Syntheses of Amide Derivatives of DL-β-Carboxy-γ-aminobutyric Acid

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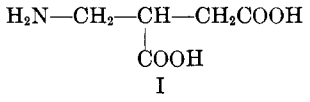
Amides of DL-β-carboxy-γ-aminobutyric acid have been synthesized, starting with itaconic anhydride. This was opened by the appropriate amine to yield 2-methylene-N-alkylsuccinamic acids, to the double bond of which one mole of benzylamine was added. Hydrogenolysis of the resulting benzylamino derivatives gave the required amides of the free amino acid. The structure of the 2-methylene-N-alkylsuccinamic acids is established.

DL-β-Carboxy-γ-aminobutyric acid (aminomethylsuccinic acid, α-carboxymethyl-β-alanine) (I) was pre-

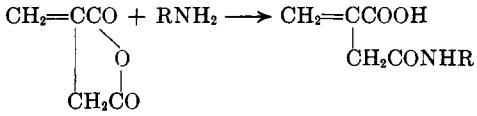


I

pared by addition of benzylamine to the double bond of itaconic acid and subsequent debenzylation.[1] It is a β-amino acid which, due to its special structure, may be a potential antagonist to aspartic acid, glutamic acid, γ-aminobutyric acid, or β-alanine, all of which have great importance in many biological processes.

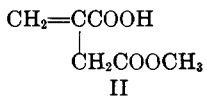
It seemed interesting to synthesize amide (peptide) derivatives of this amino acid. Itaconic anhydride was used as starting material. This on reaction with amines led to the formation of the corresponding amides. Only a few aromatic itaconamides are known[2-4] and their structure (position of the amide group) has not been identified. They were prepared either by reaction of amines with itaconic anhydride or by opening of the corresponding itaconimides with alkali. The derivatives obtained by the former method had higher melting points and our derivatives were similar to them in regards to solubility and melting points.

The following evidence proves that the opening of itaconic anhydride with amines results in the formation of 2-methylene-N-alkylsuccinamic acids (I).



I

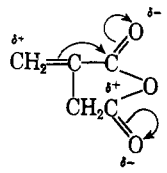
Reaction of methanol with the anhydride leads to the formation of only one monomethyl ester,[5] m.p. 70°. The structure of this ester has been proved by Hancock and Linstead[6] and shown to be 4-methyl 2-methylene succinate (II).



II

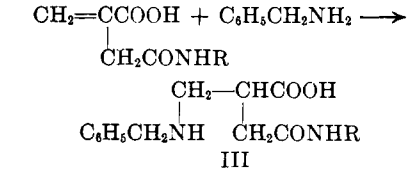
We found additional evidence from the fact that reaction of the ester with benzylamine in dioxane led to elimination of methanol and formation of N-benzyl-4-carboxy-2-pyrrolidone,[1,7] showing that the methyl ester occupied the γ-position with respect to the methylene group. The same pyrrolidone derivative was obtained on reaction of monobenzyl ester[8] (obtained similarly on reaction of benzyl alcohol with itaconic anhydride) with benzylamine, showing that opening of the anhydride leads to the exclusive formation of 4-alkyl esters of 2-methylene succinic acid. It may be mentioned that reaction of dimethyl itaconate with one mole benzylamine led to the formation of N-benzyl-4-carbomethoxy-2-pyrrolidone,[9] and with two moles of benzylamine the second methyl ester group was replaced by benzylamine to form the corresponding amide.

It is expected that opening of the anhydride with amines will lead similarly to the preferential formation of 2-methylene-N-alkylsuccinamic acids. In itaconic anhydride the two carbonyl groups are attached to an unsaturated and saturated carbon atom, respectively, thus making a decisive difference in their reaction with nucleophilic reagents. The exclusive formation of the ester or amide derivatives in a γ-position with respect to the methylene group, may be due to conjugation of C=C bond of the anhydride to the nearby C=O group, which leads to lowering of the partial positive charge of the carbon atom of the carbonyl group, whereas the remote carbonyl group is not affected.



