Amino Acids

4th Communication

Synthesis of the Homologue of L-Leucine (Synthesis of-β-Amino-δ-methyl-caproic Acid)

K. BALENOVIĆ and D. BROVET-KEGLEVIĆ

It has been shown[1]) that N-Phthalyl-derivatives of α-amino-acids can be converted into the corresponding diazoketones. We have applied this reaction to the preparation of the homologue of L-leucine by the Arndt-Eistert synthesis[2]) from the hitherto undescribed diazoketone of N-phthalyl-L-leucine (I). Methanolic solution of (I) heated with silver oxyde yielded the optically active ester of the homologous acid (II), which, refluxed with hydriodic acid, gave the free, optically active β-amino acid (III).

(I) $CH_3(CH_3)CH\ CH_2CH(R)COCHN_2$ ($R = C_6H_4(CO)_2N$-)

(II) $CH_3(CH_3)CH\ CH_2CH(R)CH_2COOCH_3$

(III) $CH_3(CH_3)CH\ CH_2CH(NH_2)\ CH_2COOH$

It is known that Arndt-Eistert homologisation of acids with the assymmetrical center on the α-carbon atom occurs without the Walden rearrangement[3]). Analogically, we can assume that the homologue (III) belongs to the L-series of amino acids.

Compound III can be regarded as β-substituted β-alanine and it shall be interesting therefore to investigate the biological properties of this amino acid, and compare them with those of β-alanine.

EXPERIMENTAL

L-Leucine. The leucine used was optically pure, ($[\alpha]_D^{20} = +15{,}3^\circ$ (± 0,3) in 20% HCl). According to literature,[4]) at the same conditions $[\alpha]_D^{20} = +15{,}5^\circ$.

N-Phthalyl-L-Leucine. The compound was synthesised for the first time by Reese[5]). However, we heated the well mixed and powdered

*) 3 rd Communication: Helv., 34 (1951) 744.

[1]) K. Balenović, Experientia, 3, (1947) 369.

[2]) Cf. e. g. W. E. Bachmann and W. S. Struve in R. Adams, Organic Reactions, Vol. 1., 1942 38—62.

[3]) Cf. e. g. J. F. Lane, J. Willenz, A. Weissenberger and E. S. Wallis, J. Org. Chem., 5, (1940) 276.

[4]) E. Fischer and O. Warburg, Ber., 38, (1905) 4022.

[5]) L. Reese, Ann., 242, (1887) 9.

equimolar amounts of phthalic anhydride and L-leucine in an oil bath at 135—140° during 1 hour, after which time the foaming of the light yellow melt ceased. After cooling it was dissolved in ethanol. The separation of crystals occurs when the ethanolic solution is slowly poured into water with constant stirring. The pure compound shows a m. p. of 118—119°, $[\alpha]_D^{0} = -22{,}0^{\circ}$ (± 0,5) (c = 5, ethanol)[5]. The yield is 85—90%.

N-Phthalyl-L-Leucinoyl-chloride. N-Phthalyl - L - leucine (50g) was heated during 1 hour under reflux with thionylchloride (40 c. c.); the mixture was dried under reduced pressure for 2 hours at 40—50°, and used without further isolation.

Diazoketone of N-Phthalyl-L-Leucine (I). The above mentioned crude chloride (0,22 mole) is dissolved in ether (250 c. c.), and added to a solution of diazomethan (1,2 mole) in ether (2 l). The ethereal solution was kept at 0° for 24 hours, the ether was then removed by evaporation under reduced pressure. The residue is a thick light yellow oil which solidifies after some time. Recrystallization from ether — light petroleum (b. p. 30—50°) gives hexagonal, yellow prisms, m. p. 81—83°, the yield being 90%. The compound was dried in a high vacuum for 2 hours at 40°.

