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Historical Essay

Robert Robinson and penicillin: an unnoticed document in the saga of its structure

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Abstract: A description and commentary is given for an unnoticed sheet of formulae and arguments in the hand of Robert Robinson concerning the structure of penicillin. In this undated document, probably of Autumn 1944, he set out his arguments for favouring a thiazolidine-oxazolone structure over the actual β-lactam structure. Copyright © 2007 European Peptide Society and John Wiley & Sons, Ltd.

Keywords: Robinson Robert; penicillin, structure of

Robert Robinson, perhaps the greatest organic chemist of the last century, made fundamental contributions to the structural analysis, synthesis, and biosynthesis of diverse natural products and to the electronic interpretation of reaction mechanisms. Alkaloids, steroids, and plant pigments were his dominant natural product themes, but he also had a little-known interest in peptides [1]. An energetic and forceful personality, he was legendary for the instinct and speed with which he solved chemical problems, and he was held in awe by his co-workers, who included several of independent fame later. His genius did not often let him down, but he stumbled over the structure of penicillin, work on which began in Oxford and was expanded to involve a large transatlantic cooperative consortium 1943–1945. Their wartime endeavours were not published in the conventional journal way, but all together, in a great treatise of over a thousand pages with hundreds of contributors, *The Chemistry of Penicillin*, in 1949 [2]. It was probably the most intensive investigation of any chemical problem ever undertaken.

We think now of antibiotics as a wonderful blessing to suffering humanity, but the immediate significance of penicillin in 1943 was the military advantage it might give in the theatres of war. The cooperative study was a secret project with the aim of discovering a means of producing it by chemical synthesis. Ironically, just as the therapeutic potential of penicillin had been proved in almost complete ignorance of its structure, the work on its chemistry played very little part in the development of its production. Culture and extraction were far from trivial, but were worked up to practicability before the structure was proved, and long before the never-to-be competitive total synthesis was achieved.

The groups in the transatlantic consortium shared results through confidential reports which were circulated only to those involved, and which were later the basis for *The Chemistry of Penicillin*. Because of the urgency with which the work was undertaken, the very large number of people and laboratories involved, and communication delays, there was much duplication of effort and discovery. It is therefore difficult to establish an exact history of the swings of opinion, and the paths to the correct structure, and to ascribe credit for the critical steps to particular groups or workers. Numerous accounts have been written which conflict in detail [3–12]. Correspondence between some of the principal chemical investigators in England - Robinson, EP Abraham, JW Cornforth, and Wilson Baker – some 30 years later [13] shows that their memories were not fully consistent, and in any case Abraham wrote then 'My own feeling is that it is seldom easy or advisable to try to analyse in detail and in retrospect the individual contributions of people who were working together' [14]. Probing is nevertheless a temptation. It was after all the most intensive chemical investigation of the century, the focal substance was of enormous importance, and many of those involved were Nobel Laureates in waiting, or of that calibre.

The structure was eventually figured out semi-independently on both sides of the Atlantic, but we concern ourselves here largely with the Oxford work. The credit for its final stages goes much more to Robinson's junior associates than to him. He was very reluctant to accept the correct β-lactam ring structure (**1**) which Abraham proposed and they also favoured. Robinson clung doggedly, and ultimately unreasonably, to his thiazolidine-oxazolone structure (**2**).

The investigation of penicillin, which had been discovered by Alexander Fleming at St Mary's Hospital in London ten years earlier, but not taken serious advantage of, was initiated in the Sir William Dunn

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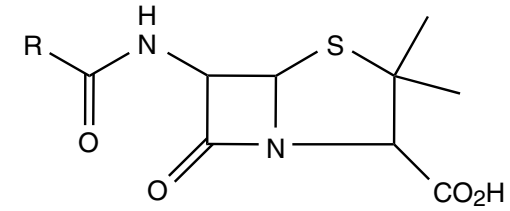

