# LETTERS TO THE EDITORS

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## Extraction and Purification of Penicillin

PUBLISHED methods for the extraction and purification of penicillin[1,2,3] are all based on the observation that penicillin can be extracted by ether, amyl acetate or chloroform from strongly acid aqueous solutions. Considerable losses of the antibacterial substance occur during these operations as penicillin is very rapidly destroyed in acid environment, particularly when shaken with air at room temperature.

A search was made for solvents which would extract penicillin from aqueous solutions at a *p*H not harmful to penicillin. It has been found that a large proportion of the antibacterial substance can be extracted by *n*-butyl alcohol from culture filtrates adjusted to *p*H 6·4, at which penicillin is most stable. When a suitable amount of ammonium sulphate was added to the culture filtrate, penicillin was then almost completely extracted by the butyl alcohol. The addition of ammonium sulphate was also of advantage as it precipitated the greater part of the inactive pigments. Penicillin was brought back into aqueous solution by the addition to the butyl alcohol extract of light petroleum ether and dilute sodium bicarbonate solution. During this procedure further purification of penicillin was effected as some impurities remained in the petroleum ether. The concentrated penicillin solution may be further purified by the usual method (ether extraction).

A typical experiment was as follows: 2,000 ml. of the crude culture fluid was adjusted with phosphoric acid to *p*H 6·4 and 800 gm. of ammonium sulphate was dissolved in it. A precipitate containing inactive proteins and pigments formed and was filtered off. 400 ml. of the filtrate was mixed with an equal volume of *n*-butyl alcohol and extracted by shaking. The same butyl alcohol extract was used for the subsequent extraction of four further 400 ml. portions of the culture fluid. To the strong butyl alcohol extract, 400 ml. in bulk, an equal volume of light petroleum ether was added. From this mixture penicillin was extracted by shaking into 200 ml. of a 2 per cent aqueous solution of sodium bicarbonate. The greater part of penicillin contained in the culture fluid was obtained in this way as an aqueous solution of the sodium salt of penicillin. The remaining penicillin was obtained by a second extraction of the butyl alcohol–petroleum ether mixture with another portion of the sodium bicarbonate solution.

The principal advantages of this method of extraction are: (1) there is no loss of penicillin during extraction as it is extracted at the *p*H point of its greatest stability; (2) the antibacterial substance is almost completely extracted from the culture fluid; and (3) considerable concentration and purification is achieved by the same process. Further advantages of the method are that extraction can be carried out at room temperature and that only relatively small quantities of solvents are required. The procedure is simpler and more efficient than other methods of purification and appears suitable for large-scale purification of penicillin.

I am much indebted to Prof. P. L. Sutherland for his advice and interest throughout the course of this investigation. My thanks are due to Mr. G. Denton for technical assistance.

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## Thiophanone Derivatives

FOLLOWING the recent papers of P. Karrer *et al.*[1,2,3,4] and the communications of E. R. Buchman and H. Cohen[5], R. B. Woodward and R. H. Eastman[6] and L. C. Cheney and J. R. Piening[7] on the synthesis of thiophanone derivatives, it seems desirable to direct attention to work carried out independently in this laboratory on the same subject.

Our method of preparing thiophanones is substantially the same as that of the above authors, and we have described it in the patent literature[8,9,10]. However, in our experiments on the ring closure of ethyl S-(β-carbethoxyethyl)-thioglycollate under the influence of powdered sodium in benzene, we found that ethyl thiophan-3-one-*2*-carboxylate always at least predominates, whereas Karrer *et al.*[1] and Buchman and Cohen[5] report the formation of the *4*-carboxylate. Our evidence for this assertion is based on the following products obtained from the Dieckmann reaction on: (i) ethyl S-(β-carbethoxyethyl)-thioglycollate, giving a cyclic keto-ester which yielded a crystalline derivative with urea[11], m.pt. 173°; (ii) methyl S-(β-carbethoxyethyl)-thioglycollate, giving a product, with urea derivative, m.pt. 222°; (iii) ethyl S-(β-carbmethoxyethyl)-thioglycollate, giving a product, with urea derivative, m.pt. 173° (admixture with that from (i) showed no depression); and finally (iv) methyl S-(β-carbmethoxyethyl)-thioglycollate, the urea derivative of the product of which melted at 222° and did not depress the melting point of that from (ii) on mixing.

That mixtures do occur was shown by subjecting *n*-butyl S-(β-carbmethoxyethyl)-thioglycollate to the same conditions (namely, powdered sodium in benzene), when *two* products were obtained. The main fraction was *n*-butyl thiophan-3-one-2-carboxylate (found: C, 52·7; H, 7·2; S, 15·6. $C_9H_{14}O_3S$ requires C, 53·4; H, 6·9; S, 15·8 per cent) and gave a derivative with urea, m.pt. 170–172° (found: C, 48·8; H, 6·3; N, 10·7. $C_{10}H_{16}O_3N_2S$ requires C, 49·2; H, 6·6; N, 11·4 per cent). The small low-boiling fraction gave a urea derivative, m.pt. 245°, the analysis of which showed it to be derived from methyl thiophan-3-one-4-carboxylate (found: N, 13·2. $C_7H_{10}O_3N_2S$ requires N, 13·9 per cent).

Although it was possible to introduce a side chain in the 2-position (as would be required to reproduce the β-biotin carbon skeleton) by condensation of the corresponding halogeno-compound with ethyl thiophan-3-one-2-carboxylate in the presence of sodium ethylate[10], nevertheless this method was soon replaced by the more satisfactory one of subjecting esters of the type $EtOOC.CH_2.CH_2.S.CHR.COOEt$ (in