dictable changes in ultrastructure. Taking into account that the losses of P could be found already after 3 min of incubation in the last mentioned media, one would not recommend them for ID.

Conclusions. (1) No changes in N and P content are found after 3 min dehydration in anhydrous ethylene glycol, glycerol and DMSO; (2) one can find considerably high losses of N and P after 30 min incubation in 50% and anhydrous ethylene glycol, 80% dextrose and DMSO; (3) especially high losses of P in 50% ethylene glycol and 80% dextrose show that these media could not be recommended for ID technique.

Résumé. Les auteurs ont examiné les possibilités de diminution du contenu en azote et phosphore dans le foie du Rat au cours de l'«inert dehydration». La diminution la plus marquée a été observée après l'incubation dans une solution de glycol éthylène à 50% et de dextrose à 80%.

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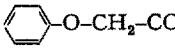
Photochemical Transformations of 6-Aminopenicillanic Acid and Phenoxymethylpenicillin

As part of an investigation concerning the influence of UV-irradiation on various penicillins, an aqueous solution of the potassium salt of 6-aminopenicillanic acid (6-APA; Ia) was irradiated in a quartz container with UV-light from a medium pressure mercury lamp (Hanovia, Type 509) at 15 °C. Bioautography of paper chromatograms of the irradiated solution on agar plates inoculated with *Staph. aureus* revealed that a new antibiotically active compound, somewhat more polar than 6-APA had been formed during the irradiation (Figure 1). The zone of inhibition due to the new compound, as well as that due to 6-APA, was considerably increased when the paper chromatograms before incubation were sprayed with aqueous $NaHCO_3$ followed by phenoxyacetylchloride or phenylacetylchloride in acetone according to BATCHELOR et al.[1].

The phenoxyacetyl derivative of the new compound, in the following called WG 942, was prepared and isolated as follows: after extraction with ether the irradiated solution was adjusted to pH 4.0 to precipitate most of the unreacted 6-APA, whereafter the filtrate was treated at pH 8 and 5–10 °C with excess of phenoxyacetylchloride in acetone. The resulting solution was washed with ether and, after acidification to pH 2.5, extracted with ethyl acetate. The extract, which in addition to WG 942 contained phenoxymethylpenicillin (Ib), phenoxyacetic acid, and other compounds, was subjected to a counter-current distribution (solvent system: citrate buffer (pH 5.0)-ethyl acetate) to separate the components. As revealed by bioautography of paper chromatograms, a complete separation of WG 942 from phenoxymethylpencillin was achieved after 40 upperphase transfers, and the compound could finally be isolated in the form of a crystalline potassium salt.

The elementary analysis of this salt corresponds well with the formula $C_{18}H_{20}N_3O_6SK$, $2H_2O$; its UV-spectrum is essentially the same as that of the potassium salt of Ib, and a strong band at 1773 cm^{-1} in the IR-spectrum (KBr) indicates the presence of a β-lactam ring. The fact that the compound is rapidly inactivated by *Bacillus cereus* penicillinase suggests that the fused thiazolidine-β-lactam ring system is intact. This assumption is supported by the NMR-spectrum[2] (D_2O) which contains signals closely corresponding to those present in the spectrum of the potassium salt of Ib: Singlets at $\delta = 1.78$ and 1.87 due to the methyl groups at C-2, a singlet at $\delta = 4.51$ due to the proton at C-3, a two-proton singlet at $\delta = 4.97$ arising from the methylene group in the grouping



an AB-system consisting of doublets centred at $\delta = 5.78$ and 5.83 ($J_{AB} = 4$ c/s) due to the protons at C-5 and C-6, and signals corresponding to 5 aromatic protons. In addition, a two-proton singlet at $\delta = 4.33$, not present in the spectrum of Ib, consistent with the presence of the grouping $-CONDCH_2CO-$[3] could be detected. The presence of latter signal suggested that WG 942 and its progenitor are represented by the structures Ic and Id, respectively. This assumption was verified by comparison of WG 942 with an authentic sample of Ic, prepared by acylation of 6-APA with phenoxyaceturic acid by the mixed anhydride method[4]. The 2 samples were identical

