The Reaction of Some N-Acylated β-Amino Acid Esters with the Grignard Reagent.

Amino Acids. XIII*

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The reactions of some β-amino acid derivatives with the Grignard reagent are described, and possible explanation of the inertness of β-amino acid derivatives against the Grignard reagent are discussed.

For our studies of the metabolism of β-amino acids it would be of considerable interest to know the configuration. By applying the Arndt-Eistert reaction to the diazoketones of some optically active N-phthaloyl amino acids, several β-amino acids have been prepared[1]. The Barbier-Wieland degradation[2] of the β-amino acid esters seemed to be a way for the direct determination of the configuration of these compounds.

Methyl-3-phthalimido-5-methyl-hexanoate (methyl ester of N-phthaloyl-β-homoleucine) [Ia] failed to react with phenyl magnesium bromide, and only resinous undentified products were obtained. The failure was interpreted by Bettzieche's[3] observation that the phthalimido group reacts easily with the Grignard reagent yielding phthalamido-derivatives Spaeth[4] also described an unsuccessful attempt to prepare tertiary alcohol from β-alanine ester and methyl magnesium iodide.

Ethyl (-) -3-acetamido-5-methyl-hexanoate [Ib], and methyl (-) -3-benzamido-5-methyl-hexanoate [Ic] were prepared. By treating them with excess phenyl magnesium bromide and refluxing the mixture one hour in anhydrous ether, no defined products were obtained. Under the same conditions, according to the procedure given by Bettzieche[3] for the inactive compound, the methyl ester of N-benzoyl-L-leucine was converted to optically active 1,1-diphenyl-2-benzamido-4-methyl-pentan-1-ol, [IIa], in a 50% yield.

In a series of investigations the molar ratio β-amino ester vs Grignard reagent, and the effect of the temperature were studied. By using one mole of methyl (-) -3-benzamido-5-methyl-hexanoate [Ic] and twenty moles of phenyl magnesium bromide, and refluxing the reaction mixture during six hours, first in ether, then in benzene, an oily reaction product was isolated. The compound obtained in 33% yield was identified as 1-phenyl-3-benzamido-5-methyl-hexan-1-one, [IIIa].

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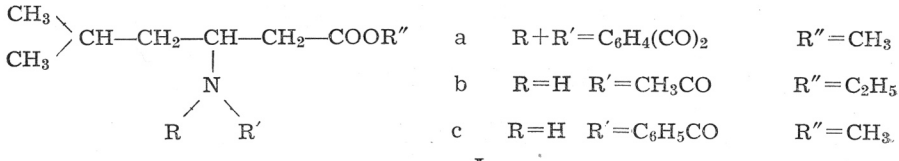
* Paper XI. S. Iskrić, *Arhiv kem.* **24** (1952) 83.
Paper XII. K. Balenović, I. Jambrešić and B. Urbas, *J. Org. Chem.* in press.

In order to establish if this type of reaction is common also to other β-amino acids, ethyl ester of N-benzoyl-β-alanine was treated under the same drastic conditions as described above. 1,1-diphenyl-3-benzamido-propan-1-ol, [IIb] in 15.7% yield and 1-phenyl-3-benzamido-propan-1-one, [IIIb] in 25% yield were isolated.



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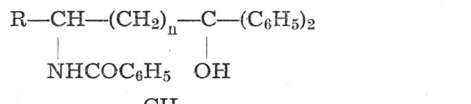

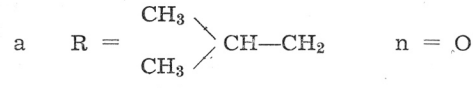

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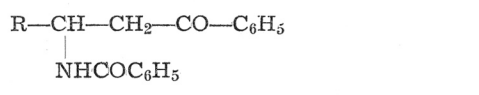

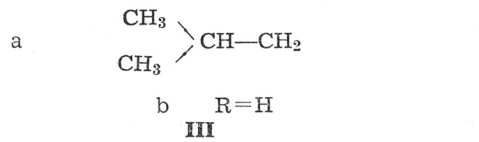

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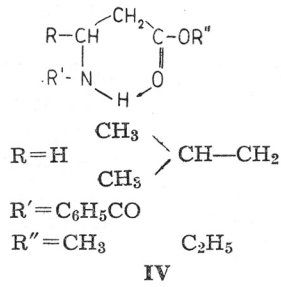

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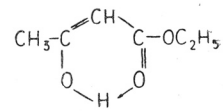

V

It is possible to explain the inertness of the β-amino acid esters in the Grignard reaction by the formation of a hydrogen bond between the doubly bound oxygen atom in the carbethoxy group and the free hydrogen in the amino group. Supposing this, a six-membered ring [IV] is formed, similar to the ring which appears in the enol form of acetoacetic ester [V]. Grignard[5] has already described the abnormal behaviour of acetoacetic ester with methyl magnesium bromide.

