Tetrahedron Letters No.39, pp. 3385-3388, 1969. Pergamon Press. Printed in Great Britain.

MERCURIC ACETATE OXIDATION OF AN ANHYDROPENICILLIN. ANHYDRO-α-PHENOXYETHYLPENICILLENE, A NOVEL ANTIBACTERIAL AGENT

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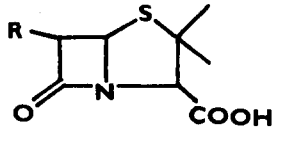
(Received in USA 9 July 1969; received in UK for publication 21 July 1969)

A penicillanic acid (1)[1] contains two more hydrogens than a Δ^3-cephem (2)[2]. In principle, therefore, a conversion of 1 into 2 would require both an oxidation and a rearrangement of the penicillanic acid nucleus. In the course of this process the two electrons lost from 1 must come ultimately from one of the methyl groups of the thiazolidine ring. A brilliant practical solution to this problem has been reported[3].



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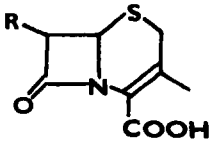

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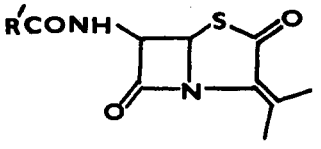

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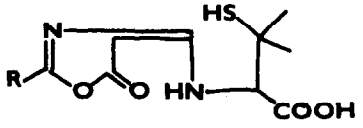
Anhydropenicillins (3)[4] appeared initially to be suitable substrates for the oxidation step, by allylic substitution on one of the methyl groups. However, these compounds have proved to be resistant to the action of lead tetraacetate and selenium dioxide, and are recovered quantitatively. In contrast, the action of mercuric acetate results in a novel oxidative rearrangement to form substances with antibacterial activity. Thus, anhydro-α-phenoxyethyl-penicillin (3, R'=$PhOCHCH_3$-)(3g) and mercuric acetate (30g) were refluxed in benzene (150 ml) for 3 hr. The mixture was filtered and the filtrate shaken with saturated sodium bisulfite until there was no further precipitation of mercury. Thin layer chromatographic analysis of the product[5] and examination under ultraviolet light revealed unchanged anhydropenicillin as a black spot at R_f 0.5 and, as well, a blue spot at R_f 0.7. An extract of the fast moving substance displayed antibacterial activity on agar plates seeded with S. lutea. Prior treatment of the extract with penicillinase did not affect the activity. The extract did not induce penicillinase production with a strain of B. subtilis[6].

Repeated preparative t.l.c. was used to obtain material for structural work. Samples of the chromatographically homogeneous active material[7] analyzed as $C_{17}H_{16}N_2O_4S \cdot 2H_2O$ (Found: C,53.6; H,4.8) or as $C_{17}H_{16}N_2O_4S$ (Found: C,59.6; H,5.1), i.e., anhydro-α-phenoxyethylpenicillin minus 2H. The substance has $[\alpha]_D$ + 6° (chloroform) (the starting material has $[\alpha]_D$ + 89° (chloroform)). The i.r. spectrum shows peaks at 2.9, 5.58, 5.9, 6.0, 6.1 and 6.7μ (KBr) (the starting material has absorption at 3.0, 5.5, 5.9, 6.0, 6.1, 6.6 and 6.7μ (KBr)). The UV spectrum ($CHCl_3$) shows absorption at 269 nm (ε 10000) and 319 nm (ε 15000) (the starting material has a maximum at 269 nm (ε 12000). The n.m.r. spectrum ($CDCl_3$) shows peaks at τ 1.8 (<1H), 2.8 (5H,m), 5.2 (1H,q), 7.8 (6H), 8.4 (3H) (the starting material has peaks at τ 2.8 (5H,m), 4.1

(1H,q), 4.3 (1H,d), 5.2 (1H,q), 7.8 (6H,d), 8.4 (3H,d)). In the product, the 6-proton absorption at 7.8 and the 3-proton absorption at 8.4 consists in each case of two doublets, indicating that the active substance is a mixture of at least two closely related compounds.

