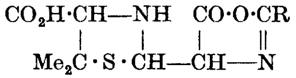
[64] E. P. Abraham, E. Chain, W. Baker, and R. Robinson, PEN 97.

[65] R. Bentley, J. R. Catch, A. H. Cook, J. A. Elvidge, I. M. Heilbron, and R. H. Hall, PEN 102, 106.

[66] R. Bentley, J. R. Catch, A. H. Cook, J. A. Elvidge, R. H. Hall, and I. M. Heilbron, PEN 114.

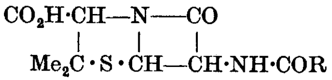
[67] See, for example, M 15c ; G. Newbery and R. Raphael, CPS 206 ; R. J. S. Beer, F. E. King, E. P. Abraham, W. Baker, E. Chain, and R. Robinson, CPS 268.

The problem of representing the penicillins was therefore one of formulating them as anhydrides of the penicilloic acids or as isomerides of the penillic acids in which the labile carboxyl group had been covered to mask any basic group, but in which the essential features of the thiazolidine system were retained. From a more physical standpoint the formulations had to be such that the characteristic light-absorbing system of the penillic acids did not appear. Three formulae—(XXXIX), (XL), and (XLI)—appeared to satisfy some if not all of these conditions though it was always considered that (XXXIX) or (XLI) might be expected to exhibit basic properties. In favour of formula (XXXIX), on the other hand, was the fact that behaviour of the penicillins towards alkalis, alcohols, and bases was exactly that to be expected of an oxazolone, whilst the penillic acids could obviously be easily derived from (XLI). Early opinion did not strongly favour (XL) as, apart from the above considerations, the instability of the penicillins was not reflected in the behaviour of known β-lactams.



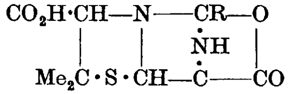
$$\begin{array}{ccccc} CO_2H\cdot CH & — & NH & CO\cdot O\cdot CR & \\ | & & | \quad | & \| & \\ Me_2C\cdot S\cdot CH & — & CH & — & N \end{array}$$

(XXXIX.)



$$\begin{array}{ccc} CO_2H\cdot CH & —N— & CO \\ | & | & | \\ Me_2C\cdot S\cdot CH & — & CH\cdot NH\cdot COR \end{array}$$

(XL.)



$$\begin{array}{cccc} CO_2H\cdot CH & —N— & CR & —O \\ | & | & \overset{\bullet}{NH} & | \\ Me_2C\cdot S\cdot CH & — & \overset{\bullet}{C} & —CO \end{array}$$

(XLI.)

The formulæ (XLI) and later (XXXIX) were ultimately excluded by *X*-ray crystallographic investigations and equally decisively by infra-red absorption measurements. Further discussion in this Review, however, is limited to purely chemical evidence, which at length heavily weighted the balance in favour of (XL), the generally accepted representation of the penicillins.

(iv) *Later Inactivation Reactions and Structure of Penicillins.*—pseudo-*Penicillin.* Four further reactions of benzylpenicillin had a direct bearing on the oxazolone or β-lactam nature of the antibiotic. These concerned the actions of anhydrous hydrogen halides, mercuric chloride, and ammonium thiocyanate, and desulphurisation studies.

It was known that oxazolones afforded addition products with, say, dry hydrogen chloride, which were for long regarded as acylamido-acid chlorides.[68] The reaction with " oxazolone " penicillin might therefore have been expected to yield a penicilloyl chloride hydrochloride, but the experiment (on methyl benzylpenicillin) in fact yielded a product, *methyl* pseudo*benzylpenicillin hydrochloride*, the properties of which were not in consonance with the aforesaid structure.[69] A return to the study of model azlactones, however, showed that the products were indeed salt-like, lost

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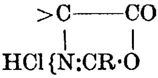
[68] E. Mohr and F. Stroschein, *Ber.*, 1909, **42**, 2521 ; E. Mohr, *J. pr. Chem.*, 1910, **81**, 49, 473.

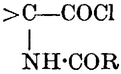
[69] M 22 ; U 10.

significant amounts of hydrogen halide in a vacuum, and generally behaved more like salts of oxazolones (XLII) than isomeric acid halides (XLIII).[70]



$$\begin{array}{l} >C\text{———}CO \\ \quad\ |\qquad\ \ | \\ HCl\{N{:}CR{\cdot}O \end{array}$$

(XLII.)

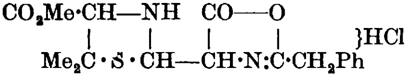


$$\begin{array}{l} >C\text{—}COCl \\ \quad | \\ NH{\cdot}COR \end{array}$$

(XLIII.)

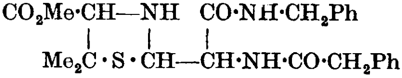
Conversely, the interaction of acylamido-acids and phosphorus halides gave " acid halides " which, on treatment with diazomethane, silver oxide, or similar reagents, were converted into undoubted oxazolones,[70, 71] so that acylamido-acid halides in general appeared to have no existence.

On these views the product from methyl benzylpenicillin and hydrogen chloride might be the corresponding oxazolone hydrochloride, which on removal of hydrogen chloride should revert to the original antibiotic. This regeneration was however not realised, though it remained reasonable to regard the primary product as in fact an oxazolone hydrochloride. The ultimate conclusion therefore was that the oxazolone formulation could not represent penicillin as it was required to represent a reaction product of the antibiotic.[72] Perhaps the strongest evidence for the intermediation of (XLIV) lay in the observation that after treatment with diazomethane the product (presumably the base XLIV) gave with benzylamine the known benzylamide (XLV) as would be expected.[73] The compound (XLV) could have come only from (XLIV) or possibly from methyl benzylpenicillenate (see later), apart from the original antibiotic which had been excluded. Methyl *pseudo*benzylpenicillin (XLIV) did not show the light absorption of methyl benzylpenicillenate which could however be obtained from it by the action of mercuric chloride.



$$\begin{array}{l} CO_2Me{\cdot}CH\text{—}NH\quad CO\text{——}O \\ \qquad |\qquad\ \ |\qquad\ |\qquad\ \ |\qquad\qquad \}HCl \\ \ Me_2C{\cdot}S{\cdot}CH\text{—}CH{\cdot}N{:}C{\cdot}CH_2Ph \end{array}$$

(XLIV.)



$$\begin{array}{l} CO_2Me{\cdot}CH\text{—}NH\quad CO{\cdot}NH{\cdot}CH_2Ph \\ \qquad |\qquad\ \ |\qquad\ | \\ \ Me_2C{\cdot}S{\cdot}CH\text{—}CH{\cdot}NH{\cdot}CO{\cdot}CH_2Ph \end{array}$$

(XLV.)

As treatment of acylamido-acids with phosphorus halides evidently yielded azlactone hydrohalides, the possibility was presented of synthesising a compound having the oxazolone structure assigned to penicillin from appropriate penicilloates.[74] The product from β-methyl benzylpenicilloate was indistinguishable from methyl *pseudo*benzylpenicillin in physical and chemical properties. The infra-red absorption of the synthetic material appeared to indicate the presence of methyl benzylpenicillin, though biological activity was only once detected.[75] It is noteworthy that the first activities in synthetic materials were observed with products of this type.

The penicillenates. Formulation (XXXIX) suggested the possible

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[70] H. J. Barber, P. Z. Gregory, R. Slack, C. E. Stickings, and A. M. Woolman, CPS 55, 234; B. D. H. Ltd., CPS 56; C 10.

[71] M 33, 50; U 13a; cf. also P. Karrer and G. Bussmann, *Helv. Chim. Acta*, 1941, **24**, 646.

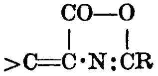
[72] U 13; see also A 15, M 49—51; P 19.

[73] A 15.

[74] U 13, 15, 16.

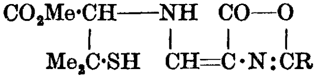
[75] Joint Rep. Univ. Michigan and Upjohn Co., 13th Nov., 1945.

degradation of benzylpenicillin to an oxazolone, especially by means of mercuric chloride—the usual reagent for cleaving thiazolidines—in organic solvents.[76] The experiment (using methyl benzylpenicillin) led to the appearance of a characteristic absorption band (also appearing under other conditions) associated with an amorphous product, *methyl benzylpenicillenate*. An absorption band in the same region also appeared in the crystalline product obtained from DL-penicillamine methyl ester and 2-phenyl-4-ethoxymethylene-oxazolone; moreover, the thiol group was unessential for such reactions, for the absorption band appeared when the penicillamine was replaced by *S*-benzylpenicillamine methyl ester or by alanine, and it was clear that all these products could only contain a 4-methyleneoxazolone group (XLVI) as the chromophoric centre. The product mentioned above from 2-phenyl-4-ethoxymethyleneoxazolone was thus formulated as (XLVII; R = Ph).[77] The existence of an oxazolone ring in methyl benzylpenicillenate of natural origin was proved chemically, since it afforded with sodium hydroxide the sodium salt of 2-benzyl-4-hydroxymethylene-oxazolone[76] (XLVIII). The degradative evidence was completed by desulphurising methyl benzylpenicillenate to a product which was still capable of furnishing (XLVIII) and must therefore have been (XLIX); the penicillenate could only thus be a *N*- and not a *S*-substituted penicillamine derivative[78] (XLVII; R = CH_2Ph). There was no doubt that the non-crystalline product from D-penicillamine and 2-benzyl-4-methoxymethyleneoxazolone was substantially the acid corresponding to (XLVII; R = CH_2Ph) in which synthetic penicillin formed a minor by-product.



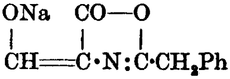
$$\begin{array}{l}\quad\ \text{CO—O}\\ \quad\ \ |\qquad |\\ >\text{C=C·N:CR}\end{array}$$

(XLVI.)



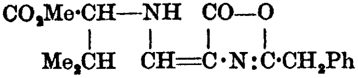
$$\begin{array}{l}\text{CO}_2\text{Me·CH——NH}\ \ \text{CO—O}\\ \qquad\ \ |\qquad\quad\ \ |\qquad |\qquad |\\ \text{Me}_2\text{C·SH}\ \ \text{CH=C·N:CR}\end{array}$$

(XLVII.)



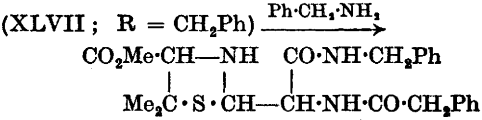
$$\begin{array}{l}\text{ONa}\ \ \text{CO—O}\\ \ \ |\qquad\ \ |\qquad |\\ \text{CH=C·N:C·CH}_2\text{Ph}\end{array}$$

(XLVIII.)



$$\begin{array}{l}\text{CO}_2\text{Me·CH—NH}\ \ \text{CO—O}\\ \qquad\ \ |\qquad\quad |\qquad |\qquad |\\ \ \text{Me}_2\text{CH}\ \ \ \text{CH=C·N:C·CH}_2\text{Ph}\end{array}$$

(XLIX.)



$$\text{(XLVII; R = CH}_2\text{Ph)}\xrightarrow{\text{Ph·CH}_2\text{·NH}_2}\begin{array}{l}\text{CO}_2\text{Me·CH—NH}\quad\text{CO·NH·CH}_2\text{Ph}\\ \qquad\ \ |\qquad\quad |\qquad\ \ |\\ \text{Me}_2\text{C·S·CH—CH·NH·CO·CH}_2\text{Ph}\end{array}$$

(L.)

Penicillenate derivatives are obtainable by cyclising appropriate penicilloate derivatives with, of course, cleavage of the thiazolidine ring,[79] and are conversely capable of affording thiazolidines; for example, methyl benzylpenicillenate furnished β-methyl penicilloate-α-benzylamide (L) with closure of the thiazolidine ring.[80]

These facts at the time of their discovery seemed to support the "oxazolone" formulation, but with later findings including that of *pseudo*-penicillin (above) they are best summed up in the view that the oxazolone-thiazolidine structure has at most a transitory existence (as *pseudo*penicillin) in the origin of the penicillenates and does not represent penicillin itself.

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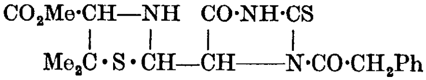
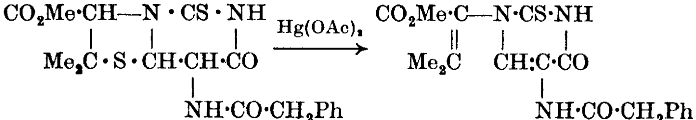
[76] M 6, 8, 46.
[77] See M 12, 15, 23, 30, 40, 46.
[78] See also M 47, 50.
[79] M 33, 37, 47.
[80] A 6, 8; see also U 16; M 46.

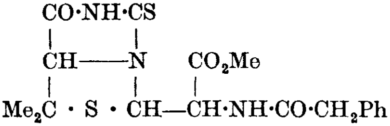
Inactivation by means of thiocyanic acid. Azlactones afford 1-acylthiohydantoins in presence of ammonium thiocyanate and acetic anhydride,[81] and the fact that methyl benzylpenicillin was found to give under these conditions a product of the expected composition [82] seemed to support an oxazolone formulation. Superficial physical evidence also suggested the structure (LI), but the product did not exhibit a basic centre and failed in other ways to behave like a thiazolidine. Without detailing the intricate experimental work, this product was ultimately recognised as the thiodihydrouracil (LII) which, like its oxidation product with mercuric acetate (LIII) and the products of alkali and acid treatment, (LIV) and (LV) respectively, was eventually synthesised. Clearly methyl benzylpenicillin was not behaving as an authentic azlactone, whereas the model β-lactam (LVI) under comparable conditions afforded what was almost certainly the dihydrothiouracil (LVII).[83] At least some β-lactams therefore appear to behave in the manner of methyl benzylpenicillin (less " reactive " methyl dethiobenzylpenicillin—see later—failed to undergo this reaction), and the observations clearly favour the β-lactam formulation for the penicillins.



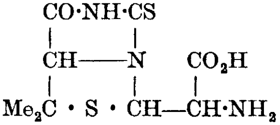
(LI.)



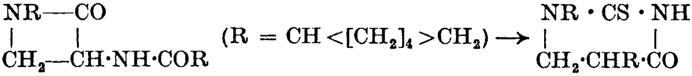
(LII.) (LIII.)



(LIV.)



(LV.)



(LVI.) (LVII.)

Desulphurisation studies. These studies on benzylpenicillin are specially noteworthy as they provided the most direct evidence in support of a β-lactam formulation. They have been restricted to the benzylpenicillin series, but it seems that desulphurisation of thiazolidines and nearly related compounds proceeds in a similar manner. For example, " natural " and

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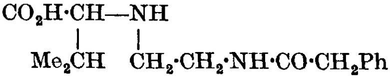
[81] A. E. Carter, " Organic Reactions ", III, 213.

[82] D 20, 22, 24, 25, 31, 34, 35 ; S 32, 34, 36, 38, 40, 42, 44, 47, 48, 50 ; cf. also M 6, 64.

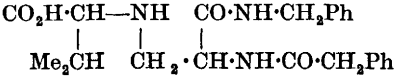
[83] M 63 ; cf. also M 66, 69.

synthetic benzylpenilloic acid afforded *N*-2-phenylacetamidoethylvaline (LVIII),[84] and stereoisomeric natural and synthetic benzylpenicilloic benzylamides were converted into a common dethio-compound (LIX).[85] Similarly, benzylpenillic acid gave dethiobenzylpenillic acid,[85] and benzylpenillamine yielded α-2-benzyl-1-iminazolyl*iso*valeric acid (LX). Occasionally further reduction and fission were experienced as in the production of *cyclo*hexylacetamidoalanine from ethyl benzylpenicilloate :[86]



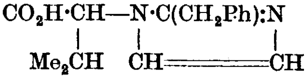
$$\begin{array}{ll}\mathrm{CO_2H{\cdot}CH{-}} & \mathrm{NH}\\ \quad | & |\\ \mathrm{Me_2CH} & \mathrm{CH_2{\cdot}CH_2{\cdot}NH{\cdot}CO{\cdot}CH_2Ph}\end{array}$$

(LVIII.)



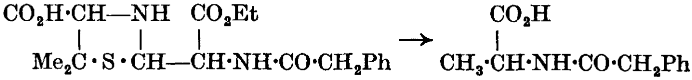
$$\begin{array}{lll}\mathrm{CO_2H{\cdot}CH{-}} & \mathrm{NH} & \mathrm{CO{\cdot}NH{\cdot}CH_2Ph}\\ \quad | & | & |\\ \mathrm{Me_2CH} & \mathrm{CH_2} & \mathrm{{\cdot}\,CH{\cdot}NH{\cdot}CO{\cdot}CH_2Ph}\end{array}$$

(LIX.)



$$\begin{array}{lll}\mathrm{CO_2H{\cdot}CH{-}} & \mathrm{N{\cdot}C(CH_2Ph)} & \mathrm{:N}\\ \quad | & | & |\\ \mathrm{Me_2CH} & \mathrm{CH} = & = \mathrm{CH}\end{array}$$

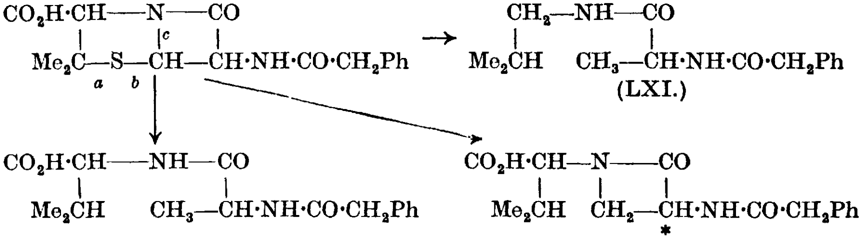
(LX.)



$$\begin{array}{llll}\mathrm{CO_2H{\cdot}CH{-}} & \mathrm{NH} & \mathrm{CO_2Et} & \\ \quad | & | & | & \\ \mathrm{Me_2C{\cdot}S{\cdot}} & \mathrm{CH{-}} & \mathrm{CH{\cdot}NH{\cdot}CO{\cdot}CH_2Ph}\end{array} \longrightarrow \begin{array}{l}\mathrm{CO_2H}\\ |\\ \mathrm{CH_3{\cdot}CH{\cdot}NH{\cdot}CO{\cdot}CH_2Ph}\end{array}$$

but these instances have mostly been disregarded here as of little structural importance.

Hydrogenolysis of sodium benzylpenicillin with Raney nickel for a short time in hot water yielded one neutral and two acidic compounds.[87] The neutral material was identical with *N*-phenylacetyl-L(+)-alanine*iso*butylamide (LXI) supposedly arising by fission of bonds *a*, *b*, and *c*, and decarboxylation ; it was synthesised from *iso*butylamine and *N*-phenylacetyl-L(+)-alanine through the azide of the latter ; the " cysteine " carbon atom (* in LXIII) thus has the " natural " configuration as is confirmed by the first of the acidic hydrogenolysis products. This was identified as *N*-phenylacetyl-L(+)-alanyl-D(−)-valine (LXII), again formed by fission of bonds *a*, *b*, and *c*, but without decarboxylation. It thus illustrates directly the N·CO bond of a β-lactam ring, and this interpretation of its origin is supported by cleavage in this sense of authentic β-lactams.[88] Compound (LXII) was



$$\begin{array}{lll}\mathrm{CO_2H{\cdot}CH{-}} & \mathrm{N{-}} & \mathrm{CO}\\ \quad | & |^{c} & |\\ \mathrm{Me_2C} \underset{a}{-} \mathrm{S} \underset{b}{-} & \mathrm{CH{-}} & \mathrm{CH{\cdot}NH{\cdot}CO{\cdot}CH_2Ph}\end{array} \longrightarrow \begin{array}{lll}\mathrm{CH_2{-}} & \mathrm{NH{-}} & \mathrm{CO}\\ | & & |\\ \mathrm{Me_2CH} & & \mathrm{CH_3{-}CH{\cdot}NH{\cdot}CO{\cdot}CH_2Ph}\end{array}$$

(LXI.)

$$\begin{array}{lll}\mathrm{CO_2H{\cdot}CH{-}} & \mathrm{NH{-}} & \mathrm{CO}\\ \quad | & & |\\ \mathrm{Me_2CH} & & \mathrm{CH_3{-}CH{\cdot}NH{\cdot}CO{\cdot}CH_2Ph}\end{array} \qquad \begin{array}{lll}\mathrm{CO_2H{\cdot}CH{-}} & \mathrm{N{-}} & \mathrm{CO}\\ \quad | & | & |\\ \mathrm{Me_2CH} & \mathrm{CH_2{-}} & \underset{*}{\mathrm{CH}}\mathrm{{\cdot}NH{\cdot}CO{\cdot}CH_2Ph}\end{array}$$

(LXII.) (LXIII.)

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[84] M 15b.

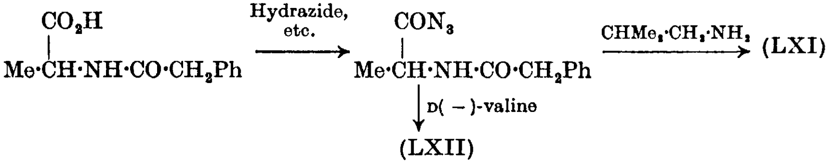
[85] M 49, 59 ; cf. also M 56 where it was obtained from dimethyl benzyl*iso*penillate.

