SYNTHESIS OF (1-^{14}C)VALINE

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SUMMARY

The method for the synthesis of DL-(1-^{14}C)valine (reaction of ethyl N-(1-phthalimido)-isobutyl-1-carbamate with K^{14}CN in ethyl alcohol, followed by acidic hydrolysis of the labelled nitrile) reported by Egyed et al. (Acta Chim. Hung. 38: 123, 1963) was studied. The yield of valine could be increased to 75-80 % by using dry isopropyl alcohol and extending the hydrolysis with hydrochloric acid-acetic acid to 36 hours. This is better than or comparable to the yields obtained with the Strecker or Bucherer methods. Since the replacement of the phthalimido ring with cyanide proved to be of the S_N1 type, a stereospecific synthesis of L- or D-(1-^{14}C)valine was not possible.

Key Words : (1-^{14}C)valine, K^{14}CN

INTRODUCTION

During our studies on the biosynthesis of penicillin, 15-20 mCi of (1-^{14}C) valine was needed for the synthesis (1) of the peptidic precursors of the β-lactam antibiotic. Although (1-^{14}C)valine is available commercially, the price for the amounts required became prohibitive. Therefore, we prepared the labelled amino acid from the less expensive K^{14}CN.

In Gaudry's study (2) on the synthesis of valine from isobutyraldehyde and cyanide, yields of only 60-65 % were obtained, both with the Strecker method and the Bucherer hydantoin synthesis. Loftfield and Eigner (3), using the Bucherer method, isolated DL-(1-^{14}C)valine in a yield of 81 %. The synthesis and hydrolysis of the hydantoin were carried out in a sealed tube at 100°C and 180°C, respectively. The procedure is rather dangerous, because of pressure build-up during these reactions, especially when applied to high amounts of radioactivity.

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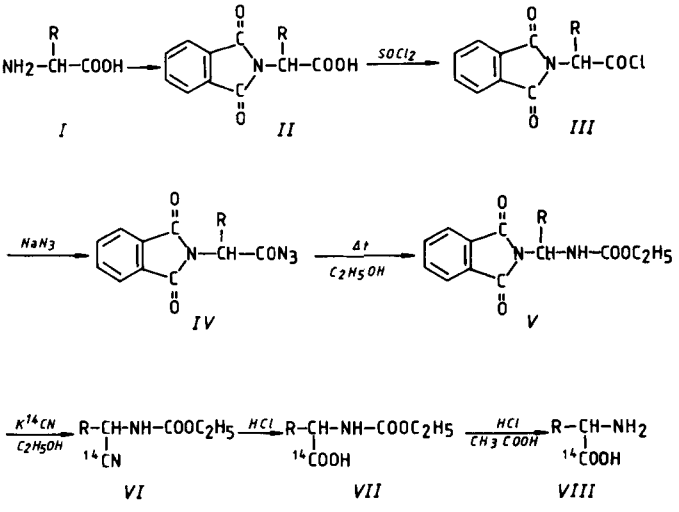
On the other hand, Egyed et al. (4) synthesized DL-(1-^{14}C)alanine, DL-(1-^{14}C)valine and DL-(1-^{14}C)phenylalanine from the corresponding unlabelled amino acids (I). The acyl azide (IV) was prepared from the N-phthaloylamino acid (II) via the acyl chloride (III). Curtius rearrangement of IV in the presence of ethyl alcohol gave the ethyl carbamate derivative (V) which was reacted with K^{14}CN in hot ethyl alcohol, yielding the labelled nitrile (VI). Hydrolysis of the latter under mild conditions (adding an equal volume of HCl 10 N to the alcoholic solution and refluxing for 2 h) gave mainly the N-carboethoxyamino acid (VII). Subsequently, in a stronger acidic medium (boiling with HCl 10 N-CH_3COOH, 3:1, for 12 h), the labelled amino acid (VIII) was formed.

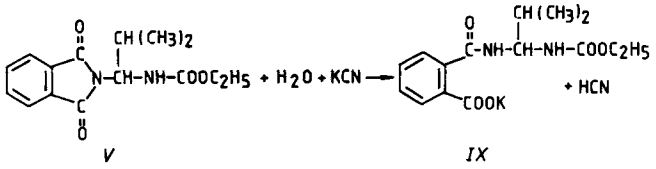


I II III IV V VI VII VIII

This synthesis is safer, since formation and hydrolysis of the nitrile are carried out at atmospheric pressure. In the reaction sequence, positions of the amino and carboxyl group are interchanged and so inversion should occur. During formation of intermediate VI, however, the nucleophilic substitution reaction causes either racemization (S_N1) or inversion (S_N2). Hence, starting from a pure enantiomer, the (1-^{14}C)amino acid with the same optical configuration will be obtained if substitution is of the S_N2 type and if the following intermediates are not racemized during acidic hydrolysis. Egyed et al. (4) did not investigate the stereospecificity of the reaction and also the yield of DL-(1-^{14}C)valine was rather low (52 %). Therefore, the method was studied, prior to the synthesis of (1-^{14}C)valine from K^{14}CN, starting with L-valine, DL-valine and DL-(2,3-^{3}H-4-^{14}C)valine.