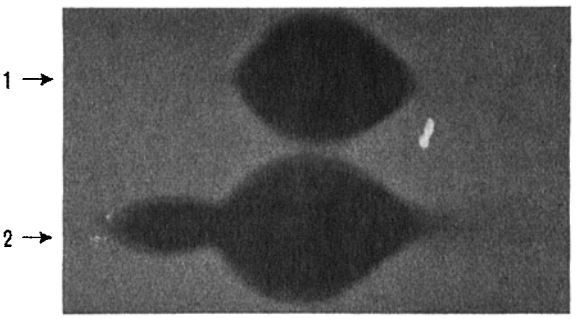


Fig. 1. Paper chromatography of an irradiated solution of 6-APA. Solvent system: *n*-Butanol-ethanol-water (5:1:4). Whatman No. 1 paper. Test organism: *Staph. aureus*. Before the incubation the paperchromatogram was sprayed with aqueous $NaHCO_3$ followed by 1% phenoxyacetylchloride in acetone. 1) 6-APA. 2) Irradiated solution of 6-APA.

[1] F. R. BATCHELOR, F. P. DOYLE, J. H. C. NAYLOR and G. N. ROLINSON, Nature, *183*, 257 (1959).

[2] The NMR-spectra were obtained with Varian HR-100 (100 Mc) and Varian A-60 (60 Mc) spectrometers, D_2O and $CDCl_3$ being used as solvents. In the former case hexamethyldisiloxane was used as external Lock signal whereas in the latter case tetramethylsilane was used as internal reference. We are indebted to Dr. J. R. ANDERSEN, University of Copenhagen and Dr. A. MELERA, Varian AG, Zurich, for the spectra.

[3] NMR-spectra Catalog (Varian Associated, Palo Alto. Calif., USA 1963) vol. 2, No. 413.

[4] Belg. Pat. No. 593,295.

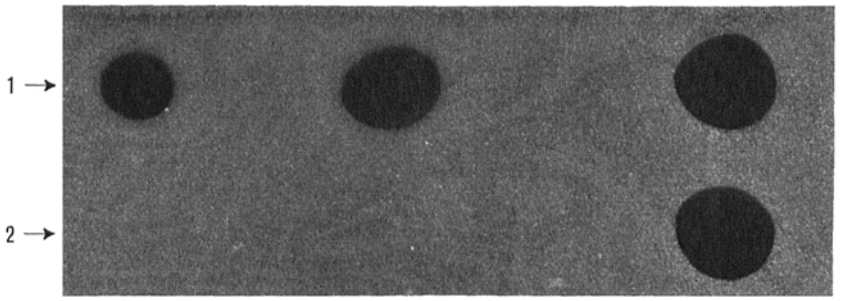
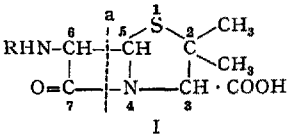


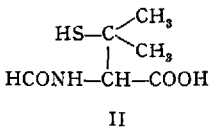
Fig. 2. Paper chromatography of an irradiated solution of potassium phenoxymethylpenicillinate. Solvent system: Acetate buffer (pH 5.6) – ethyl acetate. Whatman No. 1 paper. Test organism: *Staph. aureus*. 1) Irradiated aqueous solution of potassium phenoxymethylpenicillinate. 2) Phenoxymethylpenicillin.

in every respect (IR, UV, antimicrobial activity, Rf-values etc.).

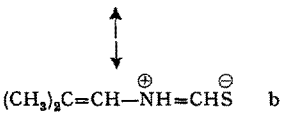


I

(a) R = H
(b) R = $C_6H_5OCH_2CO$
(c) R = $C_6H_5OCH_2CONHCH_2CO$
(d) R = H_2NCH_2CO
(e) R = $C_6H_5CH_2CO$
(f) R = *n*-C_3H_7CO
(g) R = CF_3CO
(h) R = *p*-$HOC_6H_4CH_2CO$
(i) R = *o*-$HOC_6H_4CH_2CO$



II

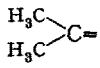


III

The formation of Id on irradiation of 6-APA must involve a fission of the β-lactam ring along *a* (Formula I), probably with formation of aminoketene or an equivalent which subsequently reacts with intact 6-APA to form Id. It is relevant in this connection that the presence of glycine and glycylglycine in the irradiated mixture has been demonstrated by thin-layer and paper chromatography.

