Synthesis and Growth-Promoting Activity of *dl-cis*-Hexahydro-4-(4-carboxybutyl)-2-cyclopentimidazolone: Carbobiotin

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Abstract ☐ A carbon analog of biotin was prepared and evaluated in three microorganism test systems. The racemic compound was found to have about 15% the growth-promoting activity of *d*-biotin under conditions where biotin limited growth.

Keyphrases ☐ Carbobiotin [*dl-cis*-hexahydro-4-(4-carboxybutyl)-2-cyclopentimidazolone]—synthesized and evaluated for growth-promoting activity ☐ *dl-cis*-Hexahydro-4-(4-carboxybutyl)-2-cyclopentimidazolone (carbobiotin)—synthesized and evaluated for growth-promoting activity ☐ Growth-promoting activity—synthesis and evaluation of carbobiotin

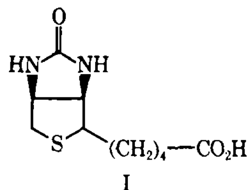
Relatively few compounds prepared to date have the ability to substitute for the cofactor biotin (I). The methyl ester shows essentially the same growth-promoting activity as free biotin in many microorganisms, whereas oxybiotin, desthiobiotin, biocytin, and biotin sulfoxide exhibit only a limited degree of activity. The majority of analogs prepared possess antivitamin properties. Furthermore, the importance of the sulfur atom is still being debated (1, 2), whereas there is little doubt that the stereochemical features of the molecule have a direct bearing on the biochemical behavior of the vitamin.

