The solvents were removed by vacuum distillation. The residual yellow solid was dissolved in 110 ml. of hot water, cooled by adding 100 g. of ice, and acidified with 30 ml. of concentrated HCl. The white solid product was filtered, washed well with water, and dried to constant weight; yield 16.1 g. (88%), m.p. 318–320°, sealed tube (copper block, corrected). This material was suitable for nitration. For purification for analysis the product was dissolved in dilute ammonium hydroxide, treated with charcoal, and reprecipitated with acid. The melting point was unchanged.

Anal. Calcd. for $C_7H_5N_3O_4$: C, 46.93; H, 2.81; N, 23.46. Found: C, 46.95, 46.95; H, 2.84, 2.86; N, 23.60, 23.63.

6-(5-Nitro-2-furyl)-*as*-triazine-3,5(2H,4H)-dione (II).—A nitration mixture was prepared by slowly mixing 8.4 ml. of 70% nitric acid containing 2 drops of concentrated sulfuric acid and 38 ml. of acetic anhydride with cooling below 25°. Solid, powdered 6-(2-furyl)-*as*-triazine-3,5(2H,4H)-dione (7.2 g.) was added in small increments with stirring and cooling to keep the temperature at 25–30°. After 1 hr., 18 g. of fused potassium acetate was added and the temperature held at 45–50° for 1 hr. The thick nitration mixture was poured into 250 ml. of ice water. The bright yellow solid was filtered, washed with water, and dried at 110°. The yield was 6.4 g. (71%), m.p. 330–331° (sealed tube, copper block, corrected). For analysis it was recrystallized from a 1:5 mixture of dimethylformamide and nitromethane and from acetonitrile; m.p. 332–333° (as above).

Anal. Calcd. for $C_7H_4N_4O_5$: C, 37.51; H, 1.80; N, 25.00. Found: C, 37.71; H, 1.92; N, 24.80, 24.88.

II is weakly acidic; ultraviolet absorption (in water) showed λ 365 mμ (ϵ_{max} 18,100); solubility in water at 25°, pH 5.5, is 45 mg./l.

Acknowledgment.—Analytical determinations were made by G. Ginther, M. Tefft, and G. Gustin.

New Compounds

Synthesis of Tripeptides of α-Aminoisobutyric Acid[1a]

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The unnatural, unmetabolizable amino acid, α-aminoisobutyric acid, is a useful model for the study of biological transport of neutral amino acids.[2] We have synthesized two new tripeptides of this amino acid, glycyl-α-aminoisobutyryl-L-alanine and glycyl-α-aminoisobutyryl-L-valine, for use as model substrates in the study of peptide transport. The uptake of glycyl-α-aminoisobutyryl-1-C^{14}-L-alanine by *Lactobacillus casei* 7469 has been reported elsewhere.[3]

The synthesis of carbobenzoxyglycyl-α-aminoisobutyric acid ethyl ester and the corresponding acylated dipeptide by the acid chloride coupling method has been reported by Bergmann, *et al.*[4] We have used the carbodiimide coupling method[5] to make the same compounds, precursors of our tripeptides.

Experimental[6]

Carbobenzoxyglycyl-α-aminoisobutyric Acid Ethyl Ester.—To a solution of 10.46 g. (0.05 mole) of carbobenzoxyglycine prepared by the usual method, 8.38 g. (0.05 mole) of α-aminoisobutyric acid ethyl ester hydrochloride, and 8 ml. (0.055 mole) of triethylamine in 200 ml. of methylene chloride was added 20.64 g. (0.10 mole) of dicyclohexylcarbodiimide. The mixture was stirred for 13 hr. at room temperature, then allowed to stand for 17 hr. at room temperature. After the addition of 1.0 ml. of glacial acetic acid, the mixture was allowed to stand in ice for 1 hr. and the dicyclohexylurea was removed by filtration. The filtrate was washed with 1 *N* HCl, 1 *N* $NaHCO_3$, and water, and dried (Na_2SO_4). Removal of methylene chloride *in vacuo* yielded 14.45 g. of crude product, m.p. 72.5–75.5°. Recrystallization from ethyl acetate–petroleum ether (b.p. 40–56°) gave 11.80 g. (73.1% yield), m.p. 80.5–83°, lit.[4] m.p. 84°. The radioactive, protected dipeptide, labeled with C^{14} in the carboxyl group of α-aminoisobutyric acid,[7] was prepared by a virtually identical procedure, as described in detail in the dissertation.[1a]

