Synthesis of *dl*-4ξ-(2-Carboxyethyl)-*cis*-hexahydropyrrolo-[3,4-*d*]imidazol-2-one (Bisnorazabiotin)

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Abstract □ Bisnorazabiotin was synthesized in a six-step sequence from 1,5-dioxo-2-carbethoxypyrrolizidine. It is anticipated that the molecule will serve as a cofactor for biotin-requiring enzymes.

Keyphrases □ Biotin analog—synthesis of *dl*-4ξ-(2-carboxyethyl)-*cis*-hexahydropyrrolo[3,4-*d*]imidazol-2-one (bisnorazabiotin), IR, UV, and NMR spectra □ Bisnorazabiotin—synthesis of biotin analog *dl*-4ξ-(2-carboxyethyl)-*cis*-hexahydropyrrolo[3,4-*d*]imidazol-2-one, IR, UV, and NMR spectral analyses

Previous articles (1–3) reported the synthesis of several analogs of biotin (I) prepared as potential cofactors for biotin-dependent enzymes. The synthesis of bisnorazabiotin, utilizing a slightly modified synthetic approach, is reported here.

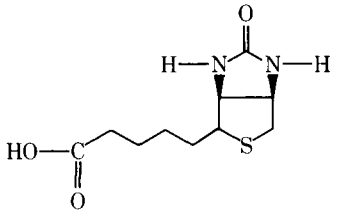
DISCUSSION

The starting point in this sequence (Scheme I) is 1,5-dioxo-2-carbethoxypyrrolizidine (II), prepared in high yield by a modification of the procedure of Gensler and Wu (4). Conversion of II to the enamine (III) was accomplished with ammonium formate in hot isopropanol. When methanol or ethanol was the solvent for this amination, the yield of enamine was considerably lower and TLC of the crude mixtures revealed the presence of an additional product of lesser polarity than either II or III.

Column chromatography of the product obtained from the amination of II in ethanol gave a 30% yield of a compound characterized as ethyl *N*-(2-carbethoxyethyl)-5-oxopyrrolidine-2-carboxylate (IV*b*) (5). The latter actually represents a reverse Dieckmann product of II. Since each stage of the Dieckmann condensation is completely reversible, the reverse reaction has been frequently used to convert β-ketoesters to open chain dicarboxylates (6–10). Under normal circumstances, a small amount of alkoxide in alcoholic solvent is sufficient to bring about the transformation. Although ammonium formate in alcohol is not a particularly basic medium, a minute amount of alkoxide might conceivably form, due to the ionization of the salt, catalyzing the reverse Dieckmann reaction.

When methanol was used as solvent, a 40% yield of methyl *N*-(2-carboxyethyl)-5-oxo-2-pyrrolidinecarboxylate (IV*a*) was obtained. A close examination of the same reaction in isopropanol failed to reveal a reverse Dieckmann product, probably indicating steric hindrance to the approach of isopropoxide anion. Treatment of III with acetic anhydride gave a crystalline enamine acetate (V) in 89% yield. Compound V should be used soon after its preparation or stored under an inert atmosphere, since exposure to air leads to the formation of the pyrrole (VI) (1, 3).

Reduction of V to *cis*-VII was effected catalytically in the presence of platinum in a Parr shaker. Conversion of VII to the tricyclic IX was carried out through the intermediacy of a hydrazide and the Curtius rearrangement of the corresponding acyl azide. Hydrolysis of the lactam ring of IX with aqueous base gave the desired amino acid (X).



I

EXPERIMENTAL[1]