The UV absorption at 319 nm is reminiscent of that observed[8] with penicillenic acids (4). Absence of the β-lactam protons from the n.m.r. spectrum and of the amide-II band from the i.r. spectrum are also consistent with the presence of this chromophoric system. The UV absorption at 269 nm together with the i.r. bands at 5.9 and 6.1μ and the n.m.r. absorption at τ 7.8 indicate that the α,β-unsaturated-γ-thiolactone survived the oxidation. Structures 5, 6 and 7 (R'= $PhOCHCH_3$-) are compatible with these spectral data and, for 5, geometrical isomers are also possible. We believe that at least two of these tautomeric structures are present and that perhaps only one is responsible for the antibacterial activity. Because of the combination of structural features in 5 the substance has been termed an anhydropenicillene.



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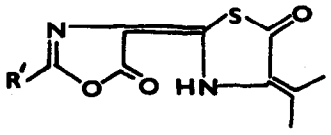

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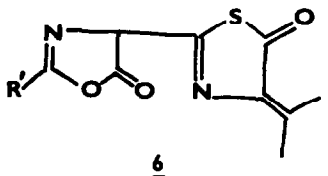

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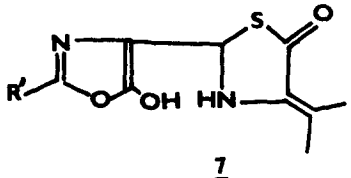

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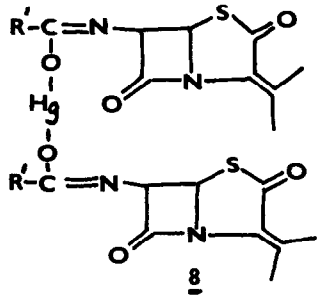
Alkaline hydrolysis (NaOH-MeOH-H_2O) of the anhydropenicillene afforded an optically active α-phenoxypropionic acid, m.p. 85-7° (Lit.[9] m.p. 87°) and racemic α-phenoxypropionic acid, m.p. 115-117° (Lit.[10] m.p. 115-116°), together with (+) α-phenoxypropionamide, m.p. 145-6° and racemic α-phenoxypropionamide, m.p.132-3° (Lit.[11] m.p. 132-3°). Alkaline hydrolysis of a penicillin or an anhydropenicillin does not produce the amide of the side chain acid.

Allylic acetoxylation by mercuric acetate is a much slower reaction[12] than the one observed here, and it is evident that the anhydropenicillin contains sites more susceptible to mercuric acetate than the carbon-carbon double bond. This is not surprising, in retrospect, since this double bond is part of an enamide[13]. Possible sites of oxidation by mercuric acetate are the amide side chain, the sulfur atom and the β-lactam nitrogen. When the oxidation of anhydro-α-phenoxyethylpenicillin was performed as described above, but for 1 hr, the products were unreacted 3 (17%), anhydropenicillene (7%) and a mercury-containing dimer, 8 (R'=$PhOCHCH_3$-), m.p. 206-208° (62%). Reaction for 0.5 hr produced unreacted 3 (38%) and 8 (53%). Thus 8 appears to be the first product of the reaction. However, 8 is not an intermediate in the formation of the anhydropenicillene; it is recovered in 80% yield after being refluxed for 2 hr in benzene and in 32% yield after being refluxed for 2 hr in toluene. Anhydropenicillene is not produced under these conditions.

Compound 8, $C_{34}H_{34}N_4O_8S_2Hg$, (Found: C,45.70; H,4.08; N, 6.15) has the following spectral data: UV (CH_2Cl_2), 272 nm (ε 21000); i.r. (KBr), 5.65, 5.91, 6.10, 6.13, 6.67μ; i.r. ($CHCl_3$) 5.65, 5.90, 6.09, 6.18, 6.69μ (i.e., no solvent dependent amide-I band). It is converted quantitatively to 3 and mercuric acetate upon treatment with acetic acid, and its formation is thus reversible.

