

CONTRIBUTIONS TO THE SYNTHESIS OF DL-p-NITROPHENYL ALANINE

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On condensating dimethyl acetylaminomalonate with p-nitrobenzyl chloride, DL-p-nitrophenyl alanine can be prepared. The synthesis can also be carried out in several steps, and the dicarboxylic acid phase can be isolated by the alkaline hydrolysis of the condensate. The thermal decarboxylation of this isolated phase yields N-acetyl-DL-aminoacid which latter affords, on acid hydrolysis, the desired aminoacid. The structure of the intermediates can be proved by acid hydrolysis in that in each case the chlorohydrate of the aminoacid forms. On liberating the aminoacid from its chlorohydrate, it was possible to isolate a stable hydrate.

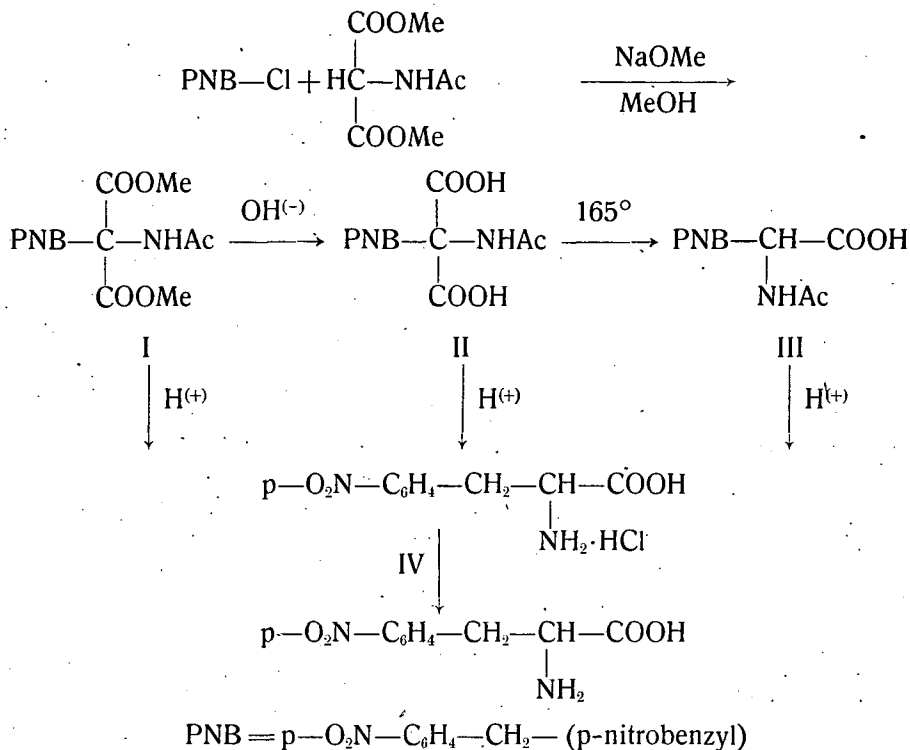
The synthesis of DL-p-nitrophenyl alanine from diethyl acetylaminomalonate by condensation with p-nitrobenzyl chloride was described by BURCKHALTER and STEPHENS [1]. The condensation product (of m. p. 193—194° C) was converted into aminoacid by hydrolysis with strong acid. However, it is also possible to conduct hydrolysis in an alkaline medium so as to isolate the intermediate phases as well, in a way already observed by TISHLER and associates [2] at the synthesis of N-acetyl-DL-triptophane. Thus, it is possible to isolate N-acetyl-DL-p-nitrophenyl alanine. This latter product is expected to be resolvable [3] and to be suited for use in an active form as a resolving agent, due to its possible capability of readily crystallizing. In the present experiments dimethyl acetamino-malonate was applied, accordingly, the obtained condensate (I) consisted of the dimethylate.

The alkaline hydrolysis of I afforded a dicarboxylic acid (II) which latter, on heating in an aqueous solution, decarboxylated and yielded N-acetyl-DL-p-nitrophenyl alanine (III). On determining the m. p. of the dicarboxylic acid it was observed that the compound melts at 152—153°C under foaming, then solidifies at about 160°C to melt again at 194—196°C. The phenomenon was subjected to examinations in detail with preparative quantities and was identified as thermal decarboxylation. The product melting at 194—196°C proved to be identical with compound III. It must be mentioned that thermal decarboxylation was carried out in excellent yields.