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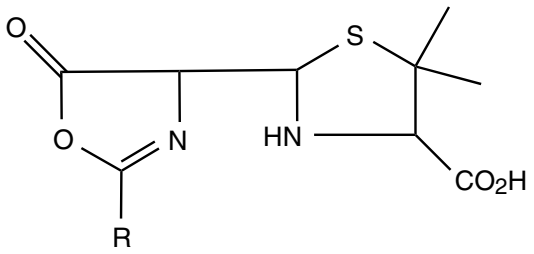

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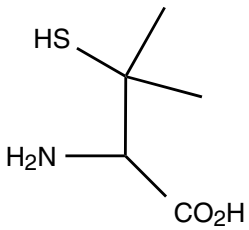
School of Pathology in Oxford by Howard Florey in 1938. Florey recruited to his team in turn Ernst Chain, a refugee from Hitler, Norman Heatley, and Abraham, a former student of Robinson's who was a Rockefeller Fellow in Stockholm when war was declared, but found his way back to Oxford. The therapeutic value of penicillin, which was at that stage a very impure and scarce preparation of largely unknown composition, against virulent infections in mice was demonstrated in 1940, and in human patients during 1941: the first patients to receive penicillin therapy were very gravely ill and died nevertheless, but only after impressive abatement or cure of their infections. It therefore became important to discover exactly what penicillin was, and to obtain useful quantities of it. Heatley engaged with the production of the material by culture and extraction, Abraham and Chain largely with the chemistry. Collaboration with Robinson, Wilson Baker, and then JW Cornforth in the nearby Dyson Perrins Laboratory began in late 1942.

Reasonably pure material was obtained by the first half of 1943, but progress was confounded for some time because the analysts had reported that there was no sulphur present, contrary to Robinson's suspicion. This was probably to do with the fact that the Oxford team were working with a barium salt. Sir John Cornforth has recently suggested [15] that in the method used for sulphur determination, after gross nitric acid oxidation of the analytical sample, the mixture was filtered to remove insolubles before the usual precipitation of any sulphur present as barium sulphate. If so, then all the sulphur would already have been lost before determination. The analysts were FB Strauss and Gerhard Weiler, who ran a semi-independent analytical business based in the Dyson Perrins Laboratory [16]. They continued there for some 30 years after the War, giving a excellent in-house service and also satisfying their external clients. A member of the Dyson Perrins sixties generation remembers well that any allegation that they had fouled up an analysis was always proved unfounded, and surreptitious submission of pure standards to check up on them always vindicated them. They were not sloppy or unreliable. Failure to find sulphur was repeated, not a one-off mistake such as any analyst might make. Probably the error was simply in failing to tell them the material was a barium salt.

Wilson Baker proved [17] by qualitative testing in mid 1943 that sulphur was in fact present in the key degradation product penicillamine (**3**), and therefore in penicillin itself. He had had discussions with Dorothy Hodgkin and Abraham, but he was alone one lunchtime when he had a eureka moment and decided the analysts must be wrong, which he confirmed in a few minutes by doing a sodium fusion test. In 1971 Abraham wrote to him [13] 'It seems clear to me that you broke the intellectual barrier set up by the reported failure of Weiler & Strauss to find sulphur in our best preparations of penicillin'. Not long after the discovery of sulphur (which was also found in America independently at about this time), Cornforth deduced and proved the structure of penicillamine synthetically [18].

Around the same time, it was reported from America by telegram [19] that the sodium salt of the penicillin being investigated there was crystalline, the Oxford antibiotic was crystallised, and the empirical formula was firmly established. As the pace quickened it also emerged that the side-chain of the American material (R = $PhCH_2$, benzylpenicillin, penicillin G) was different from that of the then Oxford material (R = pent-2-enyl), not that it made a significant difference to the relevant chemistry.