Thus attack by a nucleophilic reagent will occur at the γ-carbonyl group with formation of γ-derivatives. Steric effects of the methylene group also may favor the preferential formation of the γ-derivatives.

Indeed, the amides obtained added benzylamine to their double bonds, giving 3-carboxy-4-benzylamino butyramides (III), and without formation of pyrrolidone derivatives, which should have been formed if the carboxyl group, which is in a γ-position with respect to the methylene group, was free.



III

It is known[7,10-12] that itaconic acid very readily gives pyrrolidone derivatives on reaction with amines and,

(1) A. Zilkha, E. S. Rachman, and J. Rivlin, *J. Org. Chem.*, **26**, 376 (1961).
(2) G. Piutti, *Gaz. chim. ital.*, **40**, I, 538 (1910).
(3) R. Anschütz and F. Reuter, *Ann.*, **254**, 129 (1889).
(4) N. Bland and J. F. Thorpe, *J. Chem. Soc.*, 1496 (1912).
(5) R. Anschütz and J. Drugman, *Ber.*, **30**, 2649 (1897).
(6) J. E. H. Hancock and R. P. Linstead, *J. Chem. Soc.*, 3490 (1953).
(7) P. L. Paytash, E. Sparrow, and J. C. Gathe, *J. Am. Chem. Soc.*, **72**, 1415 (1950).
(8) R. Anschütz, *Ann.*, **461**, 190 (1928).
(9) Y. Wu and R. F. Feldkamp, *J. Org. Chem.*, **26**, 1519 (1961).
(10) P. L. Paytash, M. J. Thomson, and M. E. Fykes, *J. Am. Chem. Soc.*, **74**, 4549 (1952).
(11) P. L. Paytash, M. J. Thomson, and E. B. Clarke, *ibid.*, **76**, 3500 (1954).
(12) M. Lipp, F. Dallacker, and H. Rey, *Ber.*, **91**, 2242 (1958).

TABLE I

PREPARATION OF 2-METHYLENE-N-ALKYLSUCCINAMIC ACIDS

Substance,[h] N-alkyl-2-methylene-succinamic acid	Yield, %	M.p., °C.	Formula	Carbon, % Calcd.	Carbon, % Found	Hydrogen, % Calcd.	Hydrogen, % Found	Nitrogen, % Calcd.	Nitrogen, % Found
n-Butyl	50[a]	115	$C_9H_{15}NO_3$	58.4	58.3	8.1	7.9	7.6	7.5
Isobutyl	73	130	$C_9H_{15}NO_3$	58.4	58.3	8.1	8.1	7.6	7.4
n-Hexyl	55[a]	125	$C_{11}H_{19}NO_3$	62.0	62.4	8.9	8.8	6.6	6.7
Cyclohexyl	50[a]	153	$C_{11}H_{17}NO_3$	62.6	62.5	8.1	7.9	6.6	6.3
Benzyl	90	149	$C_{12}H_{13}NO_3$	65.8	65.7	5.9	5.9	6.4	6.3
Phenyl[b]	88	166	$C_{11}H_{11}NO_3$	64.4	63.8	5.4	5.4	6.8	6.8
4-Methoxyphenyl[c]	95	176	$C_{12}H_{13}NO_4$	61.3	62.1	5.5	5.7	6.0	6.3
2-Methoxyphenyl[d]	75	129	$C_{12}H_{13}NO_4$	61.3	60.5	5.5	5.6	6.0	5.8
4-Ethoxyphenyl[e]	95	184	$C_{13}H_{15}NO_4$	62.7	62.7	6.0	6.4	5.6	5.7
Carbethoxymethyl[f,g]	66[a]	102	$C_9H_{13}NO_5$	50.2	50.3	6.1	6.4	6.5	6.5

[a] The rather low yield was due to difficulties in crystallizing the product. [b] M.p. previously reported, 151.5°,[3] 162°.[4] [c] M.p. previously reported,[2] 167°. [d] Same m.p as previously reported.[2] [e] M.p. previously reported,[2] 166°. [f] Calcd. % of ethoxyl: 20.9. Found: 21.0. [g] Recrystallized from ethyl acetate. [h] Substances were recrystallized from water or ethanol if not indicated otherwise.