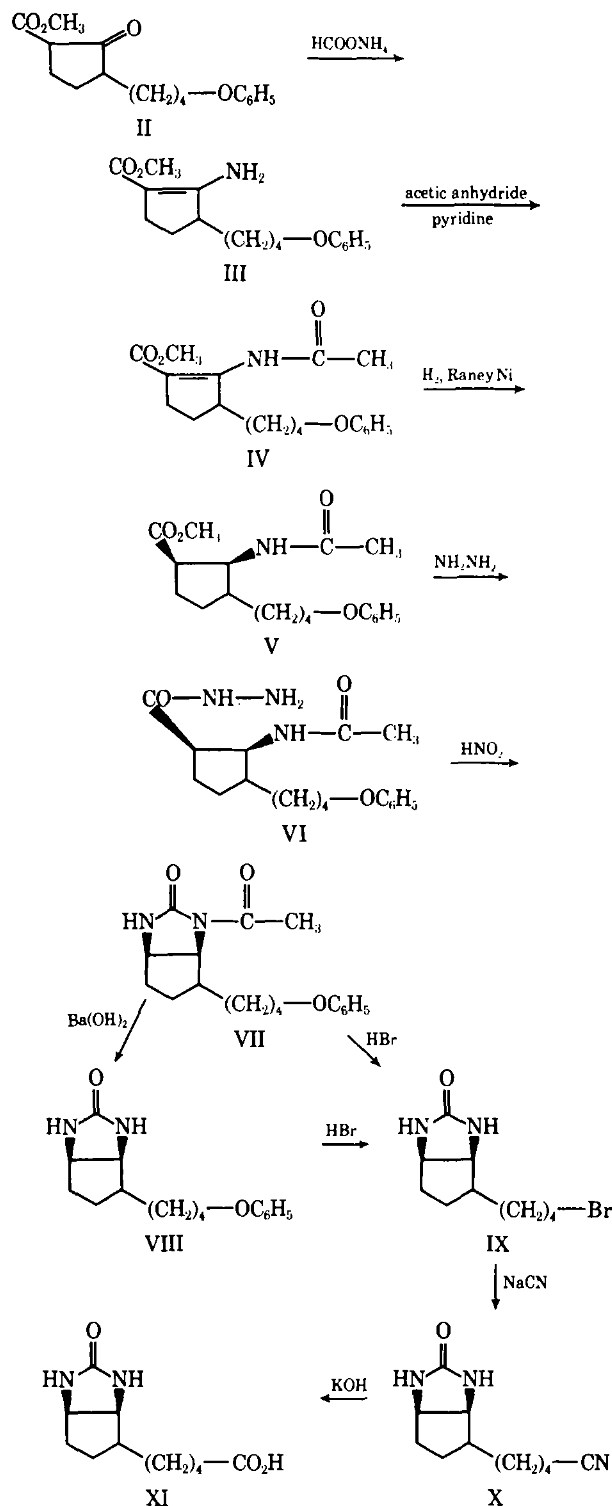
DISCUSSION

In preceding publications (3, 4), an approach to the pyrrolo-[3,4-*d*]imidazole nucleus was presented. The method described was applied in the present synthesis of the title compound, *dl-cis*-hexahydro-4-(4-carboxybutyl)-2-cyclopentimidazolone, hereafter referred to as carbobiotin (XI) (Scheme I).

3-(4-Phenoxybutyl)-2-oxocyclopentanecarboxylic acid methyl ester (II) (5) was first converted to the enamine III by condensation with ammonium formate. Following acetylation with acetic anhydride and pyridine, the cyclopentene system was reduced over freshly prepared W-2 Raney nickel to the acetamido ester V. Under similar conditions, reduction utilizing Adams' catalyst proved extremely slow and incomplete. Hydrazinolysis of the ester V was achieved in good yield by heating the ester in the presence of hydrazine hydrate at 60° for 12 hr. The reason for keeping the temperature low in this particular reaction was to prevent isomerization, since such a reaction has been known to occur during higher temperature hydrazinolysis of certain *cis*-vicinal diesters (6) and *cis*-acetamido esters (4).

Conversion of the hydrazide VI to the azide, followed by Curtius rearrangement of this intermediate, afforded the bicyclic ureide VII in 49% yield. Cleavage of the phenoxy ether linkage was carried





Scheme I

Table I—*d*-Biotin Activity of *dl*-Carbobiotin and Related Activity of Compounds in Terms of *d*-Biotin under Test Conditions

Compounds Tested	*L. plantarum* ATCC 8014 Compound Alone, %	With Oleic Acid, %	With Avidin, %	*S. cerevisiae* ATCC 7754 Compound Alone, %	With Oleic Acid, %	With Avidin, %	*M. echinata* ATCC 11973 Compound Alone, %	With Oleic Acid, %	With Avidin, %
dl-Carbobiotin	12	20	0	15	25	0	15	20	15
dl-Oxybiotin	10	23	0	11	22	0	13	25	18
dl-Desthiobiotin	0	n.t.[a]	n.t.	15	25	0	16	23	15

[a] n.t. = not tested.

out utilizing 48% hydrobromic acid in glacial acetic acid on either VII or the desacetyl VIII. The bromobutyl derivative IX was then converted to the corresponding cyanobutyl X, and base hydrolysis of the latter afforded *dl*-carbobiotin (XI) in 83% yield.

dl-Carbobiotin was evaluated as a growth factor for *d*-biotin-requiring microorganisms in comparison with *d*-biotin, *dl*-oxybiotin, and *dl*-desthiobiotin[1] (7, 8). The potency of these compounds was determined using the turbidimetric *Lactobacillus plantarum* (ATCC 8014) method (using Difco biotin assay broth) (9), *Saccharomyces cerevisiae* (ATCC 7754) [grown in the medium described by Hertz (10)], and *Memnoniella echinata* (ATCC 11973) [grown under the conditions described by Perlman (11)].

The tests (Table I) show that for these test organisms, *dl*-carbobiotin has approximately 15% of the potency of *d*-biotin. When oleic acid was present (5 mcg./l.), the organisms were more sensitive to *dl*-carbobiotin, a response previously noted by Williams and Fieger (12) for *d*-biotin effects on microorganisms. Addition of 0.13 unit of avidin[2] to tubes of media containing 10 ng. of *dl*-carbobiotin completely inhibited growth response to the carbobiotin.