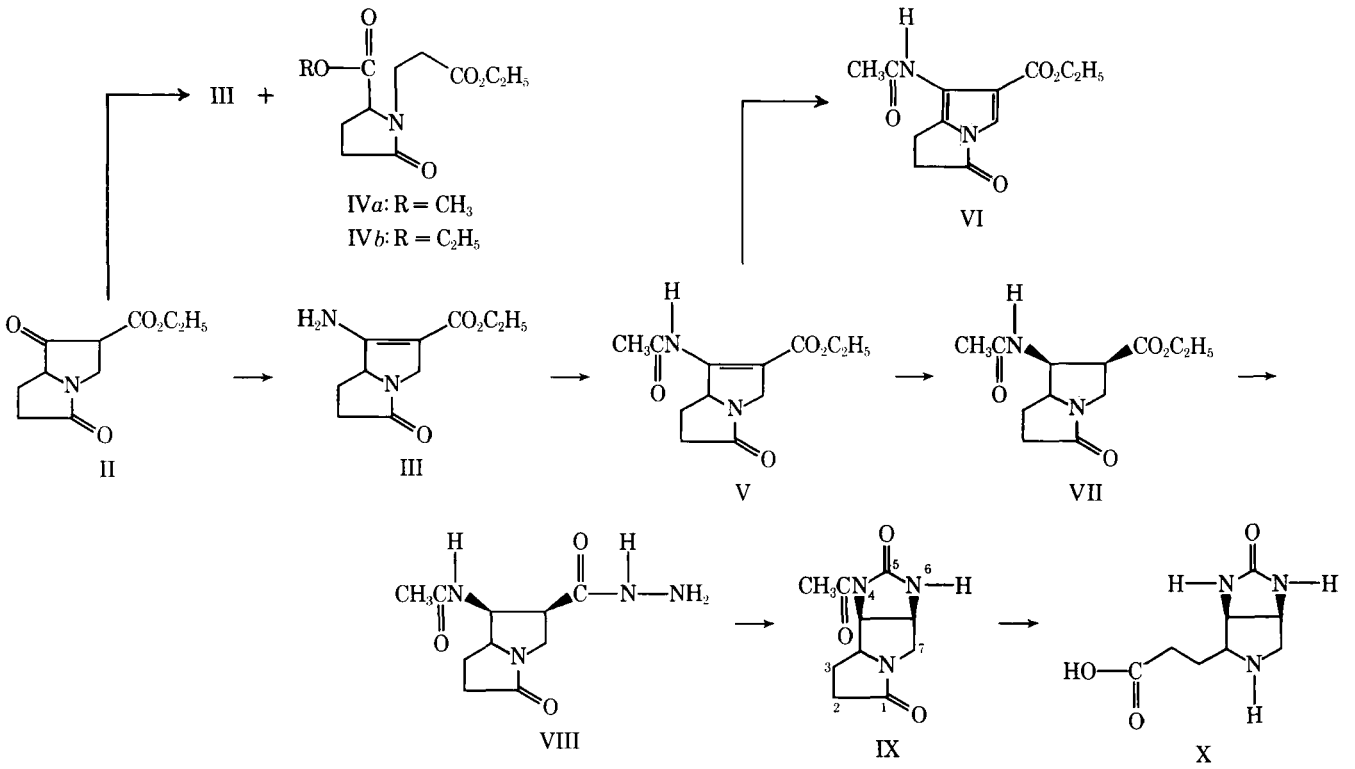
1,5-Dioxo-2-carbethoxypyrrolizidine (II) (4)—A solution of ethyl *N*-(2-carbethoxyethyl)-5-oxopyrrolidine-2-carboxylate (5) (51.46 g, 0.20 mole) in 250 ml of dry benzene was treated with sodium (4.8 g, 0.21 mole) and refluxed for 30 hr. The glassy, brown sodium enolate was filtered and dried (48.32 g, 100%). The free enol ester was released by adding the powdered enolate salt to a stirring slurry of chipped ice, concentrated hydrochloric acid, and chloroform. The chloroform solution was separated, and the aqueous phase was extracted with several portions of fresh chloroform. The combined chloroform extracts were washed with water and dried (magnesium sulfate). Evaporation of the solvent afforded 41.3 g of oily product, which showed one spot on TLC (30% methanol in ether) and gave a purple color with ferric chloride solution; IR: λ_{max} (chloroform) 5.63, 5.76, 5.87, and 6.13 μm.

Reaction of II with Ammonium Formate—*In Isopropanol*—A solution of 21.12 g (0.10 mole) of II and 12.6 g (0.20 mole) of ammonium formate in 150 ml of isopropanol was refluxed for 4 hr. The solvent was removed *in vacuo*, and the residue was partitioned between water and chloroform. The organic layer was washed with water, dried (magnesium sulfate), and concentrated to yield 20.6 g (98%) of 1-amino-5-oxo-2-carbethoxypyrrolizid-1-ene (III) as a light-brown oil. TLC on silica gel GF_{254} (10% methanol in ether) showed primarily one spot. Preparative TLC on silica gel $PF_{254+366}$, as well as column chromatography on silica gel, failed to afford a crystalline product; IR: λ_{max} (chloroform) 2.84, 2.95, 5.93, and 6.12 μm; UV: λ_{max} (ethanol) 274.5 nm; NMR: δ 1.28 (3H, t, $-OCH_2CH_3$, J = 7 Hz), 2.00–2.80 (4H, m, $-CH_2CH_2$), 4.22 (2H, q, $-OCH_2CH_3$, J = 7 Hz), and 5.97 (2H, s, $-NH_2$).

