α-Aminoisobutyric Acid, β-Hydroxyleucine, and γ-Methylproline from the Hydrolysis of a Natural Product

We have been examining the structure of an antibiotic supplied to us by the Pharmaceuticals Division of I.C.I., Ltd.; but the work has now had to be suspended for lack of material. As there will be a considerable delay before it can be resumed, some of the results, which may have a wider interest, are recorded here.

The antibiotic (I.C.I. No. 13959), which is unusually active against infections of *Trypanosoma congolense* in mice, was produced by a strain of *Paecilomyces*. It was extracted by ethyl acetate and purified by chromatography on silica and then on magnesium carbonate. The second column separated two non-crystalline fractions with antibiotic activity, eluted by benzene and methanol respectively. Most of our work has been done with the former but a cursory examination suggests that the latter has similar structural features to those reported below.

Several neutral amino-acids were produced from the antibiotic by hydrolysis with 6 *N* hydrochloric acid at 100° for 48 hr. They were separated by ascending two-dimensional paper chromatography with butan-1-ol (10) : ethanol (10) : water (5) : propionic acid (2) and then butan-1-ol (10) : acetone (10) : water (5) : di*cyclo*hexylamine (2)[1]. Five spots were produced with the following ninhydrin colours and R_F's in the two systems: *A*, blue, 0·60, 0·65; *B*, blue appearing only on heating to 100°, 0·50, 0·36; *C*, yellow, 0·45, 0·42; *D*, grey–green, 0·49, 0·86; *E*, greenish blue, 0·33, 0·35. On a semi-micro scale these amino-acids were separated almost completely by a 61-cm. column of 'Dowex'-50 × 2 cation-exchange resin[2]. They were eluted by *N* hydrochloric acid in the order *E*, *B*, *D*, *C*, *A*, and then recovered from their hydrochlorides with the aid of a short column of 'Dowex'-1 × 2 anion-exchange resin; amino-acids *A*, *B*, and *D* were obtained crystalline.

The identification of *A* with L-leucine was made by elementary analysis, infra-red spectrum, optical rotation and paper chromatography in six solvent systems. Likewise, *B* was found to be α-amino*iso*-butyric acid. This amino-acid has not previously been obtained from natural sources; its closest relatives are α-methylserine, also a component of an antibiotic[3], and 1-amino*cyclo*propane carboxylic acid, which has recently been found among the free amino-acids of pears and cowberries[4].

Like proline and hydroxyproline, amino-acid *C* gave a yellow colour with ninhydrin and a blue one with isatin, but was clearly differentiated from them by paper chromatography. It was partly crystalline but could not be recrystallized satisfactorily; the crystalline reineckate had an analysis (C, 25·4; H, 4·4; N, 21·9 per cent) not far from that required for a homologue of proline $(C_6H_{11}O_2N.Cr(SCN)_4(NH_3)_2H$ requires C, 26·8; H, 4·0; N, 21·8 per cent). As amino-acid *C* was distinguished from pipecolic acid by paper chromatography and the ninhydrin colour, the most likely structure was that of γ-methylproline, which has previously been found among the free amino-acids of apples[5]. Indeed, it appeared on paper chromatography to be identical with one component of a partially purified extract from apple juice, generously supplied by Mr. L. F. Burroughs of Long Ashton, Bristol. We therefore regard *C* as γ-methylproline; but proper identification must await comparison with a synthetic sample. Synthesis, which we are undertaking, may also answer the problems of diastereoisomerism and optical activity.

The conversions of amino-acid *D* into leucine by hydriodic acid and into glycine by sodium hydroxide[6], and its rapid destruction by periodate, were consistent with its formulation as β-hydroxyleucine. The DL-*threo* and *erythro* isomers of this amino-acid were synthesized by a method similar to one devised for the threonines[7]. As they were readily separable by paper chromatography in butan-1-ol saturated with 10 per cent aqueous diethylamine[8], it was possible to assign the structure D- or L-*threo*-β-hydroxyleucine to the natural product; its infra-red spectrum resembled that of the DL-*threo*-amino-acid but they were not identical. This is the first time that β-hydroxyleucine has been obtained from natural sources. Amino-acid *E* was shown to be β-alanine by paper chromatographic comparison and by the mixed melting point (140–141°) of its β-naphthalenesulphonyl derivative.

Hydrolysis of the antibiotic also produces basic substances; but discussion of their structures and of that of the antibiotic itself would be premature.

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3β-Methoxy-5-androsten-16β-ol: a Cytostatic and Fungistatic Steroid

In our search for a superior 'androgenic' steroid for use in human cancer of the breast, we have submitted many such steroids to study in the zebra fish-egg test[1]. Using this bioassay method for determination of cytostatic activity, intensely cytostatic compounds in the C-19 or 'androgenic' steroids are difficult to find. To date, our most active compound is 3β-methoxy-5-androsten-16β-ol[2], this steroid having *LD*50 of 1–2 parts per million. It is very interesting that this compound is the exact analogue of the steroidal œstrogen 3-methoxyœstra-1-3,5(10)-trien-16β-ol[3], which had been previously selected by us for use against cancer of the prostate, based on fish-egg cytostasis[4]. Also of much interest in steroidal cytostasis is the fact that methyl ether formation at C_3 of 5-androstene-3β,16β-diol[2] so strongly enhances the activity of this diol, which itself is virtually inactive (*LD*50 > 10 p.p.m.). Methyl ether formation at C_3 of 1,3,5(10)-œstratrien-3,16β-diol[3] had also caused an enhancement (100 per cent) of cytostatic activity in the fish-egg test.

We have also found 3β-methoxy-5-androsten-16β-ol to be among the strongest of fungistatic steroids so far tested by us. It is particularly active against