Anal. 5,330 mg substance: 12,35 mg CO_2, 2,60 mg H_2O
Calcd. for $C_{15}H_{15}O_3N_3$ (mol. wt. 285,23): C,63,14; H, 5,30
Found: C,63,23; H, 5,46

$[\alpha]_D^{20} = -98{,}3^{\circ}$ (±1°) (c = 3, ethyl acetate)

Methyl-ester of the homologue of L-Leucine (II), (dextro-β-amino-δ-methylcaproic, acid, dextro-β-iso-butyl-β-alanine). The diazoketone (55,94g, 0,195 mole) was treated with absolute methanol (90 c. c.), and refluxed. To the boiling solution a suspension of silver oxyde in methanol*) was gradually added, as soon as the nitrogen evolution ceased. At the end of the reaction, (after about 7 hours) a sample of the reaction mixture does not foam on addition of concentrated hydrochloric acid. The mixture is treated with a little charcoal, filtered, and evaporated under reduced pressure. The red-brown oily residue is extracted several times with ligroin. On volatilising the solvent, 54,8g (97%) of viscous oil is left over, from which crystals separate after standing at a low temperature. Repeated recrystallization from methanol yielded clusters of colourless needles, m. p. 54—56°. They were dried under a high vacuum for 8 hours at 30°.

Anal. 8,275 mg substance: 20,16 mg CO_2, 4,72 mg H_2O
Calcd. for $C_{16}H_{19}O_4N$ (mol. wt. 289,24): C, 66,41; H, 6,62
Found: C, 66,48; H, 6,38

$[\alpha]_D^{20} = +9{,}3^{\circ}$ (±0,6°) (c = 3, methanol)

Homologue of L-Leucine (dextro-β-amino-δ-methyl-caproic acid, dextro-β-iso-butyl-β-alanine). A solution of the methyl

*) The silver oxyde is prepared by precipitating a 10% aqueous solution of $AgNO_3$ with 10% NaOH, and washing the precipitate with water till neutral reaction. The precipitate is suspended 5 times and washed with absolute methanol.

ester (II) (62g, 0,125 mole) in concentrated acetic acid (100 c. c.) was treated with hydriodic acid (112 c. c., 56%, d. 1,7; 0,49 mole), and refluxed for 14 hours. On cooling, the phthalic acid separates, and is filtered and washed with a small amount of acetic acid (30g, 85%). The filtrate is evaporated under reduced pressure (water bath temp. 40—45°) and the residue dissolved in water (100 c. c.), then again evaporated. Through this treatment, repeated 3 times, most of the hydriodic acid was removed. The dry residue was again dissolved in about 150 c. c. of water, and shaken out three times with 100 c. c. portions of ether. The aqueous solution is diluted to 500 c. c., a determination of hydriodic acid made, and an equivalent quantity of a fresh silver oxyde suspension added. After standing for 24 hours the precipitate is filtered and washed with a little cold water. The filtrate and washings are concentrated under reduced pressure, and 23,5g of acid crystallizes (yield 75%), m. p. 203—206°. The acid is purified by dissolving in ethanol and precipitating with ether. The pure product shows a m. p. of 215—216°.

Anal. 8,370 mg substance: 17,72 mg CO_2. 7.69 mg H_2O
Calcd. for $C_7H_{15}O_2N$ (mol. wt. 145,16): C 57,89; H, 10,42
Found: C, 57,77; H, 10,28

$[\alpha]_D^{20} = +28{,}0^0$ (± 0.3) (c = 3, water)

The micro-analyses were carried out by Prof. L. Filipović in the micro-chemical laboratory of this Institute.

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IZVOD

Aminokiseline

4. saopćenje

Sinteza L-leucinu homologne β-aminokiseline

(Sinteza L-β-amino-δ-metil-kapronske kiseline)

K. BALENOVIĆ i D. BROVET-KEGLEVIĆ**)

Kako je pokazano[1]), mogu se α-aminokiseline pretvoriti preko N-ftalil derivata u odgovarajuće diazoketone. Mi smo tu reakciju sada primijenili, da iz još neopisanog diazoketona N-ftalil-L-leucina (I) priredimo pomoću Arndt-Eistert-ove sinteze[2]) L-leucinu homolognu β-aminokiselinu. Zaista je (I), grijan u metanolu sa srebrnim oksidom, prešao u optički aktivni ester homologne β-aminokiseline (II), koja je osapunjenjem sa jodovodičnom kiselinom dala slobodnu, optički aktivnu β-aminokiselinu (III).

