tyl)uridine (3.0 g) was dissolved in hot ethylene dichloride (10 ml). To the cooled solution was added a solution of diazomethane in ether (100 ml) prepared from nitrosomethylurea (10 g). The solution was allowed to stand at room temperature until no further nitrogen was evolved, and then the solution was evaporated to an oil (3.0 g). The oil was applied to a silica gel column with methanol–benzene (1:10) to give the title compound.

Anal.—Calc. for $C_{16}H_{20}N_2O_9$: C, 50.00; H, 5.46. Found: C, 49.72; H, 5.31.

2′,3′,5′-Tri-(*O*-benzyl)-β-D-arabinofuranosylurea (I)—*Procedure A*—Urea (1.0 g, 0.017 mole), 2′,3′,5′-tri-(*O*-benzyl)-β-D-arabinofuranose (1.0 g, 0.0025 mole), acetone (30 ml), water (10 ml), and concentrated sulfuric acid (0.34 g, 0.9% w/w) were heated for 48 hr at 70° under reflux with stirring. The solution was cooled and neutralized with lead carbonate. The suspension was filtered, and the filtrate was evaporated. The residue was extracted with chloroform and water, and the chloroform extracts were evaporated to yield a syrup (1.2 g). Chromatography of the syrup on silica gel with benzene–ethyl acetate (7:3) yielded I as a syrup, which was crystallized from ether (0.13 g).

Procedure B—The steps were as described under *Procedure A*, except that no water was used in the reaction mixture. The yield of I was 0.28 g (oil).

Procedure C—The steps were as described under *Procedure A*, except that 0.9% (w/w) perchloric acid was used as a catalyst instead of sulfuric acid. The yield of I was 0.21 g (crystals from ether).

Procedure D—The steps were as described under *Procedure C*, except that no water was used in the reaction mixture. The yield of I was 0.15 g (oil). This procedure was used for the preparation of II–VII. The products from Procedures A–D showed identical NMR spectra.

***N*-β-D-Arabinofuranosyl-*N*′-methylurea (VIII)**—To a solution of II (1.94 g) in methanol (500 ml) was added 5% palladium-on-charcoal (100 mg). The reaction mixture was hydrogenated at 320 psi for 120 hr and then filtered through diatomaceous earth[3], and the filtrate was evaporated to an oil. The oil was crystallized from methanol to give VIII (0.24 g), mp 150°. Compound IX was prepared by the same procedure.

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New Compounds: Total Synthesis of *dl*-3a,4,6a-*cis*-4-(4-Carboxybutyl)-hexahydropyrrolo[3,4-*d*]imidazol-2-one Hydrochloride (*dl*-Azabiotin Hydrochloride)

HENRY C. WORMSER and HANLEY N. ABRAMSON [x]

Abstract □ The total synthesis of *dl*-3a,4,6a-*cis*-4-(4-carboxybutyl)-hexahydropyrrolo[3,4-*d*]imidazol-2-one hydrochloride (*dl*-azabiotin hydrochloride) was accomplished in a seven-step sequence from 2-ethoxycarbonylazepin-7-one.

Keyphrases □ Pyrroloimidazolone, substituted—synthesized in seven-step sequence □ Azabiotin—synthesized in seven-step sequence □ Biotin analog—substituted pyrroloimidazolone synthesized in seven-step sequence

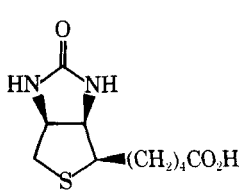
Biotin (I), a coenzyme, functions in several significant carbon dioxide fixation reactions in the cell. It is involved in carboxylation reactions catalyzed by propionyl coenzyme A carboxylase, acetyl coenzyme A carboxylase, pyruvate carboxylase, and β-methylcrotonyl coenzyme A carboxylase among others. Numerous aspects of the biochemical role of biotin were reviewed recently (1, 2).

BACKGROUND

Several analogs of biotin were synthesized, but few of these compounds substituted for biotin as a coenzyme. While biotin methyl ester and desthiobiotin show growth-promoting activity equivalent to that of biotin in many microorganisms, oxybiotin, biocytin, and biotin sulfoxide exhibit only limited activity. Many analogs possess substantial antibiotin activity.

The fact that carbobiotin (3) and oxybiotin have growth-promoting activity qualitatively, although not quantitatively, equivalent to that of biotin indicates that the sulfur atom of biotin is not essential for activity. The actual role of this sulfur atom has been a matter for debate. Glasel (4), on the basis of NMR data, postulated that the sulfur may act as a hydrogen acceptor in interactions with the protein component of biotin enzymes. This interpretation was challenged by Caplow (5), who pointed out that Glasel's observation could also be accounted for by assuming protonation of the ureide moiety.

