RESOLUTION OF DL-ALANINE

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(Received September 20, 1958)

The authors succeeded in preparing 3,5-dinitrobenzoyl-DL-alanine in yields appreciably higher than those given in literature. The resolution of the product carried out with the use of quinine, in fair yields, affording preparations of adequate optical purity.

In the course of our research work the necessity of resolving DL-alanine by a well reproducible and easily processable tehnique arose. Although a great number of chemical and biological methods are known in the literature for preparing optically active forms of alanine, these methods are far from being satisfactory, due to the difficulty of the problem. Namely, most derivatives of alanine are known to form with the conventional resolving agents partial or complete mixed crystals [7, 8] which fact involves considerable difficulties when desiring products of adequate optical purity. In certain cases no products of satisfactory optical purity are yielded by repeated recrystallizations, either. In other cases, difficulties are encountered, due to increased volumes of solvents or to the expensiveness of the resolving agent applied, mainly when processing large quantities.

In the following, a schematic survey will be given of the methods of resolving alanine, carried out by the techniques of preparative chemistry. Processes of resolution by biological methods [13, 14] will not be dealt here.

An appreciable part of the preparative chemical methods of producing optically active forms of alanine carries out the resolution of N-acyl derivatives of DL-alanine by optically active bases.

Of the methods of resolving alanine by salt formation with optically active bases, the FISCHER method deserves to be mentioned [1]. On preparing the brucine salt of benzoyl-DL-alanine, the brucine salt of benzoyl-D(—)-alanine precipitates whereas benzoyl-L(+)-alanine is obtained from the residual salt of the mother liquor, after adequate recrystallization or purifying through formation of its strychnine salt. According to investigations mentioned later, the obtained alanine cannot be considered as optically pure.

A great number of improved modifications of the FISCHER method are known. The resolution of benzoyl-DL-alanine is carried out by POPE and GIBSON [2] similarly with the use of brucine and strychnine, applying the "equilibrium technique", as follows. Two equivalents of benzoyl-DL-alanine are treated with one equivalent of strychine, in an aqueous solution contain-

ing one equivalent of sodium hydroxide. The strychnine salt of benzoyl-L(+)-alanine precipitates. The residue in the mother liquor, mainly benzoyl-D(—)-alanine, is liberated from traces of strychnine. On the addition of a quantity of brucine equivalent to that of benzoyl-D(—)-alanine calculated on the basis of rotatory power and of sodium hydroxide equivalent to the amount of benzoyl-L(+)-alanine, the brucine salt of benzoyl-D(—)-alanine precipitates from the aqueous solution.

Better results were attained by PACSU and MULLEN [3] by modifying the FISCHER method in that the precipitating alkaloids were applied in the reverse order.

COLLES and GIBSON [4] resolved DL-alanine in form of its m-nitrobenzoyl derivative, applying the previously mentioned "equilibrium technique". Metanitro-benzoyl-D(—)-alanine precipitates on the addition of quinine whilst the L(+)-modification is obtained when adding brucine. These authors carried out as well [5] the resolution of p-nitrobenzoyl-DL-alanine and β-naphtalene-sulphonyl-DL-alanine [6]. The methods suggested, whilst giving fair yields, are rather cumbersome.

GIBSON and SIMONSEN [7], on using the "equilibrium technique", decomposed p-toluene sulphonyl-DL-alanine into its antipodes, forming the brucine and strychnine salts. After an unsuccessful attempt [8], benzene sulphonyl-DL-alanine was resolved by GIBSON and LEVIN [9] with nor-d-ψ--ephedrine. However, this method is of only theoretical interest, due to difficulties in preparing nor-d-ψ-ephedrine.

Of the methods of resolving esters of DL-alanine by optically active acids, the process suggested by COLOMBANO and SANNA [10] is to be mentioned, converting DL-alanine ethylate into its antipodes by d-bromocamphor-sulphonic acid. An appreciably better method was recently published by LAGENBECK and HERBST [11], on resolving the benzylate of DL-alanine by dibenzoyl-D-tartaric acid and D-tartaric acid.

KIPPING and POPE evolved a special method for the resolution of DL-alanine [12], by reacting DL-alanine ethylate with d-hydroxy-methylene camphor. On recrystallizing the obtained product from petroleum ether, d-methylene camphor-l-alanine ethylate precipitates.

The mentioned difficulties in carriyng out the listed methods required a supervision of the resolvability of 3,5-dinitrobenzoyl-DL-alanine. Namely, on the basis of resolution methods evolved earlier in this Institute [15], and of other data of literature [16], the conclusion seems to be justified that the decomposition of amino acids into their antipodes may favourably be conducted through their 3,5-dinitrobenzoyl derivatives. The SCHOTTEN—BAUMANN acylation of the appropriate amino acid affords in fair yields the 3,5-dinitrobenzoyl derivatives of amino acids, compounds readily crystallizing. Dinitrobenzoyl groups can be removed of the molecules without any danger of racemization.