(I) $CH_3(CH_3)CH\ CH_2CH(R)\ COCHN_2$ ($R = C_6H_4(CO)_2N$-)
(II) $CH_3(CH_3)CH\ CH_2CH(R)\ CH_2COOCH_3$
(III) $CH_3(CH_3)CH\ CH_2CH(NH_2)CH_2COOH$

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*) 3. Saopćenje Helv. **34**, (1951) 744.
**) Dio disertacije D. Brovet-Keglević.
[1]) K, Balenović, Experientia, **3**, (1947) 369.

Premda za sada nismo izvršili dokaz da ova homologizacija teče bez Walden-ove pregradnje, čini nam se da smijemo iz toka do sada opisanih homologizacija kiselina sa centrom asimetrije na α-C-atomu[3]), zaključiti, da i ovdje homologni spoj pripada istom steričkom redu.

Ova se optički aktivna kiselina može smatrati i β-supstituiranim β-alaninom, pa će stoga sigurno biti interesantno pobliže ispitati biološka svojstva ovoga spoja i usporediti ih sa onima β-alanina.

EKSPERIMENTALNI DIO

L-leucin. Upotrebljeni je leucin*) bio optički čist sa $[\alpha]_D^{20} = +15{,}3^0$ ($\pm 0{,}3^0$) u 20% HCl. Prema literaturi[4]). Kod istih uvjeta, $[\alpha]_D^{20} = +15{,}5^0$.

N-Ftalil-L-leucin. Spoj je prvi priredio Reese[5]). Mi smo grijali pomiješane, dobro smrvljene, ekvimolarne količine anhidrida ftalne kiseline i L-leucina 1 sat u uljenoj kupelji na 135°—140°. Kroz to vrijeme prestaje pjenjenje svjetlo-žute taline, koja se nakon ohlađenja otopi u etanolu. Spoj kristalizira polaganim lijevanjem etanolne otopine, uz dobro miješanje, u vodu. Čist ima talište 118—119°, $[\alpha]_D^{20} = -22{,}0^0$ ($\pm 0{,}5^0$) (c = 5, etanol)[5]). Iskorištenje 85—90%.

N-Ftalil-L-leucinoil-klorid. Spoj je priređen djelovanjem tionilklorida na N-Ftalil-L-leucin i bez izolacije je dalje upotrebljavan. 50 g N-Ftalil-L-leucina je grijano 1 sat na povratnom hladilu sa 40 cm³ tionilklorida, nakon toga je smjesa sušena u vakuumu kod 40—50° 2 sata.

Diazoketon N-Ftalil-L-leucina (I). 0,22 mola sirovog, gore opisanog klorida, otopljeno je u 250 cm³ etera i dodavano je k 1,2 mola diazometana otopljenog u 2 litre etera. Eterska otopina je nakon 24 sata stajanja kod 0° otparena u vakuumu. Ostatak je svijetlo-žuto viskozno ulje koje nakon nekoga vremena počinje kristalizirati. Kristalizacija iz etera-petroletera daje šesterostrane, žute prizme. Iskorištenje je 90%. Za analizu je tvar prekristalizirana iz etera-petroletera do T. t. 81—83° i sušena 2 sata u v. v. kod 40°.

5,330 mg tvari daje 12,35 mg CO_2 i 2,60 mg H_2O
$C_{15}H_{15}O_3N_3$ (285,23) Rač. C 63,14 H 5,30
Nađ. C 63,23 H 5,46

$[\alpha]_D^{20} = -98{,}3^0$ ($\pm 1^0$) (c = 3, etilni acetat)

Metilni ester L-leucinu homologne β-amino-kiseline (II) (L-β-amino-δ-metil-kapronska kiselina, L-β-izobutil-β-alanin.) 55.4 g (0,195 mola) diazoketona I preliveno je sa 90 cm³ aps. metanola i zagrijano na povratnom hladilu. Vrelој otopini je dodavana metanolna suspenzija srebrnog oksida**), koja je dodavana uvijek u malim količinama ponovo, kada prestaje razvijanje dušika. Na koncu reakcije (nakon cca 7 sati) uzorak reakcione smjese ne pjeni se dodatkom koncentrirane solne kiseline. Reakciona smjesa se iza toga prokuha sa nešto aktivnog ugljena, filtrira i otpari u vakuumu. Crvenosmeđi uljeviti ostatak ekstrahira se nekoliko puta ligroinom. Ligroinski ekstrakt ostavlja otparavanjem u vakuumu 54,8 g (97%) viskoznog ulja, koje dužim stajanjem kod niske temperature kristalizira. Tvar prekristalizirana nekoliko puta iz me-

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[2]) Usp. n. pr. W. E. Bachmann i W. S. Struve u R. Adams, Organic Reactions, Vol. I. ,1942, str. 38—62.