CHEMISTRY

The possibility of a stereospecific synthesis of (1-^{14}C)valine was investigated using ethyl N-(1-phthalimido)-L-isobutyl-1-carbamate (V; R = -$CH(CH_3)_2$) as starting product. After boiling with KCN in absolute ethyl alcohol for 2 h, the reaction mixture was examined by TLC (Merck precoated silicagel 60 F254 plates; chloroform-acetone-formic acid, 80:20:1, detection with UV and iodine). Besides phthalimide and a trace of V (Rf = 0.86), two more products (Rf = 0.79 and 0.29) were found to be present. The ethyl alcohol was distilled off and the residue was taken up in water and extracted with ether. The organic phase contained the apolar compound (Rf = 0.79), which was identified as 2-carboethoxyamino isobutyronitrile (VI) by NMR and mass spectrometry; the optical rotation was very low, $(\alpha)_D$ = + 0.2 (c = 2; C_2H_5OH); after acidic hydrolysis, DL-valine was obtained (yield 30 %). The more polar product (Rf = 0.29) which remained in the water layer was identified by NMR and flame photometry as being the potassium salt of ethyl N-(1-phthalamoyl)-isobutyl-1-carbamate (IX). It was formed (in a yield of 40 %) by hydrolytic ring opening of the starting material (V). This reaction is promoted by the strongly alkaline potassium cyanide. Since the potassium ion of IX is derived from KCN, formation of IX results in an equivalent loss of hydrogen cyanide.



V IX

Although all precautions were taken in the following experiments to exclude moisture (e.g. by using freshly distilled super-dry ethyl alcohol), the phthalamoyl derivative IX was still present in the reaction mixture; the yield of DL-valine was increased to 50-55 %. Less hygroscopic alcohols were then tested. Preliminary experiments with dry isopropyl alcohol, isobutyl alcohol and sec-butyl alcohol showed that formation of the nitrile VI was slower in these solvents than in ethyl alcohol, but only traces of the by-product IX could be detected by TLC. After boiling for 9 h in isopropyl alcohol, potassium phthalimide was isolated in a nearly quantitative yield, indicating that the phthalimido derivative V was completely transformed into the nitrile VI. However, after hydrolysis (2 h with HCl 10 N followed by 12 h with HCl 10 N-CH_3COOH, 3:1) (4), DL-valine was obtained in a yield of only 58 %. Therefore, we studied the reaction sequence using DL-(2,3-^{3}H-4-^{14}C)valine of low specific activity as starting product. The (^{14}C)isotope was used to check the recovery of the labelled compounds or to detect the intermediates formed (TLC and scanning of the

radioactivity), whereas the $(^3H)/(^{14}C)$ ratio indicated whether tritium from carbon 2 of valine was lost or retained.

Double labelled DL-valine was prepared by mixing DL-(2-^3H)valine (5), DL-(3-^3H)valine (6) and DL-$(4\text{-}^{14}C)$valine and recrystallization from water-ethyl alcohol; specific activity (^{14}C) 2.19 μCi/mmol; specific activity (^3H) 9.62 μCi/mmol; $(^3H)/(^{14}C)$ ratio = 4.392. Tritium distribution in the labelled valine was 49.7 %, 49.7 % and 0.6 % on carbon atoms 2, 3 and 4, respectively (7,8). Subsequently, the double labelled valine was transformed (yield 75 %) into ethyl N-(1-phthalimido)-DL-$(1,2\text{-}^3H\text{-}3\text{-}^{14}C)$-isobutyl-1-carbamate (V), with the same isotope ratio as the starting valine. Boiling of 35 mmol of V with 35 mmol of KCN in 70 ml of dry isopropyl alcohol for 9 h yielded 90 % of the labelled nitrile VI (isotope ratio as in the starting product), 7 % of the by-product IX and 3 % of V. After addition of 70 ml of HCl 10 N to this solution and boiling for 20 h, only 27 % of VI was transformed into N-carboethoxyvaline (VII), as shown by TLC, scanning and mass spectrometry. In contrast, Egyed *et al.* (4) reported that this compound was the only product present after hydrolysis with HCl for 2 h. Nearly 52 % of the radioactivity was equally divided between the starting nitrile VI and a second, slightly more polar compound. The latter was identified by mass spectrometry as the isopropyl ester of VII. Furthermore, a fourth strongly polar compound, presumably the amide of VII, was detected (16 %). Since these results indicated that the conditions for acidic hydrolysis of the nitrile VI were too mild, the reaction mixture was evaporated to dryness and the residue was boiled with 160 ml of HCl 10 N-CH_3COOH, 3:1, for 20 h. This procedure yielded 98 % of valine and only 2 % of the N-carboethoxyamino acid (VII); no other intermediates were found. After recrystallization from water-ethyl alcohol, DL-valine was isolated in a yield of 75 %; the $(^3H)/(^{14}C)$ ratio was identical to that of the starting compound. Since no tritium was lost by racemization during hydrolysis of the nitrile, and since the earlier experiments with L-valine showed that this nitrile and the isolated valine did not have a significant optical rotation, we may conclude that racemization occurred during the nucleophilic substitution reaction, which therefore must be of the S_N1 type. In addition, since boiling of the alcoholic solution with HCl 10 N was inefficient to hydrolyze the nitrile, this step should be omitted and replaced by direct hydrolysis of VI with HCl 10 N-CH_3COOH, 3:1, until a nearly 100 % yield of DL-valine is obtained. These modifications were used to synthesize DL-$(1\text{-}^{14}C)$ valine from $K^{14}CN$.

