hydroxide solution, filtering the solution and acidifying with dilute hydrochloric acid. The product separated in fine white crystals which melted at 276–278°; $[\alpha]^{25}\text{D} + 56°$; c, 0.47 g./100 ml., 0.1 N sodium hydroxide.

Anal. Calcd. for $C_{16}H_{22}N_4O_4S_2$: C, 48.22; H, 5.57; N, 14.06. Found: C, 48.27; H, 5.45; N, 14.04.

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Amides and Amino Acid Derivatives of Biotin: Microbiological Studies

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Biotinamide and N-biotinyl derivatives of glycine, β-alanine, L-aspartic acid, L-glutamic acid, L-leucine, *p*-aminobenzoic acid and the diethyl esters of N-biotinyl-L-aspartic and N-biotinyl-L-glutamic acids were examined microbiologically for biotin activity. Biotinamide, N-biotinyl-*p*-aminobenzoic acid and N-biotinyl-β-alanine have a spectrum of microbiological activity quite similar to that of biocytin, a form of biotin occurring in some natural products, but may be distinguished from biocytin by differences in solubility or acid stability. Representative compounds from the group synthesized readily combine with avidin.

The chemical properties of biocytin that became apparent during the development of fractionation procedures for the isolation of this naturally-occurring complex of biotin[1] were such as to suggest that biocytin is a biotinyl derivative which might be linked through nitrogen to an amino acid-like moiety. A series of amides and amino acid derivatives of biotin have been synthesized[2] and made available for microbiological examination. The activities of these compounds in promoting growth of *Lactobacillus arabinosus* and *Lactobacillus casei* in basal media free of biotin are summarized in Table I. Biotinamide, N-biotinyl-*p*-aminobenzoic acid and N-biotinyl-β-alanine were found to have differential activity with the two strains corresponding to that of biocytin and were examined with additional organisms. The data of Table II demonstrate that, with a variety of microörganisms that depend for growth on an exogenous source of biotin, biocytin, biotinamide, N-biotinyl-*p*-aminobenzoic acid and N-biotinyl-β-alanine have an essentially similar spectrum of activity. It may be noted that two artificially-induced biotin-deficient mutants *Escherichia coli* M81-78 and *Penicillium chrysogenum* 62078 are unable to utilize biocytin as a source of biotin.

TABLE I

MICROBIOLOGICAL ACTIVITIES OF BIOTIN DERIVATIVES

Compound	Biotin activity as determined by *Lactobacillus arabinosus*	Biotin activity as determined by *Lactobacillus casei*
Biocytin	−	+
Biotinamide	−	+
N-Biotinylglycine	+	+
N-Biotinyl-β-alanine	−	+
Diethyl ester of N-biotinyl-L-aspartic acid	−	±
N-Biotinyl-L-aspartic acid	−	−
Diethyl ester of N-biotinyl-L-glutamic acid	−	±
N-Biotinyl-L-glutamic acid	−	−
Ethyl ester of N-biotinyl-L-leucine	−	−
N-Biotinyl-L-leucine	−	−
N-Biotinyl-*p*-aminobenzoic acid	−	+

+, activity essentially that of an equivalent of biotin; ±, activity in the order of 25–50% that of an equivalent of biotin; −, no activity.

TABLE II

SPECTRA OF ACTIVITY OF BIOTIN DERIVATIVES

	Biocytin	Biotinamide	N-Biotinyl-*p*-aminobenzoic acid	N-Biotinyl-β-alanine
Lactobacillus arabinosus 17-5 (8014)	−	−	−	−
Lactobacillus pentosus (8041)	− (b)	− (a)	−	−
Leuconostoc mesenteroides (8042)	−	−	−	−
Escherichia coli M81-78	−			
Penicillium chrysogenum 62078	−	− (a)	−	− (b)
Lactobacillus acidophilus (4646)	+	+	+	+
Lactobacillus acidophilus 05	+			
Lactobacillus acidophilus (314)	+	+	−	− (b)
Lactobacillus acidophilus (8530)	+	+	+	+
Lactobacillus acidophilus (4357)	+	+	−	− (b)
Lactobacillus acidophilus K	+	+	+	+
Lactobacillus brevis (8287)	+	+	−	− (b)
Lactobacillus casei (7469)	+	+	+	+
Lactobacillus delbrückii LD5 (9595)	+	+	+	+
Lactobacillus plantarum (4943)	+	+	− (a)	− (a)
Streptococcus fecalis R (8043)	+	+	− (b)	+
Neurospora crassa (9278)	+	+	−	+
Propionibacterium shermanii (8262)	+	+	+	+

+, activity essentially that of an equivalent of biotin; −(a), activity about 50% that of an equivalent of biotin; −(b), activity about 10% that of an equivalent of biotin; −, no activity.

Biotinamide and N-biotinyl-*p*-aminobenzoic acid as demonstrated by the data of Table III differ from biocytin in being much more labile to acid hydrolysis. N-Biotinyl-β-alanine differs from biocytin as demonstrated by the data of Table IV in being much more readily extractable from aqueous solution with butanol.

Avidin combinability studies on biotinamide, N-biotinyl-*p*-aminobenzoic acid, N-biotinyl-L-glutamic acid, N-biotinyl-L-leucine and N-biotinyl-β-alanine as representative derivatives have given affinity ratios of 3–6 similar to that reported previously for biocytin.[3] Until the role of avidin is more clearly defined, the significance, if any, of these affinity ratios is obscure.