However, Olah and White (6), employing conditions that hardly simulated biological media, presented unequivocal evidence that the sulfur is indeed protonated in magic acid (FSO_3H-SbF_5-SO_2). They suggested that the sulfur protonation occurs on the side *trans* to the carboxyl-



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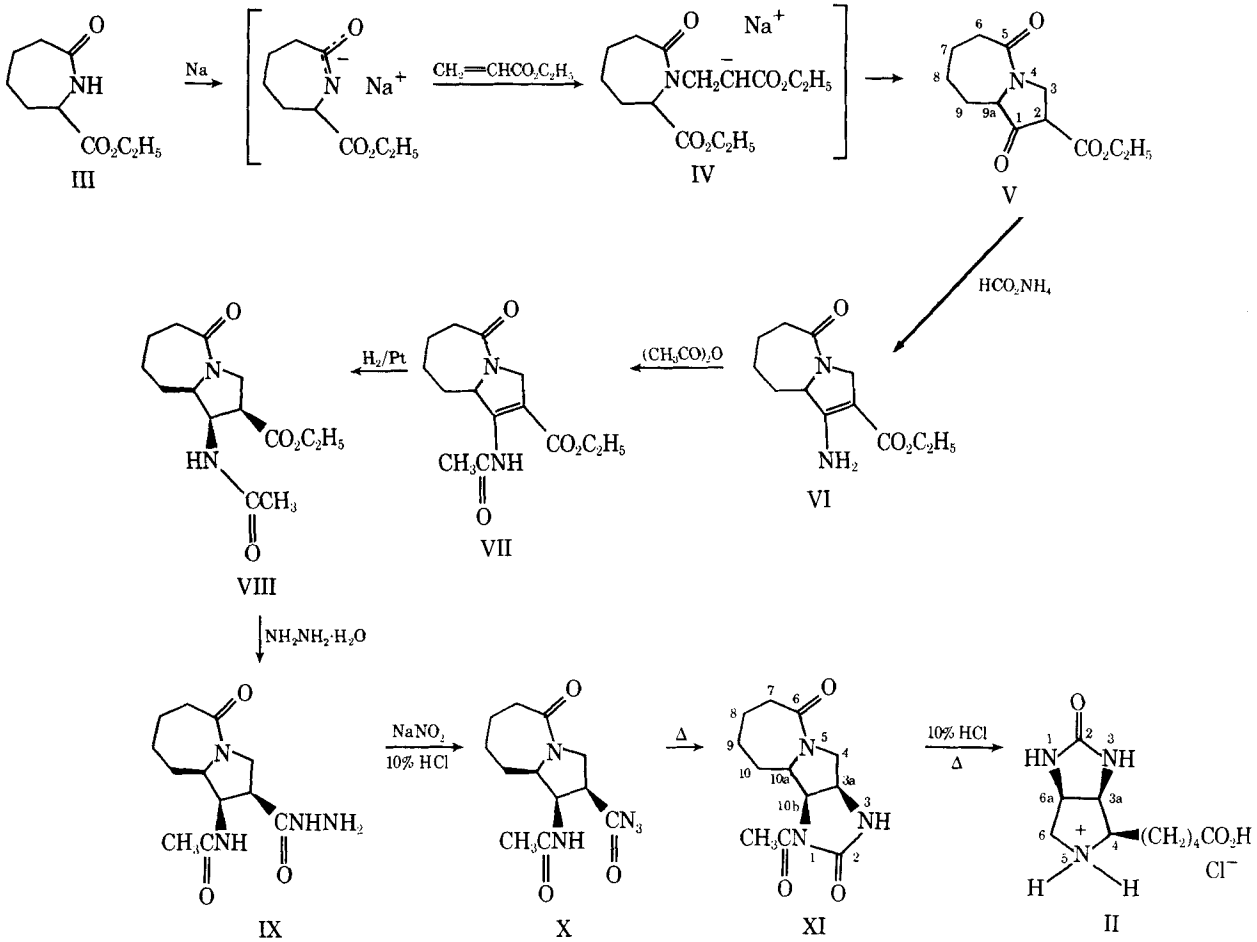

Scheme I

containing side chain, although it was subsequently demonstrated (7) that this protonation occurs *cis* to the side chain. Other studies (8) revealed that Mn^{2+} and Cu^{2+} are capable of weakly coordinating with sulfur orbitals of biotin on the side *trans* to the side chain, which might also serve to explain interaction with the protein of the holoenzyme.