Carbobenzoxyglycyl-α-aminoisobutyric Acid.—To a solution of 4.84 g. (0.015 mole) of the above protected dipeptide in a mixture of 20 ml. of water and 40 ml. of dioxane was added 2.25 ml. (0.0225 mole) of 10 *N* NaOH. The solution was stirred at 4° for 5 hr.; 4.5 ml. of 5 *N* HCl was added, giving pH 4.5; the reaction mixture was stored at 4° for 15 hr., concentrated *in vacuo*, and the crude product was recrystallized from hot water, 3.67-g. yield (83.3%), m.p. 155–158.5°, lit.[4] m.p. 155.5°. The C^{14}-labeled acylated dipeptide was prepared by a similar procedure.[1a]

Carbobenzoxyglycyl-α-aminoisobutyryl-L-alanine Benzyl Ester. Azide Method.—A modification of the procedure of Erlanger and Brand[8] was employed. To 12.89 g. (0.04 mole) of carbobenzoxyglycyl-α-aminoisobutyric acid ethyl ester dissolved in 80 ml. of hot absolute ethanol was added 5.01 g. (0.1 mole) of hydrazine hydrate. The mixture was refluxed for 2 hr. No crystals appeared on cooling to room temperature. An additional 10.0 g. (0.2 mole) of hydrazine hydrate was added; the mixture was refluxed for 2 hr. longer and left in the cold overnight. The solvent was removed *in vacuo;* the residue was resuspended in ethyl ether and the solvent was removed *in vacuo* three more times to yield 12.87 g. of crude product. Recrystallization from absolute ethanol–ethyl ether yielded 7.91 g. (64.0%) of the hydrazide, m.p. 119–121.5°.

The protected tripeptide was prepared from 3.09 g. (0.01 mole) of the above hydrazide [converted to the azide by treatment with 0.759 g. (0.11 mole) of sodium nitrite] and an ethereal solution of L-alanine benzyl ester [freshly prepared from 4.72 g. (0.014 mole) of L-alanine benzyl ester benzenesulfonate and 2.3 ml. (0.016 mole) of triethylamine]. After 55 hr. standing at room temperature, a white fibrous product was collected by filtration, washed, dried in the usual manner, and chilled to −12° to yield a second crop. Recrystallization from hot absolute ethanol yielded 2.36 g. (51.7%) of protected tripeptide, m.p. 159.5–163°, $[\alpha]^{26}D$ −22.9° (c 1, ethanol).

Anal. Calcd. for $C_{24}H_{29}N_3O_6$: C, 63.28; H, 6.42; N, 9.23. Found: C, 63.09; H, 6.10; N, 9.31.

Carbodiimide Method.—To a solution of 1.47 g. (0.005 mole) of carbobenzoxyglycyl-α-aminoisobutyric acid and 0.005 mole of L-alanine benzyl ester benzenesulfonate and 0.8 ml. (0.0055 mole) of triethylamine in 150 ml. of acetonitrile was added 2.06 g. (0.01 mole) of dicyclohexylcarbodiimide. The reaction mixture was stirred for 10 hr. at room temperature and allowed to stand at room temperature for 21 hr.; 1.0 ml. of glacial acetic acid was added and the product was worked up as usual, after replacement

(1) (a) From the Ph.D. Thesis of Ellen A. Young, University of Arkansas, 1963. This work was carried out while Ellen A. Young was a fellow supported by Training Grant 5T1-GM-551-03 from the United States Public Health Service. (b) Department of Radiology, University of Arkansas Medical Center, Little Rock, Ark.

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(6) Melting points were determined on a Fisher-Johns block and are corrected. Specific rotation was determined with the aid of a 0.01° Zeiss polarimeter, using the D line of sodium. Elementary analyses were performed by Schwarzkopf Microanalytical Laboratory, Woodside, N. Y., and by Clark Microanalytical Laboratory, Urbana, Ill.