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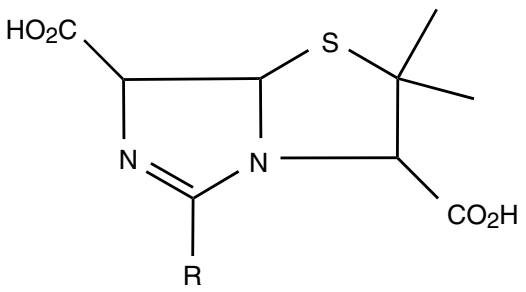

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By October 1943, sufficient information about the properties of penicillin and its various degradation products was available for Robinson to propose the thiazolidine-oxazolone structure. An important plank

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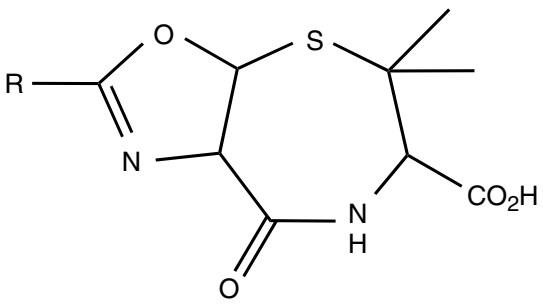
of his case was that this structure provided a simple explanation for the rearrangement of penicillin to penillic acid (**4**) under acidic conditions: see Figure 1. Abraham however was not sold on this structure, because he could not find any basic group by titration, and he put forward the β-lactam structure (**1**). Chain and Baker were swiftly convinced of its candidacy, and in Robinson's absence from Oxford this structure was included in the report which had been drafted proposing the thiazolidine-oxazolone structure (**2**), and it was submitted [20]. He was furious on his return next day, and added a note of dissent [21]. 'One of us considers the four-ring formula above somewhat improbable…' he wrote, continuing that he was 'of the opinion that the simpler constitution', the thiazolidine-oxazolone structure, 'cannot be excluded in view of the possible effect of the substituents on the basicity of the ·NH· group.' Of alternatives to his favoured structure, he preferred one with fused seven-membered and five-membered rings (**5**), which does not seem to have attracted any other credence.



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Opinion gradually firmed up during 1944 in favour of the β-lactam structure, and it was finally confirmed to the satisfaction of practically everyone except Robinson by Dorothy Hodgkin in the Spring of 1945, using X-ray crystallography [22]. His immediate reaction to the X-ray results was suspicion that they were false because the X-ray exposure had changed the penicillin. That was swiftly refuted by showing that the irradiated material retained activity, but even then he was obdurate.

In *The Chemistry of Penicillin* [23], he gave a minority view in which he wrote '…. the almost universal acceptance of the plain β-lactam structure is perhaps too complaisant', and went on to develop a laboured argument that whilst 'it was beyond question how the chief atoms of the skeleton are arranged in the crystal molecules', penicillin was not really a 'plain' β-lactam, but a mobile system comprising structures which he represented as reproduced in Figure 2. The thiazolidine-oxazolone structure XXXIX is in reversible equilibrium with a 'dipole variant electromer' XL, he suggested, and thence with a β-lactam 'protonomer' XLI; a 'mere electromeric change' with 'displacement of electrons backward or forward' providing 'a simple relation between the oxazolone and β-lactam structures'. 'A pendulum-like swing involves their interconversion', he concluded. He expounded the same theme in his Ramsden Memorial Lecture in 1950: 'the two structures are protono-electro-isomers', 'the endpoints reached by a swing like displacement' of electrons and a proton. The picture painted is reminiscent of the confusion between resonance and tautomerism sometimes suffered by present-day undergraduates.

From time to time, right to the end of his life thirty years later, he clutched at straws, pointing to perceived close similarities between the two structures and uncertainties about exactly what form the penicillin molecule had in solution [24–26].

The recent rediscovery in the Museum of the History of Science at Oxford of Robinson's own analysis of the arguments for his favoured structure versus the β-lactam structure is therefore of interest. It is a single 317 × 392 mm sheet of thin cream card written entirely on one side in ink in Robinson's hand. It was folded into four. Impressed from the back bottom left is a small circular stamp with the legend BRISTOL BOARD

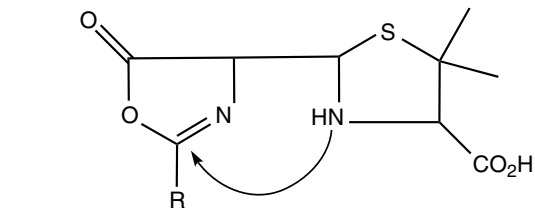

Figure 1 An internal attack in the thiazolidine-oxazolone structure (**2**) which could give penillic acid (**4**).