[86] M 22.

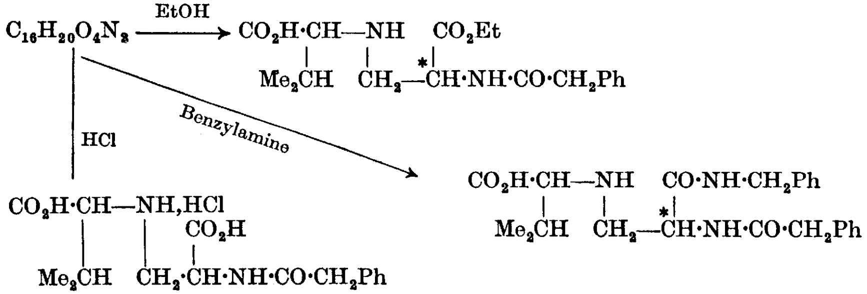
[87] M 46, 49, 62.

[88] P 22, 23.

obtained from the azide of *N*-phenylacetyl-L(+)-alanine and D(−)-valine, and also as a DL-mixture by acylating D(−)-valine with 2-benzyl-4-methyl-oxazolone, the mixture being resolved by means of cinchonidine.[89] The third significant hydrogenolysis product of sodium benzylpenicillin was a comparatively stable acid, $C_{16}H_{20}O_4N_2$. This " dethiobenzylpenicillin " afforded phenylacetic acid on hydrolysis,[90] and further evidence of a phenyl-acetamido-group was provided by infra-red spectra. Milder hydrolysis gave a dibasic acid which was identical with D-α-dethiobenzylpenicilloic acid. Other fissions of benzylpenicillin [91] were also paralleled by dethio-benzylpenicillin despite its greater stability. Thus the dethio-compound gave D-γ-dethiopenicilloic acid with hydrogen chloride, α-ethyl D-α-dethio-benzylpenicilloate with ethanol and the corresponding benzylamide with benzylamine :

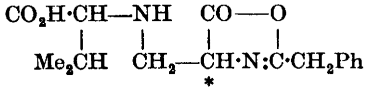


$$\begin{tikzcd} \begin{array}{l}\mathrm{CO_2H}\\ \mid\\ \mathrm{Me{\cdot}CH{\cdot}NH{\cdot}CO{\cdot}CH_2Ph}\end{array} \arrow[r, "\text{Hydrazide, etc.}"] & \begin{array}{l}\mathrm{CON_3}\\ \mid\\ \mathrm{Me{\cdot}CH{\cdot}NH{\cdot}CO{\cdot}CH_2Ph}\end{array} \arrow[r, "\mathrm{CHMe_2{\cdot}CH_2{\cdot}NH_2}"] \arrow[d, "\text{D}(-)\text{-valine}"] & \text{(LXI)} \\ & \text{(LXII)} & \end{tikzcd}$$



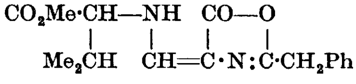
$$\begin{tikzcd} \mathrm{C_{16}H_{20}O_4N_2} \arrow[r, "\mathrm{EtOH}"] \arrow[d, "\mathrm{HCl}"] \arrow[dr, "\text{Benzylamine}"] & \begin{array}{l}\mathrm{CO_2H{\cdot}CH{-}NH\quad CO_2Et}\\ \mathrm{Me_2CH\quad CH_2{-}\overset{*}{C}H{\cdot}NH{\cdot}CO{\cdot}CH_2Ph}\end{array} \\ \begin{array}{l}\mathrm{CO_2H{\cdot}CH{-}NH,HCl}\\ \qquad\qquad\mathrm{CO_2H}\\ \mathrm{Me_2CH\quad CH_2{\cdot}CH{\cdot}NH{\cdot}CO{\cdot}CH_2Ph}\end{array} & \begin{array}{l}\mathrm{CO_2H{\cdot}CH{-}NH\quad CO{\cdot}NH{\cdot}CH_2Ph}\\ \mathrm{Me_2CH\quad CH_2{-}\overset{*}{C}H{\cdot}NH{\cdot}CO{\cdot}CH_2Ph}\end{array} \end{tikzcd}$$

The production of the γ- (" unnatural ") configuration with hydrogen chloride has a structural significance which is outlined later, but the production of compounds with the " cysteine " carbon atom (*) having the same configuration as in benzylpenicillin suggests that dethiopenicillin arises only by removal of sulphur and not by any additional rearrangement. It is indeed significant that none of the known rearrangement products of benzylpenicillin afforded the same hydrogenolysis products as did the parent compound. In particular, there were cogent arguments against structures for dethiobenzylpenicillin such as (LXIV) containing an oxazolone



$$\begin{array}{l}\mathrm{CO_2H{\cdot}CH{-}NH\quad CO{-}O}\\ \mathrm{Me_2CH\quad CH_2{-}\underset{*}{C}H{\cdot}N{:}C{\cdot}CH_2Ph}\end{array}$$

(LXIV.)



$$\begin{array}{l}\mathrm{CO_2Me{\cdot}CH{-}NH\quad CO{-}O}\\ \mathrm{Me_2CH\quad CH{=}C{\cdot}N{:}C{\cdot}CH_2Ph}\end{array}$$

(LXV.)

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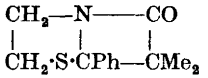
[89] M 50, 53, 62.

[90] M 49.

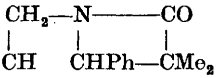
[91] M 15, 22, 49, 51, 52, 56, 59, 65.

system. Thus methyl dethiobenzylpenicillenate[92] (LXV) possessed the usual stability of 4-methyleneoxazolones, whereas (LXIV) would be expected to be highly unstable as an anhydride of an α-acylamido-acid. Incidentally, dethiobenzylpenicillin was converted into a *N*-benzyl compound which appeared to retain the original skeletal structure,[93] and methyl dethiobenzylpenicillin formed a *N*-nitroso-derivative.[94] Finally, it is well known that optically active α-acylamido-acids racemise easily on any treatment converting them into oxazolones,[95] so that on formulation (LXIV) preservation of the configuration of the asterisked carbon atom would be exceptional. On chemical grounds therefore dethiobenzylpenicillin is formulated as (LXIII), a conclusion in agreement with physical evidence. Dethiobenzylpenicillin and its methyl ester showed infra-red absorption bands at 5·78, 5·98, 6·66, and at 5·78 and 6 μ respectively. For comparison benzylpenicillin (sodium salt) and its methyl ester showed bands at 5·59—5·65, 5·89—6·0, 6·2, 6·5—6·67, and at 5·61—5·66, 5·72—5·75, 5·99—6·06, 6·51—6·67 μ respectively, depending on the mode of examination. In these and similar compounds, bands at 6·0 and *ca.* 6·6 μ appear to be associated with the *N*-monosubstituted amide group and that at *ca.* 5·7 μ in methyl dethiobenzylpenicillin to an association of the ester and lactam carbonyl groups. In this case the shift from 5·67 μ in benzylpenicillin to 5·78 μ in the dethio-compound would be a reflection of the influence of the attached thiazolidine ring in the former. Such a shift was indeed observed in a model fused thiazolidine-β-lactam.[96]



$$\begin{array}{llll} CH_2 & —N & & —CO \\ | & | & & | \\ CH_2\cdot S & \cdot CPh & & —CMe_2 \end{array}$$

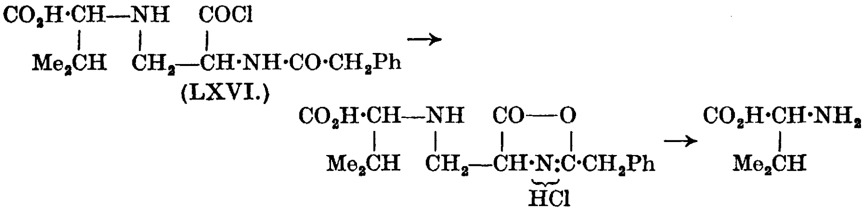
5·67 μ



$$\begin{array}{llll} CH_2 & —N & & —CO \\ | & | & & | \\ CH & CHPh & & —CMe_2 \end{array}$$

5·75 μ

On the basis of (LXIII), a number of reactions of dethiobenzylpenicillin received satisfactory explanation. Thus when treated with hydrogen chloride it gave rise to a mutarotated dethiobenzylpenicilloic acid and



$$\begin{array}{llll} CO_2H\cdot CH & —NH & COCl & \\ | & | & | & \longrightarrow \\ Me_2CH & CH_2 & —CH\cdot NH\cdot CO\cdot CH_2Ph & \end{array}$$

(LXVI.)

$$\begin{array}{lllll} CO_2H\cdot CH & —NH & CO & —O & \\ | & | & | & | & \longrightarrow \\ Me_2CH & CH_2 & —\underbrace{CH\cdot N}_{HCl} & :C\cdot CH_2Ph & \end{array} \quad \begin{array}{l} CO_2H\cdot CH\cdot NH_2 \\ | \\ Me_2CH \end{array}$$

(LXVII.)

ultimately to D(—)-valine.[97] The first product might have been the acid chloride (LXVI) which would have been tantamount to the oxazolone hydrochloride (LXVII) and so undergone mutarotation. Synthetic oxazolones of this type readily lose amine so that elimination of the valine moiety is

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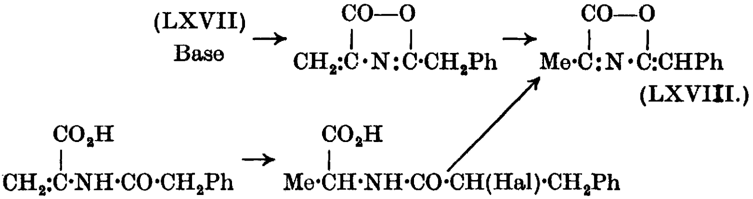
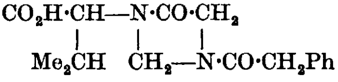
[92] M 46.
[93] M 72, 75, 80.
[94] M 54, 74, 79.
[95] Cf. S 34, 36.
[96] Sh 6, 9.
[97] M 59.

easily understood.[98] Methyl dethiobenzylpenicillin seemed to rearrange to an oxazolone on being heated in anisole, a subsequent thermal fission product rearranging in the known manner of certain 2-alkyl-4-methylene-oxazolones to give a *pseudo*oxazolone (LXVIII)[99] also obtained from α-phenylacetamidoacrylic acid or from α-chloro- or -bromo-phenylacetyl-alanine.



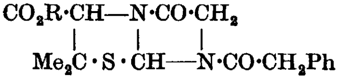
It may be useful to conclude this section of the Report with a brief review of a number of other reactions of the penicillins the study of which has had a less profound effect on structural considerations. These have contributed little to a decision between rival penicillin formulations, but their inclusion is justified by the novelty of the reactions observed. Many other inactivations and reactions of penicillins have been observed but have not been pursued with any structural objective in view.

Thermal inactivation of penicillin. Methyl benzylpenicillin underwent molecular rearrangement at > 110° in hydrocarbon solvents, especially in presence of a trace of iodine.[100] The product, *methyl benzylpenillonate*, was neutral but afforded a monobasic acid, *benzylpenillonic acid*, on mild hydrolysis. More drastic hydrolysis by various means yielded phenylacetic acid and penicillamine, and phenaceturic acid could be obtained by thermal decomposition.[101] Hydrolytic breakdown together with absence of basic properties suggested an acylthiazolidine type of structure, and this was supported by a study of the hydrogenolysis of the ester which gave methyl dethiobenzylpenillonate and thence by hydrolysis valine, phenylacetic acid, glycine, and formaldehyde.[102] Having regard to all these facts, dethiobenzylpenillonic acid was regarded as (LXIX) and benzylpenillonic acid and ester as (LXX). The ester (LXX) has been synthesised by thermal rearrangement of methyl benzylpenicillenate.



$$\begin{array}{l}CO_2H\cdot CH—N\cdot CO\cdot CH_2\\ \quad\ \ |\qquad\ |\qquad\ | \\ Me_2CH\ \ CH_2—N\cdot CO\cdot CH_2Ph\end{array}$$

(LXIX.)



$$\begin{array}{l}CO_2R\cdot CH—N\cdot CO\cdot CH_2\\ \qquad\ |\qquad\ |\qquad\ | \\ Me_2C\cdot S\cdot CH—N\cdot CO\cdot CH_2Ph\end{array}$$

(LXX.)

Inactivation of penicillin by thiols. Benzylpenicillin was inactivated in neutral aqueous solution by cysteine, many nearly related compounds such as cystine, α-amino-acids, simple thiols, or methionine being completely or

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[98] M 57.

[99] M 72 ; W 5, 14 ; E. P. Abraham, W. Baker, E. Chain, F. E. King, R. Robinson, and S. G. Whaley, CPS 272.

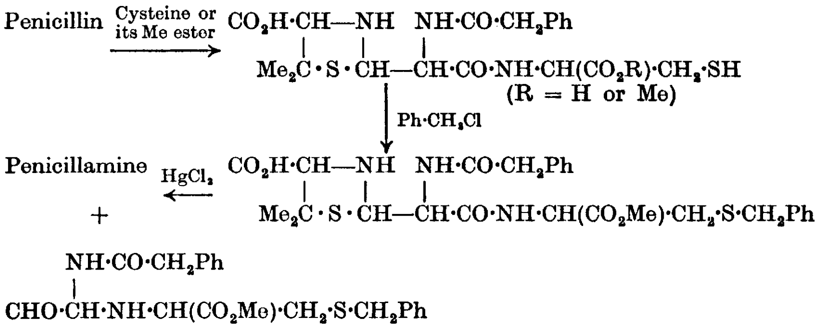
[100] M 32, 36.

[101] M 39, 46.

[102] M 50, 52, 68.

almost completely ineffective.[103] Clearly this may have bearing on the mechanism of penicillin action though its discussion in this aspect is beyond the scope of the present Report.[104] Considerable deviation from the structure of cysteine may still permit inactivation of penicillin though, inasmuch as the cysteine effect is subject to a marked pH influence and as penicillin is also inactivated by many amines, it is not always possible to distinguish the " thiol " from the " amine " effect. However, among those belonging to the cysteine-like group were cysteine esters,[105] *N*-methylcysteine [106] and amino-, dimethylamino-, and diethylamino-ethanethiols,[107] whereas among those belonging to the relatively inactive group are *N*-acylcysteines,[108] *S*-substituted cysteines,[109] etc. Primary requirements for cysteine-like inactivation thus seemed to be a free basic group and a free thiol group on the adjacent carbon atom ; there appeared to be other restrictions as penicillamine itself is inactive.[110]

Inactivation of benzylpenicillin by means of cysteine was paralleled by a fall in optical activity and formation of an acid, apparently by combination of the penicillin and cysteine in equimolecular quantities,[111] which proved to be a benzylpenicilloic acid derivative. Thus the acid contained virtually no amino-group and no characteristic light-absorbing centre, but retained a thiol group which could, in the form of the inactivation product with cysteine methyl ester, be benzylated. Mild acid hydrolysis afforded no carbon dioxide, a fact indicating penicilloamide nature, and there was good evidence of fission in the normal manner by mercuric chloride. In the latter reaction the aldehyde cleavage product remained as a mercury thiocompound when cysteine itself was used, but when cysteine methyl ester was employed and the thiol group in the primary inactivation product



$$\text{Penicillin} \xrightarrow{\text{Cysteine or its Me ester}} \begin{array}{l} \mathrm{CO_2H{\cdot}CH{-}NH\quad NH{\cdot}CO{\cdot}CH_2Ph} \\ \mathrm{Me_2C{\cdot}S{\cdot}CH{-}CH{\cdot}CO{\cdot}NH{\cdot}CH(CO_2R){\cdot}CH_2{\cdot}SH} \\ \qquad\qquad\qquad (\mathrm{R = H\ or\ Me}) \end{array}$$

$$\Big\downarrow \mathrm{Ph{\cdot}CH_2Cl}$$

$$\begin{array}{l} \text{Penicillamine} \\ + \end{array} \xleftarrow{\mathrm{HgCl_2}} \begin{array}{l} \mathrm{CO_2H{\cdot}CH{-}NH\quad NH{\cdot}CO{\cdot}CH_2Ph} \\ \mathrm{Me_2C{\cdot}S{\cdot}CH{-}CH{\cdot}CO{\cdot}NH{\cdot}CH(CO_2Me){\cdot}CH_2{\cdot}S{\cdot}CH_2Ph} \end{array}$$

$$\begin{array}{l} \qquad\mathrm{NH{\cdot}CO{\cdot}CH_2Ph} \\ \mathrm{CHO{\cdot}CH{\cdot}NH{\cdot}CH(CO_2Me){\cdot}CH_2{\cdot}S{\cdot}CH_2Ph} \end{array}$$

(LXXI.)

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[103] N. Atkinson and N. F. Stanley, *Austral. J. Exp. Biol. Med. Science*, 1943, **21,** 249, 255 ; C. J. Cavallito and J. H. Bailey, *Science*, 1944, **100,** 390 ; C. J. Cavallito, J. H. Bailey, T. H. Haskell, J. R. McCormick, and W. F. Warner, *J. Bact.*, 1945, **50,** 61 ; B. F. Chow and C. M. McKee, *Proc. Soc. Exp. Biol. Med.*, 1945, **58,** 175.

[104] See 103, and C. J. Cavallito, *J. Biol. Chem.*, 1946, **164,** 29.

[105] S 30, 32 ; W 9.

[106] M 34 ; S 40.

[107] M 25, 30 ; S 19, 38, 44 ; W 12, 13.

[108] M 30 ; S 34 ; W 9, 10, 13.

[109] W 9, 10 ; S 34, 38.

[110] M 25 ; S 9, 19, 38 ; W 13.

[111] S 5, 12, 14.

masked by benzylation, the methyl ester of *N*-benzylpenaldyl-*S*-benzylcysteine (LXXI) was obtained.[112] The inactivation of benzylpenicillin by 2-aminoethanethiol proceeded similarly, as in all probability did that with secondary amines such as *N*-methylcysteine.[113] The behaviour of tertiary bases has been less exhaustively studied, but their action is probably one of catalytic hydration, for 2-dimethylaminoethanethiol and benzylpenicillin gave benzylpenicilloic acid though there was some evidence of intermediate bonding of the thiol group.[114]

The efficacy of tertiary bases containing a thiol group on the carbon atom adjacent to that bearing the basic grouping and other facts led to a view that all these inactivations were primarily due to the thiol group,[115] as are inactivations of other antibiotics. It has been pointed out that all effective mercapto-amino-compounds are weak bases, stronger bases of $pK_\alpha > 9{\cdot}5$ being less harmful. Part at least of the rôle of the thiol group may therefore be to weaken the basic centre and thus contribute to the amount of undissociated base present, but it may also enter into transient combination.[116]

Inactivation of penicillin by mercuric acetate. It was early observed [117] that benzylpenicillin, or the corresponding α-methylpenicilloic ester or benzylamide, was rapidly oxidised by cold alcoholic mercuric acetate. It seemed from analytical data after removal of mercury by means of hydrogen sulphide that in methanol, for example, a methoxyl group was introduced; the products, to judge from their cleavage with mercuric chloride, were essentially of a penicilloic structure.[118] Reaction of methyl benzylpenicillin in pure methanol gave an apparently homogeneous though still amorphous product of composition corresponding to: methyl benzylpencillin + 2MeOH − H_2; it accordingly contained 3 methoxyl groups. Earlier work on "natural" α-methyl D(α)-benzylpenicilloate showed the presence of two methoxyl groups in the product (*i.e.*, one introduced) so that the appearance of the third methoxyl group in methyl benzylpenicillin corresponded at least superficially to normal fission of the labile ring. Cleavage of the product from methyl benzylpenicillin with mercuric chloride gave an aldehydic fragment, $C_{13}H_{15}O_5N$, containing two methoxyl groups. One of these must have been present as the ester group normally appearing in a penaldic ester, for degradation gave ultimately the bisphenylhydrazone of methyl formylglyoxylate (LXXII). The other methoxyl group must have been present as in (LXXIII) for it was removed during osazone formation and alternative positions did not warrant consideration as the precursor dinitrophenylhydrazone afforded phenylacetic acid on hydrolysis.[119]

Thiazolidines of the penicilloic or penilloic type were similarly oxidised, but other simple thiazolidines proved relatively unreactive.[120] The methanolysis of benzylpenicillin seemed to be a subsequent reaction, as the rate of formation of mercurous acetate was about the same as in the case of the

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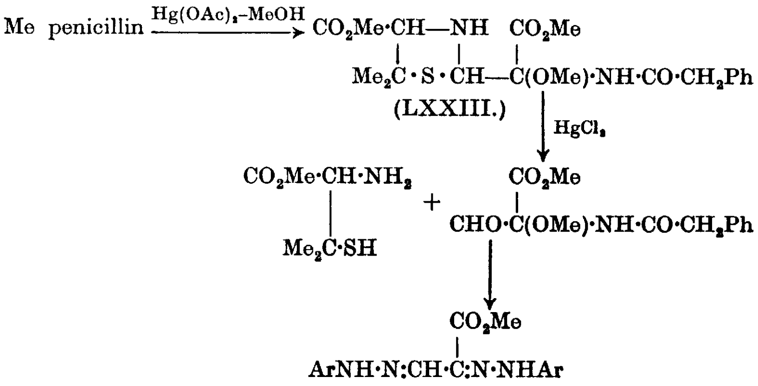
[112] S 14, 19, 28, 32, 34, 36.
[113] S 30, 36, 40.
[114] W 12, 16; S 44, 45.
[115] W 15, 16, 17.
[116] S 38, 44.
[117] S 1.
[118] S 12, 14, 19.
[119] S 38, 40.
[120] S 12, 14, 21.

