VITAMIN N BIOGENESIS AS BASIS FOR THE SEARCH FOR NEW BIOTIN PREPARATIONS

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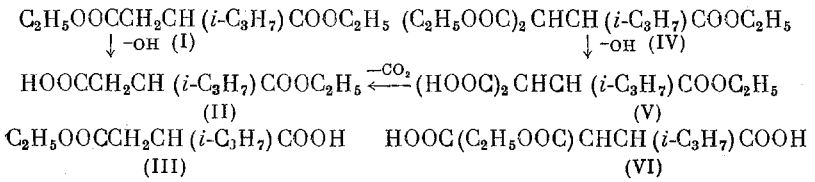
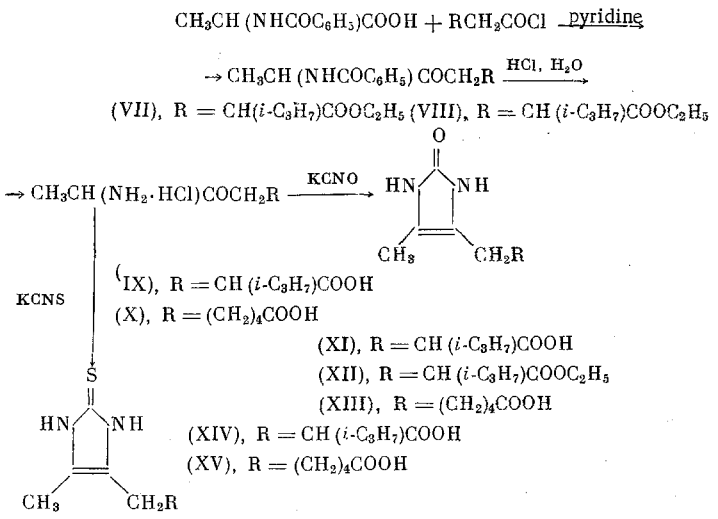
In an earlier report we described a biogenetic-like synthesis of the β-biotin (vitamin N) bioprecursors, 7-keto-8-aminopelargonic acid hydrochloride (X) and β-dehydrodesthiobiotin (XIII), with α-alanine and pimelic acid as the starter materials [1].

In this report, we used the same procedure to obtain potential precursors of the α-biotin hydrochloride of 2-isopropyl-4-keto-5-aminocaproic acid (IX) and α-dehydrodesthiobiotin (XI), as well as thioanalogs of α- and β-dehydrodesthiobiotin (XIV) and (XV). Treating the diethyl ester of isopropylsuccinic acid (I) with an alkali-alcohol solution yielded the monoethyl ester of isopropylsuccinic acid (II) which was also formed by alkaline hydrolysis of the triethyl ester of 2-carboxy-3-isopropylsuccinic acid (IV) and subsequent decarboxylation of the (isopropylcarbethoxymethyl)malinic acid (V)



Methylethyl esters prepared by reacting diazomethane with both forms of the monoester (II) yielded basically of peak on the gas-liquid chromatogram (GLC), which points to the high selectivity of (I) and (IV) saponification. In choosing between two possible structures, (II) or (III) and (V) or (VI), we decided in favor of (II) and (V), in consideration of the spatial hindrance presented by the isopropyl group with respect to the adjacent $COOC_2H_5$ group. This was confirmed by transformation of (II) into α-dehydrodesthiobiotin (XI)



The isopropylsuccinic acid monoethyl ester acid chloride (VII) was introduced into a Dakin–West reaction with α-benzoylalanine in pyridine and yielded the α-benzamidoketone (VIII) which was hydrolyzed with hydrochloric acid to the α-aminoketone hydrochloride (IX). In reaction with KCNO and KCNS, the ketone (IX) gave the corresponding α-dehydrodesthiobiotin (XI) and its thioanalog (XIV).

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TABLE 1

Compound	Activity in % of that of β-d-biotin*
Pimelic acid + α-alanine	0.1
Hydrochloride of 7-keto-8-amino-pelargonic acid	100
β-Dehydrodesthiobiotin (XIII)	10
β-Thiodehydrodesthiobiotin (XV)	100†
Hydrochloride of 2-isopropyl-4-keto-5-aminocaproic acid (IX)	0
α-Dehydrodesthiobiotin (XI)	0
α-Thiodehydrodesthiobiotin (XIV)	0

*In 0.1-1 ng/4 ml concentrations.
†In 600 ng/4 ml concentrations.

By reacting KCNS with the hydrochloride of 7-keto-8-aminopelargonic acid (X) we synthesized in the same way β-thiodehydrodesthiobiotin (XV). The structure of (XI) was shown by inverse synthesis, i.e., by hydrolysis of the ester (XII) described in the literature [2]. The structure of the thioanalogs of α- and β-dehydrodesthiobiotin (XIV) and (XV) was confirmed by comparing their IR spectra with the spectrum of the known dimethylimidazolethione [3].

