the behavior of simple systems containing *N*-carbobenzoxyamino acids and D-glucosamine. The first *N*-carbobenzoxyamino acyl derivatives of D-glucosamine were prepared by Bergman and Zervas[4] by treating the *N*-carbobenzoxyamino acid chloride with the tetra-*O*-acetyl derivative of D-glucosamine. This procedure cannot readily be applied to the coupling of proteins with polysaccharides and therefore, a new procedure had to be employed. Sheehan and Hess[5] had shown that when *N,N'*-dicyclohexylcarbodiimide was employed as the condensing agent for peptide synthesis, it was not necessary to protect hydroxyl groups. Glycopeptides have been synthesized by treating an aldonic acid with the ethyl esters of amino acids using *N,N'*-dicyclohexylcarbodiimide as the condensing agent.[6] If no free amino group is present, esterification of the primary hydroxyl of glucose with an *N*-carbobenzoxy amino acid was shown to occur in the presence of *N,N'*-dicyclohexylcarbodiimide.[7]

In the present work, in addition to carbobenzoxyglycine, two *N*-carbobenzoxyamino acids with different types of —OH groups were chosen as reactants. In both instances, esterification was not shown to occur in the condensation reaction. The product gave a positive Morgan-Elson reaction, indicative of an *N*-acyl hexosamine. The purple color produced on the chromatogram faded within a few minutes. The possibility of *O*-glycoside formation was eliminated by the positive test for reducing sugars and the *N*-acylation of glucosamine was further indicated by the absence of ninhydrin-positive material in the isolated product.

Experimental[8]

Capillary melting points were determined for all compounds and are corrected.

Chromatographic analyses were performed on Whatman No. 1 paper using a butanol–acetic acid–water (4:1:5, v./v.) solvent system. The acylated amino sugars were detected by the use of a modified Morgan-Elson procedure[9] and by alkaline permanganate containing sodium metaperiodate.[10] The acylated glucosamine derivatives were chromatographically pure. When the glucosamine derivatives were hydrolyzed and the hydrolyzates chromatographed, only the constituent amino acid and the amino sugar could be detected by ninhydrin.

N-(**Carbobenzoxyglycyl**)-D-**glucosamine.**—*N,N'*-Dicyclohexylcarbodiimide (0.015 mole, 3.09 g.) was added to a solution composed of D-glucosamine (free base,[11] 0.01 mole, 1.79 g.) and carbobenzoxyglycine[12] (0.01 mole, 2.09 g.) dissolved in 50 ml. of 25% aqueous ethanol and then stirred overnight at room temperature. The insoluble dicyclohexylurea was removed by filtration and the ethanol was removed at 60° *in vacuo*. The aqueous residue was filtered and the filtrate was evaporated *in vacuo* over potassium hydroxide and phosphorus pentoxide. The residue was extracted with boiling acetone, the solution filtered while hot, and the filtrate was evaporated to dryness. The solid was crystallized from absolute ethanol; yield 1.97 g. (53%); m.p. 178–178.5°; $[\alpha]^{25}D$ +50 (*c* 0.20, methanol); $[\alpha]^{25}D$ +25.0° (*c* 0.23, water, final) downward mutarotation, R_f = 0.60 (lit.,[4] m.p. 181; $[\alpha]^{20}D$ +40.0° (methanol).

Anal. Calcd. for $C_{16}H_{22}O_8N_2$: C, 51.9; H, 6.0; N, 7.6. Found: C, 51.4; H, 6.3; N, 7.2.

N-(*N'*-**Carbobenzoxy**-L-**tyrosyl**)-D-**glucosamine.**—*N,N'*-Dicyclohexylcarbodiimide (0.015 mole, 3.09 g.) was added to 50 ml. of 25% aqueous ethanol containing D-glucosamine (0.01 mole, 1.79 g.) and *N*-carbobenzoxy-L-tyrosine.[13] The solution was stirred overnight and then filtered. The filtrate was evaporated *in vacuo* and the residue was washed with 50 ml. of boiling water and then with 50 ml. of boiling ethyl acetate. The solid residue was crystallized from absolute ethanol; yield 2.4 g. (48%); m.p. 189–190°; $[\alpha]^{26}D$ +26.6° (*c* 0.21, ethanol), R_f = 0.75.

Anal. Calcd. for $C_{23}H_{28}O_9N_2 \cdot H_2O$: C, 55.9; H, 6.1; N, 5.7. Found: C, 56.1; H, 5.6; N, 5.6.