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TABLE III

ACID STABILITY OF BIOTIN DERIVATIVES

Compound	Per cent. availability to *Lactobacillus arabinosus* after hydrolysis at 120° for 1 hour with acid of the indicated normalities		
	0.2 *N*	1 *N*	3 *N*
Biocytin	43	91	100
N-Biotinyl-L-glutamic acid	19	69	100
N-Biotinyl-L-leucine	18	53	86
N-Biotinyl-β-alanine	26	65	100
N-Biotinyl-*p*-aminobenzoic acid	74	96	100
Biotinamide	92	99	100

TABLE IV

PARTITION OF BIOTIN AND BIOTIN DERIVATIVES BETWEEN BUTANOL AND 0.01 *N* HCl

Compound	Partition coefficient[a]
Biotin	3.0
Biocytin	0.15
Biotinyl-β-alanine	1.2

[a] Indicates solubility in butanol over solubility in aqueous phase.

Experimental

Microörganisms, Basal Media and Assay Methods.—The following species and strains were employed:

Escherichia coli M81-78 (obtained from Dr. B. D. Davis), *Lactobacillus acidophilus* (ATCC 4646), *Lactobacillus acidophilus* 05 (obtained from Dr. I. C. Gunsalus), *Lactobacillus acidophilus* (ATCC 314), *Lactobacillus acidophilus* (ATCC 8530), *Lactobacillus acidophilus* (ATCC 4357), *Lactobacillus acidophilus* K (obtained from Dr. I. C. Gunsalus), *Lactobacillus arabinosus* 17-5 (ATCC 8014), *Lactobacillus brevis* (ATCC 8287), *Lactobacillus casei* (ATCC 7469), *Lactobacillus delbrückii* LD 5[4] (ATCC 9595, obtained from Dr. David Hendlin), *Lactobacillus pentosus* (ATCC 8041), *Lactobacillus plantarum* (ATCC 4943), *Leuconostoc mesenteroides* P-60 (ATCC 8042), *Neurospora crassa* PABA-less[5] (ATCC 9278, obtained from Dr. H. K. Mitchell), *Penicillium chrysogenum* 62078[6] (obtained from Dr. H. K. Mitchell), *Propionibacterium shermanii* (ATCC 8262) and *Streptococcus fecalis* R (ATCC 8043).

The basal medium for studies with lactic acid bacteria was that previously described[7] supplemented with 5 γ of folic acid per 10 ml. where a requirement for this factor exists. When 24-hour assays with *Lactobacillus casei* were done the basal medium was supplemented further with 25 mg. of norite-treated tryptic digest of vitamin-free casein[8] per 10 ml. as a source of strepogenin. The basal medium described by Horowitz and Beadle[9] for the determination of choline was used in studies with *Neurospora crassa* PABA-less except that biotin was omitted and 250 γ of PABA per 25 ml. of medium was added. Czapek-Dox medium was used for *Penicillium chrysogenum*. A glucose–asparagine–inorganic salt medium[10] was used for *Escherichia coli*.

All determinations of microbiological activity with lactic acid bacteria and *Escherichia coli* have been read after 18–24 hours of incubation. With *Lactobacillus arabinosus* and *Lactobacillus casei* determinations also have been made after 72 hours of incubation. No increase in activity of those compounds that do not support growth of *Lactobacillus arabinosus* after 24 hours of incubation has been observed on increasing the incubation time to 72 hours. Determinations with *Neurospora crassa* and *Penicillium chrysogenum* were incubated for 4 days.

Studies of Acid Stability.—0.05 γ amounts of biotinamide, N-biotinyl-*p*-aminobenzoic acid, N-biotinyl-β-alanine, N-biotinyl-L-glutamic acid, N-biotinyl-L-leucine and biocytin in 1 ml. of water were autoclaved for 1 hour at 120° with 1-ml. portions of 0.4, 2.0, and 6.0 *N* H_2SO_4. Following hydrolysis the mixtures were neutralized, diluted and assayed for microbiological activity with *Lactobacillus arabinosus*. The hydrolyses were not all carried out at one time but repeated determinations on the acid stability of biotinamide and biocytin have demonstrated the reproducibility of the procedures. The results are summarized in Table III.

Butanol Partition Studies.—Solutions of biotin and N-biotinyl-β-alanine at a concentration of 5 γ per ml. and biocytin at a concentration of 0.05 γ per ml. in 0.01 *N* HCl were extracted once with equal volumes of water-saturated butanol. Aliquots of the biotin solution before and after extraction were assayed directly for biotin content with *Lactobacillus arabinosus*. Similar aliquots of the N-biotinyl-β-alanine and biocytin solutions before and after butanol extraction were autoclaved in 3 *N* H_2SO_4 for 1 hour at 120° prior to assay. The partition coefficients for the three compounds studied are summarized in Table IV.

Avidin Combinability Studies.—Avidin combinability studies were carried out as previously described[11,12] on biotinamide, N-biotinyl-*p*-aminobenzoic acid, N-biotinyl-L-glutamic acid, N-biotinyl-L-leucine and N-biotinyl-β-alanine. Affinity ratios varied from 3–6.

GLENOLDEN, PENNA. RECEIVED APRIL 16, 1951

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