Preparation of DL-β-Leucine. Amino Acids, XV*

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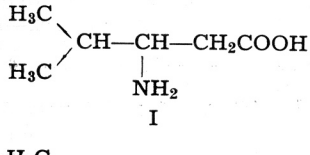
By application of the Arndt-Eistert reaction to the diazoketone of *N*-phthaloyl-DL-valine, racemic β-leucine has been prepared and tested against *Staphylococcus aureus, B. pyocyaneus, E. coli* and *Enterococcus.*

It has been shown that β-amino acids can easily be prepared by the Arndt-Eistert-homologisation of α-amino acids, the free amino group being protected by the phthaloyl group (1—6). Following this method (-)-β-leucine was prepared recently from *N*-phthaloyl-D-valine[7]. In this paper the preparation of racemic β-leucine (β-amino-γ-methyl-valeric acid, homovaline) [I] is reported, with *N*-phthaloyl-DL-valine [II] as starting material. β-Leucine was earlier prepared by hydrolizing 4-isopropyl-dihydrouracil with hydrochloric acid[8].



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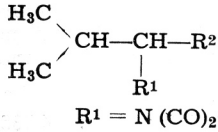

$R^1 = N(CO)_2C_6H_4$

II $R^2 = COOH$	V $R^2 = COCH_2Br$
III $R^2 = COCl$	VI $R^2 = CH_2COOCH_3$
IV $R^2 = COCHN_2$	VII $R^2 = CH_2COOH$

N-Phthaloyl-DL-valine [II], reported earlier by Billman and Hartung[9], was prepared by a slightly modified[3, 9] general method of Reese[11], described by Minard and Fox[12]. The acid chloride [III] was prepared by the action of thionyl chloride on *N*-phthaloyl-DL-valine [II]. Contrary to Foye and Hofferren[13] — who were unable to obtain the chloride in a crystalline form — the chloride is a crystalline solid which readily crystallizes from thionyl chloride (light petroleum ether) sublimed in vacuo it melts at 87—89°. The chloride [III] was converted to the diazoketone [IV], which on treatment with hydrobromic

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acid[14—16] gave the corresponding bromoketone [V]. Wolff-rearrangement of the diazoketone [IV] gave the methyl ester of *N*-phthaloyl-DL-homovaline [VI], which was partially hydrolized to the corresponding phthalimido-acid [VII]. DL-β-leucine [I] was obtained on acid hydrolysis of the phthalimido homoacid methyl ester [VI].

Of the compounds described in the present paper *N*-phthaloyl-DL-β-leucine [VII], DL-β-leucine [I] and the earlier reported (-)-β-leucine[7] were tested on *Bacillus coli, Bacillus pyocyaneus, Enterococcus* and *Staphylococcus aureus* at a dilution of 10 mg./ml. Preliminary tests showed no antibacterial activity of *N*-phthaloyl-DL-β-leucine [VII] when tested as sodium salt. While (-)-β-leucine was found to be inactive, racemic β-leucine exhibited a marked growth-stimulating effect. Since (-)-β-leucine has apparently D-configuration[17], the growth-stimulating activity of the racemate is probably due to the metabolizability of the yet undescribed L-isomer.

EXPERIMENTAL

N-Phthaloyl-DL-valine [II]

Prepared by heating DL-valine[18] (23.4 g., 0.2 mole) and phthalanhydride (29.6 g., 0.2 mole) at 140—145° for one hour, dissolving the warm clear melt in abs. ethanol (40 ml.) and precipitating the phthalimido-acid by pouring the ethanolic solution into water (230 ml.) with shaking and cooling. Yield, 92—97%. Recrystallized from carbon tetrachloride/petroleum ether the product melted at 104°. The earlier reported melting points were 101.5—102° (uncorr.)[9] and 102—103° (uncorr.)[12], respectively.

DL-*3-Methyl-2-phthalimido-butanoyl chloride* [III]

N-Phthaloyl-DL-valine [II] (24.7 g., 0.1 mole) was dissolved in thionyl chloride (77.3 g., 0.65 mole). After standing overnight at room temperature the excess of thionyl chloride was evaporated under reduced pressure and the chloride precipitated as a crystalline solid by the addition of light petroleum ether. Yield, 92%. Recrystallized from thionyl chloride/petroleum ether, and sublimed at 100%. 0,1 mm. the product melted at 87—89°.

Anal. 11.803 mg subst.: 25.320 mg CO_2, 4.735 mg. H_2O
4.239 mg. subst.: 0.20 ml. N_2 (20°, 754 mm.)
$C_{13}H_{12}ClNO_3$ (265.70) calc'd: C 58.78; H 4.55; N 5.27%
found: C 58.54; H 4.49; N 5.34%

DL-*1-Diazo-4-methyl-3-phthalimido-pentan-2-one* [IV]

The acid chloride [III] (23.8 g., 0.09 mole) was dissolved in ether (125 ml.) and gradually added to an ethereal solution (1000 ml.) of diazomethane (obtained from 70.0 g., 0.68 mole of nitriso-methyl-urea). After standing overnight the solution was evaporated to dryness under reduced pressure giving yellow crystals of the diazoketone (24.0 g., 98.5%), which after recrystallization from carbon tetrachloride/petroleum ether melted at 76.5°.