Earlier reports (3, 9–12) were directed at the synthesis of biotin analogs containing either nitrogen or carbon in place of the sulfur atom. These efforts have now culminated in the development of a facile, totally synthetic route to the nitrogen analog of biotin, referred to as azabiotin (II).

DISCUSSION

The starting material in this synthesis (Scheme I) was 2-ethoxycarbonylazepin-7-one (III), prepared by the method of Shechter and Kirk (13). Treatment of III in dry benzene with sodium sand gave the sodio derivative, which was added to ethyl acrylate to give the intermediate IV. Compound IV underwent Dieckmann cyclization under the reaction conditions to yield the β-keto ester (V) as an impure oil. When this oil was refluxed with ammonium formate in 2-propanol, the crystalline enamine (VI) was isolated in 25% overall yield from III.

The enamine (VI) was acetylated by refluxing in acetic anhydride, and the crystalline enamine acetate (VII) was smoothly hydrogenated over platinum in ethanol to yield VIII. The acetamido ester (VIII) yielded the hydrazide (IX) when warmed with hydrazine hydrate in ethanol. Treatment of IX with nitrous acid gave the intermediate acyl azide (X), which underwent cyclization to the ureide (XI) when refluxed in ethyl acetate solution. The title compound (II) was prepared by acid-catalyzed hydrolysis of XI.

The stereochemistry of II was demonstrated by X-ray crystallography[1] to be all *cis* with respect to the substituents at C-4, C-3a, and C-6a. This arrangement is the same as that found in biotin (I). The stereochemical control apparent in this synthesis was not unexpected, since the reduction of VII was presumed to proceed by *cis*-addition of the two incoming hydrogen atoms from the less hindered face of the molecule.

EXPERIMENTAL[2]

1,5-Dioxo-2-ethoxycarbonyloctahydro-1*H*-pyrrolo[1,2-*a*]azepine (V)—A suspension of 1.35 g (58.5 mmoles) of sodium sand in 30 ml of dry benzene was heated to reflux with vigorous stirring under nitrogen and treated dropwise with a solution of 7.73 g (42.0 mmoles) of 2-ethoxycarbonylazepin-7-one (III) (13) in 40 ml of dry benzene. The reaction mixture was refluxed for 1 hr after the addition was completed, cooled to room temperature, and then treated dropwise with 10 ml of ethyl acrylate. The mixture was again refluxed for 1 hr, cooled, and poured with stirring onto a mixture of ice and 50 ml of 10% hydrochloric acid. The product was extracted with chloroform.

The organic layer was washed twice with water, dried over anhydrous sodium sulfate, and concentrated to a thick oil, 10.1 g. TLC of the oil (5% methanol in chloroform) revealed one major spot at R_f 0.4 and one minor spot at R_f 0.6; both spots gave a deep-purple color with ferric chloride; IR: ν_{max} (chloroform) 3700, 3020, 2960, 2880, 1775, 1735, 1680, and 1460 cm^{-1}; UV: λ_{max} 250 nm, shifting to 279 nm with increased absorbance upon addition of base[3]. This oil decomposed upon standing in air or upon attempted distillation under high vacuum but was suitable for the subsequent amination procedure.

1-Amino-2-ethoxycarbonyl -5- oxo-5,6,7,8,9,9a-hexahydro-3*H*-pyrrolo[1,2-*a*]azepine (VI)—A solution of 10.1 g of crude V was re-

[1] Details of this study will be published elsewhere (14).

[2] Melting points were taken on a Fisher-Johns melting-point stage and are corrected. UV absorption spectra were determined in 95% ethanol on a Beckman model DK2A recording spectrophotometer. IR spectra were recorded on Beckman models 8 and 33 recording spectrophotometers. NMR spectra were determined on a Varian EM 360 spectrometer. Microanalyses were performed by Spang Microanalytical Laboratory, Ann Arbor, Mich. TLC was performed with silica gel GF_{254}.

[3] The absorptivity was not calculated because the product was not homogeneous.

fluxed with 5.20 g (82.0 mmoles) of ammonium formate and 50 ml of 2-propanol for 6 hr. The reaction mixture was concentrated to dryness, and the residue was partitioned between chloroform and water. The chloroform layer was washed with water, dried over anhydrous sodium sulfate, and concentrated to a thick oil (9.02 g).