Acknowledgment. I am indebted to Dr. L. Filipović for the microanalyses, and to Professor K. Balenović for encouragement and interest during this work.

EXPERIMENTAL*

Ethyl (-)-3-acetamido-5-methyl-hexanoate (Ethyl ester of (-)-N-acetyl-β-homoleucine) [Ib]

The reaction was carried out following the instructions for the acylation of α-amino acids, given by Cherbuliez and Plattner[6]. To 25 ccm of absolute ethanol, previously saturated with dry hydrogen chloride, 4.5 g. (0.031 mole) of optically pure (+)-β-homoleucine, $[\alpha]_D^{20}$ +28° ±1° (c, 3.00 in water) was added, followed by 20 ccm of absolute ethanol. The solution was refluxed under anhydrous conditions for two hours. After evaporation of ethanol *in vacuo*, freshly fused, powdered sodium acetate (6 g., 0.07 mole) and acetic anhydride (12 ml., 0.1 mole) were added to the oily residue. The mixture was allowed to stand for half an hour on a steam bath. The acetic acid was removed *in vacuo*, the residue extracted several times with ether, and the combined extracts dried over anhydrous sodium sulphate. After removal of the solvent, the oily residue was distilled *in vacuo*, and 5.3 g. (yield 79.4%) of a pale yellow oil, b. p. 70—80°/0.03 mm., collected. After redistillation a colourless oil of *ethyl (-)-3-acetamido-5-methyl-hexanoate* [Ib], b. p. 85—90°/0.04 mm., was obtained; $[\alpha]_D^{20}$ −30.3° ± 1° (c, 2.30 in absolute ethanol).

Anal. 10.70 mg. subst.: 24.05 mg. CO_2, 9.31 mg. H_2O
$C_{11}H_{21}NO_3$ (215.286) calc'd: C 61.36; H 9.83%
found: C 61.34; H 9.74%

Methyl (-)-3-benzamido-5-methyl-hexanoate (Methyl ester of (-)-N-benzoyl-β-homoleucine) [Ic]

The reaction was carried out following the instructions given by Karrer and Kehl for the benzoylation of L-leucine[7]. Absolute methanol (40 ml.), previously saturated with dry HCl. was poured over optically pure (+)-β-homoleucine, (5 g., 0.034 mole, $[\alpha]_D^{20}$ + 28° ± 1° (c, 3.00 in water), placed in a flask fitted with a condenser protected by a calcium chloride tube. The solution was left at room temperature for half an hour and then refluxed on a steam bath for another thirty minutes. Absolute methanol, (20 ml.) was added and the refluxing continued for two hours. Methanol was removed *in vacuo*, the residue dissolved in dry chloroform (40 ml., distilled over calcium chloride), and dry ammonia bubbled through the mixture during 20 minutes. The flask was cooled in the meantime in an ice bath and anhydrous conditions were maintained by a soda lime tube. The ammonium chloride was filtered off, washed with chloroform and the collected filtrates evaporated *in vacuo*. The remaining oil (6 g.) was dissolved in dry pyridine (10 ml.) and while the solution was stirred and cooled in an ice bath, benzoylchloride (7.5 ml., 0.045 mole) was added during half an hour, followed by dry pyridine (5 ml.). The mixture was left overnight in an ice box. In order to dissolve pyridine hydrochloride, some drops of water were added an the red solution extracted several times with ether. The combined extracts were washed with 10% hydrochloric acid, 10% sodium hydrogen carbonate and water. After drying over anhydrous sodium sulphate, the solvent was evaporated *in vacuo*, and the oily residue distilled. *Methyl (-)-3-benzamido-5-methyl-hexanoate* [Ic], (6.2 g., 68.37% yield), was collected as a pale yellow oil b. p. 121—135°/0.02 mm. A small sample was redistilled and a colourless oil b. p. 136—140°/0.03 mm. obtained. $[\alpha]_D^{18}$ −76.1° ± 1° (c, 1.08 in benzene); $[\alpha]_D^{18}$ −9.0° ± 2° (c, 2.11 in methanol).

