[CONTRIBUTION FROM THE DEPARTMENT OF ORGANIC CHEMISTRY, THE HEBREW UNIVERSITY]

# Syntheses of *N*-Alkyl-aspartic Acids and $N^2$-Alkyl-α-asparagines

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General methods for the preparation of *N*-alkyl-aspartic acids and $N^2$-alkyl-α-asparagines *via* maleic anhydride were worked out. The former were obtained on alkaline hydrolysis of their β-methyl esters, obtained by addition of amines to monomethyl maleate. Reaction of ammonia with the anhydride hydrochlorides of *N*-alkyl-aspartic acids or with their mixed anhydrides with chloroformic acid (obtained on reaction with phosgene) gave $N^2$-alkyl-α-asparagines.

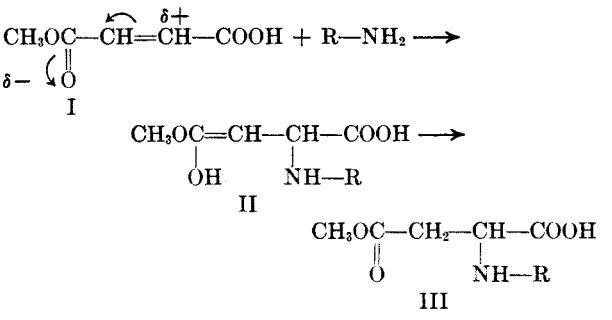
Only a few *N*-alkyl-aspartic acids are reported in the literature. They were obtained in very small yields by the action of amines on halo succinic acids,[1] or by alkylation of aspartic acid with aldehydes.[2] In a patent[3] a general method for their preparation was described; however, no melting points, analyses etc., were given. It consists of heating for a long time, maleic anhydride in aqueous solution with a large excess of amine in an autoclave. The amide of the *N*-alkyl-aspartic acid thus formed is hydrolyzed by very long refluxing with concentrated sodium hydroxide. Separation of the *N*-alkyl-aspartic acids — many of which are soluble in water — from the large amounts of salts formed, is very difficult.

A simple preparation of *N*-benzylaspartic acid had been reported recently[4] by the reaction of benzylamine with aqueous maleic acid under reflux. However, this method did not prove a general one[5] and the reaction of other amines with maleic acid either in aqueous solution or otherwise did not give the required *N*-alkyl-aspartic acid but led to the formation of amine salts of maleic acid or to unidentified products.

In continuation of this work, we found that increasing the activity of the double bond of maleic acid by the introduction of an ester group enabled a smooth nucleophilic addition of amines to the double bond. Reaction of monomethyl maleate, prepared easily from maleic anhydride and methanol, with one equivalent of amine led only to opening of the double bond, giving *N*-alkylaspartic acid β-methyl esters, without attacking the ester group; showing that addition to the double bond is preferable to amidation. Use of two equivalents of amine led both to addition to the double bond and amidation giving $N^2$-alkyl-*N*-alkyl-β-asparagines. These reactions can be carried out in methanol, dioxan, or best in pyridine. The structure of the β-methyl esters was further proved by their positive reaction with copper carbonate as is compatible with their having a free α-carboxyl group.[6]

The addition reaction proceeds probably by the following mechanism (Chart I). The ester group, being more electronegative than the carboxyl group, polarizes the double bond as shown in I and the nucleophilic amine attaches to the positive carbon atom by the unshared electron pair of the nitrogen. 1,4- addition of the amine gives the enolic form (II) which passes over to the *N*-alkyl-aspartic acid β-methyl ester (III).

CHART I



The *N*-alkyl-aspartic acids were obtained from the corresponding β-methyl esters which, contrary to the amides, are easily hydrolyzed by cold dilute alkali. Since many of the *N*-alkyl-aspartic acids are soluble in water, and thus difficult to purify them from soluble inorganic salts, hydrolysis of the β-methyl esters was carried out with barium hydroxide. Addition of an equivalent of sulfuric acid precipitated the barium as sulfate. Evaporation of the filtrate gave the *N*-alkyl-aspartic acids in almost quantitative yield. Complete hydrolysis of the ester groups was proved by negative methoxyl determinations.