In Methanol—A mixture of 4.23 g (0.02 mole) of II and 2.5 g (0.04 mole) of ammonium formate in 50 ml of methanol was stirred at room temperature for 48 hr and then refluxed for 1 hr. The separation, as described for isopropanol, gave 3.66 g of a dark-brown oil. TLC (10% methanol in ether) showed primarily two spots, with the lower R_f compound corresponding to III. The crude oil was chromatographed on 50 g of silica gel, eluting with progressively increasing concentrations of chloroform in benzene (200-ml fractions). Fractions 6–13 afforded a yellow oil (1.96 g, 40%), which proved homogeneous by TLC on silica gel G (2% methanol in ether). Fractions 17–19 afforded 1.46 g (34.7%) of a light-brown oil; IR, UV, and NMR data were identical to those of the product obtained using isopropanol (III). The less polar oil was distilled and afforded a pale-yellow oil (bp 148–149° at 0.23 mm); IR: λ_{max} (chloroform) 5.75 and 5.90 μm; NMR: δ 1.27 (3H, t, $-OCH_2CH_3$, J = 7 Hz), 2.62 [2H, t, ring $-CH_2C(=O)-$, J = 7 Hz], and 4.17 (4H, q, $-OCH_2CH_3$, J = 7 Hz); $[\alpha]_D^{25}$ 0°.

Anal.—Calc. for $C_{11}H_{17}NO_5$: C, 54.31; H, 7.04; N, 5.76. Found: C, 54.36; H, 7.06; N, 5.80.

[1] Melting points were taken on a Fisher-Johns melting-point stage and a Thomas-Hoover melting-point apparatus and are uncorrected. UV absorption spectra were determined in 95% ethanol on a Beckman recording spectrophotometer (model DK2A). IR absorption spectra were recorded on Beckman recording spectrophotometers (models 8 and 33). NMR spectra were determined in deuteriochloroform, using tetramethylsilane as the reference standard, on a Varian EM 360 spectrometer. Microanalyses were carried out by Spang Microanalytical Laboratory, Ann Arbor, Mich. TLC was carried out with silica gel G, silica gel GF_{254}, or silica gel $PF_{254+366}$. Column chromatography was performed with silica gel 60 (particle size 0.063–0.200 mm).



Scheme I

On this basis, the less polar oily product was identified as methyl *N*-(2-carbethoxyethyl)-5-oxo-2-pyrrolidine-2-carboxylate (IV*a*).

In Ethanol—A mixture of 6.24 g (0.03 mole) of II, 3.5 g (0.056 mole) of ammonium formate, and 95% ethanol (100 ml) was stirred for 48 hr at room temperature. The reaction mixture was refluxed for 1 hr, and the usual workup afforded 6.10 g of a dark-brown oil. TLC of this material was quite similar to that of the crude product obtained using methanol. The crude oil was chromatographed on 75 g of silica gel using the same elution scheme as described for the methanol method. Fractions 8–10 gave a yellow oil (2.3 g, 30%), homogeneous on TLC (2% methanol in ether; 5% methanol in ether). Distillation gave a colorless oil (bp 143–145° at 0.2 mm); IR: λ_{max} (chloroform) 5.77 and 5.92 μm; NMR: δ 1.26 (3H, t, —OCH_2CH_3, J = 7 Hz), 1.32 (3H, t, —OCH_2CH_3, J = 7 Hz), 2.61 [2H, t, ring —$CH_2C(=O)$—, J = 7 Hz], 4.16 (2H, q, —OCH_2CH_3, J = 7 Hz), and 4.26 (2H, q, —OCH_2CH_3, J = 7 Hz). This product was characterized as ethyl *N*-(2-carbethoxyethyl)-5-oxopyrrolidine-2-carboxylate (IV*b*) on the basis of superimposability of spectral data. Fractions 15–17 by TLC gave homogeneous III as a yellow oil (2.85 g, 45%).