The hydrolysis of N-acetyl-DL-p-nitrophenyl alanine (III) with hydrochloric acid afforded the chlorohydrate of DL-p-nitrophenyl alanine (IV). The same product was obtained under similar conditions by the hydrolysis

of the dicarboxylic acid (II) and of the condensate (I), respectively, with hydrochloric acid, confirming the validity of the presumed structure of the intermediates.

The hydrolysis by hydrogen bromide as described by BURCKHALTER and STEPHENS [1] proved to be more suited for hydrolyzing the condensate than the hydrolysis by hydrochloric acid. Namely, the latter is in this case an agent not strong enough.



The aminoacid can be liberated from the chlorohydrate with the use of sodium acetate buffer. On allowing the solution with aminoacid liberated in form of a fluffy precipitate to stand for some days, it recrystallized as well developed crystals of a stable hydrate. On determining its m. p., water was liberated as drops. On drying weighed amounts of the substance, defined weight decrease was experienced. On the basis of these observations and of the analytical data, the composition of the compound corresponds to that of a monohydrate. The m. p. of the monohydrate differs from that of the product previously dried, this latter being of a less elongated nature. The hydrate showed a point of decomposition of 227–238°C against 242–244°C of the aminoacid previously dried under decreased pressure at the b. p. of xylene. It must be noted, however, that BURCKHALTER and STEPHENS [1] recorded a point of decomposition of 222–240°C.

*Experimental**Dimethyl p-nitrobenzyl-acetylaminomalonate (I)*

On adding 20 g (0,105 mole) of dimethyl acetylaminomalonate to a solution of 3 g of metallic sodium in 250 ml of anhydrous methanol, the solution was slightly heated to facilitate dissolution, then heated to gentle reflux and 20 g (0,127 mole) of *p*-nitro-benzyl chloride added at this temperature in about 15 minutes. Subsequently, the reaction mixture was gently refluxed for 8 hours, then the solution evaporated to dryness under reduced pressure, the residue extracted hot with 5×50 ml of chloroform, the combined filtrates evaporated to dryness under reduced pressure and the residue (33,2 g) recrystallized from 200 ml of anhydrous ethanol. On cooling, 26,04 g brilliant white crystals of the condensate were obtained. Yield 82%, referred to dimethyl acetylaminomalonate. M. p. 162°C. On repeatedly recrystallizing from 120 ml of anhydrous ethanol, 20,4 g (64%) of product, m. p. 164–165°C.

Analysis: $C_{14}H_{16}O_7N_2$. Calcd. C: 51,97 H: 4,97 N: 8,64%
 Found C: 52,20 H: 4,82 N: 8,78%.

p-Nitrobenzyl acetylaminomalonic acid (II)

On adding 5 g (0,0154 mole) of I to 20 ml of a 10% solution of sodium hydroxide, the mixture was refluxed for 2 hours. On cooling and filtering, 10 ml of concentrated hydrochloric acid was dropwise added, the precipitated yellow crystals were filtered and washed with water. On drying, yield 3,4 g (74%), m. p. 152–153°C under foaming, solidifying at 160°C and again melting at 194–196°C.

Analysis: $C_{12}H_{12}O_7N_2$. Calcd. C: 48,65 H: 4,08 N: 9,45%
 Found C: 48,63 H: 4,00 N: 9,40%.

N-acetyl-DL-p-nitrophenyl alanine (III)

a) On adding 1 g of II to 20 ml of water, the mixture was refluxed for 3 hours, slightly clarified and filtered hot. During cooling, fine yellow crystals appeared. On filtering and drying: 0,62 g (72%), m. p. 193–196°C.

Analysis: $C_{11}H_{12}O_5N_2$. Calcd. C: 52,37 H: 4,79 N: 11,10%
 Found C: 52,57 H: 4,60 N: 11,43%.

b) On cautiously heating 2 g of II, it was melted in an oil bath at 165°C, kept at this temperature for further 10 minutes and allowed to cool. On solidification, it was dissolved hot in 30 ml 50% aqueous ethanol, clarified and filtered. During cooling, orange crystals appeared. On filtering and drying: 1,48 g (86%), m. p. 194–196°C.

