THE PROBLEMS OF THE OPTICAL RESOLUTION OF ASPARAGINE AND ASPARTIC ACID

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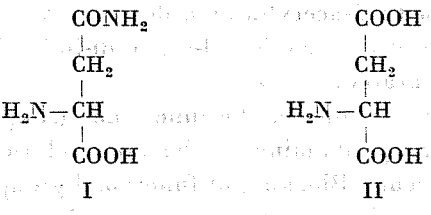
Introduction

The classical Pasteur's procedure — "mechanical separation of crystals" was applied by Putti [1] for the first resolution of racemic asparagine.

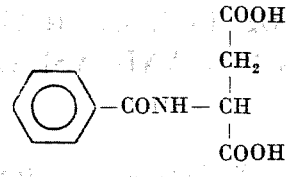
Resolution of amino acids is different from that of the other compounds. This is partly due to the bifunctionality of amino acids. The most widely applied method of resolution of amino acids is the spontaneous or induced crystallization of the free amino acid or its salt.

Many varieties of this procedure are known, but the yield of one crystallization is not more than 20% and after some repetitions of the crystallization, the compounds has to be processed. Harada [2] suggested a method of spontaneous crystallization in the presence of ammonium formate for resolving the racemic asparagine (I) and aspartic acid (II); I and II were obtained in 10– to 15%.



The optical isomers of asparagine (I) were isolated by Ostromislenszky [3] from the supersaturated aqueous solution of the racemic compound. Harada and Fox [4] crystallized the aspartic acid (II) in form of its copper complex, from aqueous solution. Contimous spontaneous crystallization are also known in the literature but in all cases (e. g. by recrystallization) the isomers obtained in this manner must be purified.

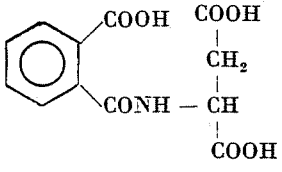
The enzymatic optical activation of aspartic acid (II) is also known (e. g.: the N-benzoyl derivative (III) of aspartic acid (II) is resolvable the enzymatic way [5]).



III

The most economical procedures are the resolutions via diastereomer formations. In this manner the products are obtained in the highest yield and purity. In many cases the amino acids are resolved after blocking one of their functional groups, mostly the amino group is blocked by acylation and the N-substituted derivative is resolvable with resolving bases. After the resolution the acyl group is eliminated by hydrolysis.

The N-phtaloyl-DL-aspartic acid (IV) has been resolved by DWYER [6]. The resolving agent was L-cysteine-dinitro-bis-etylene-diamino/Co^{III} acetate.



IV

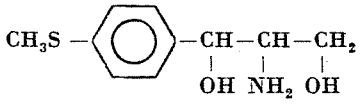
Another possibility is the conversion of the carboxyl group to ester, amide, hydrazide etc. In these cases the compounds are resolvable as acids.

Sometimes the methods may be combined (e. g.: the amino acid is N-acylated, esterified and converted to amide).

YAMAMOTO and TSUKAMOTO [7] N-acetylated, esterified and condensed the aspartic acid (II) with 2-acetyl-amido-desoxy-3,4,6-tri-O-acetyl-D-glucose--amin, in the presence of di-cyclo-hexyl-carbo-di-imide. The obtained diastereoisomers could be separated.

In most lucky cases none of the functional groups must be blocked, as the other functional groups of amino acid lead to resolution without temporarily transforming the molecule. Blocking of functional groups of amino acids with more amino than carboxyl groups (or vice versa) is needless, they can be resolved as bases (or acids).

A good resolving agent for II is the L-threo-2-amino-(p-methyl-mercapto-phenyl)-propan-1.3-diol (V). Isomers are separated through the fractional crystallization of diastereomers [8].



V

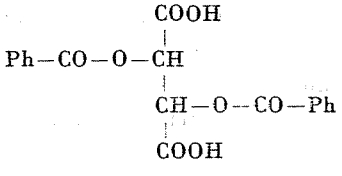
The molecule having one amino and one carboxyl group can be resolved with basic / or acidic resolving agent if the isoelectric point of the molecule is near to pH:1 (or pH:7). This principle has been applied to resolve the asparagine (I) with isoelectric point of 5,9.

The asparagine (I) is resolvable without transforming the compound [9]. The resolving agent must be acidic. In the resolution of asparagine (I) this possibility is very advantageous because the amide group is readly hydrolyzed and it is uneconomical to recover the asparagine (I) from the resulting aspartic acid (II).

Thus, asparagine (I) can be resolved without transforming the molecule.

Discussion

Separation of diastereoisomers has been attempted with several resolving agents. The best results were obtained with dibenzoyl-d-tartaric acid (VI) [9].