In these bioassay systems, *dl*-oxybiotin had about 10% the activity of *d*-biotin for these test organisms [as was previously reported (7, 11)], and *dl*-desthiobiotin promoted the growth of the yeast and the mold but not the *L. plantarum*. Addition of oleic acid to the medium enhanced the growth-promoting activity of oxybiotin and of desthiobiotin, while avidin counteracted the activity of the oxybiotin for the yeast and the bacteria but not for the mold.

These studies show that either carbon or oxygen can replace the sulfur of biotin without affecting qualitatively its growth-promoting activity for microorganisms. Examination of the growth-promoting factor in *L. plantarum* cells grown on *dl*-carbobiotin-containing media showed the presence of carbobiotin-like substances as determined by bioautographs of thin-layer chromatograms.

EXPERIMENTAL[3]

3-(4-Phenoxybutyl)-2-amino-1-cyclopentenecarboxylic Acid Methyl Ester (III)—A solution of 5.91 g. (0.02 mole) of 3-(4-phenoxybutyl)-2-oxocyclopentanecarboxylic acid methyl ester (II) (5) and 10.0 g. (0.16 mole) of ammonium formate in 100 ml. of absolute ethanol was refluxed for 24 hr. Evaporation of the solvent under reduced pressure afforded an oily crystalline residue. The product was treated with 50 ml. of water to dissolve the excess unreacted ammonium formate, and the reaction product was extracted with chloroform. The chloroform extract was washed with water, saturated sodium chloride solution, and dried over anhydrous sodium sulfate. Evaporation of the solvent afforded 5.18 g. (88%) of a light-yellow viscous oil which resisted crystallization. The UV spectrum showed $\lambda_{max.}^{EtOH}$ 278 nm. (ϵ 15,786). The IR spectrum showed bands at 3521, 3356, 1724, and 1667 cm.$^{-1}$.

3-(4-Phenoxybutyl)-2-acetamido-1-cyclopentenecarboxylic Acid Methyl Ester (IV)—A solution of 4.25 g. (0.015 mole) of III in 10 ml. of acetic anhydride containing 5 ml. of dry pyridine was heated on a steam bath for 2 hr. The excess reagents were removed *in vacuo*, and the brown oily residue was dissolved in chloroform and washed successively with 2 *N* hydrochloric acid, 10% sodium carbonate, and water. Following drying over anhydrous sodium sulfate, the chloroform solution was evaporated to dryness, leaving a viscous yellow oil (4.5 g.). Column chromatography on silica gel, using benzene as the eluent, yielded 2.77 g. (57%) of a pale-yellow oil. This oil, which showed a single spot on TLC, could not be crystallized. The UV spectrum showed $\lambda_{max.}^{EtOH}$ 279 nm. (ϵ 16,000). The IR spectrum showed bands at 3311, 1695, 1667, and 1618 cm.$^{-1}$.

***cis*-3-(4-Phenoxybutyl)-2-acetamidocyclopentanecarboxylic Acid Methyl Ester (V)**—A solution of IV (1.30 g., 3.92 mmoles) in 100 ml. of absolute ethanol was hydrogenated in a Parr shaker over approximately 3 g. of freshly prepared W-2 Raney nickel for 50 hr. Following filtration of the catalyst and evaporation of the solvent under reduced pressure, 1.10 g. of a thick, colorless oil was obtained. The reduction product showed a single spot on TLC (2% methanol in chloroform). Crystallization from ether afforded 0.96 g. (75%) of colorless crystals, m.p. 106–109°. The UV spectrum showed $\lambda_{max.}^{EtOH}$ 271 nm. (ϵ 1635). The IR spectrum showed bands at 3413, 1727, and 1669 cm.$^{-1}$.

Anal.—Calc. for $C_{19}H_{27}NO_4$: C, 68.43; H, 8.18; N, 4.20. Found: C, 68.40; H, 8.17; N, 4.15.