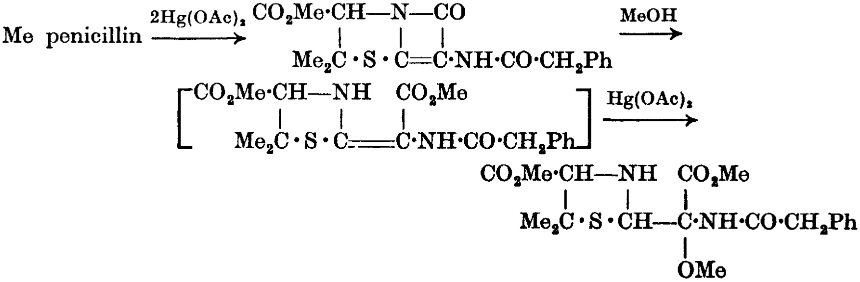


$$\text{Me penicillin} \xrightarrow{\mathrm{Hg(OAc)_2\text{–}MeOH}} \begin{array}{l} \mathrm{CO_2Me\cdot CH\text{—}NH \quad CO_2Me} \\ \mathrm{Me_2C\cdot S\cdot CH\text{—}C(OMe)\cdot NH\cdot CO\cdot CH_2Ph} \end{array} \xrightarrow{\mathrm{HgCl_2}} \begin{array}{l} \mathrm{CO_2Me\cdot CH\cdot NH_2} \\ \mathrm{Me_2C\cdot SH} \end{array} + \begin{array}{l} \mathrm{CO_2Me} \\ \mathrm{CHO\cdot C(OMe)\cdot NH\cdot CO\cdot CH_2Ph} \end{array} \longrightarrow \begin{array}{l} \mathrm{CO_2Me} \\ \mathrm{ArNH\cdot N{:}CH\cdot C{:}N\cdot NHAr} \end{array}$$

(LXXIII.)

(LXXII.)

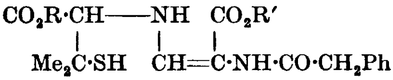
thiazolidines, and there was evidence that mercuric salts as such did not influence the normal alcoholysis of benzylpenicillin. The reaction might thus be represented as follows, the whole process requiring 3 molecular proportions of mercuric acetate in accordance with experiment. The



$$\text{Me penicillin} \xrightarrow{\mathrm{2Hg(OAc)_2}} \begin{array}{l} \mathrm{CO_2Me\cdot CH\text{—}N\text{—}CO} \\ \mathrm{Me_2C\cdot S\cdot C{=}C\cdot NH\cdot CO\cdot CH_2Ph} \end{array} \xrightarrow{\mathrm{MeOH}} \left[\begin{array}{l} \mathrm{CO_2Me\cdot CH\text{—}NH \quad CO_2Me} \\ \mathrm{Me_2C\cdot S\cdot C{=\!=}C\cdot NH\cdot CO\cdot CH_2Ph} \end{array}\right] \xrightarrow{\mathrm{Hg(OAc)_2}} \begin{array}{l} \mathrm{CO_2Me\cdot CH\text{—}NH \quad CO_2Me} \\ \mathrm{Me_2C\cdot S\cdot CH\text{—}C\cdot NH\cdot CO\cdot CH_2Ph} \\ \mathrm{\qquad\qquad\qquad\quad OMe} \end{array}$$

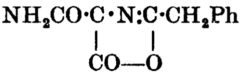
mechanism of reaction of various thiazolidines is possibly similar though the double bond may have a different genesis as in (LXXIV); the positive thiol reactions given by some thiazolidines in solution, and the mutarotation of optically active representatives provide evidence for the reality of such structures.

Miscellaneous inactivations of penicillin. Sodium benzylpenicillin was readily oxidised by ammoniacal silver oxide to a stable, weakly acidic



$$\begin{array}{l} \mathrm{CO_2R\cdot CH\text{——}NH \quad CO_2R'} \\ \mathrm{Me_2C\cdot SH \quad CH{=}C\cdot NH\cdot CO\cdot CH_2Ph} \end{array}$$

(LXXIV.)

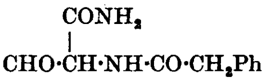


$$\begin{array}{l} \mathrm{NH_2CO\cdot C\cdot N{:}C\cdot CH_2Ph} \\ \mathrm{\qquad\quad CO\text{—}O} \end{array}$$

(LXXV.)

$$\begin{array}{l} \mathrm{NH\text{—}CO} \\ \mathrm{CO\text{—}CH\cdot NH\cdot CO\cdot CH_2Ph} \end{array}$$

(LXXVI.)



$$\begin{array}{l} \mathrm{\qquad CONH_2} \\ \mathrm{CHO\cdot CH\cdot NH\cdot CO\cdot CH_2Ph} \end{array}$$

(LXXVII.)

product ultimately accepted as $C_{10}H_{8\text{ or }10}O_2N_2$.[121] Of structures based

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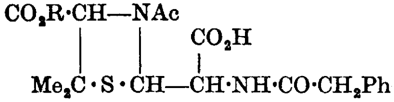
[121] S 3a, 9.

on an earlier formulation, $C_{11}H_{10\ \mathrm{or}\ 12}O_3N_2$, (LXXV) and (LXXVI) were discredited on the grounds of the probable instability of such compounds.[122] Other structures (*e.g.*, LXXVII) based on the revised formulation were synthesised without identity being established, [123] and the nature of the product remains unknown.

Oxidation of methyl benzylpenicillin with sodium metaperiodate resulted in addition of 1 oxygen atom,[124] whereas similar oxidation with potassium permanganate in organic solvents added 2 oxygen atoms,[125] the products being formulated as a sulphoxide and sulphone respectively. These findings had considerable bearing on penicillin formulations, as a thiazolidine carrying an :NH group, as the " oxazolone " formulation represented penicillin to be, should from model investigations have afforded only oxidation products of the thiolamine cleavage product ; on the other hand *N*-acylthiazolidines were known to be oxidisable on the sulphur atom without ring cleavage, and the behaviour of penicillin in this respect was thus more in accord with the β-lactam formulation. The ring-fission reactions of the sulphone were generally similar to those of benzylpenicillin though proceeding more difficultly, and generally the sulphone was more stable than methyl benzylpenicillin. Neither the sulphone nor the sulphoxide had any considerable biological activity.

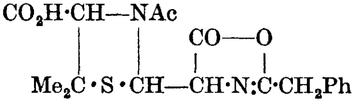
It might have been supposed that inactivation of benzylpenicillin by organic acids would have been similar to its inactivation *via* benzylpenillic acid, but this did not appear to be invariably so. Thus benzylpenicillin in presence of excess of aqueous phenylacetic acid gave a product which, after precipitation with mercuric chloride, gave benzylpenillamine,[126] the penillic acid presumably being an intermediate. However, fusion of sodium or methyl benzylpenicillin with phenylacetic acid gave products which were not precipitated by mercuric chloride,[127] and inactivation with acetic acid presumably proceeded similarly.

Sodium or methyl benzylpenicillin quickly lost their activity in glacial acetic acid to give a mixture the main component of which was not precipitated by mercuric chloride. The main product from penicillin itself at this stage was a dicarboxylic acid of composition corresponding to equimolecular quantities of penicillin and acetic acid ; fractionation of its benzylamine salt showed it to consist of the D(α)- and D(γ)-isomerides of N^4-acetylbenzylpenicilloic acid [128] (LXXVIII ; R = H), the second at least of which was



$$\begin{array}{l} \mathrm{CO_2R{\cdot}CH{-}NAc} \\ \quad\ \ |\qquad |\qquad \mathrm{CO_2H} \\ \quad\ \ |\qquad |\qquad\quad | \\ \mathrm{Me_2C{\cdot}S{\cdot}CH{-}CH{\cdot}NH{\cdot}CO{\cdot}CH_2Ph} \end{array}$$

(LXXVIII.)



$$\begin{array}{l} \mathrm{CO_2H{\cdot}CH{-}NAc} \\ \quad\ \ |\qquad |\qquad \mathrm{CO{-}O} \\ \quad\ \ |\qquad |\qquad\ \ |\qquad | \\ \mathrm{Me_2C{\cdot}S{\cdot}CH{-}CH{\cdot}N{:}C{\cdot}CH_2Ph} \end{array}$$

(LXXIX.)

identified with a synthetic specimen. The corresponding product from methyl benzylpenicillin was probably the ester (LXXVIII ; R = Me).[129]

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[122] M 8, 12b ; S 9 ; L 4.

[123] S 9, 12, 19.

[124] P. Sykes and A. R. Todd, CPS 526.

[125] M 56, 59, 64.

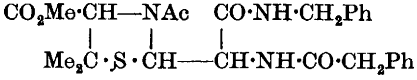
[126] S 32 ; cf. also M 41.

[127] S 30, 32.

[128] S 19, 21, 24, 28, 36.

[129] S 21, 24.

It is possible that attempts to cyclise these penicilloates containing an α-carboxyl group stabilised by the *N*-acetyl group may have given the azlactone (LXXIX); when the product obtained by the use of acetic anhydride was treated with aniline or benzylamine, the anilide or benzylamide of phenylacetic acid was produced, a reaction paralleled by that of some simple oxazolones.[130] Perhaps more convincing was the formation of a benzylamide regarded as (LXXX) from methyl benzylpenicillin in acetic acid–acetic anhydride, though stereochemical considerations render the significance of these changes obscure in relation to penicillin structure.[131]

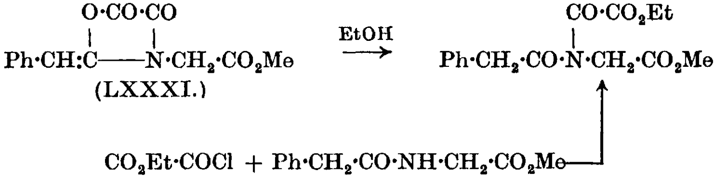


(LXXX.)

$$\begin{array}{l} CO_2Me\cdot CH\text{—}NAc \quad CO\cdot NH\cdot CH_2Ph \\ Me_2C\cdot S\cdot CH\text{——}CH\cdot NH\cdot CO\cdot CH_2Ph \end{array}$$

Sodium benzylpenicillin was rapidly and completely inactivated by neutral aqueous sodium sulphite. The product appeared to be benzylpenicilloic acid, part of which was decarboxylated during subsequent treatment. Even when an equimolecular quantity of sulphite was used, a large part could be recovered unchanged and the effect was essentially a catalysed hydrolysis.[132] A similar inactivation seemed to take place in presence of many neutral organic salts.[133]

Methyl benzylpenicillin was quickly inactivated by oxalyl chloride,[134] and though the products were not well defined it seemed probable that they arose by reaction with the phenylacetamido-group. Thus certain (but not all) phenylacetamido-compounds reacted with oxalyl chloride to give oxazolidiones such as (LXXXI). Their structures were indicated by alcoholysis of the representative (LXXXI) to give a *N*-ethoxalyl compound



(LXXXI.)

$$\begin{array}{l} O\cdot CO\cdot CO \qquad\qquad\qquad\qquad\qquad CO\cdot CO_2Et \\ Ph\cdot CH{:}C\text{——}N\cdot CH_2\cdot CO_2Me \xrightarrow{EtOH} Ph\cdot CH_2\cdot CO\cdot N\cdot CH_2\cdot CO_2Me \\ \qquad\qquad CO_2Et\cdot COCl + Ph\cdot CH_2\cdot CO\cdot NH\cdot CH_2\cdot CO_2Me \uparrow \end{array}$$

which could be obtained directly from methyl phenaceturate and ethoxalyl chloride.[135]

II. *Synthetic Studies in the Penicillin Field*

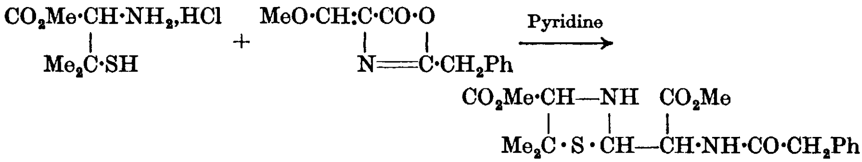
It will already be obvious that the chemistry of attempted syntheses of penicillins is inextricably bound up with the chemistry of thiazolidines. During an earlier phase much fundamental work on oxazolones was carried out, and with emphasis shifting to the β-lactam formulation the behaviour of this ring-system was also investigated almost systematically. It will

[130] S. 30; cf. S 40; M 56.
[131] C 20.
[132] S 40, 42.
[133] A. Campbell, J. O. Harris, H. Harrison, and G. Newbery, CPS 350.
[134] A 17, 18.
[135] A 17, 18, 20; M 60, 61, 65; D 32.

be convenient therefore to review these general advances before considering attempts to synthesise penicillins and their analogues.

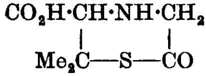
(i) *The Chemistry of Thiazolidines and Thiazolines.*—The penicilloates were referred to above as thiazolidine inactivation products of the various penicillins. They were obtained by the use of alkali or alcohols and later of benzylamine or other amines, the products being penicilloic salts, esters, or amides respectively; these were functional derivatives of the "labile" carboxyl group of penicillin and its near relatives. The simpler penilloic acids lacked the labile grouping or the derived grouping. The study of these important thiazolidines was supported by the examination of a great variety of others with this ring system.

Before work on penicillin, the thiazolidines had been rather neglected. They are now known to be generally and easily available by the condensation of α-amino-β-thiols or their hydrochlorides (*e.g.*, aminoethylthiol, cysteine, penicillamine and its esters) with carbonyl compounds in the usual solvents, sometimes with advantage under anhydrous conditions in presence of hydrogen halides. 4-Alkoxy- or -hydroxy-methyleneoxazolones (see later) function as potential formyl compounds and condense with penicillamine or cysteine to give 2-acylamidomethylthiazolidines [1] :



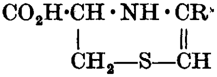
$$\begin{array}{l}CO_2Me\cdot CH\cdot NH_2,HCl\\ \quad |\\ Me_2C\cdot SH\end{array} + \begin{array}{l}MeO\cdot CH{:}C\cdot CO\cdot O\\ \qquad\quad |\quad\ \ |\\ \qquad\ N{=\!=}C\cdot CH_2Ph\end{array} \xrightarrow{\text{Pyridine}} \begin{array}{l}CO_2Me\cdot CH—NH\quad CO_2Me\\ \qquad\quad |\qquad\ |\qquad\ |\\ Me_2C\cdot S\cdot CH—CH\cdot NH\cdot CO\cdot CH_2Ph\end{array}$$

A few carbonyl compounds of exceptional types have failed to yield thiazolidines or have given other ring systems. Examples are glucosamine and phenylacetylglucosamine which failed to yield definite products,[2] dihydroxytartaric acid which afforded probably the thiazane compound (I) [3] and some chloroformyl compounds yielding probably dihydrothiazines (II).[4]



$$\begin{array}{l}CO_2H\cdot CH\cdot NH\cdot CH_2\\ \qquad\ |\qquad\qquad |\\ \ Me_2C—S—CO\end{array}$$

(I.)

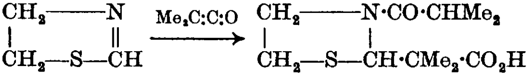


$$\begin{array}{l}CO_2H\cdot CH\cdot NH\cdot CR\\ \qquad |\qquad\qquad \|\\ \ CH_2—S—CH\end{array}$$

(R = CHO or CO_2Et)

(II.)

Thiazolidines have also been prepared from thiazolines by reduction with aluminium amalgam,[5] and *N*-acylthiazolidines by adding ketens to thiazolines,[6] *e.g.*:



$$\begin{array}{l}CH_2——N\\ |\qquad\quad \|\\ CH_2—S—CH\end{array} \xrightarrow{Me_2C{:}C{:}O} \begin{array}{l}CH_2——N\cdot CO\cdot CHMe_2\\ |\qquad\quad |\\ CH_2—S—CH\cdot CMe_2\cdot CO_2H\end{array}$$

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[1] M 15c ; cf. F. C. Copp, W. M. Duffin, S. Smith, and S. Wilkinson, CPS 388 ; B 3.

[2] P 7.

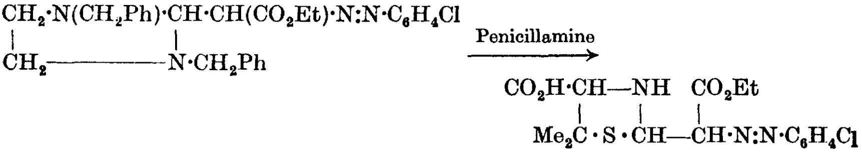
[3] J. Elks, B. A. Hems, F. A. Robinson, and B. E. Ryman, CPS 510.

[4] S 9.

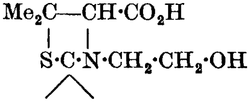
[5] R. Bentley, J. R. Catch, A. H. Cook, I. M. Heilbron, and G. Shaw, CPS 267 ; see also J. A. Barltrop, E. P. Abraham, W. Baker, E. Chain, and R. Robinson, CPS 463 ; B 10, 12.

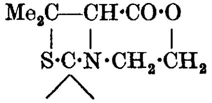
[6] M 66 ; Sh 14.

Finally, certain more complex thiazolidines have been prepared from other ring systems, particularly oxazolidines, iminazolidines, and hexahydropyrimidines, by exchange reactions in presence of mercapto-amines[7]:

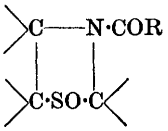


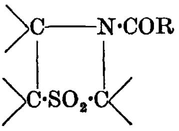
N-Unsubstituted thiazolidines are normal bases which can be acylated and alkylated in the usual ways. For example, reaction of a variety of thiazolidines derived from penicillamine with ethylene oxide led to *N*-hydroxyethylthiazolidines (III) which readily formed lactones (IV).[8] The thiazolidine ring is rather easily cleaved hydrolytically or by other means. Thus reaction of the compounds derived from penicillamine or cysteine with sodium in liquid ammonia led to formation of *N*-alkylpenicillamine or cysteine derivatives,[9] and simple hydrolysis led almost invariably to regeneration of the carbonyl and mercapto-amine components. For this reason thiazolidines give, with varying readiness, thiol reactions with ferric chloride, and sodium azide and iodine,[10] and the carbonyl component of one thiazolidine can often be displaced by another.[11] Similarly, oxidation of simple thiazolidines, for example by sodium periodate or hydrogen peroxide, appeared to consist in ring fission followed by oxidation.[12]


(III.)


(IV.)

Many of the above statements require modification in the case of *N*-acylthiazolidines. These are more stable to simple hydrolysis and to fission by metal reagents such as mercuric or cadmium chloride. On oxidation with sodium periodate or permanganate they are converted into the corresponding sulphoxides (V)[12] or sulphones (VI)[13] respectively. The interest of these observations was enhanced when it was shown that penicillin


(V.)


(VI.)

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[7] J. Baddiley, B. A. Kilby, and A. R. Todd, CPS 389, 544.

[8] R. Bentley, A. H. Cook, and I. M. Heilbron, CPS 327.

[9] U 18, 19, 24.

[10] J. C. Clayton, J. Elks, B. A. Hems, and F. A. Robinson, CPS 201, 223.

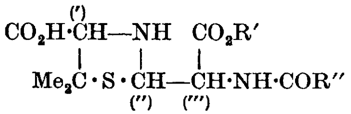
[11] W. Bradley and M. Gayler, CPS 627.

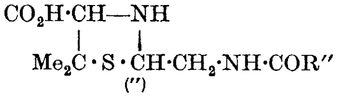
[12] See, for example, P. Sykes and A. R. Todd, CPS 526; CPS 677.

[13] M 56, 59, 64.

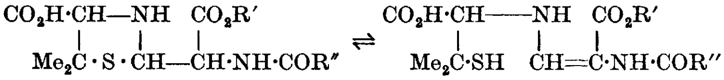
itself was stable to iodine under mild conditions [14] and that benzylpenicillin could be oxidised to a sulphoxide and a sulphone (Section I) ; these reactions may be regarded as the turning point of the chemical evidence away from the thiazolidine–oxazolone and towards the *β*-lactam formulation.

Although certain penilloic and penicilloic derivatives, notably of the *n*-amyl series,[15] had been made immediately the structural outlines of the penicillins had become clear, it was soon obvious that closer attention might have to be paid to the stereochemical configuration of the compounds of natural origin if the best chance of successful synthesis were to be ensured. The penicilloates (VII) have three asymmetric centres, and it seemed that the choice of configuration about these might be a deciding factor in the ultimate biological activity and even the feasibility of the cyclisation reactions contemplated. The absolute configuration of C′ in the compounds


(VII.)