In addition to Id several other compounds are formed on irradiation of aqueous solutions of 6-APA as revealed by paper and thin-layer chromatography. 2 of these have been isolated in a crystalline state. One is an optically active carboxylic acid $C_6H_{11}NO_3S$, m.p. 154 °C, $[\alpha]_D^{20} + 64.6°$ (*c* 1, pyridine). IR-studies suggested this compound to be the known N-formyl-D-penicillamine (II)[5], an assumption which was verified by comparison of its IR-spectrum with that of an authentic sample of N-formyl-D,L-penicillamine prepared by formylation of D,L-penicillamine according to JOHNSON and PANETTA[6]. Its specific rotation is in accordance with the reported value[6].

The other compound obtained in a crystalline state is an optically inactive compound, C_5H_9NS, m.p. 90 °C, which exhibits a strong UV-maximum at 304 nm (ε = 20,500). Its monomeric nature was certified by mass-spectrometric determination of the mol-weight. The NMR-spectrum ($CDCl_3$) shows the presence of the grouping



(singlets at δ = 1.78 and 1.81),

and contains doublets centered at δ = 7.11 (J = 10 c/s) and 9.19 (J = 7.5 c/s), each corresponding to one proton. In addition, a broad, ill-defined signal consistent with the presence of an –NH– group could be detected. After shaking the $CDCl_3$ solution with D_2O the latter signal disappeared, and the doublets at δ = 7.11 and 9.19 collapsed into singlets indicating that the corresponding 2 protons both couple with a proton, exchangeable by deuterium. In view of these observations this compound may be depicted as the hybride III.

According to bioautography of paper chromatograms on agar plates seeded with *Staph. aureus* irradiation of benzylpenicillin (Ie), *n*-propylpenicillin (If), and trifluoromethylpenicillin (Ig) does not result in the formation of new antibiotically active compounds but is accompanied by a gradual decrease of the antibacterial activity[7]. In contrast, irradiation of phenoxymethylpenicillin (Ib) rapidly gave rise to the formation of 2 antibiotically active compounds with lower Rf-values than their progenitor (Figure 2). The 3 compounds were readily separated by counter-current distribution in the solvent system: citrate buffer (pH 5.0) – ethyl acetate. The fact that both of the irradiation products were inactivated by *Bacillus cereus* penicillinase suggested that they were penicillins. The most polar could be identified as *p*-hydroxybenzylpenicillin (X-penicillin) (Ih) by comparison with an authentic sample. The less polar, which is the main product, gave *o*-hydroxyphenylacetic acid on acid hydrolysis and can therefore be assigned structure Ii. The reaction is consequently a photoarrangement of the Claisen type, analogous to those previously reported for aryl allyl and aryl benzyl ethers[9], aryloxyacetic acids[10], and aryloxyacetones[11].

Zusammenfassung. Bei der Bestrahlung von 6-Aminopenicillansäure mit UV-Licht entstehen Aminomethylpenicillin (Id), N-Formylpenicillamin (II) und ein Stoff der Summenformel C_5H_9NS, der vermutlich Struktur III besitzt. Phenoxymethylpenicillin wird bei der UV-Bestrahlung in ein Gemisch von *p*- und *o*-Hydroxybenzylpenicillin umgewandelt.

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Leo Pharmaceutical Products, Ballerup (Denmark), 23rd December 1966.

[5] *The Chemistry of Penicillin* (Ed. H. T. CLARKE, J. R. JOHNSON and R. ROBINSON; Princeton University Press, Princeton, N.J. 1949) p. 467.
[6] D. A. JOHNSON and C. A. PANETTA, J. org. Chem. *29*, 1826 (1964).
[7] A similar decrease in the activity of cephalosporin C on exposure to UV-light has recently been reported[8]. Although the chemical nature of this deactivation was not determined, it is reasonable to assume that it is related to the photochemical degradation of 6-APA described above.
[8] A. L. DEMAIN, Nature *210*, 426 (1966).
[9] M. S. KHARASCH, G. STAMPA and W. NUDENBERG, Science *116*, 309 (1952).
[10] D. P. KELLY and J. T. PINHEY, Tetrahedron Lett. *1964*, 3427.
[11] J. HILL, Chem. Comm. *1966*, 260.