TABLE II

PREPARATION OF DL-3-CARBOXY-4-BENZYLAMINO-N-ALKYLBUTYRAMIDES

Substance,[c] 3-carboxy-4-benzylamino-N-alkyl-butyramide	Yield, %	M.p., °C.	Formula	Carbon, % Calcd.	Carbon, % Found	Hydrogen, % Calcd.	Hydrogen, % Found	Nitrogen, % Calcd.	Nitrogen, % Found
n-Butyl	60	184	$C_{16}H_{24}N_2O_3$	65.8	66.0	8.2	8.2	9.6	9.4
Isobutyl	55	182	$C_{16}H_{24}N_2O_3$	65.8	65.6	8.2	8.4	9.6	9.6
n-Hexyl	80	185	$C_{18}H_{28}N_2O_3$	67.5	67.9	8.8	8.7	8.8	8.9
Cyclohexyl	87	202	$C_{18}H_{26}N_2O_3$	67.9	67.7	8.2	8.1	8.8	8.5
Benzyl	75	188	$C_{19}H_{22}N_2O_3$	69.9	70.2	6.8	6.8	8.6	8.7
Phenyl	90	200	$C_{18}H_{20}N_2O_3$	69.2	70.1	6.4	6.0	9.0	8.7
4-Methoxyphenyl	10[a]	161	$C_{19}H_{22}N_2O_4$	66.7	66.6	6.4	6.2	8.2	8.1
2-Methoxyphenyl	35	145	$C_{19}H_{22}N_2O_4$	66.7	66.7	6.4	6.5	8.2	8.1
4-Ethoxyphenyl	10[a]	175	$C_{20}H_{24}N_2O_4$	67.4	68.4	6.7	6.7	7.9	7.7
Carbethoxymethyl[b]	70	194	$C_{16}H_{22}N_2O_5$	59.6	59.5	6.8	7.0	8.7	8.5

[a] Yield of substance that crystallized directly from the reaction mixture. From the filtrate it was not possible to isolate another crop. [b] Calcd. % of ethoxyl: 14.0. Found: 14.3. [c] Substances were recrystallized from alcohol or water.

only under very careful conditions, have we previously found[1] that it is possible to add benzylamine to the double bond without causing cyclization (pyrrolidone formation). It may be mentioned that 2-methylene-N-phenylsuccinamic acid did not cyclize to the corresponding pyrrolidone derivative even on heating in dioxane solution for several hours.

Several aliphatic and aromatic amides of itaconic acid were obtained in good yield (Table I) by reaction of itaconic anhydride with amines in chloroform solution; with ethyl glycinate the derivative crystallized with difficulty. 2-Methylenesuccinamic acid was obtained on reaction of gaseous ammonia with itaconic anhydride in chloroform.

Addition of one mole of benzylamine to the double bond of the 2-methylenesuccinamic acids led to the formation of 3-carboxy-4-benzylamino-N-alkylbutyramides (Table II). These gave a positive reaction with ninhydrin on paper chromatograms and were reduced, in the presence of palladium chloride on charcoal (30%), to the corresponding debenzylated amino acids (Table III), contrary to N-benzyl-4-carboxy-2-pyrrolidone; which being an N-benzylamide did not give these reactions. With 2 - methylene - N - carbethoxymethylsuccinamic acid, smooth addition of benzylamine to the double bond occurred without attack of the ester group; showing that addition to the double bond is preferable to amidation.[13]

The double bond of the 2-methylenesuccinamic acids is reactive and other amines besides benzylamine add to the double bond.

The dipeptide ester, DL-ethyl-3-carboxy-4-aminobutyryl glycinate, was similarly obtained. We tried to obtain the free dipeptide by preferential saponification of the ester group with dilute alkali,[14] but the peptide bond also was disrupted leading to the formation of the free amino acids.