1-Acetamido-5-oxo-2-carbethoxypyrrolizid-1-ene (V)—A solution of 12.6 g (0.05 mole) of III in 150 ml of acetic anhydride was heated on an oil bath at 90–100° for 3 hr. The excess acetic anhydride was removed under vacuum to give 13.4 g of a dark-brown oil, which crystallized upon standing at room temperature for several days. Recrystallization from ether gave colorless crystals (11.2 g, 89%), mp 123–126°. Two additional recrystallizations from ether gave the analytical sample, mp 127–129°; IR: λ_{max} (chloroform) 3.01, 5.87, 5.95, and 6.13 μm; UV: λ_{max} (ethanol) 274 nm (ε = 23,300); NMR: δ 1.31 (3H, t, —OCH_2CH_3, J = 7 Hz), 2.18 [3H, s, N—(C=O)CH_3], 2.00–2.90 (4H, m, —CH_2CH_2—), 4.26 (2H, q, —OCH_2CH_3, J = 7 Hz), and 5.25 (1H, s, NH).

Anal.—Calc. for $C_{12}H_{16}N_2O_4$: C, 57.13; H, 6.39; N, 11.10. Found: C, 57.10; H, 6.40; N, 11.06.

1-Acetamido-5-oxo-2-carbethoxy-6,7-dihydro-5*H*-pyrrolizine (VI)—A thin film of V (0.25 g, 1.0 mmole) on a watchglass was allowed to remain at room temperature while exposed to the air for 5 weeks. TLC on silica gel GF_{254} (10% methanol in ether) showed the appearance of a new spot with a higher R_f than V and very little remaining starting material. Crystallization from ether gave colorless crystals, mp 108–110°; IR: λ_{max} (chloroform) 2.96, 3.14, 5.68, and 5.92 μm; UV: λ_{max} (ethanol) 240 nm (ε = 31,300); NMR: δ 1.37 (3H, t, —OCH_2CH_3, J = 7 Hz), 2.17 [3H, s, NC(=O)CH_3], 4.37 (2H, q, —OCH_2CH_3, J = 7 Hz), 7.54 (1H, s, =CH), and 9.06 (1H, s, NH).

Anal.—Calc. for $C_{12}H_{14}N_2O_4$: C, 57.59; H, 5.64; N, 11.19. Found: C, 57.53; H, 5.79; N, 11.21.

1-Acetamido-5-oxo-2-carbethoxypyrrolizidine (VII)—Compound V (0.85 g, 3.37 mmoles), dissolved in 50 ml of absolute ethanol, was hydrogenated at 60 psi in a Parr shaker over platinum (100 mg of platinum oxide). The catalyst was filtered, and the ethanol was evaporated *in vacuo*, affording a colorless viscous oil (0.84 g, 98%). TLC on silica gel G (10% methanol in ether) showed disappearance of V and a new spot of lower R_f; IR: λ_{max} (chloroform) 2.91, 5.85, and 5.97 μm; NMR: δ 1.30 (3H, t, —OCH_2CH_3, J = 7 Hz), 2.02 [3H, s, NC(=O)CH_3], 4.24 (2H, q, —OCH_2CH_3, J = 7 Hz), and 6.85 (1H, d, NH, J = 7 Hz). All attempts to crystallize the compound failed.

6-Acetyldecahydroimidazo[4,5-*a*]pyrrolizine-1,5-dione (IX)—Compound VII (5.4 g, 0.021 mole), dissolved in 100 ml of absolute ethanol, was treated with 10 ml of hydrazine hydrate and kept at room temperature for 6 hr. The excess reagent and solvent were removed *in vacuo*, and the oily residue was subjected to vacuum for an additional 24 hr. A foamy, hygroscopic residue was obtained (5.14 g). This hydrazide, dissolved in 70 ml of 20% hydrochloric acid, was cooled to 3° and treated dropwise with sodium nitrite (2.53 g, 0.037 mole) in 10 ml of water, keeping the temperature near 0°. The reaction mixture was extracted with ethyl acetate (5 × 50 ml), the combined fractions were dried (magnesium sulfate), and the ethyl acetate solution was refluxed for 3 hr. Evaporation of the solvent gave a yellow oil, which crystallized upon the addition of 95% ethanol (3.2 g, 68%), mp 250–254°. Two recrystallizations from ethanol gave the analytical sample, mp 253–254°; IR: λ_{max} (mineral oil) 3.01, 3.13, 5.75, 5.93, and 6.01 μm.

Anal.—Calc. for $C_{10}H_{13}N_3O_3$: C, 53.81; H, 5.87; N, 18.82. Found: C, 53.75; H, 5.80; N, 18.79.

