

of it being extractable by cold water. It is not at present clear whether perhaps this portion was excreted through the skin and only adhered to the hair, the other half having been deposited in the hair keratin. Whatever the exact mechanism, it seems fairly certain that the eradication of dermatophytic infections by griseofulvin is due to its incorporation somehow in keratinous tissues.

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Effects of Deficiency of Pantothenic Acid on Oxygen Poisoning in the Rat

It has been known for many years¹ that a close connexion exists between pantothenic acid and the suprarenal glands, atrophy of which may result from a deficiency of pantothenic acid. More recently, Hurley and Morgan², arguing that animals in the final stages of pantothenic acid deficiency should behave as though adrenalectomized, showed that rats on such a diet were less resistant to the stress of anoxia than were normal controls.

It has, however, been shown that when the 'stress' is exposure to pure oxygen under high pressure, adrenalectomized rats fare better than do normal animals³⁻⁵. I found⁶ that the variability in the time of appearance of nervous system effects in oxygen poisoning was very great in adrenalectomized rats and could not be correlated with the presence of small amounts of residual cortical tissue. Since it seemed reasonable to assume that a dietary deficiency would affect all susceptible tissue, irrespective of its site in the body, it followed that a pantothenic acid deficiency might well dispose of aberrant cortical tissue in a way that operation could never do. If such deficient animals were at least as resistant as operated animals and showed less variability of response, the result would tend to confirm the hypothesis that in operated animals the variability was in fact due to aberrant cortical tissue.

A series of experiments was carried out to test the effects of exposure to pure oxygen under a pressure of 6 atm. on rats reared on a pantothenic acid-deficient diet. Eight litters of eight rats, four of each sex, were used. The basic diet was that already described by me⁷. Appropriate vitamin supplements other than pantothenic acid were added to it, and it was fed to the mothers from 12 days before weaning. On weaning, the young rats were divided into 4 groups as follows: *A*, rats fed the deficient diet *ad lib.*; *B*, rats given the basic diet with vitamin supplements, including pantothenic acid (that is, the control diet), and paired within litters with those of *A*; *C*, rats from which both adrenals were removed four days before exposure to oxygen and fed the control diet *ad lib.*; *D*, rats fed the control diet *ad lib.*

At the age of approximately 8 weeks the rats were exposed to oxygen under 6 atm. in a way already described⁸. Four male and two female rats in group *A* died before exposure to oxygen.

The mean times to the onset of severe poisoning, with standard errors, were, for the four groups, as follows: *A*, 49.75 ± 4.46 min.; *B*, 37.50 ± 1.94

min.; *C*, 45.12 ± 2.78 min.; *D*, 30.50 ± 1.09 min. Both the deficient and the operated rats (*A* and *C*) were more resistant than groups *B* and *D*, although, of these, the pair-fed rats *B* were more resistant than the controls fed *ad lib.* (*D*). There was no significant difference in resistance between groups *A* and *C*. Groups *A* and *C* were more variable than group *B*, itself more variable than the controls fed *ad lib.* (*D*).

The effects of pantothenic acid deficiency on oxygen poisoning thus resemble those of adrenalectomy. The problem of the variability of response in these animals remains unsolved. That the diet was effective was shown by the six deaths, by greying of the fur and by the relative failure of the rats to grow; but there was no evidence that time to onset of severe poisoning was related to body-weight. There was no relationship between weight of suprarenal tissue and time to onset of severe poisoning in group *A*, but three of the four most resistant animals had glands which were obviously haemorrhagic. It is not clear how to determine precisely, in terms of cortical function, the response of individual rats to the pantothenic acid-deficient diet, and thus one source of variability may have been substituted for another. Or it may be that the variability is due to a highly complex relationship between suprarenal cortical function and response to exposure to oxygen under high pressure.

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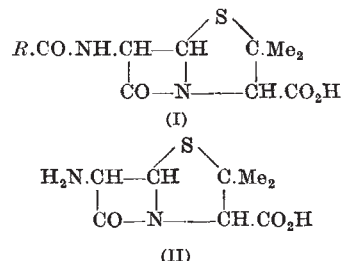
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Synthesis of Penicillin : 6-Aminopenicillanic Acid in Penicillin Fermentations

THE work reported here stemmed from the experiments described in an earlier communication¹ on *p*-aminobenzylpenicillin, in which discrepancies were observed between chemical and biological methods of penicillin assay when a precursor was omitted from penicillin fermentations.

The various penicillins (formula I) may all be regarded as acyl derivatives of a common parent amine, 6-amino-penicillanic acid (formula II: for nomenclature see Sheehan *et al.*²). We wish to report the isolation of pure 6-amino-penicillanic acid from penicillin fermentations carried out in the absence of added side-chain precursor, the culture used being an isolate from *Penicillium chrysogenum* W.51.20.