N-(*N'*-**Carbobenzoxyhydroxy**-L-**prolyl**)-D-**glucosamine.**—*N,N'*-Dicyclohexylcarbodiimide (0.015 mole, 3.09 g.) was added to 50 ml. of 25% aqueous ethanol containing D-glucosamine (free base, 0.01 mole, 1.79 g.) and *N*-carbobenzoxyhydroxy-L-proline[14] (0.01 mole, 2.65 g.) and the solution stirred at room temperature overnight. The remainder of the procedure was similar to that described for the preparation of *N*-(*N'*-carbobenzoxyglycyl)-D-glucosamine. Yield 2.04 g. (46%); m.p. 155–156°; $[\alpha]^{31}D$ −13.5° (*c* 0.25, water, final) downward mutarotation, R_f = 0.56.

Anal. Calcd. for $C_{19}H_{26}O_9N_2 \cdot H_2O$: C, 51.4; H, 6.3; N, 6.3. Found: C, 51.8; H, 6.2; N, 6.4.

Acknowledgment.—We wish to thank Mr. John Humes for the polarimetric analysis.

(2) A preliminary report of this study was presented in part before the American Society of Biological Chemists, Chicago, Illinois, April, 1961, *Fed. Proc.*, **19**, 78 (1961).

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A New Synthesis of *threo*-β-Methylaspartic Acid

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For a study of the stereospecificity of a certain bacterial deaminase,[1] we required a sample of β-methylaspartic acid, preferably the *erythro* isomer. Although syntheses of the amino acid were reported in the literature,[2] the methods seemed

(1) V. R. Williams and J. G. Traynham, *Federation Proc.*, **21**, 247 (1962).

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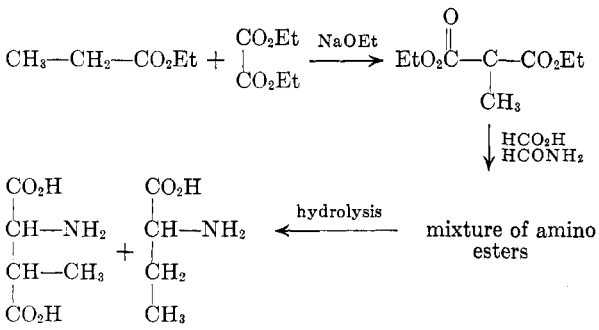
lengthy or were reported to give almost exclusively the *threo* isomer. We undertook a new synthesis of this amino acid and report the results here.[3]

Diethyl α-keto-β-methylsuccinate was prepared in good yield by a Claisen condensation between ethyl propionate and diethyl oxalate. This keto ester was then transformed directly into a mixture of amino esters by a Leuckart reaction. Although the Leuckart reaction has been used with many



aldehydes and ketones,[4] we are unaware of any previous use with a keto ester. After acid-catalyzed hydrolysis of the reaction mixture and removal of formic acid, a mixture of amino acids was obtained. These amino acids, identified by paper chromatography and paper electrophoresis, were α-aminobutyric acid and *threo*-β-methylaspartic acid.[5]

Particularly interesting (and disappointing) to us is the apparently exclusive formation of the *threo* isomer of β-methylaspartic acid. No *erythro* isomer could be detected among the reaction products by paper electrophoresis. Further, enzymatic resolution of our β-methyl-DL-aspartic acid (as well as the commercial sample[3]) gave β-methyl-D-aspartic acid with specific rotation at least as high as previously reported for *threo*-β-methyl-D-aspartic acid.[6] *erythro*-β-Methyl-D-aspartic acid has a large specific rotation of opposite sign,[6] and even small amounts of that isomer in our product would have reduced the observed rotation substantially.