DIPOLE VARIANT ELECTROMER OF THIAZOLIDINE-OXAZOLONE (**XXXIX**); PROTONOMER OF β-LACTAM.

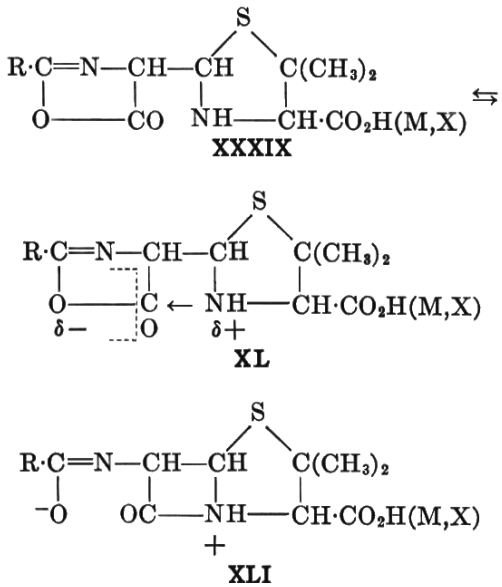

Figure 2 Robinson's post-War formulations of penicillin, reproduced directly from *The Chemistry of Penicillin* (1949).

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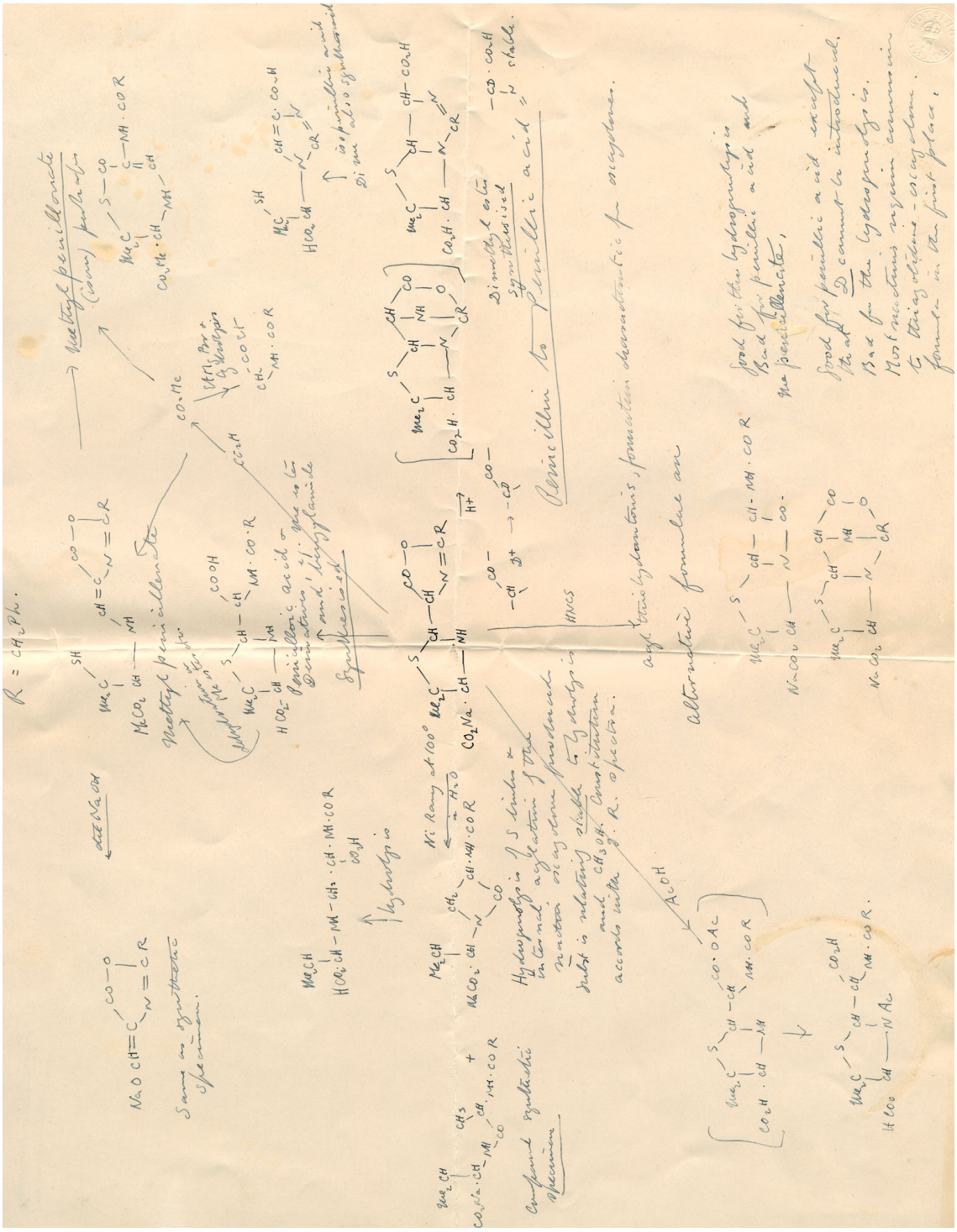