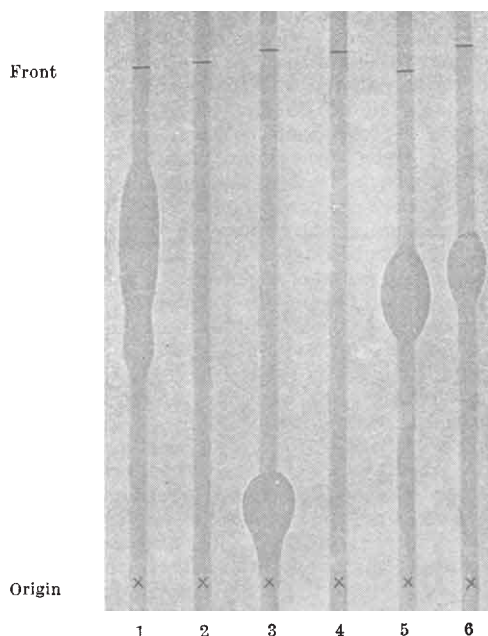


Fig. 1. Chromatograms of fermentation liquors. 1, Filtrate of fermentation carried out in the absence of side-chain precursors; 2, filtrate as (1) after extraction with butyl acetate at pH 2.0; 3, as (2) but the chromatogram sprayed with aqueous sodium bicarbonate and an acetone solution of phenylacetyl chloride before developing on the seeded agar plate; 4, solvent-extracted filtrate as (2) treated with penicillinase before chromatography and then sprayed with the solutions of sodium bicarbonate and phenylacetyl chloride as in (3); 5, solvent-extracted filtrate, as (2), treated with sodium bicarbonate and phenylacetyl chloride before chromatography; 6, penicillin G. Solvent: butanol/ethanol/water, 40:10:50, top layer; Whatman No. 1, running time 15 hr.

The presence of (II) was first indicated when such a fermentation liquor, after removal of natural penicillins by solvent extraction at low pH, was treated with an excess of phenylacetyl chloride in the presence of a weak base such as sodium bicarbonate. This resulted in the formation of an antibiotic substance which was readily destroyed by penicillinase and which behaved similarly to penicillin G on paper chromatograms. Similarly, reaction with phenoxyacetyl chloride resulted in the formation of an antibiotic resembling penicillin V in its stability in acid solution as well as its behaviour on paper chromatograms. The reaction with phenylacetyl chloride in the presence of sodium bicarbonate was readily adapted to the detection of (II) on paper chromatograms (Fig. 1) which were sprayed with the appropriate reagents before plating on agar seeded with a sensitive bacterium in the usual way. This conversion of (II) to benzylpenicillin on paper strips also provided a convenient method of assay, being similar to the well-known paper disk method.

Pure crystalline 6-aminopenicillanic acid, details of the isolation of which will be reported at a later date, had m.p. 208–209° C. (decomp.) (found: C, 44.6; H, 5.7; N, 13.1; S, 14.1 per cent; $C_8H_{12}O_3N_2S$ requires C, 44.4; H, 5.6; N, 13.0; S, 14.8 per cent). Phenylacetylation and subsequent biological assay on paper as outlined above gave 2,740 units of benzylpenicillin per mgm. of starting material (theory 2,752 units/mgm.). 6-Aminopenicillanic acid itself possesses definite antibacterial properties, but these are mostly of a much lower order than those of benzylpenicillin, and the spectrum is of a different type. It is destroyed by penicillinase

(*Bacillus cereus* broth filtrate) but at a much slower rate than is benzylpenicillin. Like the penicillins it is rapidly decomposed by strong alkali, but as would be expected from its structural inability to undergo rearrangement to a penillic acid, it is relatively stable to acids. The compound is itself a surprisingly strong acid, the isoelectric point being about 4.3.

Chemical evidence supporting the structure (II) has been obtained by treatment of the substance with ethyl chloroformate to give a crude N-carbethoxy derivative (I; $R = EtO$) which was degraded by the general method of Dutcher *et al.*³ to N-carbethoxy-aminoacetaldehyde 2:4-dinitrophenylhydrazone (51 per cent overall yield), bright yellow micro-needles from alcohol, m.p. 174–175° C., not depressed by admixture with a specimen synthesized from aminoacetal (found: C, 42.2; H, 4.2 per cent; $C_{11}H_{15}O_6N_5$ requires C, 42.4; H, 4.2 per cent).

Furthermore, the benzylpenicillin which had been prepared by phenylacetylation of (II) was isolated as the N:N'-dibenzylethylenediamine salt, which after crystallization from acetone had m.p. 114–115° C., not depressed by admixture with an authentic specimen⁴ (found: C, 59.4; H, 6.8; N, 9.0; S, 6.5 per cent; calc. for $C_{48}H_{56}O_8N_6S_2 \cdot 3H_2O$: C, 59.8; H, 6.5; N, 8.7; S, 6.7 per cent). A portion of this same phenylacetylation product was degraded to benzylpenilloaldehyde, which was isolated as the 2:4-dinitrophenylhydrazone, m.p. 196° C., not depressed by admixture with an authentic specimen⁵.

Various authors have expressed the view that the last stage in penicillin biosynthesis is the introduction of the acyl side-chain⁵. Our isolation of 6-aminopenicillanic acid is consistent with this view, but does not preclude the possibility of it arising during fermentation, either as an alternative metabolite or by enzymatic hydrolysis of a penicillin⁶. The substance is nevertheless a most useful chemical intermediate for the preparation of new penicillins and related compounds which are not easily accessible in other ways. The possible therapeutic usefulness of such derivatives is being investigated in these Laboratories (patent application pending, 1957).

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