Synthesis of *N*-Alkyl Biotin Amides as Models for Biotin-Dependent Enzymes (1)

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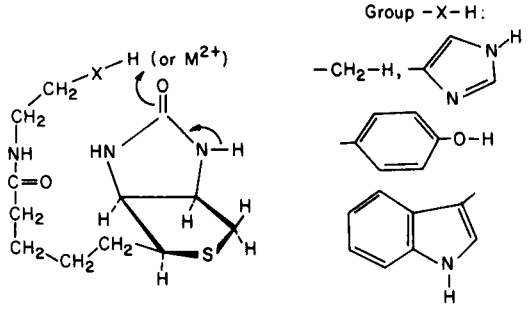
Many biological carboxylations require the vitamin, biotin, which functions as a coenzyme when covalently attached *via* an amide linkage to an enzyme. The sterically less impeded ring nitrogen 1 of the ureido portion of the bicyclic coenzyme in the protein milieu acts as a nucleophile toward an activated form of carbon dioxide to form N^1-carboxybiotin (2). As the nucleophilicity of the ureido nitrogens of free biotin and related 2-imidazolidones is low (3-6), it is clear that the protein surrounding the coenzymatically operable biotin must enhance the reactivity of nitrogen 1, undoubtedly through polarization of the 2-carbonyl double bond toward the tautomeric structure. Indeed, it has been shown that a model for a biotin tautomer, 2-methoxy-2-imidazoline, is much more nucleophilic toward an sp^2 carbonyl carbon than 2-imidazolidone (7). A likely way in which such polarization of enzyme-bound biotin can occur is by hydrogen bonding of the 2-carbonyl oxygen of the coenzyme to those neighboring amino acid residues that can serve as a hydrogen donor. It is also reasonable that such amino acid moieties coordinate a divalent cation, additionally required by carboxylases and transcarboxylases, thereby generating a complex similarly able to polarize the ureido carbonyl of the coenzyme.

To investigate the role that specific amino acid residues in biotin-containing enzymes can play in affecting the reactivity of the coenzyme, appropriate models were needed. The present paper describes the syntheses of amides of natural *d*-biotin with propylamine, histamine, tryptamine, and tyramine. The structures of these biotin amides is given in the following formulas, which also indicate how the nucleophilicity of ureido nitrogen 1 is affected by polarization of the carbonyl function in either hydrogen bonding or metal ion coordination.



Syntheses were accomplished by converting biotin to its *p*-nitrophenyl ester and allowing it to react with the appropriate amine in dimethyl sulfoxide. The crude product was purified by passage through an anion-exchange column to remove contaminating biotin and by recrystallization from organic solvents to remove any unreacted amine. Elemental analyses calculated and found for the *N*-alkyl biotin amides obtained are given in Table I.

In addition, quantitative colorimetric assay of the biotin moiety of each amide was made by reaction with *p*-dimethylaminocinnamaldehyde in acidified ethanol (8). The resulting red Schiff bases of the biotin amides had a λ max at 533 nm and gave values of 100-110% relative to biotin. The biotin amide of tryptamine also gave a more rapidly fading purple (λ max $\approx$ 590 nm) due to an Ehrlich-type of reaction between the aldehyde and the indole ring. Purity of all amides was also checked by spraying developed chromatograms with the acidified aldehyde reagent to locate the amide and any contaminating biotin, and with ninhydrin to locate any contaminating amine. Using both thin-layer (Brinkman MN Silica Gel S-HR) and paper (Whatman No. 1) chromatograms, with ascending butanolic solvents, absolute purities were ascertained. Mobilities of biotin and the derived amides in these systems are shown by the data in Table II.

Finally, spectra helped establish the chemical identity of each amide. The presence of the *p*-hydroxyphenyl and especially 3-indolyl moieties in aqueous solutions was confirmed by ultraviolet absorption near 280 nm. Infrared absorbancies in potassium bromide pellets not only confirmed the functional groups present in each interacted partner (biotin and amine) but also indicated the amide nature of the linkage, since the CO(NH) stretching (amide I) absorbances were found at 1625 to 1635 cm^{-1} and NH deformation (amide II) absorbances were found at 1535 cm^{-1}. Proton magnetic resonance spectra in deuterated dimethyl sulfoxide confirmed the structures.

TABLE I

Elemental Analyses of *N*-Alkyl Biotin Amides

N-Substituent	Composition	C	H	N	S
n-Propyl	$C_{13}H_{23}N_3O_2S$				
Calcd.		54.71	8.12	14.72	11.23
Found		54.65	8.19	14.63	11.10
β-(4-Imidazolyl)ethyl	$C_{15}H_{23}N_5O_2S$				
Calcd.		53.39	6.87	20.76	9.50
Found		53.25	7.02	20.48	9.44
β-(4-Hydroxyphenyl)ethyl	$C_{18}H_{25}N_3O_3S$				
Calcd.		59.48	6.88	11.56	8.82
Found		59.12	6.92	11.68	8.87
β-(3-Indolyl)ethyl	$C_{20}H_{26}N_4O_2S$				
Calcd.		62.15	6.75	14.50	8.30
Found		62.07	7.05	14.60	8.35

TABLE II

Mobilities of Biotin and Amides on Thin-Layer and Paper Chromatograms

Compound	R_f values	
	Thin-layer (a)	Paper (b)
Biotin	0.36	0.82
N-(*n*-Propyl) biotin amide	0.61	0.87
N-[β-(4-Imidazolyl)ethyl] biotin amide	0.53	0.72
N-[β-(4-Hydroxyphenyl)ethyl] biotin amide	0.67	0.84
N-[β-(3-Indolyl)ethyl] biotin amide	0.72	0.84

(a) Developed with *n*-butyl alcohol:2 *N* ammonium hydroxide:ethanol (3:1:1, v/v/v). (b) Developed with *n*-butyl alcohol:acetic acid:water (2:1:1, v/v/v).