Figure 3 Robinson's penicillin scheme (1944), reproduced directly from the original (MS Museum 356) in the Museum of the History of Science, Oxford.

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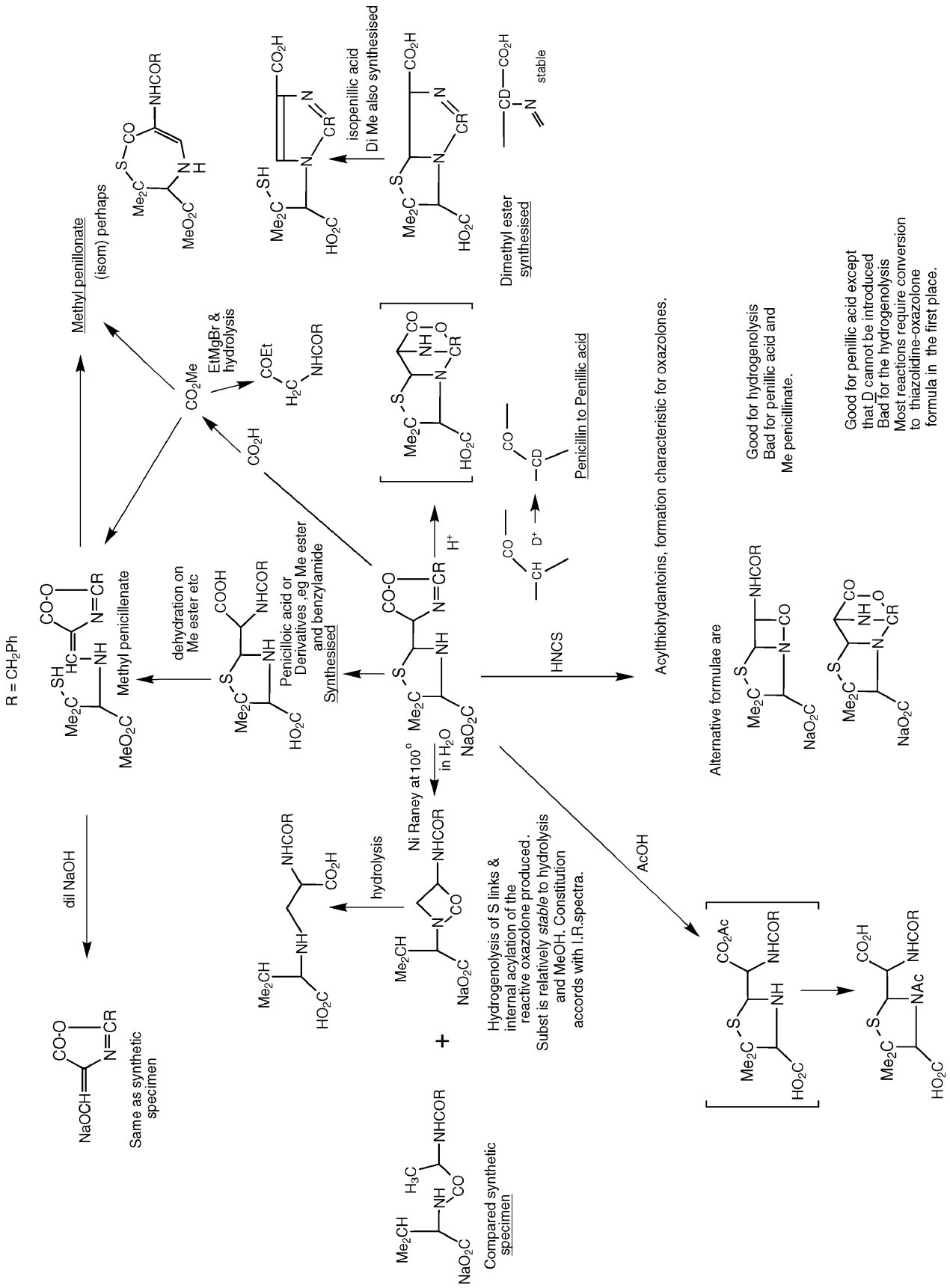