(VIII.)

obtained from penicillin itself is that of the derived penicillamine, *i.e.*, the D- or " unnatural " configuration. Theoretically the remaining asymmetric centres, C″, C‴, give rise to four stereoisomeric penicilloates derived from D-penicillamine alone. In the synthetic series these have been arbitrarily termed *α*-, *β*-, *γ*-, and *δ*-isomerides. In most cases the complete configuration remains undetermined as the compounds are usually made by condensing an enolic formyl (hydroxymethylene) compound or its equivalent with penicillamine so that the asymmetry about both C″ and C‴ is introduced in a single stage ; in certain instances the configuration about C‴ has been determined (see for example Section I, iv). Further, in some cases isomerides with respect to C″, C‴ have given equilibrium mixtures in solution (mutarotation) presumably because of ring-fission : by crystallisation of



the free compounds or their salts with bases such as ephedrine, and by taking advantage of this mutarotation, several compounds have been obtained in the required number of isomeric forms.[16]

It is noteworthy that the same stereoisomerides are not invariably obtained from penicillin. Thus benzylpenicillin and alkali, or the action of alkali on methanol-inactivated benzylpenicillin, yielded one (*α*-) isomeride of the D-benzylpenicilloate, whereas inactivation of the same penicillin by means of copper sulphate afforded another (*δ*-) D-benzylpenicilloic acid.[17]

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[14] See, for example, U 13, 14 ; L. 19.

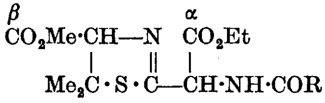
[15] R. Bentley, J. R. Catch, A. H. Cook, J. A. Elvidge, R. H. Hall, and I. M. Heilbron, PEN 114.

[16] See, for example, M 12a and 12c, 15a, 29, 33.

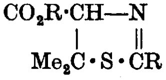
[17] S 21, 24, 29, 30, 32, 36, 38.

The penilloates have only two asymmetric centres (C′, C″). Like the penicilloates, the "natural" penilloates have C′ in the D-configuration, that about C″ again being undecided. These compounds also undergo mutarotation, but the essential identity of the natural and synthetic compounds (in the benzyl series) was left in no doubt by their desulphurisation [18] to the same product.

A wide range of penilloate derivatives is known. Penicilloic acids in some variety have been prepared by saponification of esters, care being necessary as the α- (labile) carboxyl is easily removed under acid conditions. Where both α- and β- (penicillamine) carboxyl groups are esterified, the former is the first to be attacked on saponification, though this regularity is broken in the dehydropenicilloate (IX) (thiazoline) series, the penicillamine ester being first saponified.[19] Numerous penicilloate derivatives in which the α-carboxyl group has been stabilised by esterification or amide formation have been synthesised from penaldate derivatives, themselves prepared by formylating acylglycine esters or by equivalent methods or from hydroxy- or alkoxy-methyleneoxazolones.



(IX.)



(X.)

Thiazolines (X) derived from penicillamine or its near derivatives have been the features of several approaches to penicillins,[20] and have been prepared by several methods. Thus the esters (R = Et, R′ = Me or $CH_2{\cdot}CO_2Et$) were obtained by heating penicillamine ethyl ester with thioacetamide or ethyl malonate respectively.[21] Many others have been prepared by dehydrating acylpenicillamines with ethereal hydrogen chloride,[22] though cyclisation is sometimes almost spontaneous.[23] Perhaps the most useful ring synthesis consists in the interaction of an αβ-mercapto-amine such as a penicillamine ester and an imino-ether.[24] The thiazolines are often weaker bases than the corresponding thiazolidines, are easily cleaved to mercapto-acylamido-compounds and to mercapto-amines, and can be reduced to thiazolidines by aluminium amalgam.

(ii) *The Chemistry of Oxazoles and Oxazolones.*—An attempt to obtain penillic acids by condensing penicillamine with appropriate formyl esters, $R{\cdot}C(OEt){:}N{\cdot}C({:}CH{\cdot}OK){\cdot}(CO_2Et)$, was itself unsuccessful but provided a new synthesis of oxazoles and iminazoles by the following steps: [25]

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[18] M 15b.

[19] A. H. Cook, J. A. Elvidge, and I. M. Heilbron, CPS 680.

[20] *E.g.*, A. H. Cook and I. M. Heilbron, CPS 44.

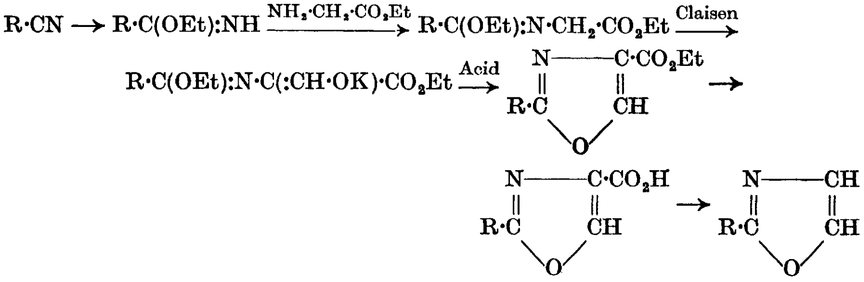
[21] R. Bentley, J. R. Catch, A. H. Cook, I. M. Heilbron, and G. Shaw, CPS 267; A. H. Cook, J. A. Elvidge, I. M. Heilbron, and A. L. Levy, CPS 443.

[22] M 35, 15c.

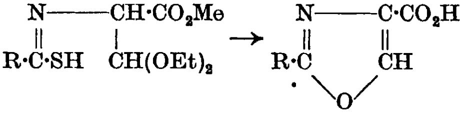
[23] M 25.

[24] J. A. Barltrop, E. P. Abraham, W. Baker, E. Chain, and R. Robinson, CPS 463; see also H. J. Barber, P. Z. Gregory, K. N. Langford, R. Slack, C. E. Stickings, and A. M. Woolman, CPS 385.

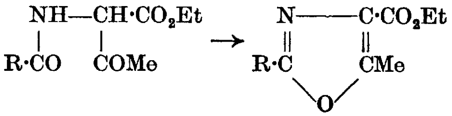
[25] J. W. and R. H. Cornforth, CPS 57, 423; *J.*, 1947, 96.



Similar reactions were used to prepare 2-phenyloxazole [26] and 2-benzyliminazole derivatives by reaction of the formyl esters with bases [25, 27] Comparable also was the removal of ethylthiol and ethanol from the thioacylamido-acetal (XI) by means of silver benzenesulphonate,[28] and the cyclisation of ethyl α-benzamidoacetoacetate (XII).[29] In this connection it may be noted that dehydration of α-acylamido-esters or equivalent processes such as removal of hydrogen sulphide from thioacylamido-esters [28] affords 5-alkoxyoxazoles, these reactions and that typified by the cyclisation of (XII) being the analogues of the Piloty and Knorr reactions in the pyrrole series. The dehydration processes were all based on the type reactions described earlier [30] employing reagents such as phosphorus pentachloride or thionyl chloride.[31]

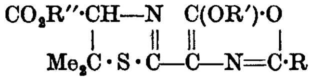


(XI.)

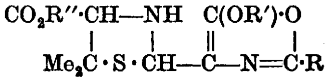


(XII.)

Much of the interest of these reactions resided in their potential application to derivatives of the thiazoline and thiazolidine series, *e.g.*, (XIII) and (XIV), it having been intended to subject the former products to reduction



(XIII.)



(XIV.)

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[26] H. J. Barber, P. Z. Gregory, R. Slack, C. E. Stickings, and A. M. Woolman, CPS 66.

[27] M 40.

[28] C. I. Brodrick, D. A. Peak, F. F. Whitmont, and W. Wilson, CPS 592.

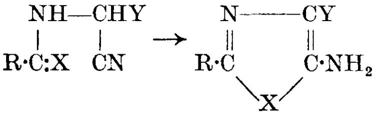
[29] D. F. Elliott, B. A. Hems, and F. A. Robinson, CPS 593.

[30] P. Karrer and Ch. Granacher, *Helv. Chim. Acta*, 1924, **7**, 763.

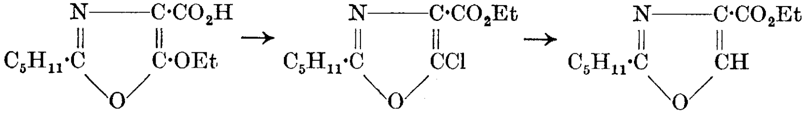
[31] Cf. H. C. Carrington, J. S. H. Davies, W. G. M. Jones, G. R. Ramage, and W. A. Sexton, CPS 274 ; I.C.I. Ltd., CPS 479, 634.

at a suitable stage. This approach was especially attractive when it was shown that 5-benzyloxyoxazoles could be reductively debenzylated in presence of palladium to give oxazolones [32] thus supporting the feasibility of converting (XIV; $R' = CH_2Ph$) into "oxazolone" penicillin; this theme is mentioned again later. In connection with the transformation of 5-alkoxyoxazoles into oxazolones, it is interesting to note that several oxazolones have been alkylated by means of diazohydrocarbons.[33]

Just as α-acylamido-esters afford 5-alkoxyazoles, so α-acylamido-nitriles afford 5-amino-oxazoles, and the corresponding thio-analogues afford 5-aminothiazoles; [34] sometimes in the *O*-series the cyclisation took place on melting, and sometimes an equilibrium mixture was encountered.



Mostly these new oxazole derivatives call for no special comment, but occasionally unexpected rearrangements have been observed. Thus in attempts to translate this experience to the preparation of appropriate thiazolidines it became desirable to prepare suitable 5-alkoxy-4-formyl-oxazoles, and in this connection the oxazole (XV) was converted supposedly into the acid chloride. The product, however, was hydrogenated to ethyl 2-*n*-amyl-4-oxazolecarboxylate and was evidently the chloro-oxazole (XVI).[35] Several similar changes have been observed, and certain 5-amino-oxazoles and 5-hydroxy-oxazoles (oxazolones) undergo such reactions.



(XV.) (XVI.)

Before attempts to synthesise "oxazolone" penicillins only a few oxazolones were known, having been mostly prepared by dehydrating α-acylamido-acids by means of acetic anhydride. 2-Phenyl-5-oxazolone had been obtained [36] by removing hydrogen chloride from "hippuryl chloride" with diazomethane, though α-acylamido-acid halides are better regarded as oxazolone hydrohalides (Section I, iv), and the preparation of the cyclic compounds consists merely in liberating the bases from their salts.[37] Other

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[32] H. C. Carrington, J. S. H. Davies, W. G. M. Jones, G. R. Ramage, and W. A. Sexton, CPS 274; I.C.I. Ltd., CPS 422, 793.

[33] M 23; I.C.I. Ltd., CPS 346, 693.

[34] R. Bentley, J. R. Catch, A. H. Cook, I. M. Heilbron, and G. Shaw, CPS 267; I.C.I. Ltd., CPS 346, 479, 634, 693; J. W. and R. H. Cornforth, CPS 689.

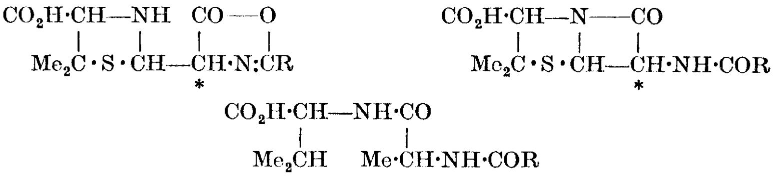
[35] *Idem,* CPS 492; cf. L. J. Goldsworthy and R. Robinson, CPS 650; I.C.I. Ltd., CPS 693.

[36] P. Karrer and R. Widmer, *Helv. Chim. Acta,* 1925, **8,** 203; P. Karrer and G. Bussmann, *ibid.,* 1941, **24,** 645.

[37] H. J. Barber, P. Z. Gregory, R. Slack, C. E. Stickings, and A. M. Woolman, CPS 234.

reagents such as sodium acetate or pyridine[38] can replace diazomethane; 2 : 4-diphenyl- and 2-phenyl-4-benzyl-oxazolone have been prepared in this way though the method is not invariably successful, 2-benzyloxazolone, for example, being apparently unobtainable by such methods. The use of acetic anhydride to dehydrate α-acylamido-acids seems more generally successful.[39]

The dehydration of optically active acylamido-acids to active oxazolones was a consideration of some importance if "oxazolone" (or even β-lactam) penicillins with possible stereochemical limitations with respect to the (*) carbon atom were to result from attempts to cyclise penicilloates. The configuration of this carbon atom was known, for example, by desulphurisation experiments leading to (XVII) to be the L- or "natural" one, whereas treatment of α-acylamido-acids with dehydrating agents led usually to racemisation. Under the usual conditions of oxazolone formation (*e.g.*, in boiling acetic anhydride) this generalisation remains true, and it is often so under milder conditions. However, oxazolone formation, in certain instances at least, proceeds at room temperature, and so a number of acyl-L-leucines afforded optically active (though probably optically impure) 4-*iso*butyloxazolones. The solvent had a marked effect on promoting or retarding racemisation. Often on attempted confirmation of the activity by ring-fission, racemisation continued at a more rapid rate than ring-opening, the product being optically inactive. On the other hand, reaction with thiocyanic acid was rapid and with active 2-phenyl-4-*iso*butyloxazolone the optically active 4-*iso*butyl-2-thiohydantion resulted.[40]



$$\begin{matrix}\mathrm{CO_2H{\cdot}CH{-}NH\quad CO{-}O}\\ |\qquad\;\; |\qquad |\qquad\; |\\ \mathrm{Me_2C{\cdot}S{\cdot}CH{-}\underset{*}{C}H{\cdot}N{:}CR}\end{matrix}\qquad\qquad \begin{matrix}\mathrm{CO_2H{\cdot}CH{-}N{-}CO}\\ |\qquad\;\; |\quad\;\; |\\ \mathrm{Me_2C{\cdot}S{\cdot}CH{-}\underset{*}{C}H{\cdot}NH{\cdot}COR}\end{matrix}$$

$$\begin{matrix}\mathrm{CO_2H{\cdot}CH{-}NH{\cdot}CO}\\ |\qquad\qquad |\\ \mathrm{Me_2CH\quad Me{\cdot}CH{\cdot}NH{\cdot}COR}\end{matrix}$$

(XVII.)

Most of the oxazolones known in the earlier orthodox literature contained a 4-alkylidene or -arylidene group and were available by the Erlenmeyer reaction between carbonyl compounds and acylglycines. As this reaction is usually applied employing hippuric acid, it seems that 2-phenyloxazolone is first formed and that this condenses readily either in the original reaction medium or under other conditions with the carbonyl reactant.[41] Using acyl residues other than the benzoyl group the products isolated were sometimes the acylamido-acids (XVIII) though these could be converted into

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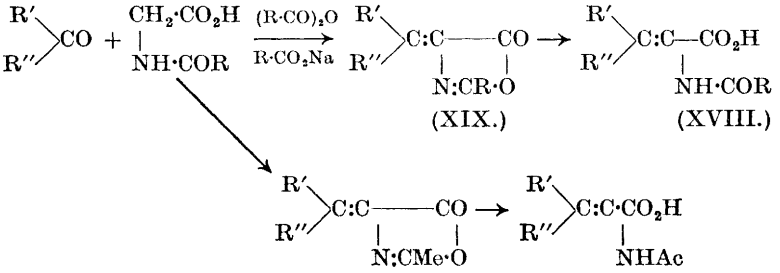
[38] *E.g.*, M 37.

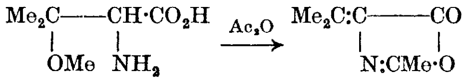
[39] See, for examples, Sh 1, 2, 5; M 10, 12, 23, 39; S 21, 38, 40.

[40] M 40; S 32, 33, 34, 36, 38, 40.

[41] B.H.D. Ltd., CPS 56; J. L. Bailey, W. Bradley, M. Davis, A. A. Evans, and J. A. R. Maclean, CPS 458; M 32.

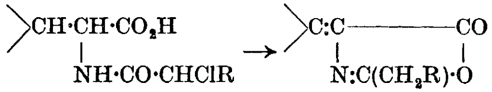

(XIX.) (XVIII.)

the oxazolones (XIX) which were probably intermediates.[42] In such cases ($R = C_5H_{11}$ or CH_2Ph) use of acetic anhydride led to extensive transacylation which, however, could be avoided by using the appropriate acid (*e.g.*, hexoic) anhydride–salt mixture.[43] Formally similar to the method of obtaining 4-alkylidine- or -arylidine-oxazolones is that of dehydrating α-acylamido-β-hydroxy-acids or their derivatives, *e.g.*[44]:

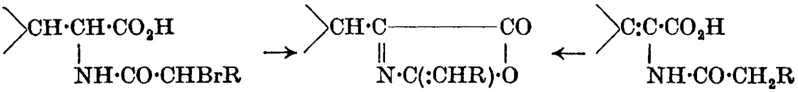


$$\underset{\text{OMe}}{\text{Me}_2\text{C}}\text{---}\underset{\text{NH}_2}{\text{CH}}\cdot\text{CO}_2\text{H} \xrightarrow{\text{Ac}_2\text{O}} \text{Me}_2\text{C:}\underset{\text{N:CMe}\cdot\text{O}}{\text{C---CO}}$$

Finally, compounds of this type have also been prepared by treating α-haloacylamido-acids with acetic anhydride–base mixtures:[45]



This method has sometimes been found to lead to *pseudo*-oxazolones[46] which were also found to be obtainable from appropriate acylamidoacrylic acids or their equivalent.[47] Thus 2-benzylidene-4-methyl-*pseudo*-oxazolone arose from the pyrolysis of methyl dethiobenzylpenicillin.



Certain 4-alkylidene-(arylidene-)oxazolones have been obtained by removing the elements of hydrazoic acid from the corresponding acid azide, a method which is clearly similar to that *via* " acylamido-acid halides "[48]

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[42] P 17; D. F. Elliott, B. A. Hems, and F. A. Robinson, CPS 490.

[43] *Idem*, CPS 52.

[44] H. E. Carter, P. Handler, and D. B. Melville, *J. Biol. Chem.*, 1939, **129**, 364; C. I. Brodrick, D. A. Peak, and W. Wilson, CPS 417; M 10, 26.

[45] M. Bergmann and F. Stern, *Annalen*, 1926, **448**, 20; D. G. Doherty, J. E. Tietzmann, and M. Bergmann, *J. Biol. Chem.*, 1943, **147**, 617; see also D 1, 8; M 26; A 13, and " dehydrobenzylpenicillin " (later).

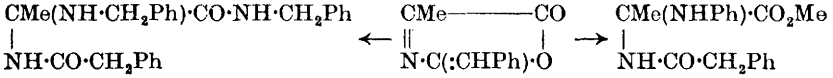
[46] S. G. Waley and F. E. King, CPS 68, 272, 491; F. C. Copp, W. M. Duffin, S. Smith, and S. Wilkinson CPS 547; W 5, 14.

[47] M 72.

[48] J. P. Wilson, E. P. Abraham, W. Baker, E. Chain, and R. Robinson, CPS 91, 445.

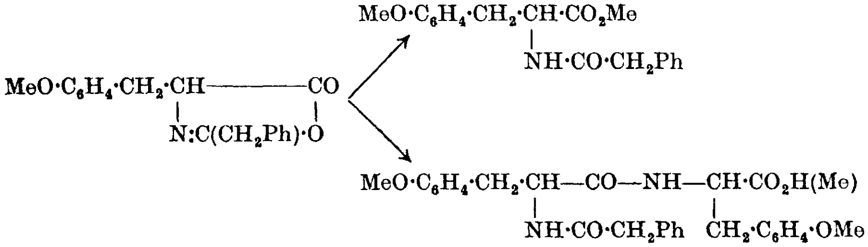
and which has been extensively explored as a possible means of cyclising penicilloate derivatives though largely without success.

