ACTA CHEMICA SCANDINAVICA 17 (1963) 933–936

Synthesis of ^{14}C-Labelled α-Aminobenzylpenicillin

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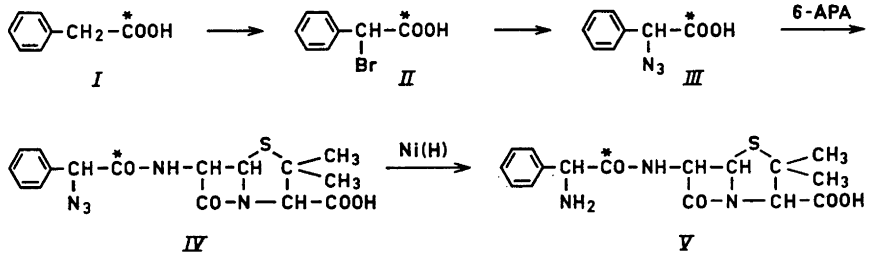
The synthesis of 6-(α-amino-phenyl-[1-^{14}C]acetamido)penicillanic acid is described. The aminopenicillin was obtained by hydrogenation over nickel of 6-(α-azido-phenyl-[1-^{14}C]acetamido)penicillanic acid. The labelled sidechain was prepared by a series of reactions starting from barium [^{14}C]carbonate.

α-Aminobenzyl penicillin is the first of the semisynthetic penicillins to show appreciable activity against Gram-negative organisms,[1] its enhanced activity over penicillin G obviously being due to the α-amino group in the side-chain [2]. The new penicillin is bactericidal, its activity against Gram-positive organisms equals that of penicillin G and it has an activity against various Gram-negative bacteria comparable to that of the broad-spectrum antibiotics such as the tetracyclines, and chloramphenicol [3]. Other advantages include high acid stability, rapid and good peroral absorption,[4] and low toxicity [5].

The mechanism of action and selective toxicity to bacteria of penicillins are related to an inhibition of the biosynthesis of the bacterial cell wall. A pertinent review has recently appeared [6]. It was of interest to see if this new broad-spectrum amino-penicillin has the same mode of action as previously tested penicillins and to compare its mechanism of action in both Gram-negative and Gram-positive bacteria. We therefore have prepared radioactive α-aminobenzylpenicillin.

Penicillins labelled with ^{35}S,[7] with ^{14}C in the nucleus [8] or in the side-chain [9] have been prepared by biosynthesis. For certain biological studies it is important to have the penicillin labelled exclusively in the side-chain. However, in fermentation using phenyl-[1-^{14}C]acetic acid as precursor, some of the radioactivity will also be found in the 6-aminopenicillanic acid moiety [9]. Biosynthetic methods are almost exclusively limited to the incorporation of monosubstituted acetic acids [10] and these methods are often complicated when high specific activities are required [11]. However, radioactive penicillins are more readily prepared synthetically by coupling appropriately labelled organic acids with 6-aminopenicillanic acid now available in quantities [12]. Furthermore, this method allows the preparation of penicillins not obtainable by fermentation.

Doyle *et al.*[2] prepared α-aminobenzylpenicillin by coupling α-benzyloxycarbonylaminophenylacetic acid with 6-aminopenicillanic acid followed by hydrogenolysis. We have found that the α-aminobenzylpenicillin can more readily be prepared from the corresponding azido-penicillin by catalytic reduction over Raney-nickel[13].



I *II* *III* *IV* *V*

The starting material for our synthesis of the radioactive side-chain was benzyl chloride whose Grignard derivative was carbonated with carbon dioxide generated from barium [^{14}C]carbonate using a simple vacuum line technique described by Tegnér and Domeij[14]. Bromination of the phenylacetic acid (I) and treatment of the α-bromo derivative (II) with sodium azide furnished α-azidophenyl-[1-^{14}C]acetic acid (III) whose acid chloride was condensed with 6-aminopenicillanic acid in aqueous solution. The resulting azido-penicillin (IV) was not isolated from the solution but hydrogenated directly over Raney nickel to the required α-aminobenzylpenicillin (V) which was isolated by adjustment of the pH to the isoelectric point and concentration of the solution until crystallization. The purity of the resulting product was 84 % according to the hydroxamate method,[15] the overall yield 28 % based on barium carbonate, and the activity 910 μC/mmole. The substance was further purified by careful base-acid reprecipitation and assayed then for 89 % penicillin, the main other component being water. The purity of this product was further ascertained by chromatography.

EXPERIMENTAL

Phenyl-[1-^{14}C]acetic acid (I). The apparatus used in this synthesis consisted of a 25 ml and a 50 ml round bottom flask interconnected by an inverted U-tube to which had also been fused a 25 ml dropping funnel[14].