Figure 4 A transcript of the above, with minimal editing for clarity.

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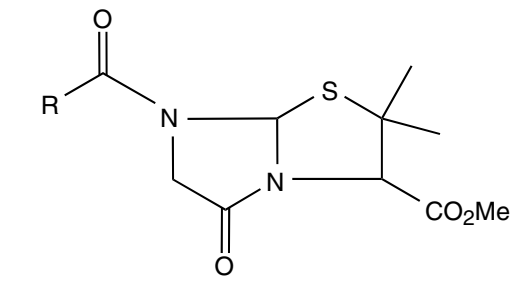
round the edge and a crown over a capital R in the middle. No watermark is discernable. It is slightly foxed and somewhat stained with cup-marks. It is reproduced here as Figure 3, together with a transcript in Figure 4. The structures are all drawn backwards according to the convention which was established soon afterwards and is still followed. If we regard penicillin as a tripeptide derivative, which we of course now know it is, then Robinson's representations place the *N*-terminal on the right instead of the left. The document is endorsed in the hand, which is very familiar to JHJ, of Sir Ewart Jones: 'RR notes on Penicillin structure'. Jones, who succeeded Robinson as Waynflete Professor of Chemistry at Oxford in 1955, was something of an archival squirrel, and collected miscellaneous items of historical interest in connection with the Dyson Perrins Laboratory which eventually found their way in no particular order to the Museum. Unfortunately Jones did not record how he came by the penicillin document, but we can safely conjecture that he found it left behind in the Oxford professorial office when Robinson retired. At any rate there is no doubt about its authenticity and identification.

The document is undated, but can be placed in the Autumn of 1944 on internal evidence concerning chemistry established in America, which became available to Robinson through the secret reports. It cannot postdate late 1944: the correct structure (**6**) for methyl penillonate, differing from that in Robinson's scheme top right, had been established, confirmed by synthesis, and published within the secret circle by then [27]. Nor can it predate the Summer of that year: that was roughly when the Raney Nickel desulphurisation work, which appears in Robinson's scheme mid left, began [28]. Further confirmation of the date can be inferred from the observation in the scheme that the penicillin system gives acylthiohydantoins, a reaction characteristic of oxazolones, on treatment with HNCS. The reaction of benzylpenicillin methyl ester with HNCS was reported in October 1944 to give the acylthiohydantoin (**7**) [29]; but this structure was in serious question by January1945, and proof that the derivative was in fact a thiouracil (**8**) was reported in mid 1945 [30]. The appearance of the tricyclic structure bottom right of centre in Robinson's scheme is also consistent with the proposed dating; although never a front-runner, it was on the table and had not been ruled out.