No general method had been given for the preparation of $N^2$-alkyl-α-asparagines. A difficult procedure for the preparation of $N^2$-methyl-α-asparagine had been reported.[7] Their preparation was carried out either by action of dry ammonia on the α-mixed anhydrides of the *N*-alkyl-aspartic acids

(1) O. Lutz, *Ber.*, **67**, 648 (1934).
(2) S. Kano, *J. Pharm. Soc., Japan*, **66**, 4 (1946).
(3) W. Reppe and H. Ufer, U. S. Patent **2,200,220** (1940); *cf.* I. G. Farbenind, French Patent **793,504** (1936).
(4) M. Frankel, Y. Liwschitz, and Y. Amiel, *J. Am. Chem. Soc.*, **75**, 330 (1953).
(5) Y. Liwschitz and R. D. Irsay, private communication.
(6) P. Desnuelle and G. Bonjour, *Biochim. et Biophys. Acta*, **9**, 356 (1952); *cf.* also ref. 8.
(7) G. Körner and A. Menozzi, *Gazz. chim. ital.*, **19**, 427 (1889); Beilstein, *Organische Chemie* **IV**, 485.

TABLE I

PREPARATION OF *N*-ALKYL-DL-ASPARTIC ACIDS β-METHYL ESTERS

| Substance, *N*-Alkyl-Aspartic Acid β-Methyl Ester | Yield, % | M.P., °C. | Formula | Carbon, % | | Hydrogen, % | | Nitrogen, % | |
|---|---|---|---|---|---|---|---|---|---|
| | | | | Calcd. | Found | Calcd. | Found | Calcd. | Found |
| Cyclohexyl- | 55[a] | 216 | $C_{11}H_{19}NO_4$ | 57.7 | 57.9 | 8.3 | 8.3 | 6.1 | 6.1 |
| *n*-Hexyl- | 69 | 213 | $C_{11}H_{21}NO_4$ | 57.1 | 57.5 | 9.1 | 9.1 | 6.1 | 5.9 |
| *n*-Butyl- | 68 | 220 | $C_9H_{17}NO_4$ | 53.2 | 53.1 | 8.4 | 8.2 | 6.9 | 6.7 |
| Allyl- | 66 | 213 | $C_8H_{13}NO_4$ | 51.3 | 51.9 | 7.0 | 7.2 | 7.5 | 7.2 |
| Isobutyl-[b] | 85 | 215 | $C_9H_{17}NO_4$ | 53.2 | 53.4 | 8.4 | 8.5 | 6.9 | 7.1 |
| Benzyl-[c] | 65 | 219 | $C_{12}H_{15}NO_4$ | 60.8 | 60.8 | 6.4 | 6.6 | 5.9 | 5.8 |
| Methyl-[d] | 57 | 206 | $C_6H_{11}NO_4$ | | | | | 8.7 | 9.0 |

[a] Reaction carried out in dioxan. [b] Using 2 equivalents of isobutylamine gave $N^2$-isobutyl-*N*-isobutyl-DL-β-asparagine, crystallized from water, m.p. 238°. *Anal.* Calcd. for $C_{12}H_{24}N_2O_4$: C, 59.0; H, 9.8; N, 11.5. Found: C, 59.4; H, 9.8; N, 11.3. [c] Using 2 equivalents of benzylamine gave $N^2$-benzyl-*N*-benzyl-β-DL-asparagine, crystallized from water, m.p. 216°. Identical with substance prepared by different method (cf. ref. 4). [d] Methylamine 33% alcoholic solution was used, and this added to the pyridine solution of the monomethyl maleate.

with chloroformic acid, obtained by reaction of phosgene with the *N*-alkyl-aspartic acids[8,9]; or by the action of aqueous ammonia on the *N*-alkyl-aspartic anhydride hydrochlorides formed in the cold by elimination of water from *N*-alkyl-aspartic acids by a (1:1) mixture of acetyl chloride–acetic acid.[10] The first method is to be preferred as it gave purer compounds. Considering the similar solubilities of the $N^2$-alkyl-α-asparagines and ammonium chloride we found that the most efficient method of purification was by cation exchange resins.[11]

The $N^2$-alkyl-α-asparagines are soluble in water and in hot ethanol, and insoluble in acetone. They give a positive sensitive red biuret reaction; and a negative reaction with copper carbonate when this is added to their boiling aqueous solutions[6] contrary to their β-isomers.[9] Having no α-carboxyl group available for zwitterion formation with the α-amino group, they have lower melting points than the β-isomers reported.[9]

## EXPERIMENTAL[12]

*Preparation of N-alkyl-aspartic acids β-methyl esters.* Maleic anhydride (0.054 mole) was dissolved in 15 ml. absolute methanol and refluxed for 30 min., excess methanol distilled *in vacuo*. The monomethyl maleate thus obtained was cooled in ice water, 10 ml. pyridine followed by 0.05 mole of amine added, and refluxed at 110–120° (oil bath) for about 1 hr. The reaction mixture assumed a brownish red color and the reaction product generally started to precipitate within 15 min. It was purified by trituration with acetone or ether-acetone (1:1), to remove the color of the mother pyridine solution, and filtered.