Anal. 9.390 mg. subst.: 21.29 mg. CO_2, 4.09 mg. H_2O
$C_{14}H_{13}N_3O_3$ (271.26) calc'd: C 62.00; H 4.83%
found: C 61.87; H 4.87%

DL-*1-Bromo-4-methyl-3-phthalimido-pentan-2-one* [V]

To a solution of the diazoketone [IV] (15.3 g., 0.056 mole) in glacial acetic acid (75 ml.), 48% hydrobromic acid (14.5 ml.) was gradually added with stirring and

* The melting points were determined with a Kofler micro melting point apparatus.

cooling. After standing for one hour at room temperature the bromoketone (15.4 g., 84.1%) was precipitated by the addition of water (820 ml.). Recrystallization from carbon tetrachloride/petroleum ether gave pale yellow prisms of the bromoketone melting at 83—86°.

Anal. 9.930 mg. subst.: 19.063 mg. CO_2, 3.818 mg. H_2
$C_{14}H_{14}BrNO_3$ (324.18) calc'd: C 51.88; H 4.35%
found: C 52.39; H 4.30%

Methyl DL-*4-methyl-3-phthalimido-pentanoate* [VI]

A freshly prepared methanolic suspension of silver oxide (obtained from 3.0 g. of silver nitrate) was gradually added to a boiling solution of the diazoketone [IV] (41.0 g., 0,15 mole) in methanol (73 ml.) nutil the evolution of nitrogen ceased (3 hours). After refluxing for additional four hours, charcoal was added, the suspension filtered and the filtrate evaporated to dryness under reduced pressure giving the crystalline homoester [VI] (35.5 g., 86%), which after recrystallization from carbon tetrachloride/petroleum ether melted at 79°.

Anal. 8.760 mg. subst.: 21.07 mg. CO_2, 4.88 mg. H_2O
$C_{15}H_{17}NO_4$ (275.30) calc'd: C 65.43; H 6.23%
found: C 65.64; H 6.23%

DL-*4-Methyl-3-phthalimido-pentanoic acid* [VII]

A mixture of the homo-ester [VI] (2.75 g., 0.01 mole), 48% hydrobromic acid (14.5 ml.) and glacial acetic acid (8.7 ml.) was warmed at 40—50° for 2.5 hours. The cooled clear solution was poured into water (35 ml.) and the separated oil again suspended in water (50 ml.). The water layer was decanted and the residue dissolved in a few drops of absolute ethanol. On standing in a dessicator the oily phthalimido-acid [VII] solidified (1.9 g, 72.8%. Recrystallized from carbon tetrachloride / petroleum ether, it melted at 88—90°.

Anal. 9.010 mg. subst.: 21.29 mg. Co_2, 4.70 mg. H_2O
$C_{14}H_{15}NO_4$ (261.27) calc'd: C 64.37; H 5.79%
found: C 65.64; H 6.23%

DL-*3-Amino-4-methyl-pentanoic acid,* DL-β-*leucine* [I]

A solution of the homo-ester [VI] (2.2 g., 0.008 mols) in glacial acetic acid (5.5 ml.) and 46% hydriodic acid (6.9 ml.) was heated under reflux for 14 hours. After cooling the phthalic acid (1.05 g., 79.1%), which separated on scratching, was filtered off, washed with acetic acid and the combined filtrate evaporated to dryness under reduced pressure. The residue was dissolved in water (25 ml.) and evaporated to dryness under reduced pressure. This treatment was repeated for two times more. The residue was then dissolved in water (25 ml.), extracted with ether (3×25 ml.) and evaporated to dryness. The solid hydriodide of β-leucine was dissolved in water (700 ml.) and passed through a column of Amberlite IR-4B (10 ml.). The column was washed with water (350 ml.) and the combined washings evaporated to dryness under reduced pressure at 35—40°, giving solid β-leucine [I] (0.80 g., 76.8%), which after several recrystallizations from methanol / ether melted at 196°. The earlier reported melting point was 197—197.5° (uncorr.)[8].

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IZVOD

Priprema DL-β-leucina. Amino kiseline. XV*

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Poznato je, da se β-amino kiseline mogu lako pripremiti Arndt-Eistertovom homologizacijom α-amino kiselina, kojima je amino grupa zaštićena ftaloilnom grupom[1—7]. Istom metodom, t. j. homologizacijom *N*-ftaloil-DL-valina [II], sada je pripremljen DL-β-leucin, t. j. racemični homovalin [I].

Prethodna mikrobiološka ispitivanja na *B. coli, B. pyocyaneus, Enterococcus* i *Staphylococcus aureus* pokazala su, da su — kod koncentracije od 10 mg/ml — natrijska sol *N*-ftaloil-DL-β-leucina [VII] i (-)-β-leucin (7) inaktivni, a DL-β-leucin da vidljivo stimulira rast. Budući da je (-)-β-leucin vjerojatno D-konfiguracije[17], može se stimulatorni efekt pripisati metabolizabilnosti neopisanog L-izomera.

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