It was of interest to prepare the N,N-dibenzyl derivative of 3-carboxy-4-aminobutyric acid. The N,N-dibenzyl group can act as a protecting group for the synthesis of peptides of this amino acid (it can be removed by hydrogenolysis). It has no secondary hydrogen available on the nitrogen for lactam formation and, in this respect, offers advantage over the N-benzyl group where the secondary amino group can easily cyclize to a γ-lactam, in cases where the 1-carboxyl group of the amino acid is free. 3-Carboxy-4-dibenzylaminobutyric acid was obtained by heating itaconic acid with dibenzylamine in dioxane.

Experimental

Melting points were determined in a Fisher-Johns apparatus. The ascending method of paper chromatography (80% phenol) was used.

(13) A. Zilkha and M. D. Bachi, *J. Org. Chem.*, **24**, 1096 (1959).

TABLE III

PREPARATION OF DL-3-CARBOXY-4-AMINO-N-ALKYLBUTYRAMIDES

Substance,[d] 3-carboxy-4-Amino-N-alkyl-butyramide	M.p., °C.	R_f	Formula	Carbon, % Calcd.	Carbon, % Found	Hydrogen, % Calcd.	Hydrogen, % Found	Nitrogen, % Calcd.	Nitrogen, % Found
n-Butyl[a]	220	0.92	$C_9H_{18}N_2O_3$	53.5	53.2	8.9	9.1	13.9	14.1
Isobutyl[a]	232	0.90	$C_9H_{18}N_2O_3$	53.5	52.9	8.9	9.0	13.9	13.9
n-Hexyl[a]	234	0.94	$C_{11}H_{22}N_2O_3$	57.4	56.9	9.6	9.3	12.2	12.1
Cyclohexyl[a]	231	0.94	$C_{11}H_{20}N_2O_3$	57.9	58.6	8.8	9.0	12.3	12.4
Carbethoxymethyl[b,c]	202	0.94	$C_9H_{16}N_2O_5$	46.6	46.2	6.9	7.0	12.1	12.3

[a] Obtained in approximately quantitative yield. [b] Recrystallized from water–acetone. [c] Obtained in 80% yield. [d] Substances were recrystallized from water if not indicated otherwise.

Itaconic anhydride was prepared in 92% yield by heating itaconic acid with acetyl chloride,[15] m.p. 65°.

Preparation of 2-Methylene-N-alkylsuccinamic Acids.—To an ice-cooled solution of 0.1 mole of itaconic anhydride in 60 ml. of dry chloroform, 0.1 mole of amine was added dropwise with mechanical stirring during 15 min. The reaction mixture was stirred for 2 hr. at room temperature, and the product was filtered and washed with chloroform. Another crop (5–20% yield) was obtained on evaporation of the filtrate.

With amino acid esters, the reaction mixture was stirred for another hour at 40°. The chloroform was evaporated *in vacuo;* the residue was dissolved in a small volume of ethyl acetate and left to crystallize in a refrigerator.

The products gave a positive reaction for double bonds with aqueous permanganate solution and a negative reaction with ninhydrin.

2-Methylenesuccinamic Acid.—Excess dry gaseous ammonia was passed into an ice-cooled solution of itaconic anhydride in chloroform. The ammonium salt which precipitated was filtered, dissolved in water, and heated for a few minutes to expell excess ammonia. The solution was then passed through a column packed with cation exchange resin (nuclear sulfonic acid type resin, Amberlite IR-120) and evaporated *in vacuo*. The product which crystallized was obtained in 40% yield. It was recrystallized from ethanol, m.p. 152°.

Anal. Calcd. for $C_5H_7NO_3$: C, 46.5; H, 5.4; N, 10.8. Found: C, 46.9; H, 5.5; N, 10.8.

Preparation of DL-3-Carboxy-4-benzylamino-N-alkylbutyramides.—2-Methylene-N-alkylsuccinamic acid (0.1 mole) was suspended in 70 ml. of dry dioxane and heated under reflux and mechanical stirring until it dissolved. Benzylamine (0.1 mole) was then added and the reaction mixture heated in an oil bath at 120° for 2–3 hr. The reaction product generally started to precipitate within 15–30 min. The reaction mixture was cooled and the product was filtered and washed with acetone. An additional crop was obtained on evaporation of the filtrate and recrystallization of the residue from alcohol, acetone, or water.

The pure products gave negative permanganate and ninhydrin reactions.