VI

Resolution of asparagine (I) was done by adding 1 mol of VI. to 1 mol of racemic I. The experiments were carried out in aqueous solution. The precipitate was an L-isomer salt. After recrystallization the obtained salt was decomposed to yield high-purity of L—I. D-isomer of I obtained from the mother liquor. at a yield of 60% for both isomers.

Possibilities of increasing the yield, decreasing the number of processes and improving their economy has been considered, and found to be preconditioned by the basicity of asparagine.

The amount of resolving agent was reduced to 0.5 mol and 0.5 mol of aq. HCl was added to the mixture to keep the remaining 0.5 mol of asparagine (I) in solution. True to expectation the L-isomer salt precipitated, but in a higher yield and purity than in the previous experiments. The yield of the separated L-asparagine is 80%, and it is of high purity.

The D-isomer of asparagine (I) is obtained from the mother liquor in a good yield and quality.

This principle (POPE-PEACHEY [10] equilibrum method, often successfully applied without noting or even knowing its authors [11]) permits resolution of better yield and purity when the substrate to be resolved is treated with

half of the usual amount of resolving agent, and an achiral base (or acid, depending upon the chemical character of the resolving agent) is added to the mixture.

In ideal cases the separation does not depend upon the stability differences of diastereomers but upon the solubility differencies one diastereomeric salt and a salt of the substrate and of the inactive co-base (or co-acid).

The inactive co-base (co-acid) can be selected so that its salt remains in solution.

In our procedure several problems awaited solution.

For a 1:1 molar ratio of the two components by themselves in water must be heated together to achieve hot solution of the obtained salts.

Upon cooling the L-isomer salt precipitated, but not purely. During heating both the ester groups of VI and amide group of I may hydrolize, to the detriment of yield:

a) *hydrolysed products are useless*, and

b) *disturb the crystallization*

Recrystallization for purifying the salt is another loss, due to both solubility differencies and hydrolysis. Mixed else than in solution they yield non pure isomer salt. Difficulties can be bypassed by applying a 1:0.5 molar ratio of racemic asparagine to dibenzoyl-d-tartaric acid, because the racemic asparagine (I) is soluble at 60 °C in the presence of 0.5 mol of HCl, without hydrolyzing. VI is very soluble in methanol. In this manner two solutions are mixed satisfactory by eliminating demands heating to 90 °C and yielding a clear solution at 50 °C, causing the L-isomer salt to precipitate pure from this solution.

Also isolation of stereoisomers is an interesting point, because the salt decomposes in both acidic and basic aqueous media, but under acidic condition the precipitated VI crystallizes poorly and contains much of L—I. In this case the D-isomer yield mother liqour depends upon the pH setting.

Salt decomposed in a basic medium yield easy crystallizing L—I monohydrate precipitate without VI impurity.

The salt can be decomposed by boiling in methanol, because the L—I, as a "zwitter-ion" is insoluble in methanol, and less soluble than the diastereomeric salt. Also the L—I is filtrable, and VI remains in solution.

The pure D—I monohydrate is obtained in good yield from the mother liquor at the required pH.

In this procedure it is sufficient to recrystallize the isolated optical isomers (or even may be ommitted).

The last problem is the regeneration of resolving agent (VI). For a molar ratio of 1:1, the hydrolysis prevents it from purely regenerating at a good yield, to be reutilized.

For a molar ratio of 1:0.5, the resolving agent can purely be regenerated, to a high yield and apt to be re-used.

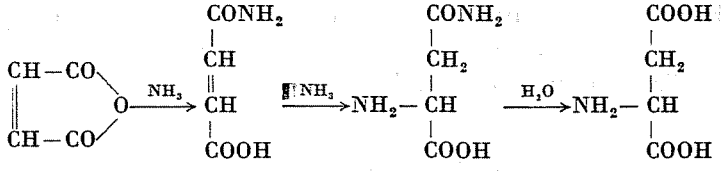
For the sake of comparaison the possibility of production of the optical isomers of aspartic acid (II) was considered. The isoelectric point of aspartic acid (II) being 2.9, the resolution of II was likely to be carried out with a basic resolving agent, without the transformation of the molecule. (Since the molecule contains two carboxyl and one amino groups, one of the carboxyl groups is not neutralised, therefore the compound can be considered as an acid). Indeed, it could be resolved with threo-L-2-amino-1-(p-methyl-mercapto--phenyl)-propane-1.3-diol (V), at a yield of 60% [8], still insufficient for industrial applications.

The resolution of aspartic acid (II) with other amines was considered but with still poorer results.

The aspartic acid (II) is not resolvable by the same way as the asparagine (I). The aspartic acid (II) is too week an acid to be resolved as an acid, its isoelectric point is too high for it.

To separate the optical isomers of II in high yield, the carboxyl or the amino group have to be blocked and the obtained product resolved. One of these derivatives is asparagine (I), it is the half-amide of aspartic acid (II), to be resolved as above.