4-Alkylidene- or -arylidene-oxazolones are stable, crystalline compounds. Some arylideneoxazolones (as well as *pseudo*-oxazolones) have however been hydrogenated, 2-phenyl-4-benzylideneoxazolone, for example, having been converted into 2-phenyl-4-benzyloxazolone.[49] It may be mentioned here that *pseudo*-oxazolones are perhaps best regarded as only tautomeric forms of the oxazolones and for this reason they received some attention as potential intermediates for penicillin synthesis.[50] Nevertheless *pseudo*-oxazolones contain a more stable ring system than oxazolones and only react with bases, for example, with some difficulty, two molecules of adduct being taken up,[51] *e.g.* :



$$\begin{array}{l}\mathrm{CMe(NH{\cdot}CH_2Ph){\cdot}CO{\cdot}NH{\cdot}CH_2Ph}\\ |\\ \mathrm{NH{\cdot}CO{\cdot}CH_2Ph}\end{array} \longleftarrow \begin{array}{l}\mathrm{CMe}\text{———}\mathrm{CO}\\ \| \qquad\qquad |\\ \mathrm{N{\cdot}C(:CHPh){\cdot}O}\end{array} \longrightarrow \begin{array}{l}\mathrm{CMe(NHPh){\cdot}CO_2Me}\\ |\\ \mathrm{NH{\cdot}CO{\cdot}CH_2Ph}\end{array}$$

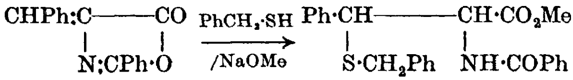
Oxazolones lacking a 4-alkylidene or -arylidene group undergo ring fissions extremely easily in presence of water, acid or alkalis, alcohols, primary or secondary amines, etc., to give α-acylamido-acids or their salts, esters, or amides respectively ; such reactions permit the characterisation of oxazolones. The reaction of some oxazolones with alcohols has led to dipeptides and their esters,[52] *e.g.* :



$$\begin{array}{lcl} & & \begin{array}{l}\mathrm{MeO{\cdot}C_6H_4{\cdot}CH_2{\cdot}CH{\cdot}CO_2Me}\\ \qquad\qquad\qquad\quad |\\ \qquad\qquad\qquad\quad \mathrm{NH{\cdot}CO{\cdot}CH_2Ph}\end{array}\\ & \nearrow & \\ \begin{array}{l}\mathrm{MeO{\cdot}C_6H_4{\cdot}CH_2{\cdot}CH}\text{———}\mathrm{CO}\\ \qquad\qquad\qquad\quad | \qquad\quad |\\ \qquad\qquad\qquad\quad \mathrm{N{:}C(CH_2Ph){\cdot}O}\end{array} & & \\ & \searrow & \\ & & \begin{array}{l}\mathrm{MeO{\cdot}C_6H_4{\cdot}CH_2{\cdot}CH{-}CO{-}NH{-}CH{\cdot}CO_2H(Me)}\\ \qquad\qquad\qquad\quad | \qquad\qquad\qquad\quad |\\ \qquad\qquad\qquad\quad \mathrm{NH{\cdot}CO{\cdot}CH_2Ph} \quad \mathrm{CH_2{\cdot}C_6H_4{\cdot}OMe}\end{array}\end{array}$$

though the mechanism of these reactions is obscure.

The addition of hydrogen sulphide or thiols, particularly to 4-*iso*propylideneoxazolones with simultaneous ring fission, has been briefly reviewed above in connection with penicillamine, though such reactions have also been effected with rather different oxazolones : [53]



$$\begin{array}{l}\mathrm{CHPh{:}C}\text{——}\mathrm{CO}\\ \qquad\quad | \qquad |\\ \qquad\quad \mathrm{N{:}CPh{\cdot}O}\end{array} \xrightarrow[\mathrm{/NaOMe}]{\mathrm{PhCH_2{\cdot}SH}} \begin{array}{l}\mathrm{Ph{\cdot}CH}\text{———}\mathrm{CH{\cdot}CO_2Me}\\ \quad | \qquad\qquad\quad |\\ \mathrm{S{\cdot}CH_2Ph} \quad \mathrm{NH{\cdot}COPh}\end{array}$$

2-Phenyl-4-*iso*propylideneoxazolone added directly 1 mol. of benzylthiol or thioacetic acid [54] to give products which readily reverted to the original

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[49] P 17, 18.

[50] *E.g.*, W 6, 7.

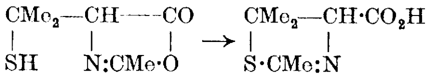
[51] M 76, 78.

[52] Sh 3 ; S 38, 40.

[53] A. H. Cook, A. R. Graham, G. Harris, and I. M. Heilbron, CPS 462.

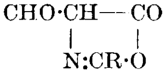
[54] A. H. Cook, J. A. Elvidge, R. H. Hall, I. M. Heilbron, and G. Shaw, CPS 270.

oxazolone. In similar additions hydrogen sulphide gave thiazolines evidently by a two-stage reaction : [55]



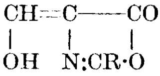
$$\begin{array}{lll} CMe_2\text{—}CH\text{——}CO & & CMe_2\text{—}CH\cdot CO_2H \\ \;| \qquad\quad | \qquad\quad | & \rightarrow & \;| \qquad\qquad\; | \\ SH \quad N{:}CMe\cdot O & & S\cdot CMe{:}N \end{array}$$

Initially, interest in the oxazolones was concerned almost entirely with their possible conversion into " penicillins " *via* 4-formyloxazolones (XX).



$$\begin{array}{l} CHO\cdot CH\text{—}CO \\ \qquad\;\; | \qquad\;\; | \\ \qquad N{:}CR\cdot O \end{array}$$

(XX.)



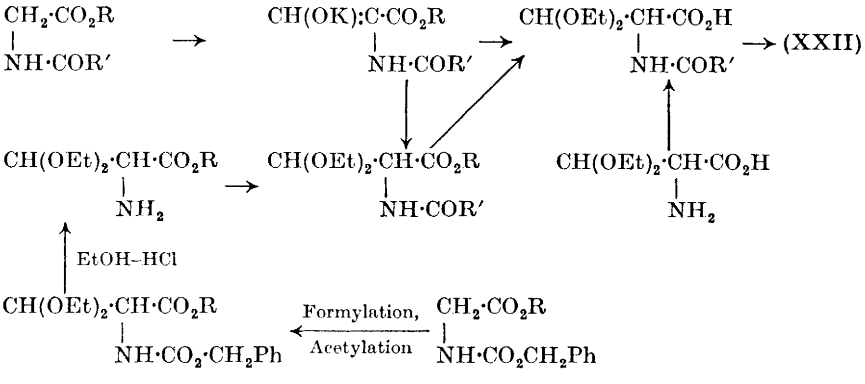
$$\begin{array}{l} CH{=}C\text{——}CO \\ | \qquad | \qquad\;\; | \\ OH \;\; N{:}CR\cdot O \end{array}$$

(XXI.)

$$\begin{array}{l} CH{=}C\text{——}CO \\ | \qquad\; | \qquad\;\; | \\ OEt \;\; N{:}CR\cdot O \end{array}$$

(XXII.)

Many attempts to make compounds of this type invariably failed to yield products with carbonyl reactivity, and " formyloxazolones " are in fact best regarded as 4-hydroxymethyleneoxazolones (XXI). One of the early routes to these compounds consisted in condensing an alkyl orthoformate with an acylglycine (or the corresponding oxazolone), and hydrolysing the resulting 4-alkoxy-(usually ethoxy-)methyleneoxazolone (XXII).[56] A second method of wider applicability involved cyclisation of an acyl-$\beta\beta$-dialkoxyalanine : [57]



It is convenient to use phosphorus tribromide in the final reaction, the first product being probably the oxazolone hydrobromide rather than a α-acylamido-β-ethoxyacrylyl bromide (XXIII) as it was first formulated.[58] The question of the separate existence of compounds of type (XXIII) has not been finally answered, however, and they may be in equilibrium with the oxazolone hydrohalides. Under mild conditions cyclisation of $\beta\beta$-dialkoxyacylalanines (penaldic acetals) apparently afforded 4-dialkoxymethyl-

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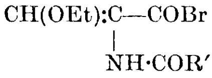
[55] M 12d, 26 ; I.C.I. Ltd., CPS 525, 634.

[56] For examples see J. P. Wilson, J. B. Jepson, and G. M. Robinson, CPS **26** ; H. J. Barber and R. Slack, CPS 40 ; A 3 ; D 4 ; M 10, 20.

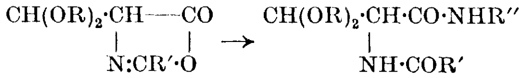
[57] For early examples see M 10 ; B 1.

[58] M 12b, 32 ; U 13 ; H. J. Barber, P. Z. Gregory, R. Slack, C. E. Stickings, and A. M. Woolman, CPS 234.



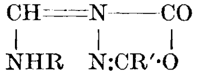
$$\begin{array}{l} \mathrm{CH(OEt){:}C{-}COBr} \\ \qquad\quad | \\ \qquad\mathrm{NH{\cdot}COR'} \end{array}$$

(XXIII.)



$$\begin{array}{l} \mathrm{CH(OR)_2{\cdot}CH{-}CO} \\ \qquad\qquad\; | \qquad | \\ \qquad\quad\mathrm{N{:}CR'{\cdot}O} \end{array} \longrightarrow \begin{array}{l} \mathrm{CH(OR)_2{\cdot}CH{\cdot}CO{\cdot}NHR''} \\ \qquad\qquad\; | \\ \qquad\quad\mathrm{NH{\cdot}COR'} \end{array}$$

(XXIV.) (XXV.)

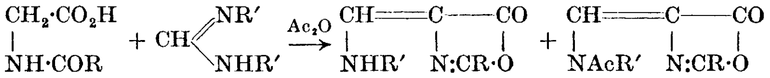


$$\begin{array}{lll} \mathrm{CH} & \!\!=\!\!=\mathrm{N} & \!\!-\!\!-\mathrm{CO} \\ | & \;\;| & \quad\;\; | \\ \mathrm{NHR} & \mathrm{N{:}CR'} & \!\!\!\!{\cdot}\mathrm{O} \end{array}$$

(XXVI.)

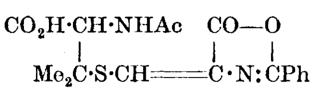
oxazolones (XXIV) in solution at least for a short time, for the products yielded penaldic acetal amides (XXV) on treatment with amines [59]; nevertheless, no appreciable antibiotic activity resulted in condensing these " acetals " with penicillamine with the object of elaborating the " thiazolidine–oxazolone " formulation.

Hydroxyalkylideneoxazolones, so far from behaving as formyl compounds, are relatively strong acids, the corresponding alkoxy- and aminomethylene compounds (*e.g.*, XXVI) behaving as esters and amides respectively. The latter are often obtainable by mild reaction of ethoxy- or hydroxy-methyleneoxazolones with amines. It will be recalled that the penicillenates are of type (XXVI) and that they, like others of the group, are hydrolysed by alkali to the hydroxymethyleneoxazolones. Certain aminomethylene derivatives of oxazolones have been obtained by condensing various *N*-substituted formamidines with appropriate oxazolones or their equivalent, diphenyl-, dibenzyl-, and diethyl-formamidine having been used in this way : [60]



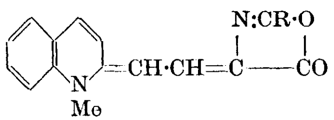
$$\begin{array}{l} \mathrm{CH_2{\cdot}CO_2H} \\ \;| \\ \mathrm{NH{\cdot}COR} \end{array} + \mathrm{CH}\begin{array}{l} \nearrow\mathrm{NR'} \\ \searrow\mathrm{NHR'} \end{array} \xrightarrow{Ac_2O} \begin{array}{lll} \mathrm{CH} & \!\!=\!\!=\mathrm{C} & \!\!-\!\!-\mathrm{CO} \\ | & \;\;| & \quad\;\; | \\ \mathrm{NHR'} & \mathrm{N{:}CR} & \!\!\!\!{\cdot}\mathrm{O} \end{array} + \begin{array}{lll} \mathrm{CH} & \!\!=\!\!=\mathrm{C} & \!\!-\!\!-\mathrm{CO} \\ | & \;\;| & \quad\;\; | \\ \mathrm{NAcR'} & \mathrm{N{:}CR} & \!\!\!\!{\cdot}\mathrm{O} \end{array}$$

The ethoxyl group in ethoxymethyleneoxazolones is replaceable by –SH or –SR groups by reaction with hydrogen sulphide or thiols.[61] The reaction was closely studied because of the possibility of bringing appropriate oxazolones into reaction with penicillamine derivatives so that the first linking was made through the sulphur atom as in (XXVII),[62] perhaps facilitating subsequent formation of " oxazolone " penicillin.



$$\begin{array}{ll} \mathrm{CO_2H{\cdot}CH{\cdot}NHAc} & \mathrm{CO{-}O} \\ \qquad\quad | & \;\;|\quad\; | \\ \;\;\mathrm{Me_2C{\cdot}S{\cdot}CH} & \!\!\!=\!\!=\mathrm{C{\cdot}N{:}CPh} \end{array}$$

(XXVII.)



(XXVIII.)

Finally, 4-alkoxymethyleneoxazolones react readily with compounds

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[59] M 23, 33, 37 ; S 15.

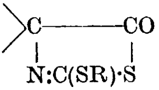
[60] J. S. H. Davies and W. G. M. Jones, CPS 41 ; J. A. Barltrop, and G. M. and R. Robinson, CPS 48 ; W. G. M. Jones, CPS 89 ; I.C.I. Ltd., CPS 479.

[61] For simple examples see H. J. Barber, P. Z. Gregory, R. Slack, C. E. Stickings, and A. M. Woolman, CPS 234 ; F. C. Copp, W. M. Duffin, S. Smith, and S. Wilkinson, CPS 341.

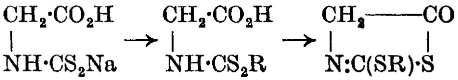
[62] *Idem*, CPS 388.

containing " reactive " methyl or methylene groups, a series of dyes typified by (XXVIII) being obtained by using components such as quinaldine quaternary salts.[63]

Similar to the last compounds are deeply coloured condensation products containing the analogous ring system (XXIX) derived from 2-alkylthiothiazolones. The latter, which were investigated because of a probably superior stability over that of the oxazolones, were easily prepared from glycine and showed many of the properties of the analogous oxazolones including formation of 4-ethoxymethylene, -hydroxymethylene, and -anilinomethylene derivatives, and a penicillenate analogue by reaction of the ethoxymethylene compound with penicillamine.[64]



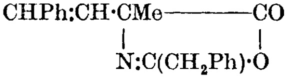
(XXIX.)



$$\begin{array}{l}\text{CH}_2\text{·CO}_2\text{H}\\ |\\ \text{NH·CS}_2\text{Na}\end{array} \rightarrow \begin{array}{l}\text{CH}_2\text{·CO}_2\text{H}\\ |\\ \text{NH·CS}_2\text{R}\end{array} \rightarrow \begin{array}{l}\text{CH}_2\text{——CO}\\ |\qquad\quad |\\ \text{N:C(SR)·S}\end{array}$$

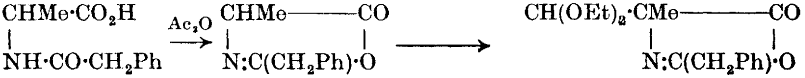
(XXX.)

In view of the above behaviour of oxazolone derivatives, and repeated failures to obtain true 4-formyloxazolones, attempts were made to synthesise compounds of the latter kind carrying a second substituent in the 4-position to prevent enolisation of the formyl group. Attempts to cleave 2-benzyl-4-styryl-4-methyloxazolone (XXXI) by ozonolysis in the desired manner were indecisive,[65] but the acetal of the required oxazolone (XXXII) could be prepared as indicated.[66] Though this and similar oxazolones were normal as oxazolones, they were remarkably inert as acetals ; despite some reaction with penicillamine it was doubtful whether the products consisted to any appreciable extent of " thiazolidine–oxazolones ", and certainly they had at most only slight antibacterial activity.[67]



$$\begin{array}{r}\text{CHPh:CH·CMe———CO}\\ |\qquad\qquad |\\ \text{N:C(CH}_2\text{Ph)·O}\end{array}$$

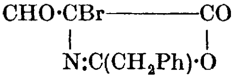
(XXXI.)



$$\begin{array}{l}\text{CHMe·CO}_2\text{H}\\ |\\ \text{NH·CO·CH}_2\text{Ph}\end{array} \xrightarrow{\text{Ac}_2\text{O}} \begin{array}{l}\text{CHMe———CO}\\ |\qquad\qquad |\\ \text{N:C(CH}_2\text{Ph)·O}\end{array} \longrightarrow \begin{array}{r}\text{CH(OEt)}_2\text{·CMe———CO}\\ |\qquad\qquad |\\ \text{N:C(CH}_2\text{Ph)·O}\end{array}$$

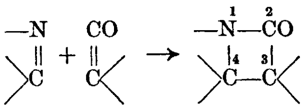
(XXXII.)

Similar in object were several attempts to obtain halogenated formyloxazolones (*e.g.*, XXXIII) by halogenation of hydroxymethylene- and other



$$\begin{array}{l}\text{CHO·CBr———CO}\\ \quad|\qquad\qquad |\\ \quad\text{N:C(CH}_2\text{Ph)·O}\end{array}$$

(XXXIII.)



(XXXIV.)

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[63] A. H. Cook, G. Harris, I. M. Heilbron, and G. Shaw, CPS 584.

[64] *Idem*, CPS 440, 500.

[65] J. R. Catch, A. H. Cook, and I. M. Heilbron, CPS 105.

[66] See 65 ; also S. 27 ; D. 28 ; cf. also similar 4-*n*-butyl derivatives, A. H. Cook, A. R. Graham, and I. M. Heilbron, CPS 552.

[67] See G 5 ; also F. C. Copp, W. M. Duffin, S. Smith, and S. Wilkinson, CPS 341.

oxazolones ; these reactions appeared, however, to result either in hydrohalides of the oxazolones or in unidentified products.[68] It was in related attempts to introduce 4-substituents that it was found [69] that treatment of 2-phenyloxazolone with Grignard reagents resulted in dimerisation to an acid. Simultaneously the same acid, $C_{18}H_{14}O_4N_2$, was obtained in other ways [70] and now seems identical with one obtained long before in various ways.[71]

(iii) *The Chemistry of β-Lactams.*—The emergence of the β-lactam formula for penicillins led to considerable development of the chemistry of β-lactams or " 2-azetidinones " [72] (XXXIV). Much effort was devoted to obtaining model structures containing appropriately fused thiazolidine and β-lactam rings to provide infra-red absorption data and to examine any chemical similarities to penicillin. Before work on penicillin, only relatively simple non-fused β-lactams were known, if compounds of doubtful structure be disregarded. Many of these had been prepared by Staudinger by the interaction of ketens and imines, but, owing to the complexity of the necessary reactants, this method has not provided any very useful advance towards penicillin or its models.

A large number of experiments were directed towards using reagents which might furnish ketens intermediately and so permit the ultimate use of complex reactants. Thus benzylideneaniline was heated with ethyl iodomalonate in presence of metals, with keten acetal, or with diazoacetone in presence of silver oxide,[73] but in no case was the β-lactam isolated. Attempts to modify the reaction to afford model bicyclic lactams by bringing various thiazolines and ketens into combination were disappointing. Thus keten or dimethylketen and various thiazolines (most of them derived from penicillamine methyl ester) gave either obscure products or fused thiazolidine–piperidinediones [74] (see later). Diphenylketen usually behaved analogously and in one instance only (XXXV) was the anticipated fused lactam structure obtained : [75] its structure is based on analytical data and infra-red absorption, on its stability as compared with (stable) β-lactams and with probably unstable ketotrimethyleneimines (a structure which would arise from alternative addition of keten to the N:C bond), and on analogy with a second thiazolidine-β-lactam (see later) the structure of which has been confirmed.

In this connection phenylacetyl *iso*cyanate reacted with hydrogen chloride to give phenylacetylcarbamyl chloride (XXXVI) which was treated with diazomethane to give supposedly (XXXVII) which was allowed to

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[68] C 7 ; S. 12b, 15, 20, 22 ; H. J. Barber, P. Z. Gregory, K. N. Langford, R. Slack, C. E. Stickings, and A. M. Woolman, CPS 385.

[69] A. H. Cook, J. A. Elvidge, I. M. Heilbron, and A. L. Levy, CPS 443.

[70] J. L. Bailey, B. Baker, and W. Bradley, CPS 444.

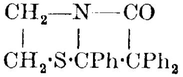
[71] P. Karrer and V. Itschner, *Helv. Chim. Acta*, 1932, **15,** 1420 ; T. Curtius and W. Lenhard, *J. pr. Chem.*, 1904, **70,** 240 ; L. Rügheimer, *Ber.*, 1888, **21,** 3325.

[72] *Chem. Abs.*, 1944, **38,** 7061.

[73] H 13, 14 ; cf. also P 22.

[74] Sh 8, 10, 14 ; B 15, 17, 19 ; P 29, 32, 33 ; U 21, 22, 24 ; cf. also Sh 9 ; B 16.

[75] Sh 7.

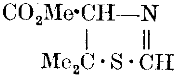


$$\begin{array}{ccc} \mathrm{CH_2} & \mathrm{—N—} & \mathrm{CO} \\ | & | & | \\ \mathrm{CH_2{\cdot}S{\cdot}} & \mathrm{CPh{\cdot}} & \mathrm{CPh_2} \end{array}$$

(XXXV.)

$\mathrm{Ph{\cdot}CO{\cdot}N{:}C{:}O \rightarrow Ph{\cdot}CO{\cdot}NH{\cdot}COCl \rightarrow}$
(XXXVI.)

$\mathrm{Ph{\cdot}CO{\cdot}NH{\cdot}CO{\cdot}CHN_2 \longrightarrow [Ph{\cdot}CO{\cdot}NH{\cdot}CH{\cdot}C{:}O]}$
(XXXVII.) (XXXVIII.)



$$\begin{array}{cc} \mathrm{CO_2Me{\cdot}CH} & \mathrm{—N} \\ | & \| \\ \mathrm{Me_2C{\cdot}S{\cdot}} & \mathrm{CH} \end{array}$$

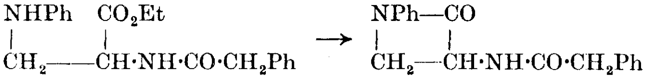
(XXXIX.)

react with the thiazoline (XXXIX). It was hoped that rearrangement of the free radical resulting potentially from (XXXVII) might have led to (XXXVIII) which with (XXXIX) might have given methyl benzylpenicillin directly.[76] The resulting antibiotic activities were unfortunately erratic, but some were quite high.