SAUNDERS [17] prescribes two minutes as the duration of reaction when preparing the 3,5-dinitrobenzoyl derivatives of DL-alanine and L-alanine. However, the yields given by this author are unsatisfactory. On supervising the data of SAUNDERS, TOWN states 30 minutes as the time of reactions [18], obtaining rather low yields, either. At the dinitrobenzoylation of DL-alanine

and L-alanine, respectively, the yields ranged 63% with respect to the crude product and 49,5% with respect to recrystallized one.

By altering the conditions of reaction, we succeeded in our experiments to obtain a yield of 89,4% with respect to crude product and of 79,8% with respect to recrystallized pure substance. This could be attained, in addition to applying elongated times of reaction, by adding 3,5-dinitrobenzoyl chloride in a benzene solution and by the stepwise addition of a quantity of sodium hydroxide slightly exceeding the theoretic one. In connection with the resolution it is of interest to note that 3,5-dinitrobenzoyl-DL-alanine could not be resolved by brucine whereas the benzoyl [1] and the m-nitrobenzoyl [4] derivatives of alanine were readily resolved by brucine.

After a great number of unsuccessful attempts with many alkaloids from various solvents, the recrystallization of the quinine salt of 3,5-dinitrobenzoyl-DL-alanine from acetone was found best suited. First the quininate of 3,5-dinitrobenzoyl-D(—)-alanine precipitated as poorly soluble salt, affording on repeated recrystallizations from acetone optically pure 3,5-dinitrobenzoyl-D(—)-alanine quininate. From this latter, 3,5-dinitrobenzoyl-D(—)-alanine was liberated by the usual way. The rotatory power of the product was identical to that of an authentic preparation.

The L(+)-modification was liberated from the residual quininate of the mother liquor, by appropriate processing.

Experimental

3,5-Dinitrobenzoyl-DL-alanine

In a three-neck round-bottomed flask of 1000 ml, 10,6 g (0,119 mole) of DL-alanine (analytical grade, Chinoin make) was dissolved in a mixture of 50 ml of benzene, 150 ml of water and 5,2 g (0,130 mole) of sodium hydroxide. On keeping the contents of the flask at a temperature below +10°, the solution of 27,5 g (0,119 mole) of 3,5-dinitrobenzoyl chloride in 150 ml of benzene and a solution of 5,0 g (0,125 mole) of sodium hydroxide in 150 ml of water were added, during continuous stirring, in a period of one hour. Stirring was then continued for further an hour at room temperature. On separating the benzene phase in a separating funnel, the aqueous solution was treated, under continuous stirring, with 20 ml of concentrated hydrochloric acid added dropwise. The precipitated 3,5-dinitrobenzoyl-DL-alanine was allowed to stand for an hour at room temperature and for two hours at +4°. On filtering, 30,2 g (89,4%) of white crystalline subsance was obtained. Recrystallization from water (about 600 ml) afforded 26,9 g (79,8%) of completely pure compound, m. p. 176—177° [18].

Preliminary experiments on resolution

In these experiments the acetonic solution of 0,005 mole of 3,5-dinitrobenzoyl-DL-alanine and the acetonic or chloroformic solution of 0,005 mole of a base generally used for resolution were combined, evaporated to dryness and dissolved by an adequate solvent, adding the solvent in small portions. The solution was allowed to stand at room temperature or cooled when re-

quired, until crystals or an oil formed. On separating the crystals by filtration, they were dissolved in water, the resolving base liberated by adding 1,0 N sodium hydroxide, then filtered. The solution was extracted by chloroform, acidified with 1,0 N hydrochloric acid, and the rotatory power of 3,5-dinitrobenzoyl-alanine, precipitated on acidifying the solution, measured in an acetone medium.

3,5-Dinitrobenzoyl-DL-alanine gave with brucine a salt readily crystallizing from water, from a 3:1 mixture of water and methanol, further from methanol. The prepared brucine salt (m. p. 151—153°) proved to be a complete mixed crystal. The liberated 3,5-dinitrobenzoyl-alanine did not show any rotatory power.

Treatment of the aqueous solution of 3,5-dinitrobenzoyl-DL-alanine with cinchonine gave an oil.

On reacting 3,5-dinitrobenzoyl-DL-alanine with strichnine in an aqueous medium afforded an oil. A readily crystallizing substance was obtained in an acetone solution. However, the liberated 3,5-dinitrobenzoyl-alanine proved to be inactive.

The salt formed with quinine separated from an equeous solution in form of an oil. On recrystallizing from methanol, a crystalline product was obtained. 3,5-Dinitrobenzoyl-alanine liberated from this product was found to be slightly levorotatory. On recrystallizing from an acetone solution, fair resolution was attained. The quininate of 3,5-dinitrobenzoyl-D(—)-alanine precipitated first.