To synthetise the asparagine (I) from aspartic acid (II) is however, of poor economy, still feasible for preparing the optical isomers of II, namely:



Asparagine (I) is an intermediary of the economical industrial synthesis starting from maleic anhydrid of aspartic acid (II) [12]. Resolving the arising asparagine (I) and hydrolysing yields optical isomers of aspartic acid (II).

Experimental

1. 18.8 g (0.05 mol) of dibenzoyl-d tartaric acid monohydrate are solved in 500 ml of water, during continuous heating and stirring, 7,5 g (0,05 mol) of DL-asparagine monohydrate.

The temperature of mixture must not exceed 90 °C. After dissolving the solution is allowed to cool to room temperature, and kept at 0 °C overnight. The precipitated crystals were filtered, solved in 50 ml of water during

heating, and kept at room temperature overnight, filtered at room temperature. The obtained salt is L-asparagine-dibenzoyl-d-hemitartarate at a yield of 13.6 g.

2. The obtained salt (13.6 g of L—I—VI) is refluxed in 50 ml of anhydrous methanol for 1 hour, then filtered. The obtained product is crude L—asparagine L—(I) of 1.9 g (50.8%)

$$[\alpha]_D^{20}: + 29.3^0 \text{ (c:10; nHCl)}$$

3. The combined mother liqours of 1 and 2 are concentrated in vacuo to 50 ml, basified to pH:6 with NH_4OH. The precipitate of crude D-asparagine (D—I) is recrystallized in 10 ml of water.
Yield: 1.95 g 52%

$$[\alpha]_D^{20}: -29.0^0 \text{ (c: 10; nHCl)}$$

4. 7,5 g (0.05 mol) of DL-asparagine (I) monohydrate are suspended in 12.7 ml of distilled water, adding 2.08 ml (0.025 mol) of aq. HCl (c:37%; pw: 1.19 g/cm^3), then heating to about 80 °C. 9.58 g (0.025 mol) of dibenzoyl-d-tartaric acid (VI) solved in 12.7 ml of methanol are added. These solutions combined at about 40 °C are allowed to cool during stirring, start crystallization. Left at 10 °C overnight, the other day the crystals are separated by filtration, washed with 2×2 ml of methanol (50%). The obtained salt was L-asparagine-dibenzoyl-d-hemitartarat (L—I—VI).

Yield: 11,1 g

5. The combined filtrates of methanol-water are basified to pH:6 with cc. NH_4OH. The crystallization is completed at 10 °C overnight. The precipitated D-asparagine-monohydrate is filtered, washed with 2×1 ml of methanol and recrystallized in 10 ml of distilled water.

Yield: 3.0 g (80.2%)

$$[\alpha]_D^{20}: -30.2^\circ \text{ (c:10; nHCl)}$$

6. 11.1 g of L—I—VI are suspended in 15 ml of distilled water and neutralised to pH:7 with cc. NH_4OH, kept overnight at 10 °C. The precipitated crystals — crude L-asparagine monohydrate — are filtered, recrystallized from 10 ml of distilled water.

Yield: 3.05 g (81.3%)

$$[\alpha]_D^{20}: -31.2^\circ \text{ (c: 10; nHCl)}$$

7. Mother liquors 4,5,6 are combined, methanol distilled and the residue stirred and acidified to pH:1 with ccHCl at 10 °C. The precipitated dibenzoyl-d-tartaric acid (VI) is filtered and washed with 2×5 ml of water.

Yield: 8.2 g (85.8%)

mp.: 86—88 °C

The obtained VI can be re-used.

8. The obtained 3 g of D-asparagine monohydrate are boiled in the mixture of 10 ml of distilled water and 4.5 ml of cc HCl for two hours. The obtained solution is basified to pH:3, with NH_4OH, and left overnight at 10 °C.

The precipitated D-asparagine is filtered, washed with 3×1 ml of water.

Yield: 2.38 g (90.0%)

$$[\alpha]_D^{20}: +29.8° \text{ (c:10; 6nHCl)}$$

9. The obtained 3.05 g of L-asparagine is hydrolysed as above.

Yield: 2.43 g (90,0%)

$$[\alpha]_D^{20}: -30° \text{ (c:10; 6nHCl)}$$

Summary

Optical isomers of asparagine were prepared from racemic asparagine by a new method. The resolution was carried out with dibenzoyl-d-tartaric acid, in water. The molar ratios of racemic bases to resolving agent were 1 : 1 and 1 : 0,5. In the last case the mixture was neutralised with 0,5 mol of HCl.

In both procedures the L-asparagine-dibenzoyl-d-hemitartarat precipitated and the D-isomer arose from the mother liquor neutralised to pH: 6.

The optical isomers of aspartic acid were prepared by hydrolysis from asparagine's optical isomers.

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