Anal. 11.11 mg. subst.: 27.99 mg. CO_2, 7.89 mg. H_2O
$C_{15}H_{21}NO_3$ (263.260) calc'd: C 68.40; H 8.04%
found: C 68.75; H 7.95%

L-*1.1-diphenyl-2-benzamido-4-methyl-pentan-1-ol* [IIa]

The reaction was carried out following exactly the instructions of Bettzieche and al.[3] for the preparation of the inactive compound. Optically pure methyl ester

* All melting points are uncorrected.

of N-benzoyl-L-leucine (2.0 g., 0.008 mole), $[\alpha]_D^{18}$ $-20.89^0 \pm 1^0$ (c, 2.63 in methanol), yielded 1.5 g. (50%) of optically active *1,1-diphenyl-2-benzamido-4-methyl-pentan-1-ol*, m. p. 192—194°, $[\alpha]_D^{18}$ $-67.9^0 \pm 1^0$ (c, 1.324 in ethanol). Bettzieche[3] reported m. p. 184° and yield 52%.

Anal. 8.50 mg. subst.:25.09 mg. CO_2, 5.41 mg. H_2O
$C_{25}H_{27}NO_2$ (373.360) calc'd: C 80.39; H 7.29%
found: C 80.55; H 7.12%

(-)-1-phenyl-3-benzamido-5-methyl-hexan-1-one [IIIa]

A solution of benzylmagnesium bromide was prepared by standard technique from magnesium turnings (9.65 g., 0.39 mole) and benzylbromide (76 g., 0.485 mole) in dry ether (170 ml.). The flask was cooled in an ice bath and a solution of methyl (-)-3-benzamido-5-methyl-hexanoate [Ic], (5.7 g., 0.022 mole) in dry ether (40 ml.) was added during 15 minutes. The mixture was refluxed for two hours, the ether evaporated *in vacuo*, dry benzene (75 ml.) poured on the residue and the solution refluxed again for two hours. The benzene was removed by distillation, raising gradually the temperature of the oil bath to 110° during one hour. After cooling, the content was decomposed by gradual addition of cracked ice (100 g.), followed by diluted hydrochloric acid. The mixture was extracted several times with benzene, then with anhydrous ether, the combined extracts washed with water and dried over anhydrous sodium sulphate. After removal of the solvent, the residue was steam-distilled *in vacuo* during six hours, in order to remove diphenyl. The remaining brown oil was distilled *in vacuo*, and the fraction b. p. 138—168°/0.03 mm. collected. An viscous yellow oil (5.6 g.) distilled, from which a sample (1.756 g.) was dissolved in benzene (5 ml.) and chromatographed through an alumina column (17 g., activity 4.) eluted with benzene. The oils obtained from the fractions 3. (144 mg.) and 4. (49 mg.) crystallized completely. From the fraction 2., after precipitating the benzene solution by petroleum-ether, an additional amount (500 mg.) was obtained. White needles of *(-)-1-phenyl-3-benzamido-5-methyl-hexan-1-one*, [IIIa], (693 mg., yield 33.07% based on methyl (-)-3-benzamido-5-methyl-hexanoate [Ic]. After recrystallization from benzene-petroleum ether and resublimation at 150—160°/0.02 mm., m. p. 111—112° and $[\alpha]_D^{17}$ $-85.2^0 \pm 2^0$ (c, 1.420 in benzene).

Anal. 5.785 mg. subst.: 16.48 mg. CO_2, 3.83 mg. H_2O
7.340 mg. subst.: 0.284 ml. N_2 (19°, 757 mm)
$C_{20}H_{23}NO_2$ (309.392) calc'd: C 77.63; H 7.49; N 4.53%
found: C 77.74; H 7.41; N 4.51%

A sample was converted by standard technique to the semicarbazone. Recrystallized from methanol, the white plates had m. p. 205—207°.