(7) Carboxyl-labeled α-aminoisobutyric acid, 1.238 mc./mmole, was obtained from Tracerlab, Inc., Waltham, Mass.

(8) B. F. Erlanger and E. Brand, *J. Am. Chem. Soc.*, **73**, 3508 (1951).

of the acetonitrile by ethyl acetate; 1.22-g. yield (53.5%), m.p. 158.5–160.5°. The C^{14}-labeled protected tripeptide was prepared by the carbodiimide method also.[1a]

Glycyl-α-aminoisobutyryl-L-alanine.—Hydrogen was passed through a solution of 1.37 g. (0.03 mole) of the protected tripeptide in 50 ml. of absolute ethanol containing 2.0 ml. of glacial acetic acid for 4 hr. in the presence of 0.5 g. of fresh palladium black with agitation by means of a Vibro-Mixer.[9] The product was obtained as shiny plates and recrystallized from water–acetone; 0.66-g. yield (95%), m.p. 238.5–240° dec., $[\alpha]^{26}D$ −26.3° (*c* 0.457, 1 *N* HCl); R_f 0.34 in 2-butanol–formic acid–water (75:15:10) and 0.70 in phenol–water (80:20), descending 15 hr., Whatman No. 1 paper.

Anal. Calcd. for $C_7H_{17}N_3O_4$: C, 46.74; H, 7.41; N, 18.17. Found: C, 46.78; H, 7.56; N, 17.57.[10]

The C^{14}-labeled free tripeptide was prepared by a similar procedure; specific activity was 16.8 μc./mmole.[1a]

Carbobenzoxyglycyl-α-aminoisobutyryl-L-valine Benzyl Ester.—To a solution of 1.47 g. (0.005 mole) of carbobenzoxyglycyl-α-aminoisobutyric acid in 100 ml. of acetonitrile was added 1.03 g. (0.005 mole) of dicyclohexylcarbodiimide; the mixture was stirred at room temperature for 1 hr. A fresh filtered solution of L-valine benzyl ester in acetonitrile [from 1.83 g. (0.005 mole) of L-valine benzyl ester benzenesulfonate and 0.75 ml. (0.005 mole) of triethylamine] was added; stirring was continued for 20 hr. at room temperature. The acetonitrile was removed *in vacuo*, and the residue was taken up in ethyl acetate and worked up as usual. Recrystallization from hot absolute ethanol and ethanol–ether yielded 1.60 g. (66%) of protected tripeptide, m.p. 114–116.5°, $[\alpha]^{26}D$ −26.7° (*c* 0.457, ethanol). A portion was recrystallized from ethanol–ether for analysis, m.p. 115.5–118°.

Anal. Calcd. for $C_{28}H_{33}N_3O_6$: C, 64.58; H, 6.88; N, 8.69. Found: C, 64.66; H, 6.98; N, 8.59.

Glycyl-α-aminoisobutyryl-L-valine.—Catalytic hydrogenolysis was carried out as described above on 0.97 g. (0.002 mole) of the protected tripeptide. To remove any acetate associated with the free tripeptide obtained, the product was dissolved in 20 ml. of water containing a sixfold molar excess of ammonia, lyophilized, redissolved in water, and lyophilized three more times. Recrystallization from water–alcohol–acetone yielded 0.50 g. (96%) of shiny plates, m.p. 240.5–241.5°, $[\alpha]^{26}D$ −11.0° (*c* 1.486, 1 *N* HCl); R_f 0.49 in 2-butanol–formic acid–water (75:15:10) and 0.86 in phenol–water (80:20), descending 15 hr.

Anal. Calcd. for $C_{11}H_{21}N_3O_{24}$: C, 50.95; H, 8.94; N, 16.21. Found: C, 50.99; H, 8.70; N, 16.02.

(9) The Vibro-Mixer was obtained from Fisher Scientific Co.

(10) A second portion of the carbobenzoxytripeptide benzyl ester was dissolved in methanol and subjected to hydrogenolysis as above, but in the absence of acetic acid. The melting point of the free tripeptide obtained was identical with that of the analytical sample and with that of an equal mixture of the two preparations.