Among other known syntheses of β-lactams was the Reformatsky reaction between an α-bromo-ester and an imine,[77] *e.g.* : [78]

$$\mathrm{Ph{\cdot}CH_2{\cdot}N{:}CHPh + CHMeBr{\cdot}CO_2Et + Zn \rightarrow} \begin{array}{cc} \mathrm{CH_2Ph{\cdot}N} & \mathrm{———CO} \\ | & | \\ \mathrm{CHPh} & \mathrm{—CHMe} \end{array}$$

This reaction, however, proved suitable only for model lactams, and so did the direct cyclisation of β-amino-acids with acetyl chloride [79] or phosphorus trichloride.[80] The cyclisation of β-amino-esters falls into the same class, alcohol being removed by an organometallic compound (methylmagnesium iodide, phenyl-lithium, etc.),[81] *e.g.* :



$$\begin{array}{cc} \mathrm{NHPh} & \mathrm{CO_2Et} \\ | & | \\ \mathrm{CH_2} & \mathrm{——CH{\cdot}NH{\cdot}CO{\cdot}CH_2Ph} \end{array} \longrightarrow \begin{array}{cc} \mathrm{NPh} & \mathrm{—CO} \\ | & | \\ \mathrm{CH_2} & \mathrm{—CH{\cdot}NH{\cdot}CO{\cdot}CH_2Ph} \end{array}$$

Numerous attempts to apply this reaction to the preparation of fused thiazolidine-β-lactams led to no definite results, nor did thiazolidines which might have yielded " penicillins " by such treatment produce any biological activity.[81]

It had been observed by Staudinger that *N*-benzylidenemethylamine and dimethylketen gave predominantly a product containing two molecules of keten and formulated as a piperidinedione. Similar reactions were effected with *N*-benzylidene-ethylamine and with *N*-benzylidenebenzylamine (XL), it being shown that the dione (XLI) underwent easy hydrolysis to a β-acylamido-acid ; compounds of the above type may still, however, be intermediates in the normal reaction, as the acids so formed decompose into β-lactams by thermal elimination of fatty acid : [82]

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[76] B 15, 16.

[77] H. Gilman and M. Speeter, *J. Amer. Chem. Soc.*, 1943, **65**, 2255.

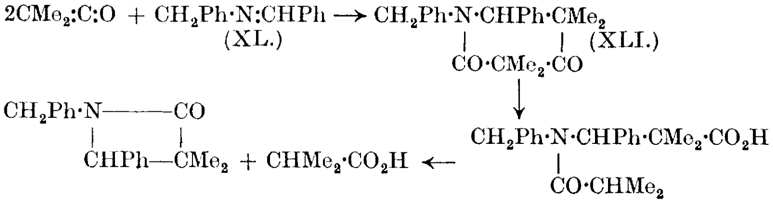
[78] M 58.

[79] H. Staudinger, H. W. Klever, and P. Kober, *Annalen*, 1910, **374**, 1.

[80] P 24.

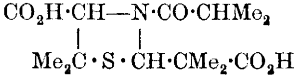
[81] See, for example, Sh 13, 14 ; S 50 ; U 20 ; M 63, 81 ; PD 22.

[82] Sh 9, 10 ; cf. Sh 7, 8.



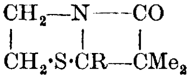
$$2\mathrm{CMe_2{:}C{:}O} + \underset{\mathrm{(XL.)}}{\mathrm{CH_2Ph{\cdot}N{:}CHPh}} \longrightarrow \underset{\mathrm{CO{\cdot}CMe_2{\cdot}CO}}{\mathrm{CH_2Ph{\cdot}N{\cdot}CHPh{\cdot}CMe_2}}\ \mathrm{(XLI.)} \longrightarrow \underset{\mathrm{CO{\cdot}CHMe_2}}{\mathrm{CH_2Ph{\cdot}N{\cdot}CHPh{\cdot}CMe_2{\cdot}CO_2H}} \longrightarrow \begin{matrix}\mathrm{CH_2Ph{\cdot}N}\text{---}\mathrm{CO}\\ \mathrm{CHPh}\text{---}\mathrm{CMe_2}\end{matrix} + \mathrm{CHMe_2{\cdot}CO_2H}$$

In view of these reactions it was disappointing that analogous conditions applied to various *N-iso*butyrylthiazolidines such as (XLII) led mostly not to bicyclic β-lactams but only to decarboxylation and unelucidated



$$\begin{matrix}\mathrm{CO_2H{\cdot}CH}\text{---}\mathrm{N{\cdot}CO{\cdot}CHMe_2}\\ \mathrm{Me_2C\cdot S\cdot CH{\cdot}CMe_2{\cdot}CO_2H}\end{matrix}$$

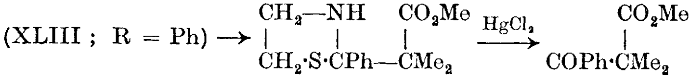
(XLII.)



$$\begin{matrix}\mathrm{CH_2}\text{---}\mathrm{N}\text{---}\mathrm{CO}\\ \mathrm{CH_2{\cdot}S{\cdot}CR}\text{---}\mathrm{CMe_2}\end{matrix}$$

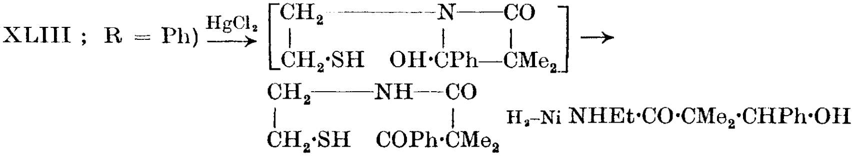
(XLIII.)

reactions.[83] Nevertheless, 2-phenyl- and 2-methyl-thiazoline gave products with dimethylketen by this method which seemed to be the required β-lactams (XLIII; R = Ph or Me).[84] Thus the first product underwent methanolysis in presence of sodium methoxide, and the resulting thiazolidine was cleaved by mercuric chloride to give methyl α-benzoyl*iso*butyrate :



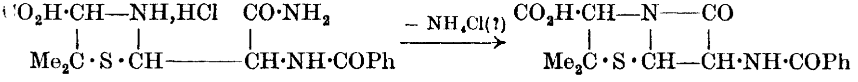
$$\mathrm{(XLIII;\ R = Ph)} \longrightarrow \begin{matrix}\mathrm{CH_2}\text{---}\mathrm{NH} \quad \mathrm{CO_2Me}\\ \mathrm{CH_2{\cdot}S{\cdot}CPh}\text{---}\mathrm{CMe_2}\end{matrix} \xrightarrow{\mathrm{HgCl_2}} \begin{matrix}\mathrm{CO_2Me}\\ \mathrm{COPh{\cdot}CMe_2}\end{matrix}$$

The lactam structure was moreover supported by its behaviour towards mercuric chloride followed by hydrogenolysis, leading apparently to the following changes :



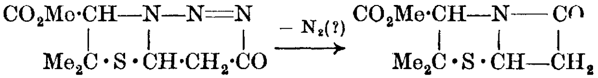
$$\mathrm{(XLIII;\ R = Ph)} \xrightarrow{\mathrm{HgCl_2}} \left[\begin{matrix}\mathrm{CH_2}\text{------}\mathrm{N}\text{---}\mathrm{CO}\\ \mathrm{CH_2{\cdot}SH \quad OH{\cdot}CPh}\text{---}\mathrm{CMe_2}\end{matrix}\right] \longrightarrow \begin{matrix}\mathrm{CH_2}\text{------}\mathrm{NH}\text{---}\mathrm{CO}\\ \mathrm{CH_2{\cdot}SH \quad COPh{\cdot}CMe_2}\end{matrix} \xrightarrow{\mathrm{H_2\text{-}Ni}} \mathrm{NHEt{\cdot}CO{\cdot}CMe_2{\cdot}CHPh{\cdot}OH}$$

Considerable ingenuity was devoted to other possible syntheses of fused thiazolidine-β-lactams. Most were directed towards effecting ring-closure of substituted thiazolidine-2-acetic acids, though the results were often indeterminate. Thus the thiazolidine (XLIV) could not be made to lose ammonium chloride ;[85] again, although the triazine (XLV) was obtained



$$\underset{\mathrm{(XLIV.)}}{\begin{matrix}\mathrm{CO_2H{\cdot}CH}\text{---}\mathrm{NH,HCl} \quad \mathrm{CO{\cdot}NH_2}\\ \mathrm{Me_2C\cdot S\cdot CH}\text{------}\mathrm{CH{\cdot}NH{\cdot}COPh}\end{matrix}} \xrightarrow{-\ \mathrm{NH_4Cl(?)}} \begin{matrix}\mathrm{CO_2H{\cdot}CH}\text{---}\mathrm{N}\text{---}\mathrm{CO}\\ \mathrm{Me_2C\cdot S\cdot CH}\text{---}\mathrm{CH{\cdot}NH{\cdot}COPh}\end{matrix}$$

(XLIV.)



$$\begin{matrix}\mathrm{CO_2Me{\cdot}CH}\text{---}\mathrm{N}\text{---}\mathrm{N}{=}\mathrm{N}\\ \mathrm{Me_2C\cdot S\cdot CH{\cdot}CH_2{\cdot}CO}\end{matrix} \xrightarrow{-\ \mathrm{N_2(?)}} \begin{matrix}\mathrm{CO_2Me{\cdot}CH}\text{---}\mathrm{N}\text{---}\mathrm{CO}\\ \mathrm{Me_2C\cdot S\cdot CH}\text{---}\mathrm{CH_2}\end{matrix}$$

(XLV.)

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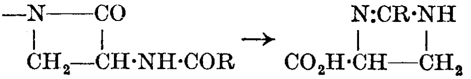
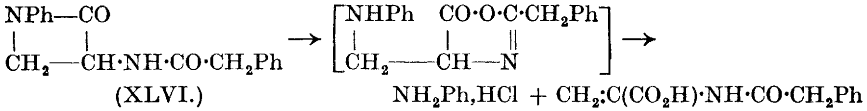
[83] Sh 10, 14, 15; M 66. [84] Sh 8, 14.

[85] G. Newbery, A. W. Nineham, and R. Raphael, CPS 674.

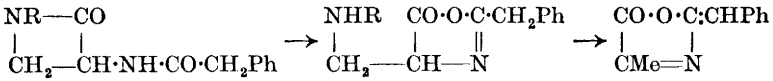
and appeared to lose nitrogen on heating, its conversion into a β-lactam was doubtful.[86] It remains then to review more closely how far the behaviour of monocyclic and fused thiazolidine β-lactams paralleled that of penicillins.

All non-fused β-lactams appeared more stable than penicillins to hydrolysis by alkalis, though differing widely in stability among themselves. Thus 3-phenylacetamido-1-phenyl- and 3-*cyclo*hexylacetamido-1-*cyclo*hexyl-2-azetidinone (the latter obtained by catalytic hydrogenation of the former [87] and both more closely comparable than simple alkyl- and aryl-azetidinones with penicillins) seemed from infra-red data to be hydrolysed by 1N-sodium hydroxide at 100° for 90 minutes or by butanolic sodium butoxide at 100° for 1 minute, whereas Staudinger records [88] that 1 : 3 : 3 : 4-tetraphenylazetidinone is only very slightly attacked by 0·5N-alkali in boiling methanol in 25 hours. Thus, disregarding problematical steric and electronic effects of aryl and other groups, it appears that a 3-acylamido-group confers lability on the lactam ring. Relative to penicillin the model thiazolidine β-lactams were, as far as they were studied, also more stable towards alkali and comparable with simple alkyl- and aryl-azetidinones.[89] Monocyclic β-lactams were fairly resistant to hydrolysis by acid,[90] though again a 3-acylamido-group may have made for instability No formal parallel to the penillic acid change was observed, but this was not surprising as even dethiobenzylpenicillin was sharply marked off from penicillin in reactivity. It has incidentally been suggested [91] that the fission of the azetidinone (XLVI) by hydrogen chloride in dioxan leading to aniline, and related observations, may have some bearing on the penillic acid reaction. However, these reactions present only a remote and partial analogy especially as they are carried out under conditions which, with penicillin, lead to *pseudo*penicillin (Section I, iv). A correlation among synthetic compounds between the " lactam " and " oxazolone " reactions of penicillins lies in the thermal conversion of β-lactams, including dethiobenzylpenicillin, into *pseudo*-oxazolones :





(XLVI.)



(R = Ph or $CHMe_2·CH·CO_2Me$.)

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[86] S 50.

[87] M 63, 64.

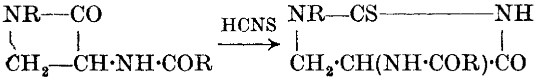
[88] H. Staudinger, " Die Ketene ", 1912.

[89] Sh 7, 8, 11.

[90] M 51, 55, 63, 64, 67 ; P 8.

[91] M 57.

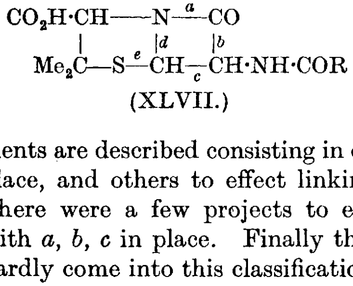
Several monocyclic β-lactams suffered ring-fission on being heated with benzylamine or hydrazine, but reaction was always less ready than with benzylpenicillin.[92] Finally, it was notable that reaction of an azetidinone with thiocyanic acid recalled that of benzylpenicillin in giving a dihydrothiouracil : [93]



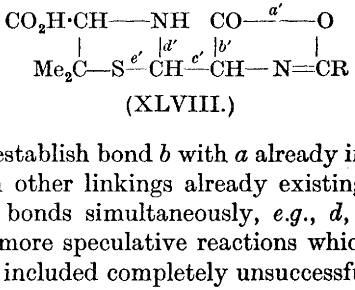
The outcome of these comparisons therefore is that while there is nothing in the behaviour of penicillin which is not consonant with a β-lactam structure, its lability remains outstanding and is perhaps most influenced by the acylamido-group.

(iv) *Studies in the Synthesis of Penicillins.*—Among the earliest approaches were attempts to cyclise penicilloic acids or esters. These were at first regarded as approaches to the tricyclic or oxazolone structures though they were later recognised as potential routes to " lactam " penicillin also.

This view is related not only to what often appear formally as alternative ring syntheses, but also to the acylating properties of oxazolones by which any thiazolidine–oxazolone formed transiently might be expected to assume the β-lactam form by internal self-acylation. The difficulty resides in the apparent incompatibility of the thiazolidine and oxazolone or β-lactam rings. Viewed in retrospect, all these early attempts may be regarded as efforts to establish bond *a* in (XLVII) or *a′* in (XLVIII), with some representing schemes to effect linkings *a* and *b* (or *a′* and *b′*) simultaneously. Later, experiments are described consisting in efforts to establish bond *b* with *a* already in place, and others to effect linking *c′* with other linkings already existing. There were a few projects to effect two bonds simultaneously, *e.g.*, *d*, *e* with *a*, *b*, *c* in place. Finally there were more speculative reactions which hardly come into this classification ; these included completely unsuccessful attempts to " reactivate " penillic acids [94] and to convert penicillenates into penicillins.[95] Many of these approaches were chemically inconclusive, and were abandoned when no useful activities were observed without the actual course of reaction being elucidated.



(XLVII.)



(XLVIII.)

Attempts to form bond a(a′). Simple methods of removing water or alcohols, such as treatment of appropriate penicilloic derivatives with phosphorus halides, boron halides, or various dehydrating agents mostly failed to afford definite products or any considerable activity ; small activities produced in this way represent the first active material to be

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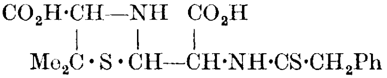
[92] B 16.

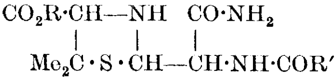
[93] M 62, 63.

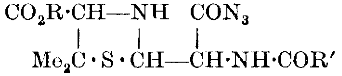
[94] See, for example, L 9 ; M 53 (Section I, ref. 60).

[95] M 23.

obtained in penicillin studies,[96] while in other instances benzylpenicillenates were isolated in small yield indicating some degree of azlactonisation. β-Methyl benzylpenicilloate and phosphorus tribromide afforded methyl *pseudo*penicillin (Section I, iv) which appeared to be mainly the corresponding oxazolone hydrobromide. Similar attempts to remove hydrogen sulphide from the thiopenicilloic acid (XLIX) were unavailing, though model experiments indicated this to be a means of closing the oxazolone ring.[97]

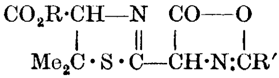

(XLIX.)

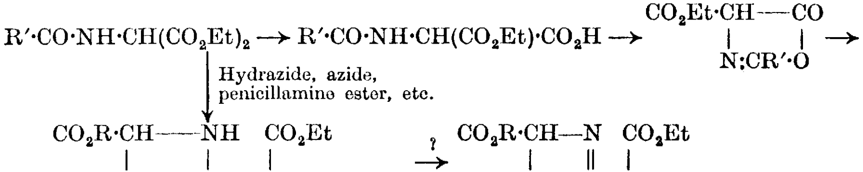

(L.)


(LI.)

Many indirect attempts from penicilloate derivatives were conceivably directed towards the β-lactam structure. Thus unsuccessful attempts to remove the elements of alcohols from α-penicilloic esters by means of Grignard reagents [98] were based on a known method of forming the β-lactam ring (see above). Efforts to remove the elements of ammonia from penicilloic α-amides (L) were also fruitless,[99] as were also many experiments to remove hydrazoic acid from penicilloic α-azides (LI).[100]

Perhaps the approaches which are most satisfying from the chemical standpoint, though as yet they provide no synthesis of penicillins, are those which have sought to circumvent the incompatibility of the thiazolidine and oxazolone rings by elaborating thiazolineoxazolones (LII) which offer the possibility of reduction at a suitable stage in the known manner of thiazolines.[101] The oxazolone moiety was at first approached *via* acyl-


(LII.)



(LIII.)

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[96] J. R. Catch, A. H. Cook, R. H. Hall, and I. M. Heilbron, CPS 5.

[97] E. P. Abraham, E. Chain, W. Baker, and R. Robinson, CPS 43; C. I. Broderick, D. A. Peak, F. F. Whitmont, and W. Wilson, CPS 592.

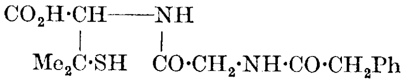
[98] See, for example, U 20.

[99] M 55; cf. also G. Newbery, A. W. Nineham, and R. Raphael, CPS 674.

[100] B 12, 13; M 60; F. C. Copp, W. M. Duffin, S. Smith, and S. Wilkinson, CPS 424, 642.

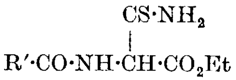
[101] See, for example, R. Bentley, J. R. Catch, A. H. Cook, J. A. Elvidge, I. M. Heilbron, and G. Shaw, CPS 44, 63.

amidomalonic acid derivatives[102, 103] to give acylpenicillamines; the latter, however, could not be converted satisfactorily into thiazolines despite the successful cyclisation of almost equally complex compounds



$$\begin{array}{ll}\mathrm{CO_2H{\cdot}CH}\text{——} & \mathrm{NH} \\ \quad | & \ | \\ \mathrm{Me_2C{\cdot}SH} & \mathrm{CO{\cdot}CH_2{\cdot}NH{\cdot}CO{\cdot}CH_2Ph}\end{array}$$

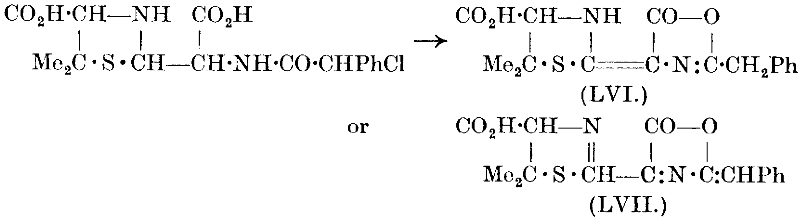
(LIV.)



$$\begin{array}{c}\mathrm{CS{\cdot}NH_2} \\ | \\ \mathrm{R'{\cdot}CO{\cdot}NH{\cdot}CH{\cdot}CO_2Et}\end{array}$$

(LV.)

such as (LIV). Again, efforts to use acylamidocyanoacetates by converting them into thioamides (LV) which might have afforded the required thiazolines were unavailing despite model reactions of this kind.



$$\begin{array}{l}\mathrm{CO_2H{\cdot}CH{-}NH\quad CO_2H} \\ \mathrm{Me_2C{\cdot}S{\cdot}CH{-}CH{\cdot}NH{\cdot}CO{\cdot}CHPhCl}\end{array} \longrightarrow \begin{array}{l}\mathrm{CO_2H{\cdot}CH{-}NH\quad CO{-}O} \\ \mathrm{Me_2C{\cdot}S{\cdot}C{=}C{\cdot}N{:}C{\cdot}CH_2Ph}\end{array}$$

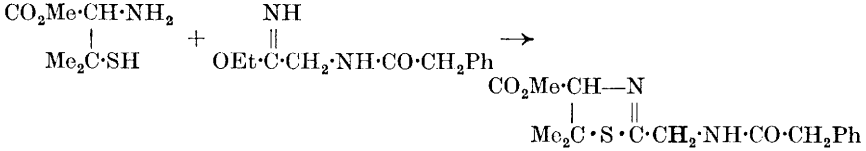
(LVI.)

or

$$\begin{array}{l}\mathrm{CO_2H{\cdot}CH{-}N\quad CO{-}O} \\ \mathrm{Me_2C{\cdot}S{\cdot}CH{-}C{:}N{\cdot}C{:}CHPh}\end{array}$$

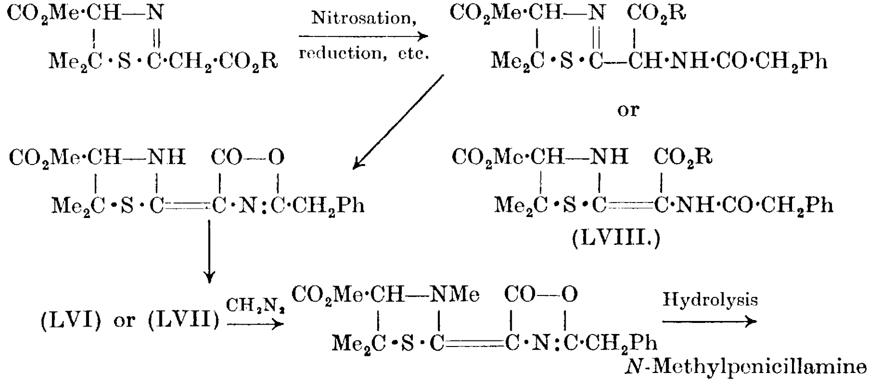
(LVII.)