The esters were generally crystallized from a minimum volume of hot water, and acetone added to complete precipitation. The white crystalline material thus obtained gave negative reaction with aqueous potassium permanganate indicating the absence of double bonds.

The *N*-alkyl-aspartic acids β-methyl esters thus prepared are listed in Table I.

*Preparation of N-alkyl-aspartic acids.* *N*-Alkyl-aspartic acid β-methyl ester (0.05 mole) was dissolved in 0.125 mole barium hydroxide solution (0.35*N*) and left for 2 hr. at room temperature. The solution was heated for 10 min. near the boiling point and an exact equivalent of hot sulfuric acid (1*N*) was added portionwise with shaking to get an easily filtrable precipitate of barium sulfate. The solution was filtered through a sintered-glass Büchner funnel with fine perforations and evaporated to dryness *in vacuo* from a water bath. The *N*-alkyl-aspartic acid, which usually crystallized, was triturated with acetone and filtered. Recrystallization was carried out from a minimum volume of hot ethanol-water (2:1), more ethanol was then added to complete precipitation.

The *N*-alkyl-aspartic acids thus prepared are listed in Table II.

*Preparation of $N^2$-alkyl-α-asparagines. Method I.* Dry *N*-alkyl-aspartic acid (2 g.) was suspended in 100 ml. dry dioxan in a 3-necked flask equipped with a gas leading tube, reflux condenser connected to a calcium chloride tube, and a mechanical stirrer. Phosgene, dried over concentrated sulfuric acid, was bubbled in with stirring for 1 hr. and the temperature maintained at 60°. The substance usually dissolved within the first 15 min. Excess phosgene was removed *in vacuo* at 30°. The solution of the mixed anhydride with chloroformic acid thus obtained was transferred to a 3-necked flask equipped with a gas leading tube and a mechanical stirrer, and dry ammonia gas passed in with stirring and cooling for 15 min. The sticky precipitate formed was separated from the dioxan by decantation and dissolved in water. The solution was evaporated to dryness on a water bath to remove excess ammonia and the residue dissolved again in water. To remove the ammonium chloride the solution was passed through a column packed with (about 2.5–3 equivalents) cation exchange resin (nuclear sulfonic acid type resin, Amberlite IR-120). The course of the absorption and desorption of the $N^2$-alkyl-α-asparagines was followed conveniently by their sensitive reaction with biuret reagent. The ammonium ions and the $N^2$-alkyl-α-asparagine were held on the column while the chloride ions were removed as hydrochloric acid. The column was washed with water till the effluent liquid gave a negative reaction with silver nitrate. The column was eluted with ammonia (4–5%) till the eluent gave a negative red biuret reaction. The $N^2$-alkyl-α-asparagine, which crystallized on evaporation to

(8) Y. Liwschitz and A. Zilkha, *J. Am. Chem. Soc.*, **76**, 3698 (1954).

(9) Y. Liwschitz, Y. Edlitz-Pfeffermann, and Y. Lapidoth, *J. Am. Chem. Soc.*, **78**, 3069 (1956).

(10) A. Zilkha and Y. Liwschitz, *J. Chem. Soc.*, 4397 (1957).

(11) E. I. Vasilyeva and R. Kh. Freidlina, *Bull. Acad. Sci., U.S.S.R., Div. Chem. Sci.* (*English Translation*), **2**, 169 (1956).

(12) Microcombustion analyses were carried out by Drs. Weiler and Strauss. Melting points were determined in a Fisher-Johns apparatus.