3,6-Bis-*p*-dimethylaminobenzylidene-2,5-diketopiperazine[1]

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In preparing compounds containing systems of conjugated double bonds by reaction of dialkylaminobenzaldehyde with suitable ring compounds,[2] we attempted unsuccessfully to condense *p*-dimethylaminobenzaldehyde with 2,5-diketopiperazine. The desired compound was obtained, however, by use of the methiodide salt of this aldehyde. A mixture of 11.6 g. of the quaternary salt, 2.2 g. of 2,5-diketopiperazine, 4.0 g. of sodium acetate, and 12.0 g. of acetic anhydride was heated 3 hr. in an oil bath at 170°. The resulting insoluble product was washed with hot water and with hot methanol, then recrystallized twice from dimethylformamide. The tan crystals melted at 340° [3]; they formed an orange solution in acetic acid which became colorless on addition of a little concentrated HCl.

Anal. Calcd. for $C_{22}H_{24}N_4O_2$: C, 70.20; H, 6.41. Found: C, 69.98; H, 6.18.[4]

Walker 256 tumor screening test showed C/T 0.85 at 400 mg./kg.[5]; tissue culture screening test against KB cells *in vitro*: ED_{54}, 43 γ/ml.[6]

(1) This research was supported by a United States Public Health Service Grant CA-03717-5 from the National Cancer Institute.

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(3) Corrected for stem exposure; determined by use of Thiele tube.

(4) Average of two analyses by Weiler and Strauss, Oxford, England.

(5) We are grateful to Professor Alexander Haddow, Mr. J. E. Everett, and Mr. B. C. V. Mitchley of the Chester Beatty Research Institute for data on toxicity and activity against the Walker 256 tumor in rats weighing 200–250 g. Each compound was administered as a single i.p. injection in arachis oil on the day following tumor implantation or on the first day of the toxicity observation. Tumor bearing animals were sacrificed approximately 8 days later and the average weights of tumors in treated and untreated hosts reported as the ratio C/T.

(6) Results of the standard KB tumor cell inhibition tests carried out under sponsorship of the Cancer Chemotherapy National Service Center.

The Preparation of 4-Substituted Benzothiadiazines

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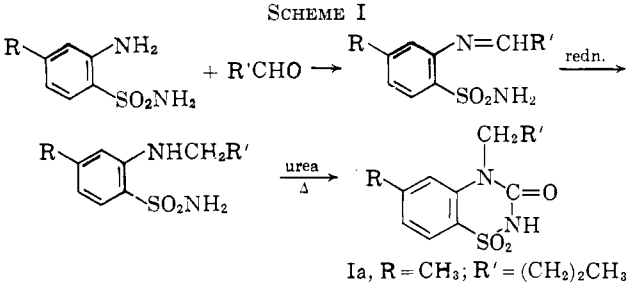
During our attempts to prepare cyclic analogs (I) of tolbutamide, a publication[2] appeared setting forth the synthesis of Ia. We have prepared Ia and twelve other 4-substituted analogs by an alternate route according to Scheme I.

SCHEME I



Ia, R = CH_3; R′ = $(CH_2)_2CH_3$

In only one instance (**15**), when using 10% palladium on carbon in acetic acid solution, was catalytic reduction of a Schiff base successful. When the Schiff bases were refluxed in acetic acid with either di- or trimethylamine borane for 0.5 hr., pure reduction products were obtained in yields ranging from 84–99%. Schiff bases (**12–14**) prepared from *para*-substituted benzaldehydes did not give pure products by this procedure.

The 4-pyridylethyl compound (**21**) was prepared by addition of 4-vinylpyridine to *o*-aminobenzenesulfonamide. All cyclizations were carried out by heating the N-substituted sulfonamides with urea at 200–205°.[3] The N-pyridylethylsulfonamide was heated at lower temperatures (*ca.* 170°) since at 200° decomposition occurred with loss of 4-vinylpyridine.

Experimental[4]

Schiff Bases.—These were prepared by mixing equimolar amounts of the appropriate aniline and aldehyde in ethanol. The mixtures were allowed to stand from 7–24 hr. at room temperature, and the solvent was removed. Yields, analyses, recrystallization solvents, and melting points are indicated in Table I.

(1) Author to whom inquiries should be addressed.

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(4) Melting points were determined on Fisher-Johns block with a calibrated thermometer. Analyses were performed by Midwest Microlab, Inc.