In tests [4] with yeasts (Saccharomyces cerevisiae), the bioprecursors of β-biotin and β-thiodehydrodesthiobiotin (XV) exhibited biotin activity (Table 1). The most interesting compounds are 7-keto-8-aminopelargonic acid hydrochloride (X) and β-thiodehydrodesthiobiotin (XV) whose growth stimulatory effect approximately equals that of natural biotin. In growing the yeasts under semiindustrial conditions on molasses [4], the activity of (X) decreased to 68% of the natural biotin.

The potential precursors of α-biotin (IX) and (XI) as well as α-thiodehydrodesthiobiotin (XIV) proved inactive.

EXPERIMENTAL

Monoethyl Ester of Isopropylsuccinic Acid (II). A mixture of 12 g diethyl ester of isopropylsuccinic acid (I) (bp 126-130°C at 18 mm; n_D^{20} 1.4250) [5] and 3 g KOH in 150 ml alcohol were boiled for 4 h. After removing the alcohol, the residue was washed with water and the nonreacted (I) was extracted with ether. The aqueous layer was acidified with hydrochloric acid (Congo indicator), and 6 g (68%) of (II) were extracted with chloroform, bp 133-135°C (2 mm); n_D^{18} 1.4378; R_f 0.53. Now and later we used thin-layer chromatography on silica gel G, system $CHCl_3$–acetone 9 : 1, and treated the spots with iodine. Found %: C 57.67, 57.56; H 8.42, 8.33. $C_9H_{16}O$. Calculated %: C 57.43; H 8.37.

A mixture of 80 g triethyl ester of 2-carboxy-3-isopropylsuccinic acid (IV) with bp 170-173°C (18 mm) [6] and 37.2 g KOH in 350 ml alcohol was left to stand for 12 h at ~20°C. The K-salt precipitate was then filtered off, treated with an excess of diluted hydrochloric acid, and 66 g of (isopropylcarbethoxymethyl) malonic acid (V) were extracted with chloroform. Under vacuum distillation, the latter was subjected to decarboxylation and transformed into (II). Yield of (II)was 25 g (48%); bp 133-135° (2 mm); n_D^{18} 1.4378; R_f 0.53. The methyl esters prepared by treatment of the above described (II) specimens with diazomethane gave identical peaks on the GLC [7].

Acid Chloride of the Monoethyl Ester of Isopropylsuccinic Acid (VII). A mixture of 18 g (II) and 36 ml $SOCl_2$ was boiled for 1 h. After removal of the excess $SOCl_2$, the residue was vacuum-distilled. We obtained 14 g (70%) of (VII) with bp 96-97° (7 mm); n_D^{18} 1.4470. Found %: C 52.10, 52.37; H 7.01, 7.05; Cl 17.30, 17.39. $C_9H_{13}O_3Cl$. Calculated %: C 52.24; H 7.31; Cl 17.16.

Anilide (II). A solution of 0.45 g aniline in 2 ml benzene is added dropwise with a solution of 0.5 g of (VII) in 2 ml benzene. After washing with water, 0.5 g (78%) of the anilide (II) is isolated from the benzene solution; bp 60-62°C (washed with a mixture of ether and heptane). Found %: N 5.50, 5.34. $C_{15}H_{21}O_3N$. Calculated %: N 5.30.

Ethyl Ester of 2-Isopropyl-4-keto-5-benzamidocaproic Acid (VIII). A solution of 2.84 g α-benzoylalanine in 50 ml pyridine is added dropwise with 6.4 g (VII). The reaction mixture is heated in a water bath for 1 h, then evaporated under vacuum. The residue is treated with a saturated solution of $NaHCO_3$, the reaction product is isolated with benzene and subjected to chromatography on Al_2O_3 (active III). A mixture of benzene–ethylacetate, 4 : 1, served to elute 3.8 g (80%) of (VIII), as a viscid fatty substance, R_f 0.58 (system benzene–ethylacetate, 3 : 2). IR spectrum: 1720, 1645 cm^{-1}.*

*Here and later IR spectra were taken on the UR-10 in a pellet with KBr.

Hydrochloride of 2-Isopropyl-4-keto-5-aminocaproic Acid (IX). A mixture of 2.4 g (VIII) and 45 ml concentrated HCl are boiled for 2 h. After cooling, the precipitated benzoic acid is filtered off, and the mother liquor is evaporated under vacuum. The residue is treated with 5 ml water, benzoic acid residues are removed, and the solution is again evaporated under vacuum to dryness. The (IX) obtained as a fatty mass (1.5 g) is purified by chromatography on SiO_2 (50-100 mesh). A mixture acetone–water (4 : 1) served to elute 1.2 g (75%) (IX) with R_f 0.63 (system acetone–water, 4 : 1).