Preparation of DL-3-Carboxy-4-amino-N-alkylbutyramides.—DL-3-Carboxy-4-benzylamino-N-alkylbutyramide (0.02 mole) was suspended in 120 ml. of absolute ethanol and 0.4 g. of catalyst (palladium chloride on charcoal, 30%, was added). The hydrogenolysis was carried out in a Parr low pressure hydrogenation apparatus for about 16 hr. at 50–60°. The product generally precipitated on the catalyst from which it was extracted with boiling water. The free amino acids generally crystallized out on cooling.

DL-3-Carboxy-4-dibenzylaminobutyric Acid.—Itaconic acid (13 g., 0.1 mole) was dissolved in 50 ml. of dry dioxane, dibenzylamine (19.7 g., 0.1 mole) was added, and the reaction mixture was heated for 4 hr. at 120°. The dioxane was evaporated *in vacuo;* the residue was dissolved in a small volume of ethanol and left to crystallize in a refrigerator; yield, 19 g. (58%); m.p. 148°, on recrystallization from ethanol.

Anal. Calcd. for $C_{19}H_{21}NO_4$: C, 69.7; H, 6.4; N, 4.3. Found: C, 70.3; H, 6.6; N, 4.1.

N-Benzyl-4-carboxy-2-pyrrolidone.—To a solution of 2.9 g. (0.02 mole) of monomethyl ester of itaconic acid, m.p. 70°, in 20 ml. of dioxane was added 2.1 g. (0.02 mole) of benzylamine. The solution was heated in an oil bath at 110–120° for 2 hr. The solvent was removed *in vacuo* and the pyrrolidone crystallized from water, m.p. and m.m.p.,[1] 144°.

Anal. Calcd. for $C_{12}H_{13}NO_3$: C, 65.7; H, 5.9; N, 6.4. Found: C, 66.1; H, 6.1; N, 6.6.

On using 2 moles of benzylamine, the benzylamine salt of the pyrrolidone was obtained; m.p. and m.m.p.,[1] 111°.

Benzylamide Derivative of N-Benzyl-4-carboxy-2-pyrrolidone.—To a solution of 5.2 g. (0.033 mole) of dimethyl itaconate in 20 ml. of dioxane was added 7.1 g. (0.066 mole) of benzylamine. The solution was heated in an oil bath at 110–120° for 2 hr. The solvent was removed *in vacuo*, and the product crystallized from water; yield, 3 g. (30%); m.p. 104°. It contained no methoxyl groups.

Anal. Calcd. for $C_{19}H_{20}N_2O_2$: N, 9.1. Found: N, 9.2.

(14) Y. Liwschitz and A. Zilkha, *J. Am. Chem. Soc.*, **76**, 3698 (1954); *J. Chem. Soc.*, 4394 (1954).

(15) A. Anschütz and W. Petri, *Ber.*, **13**, 1539 (1880).

Epoxide Studies. I. The Ring Opening of *cis*- and *trans*-N,N-Diethylphenylglycidamide

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Treatment of *trans*-N,N-diethyl-3-phenylglycidamide (*trans*-I) with hydrogen chloride in benzene gave the *threo*-chlorohydrin II with retention of configuration whereas with hydrogen chloride in methanol, the *erythro*-chlorohydrin III was formed. *cis*-Glycidamide (*cis*-I) with either of these reagents afforded only II. In the presence of base, II gave mixtures of *cis–trans*-I and III gave only *trans*-I. The stereochemistry and mechanisms of these transformations are reported.

The opening of an epoxide ring by nucleophilic reagents has been regarded generally as a bimolecular nucleophilic displacement (SN2) on carbon proceeding with inversion of configuration.[1] For example, the reaction of *cis*- and *trans*-stilbene oxides with hydrogen

(1) (a) R. E. Parker and N. S. Isaacs, *Chem. Rev.*, **59**, 737–799 (1959); (b) S. Winstein and R. B. Henderson, "Heterocyclic Compounds," Vol. I, R. C. Elderfield, Ed., John Wiley and Sons, Inc., New York, N. Y., 1950, Chap. 1, pp. 27.