EXPERIMENTAL

Synthesis of ethyl N-(1-phthalimido)-isobutyl-1-carbamate

Phthaloyl-L-valine was synthesized via N-(ethoxycarbonyl)phthalimide (9) as reported previously (10); yield 75 %, m.p. 115-117°C; $(\alpha)_D$ = - 68.0° (c = 2; C_2H_5OH). Phthaloyl-L-valine was dissolved in an excess of thionyl chloride and stored overnight at room temperature; after crystallization from benzene-petroleum ether, 1:9, phthaloyl-L-valyl chloride was isolated in a yield of 95-98 %; m.p. 121-122°C. A solution of the acyl chloride in acetone-ether, 1:3, was stirred with a slight excess of NaN_3 in water for 2.5 h at 0-2°C. The organic layer containing the phthaloyl-L-valyl azide was evaporated to dryness and the residue was boiled for 30 min with 15 % ethyl alcohol in toluene. After evaporation of the solvents, ethyl N-(1-phthalimido)-L-isobutyl-1-carbamate was crystallized from petroleum ether; yield 85-90 %. This product can be used as such or after purification by column chromatography on silicagel (elution with a gradient of acetone in chloroform); m.p. 72-73°C; $(\alpha)_D$ = + 41.8° (c = 2; C_2H_5OH).

Phthaloyl-DL-valine was prepared by fusion of DL-valine with phthalic anhydride as described by Sheehan *et al.* (11,12) and Fling *et al.* (13); after crystallization from benzene-petroleum ether, 1:9, yields of 85-95 % were obtained; m.p. 101-102°C. The corresponding derivatives of phthaloyl-DL-valine were prepared in a similar way.

Synthesis of DL-(1-^{14}C)valine

Ethyl N-(1-phthalimido)-DL-isobutyl-1-carbamate (V) (7.25 g; 25 mmol), KCN (95 %; 1.71 g; 25 mmol) and 2 g of molecular sieve 3 Å (Merck) were weighed in a flask of 250 ml and dried in a vacuum desiccator over P_2O_5 for 24 h. Isopropyl alcohol (60 ml; dried over molecular sieve) and approximately 22 mCi of $K^{14}CN$ (The Radiochemical Centre, Amersham, England; specific activity 59.3 mCi/mmol) were added and the mixture was refluxed for 9 h under anhydrous conditions. After cooling in ice for 2 h, the potassium phthalimide formed (together with 1.22 mCi of unreacted $K^{14}CN$) was filtered off; yield 82.5 %. The filtrate, which contained 18.43 mCi of the pure nitrile VI, was evaporated to dryness and refluxed with HCl 10 N (120 ml) and acetic acid (40 ml) for 36 h. TLC (silicagel; n-butanol-acetic acid-water, 2:1:1) and scanning showed that hydrolysis of the nitrile into valine was complete. After evaporation of the acids, the residue was dissolved in water (500 ml) and loaded on a column of Dowex 50W X8 (H^+; 200-400 mesh; 16 x 2.5 cm). The resin was washed with water (250 ml) and the valine was eluted with NH_3 2 N (200 ml). The solvent was removed in vacuum and the residue was recrystallized from 15 ml of water and 150 ml of ethyl alcohol. Yield of DL-(1-^{14}C)valine = 2.24 g = 16.42 mCi; specific activity 860 µCi/mmol. From the mother liquor a second fraction was isolated after addition of unlabel-

led DL-valine (468 mg; 4 mmol) and recrystallization from water-ethyl alcohol; yield 595 mg = 1.73 mCi; specific activity 341 µCi/mmol. Total radiochemical yield 18.15 mCi = 82.5 %. TLC (scanning and reaction with ninhydrin) showed that both fractions were pure.

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