A compound of composition corresponding to a "dehydrobenzylpenicillin" (LVI) was obtained by a recognised method of forming oxazolones[104] though it may well have been (LVII) and its nature has been questioned more



$$\begin{array}{l}\mathrm{CO_2Me{\cdot}CH{\cdot}NH_2} \\ \mathrm{Me_2C{\cdot}SH}\end{array} + \begin{array}{c}\mathrm{NH} \\ \| \\ \mathrm{OEt{\cdot}C{\cdot}CH_2{\cdot}NH{\cdot}CO{\cdot}CH_2Ph}\end{array} \longrightarrow \begin{array}{l}\mathrm{CO_2Me{\cdot}CH{-}N} \\ \mathrm{Me_2C{\cdot}S{\cdot}C{\cdot}CH_2{\cdot}NH{\cdot}CO{\cdot}CH_2Ph}\end{array}$$

recently.[105] Particularly timely, however, was the finding that thiazolines were obtainable by the interaction of imino-ethers and penicillamine



$$\begin{array}{l}\mathrm{CO_2Me{\cdot}CH{-}N} \\ \mathrm{Me_2C{\cdot}S{\cdot}C{\cdot}CH_2{\cdot}CO_2R}\end{array} \xrightarrow[\text{reduction, etc.}]{\text{Nitrosation,}} \begin{array}{l}\mathrm{CO_2Me{\cdot}CH{-}N\quad CO_2R} \\ \mathrm{Me_2C{\cdot}S{\cdot}C{-}CH{\cdot}NH{\cdot}CO{\cdot}CH_2Ph}\end{array}$$

or

$$\begin{array}{l}\mathrm{CO_2Me{\cdot}CH{-}NH\quad CO_2R} \\ \mathrm{Me_2C{\cdot}S{\cdot}C{=}C{\cdot}NH{\cdot}CO{\cdot}CH_2Ph}\end{array}$$

(LVIII.)

$$\begin{array}{l}\mathrm{CO_2Me{\cdot}CH{-}NH\quad CO{-}O} \\ \mathrm{Me_2C{\cdot}S{\cdot}C{=}C{\cdot}N{:}C{\cdot}CH_2Ph}\end{array}$$

$$\text{(LVI) or (LVII)} \xrightarrow{\mathrm{CH_2N_2}} \begin{array}{l}\mathrm{CO_2Me{\cdot}CH{-}NMe\quad CO{-}O} \\ \mathrm{Me_2C{\cdot}S{\cdot}C{=}C{\cdot}N{:}C{\cdot}CH_2Ph}\end{array} \xrightarrow{\text{Hydrolysis}} N\text{-Methylpenicillamine}$$

---

[102] See 101; R. Bentley, A. H. Cook, G. Harris, and I. M. Heilbron, CPS 386.

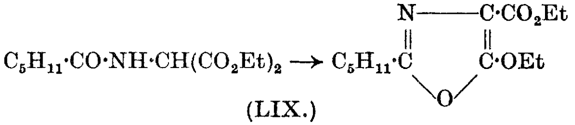
[103] B 8, 10; for experiments in the benzamidomalonic acid series, see I.C.I. Ltd., CPS 634.

[104] G. Newbery and R. Raphael, CPS 378, 478; cf. CPS 674.

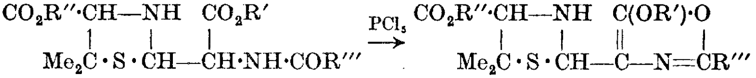
[105] P 31, 32, 33.

esters.[106] With this aid a compound of structure (LVI) was made by rational means and its nature confirmed by degradation ; a number of other compounds, *e.g.*, of the " amylpenicillin " series were similarly synthesised,[107] but all attempts to reduce these under conditions which might have afforded penicillin failed to yield any significant antibiotic activity.

It was not at first anticipated that the esters (LVIII) would prove directly convertible into oxazolones, as simpler cyclisations of this kind had resulted in the formation of alkoxyoxazoles,[108] *e.g.*, (LIX), and were

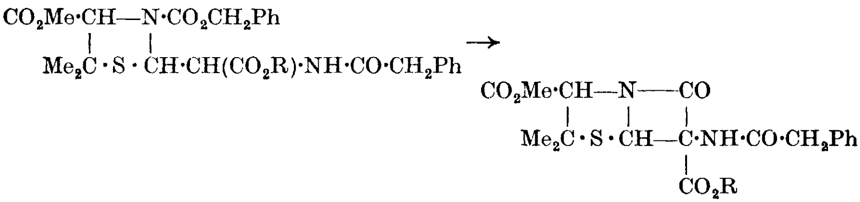

(LIX.)

later applied to the synthesis of thiazolidineoxazoles, particularly in the " phenylpenicillin " series (R‴ = Ph) : [109]



It appeared that with, for example, R″ = R‴ = CH_2Ph it might be possible to remove the benzyl groups by reduction and achieve a synthesis of a penicillin by spontaneous rearrangement of the resulting thiazolidine-oxazolone. Reduction of the benzyloxyoxazole seemed to occur, but its precise course was obscure and no significant biological activities were attained.

Attempts to form bond b. A number of approaches were designed to force β-lactam formation by appropriate substitution on the penicillamine nitrogen atom, though similar reactions had been regarded as offering routes to the oxazolone structure. In the latter group were attempts to cyclise *N*-carbobenzyloxypenicilloic acids or esters (LX) to oxazolones which might then be converted into penicillins by catalytic removal of the carbobenzyloxy-group : [110]


(LX.) (R = H or alkyl) (LXI.)

such esters (LX) failed also to afford β-lactams on attempted removal of the elements of benzyl alcohol to give (LXI) in presence of Grignard reagents.[111]

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[106] E. P. Abraham, W. Baker, E. Chain, and R. Robinson, CPS 463.

[107] A. H. Cook, J. A. Elvidge, I. M. Heilbron and A. L. Levy, CPS 443, 529, 680.

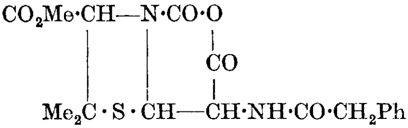
[108] C. I. Broderick, D. A. Peak, W. F. Short, F. F. Whitmont, and W. Wilson, CPS 305 ; see also Section II, ii.

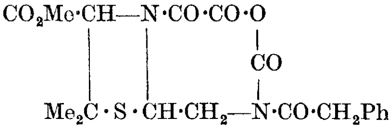
[109] I.C.I. Ltd., CPS 634, 693.

[110] D 19.

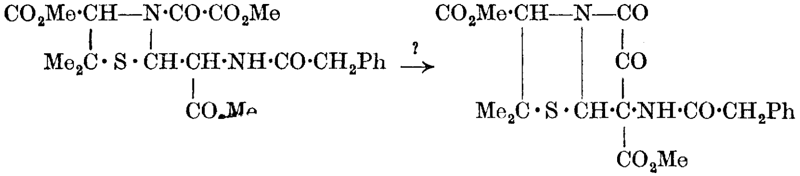
[111] M 55.

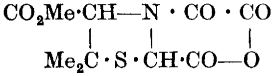
Carbonyl chloride failed to react satisfactorily with β-methyl benzylpenicilloate or a near derivative to give (LXII), so that experiments to eliminate carbon dioxide could not be made.[112] Likewise, oxalyl chloride

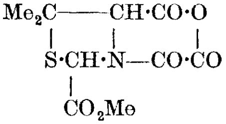

(LXII.)

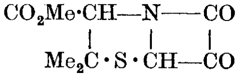

(LXIII.)

could not be made to give (LXIII) by reaction with β-methyl benzylpenicilloate [113] nor could (LXIV) be cyclised to (LXV).[114] In this connection the thiazolidines (LXVI) and (LXVII) were made, but despite

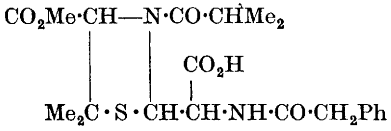

(LXIV.) (LXV.)

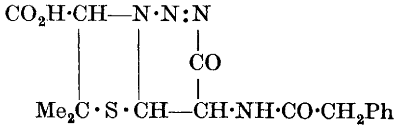

(LXVI.)


{LXVII.)


(LXVIII.)

detailed study gave no clear indication of losing carbon dioxide to afford model compounds such as (LXVIII).[115] A number of experiments which may be included here concerned attempts to expel the elements of a fatty acid from *N*-acylpenicilloic acids (LXIX) by pyrolysis, though the chance


(LXIX.)


(LXX.)

of success was always slight owing to the ease of decarboxylation of penicilloic acids.[116] Similar in conception were attempts to form a cyclic triazine (LXX) by the action of nitrous acid on a penicilloamide, followed by elimination of nitrogen, but these attempts also were inconclusive,[117] as were attempts to eliminate benzaldehyde from the supposed intermediate (LXXI),[118] or formaldehyde from the cyclised reaction product of (LXXII).

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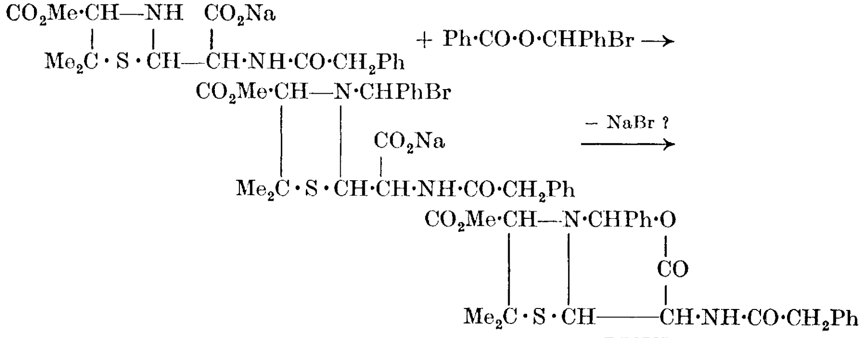
[112] PD 22. [113] M 57. [114] M 63.

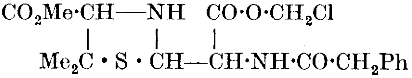
[115] R. Bentley, A. H. Cook, J. A. Elvidge, I. M. Heilbron, and G. Shaw, unpublished work.

[116] Sh 9 ; M 66 ; F. C. Copp, W. M. Duffin, S. Smith, and S. Wilkinson, CPS 642.

[117] S 42, 44, 46, 47 ; B 15. [118] B 15.

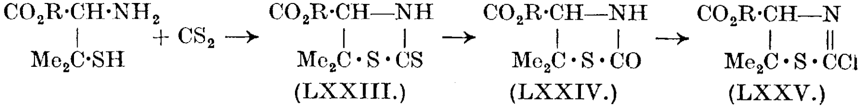


(LXXI.)

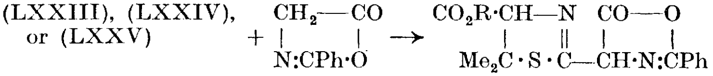


(LXXII.)

Attempts to form bond c. The thiazolidine (LXXIII) was obtained in several ways, of which the most convenient is shown, and eventually converted into (LXXIV) and (LXXV). It was hoped to condense one of these three with appropriate oxazolones or their equivalents with elimination of hydrogen sulphide, water, or hydrogen chloride to obtain the " dehydropenicillins " (LXXVI), but, in general, the thiazoline intermediates proved unexpectedly stable;[119] it has been mentioned above that the required condensation products were obtained in other ways, but they in turn had to be discarded.

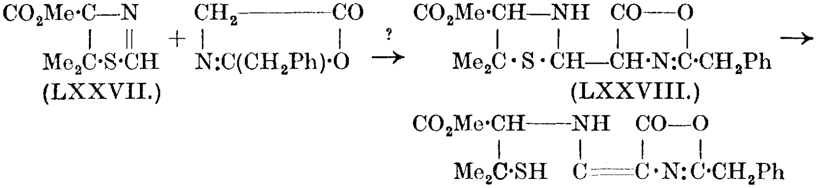


(LXXIII.) (LXXIV.) (LXXV.)



(LXXVI.)

A resemblance between keten acetals and oxazolones or 5-alkoxyoxazoles suggested that the latter might add directly to thiazolines,[120] and some justification for this opinion was to be found in the reaction between 2-benzyl-5-oxazolone and the thiazoline (LXXVII) which afforded



(LXXVII.) (LXXVIII.)

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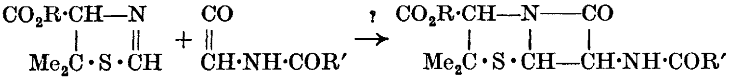
[119] A. H. Cook, J. A. Elvidge, I. M. Heilbron, A. L. Levy, and G. Shaw, CPS 311, 443.

[120] M 63.

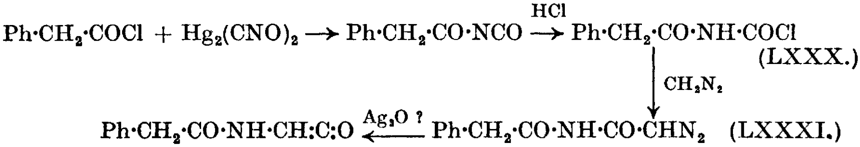
methyl benzylpenicillenate, presumably *via* the thiazolidine oxazolone (LXXVIII).[121]

Simple ketens and thiazolines eventually yielded "thiazolidine-β-lactams" (Section II, iii), and the formal application of this reaction to the synthesis of "lactam" penicillin required the unknown acylamido-ketens (LXXIX). As an approximation to these compounds phenylacetylcarbamyl chloride (LXXX) was converted supposedly into the diazoketone



$$\begin{array}{l}CO_2R\cdot CH-N \\ \ \ \ |\qquad\quad \| \\ Me_2C\cdot S\cdot CH\end{array} + \begin{array}{l}CO\\ \| \\ CH\cdot NH\cdot COR'\end{array} \xrightarrow{?} \begin{array}{l}CO_2R\cdot CH-N-CO\\ \ \ \ |\qquad\quad |\qquad | \\ Me_2C\cdot S\cdot CH-CH\cdot NH\cdot COR'\end{array}$$

(LXXIX.)



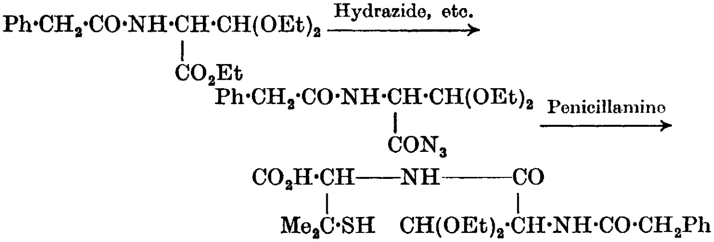
$$Ph\cdot CH_2\cdot COCl + Hg_2(CNO)_2 \rightarrow Ph\cdot CH_2\cdot CO\cdot NCO \xrightarrow{HCl} Ph\cdot CH_2\cdot CO\cdot NH\cdot COCl \quad (LXXX.)$$

$$\downarrow CH_2N_2$$

$$Ph\cdot CH_2\cdot CO\cdot NH\cdot CH{:}C{:}O \xleftarrow{Ag_2O\ ?} Ph\cdot CH_2\cdot CO\cdot NH\cdot CO\cdot CHN_2 \quad (LXXXI.)$$

(LXXXI) which in presence of silver oxide might have rearranged, and, with 4-carbomethoxy-5 : 5-dimethylthiazoline, have given methyl benzylpenicillin.[122] After suitable hydrolysis the products showed variable but sometimes relatively high activity. However, another study [123] demonstrated that the product at first regarded as (LXXXI) was in fact devoid of the reactions of diazoketones. Experiments in the *n*-hexoyl series were equally inconclusive, and the earlier biological activities seem probably to have been due to unconsidered products rather than penicillin itself.

Attempts to form bonds d, e. Some of these attempts are represented in the following reaction scheme: [124]



$$\begin{array}{l}Ph\cdot CH_2\cdot CO\cdot NH\cdot CH\cdot CH(OEt)_2\\ \qquad\qquad\qquad\quad |\\ \qquad\qquad\qquad CO_2Et\end{array} \xrightarrow{\text{Hydrazide, etc.}} \begin{array}{l}Ph\cdot CH_2\cdot CO\cdot NH\cdot CH\cdot CH(OEt)_2\\ \qquad\qquad\qquad\quad |\\ \qquad\qquad\qquad CON_3\end{array} \xrightarrow{\text{Penicillamine}}$$

$$\begin{array}{l}CO_2H\cdot CH-NH-CO\\ \qquad\ \ |\qquad\qquad\quad |\\ Me_2C\cdot SH\quad CH(OEt)_2\cdot CH\cdot NH\cdot CO\cdot CH_2Ph\end{array}$$

(LXXXII.)

The acetal (LXXXII) generated no marked biological activity when heated, and attempts to hydrolyse it to the corresponding aldehyde were inconclusive. Other acetals of this type gave small, hardly significant antibacterial activities.[125] Deliberate dehydration of one such compound resulted not in thiazolidine formation but in removal of water probably to give the oxazolone (LXXXIII).[126]

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[121] M 50, 63, 69.

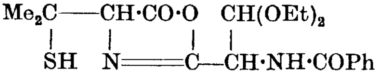
[122] B 16

[123] W. Baker, P. G. Jones, E. P. Abraham, E. Chain, and R. Robinson, CPS 1/1946.

[124] B 12.

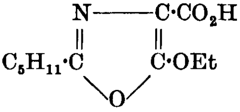
[125] S 37, 39.

[126] F. C. Copp, W. M. Duffin, S. Smith, and S. Wilkinson, CPS 340.

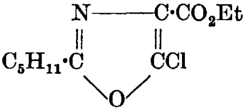


(LXXXIII.)

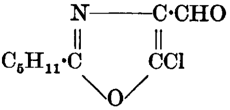
Of outstanding interest in this connection was the reaction between alkoxy- or hydroxy-methyleneoxazolones and penicillamine derivatives, but it will be convenient first to consider some related reactions. It was observed that attempts to convert (LXXXIV) into its acid chloride, which might have been reduced to the aldehyde and then condensed with penicillamine, led actually to the oxazole ester (LXXXV) (Section II, ii above). The latter was, however, still potentially useful in offering a route to (LXXXVI) which might have afforded a penicillin by condensation with penicillamine followed by removal of the halogen atom. The acid chloride corresponding to (LXXXV) could only be reduced to (LXXXVII), but the related compound (LXXXVIII) was prepared by reducing the nitrile (LXXXIX) obtained *via* the corresponding ester and amide.[127] Incidentally, the amide (XC) exemplified again the transformations of oxazoles mentioned earlier in rearranging on heating into (XCI), the structure of which was proved by alternative syntheses.[128] When (LXXXVIII) was condensed with penicillamine and the product warmed in pyridine, small activities were produced which were tentatively regarded as due to a " phenylpenicillin ".[128] Similar syntheses in the benzyl series do not appear to have led to active materials,[129] while in the *n*-amyl series the reduction of the nitrile corresponding to (LXXXIX) was not achieved.[130]



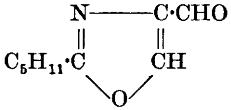
(LXXXIV.)



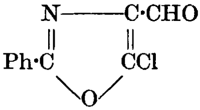
(LXXXV.)



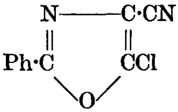
(LXXXVI.)



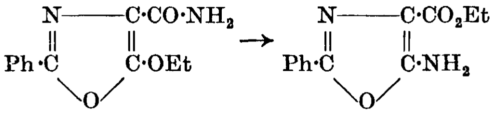
(LXXXVII.)



(LXXXVIII.)



(LXXXIX.)



(XC.) (XCI.)