On the basis of Cram's rule of asymmetric induction,[7] in spite of uncertainties about the mechanism of the Leuckart reaction,[4] one has difficulty avoiding the expectation that the reaction of the keto ester with formamide should lead predominately to the *erythro* isomer, unless the mechanism operating is reduction of an intermediate amino alcohol with retention of configuration (Fig. 1 and 2). The apparent exclusion of *erythro* isomer may

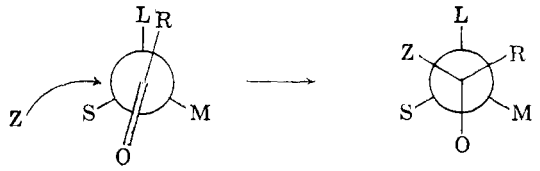


Figure 1

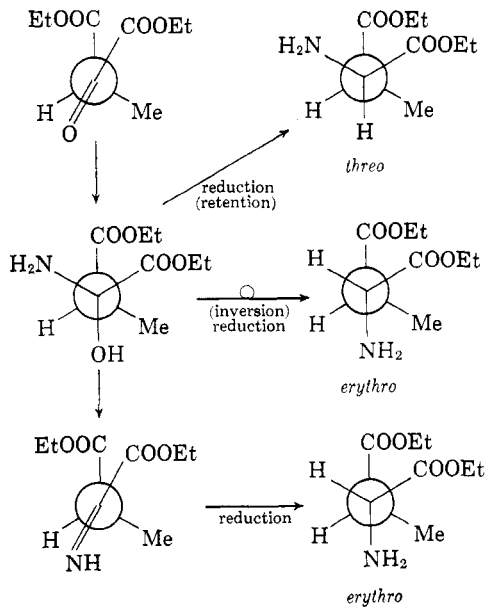


Figure 2

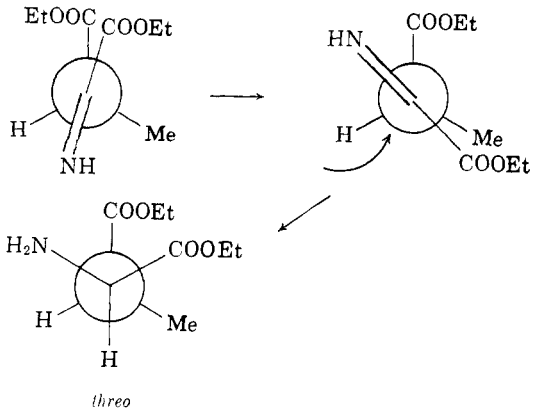


Figure 3

well stem from the repulsive forces between the two carbethoxy groups which force the keto ester to adopt a preferred conformation other than that indicated in Fig. 2 (see Fig. 3). Although the low yield of β-methylaspartic acid isolated in our synthesis weakens the claim that no *erythro* isomer is formed, our data strongly support the inference that, at most, this isomer is a very minor part of the product mixture.

The low yields of β-methylaspartic acid isolated from our syntheses probably result, in part, from losses during purification. The product mixtures

(3) After our work was substantially complete, we learned that *threo*-β-methylaspartic acid had become commercially available from Nutritional Biochemicals Corp.

(4) M. L. Moore, "The Leuckart Reaction," in *Org. Reactions*, **V**, 301 (1949).

(5) In another experiment, when the hydrolysis was carried out with aqueous sodium hydroxide, only α-aminobutyric acid and no β-methylaspartic acid was obtained from the mixture.

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contained a brown, viscous sirup with odor of caramel which made crystallization difficult. Subsequent recrystallizations of the commercial β-methylaspartic acid with the same conditions used for our product resulted in poor recovery of amino acid. The simplicity of the synthesis, however, offsets to some extent the low yield of pure product obtained.

Experimental

Diethyl β-methyl-α-ketosuccinate was prepared by the condensation of diethyl oxalate (4 moles) with ethyl propionate (1 mole) in the presence of sodium ethoxide (1 mole).[8] The product distilled at 85–88° (2 mm.). Attempts to prepare an oxime derivative were unsuccessful, but prolonged treatment with phenylhydrazine on a steam bath led to the formation of the pyrazolone derivative[8] (ethyl 1-phenyl-4-methyl-5-pyrazolone-3-carboxylate), m.p. 148–148.1° (white crystals from chloroform–petroleum ether).

The keto ester gave qualitative reactions which indicated a high enol content: It rapidly decolorized bromine in carbon tetrachloride and gave a deep red coloration with ferric chloride solution.

Preparation of Amino Acid.—A mixture of diethyl β-methyl-α-ketosuccinate (20.2 g., 0.10 mole), 90% formic acid (18.5 g., 0.36 mole), and 99% formamide (18.0 g., 0.40 mole) was heated under reflux for 17 hr. During the first half hour, some volatile liquid with an ester odor (ethyl formate?) was removed to keep the temperature near 135°, where it remained throughout the refluxing period. Concentrated hydrochloric acid (20 ml.) was added, and heating was continued to hydrolyze formamide and ester. After 3 hr., 30 ml. of water was added; the pH of the mixture was about 4–5. More acid (20 ml. of conc. hydrochloric acid and 10 ml. of water) was added to lower the pH to about 2, and the brown mixture was then boiled for 8 hr. longer.