There is no clue to the purpose for which Robinson prepared his scheme; perhaps for a discussion such as that shown in the familiar [31] conversation piece photograph of him in his study with Abraham, Baker, and Chain.

How did Robinson come to be barking up the wrong tree here? Well, he had had thirty odd years of being right, and was a man whose personality by all accounts brooked little argument. It seems he was overconfident of his own judgement here, and whereas the thiazolidine-oxazolone structure fitted with his ideas



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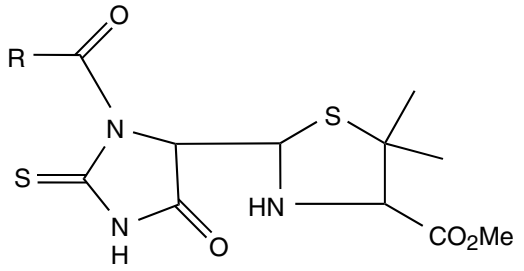

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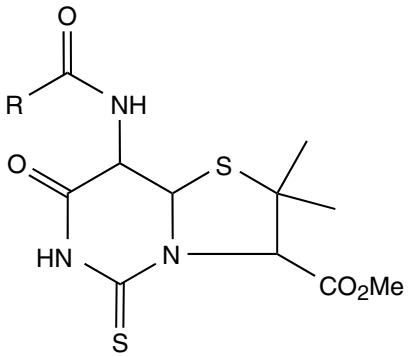

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about reaction mechanisms and reactivity, the β-lactam structure did not. Few β-lactams were known at the time, none had been found in Nature, all were monocyclic, and they were not easily hydrolysed. Rather than bend his ideas to the facts, he preferred to stick with his instincts, even when that meant brushing aside objections such as those based on acid-base properties. The thiazolidine-oxazolone structure is not only unsatisfactory because no basic group was found, but also because the pK_a of the acidic group in penicillin is too low at 2.7 [32] for it to be a simple thiazolidine-4-carboxylic acid, and implies a nearby strong electron-withdrawing influence, such as is there in the β-lactam structure (which is also an *N*-acylthiazolidine). It is noticeable that Robinson's scheme does not introduce these points at all. And the penicillin-penillic acid change which so concerned him can, as outlined by Woodward in *The Chemistry of Penicillin* [33], be accommodated easily by the β-lactam structure when it is appreciated that the strained system is poised for internal acylation: Figure 5. As Sheehan wrote [34] of Robinson later, 'Even the most brilliant people have their blindspots.' Dorothy Hodgkin, also much later [26], compared Robinson's persistent belief in the

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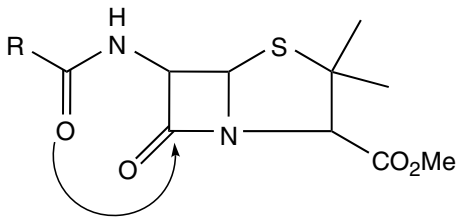

Figure 5 An internal attack in the β-lactam structure (**1**) which could give penillic acid (**4**) after further intramolecular reaction.

thiazolidine-oxazolone structure with Dorothy Wrinch's obsession with the cyclol hypothesis [1].

There may be something in the analogy, which would probably have infuriated Robinson. But whereas Wrinch's reputation was damned for good, Robinson's abberation has rightly been completely overshadowed by all his other achievements. In any case, although his stance may have hindered progress a bit, it probably also stimulated the others involved to prove the great man wrong.

Acknowledgements

We are grateful to Tony Simcock of the Oxford Museum of the History of Science for kindly supplying a scan of the document and in other ways, to Sir John Cornforth CBE FRS for patient and helpful correspondence, and to Georgina Ferry, Margery Ord, Marjorie Senechal, and Lloyd Stocken for assistance on various points; but all the opinions and deductions herein are entirely our responsibility.

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