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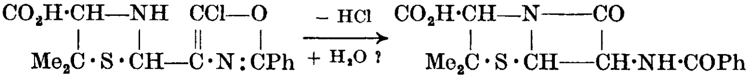
[127] J. W. and R. H. Cornforth, E. P. Abraham, W. Baker, E. Chain, and R. Robinson, CPS 492.

[128] E. P. Abraham, W. Baker, E. Chain, L. J. Goldsworthy, and R. Robinson, CPS 650.

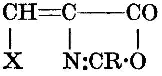
[129] L. J. Goldsworthy and R. Robinson, CPS 695.

[130] E. P. Abraham, W. Baker, E. Chain, J. W. and R. H. Cornforth, and R. Robinson, CPS 689.



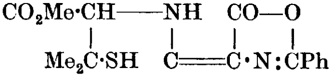
$$\begin{array}{l}\mathrm{CO_2H{\cdot}CH{-}NH\quad CCl{-}O}\\ \mathrm{Me_2C{\cdot}S{\cdot}CH{-}C{\cdot}N{:}CPh}\end{array}\xrightarrow[+\,H_2O\,?]{-\,HCl}\begin{array}{l}\mathrm{CO_2H{\cdot}CH{-}N{-}CO}\\ \mathrm{Me_2C{\cdot}S{\cdot}CH{-}CH{\cdot}NH{\cdot}COPh}\end{array}$$

Most experiments in the present category concerned attempts to cause penicillamine or other α-amino-β-mercapto-acids such as cysteine, or their derivatives, to react with appropriate oxazolones. At first the aim was by the interaction of, say, penicillamine with an oxazolone-4-aldehyde, to obtain " oxazolone " penicillin. As outlined earlier, it became clear that the required oxazolone-4-aldehydes were not obtained, all approaches leading to derivatives of 4-methyleneoxazolones. Thus in place of the required aldehydes or their acetals, thioacetals, or Schiff bases, there were obtained hydroxy-, alkoxy-, thioalkoxy-, or amino-methyleneoxazolones (XCII)—(XCV) respectively.



$$\begin{array}{l}\mathrm{CH{=}C{-}CO}\\ \mathrm{X\quad N{:}CR{\cdot}O}\end{array}$$

(XCII.) (X = OH)
(XCIII.) (X = OAlk)
(XCIV.) (X = SAlk)
(XCV.) (X = NHR)



$$\begin{array}{l}\mathrm{CO_2Me{\cdot}CH{-}NH\quad CO{-}O}\\ \mathrm{Me_2C{\cdot}SH\quad C{=}C{\cdot}N{:}CPh}\end{array}$$

(XCVI.)

Despite this lack of carbonyl or near-carbonyl nature there was still the possibility that these compounds might afford thiazolidines and thus penicillins, and an exceedingly large number of experiments were made to test this hypothesis. Most of these were concerned with the effect of empirical changes in reaction conditions on the possible development of biological activities, and in only a few was the chemical nature of the products established. The empirical nature of these experiments was enhanced when it was realised that penicillins were structurally more remote from the oxazolone intermediates than was at first appreciated, though as is outlined below this approach represents the sole demonstrable synthesis of penicillin.

Perhaps the earliest experiments of this kind were attempts to condense D(—)-penicillamine with the azlactone (XCIII ; X = OMe, R = CH_2Ph), a reaction which was stated to afford a product having an *in vitro* activity of 0·5 unit/mg., and also an *in vivo* activity.[131] In a similar reaction between 2-phenyl-4-ethoxymethyleneoxazolone and penicillamine methyl ester a crystalline product was isolated which was probably the penicillenate (XCVI) though it was at the time regarded as the isomeric thiazolidine–oxazolone. These results were not received in Great Britain until some time later, and meanwhile various other ethoxymethyleneoxazolones had been prepared. Of these 2-styryl-4-ethoxymethyleneoxazolone on condensation with penicillamine afforded a product also thought to be a thiazolidine-oxazolone which had a small bacteriostatic activity,[132] and from this time on attempts were extended to other oxazolones, cysteine

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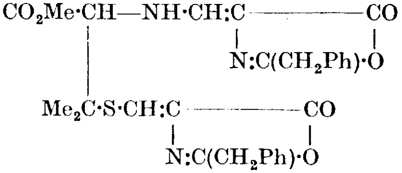
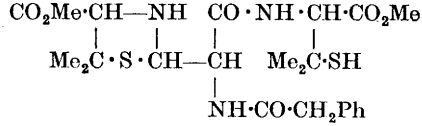
[131] M 10.

[132] E. P. Abraham, W. Baker, E. Chain, J. B. Jepson, G. M. and R. Robinson, and J. P. Wilson, CPS 26, 30, 35.

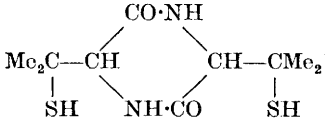
derivatives, etc. When using hydroxymethyleneoxazolones and D- or DL-penicillamine or its ester in aqueous media at varying pH, in dioxan, pyridine, or ester solvents at room temperature or slightly elevated temperatures (choosing conditions which would not preclude the persistence of any penicillin formed), the activities observed were at most only slight. In several instances the main products were isolated. Thus under slightly acid conditions a mixture containing penicillamine methyl ester and 2-benzyl-4-hydroxymethyleneoxazolone lost carbon dioxide,[133] presumably with the formation of a penilloic ester, as in similar experiments in other series.[134] Under rather different conditions condensation between equimolecular quantities of the reactants gave the corresponding penicillenate.[135] Again, in similar reactions condensation products between 2 mols. of oxazolone and 1 mol. of the mercapto-amine,[136] and between 1 mol. of oxazolone and 2 mols. of mercapto-amine,[133] have been isolated; these are regarded as (XCVII) and (XCVIII) respectively. Lastly it appears that oxazolones, having anhydride-like properties, are capable of converting the mercapto-amino-ester component into a diketopiperazine[137] (XCIX); diketopiperazine formation sometimes occurs very easily in this series.[138]



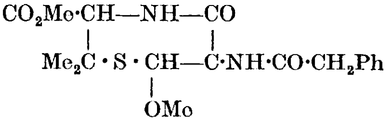
(XCVII.)



(XCVIII.)



(XCIX.)



(C.)

The reactions of alkoxy- (mostly ethoxy-) methyleneoxazolones with penicillamine and its esters present a similar picture. In acid media, and probably on fusion, penicilloic acids or their derivatives were formed,[139] whereas in neutral or basic (pyridine) media the main isolated products were penicillenates.[140] Occasionally products of other kinds were isolated such as those regarded as (C),[141] reflecting the acylating properties of oxazolones.

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[133] M 23.

[134] B 2; J. Attenburrow, D. F. Elliott, B. A. Hems, and F. A. Robinson, CPS 383.

[135] F. C. Copp, W. M. Duffin, S. Smith, and S. Wilkinson, CPS 542.

[136] B 4, 5.

[137] B 3.

[138] A. H. Cook, J. A. Elvidge, R. H. Hall, I. M. Heilbron, and G. Shaw, CPS 5, 273.

[139] H. J. Barber, R. Slack, P. Z. Gregory, C. E. Stickings, and A. M. Woolman, CPS 40, 66.

[140] See, for examples, M 10, 12, 15a and c, 23, 37, 47, 50, 76; I.C.I. Ltd., CPS 693; F. C. Copp, W. M. Duffin, S. Smith, and S. Wilkinson, CPS 424, 542, etc.

[141] M 37, 40, 47.

Clearly the small activities observed in such experiments were the result of side-reactions. They did not vary markedly on using different oxazolones (which included 2-methyl-, -propyl-, -*n*-amyl-, -phenyl-, -styryl-, -phenylethyl-, -*p*-nitrophenyl and -*p*-nitrobenzyl-, -*p*-nitrostyryl-4-alkoxymethylene [142, 143] -oxazolones); on the other hand DL-penicillamine gave lower activities than did D-penicillamine, and L-penicillamine afforded no activity, stereochemical specificity being thus indicated.[144] Mercapto-amino-acids other than penicillamine were also used with 2-benzyl-4-alkoxymethylene-oxazolones, the resultant small activities again being ascribed to "un-natural" penicillins; the acids so far used in this way were D- and DL-cysteine, DL-thiothreonine and DL-β-mercaptoleucine.[145]

Unpromising as were the small activities obtained with D-penicillamine and 2-benzyl-4-alkoxymethyleneoxazolones—the calculated yield of penicillin was about 0·06%—accumulating evidence pointed to their being due to that antibiotic. It should be mentioned that thiazolidines derived from penicillamine, penicillamine itself, and many of the reactants used showed antibacterial though not penicillin-like action ; indeed certain penicillamine esters exhibited a rather high degree of activity which, however, was useless for therapeutic purposes.[143] The penicillin-like activity mentioned above possessed the same instability as natural benzylpenicillin towards, for example, methanol or acid,[145] and towards the specific penicillin-destroying enzyme, penicillinase ; [146] further, it had the same relative activity towards a range of bacteria.[145] Ingenious evidence for the identity of the synthetic and natural antibiotics was provided by carrying out the reaction with DL-penicillamine containing radioactive sulphur, "diluting" the resulting activity with natural benzylpenicillin, and submitting the dilution to the normal concentration procedure. The recovered benzylpenicillin salts as well as the derived benzylpenillic acid contained an almost constant proportion of radioactive sulphur ; the radioactivity must almost certainly have been partitioned by the various operations if the synthetic activity had been due to a product of constitution other than penicillin.[147]

The first attempts to concentrate the activity by known methods of penicillin concentration,[148] aided by use of a semi-continuous counter-current extraction method,[149] gave materials containing 30—50 units/mg. and exhibiting the infra-red absorption band at 5·63 μ characteristic of benzylpenicillin. By repeated counter-current extraction from slightly acid solution the active material was finally obtained as its crystalline triethylammonium salt which was completely identical with the material

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[142] D. 32 ; E. P. Abraham, W. Baker, E. Chain, J. W. and R. H. Cornforth, and G. M. and R. Robinson, CPS 26, 35, 58, 59, 648, 649.

[143] F. C. Copp, W. M. Duffin, S. Smith, and S. Wilkinson, CPS 341.

[144] See, for example, M 44 ; E. P. Abraham, W. Baker, E. Chain, and R. Robinson, CPS 648.

[145] D 27, 37 ; M 78.

[146] E. P. Abraham, W. Baker, E. Chain, and R. Robinson, CPS 648.

[147] D 36.

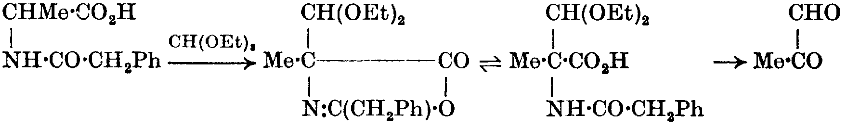
[148] M 15c ; U 26 ; D 38.

[149] L. C. Craig, *J. Biol. Chem.*, 1944, **155,** 519.

of natural origin.[150] Satisfactory as is the culmination of this synthesis, it must not be forgotten that the initial yield remains unsurpassed at *ca.* 0·1% and that the course of reaction has not been rationally elucidated; a synthesis of practical value thus remains as a most important objective.

(v) *Studies in the Synthesis of Penicillin Analogues.*—Some attempted modifications in the features of natural penicillins, such as the use of mercapto-amino-acids other than penicillamine and the inclusion of groups other than benzyl, amyl, etc., have already been mentioned or implied, but some more remote possibilities also warrant mention.

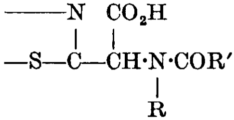
Following many attempts to prepare true 4-formyloxazolones efforts were made to enforce carbonyl activity on such products not only by inserting a 4-halogeno-atom as mentioned previously but also by inserting an alkyl grouping as shown at (CI).[151] The oxazolone structure was indicated



$$\begin{array}{l}\text{CHMe·CO}_2\text{H}\\ |\\ \text{NH·CO·CH}_2\text{Ph}\end{array} \xrightarrow{\text{CH(OEt)}_3} \begin{array}{l}\text{CH(OEt)}_2\\ |\\ \text{Me·C}\text{————}\text{CO}\\ |\qquad\qquad |\\ \text{N:C(CH}_2\text{Ph)·O}\end{array} \rightleftharpoons \begin{array}{l}\text{CH(OEt)}_2\\ |\\ \text{Me·C·CO}_2\text{H}\\ |\\ \text{NH·CO·CH}_2\text{Ph}\end{array} \longrightarrow \begin{array}{l}\text{CHO}\\ |\\ \text{Me·CO}\end{array}$$

(CI.) (CII.)

by the reactions shown, but acetals in this series were curiously inert; they could not be converted into corresponding aldehydes, and perhaps for this reason afforded no definite products with penicillamine.[152] Experience using norleucine instead of alanine derivatives was essentially similar except that a small degree of antibacterial activity was observed.[153] Experiments to convert the penicilloate analogues derived, say, from (CII) were subject to the same limitations as those with true penicilloates and were equally abortive.

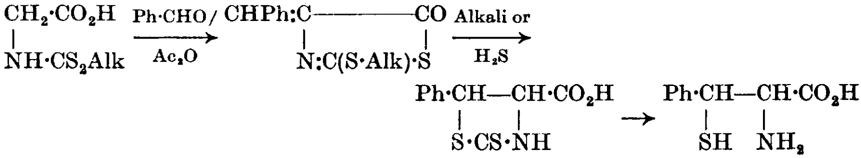


$$\begin{array}{l}\text{——N}\quad\text{CO}_2\text{H}\\ \qquad\ |\qquad |\\ \text{—S—C—CH·N·COR}'\\ \qquad\qquad\quad |\\ \qquad\qquad\quad \text{R}\end{array}$$

(CIII.)

Later, the device of inserting blocking substituents into the penaldates and derived thiazolidines as in (CIII) was considered as a means of enforcing lactam formation.[154] Orthodox synthetic methods were used and no outstanding activities were achieved.

Another exploratory effort included the use in place of penicillamine of β-phenylcysteine synthesised in unexpected fashion:[155]



$$\begin{array}{l}\text{CH}_2\text{·CO}_2\text{H}\\ |\\ \text{NH·CS}_2\text{Alk}\end{array} \xrightarrow[\text{Ac}_2\text{O}]{\text{Ph·CHO/}} \begin{array}{l}\text{CHPh:C————CO}\\ |\qquad\qquad\quad |\\ \text{N:C(S·Alk)·S}\end{array} \xrightarrow[\text{H}_2\text{S}]{\text{Alkali or}} \begin{array}{l}\text{Ph·CH—CH·CO}_2\text{H}\\ |\qquad |\\ \text{S·CS·NH}\end{array} \rightarrow \begin{array}{l}\text{Ph·CH—CH·CO}_2\text{H}\\ |\qquad |\\ \text{SH}\quad \text{NH}_2\end{array}$$

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[150] V. du Vigneaud, F. H. Carpenter, R. W. Holby, A. H. Livermore, and J. R. Rachel, *Science*, 1946, **104**, 431.

[151] See 65; also S 27, 31; D 28.

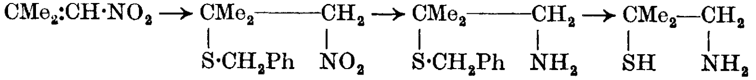
[152] See 151; F. C. Copp, W. M. Duffin, S. Smith, and S. Wilkinson, CPS 341.

[153] A. H. Cook, A. R. Graham, and I. M. Heilbron, CPS 552.

[154] U 18; M 63; cf. F. C. Copp, W. M. Duffin, S. Smith, and S. Wilkinson, CPS 642.

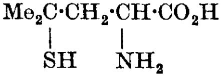
[155] A. H. Cook, A. R. Graham, G. Harris, and I. M. Heilbron, CPS 462, 583.

They included also the use of " decarboxypenicillamine " (CIV) prepared from α-nitro*iso*butene,[156] and of " homopenicillamine " (CV) which is formally capable of affording a 6-membered ring homologue of penicillin.[157] Still more remote analogues included the thiazolidine*iso*oxazolones (CVI), which were isomeric with " oxazolone " penicillin but biologically quite inactive.[158]



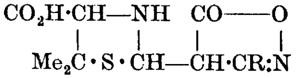
$$\mathrm{CMe_2{:}CH{\cdot}NO_2} \rightarrow \begin{array}{ll}\mathrm{CMe_2}\text{———}&\mathrm{CH_2}\\ | & | \\ \mathrm{S{\cdot}CH_2Ph} & \mathrm{NO_2}\end{array} \rightarrow \begin{array}{ll}\mathrm{CMe_2}\text{———}&\mathrm{CH_2}\\ | & | \\ \mathrm{S{\cdot}CH_2Ph} & \mathrm{NH_2}\end{array} \rightarrow \begin{array}{ll}\mathrm{CMe_2}\text{—}&\mathrm{CH_2}\\ | & | \\ \mathrm{SH} & \mathrm{NH_2}\end{array}$$

(CIV.)



$$\begin{array}{lll}\mathrm{Me_2C{\cdot}CH_2{\cdot}} & \mathrm{CH{\cdot}CO_2H} \\ | & | \\ \mathrm{SH} & \mathrm{NH_2}\end{array}$$

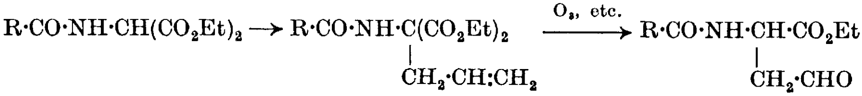
(CV.)



$$\begin{array}{llll}\mathrm{CO_2H{\cdot}CH} & \text{—NH} & \mathrm{CO} & \text{——O}\\ | & | & | & | \\ \mathrm{Me_2C{\cdot}S{\cdot}CH} & \text{—} & \mathrm{CH{\cdot}CR} & \mathrm{{:}N}\end{array}$$

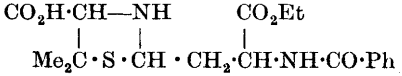
(CVI.)

The preparation of ethyl " homopenaldate " and " norhomopenaldate " (CVII ; R = CH_2Ph and Ph respectively) was accomplished,[159] and the products were condensed with cysteine or penicillamine to give, for example, (CVIII). When these thiazolidines were heated in quinoline or in other ways they lost the elements of ethanol just as glutamic ester affords pyrrolidonecarboxylic ester, so that the products were presumed to be γ-lactams of type (CIX).[160] That they did in fact have that structure was illustrated most convincingly by their desulphurisation to give acids of composition corresponding to type (CX). All these analogues were however devoid of the biological properties and characteristic physical and chemical properties of true penicillins.



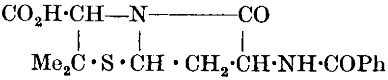
$$\mathrm{R{\cdot}CO{\cdot}NH{\cdot}CH(CO_2Et)_2} \rightarrow \begin{array}{l}\mathrm{R{\cdot}CO{\cdot}NH{\cdot}C(CO_2Et)_2}\\ \quad | \\ \mathrm{\quad CH_2{\cdot}CH{:}CH_2}\end{array} \xrightarrow{\mathrm{O_3,\ etc.}} \begin{array}{l}\mathrm{R{\cdot}CO{\cdot}NH{\cdot}CH{\cdot}CO_2Et}\\ \qquad\qquad | \\ \qquad\qquad \mathrm{CH_2{\cdot}CHO}\end{array}$$

(CVII.)



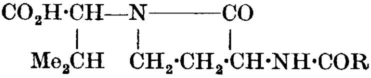
$$\begin{array}{llll}\mathrm{CO_2H{\cdot}CH} & \text{—NH} & & \mathrm{CO_2Et}\\ | & | & & | \\ \mathrm{Me_2C{\cdot}S} & \mathrm{{\cdot}CH} & \mathrm{{\cdot}CH_2{\cdot}} & \mathrm{CH{\cdot}NH{\cdot}CO{\cdot}Ph}\end{array}$$

(CVIII.)



$$\begin{array}{llll}\mathrm{CO_2H{\cdot}CH} & \text{—N} & \text{————} & \mathrm{CO}\\ | & | & & | \\ \mathrm{Me_2C{\cdot}S} & \mathrm{{\cdot}CH} & \mathrm{{\cdot}CH_2{\cdot}} & \mathrm{CH{\cdot}NH{\cdot}COPh}\end{array}$$

(CIX.)



$$\begin{array}{llll}\mathrm{CO_2H{\cdot}CH} & \text{—N} & \text{———} & \mathrm{CO}\\ | & | & & | \\ \mathrm{Me_2CH} & \mathrm{CH_2{\cdot}} & \mathrm{CH_2{\cdot}} & \mathrm{CH{\cdot}NH{\cdot}COR}\end{array}$$

(CX.)

The author thanks Sir Ian Heilbron, D.S.O., F.R.S., for his interest and encouragement, and Dr. G. Harris for invaluable help in checking the manuscript of this Review.

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[156] H. R. V. Arnstein, A. H. Cook, and I. M. Heilbron, CPS 640.

[157] J. R. Catch, A. H. Cook, and I. M. Heilbron, CPS 639.

[158] A. H. Cook, I. M. Heilbron, and G. Shaw, CPS 106, 204.

[159] D 21.

[160] D 26, 33 ; see also P. Sykes and A. R. Todd, CPS 677.