3,5-Dinitrobenzoyl-D(—)-alanine quininate

The solution of 5,66 g (0,02 mole) of 3,5-dinitrobenzoyl-DL-alanine in 25 ml methanol was treated with the solution of 6,49 g (0,02 mole) of water-free quinine in 25 ml of methanol, the solvent removed by distillation, the residual yellowish substance dissolved hot in 70 ml of acetone and the filtered solution allowed to stand for eight hours at room temperature, for four hours at $+4°$ and for further four hours at $-20°$. Yield on filtering: 3,94 g of sucrose-like yellowish-green crystals, m. p. 202—204°, $[\alpha]_D^{26}$: $-101,7°$ ($c = 1,0$; ethylacetate). On evaporating the filtrate to 45 ml, further 1,27 g of substance of identical physical constants was obtained.

Combining both fractions, 5,21 g of substance (85,8%) was repeatedly recrystallized from acetone, affording 4,05 g (66,7%) of optically pure 3,5-dinitrobenzoyl-D(—)-alanine quininate, m. p. 208—209°, $[\alpha]_D^{26}$: $-115,3°$ ($c = 1,0$; ethylacetate). Analysis of airdry substance: N 11,5% (calculated on the basis of formula $C_{30}H_{33}O_9N_5$). Found N 10,9%.

3,5-Dinitrobenzoyl-D(—)-alanine

3,0 g of 3,5-dinitrobenzoyl-D(—)-alanine quininate (repeatedly recrystallized from acetone $[\alpha]_D^{26}$: $-115,3°$; ($c = 1,0$; ethylacetate), m. p. 207—208°) was dissolved in 260 ml of hot water, 1,0 N sodium hydroxide added to decompose the salt, then the slightly alkalified solution allowed to stand at $+4°$ for an hour. On removing the precipitated quinine base (1,7 g) by fil-

tering, traces of quinine were separated by extraction with 2×100 ml of ether, the solution acidified with 2,0 N hydrochloric acid, evaporated to 60 ml under a pressure of 20 mm Hg, and allowed to stand for four hours at room temperature and for further four hours at $+4°$. Yield: 0,87 g of long white needles of 3,5-dinitrobenzoyl-D(—)-alanine of analytical purity. When reducing the volume of the solution to 20 ml by evaporation, further 0,45 g of pure product was obtained. Total yield 1,32 (92,5%), m. p. 178—178,5° [18], $[\alpha]_D^{26}$: $-22,1°$ ($c = 3,2$; acetone). Analysis: N calculated on the basis of formula $C_{10}H_9N_3O_7$ 14,84%. N found 14,70%.

Authentic 3,5-dinitrobenzoyl-D(—)-alanine

The solution of 0,50 g (0,00562 mole) of D(—)-alanine (Fluka; analytical grade $[\alpha]_D^{26}$: $-14,5$; $c = 2,0$; 6 N HCl) in 7 ml of 1,0 N sodium hydroxide was cooled to $+5°$ and treated under continuous shaking with small portions of a solution of a solution of 1,29 g (0,00562 mole) of 3,5-dinitrobenzoyl chloride in 4 ml of benzene, further of 6 ml of 1,0 N sodium hydroxide, in 20 minutes. On mechanical shaking for 1,5 hours at room temperature, the mixture was slightly acidified with 1,0 N hydrochloric acid and allowed to stand for two hours at $+4°$. Yield: 1,37 g (86,2%) of 3,5-dinitrobenzoyl-D(—)-alanine, m. p. 172—173°. On recrystallizing repeatedly from water, m. p. 177°, $[\alpha]_D^{26}$: $-21,8°$ ($c = 2,4$; acetone). Analysis: N calculated on the basis of formula $C_{10}H_9N_3O_7$ 14,84%. N found 15,19%.

3,5-Dinitrobenzoyl-L(+)-alanine

On reducing, by evaporation, the volume of the mother liquor obtained after filtration of the second fraction of 3,5-dinitrobenzoyl-D(—)-alanine quininate to about 30 ml, and allowing the solution to stand at room temperature, 1,45 g of crystalline substance was obtained, mainly consisting of 3,5-dinitrobenzoyl-D(—)-alanine quininate. The mother liquor was evaporated to dryness, the residue dissolved in 40 ml of ethylacetate and the solution allowed to stand for four hours at room temperature, then for further four hours at $+4°$, to afford 3,15 g of greenish yellow microcrystals (mainly consisting of the quininate of 3,5-dinitrobenzoyl-L(+)-alanine m. p. 112—114°. The evaporation of the mother liquor to half volume gave further 1,30 g of substance with identical m. p., raising thus the yield to 4,45 g (73,2%).

Liberation of 3,5-dinitrobenzol-L(+)-alanine from the quininate was carried out by the way described at 3,5-dinitrobenzoyl-D(—)-alanine, 1,10 g (87,4%) of 3,5-dinitrobenzoyl-L(+)-alanine being obtained from 2,7 g of 3,5-dinitrobenzoyl-L(+)-alanine quininate. Recrystallization from 20 ml of water gave 0,88 g of optically pure substance, m. p. 177°; $[\alpha]_D^{25}$: $+21,7°$ ($c = 1,6$; acetone).

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The authors express their thanks to Mrs. G. BARTÓK—BOZÓKI and to Mrs. M. CSILLIK—SOMFAY for carrying out the microanalyses.

This work has been sponsored by the Factory of Fine Chemicals Reanal, Budapest.

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