***cis*-Hexahydro-3-acetyl-4-(4-phenoxybutyl)-2-cyclopentimidazolone (VII)**—A solution of 2.0 g. (6.0 mmoles) of the ester V in 15 ml. of 95% ethanol was treated with 5 ml. of hydrazine hydrate at 60° for 12 hr. Evaporation of the solvent and excess hydrazine hydrate under reduced pressure afforded 1.80 g. (90%) of a white product, m.p. 87–93°. TLC (30% methanol in chloroform) showed a single spot. The IR spectrum (mineral oil) showed bands at 3279 and 1639 cm.$^{-1}$.

The hydrazide VI was used in the following step without additional purification. To a solution of VI (0.20 g., 0.60 mmole) in 2 ml. of cold 2 *N* hydrochloric acid was added, with stirring, 0.08 g. (1.2 mmoles) of sodium nitrite in 1 ml. of water. The oily azide was extracted with cold ether, and the ethereal extract was washed with 10% sodium bicarbonate and water and dried over anhydrous sodium sulfate. Evaporation of the ether afforded 0.14 g. of a yellow oil. The IR spectrum (neat) of this material showed a pronounced band at 2114 cm.$^{-1}$, indicative of the azide functionality. The yellow oil was refluxed in 10 ml. of benzene for 2 hr. Evaporation of the solvent gave 0.09 g. (49%) of a crystalline product, m.p. 109–112°. Recrystallization from a mixture of ethyl acetate and petroleum ether (b.p. 30–60°) afforded the analytical sample, m.p. 116–118°. The UV spectrum showed $\lambda_{max.}^{EtOH}$ 271 nm. (ϵ 1774). The IR spectrum showed bands at 3448, 1724, and 1681 cm.$^{-1}$.

Anal.—Calc. for $C_{18}H_{24}N_2O_3$: C, 68.32; H, 7.66; N, 8.85. Found: C, 68.48; H, 7.74; N, 8.81.

***cis*-Hexahydro-4-(4-phenoxybutyl)-2-cyclopentimidazolone (VIII)**—A suspension of 0.20 g. (0.63 mmole) of VII and 0.75 g. (2.37 mmoles) of barium hydroxide octahydrate in 7.5 ml. of water and 20 ml. of methanol was refluxed for 3 hr. The suspension was cooled to room temperature, and carbon dioxide was introduced into it for 1 hr. The formed barium carbonate was filtered, and the clear filtrate was concentrated under reduced pressure. Preparative TLC of the oily residue on silica gel GF_{254} (10% methanol in chloroform) afforded 0.08 g. (46%) of a pale-yellow crystalline product, m.p. 87–93°. Several recrystallizations from ether gave the analytical sample as colorless microcrystals, m.p. 103–106°. The UV spectrum showed $\lambda_{max.}^{EtOH}$ 278 nm. (ϵ 1894). The IR spectrum showed bands at 3448 and 1695 cm.$^{-1}$.

Anal.—Calc. for $C_{16}H_{22}N_2O_2$: C, 70.03; H, 8.10; N, 10.21. Found: C, 70.00; H, 8.17; N, 10.10.

***cis*-Hexahydro-4-(4-bromobutyl)-2-cyclopentimidazolone (IX)**—*Method A*—A solution of 0.27 g. (1.0 mmole) of VIII and 2.0 ml. of 48% hydrobromic acid in 5.0 ml. of acetic acid was heated at 90° for 3 hr. The red solution was concentrated *in vacuo*, and the remaining

[1] *d*-Biotin, *dl*-oxybiotin, and *dl*-desthiobiotin were gifts of Dr. W. R. Sullivan, Hoffmann-La Roche, Inc., Nutley, N. J.

[2] Sigma Chemical Co., St. Louis, Mo.