[3]) Usp. n. pr. J. F. Lane, J. Willenz, A. Weissenberger i E. S. Wallis u J. Org. Chem., 5, (1940) 276.

[4]) E. Fischer i O. Warburg, Ber., 38, (1905) 4022 .

[5]) L. Reese, Ann., 242, (1887) 9. T. t. 115—116° $[\alpha]_C^{20} = -21{,}87^0$ (c = 5, etanol).

* Hidrolizat goveđih eritrocita, iz kojeg je izoliran L-leucin, zahvaljujemo tvornici »Pliva«, Zagreb. Kod izolacije je intenzivno pomagao V. Tomičić.

**) Srebrni oksid je priređen taloženjem 10% vodene otopine $AgNO_3$ sa 10% NaOH, te pranjem vodom i dekantiranjem do neutralne reakcije. Talog je nakon toga još 5 puta suspendiran i pran sa aps. metanolom.

tanola, daje snopove bezbojnih igala sa T. t. 54—56°. Za analizu je tvar kristalizirana iz metanola i sušena u v. v. kod 30°, 8 sati.

8,275 mg tvari daje 20,16 mg CO_2 i 4,72 mg H_2O.
$C_{16}H_{19}O_4N$ (289,24) Rač. C 66,41 H 6,62
Nađ. C 66,48 H 6,38

$[\alpha]_D^{20} = +9{,}3^0$ ($\pm$ 0,6°) (c = 3, metanol)

L-leucinu homologna β-aminokiselina (III). (L-β--amino-δ-metil-kapronska kiselina, L-β-izobutil-β-alanin.) 62 g metilnog estera II (0,215 mola) otopljeno je u 100 cm³ konc. octene kiseline. Otopini je dodano 112 cm³ jodovodične kiseline (56%, sp. tež. 1,7; 0,49 mola), pa je sve grijano 14 sati na povratnom hladilu, i iza toga ohlađeno. Tako iskristalizira ftalna kiselina, koja se filtrira i opere sa malo octene kiseline (30 g, 85%). Filtrat je otparen u vakuumu (vod. kup. 40—45°), a ostatak otopljen u 100 cm³ vode i ponovno otparen u vakuumu. Ovakovim postupkom, ponovljenim 3 puta, mogla je biti uklonjena glavna količina jodovodične kiseline. Suhi ostatak ponovno je otopljen u cca 150 cm³ vode i ekstrahiran tri puta sa po 100 cm³ etera. Vodena otopina razrijeđena je na 500 cm³, i dodana joj je prema titraciji jod-jona ekvivalentna količina svježe pripravljene suspenzije srebrnog oksida. Nakon 24 sata dekantira se bistra otopina od taloga srebrnog jodida, koji se dobro opere sa malo hladne vode. Vodene otopine se upare u vakuumu kod što niže temperature na 100 cm³. Uvođenjem sumporovodika istaloži se preostali Ag-jon, doda se malo aktivnog ugljena, filtrira i pere sa malo vode. Filtrat, otparen u vakuumu, ostavlja 23,5 g kiseline T. t. 203—206° (75% teor.). Kiselina se dade čistiti otapanjem u etanolu i taloženjem sa eterom. Sasvim čista ima T. t. 215—216°.

8,370 mg tvari daje 17,72 mg CO_2 i 7,69 mg H_2O
$C_7H_{15}O_2N$ (145,16) Rač. C 57,89 H 10,42
Nađ. C 57,77 H 10,28

$[\alpha]_D^{20} = +28{,}0^0$ ($\pm$ 0,3°) (c = 3, voda)

Mikroanalize je izvršio prof. L. Filipović u mikro-kemijskom laboratoriju instituta.

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