TABLE II

PREPARATION OF *N*-ALKYL-DL-ASPARTIC ACIDS

| Substance *N*-Alkyl-Aspartic Acid[a] | M.P., °C. | Formula | Carbon, % | | Hydrogen, % | | Nitrogen, % | |
|---|---|---|---|---|---|---|---|---|
| | | | Calcd. | Found | Calcd. | Found | Calcd. | Found |
| Cyclohexyl- | 216 | $C_{10}H_{17}NO_4$ | 55.8 | 55.8 | 8.0 | 8.0 | 6.5 | 6.5 |
| *n*-Hexyl-[b] | 168 | $C_{10}H_{19}NO_4$ | 55.3 | 55.5 | 8.7 | 8.7 | 6.4 | 6.2 |
| *n*-Butyl- | 163 | $C_8H_{15}NO_4$ | 50.8 | 51.0 | 7.9 | 8.1 | 7.4 | 7.0 |
| Allyl-[c] | 184 | $C_7H_{11}NO_4$ | 48.6 | 48.7 | 6.4 | 6.5 | 8.1 | 8.1 |
| Isobutyl- | 191 | $C_8H_{15}NO_4$ | 50.8 | 51.2 | 7.9 | 7.7 | 7.4 | 7.6 |
| Methyl-[d] | 178 | $C_5H_9NO_4$ | | | | | 9.5 | 9.5 |

[a] The *N*-alkyl-aspartic acids were obtained in nearly quantitative yield from their esters. [b] Insoluble in water; to prevent precipitation with barium sulfate, its solution after hydrolysis must be filtered hot. May be hydrolyzed at room temperature with (2*N*) sodium hydroxide. [c] Evaporation of the solution after hydrolysis left an oil which crystallized from hot water–methanol and the addition of acetone. [d] Same m.p. as reported in Beilstein, *Organische Chemie* (*cf.* ref. 6).

TABLE III

PREPARATION OF $N^2$-ALKYL-DL-α-ASPARAGINES

| Substance, $N^2$-Alkyl-DL-α-asparagine | Yield,[a] % | M.P., °C. | Formula | Carbon, % | | Hydrogen, % | | Nitrogen, % | |
|---|---|---|---|---|---|---|---|---|---|
| | | | | Calcd. | Found | Calcd. | Found | Calcd. | Found |
| Cyclohexyl- | 71 | 181 | $C_{10}H_{18}N_2O_3$ | 56.0 | 55.8 | 8.4 | 8.5 | 13.1 | 12.5 |
| *n*-Hexyl-[b] | 85 | 160 | $C_{10}H_{20}N_2O_3$ | 55.5 | 55.1 | 9.3 | 9.1 | 12.9 | 12.4 |
| *n*-Butyl- | 73 | 193 | $C_8H_{16}N_2O_3$ | 51.1 | 51.1 | 8.5 | 8.5 | 14.9 | 14.7 |
| Allyl- | 76 | 175 | $C_7H_{12}N_2O_3$ | 48.8 | 48.4 | 6.9 | 6.9 | 16.2 | 15.7 |
| Isobutyl- | 83 | 170 | $C_8H_{16}N_2O_2$ | 51.0 | 51.0 | 8.5 | 8.5 | 14.9 | 14.7 |
| Methyl-[c] | 80 | 191 | $C_5H_{10}N_2O_3 + H_2O$ | | | | | 17.1 | 17.1 |

[a] Yields reported are from the "mixed anhydride" method. [b] Using the "anhydride hydrochloride" method gave 44% yield. [c] The substance sinters around 115°, due to loss of water of crystallization, resolidifies and melts at 191°. Same m.p as reported in *Beilstein*, Organische Chemie (*cf.* ref. 6).

dryness of the ammonia solution on a water bath, was triturated with acetone and filtered. The substances were recrystallized from a minimum quantity of hot water, a large volume of acetone was added and left overnight in an ice box to complete precipitation. (For the $N^2$-cyclohexyl-α-asparagine ether was also added to help crystallization.)

*Method II.* To dry *N*-alkyl-aspartic acid (1 g.) held in a glass-stoppered flask, 5 ml. acetic acid and 5 ml. acetyl chloride were added, shaken until solution was complete, and left overnight at room temperature. Generally, the anhydride hydrochloride precipitated, otherwise dry ether was added. It was filtered, washed with dry ether, and dried in a vacuum desiccator for a short time. The dry substance was added to 20 ml. concentrated ammonia solution, left at room temperature for 20 min. and evaporated on a water bath. Purification of the $N^2$-alkyl-α-asparagine was carried out as before.

The $N^2$-alkyl-α-asparagines thus prepared are listed in Table III.

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