When the oil was scratched under isopropyl ether, VI precipitated out as fine needles, 2.50 g, mp 210–214°. Recrystallization from ethanol–water gave the analytical sample, mp 226–227°; IR: ν_{max} (chloroform) 3520, 3460, 3380, 3010, 2950, 2880, 1730, 1685, and 1639 cm^{-1}; UV: λ_{max} 276 nm (ϵ 20,400); NMR[4] ($CDCl_3$): δ 6.11 (s, 2H, NH_2), 4.60 (m, 1H, NCHC=C), 4.33 (s, 2H, NCH_2C=C), 4.19 (q, 2H, J = 7 Hz, OCH_2CH_3), 2.55 [m, 2H, CH_2C(=O)N], 1.60–2.33 [m, 6H, $(CH_2)_3$], and 1.28 (t, 3H, J = 7 Hz, OCH_2CH_3) ppm.

Anal.—Calc. for $C_{12}H_{18}N_2O_3$: C, 60.49; H, 7.61; N, 11.76. Found: C, 60.48; H, 7.67; N, 11.70.

1-Acetamido-2-ethoxycarbonyl -5- oxo-5,6,7,8,9,9a-hexahydro-3*H*-pyrrolo[1,2-*a*]azepine (VII)—A solution of 3.68 g (15.4 mmoles) of VI in 50 ml of acetic anhydride was refluxed for 2 hr. The solvent was removed *in vacuo*, and the residual oil was scratched under isopropyl ether. The resulting solid was removed by filtration and washed with isopropyl ether to give 3.01 g (70% yield) of light-brown powder, mp 112–113°. Recrystallization from isopropyl ether gave the analytical sample, mp 116–116.5°; IR: ν_{max} (chloroform) 3320, 3010, 2950, 2880, 1715, 1680, and 1640 cm^{-1}; UV: λ_{max} 274 nm (ϵ 20,500); NMR[4] ($CDCl_3$): δ 10.16 (s, 1H, NH), 5.48 (m, 1H, NCHC=C), 4.35 (s, 2H, NCH_2C=C), 4.28 (q, 2H, J = 7 Hz, OCH_2CH_3), 2.50–2.80 [m, 2H, CH_2C(=O)N], 2.25 [s, 3H, NC(=O)CH_3], 1.80–2.20 [m, 6H, $(CH_2)_3$], and 1.33 (t, 3H, J = 7 Hz, OCH_2CH_3) ppm.

Anal.—Calc. for $C_{14}H_{20}N_2O_4$: C, 59.99; H, 7.19; N, 9.99. Found: C, 60.03; H, 7.13; N, 10.02.

1-Acetamido-2-ethoxycarbonyl -5- oxooctahydro-1,2,9a-*cis*-1*H*-pyrrolo[1,2-*a*]azepine (VIII)—A solution of 1.73 g (6.30 mmoles) of VII in 50 ml of absolute alcohol was treated with 1.0 g of platinum oxide and hydrogenated at 45 psi for 21 hr. The catalyst was removed by filtration and washed thoroughly with absolute alcohol. The ethanolic solution was concentrated to dryness to give 1.72 g (97% yield) of crystals, mp 209–210°. Recrystallization from methanol gave the analytical sample, mp 209–210°; IR: ν_{max} (chloroform) 3440, 3300, 3000, 2940, 2870, 1730, 1676, and 1625 cm^{-1}; NMR[4] ($CDCl_3$): δ 7.40 (d, 1H, J = 10 Hz, NH), 4.19 (q, 2H, J = 7 Hz, OCH_2CH_3), 3.64–4.00 (m, 2H, C-1 and C-9a H's), 3.10–3.25 [m, 2H, CH_2NC(=O)], 2.70–3.00 (m, 1H, $CHCO_2C_2H_5$), 2.33–2.70 [m, 2H, CH_2C(=O)], 2.05 [s, 3H, CH_3C(=O)N], 1.48–1.90 [m, 6H, $(CH_2)_3$], and 1.26 (t, 3H, J = 7 Hz, OCH_2CH_3) ppm.

Anal.—Calc. for $C_{14}H_{22}N_2O_4$: C, 59.56; H, 7.85; N, 9.92. Found: C, 59.65; H, 7.53; N, 9.91.