***dl*-4ξ-(2-Carboxyethyl)-*cis*-hexahydropyrrolo[3,4-*d*]imidazol-2-one (X)**—A suspension of IX (0.095 g, 0.43 mmole) in 3 ml of 10% sodium hydroxide was heated at 120° in a sealed tube. The colorless solution was evaporated to dryness, and the residual powder was neutralized with ethanolic hydrogen chloride. The crystalline product obtained (mp 174–176°, resolidifies at 177°, and then mp 252–254°) was recrystallized from aqueous ethanol. TLC on silica gel G in the following systems showed the product to be ho-

mogeneous: *n*-butanol–acetic acid–water (3:1:1), 95% ethanol–water (7:3), *n*-propanol–34% ammonia (7:3), 95% ethanol–34% ammonia (7:3), and *n*-propanol–water (1:1). One additional recrystallization from aqueous ethanol gave the analytical sample (mp 178–180°, resolidifies, and then mp 253–255°); IR: λ_{max} (mineral oil) 2.86, 2.91, and 5.92 μm.

Anal.—Calc. for $C_8H_{13}N_3O_3 \cdot H_2O$: C, 44.23; H, 6.96; N, 19.34. Found: C, 44.30; H, 7.00; N, 19.44.

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Sodium Chloride Equivalents, Cryoscopic Properties, and Hemolytic Effects of Certain Medicinals in Aqueous Solution III: Supplemental Values

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Abstract □ A supplemental table of sodium chloride equivalents and freezing-point depressions at various concentrations for 44 different substances in aqueous solution is presented. Also given in the table is the isosmotic concentration of each material that can form such a solution. The degree of hemolysis of human erythrocytes was determined in 24 different isosmotic solutions, and the data are presented in a table to supplement the previously published values. Eleven isosmotic solutions prevented hemolysis, and 13 others failed to prevent hemolysis.

Keyphrases □ Sodium chloride equivalents—data for 44 drugs □ Cryoscopic properties—data for 44 drugs □ Hemolytic effects—data for 44 drugs □ Drug substances—sodium chloride equivalents, cryoscopic properties, and hemolytic effects determined for 44 drugs

The sodium chloride equivalents and freezing-point depressions for 456 substances in aqueous solution were determined experimentally and reported previously (1–4). Furthermore, the degree of hemolysis of fresh human erythrocytes in certain aqueous isosmotic solutions was determined using the hemolytic method (3–5).

The objectives of the current investigation were to study, using the same methods, some additional available substances not included in the earlier cryoscopic and hemolytic investigations and to present these data in suitable tables to supplement the previous data.

EXPERIMENTAL

Cryoscopic Measurements—The method used for the measurements of the freezing points of the solutions was the same as that already reported; all freezing-point data were obtained with a cryoscopic osmometer (4).

The freezing-point measurements were corrected for the amount of disengaged ice, and −0.52° was used as the comparative freezing point for aqueous 0.9% reagent grade sodium chloride solution, which is isotonic and isosmotic with blood and tears. The materials studied were of the official grade of purity or better, and the grade of purity of the donated specialty preparations complied with the manufacturer's specifications.

Hemolysis of Human Erythrocytes—Colorimetric hemoglobin determinations were made to indicate the degree of hemolysis for solutions that could be made isosmotic. The method, utilizing a 45-min incubation period of erythrocytes in the isosmotic solution followed by centrifugation of the erythrocytes and ghosts and determination of absorbance *versus* a standard at 520 nm in a colorimeter, was reported previously in detail (3–5).

RESULTS AND DISCUSSION

Table I lists the sodium chloride equivalents and freezing-point depressions at various concentrations for the 44 currently studied substances. To use these data, one should employ the sodium chloride equivalent that represents the concentration nearest to the desired final concentration of medicinal substance used. Because of general interest in the colligative properties of medicinal solutions, the freezing-point depressions and sodium chloride equivalents are included for several substances that are not necessarily used as isotonic or isosmotic solutions. The sodium chloride equivalents and isosmotic concentrations are reported to the nearest 0.01.

The percent of hemolysis found for the 24 compounds studied is listed in Table II in addition to the isosmotic concentration used for each and the solution's approximate pH. For solutions that developed a color or cloudiness upon the addition of blood, the proportional decrease in the volume of the packed, nonhemolyzed centrifuged erythrocytes was estimated visually. Any noticeable change in appearance of the erythrocytes or the solution is referred to in the footnotes for Table II. Of the compounds studied, 11 isosmotic solutions prevented hemolysis of human erythrocytes and 13 failed to prevent hemolysis.

A compilation of the 293 substances whose isosmotic solutions were studied using the same hemolytic method in this laboratory