α-Dehydrodesthiobiotin (XI). A mixture of 1.1 g (IX) in 2 ml water is added with 0.44 g KCNO in 2 ml water and heated on a boiling water bath for 15 min. After cooling, the reaction mixture is acidified with HCl and the precipitating fatty substance is extracted with $CHCl_3$. After removal of the solvent, the residue is chromatographed on SiO_2 (50-100 mesh). A semicrystalline product is eluted with acetone; after washing with ether this yields 0.1 g (XI), R_f 0.41 (system acetone–water, 9 : 1); mp 178-180°C. Found %: C 54.10, 54.22; H 7.38, 7.37; N 12.74, 12.89. $C_{10}H_{16}O_3N_2 \cdot \frac{1}{2} H_2O$. Calculated %: C 54.30; H 7.00; N 12.67. IR spectrum: 3300, 2600, 1700, 1680, 1650 cm^{-1}.

A dehydrated sample of (XI) is obtained by drying the semihydrate over P_2O_5 at 140°C; mp 231-233°C. Found %: C 57.53, 56.98; H 7.63, 7.49; N 13.04, 13.19. $C_{10}H_{16}O_3N_2$. Calculated %: C 56.69; H 7.60; N 13.20.

A mixture of 0.1 g of the α-dehydrodesthiobiotin ethyl ester (XII) (mp 118-120°C) [2] and 10 ml concentrated HCl are boiled for 10 h. After evaporation under vacuum the residue is chromatographed on SiO_2 (50-100 mesh). Product (XI) is eluted with acetone (mp 203-204°C, R_f 0.41); no decrease of melting point was observed in a mixed sample with the above-described (XI), and both had the same IR spectrum.

α-Thiodehydrodesthiobiotin (XIV). A mixture of 0.43 g (IX) in 1 ml water is added with 0.4 g KCNS. The reaction mixture is heated in a boiling water bath for 2 h and left to stand for 12 h at above 20°C. The precipitate is then filtered off and washed with water. We obtained 0.15 g (30%) of (XIV) with mp 280-282°C (from water); R_f 0.78 (system acetone–heptane, 4 : 1). Found %: C 50.91, 51.04; H 6.98, 6.76; S 13.92, 14.06. $C_{10}H_{16}N_2SO_2 \cdot \frac{1}{2} H_2O$. Calculated %: C 50.67; H 6.79; S 13.52. IR spectrum: 3200-2900, 1705, 1660, 1510, 1200, 800 cm^{-1}.

A dehydrated sample of (XIV) was obtained upon drying the semihydrate over P_2O_5 at 140°; mp 290 to 292°C. Found %: C 51.96, 52.24; H 7.02, 7.01; S 13.81, 14.42; N 12.35, 12.30. $C_{10}H_{16}N_2SO_2$. Calculated %: C 52.60; H 7.06; S 14.06; N 12.28. IR spectrum of 4,5-dimethylimidazolethione [3]: 3200-2900, 1665, 1520, 1210, 805 cm^{-1}.

β-Thiodehydrodesthiobiotin (XV). A solution of 0.6 g 7-keto-8-aminopelargonic acid hydrochloride (X) [1] in 3 ml water is added with 1.0 g KCNS. The reaction mixture is heated in a boiling water bath for 2 h and left to stand for 12 h at about 20°. The precipitate is then filtered off and washed with water. Yield is 0.11 g (17%) of (XV) with mp 183-184°C (from water); R_f 0.70 (system acetone–heptane, 4 : 1). Found %: C 52.94, 50.91; H 7.30, 7.32; S 13.90, 13.94; N 12.28, 12.17. $C_{10}H_{16}N_2O_2S$. Calculated %: C 52.6; H 7.06; S 14.02; N 12.28. IR spectrum: 3200-2900, 1720, 1655, 1520, 1190, 790 cm^{-1}.

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CONCLUSIONS

1. A synthesis resembling that of biogenesis was conducted to obtain potential precursors of α-biotin, i.e., 2-isopropyl-4-keto-5-aminocaproic HCl and α-dehydrodesthiobiotin HCl.

2. By reacting potassium rhodanate with the hydrochlorides of 2-isopropyl-4-keto-5-aminocaproic acid and 7-keto-8-aminopelargonic acid, we obtained the respective α- and β-thiodehydrodesthiobiotins.

3. Biologic tests with precursors and analogs of α- and β-biotins revealed a high biotin activity in 7-keto-8-aminopelargonic acid hydrochloride and β-thiodehydrodesthiobiotin.

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