Barium [^{14}C]carbonate (1.974 g, 10 mmole; activity about 1 mC/mmole) was placed in the 50 ml flask together with a few glass beads, the apparatus evacuated to 10 mm Hg and then refilled with nitrogen. A freshly prepared solution of benzylmagnesium bromide (25 mmole) in ether (25 ml) was transferred under nitrogen to the 25 ml flask containing a few glass beads and the flask cooled down in dry ice/ethanol. The apparatus was then twice evacuated to 10 mm Hg and refilled with nitrogen before finally being evacuated to 10 mm Hg. The reaction vessel was shaken gently while concentrated sulphuric acid (17 ml) was added dropwise over 40 min to the barium carbonate with the Grignard solution immersed in the cooling-bath. The last traces of the carbon dioxide was driven over into the Grignard reagent by warming the carbon dioxide generator slightly. After another 90 min in the cooling-bath the reaction flask was allowed to warm up to room temperature with shaking before again being immersed in the cooling-bath. 2 N sulphuric acid was added slowly with shaking, the reaction mixture again allowed to reach room tem-

perature, the ether layer separated and the aqueous layer extracted with ether (4 × 25 ml). The combined ether solution and extracts were concentrated to about 25 ml and extracted with 2 N sodium hydroxide (2 × 2.5 ml). The aqueous extract upon neutralization with hydrochloric acid and standing precipitated the required phenylacetic acid (1.08 g, 80 %) as white needles, m.p. 76—77° (lit.[16] 76.5—78°).

α-Bromo-phenyl-[1-^{14}C]acetic acid (II). A solution of phenyl-[1-^{14}C]acetic acid (1.08 g, 8 mmole) and thionyl chloride (2.2 ml) was kept at 60—70° for one hour before the excess thionyl chloride was distilled off *in vacuo*. Bromine (0.7 ml, 12 mmole) was added over one hour to the acid chloride at 100° and the reaction flask kept at 100° for another 3 h. Removal of excess bromine *in vacuo* and addition of ice-water (8 ml) furnished an oily mixture which was extracted with ether (4 × 25 ml). Evaporation of the magnesium sulphate dried ether solution left a colourless oil which crystallized spontaneously to a white solid (1.65 g), m.p. 73—75°. One recrystallization from 2 ml of ligroin (b.p. 96—100°) gave the required compound (1.45 g, 83 %), m.p. 78—80° (lit.[17] m.p. 84°).

α-Azido-phenyl-[1-^{14}C]acetic acid (III). To a solution of α-bromo-phenyl-[1-^{14}C]acetic acid (1.44 g, 7 mmole) in water (2 ml) and acetone (9 ml) were added sodium azide (0.44 g, 7 mmole) and sodium carbonate (0.36 g, 3.5 mmole) and the resultant solution stirred at room temperature overnight. The acetone was then distilled off at reduced pressure, water (8 ml) added and the solution weakly acidified with dilute sulphuric acid. Ether extraction (4 × 25 ml) of the resultant oily mixture, and evaporation of the dried ether solution left a colourless oil which crystallized spontaneously on drying *in vacuo*, yield 1.05 g (88 %), m.p. 96—98°. One recrystallization from ligroin (b.p. 96—100°) furnished white needles (0.96 g, 81 %), m.p. 98—99° (lit. [18] 98—102°).

6-(α-Amino-phenyl-[1-^{14}C]acetamido)penicillanic acid (V). A solution of α-azido-phenyl-[1-^{14}C]acetic acid (0.90 g, 5 mmole) in thionyl chloride (2.2 ml) was warmed at 60° for 2 h before excess thionyl chloride was removed *in vacuo*. The resultant acid chloride was dissolved in anhydrous acetone (5 ml) and added dropwise over 15 min at 0° to a vigorously stirred solution resulting from 6-aminopenicillanic acid (1.7 g, 8 mmole) and sodium bicarbonate (0.7 g, 8 mmole) in water (15 ml) and acetone (5 ml). The pH of the solution was kept at 6—7 during the addition of the acid chloride by additions of sodium bicarbonate. After stirring for another 20 min, the solution was filtered through a little "Hyflo" and extracted with ether (2 × 30 ml). The aqueous layer was adjusted to pH 2 with hydrochloric acid and the resultant oily mixture extracted with ether (3 × 20 ml). After washing with water (5 ml), the ether solution was extracted with small volumes of 0.5 M potassium bicarbonate (16 ml) until the pH of the extracts had reached 6.5—7. The aqueous solution was diluted to 50 ml and hydrogenated over Raney nickel (2 g) at 60 lbs./sq.inch at room temperature for 45 min. The reaction mixture was then filtered through "Hyflo". The filtrate (pH 8—9) was adjusted to pH 2.0 with dilute hydrochloric acid and extracted twice with ether. Dilute sodium hydroxide was carefully added to the solution to bring the pH up to 5.0. By concentrating the solution *in vacuo* the α-aminobenzylpenicillin was obtained in the form of a white powder. The crystalline material was filtered off and dried *in vacuo* at room temperature; yield 0.94 g (51 %), purity 84 %, specific activity 910 μC/mmole. An analytical sample was prepared by dissolving some of the crude product in water at pH 7.5 (dilute sodium hydroxide), extracting this solution with butylacetate and readjusting the pH of the aqueous solution to 4—5 with dilute sulphuric acid when very fine, white crystals slowly appeared and were filtered off after standing in an ice-bath for 1 h. After being dried in a vacuum desiccator over phosphorus pentoxide for two days, this material had m.p. 210° (decomp.) and was found to contain 89 % penicillin by the hydroxamate method [15] the remaining 11 % apparently being water as this material was homogeneous on chromatography. After prolonged drying *in vacuo* this material analysed as monohydrate. (Found: C 52.2; H 5.79; N 11.4. Calc. for $C_{16}H_{19}N_3O_4S \cdot H_2O$: C 52.30; H 5.67; N 11.44).

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Received November 27, 1962.