[3] Melting points were determined on a Fisher-Johns melting-point stage and a Thomas-Hoover melting-point apparatus which had been calibrated with standard samples. UV absorption spectra were determined in 95% ethanol on a Beckman model DK2A recording spectrophotometer. IR absorption spectra were recorded in chloroform (unless otherwise specified) on a Beckman model 8 recording spectrophotometer. Microanalyses were carried out by Spang Microanalytical Laboratory, Ann Arbor, Mich. TLC was carried out with silica gel G and silica gel $HF_{254+366}$ (Brinkmann Instruments).

oil was subjected to preparative TLC on silica gel $HF_{254+366}$ (ethyl acetate). The higher R_f band gave 0.042 g. of an oily product, which was characterized as phenol on the basis of IR and UV spectra as well as TLC. The lower R_f band gave the desired product, IX, as an oil, 0.21 g. (82%). The IR spectrum showed bands at 3472, 3333, and 1681 cm.$^{-1}$. There was no UV absorption in the 240–290-nm. region.

Method B—Compound VII (0.60 g., 1.89 mmoles) was subjected to hydrolytic conditions identical to those outlined in Method A. The product obtained following workup was identical to the one obtained by the previous method (0.42 g., 85%).

***cis*-Hexahydro-4-(4-cyanobutyl)-2-cyclopentimidazolone (X)**—A solution of 0.25 g. (5.0 mmoles) of sodium cyanide in 1.0 ml. of water was added to a solution of 0.13 g. (0.5 mmole) of the bromo derivative IX in 5.0 ml. of methanol. The reaction was refluxed for 20 hr., the solvent was evaporated to dryness, and the oily residue was purified by preparative TLC (25% methanol in chloroform). The nitrile was obtained as a colorless oil (0.097 g., 94%). The IR spectrum showed bands at 3472, 3247, 2247, and 1681 cm.$^{-1}$.

Hexahydro-4-(4-carboxybutyl)-2-cyclopentimidazolone (Carbobiotin) (XI)—Compound X (0.21 g., 1.0 mmole), dissolved in a mixture of 15 ml. of methanol and 8 ml. of 3 *N* aqueous potassium hydroxide, was heated on the steam bath for 5 hr. The reaction mixture was concentrated to one-third of its original volume and acidified with 10% hydrochloric acid (Congo Red). The white precipitate was filtered and dried, affording 0.19 g. (83%) of crude product, m.p. 187–195°. Several recrystallizations from 95% ethanol gave colorless crystals of XI, m.p. 211–213°. The IR spectrum (mineral oil) showed bands at 3257 and 1695 cm.$^{-1}$.

Anal.—Calc. for $C_{11}H_{18}N_2O_3$: C, 58.40; H, 8.02; N, 12.38. Found: C, 58.48; H, 7.89; N, 12.47.

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COMMUNICATIONS

Effect of Volume of Distribution on Plasma Levels of Total Radioactivity

Keyphrases ☐ Volume of distribution—effect on plasma levels of total radioactivity, intravenous haloperidol-^{3}H, man ☐ Plasma profile of radioactivity—atypical appearance after intravenous haloperidol-^{3}H administration, man, effect of volume of distribution ☐ Haloperidol-^{3}H, intravenous, man—effect of volume of distribution on plasma levels of total radioactivity, atypical profile discussed ☐ Radiolabeled haloperidol—effect of volume of distribution on plasma levels, atypical profile discussed, intravenous administration, man

Sir:

An unusual but, in retrospect, not unexpected pharmacokinetic phenomenon was observed following intravenous administration of haloperidol-^{3}H (generally labeled). Figure 1 shows the mean plasma level profile of total radioactivity obtained in three healthy male subjects following intravenous administration of 2 mg. of haloperidol-^{3}H. The concentration of tritium in each sample was determined using a liquid scintillation counter[1] and internal standards. All samples were counted to less than a 1% counting error. The purity of the haloperidol-^{3}H used was 99+% as determined by solvent extraction and TLC. Heparinized plasma samples were removed at 0 (predose), 10, 20, 40, and 60 min. and at 2, 4, 6, 8, 12, 24, 48, 72, and 96 hr.

The profile of Fig. 1 is not what one would expect following intravenous administration. Instead of the expected maximum at the first sample time followed by a decline, the plasma levels of total radioactivity increased over the 96-hr. period of observation. The cumulative urinary excretion profile (Fig. 2) shows that significant excretion of radioactivity occurred

[1] Beckman LS200 B.