Preliminary nmr studies, which have already been reported (9), indicate that coordination can occur between Mn^{2+} and the ureido group of the biotin moiety in each case and also between this divalent cation and the imidazole group of the histamine moiety. A more detailed investigation of the interactions of metal ion and hydrogen bonding, which can occur within these models of biotin-containing enzymes, will be reported (10). It is now clear that such reactions do occur and can account for the increased nucleophilicity of ureido nitrogen 1 of enzyme-bound biotin.

EXPERIMENTAL

Syntheses.

The amides of *d*-biotin with propylamine, histamine, tryptamine, and tyramine were synthesized by way of the *p*-nitrophenyl ester of biotin as follows: Biotin (6.1 g., 25 mmoles) and *p*-nitrophenyl trifluoroacetate (11.8 g., 50 mmoles), freshly prepared by refluxing equivalent amounts of *p*-nitrophenol and trifluoroacetic anhydride and evaporating off trifluoroacetic acid formed (11), were stirred into 400 ml. of anhydrous pyridine. The mixture was kept at 50° for an hour. The volume was reduced to about one-tenth by warming under reduced pressure, and the concentrate was stirred rapidly into a mixture of 250 ml., each, of diethyl ether and water near 0°. The precipitate was filtered off and washed successively with 50-ml. portions of 1% aqueous sodium bicarbonate, water, ether:acetone (4:1, v/v), and ether. The crude *p*-nitrophenyl biotinate was dried over phosphorus pentoxide *in vacuo* for 6.4 g. (70% yield). This reactive biotin ester (1.8 g., 5 mmoles) and the appropriate free amine (5 mmoles), in the cases of tryptamine and tyramine prepared from hydrochloride salts by precipitation with dilute ammonium hydroxide and drying, were stirred into 15 ml. of anhydrous dimethyl sulfoxide. This solution was stored overnight at room temperature, stirred into 35 ml. of acetone, and 50 ml. of ether was added. After a few hours in the refrigerator, the crude product was filtered, dissolved in 100 ml. of 50% (v/v) aqueous methanol, and poured over a column (2.5 x 20 cm.) of Dowex 1 X2 (formate) to remove some contaminating biotin and traces of trifluoroacetic acid. The amide was washed through with a further 500 ml. of 50% methanol and solvent removed by warming under reduced pressure. To remove traces of unreacted amine, the residue was taken up in 10 ml. of ethanol and product precipitated with 15 ml. of acetone and 25 ml. of ether, filtered, and dried over phosphorus pentoxide *in vacuo* for 0.3 to 0.8 g. (25 to 50% yields) of compound with mp ranges, for tryptamyl, tyramyl, propamyl, and histamyl derivatives, of 135-140°, 190-195°, 195-200°, and 200-205°, respectively.

Analyses.

Elemental analyses on the amides of biotin were done by Schwarzkopf Microanalytical Laboratory, Woodside, N. Y. Visible absorbances were determined with a Beckman DU spectrophotometer; ultraviolet spectra with a Cary Model 14 recording spectrophotometer. Infrared spectra were taken with a Perkin-Elmer Infracord. Nuclear magnetic resonance spectra were made with Varian 30 and 60 MHz spectrometers. Melting points were determined with a Fisher-Johns melting point apparatus.

REFERENCES

(1) This work was supported in part by Research Grant AM-04585 from the National Institutes of Health, U.S.P.H.S., and in part by funds made available through the State University of New York.

(2) J. Knappe, *Ann. Rev. Biochem.*, **39**, 757 (1970).

(3) J. Knappe, *Proc. Int. Congr. Biochem.*, 6th, **32**, 355 (1964).

(4) M. Caplow, *J. Am. Chem. Soc.*, **87**, 5774 (1965).

(5) M. Caplow and M. Yager, *ibid.*, **89**, 4513 (1967).

(6) M. Caplow, *ibid.*, **90**, 6795 (1968).

(7) A. F. Hegarty, T. C. Bruice, and S. J. Benkovic, *Chem. Commun.*, 1173 (1969).

(8) D. B. McCormick and J. A. Roth, *Anal. Biochem.*, **34**, 226 (1970).

(9) R. Griesser, H. Sigel, L. D. Wright, and D. B. McCormick, *Federation Proc.*, **30**, 1088 (1971).

(10) R. Griesser, H. Sigel, L. D. Wright, and D. B. McCormick, to be published (1973).

(11) L. F. Fieser and M. Fieser, "Reagents for Organic Synthesis", John Wiley and Sons, New York, p. 745 (1967).