Analysis: C: 52,48 H: 4,82 N: 11,32%.

DL-p-Nitrophenylalanine chlorohydrate (IV)

a) On adding 15 ml of concentrated hydrochloric acid to 3,24 g (0,01 mole) of I, the mixture was boiled under reflux for 6 hours, rapidly filtered

while hot and allowed to cool when fine needles crystallized. On filtering and drying: 2,3 g (93%). Recrystallized from 50% aqueous ethanol or from a mixture of anhydrous ethanol and anhydrous ether: m. p. 227—230°C (decomp.).

Analysis of substance dried over P_2O_5 , at the b. p. of xylene under a pressure of 5 mm Hg

$C_9H_{11}O_4N_2Cl$ Calcd. C: 43,82 H: 4,49 N: 11,35 Cl: 14,37%

Found C: 44,02 H: 4,30 N: 11,50 Cl: 14,22%.

b) On hydrolyzing 2,96 g (0,01 mole) of II with concentrated hydrochloric acid in the way described under a), and recrystallizing the product, yield 1,91 g (78%), m. p. 227—230°C (decomp.).

Analysis: C: 43,98 H: 4,41 N: 11,57 Cl: 14,09%.

c) On hydrolyzing 2,52 g (0,01 mole) of III with concentrated hydrochloric acid in the way described under a) and recrystallizing the product, yield 1,69 g (69%), m. p. 227—230°C (decomp.).

Analysis: C: 43,65 H: 4,51 N: 11,43 Cl: 14,11%.

DL-p-Nitrophenylalanine (V)

On dissolving 1 g of DL-p-nitrophenylalanine chlorohydrate in 10 ml hot water, 1 g of pulverized sodium acetate was added to the solution while hot, the precipitated crystalline substance was filtered, washed with some water, ethanol and finally with ether. On drying, yield 0,68 g (80%), m. p. 242—244°C (decomp.).

Analysis of substance dried over P_2O_5 for 3 hours at the b. p. of xylene under a pressure of 5 mm Hg

$C_9H_{10}O_4N_2$ Calcd. C: 51,42 H: 4,79 N: 13,32%

Found C: 51,21 H: 4,90 N: 13,23%.

DL-p-Nitrophenylalanine hydrate

On adding 5 ml of a solution of sodium acetate saturated at room temperature to a solution of 1 g of DL-p-nitrophenylalanine chlorohydrate in 5 ml of water, the mixture was allowed to stand for two days. The flocculated substance which appeared at the beginning, slowly converted into well developed fine platelets-needles. On filtering and washing the crystals with some water, ethanol and ether, and drying, yield: 0,78 g (83%), m. p. 227—238°C (decomp.).

Analysis: $C_9H_{12}O_5N_2$ (monohydrate) Calcd. C: 47,20 H: 5,28 N: 12,23%

Found C: 47,18 H: 5,04 N: 12,30%.

Dehydration

On drying 0,1132 g of DL-p-nitrophenylalanine hydrate over P_2O_5 for 6 hours at the boiling point of xylene under a pressure of 5 mm Hg, the actually weighed decrease of weight amounted to 0,101 g, against 0,0090 g calculated with respect to monohydrate.

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ДАНИЕ К СИНТЕЗУ DL-p-НИТРОФЕНИЛАЛАНИНА

М. Гальмош

Конденсацией диметилового эфира ацетиламиномалоновой кислоты и хлористого p-нитробензила может быть получен DL-p-нитрофенилаланин. Синтез может быть разложен на этапы: щелочным гидролизом конденсата может быть изолирована дикарбоно-кислотная фаза, последняя может быть декарбоксилирована термически в N-ацетил-DL-аминокислоту. Аминокислота получается из последней кислотным гидролизом. Структура промежуточных продуктов может быть доказана кислотным гидролизом, из каждого из них образуется соляно-кислотная соль аминокислоты. При освобождении аминокислоты из ее соляно-кислотной соли удалось изолировать устойчивый гидрат.