Anal. 11.160 mg. subst.: 28.01 mg. CO_2, 7.27 mg. H_2O
$C_{21}H_{26}N_4O_2$ (366.45) calc'd: C 68.83; H 7.15%
found: C 68.49; H 7.29%

1,1-diphenyl-3-benzamido-propan-1-ol [IIb]

In a Grignard solution prepared from magnesium turnings (10.5 g., 0.43 mole), and bromobenzene (84 g., 0.53 mole) in anhydrous ether (118 ml.), ethyl ester of N-benzoyl-β-alanine (5.3 g., 0.024 mole) dissolved in anhydrous ether (30 ml.) was gradually added under cooling. The mixture was treated exactly as described for (—)-1-phenyl-3-benzamido-5-methyl-hexan-1-one [IIIa]. After the removal of the diphenyl, the remaining oil (8.3 g.) was dissolved in benzene (10 ml.) and chromatographed through an alumina column (50 g., activity 4.) using benzene as the eluent. Fraction 6. (76 mg.) crystallized quantitatively, and from the fractions 3. (200 mg.), 4. (980 mg.), and 5. (244 mg.) an additional amount was obtained, after redissolving the oils in

benzene and precipitating them with petroleum ether: *The 1,1-diphenyl-3-benzamido-propan-1-ol* [IIb] (1.5 g., yield 15.7%) crystallized in white platelets and had after recrystallization from benzene-petroleum ether m. p. 148—150°.

Anal. 10.710 mg. subst.: 31,38 mg. CO_2, 6,14 mg. H_2O
$C_{22}H_{21}NO_2$ (331.396) calc'd: C 79,73; H 6,39%
found: C 79,96; H 6,42%

1-phenyl-3-benzamido-propan-1-one [IIIb]

The substance was isolated from the benzene fractions, after the removal of [IIb]. Fraction 2. and evaporated mother liquors from the fractions 3, 4, and 5, were collected, evaporated to dryness and the resulting oil (5.5 g.) dissolved in benzene (5 ml.) and passed through an alumina column (50 g., activity 4.) using benzene as the eluent. After removing benzene, the oils from the fractions 5. (275 mg.) and 6. (184 mg.) crystallized quantitatively after standing overnight in an ice box. Redissolved in benzene and precipitated by petroleum ether, partially crystallised fractions 3. (231 mg.) and 4. (820 mg.). *1-phenyl-3-benzamido-propan-1-one* [IIIb] (1.51 g., yield 25%) had after the purification from benzene-petroleum ether m. p. 92—93°. White needles.

Anal. 7.420 mg. subst.: 20,63 mg. CO_2, 4,02 mg. H_2O
$C_{16}H_{15}NO_2$ (253.288) calc'd: C 75.87; H 5.97%
found: C 75,87; H 6,06%

A semicarbazone was prepared by standard technique. After the recrystallization from methanol the white prisms had m. p. 192—193°.

Anal. 12.510 mg. subst.: 30,21 mg. CO_2, 6,60 mg. H_2O
$C_{17}H_{18}N_4O_2$ (310.346) calc'd: C 65,79; H 5,85%
found: C 65.90; H 5,90%

REFERENCES

1. K. Balenović, *Experientia* **3** (1947) 369.
2. Ph. Barbier and R. Locquin, *Compt. rend.* **156** (1913) 1443.
H. Wieland, O. Schlichting and R. Jacobi, *Z. physiol. Chem.* **161** (1926) 79.
3. F. Bettzieche, R. Menger and K. Wolf, *Z. physiol. Chem.* **160** (1926) 270.
4. E. Spaeth and W. Spitzy, *Ber.* **58** (1925) 2273.
5. V. Grignard, *Compt. rend.* **134** (1902) 849.
6. E. Cherbuliez, Pl. Plattner and S. Ariel, *Helv. Chim. Acta* **13** (1930) 1390.
7. P. Karrer and W. Kehl, *Helv. Chim. Acta* **13** (1930) 50.

IZVOD

Reakcija nekih estera N-aciliranih β-aminokiselina sa Grignardovim reagensom Aminokiseline. XIII

D. Keglević

Na neke estere β-aminokiselina djelovali smo fenil magnezijskim bromidom. Reakcija teče abnormalno sporo, a kao konačni produkti izolirani su ketoni. Pretpostavlja se, da je uzrok te tromosti reagiranja vodikov most.

INSTITUT »RUĐER BOŠKOVIĆ«
BIOKEMIJSKI ODJEL
ZAGREB

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