1-Acetamido-2-hydrazinocarbonyl -5- oxooctahydro-1,2,9a-*cis*-1*H*-pyrrolo[1,2-*a*]azepine (IX)—A solution of 500 mg (1.91 mmoles) of VIII in 10 ml of absolute ethanol was treated with 2 ml of hydrazine hydrate and stirred at 40–50° for 7 hr. The reaction mixture was cooled in the refrigerator overnight and filtered to give 260 mg of colorless crystals, mp 278–279°. An additional 75 mg of this solid, mp 276–278°, was obtained when the mother liquor was concentrated to about half of the original volume, giving a total yield of 335 mg (65%) of pure IX. Recrystallization from aqueous methanol gave the analytical sample, mp 280–281°; IR: ν_{max} (mineral oil) 3280, 1670, and 1600 cm^{-1}; NMR[5] (D_2O): δ 3.38–3.83 (m, 2H, C-1 and C-9a H's), 3.02–3.35 [m, 2H, CH_2NC(=O)], 2.65–2.98 (m, 1H, $CHCO_2C_2H_5$), 2.10–2.48 [m, 2H, CH_2C(=O)N], 2.03 [s, 3H, NC(=O)CH_3], and 1.20–1.67 [m, 6H, $(CH_2)_3$] ppm.

Anal.—Calc. for $C_{12}H_{20}N_4O_3$: C, 53.72; H, 7.51; N, 20.88. Found: C, 53.62; H, 7.41; N, 20.80.

1-Acetyl-2,6-dioxododecahydro- 10a,10b,3a- *cis*-imidazo[4,5-*c*]pyrrolo[1,2-*a*]azepine (XI)—A solution of 143 mg (0.53 mmole) of IX in 5 ml of 10% hydrochloric acid was cooled to 0° and treated dropwise with a solution of 130 mg of sodium nitrite in 3 ml of water. The solution was stirred at 0° for 30 min and then extracted with six 50-ml portions of ethyl acetate. The organic extract was dried over anhydrous magnesium sulfate and concentrated *in vacuo* to give 120 mg of the crude azide (X) as a yellow oil; IR: ν_{max} (neat) 3300, 2920, 2850, 2160, 1710, 1660, and 1610 cm^{-1}.

The azide was redissolved in 25 ml of ethyl acetate and refluxed for 2 hr. Then the reaction mixture was concentrated to dryness to give an oil, which solidified when scratched under ether. The solid was removed by filtration and washed with ether to give 103 mg (77% yield from IX), mp 233–235°. Recrystallization from ethyl acetate–acetone gave the analytical sample, mp 237–238°; IR: ν_{max} (mineral oil) 3280, 1750, 1740, and 1640 cm^{-1}; NMR[5] (CD_3SOCD_3): δ 8.00 (s, 1H, NH), 4.98 (t, 1H, C-10b H, $J_{10a-10b}$ = 8 Hz, J_{10b-3a} = 8 Hz), 4.01–4.37 (m, 2H, C-3a and C-10a H's), 3.10–3.35 (m, 2H, C-4 H), 2.43–2.60 [m, 2H, CH_2C(=O)N], 2.40 [s, 3H, NC(=O)CH_3], and 1.20–2.08 [m, 6H, $(CH_2)_3$] ppm.

Anal.—Calc. for $C_{12}H_{17}N_3O_3$: C, 57.36; H, 6.82; N, 16.72. Found: C, 57.36; H, 6.78; N, 16.68.

***dl*-3a,4,6a-*cis*-4-(4-Carboxybutyl)octahydropyrrolo[3,4-*d*]imidazol-2-one Hydrochloride (II)**—A solution of 100 mg (0.40 mmole) of XI in 0.4 ml of 10% hydrochloric acid was heated in a sealed tube at 120° for 16 hr. The reaction mixture was cooled and concentrated to dryness *in vacuo*. The residue was scratched with ether to give a light-brown solid, which was filtered off and washed with ether. The crude product weighed 65 mg (62% yield) and was homogeneous on TLC [1-propanol–water (7:3) or 1-propanol–water–concentrated ammonium hydroxide (8:1:1)].

The analytical sample was prepared by recrystallization from ethanol; this solid melted at 132°, slowly resolidified at 140–150°, and melted again at 163–164°; IR: ν_{max} (mineral oil) 3450, 3300, 3230, 1690, and 1600 cm^{-1}; NMR[5] (D_2O): δ 4.50–4.72 (m, 2H, C-3a and C-6a H's), 3.50–3.68 (m, 1H, C-4 H), 3.28–3.46 (m, 2H, C-6 H), 2.42 (t, 2H, J = 6 Hz, CH_2CO_2H), and 1.32–1.80 [m, 6H, $(CH_2)_3$] ppm.

Anal.—Calc. for $C_{10}H_{18}ClN_3O_3$: C, 45.53; H, 6.87; N, 15.93. Found: C, 45.53; H, 7.01; N, 15.77.

[4] Tetramethylsilane as the internal standard.

[5] Sodium 2,2-dimethyl-2-silapentanesulfonate as the internal standard.

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