It was poured into a crystallizing dish and set on a steam bath to evaporate. A grayish tan crystalline residue with some sticky material remained; weight, 31.7 g. A portion (26.8 g.) of this crude material was dissolved in water. When the solution was concentrated on a steam bath, 11 g. of pure ammonium chloride was obtained. The filtrate was diluted with 95% ethyl alcohol and was chilled at 4° for a week. White crystals, which proved to be essentially pure β-methylaspartic acid, separated from the solution and were collected with suction; yield 2.0 g. (16%). An appreciable amount of β-methylaspartic acid remained in the filtrate. A paper chromatogram of the filtrate gave strongly positive ninhydrin tests for both β-methylaspartic acid and α-aminobutyric acid (solvent, *n*-butyl alcohol–formic acid–water, 4:1:1 v./v.).

Identification of β-Methylaspartic Acid Product.—The white crystals obtained from the alcohol solution showed 100% amino acid in a quantitative ninhydrin test, with both L-aspartic acid and commercial β-methyl-DL-aspartic acid being used as standards. Paper chromatography R_f values for both the amino acid and its *N*-(2,4-dinitrophenyl) derivative were identical with those for known standards. (Chromatography solvent for amino acid was *n*-butyl alcohol–formic acid–water, 4:1:1 v./v.; that for the *N*-(2,4-dinitrophenyl) derivative was *t*-amyl alcohol saturated with 0.1 *M* phthalate buffer, pH 6.) This same comparison enabled us to identify both β-methylaspartic acid and α-aminobutyric acid in the alcoholic filtrate.

Anal.[9] Calcd. for $C_5H_9NO_4$: C, 40.81; H, 6.17; N, 9.52. Found: C, 40.27; H, 6.26; N, 9.42.

The synthesized β-methylaspartic acid was shown to have the *threo* configuration by enzymatic resolution of the DL-acid with β-methylaspartase prepared from sonic lysates of *Bacterium cadaveris*.[1] This enzyme deaminates only the L-isomer. β-Methyl-D-aspartic acid was recovered from the mixture; $[\alpha]^{22}D$ +14.8° (*c* 1.42 mg./ml., in water). Repetition of the resolution with a commercial sample of *threo*-β-methyl-DL-aspartic acid[3] gave D-acid with +12.6° (*c* 3.88 mg./ml., in water). The specific rotation reported for *threo*-β-methyl-D-aspartic acid is $[\alpha]^{25}D$ +11.7 ± 2°[6]; that for *threo*-β-methyl-L-aspartic acid is −12.4°[10]; and that for *erythro*-β-methyl-D-aspartic acid is −24.5 ± 3°,[6] all in water solutions.

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Preparation and Polymerization of 6-*O*-Vinyl-1,2;3,4-di-*O*-isopropylidene-D-galactopyranose[1]

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1 - *O* - Vinyl - 2,3;4,5 - di - *O* - isopropylidene-D-fructose[4] has been prepared by treating in an autoclave 2,3;4,5-di-*O*-isopropylidene-D-fructose with acetylene diluted with nitrogen in the presence of small amounts of potassium hydroxide catalyst. Best reactions occurred at 150–160° during a period of 18.5 hours. Similarly there has been obtained 3,5,6-tri-*O*-vinyl-1,2-isopropylidene-D-glucose[5] and 3-*O*-vinyl-1,2;5,6-di-*O*-isopropylidene-D-glucose.[6] Acetylene has been found to react readily at 100° and atmospheric pressure with fatty alcohols to produce the corresponding vinyl ethers.[7] The vinylation of methyl α-D-glucopyranoside with acetylene at 150° and 375 p.s.i.g. has recently been discussed.[8]

Herein we describe the vinylation of 1,2;3,4-di-*O*-isopropylidene-D-galactopyranose to obtain the 6-*O*-vinyl derivative. Reaction is accomplished by bubbling acetylene through the melt for 16 hours at atmospheric pressure in the presence of dry, powdered potassium hydroxide. Identity of the product is established by reduction to the 6-*O*-ethyl derivative which can be hydrolyzed to

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