We suggest that the anhydropenicillene is formed via attack of mercuric acetate on the β-lactam nitrogen of the anhydropenicillin. Subsequent azlactonization would produce the mercurated oxazolone 9; loss of a proton and mercurous acetate from 9[14] would generate one of the proposed tautomeric forms of the anhydropenicillene. To test this proposal, anhydro-α-phenoxyethylpenicillin was treated in methylene chloride at 0° with a molar equivalent of boron trifluoride etherate. During a 1 hr period the N-H,β-lactam, and amide-I and II peaks gradually disappeared from the infrared spectrum. The product isolated at this time is assigned the anhydro oxazolone structure 10: i.r. (CH_2Cl_2), 2.93, 5.67, 5.88, 6.06, 6.52, 6.69μ; n.m.r. ($CDCl_3$), τ 2.5-3.3 (5H,m), 5.25 (1H,d,J=7Hz), 5.3-5.7 (1H,q), 5.95 (1H,d,J=7Hz), 7.6 (1H, exchanges slowly with D_2O), 8.37 (3H), 8.5 (3H), 8.7 (3H,d). Refluxing 10 with mercuric acetate in benzene produced the anhydropenicillene, identified by t.l.c. and by its UV and antibacterial spectra. The proposed mechanism for the mercuric acetate oxidation is further supported by the finding that anhydro-6-N-phthaloylaminopenicillin, which is incapable of azlactonization, does not react with mercuric acetate.



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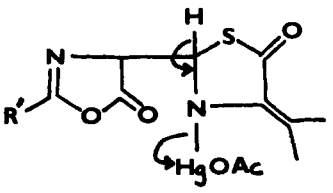

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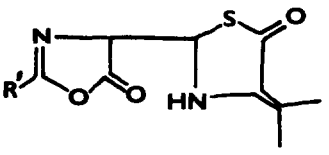

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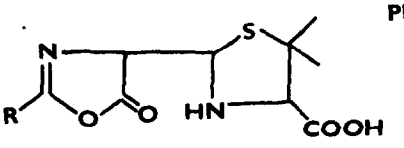
Anhydro-α-phenoxyethylpenicillene is therefore accessible from α-phenoxyethylpenicillin by a combination of two rearrangements and an oxidation. Since penicillins are convertible, under appropriate conditions, to thiazolidine-oxazolones (e.g., 11)[15] the possibility of modifying the timing of the two rearrangements was considered. Since azlactonization of penicillins is acid-catalyzed, and penicillin anhydrides will undergo the anhydropenicillin rearrangement[13], it was thought that the two rearrangements might occur concurrently in acetic anhydride. Penicillin V (5g) was therefore refluxed in acetic anhydride (50 ml) for one hour. Phenoxyacetic acid was obtained from the acidic portion of the reaction mixture; the neutral product (1.83g) yielded two isomeric compounds $C_{18}H_{18}N_2O_5S$ by chromatography on alumina (Woelm, grade III). One of these compounds melted at 172-175° and the other at 180-185°. Both compounds are optically inactive and their spectral characteristics are identical with those of the compounds 12 (two geometrical isomers) reported by Kukolja, Cooper and Morin[16]. Identity of the lower melting isomer prepared in the two laboratories has been confirmed by direct comparison.



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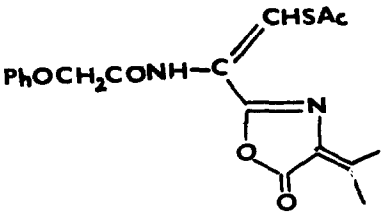

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Acknowledgments - We thank Bristol Laboratories for financial support of this work and Drs. Kukolja, Cooper and Morin for informing us of their related work in advance of publication.

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5. The plates were coated with Avicel, and were eluted with benzene-petroleum ether (30-60°), 1:2.

6. Anhydro-α-phenoxyethylpenicillin does induce penicillinase production under these conditions. We thank Dr. J.F. Collins for providing us with the procedure.

7. The following MIC values were obtained (γ/ml): Staph. aureus Smith, 0.5; S. aureus Smith after penicillinase treatment, 0.39; S. aureus 1633-2 (benzylpenicillin resistant), 3.13; S. aureus 52-75 (benzylpenicillin resistant), 12.5; Kleb. pneum., 3.13.

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