958. *A Search for Biotin Antagonists and the Isolation of γ-Biotin.*

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Some *N*-substituted derivatives of biotin were synthesised in the hope that, because they could not form allophanate ions of the type (I; R = CO_2^-, R′ = R″ = H) which are probably essential for normal biochemical function, they might act as antimetabolites.

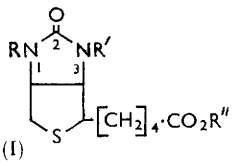
The isolation is reported of a new vitamin, designated γ-biotin, which is formulated as an *N*-phenylbiotin.

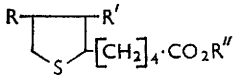
Since the determination of the structure of biotin * (I; R = R′ = R″ = H) by du Vigneaud [2] in 1942, various analogues [3] have been synthesised for evaluation as antagonists. The modifications examined, some of which inhibited reversibly the growth of biotin-dependent micro-organisms, have included variation of the length of the side chain, oxidation of the sulphide group to a sulphone, rupture of the tetrahydrothiophen ring by hydrogenolysis, and of the imidazolidone ring by hydrolysis. A possible basis, however, for a more rational design of antimetabolites of the vitamin can now be discerned in the recent studies of Lynen and his co-workers [4] on the reaction, CoA·S·CO·CH:CMe_2 ⟶ CoA·S·CO·CH:CMe·CH_2·CO_2H,† promoted by β-methylcrotonyl carboxylase derived from *Mycobacterium* and *Achromobacter* spp. They have presented evidence that biotin, probably attached to the enzyme by its carboxyl group, is the active portion which transports carbon dioxide by forming an allophanate-type ion (I; R = CO_2^-, R′ = R″ = H). With the mechanism of a mode of action (perhaps the general one) of the vitamin elucidated, it appears that the requirement for an effective antagonist is some modification of the imidazolidone system sufficient to prevent its reaction with carbon dioxide but not to diminish the affinity of the vitamin for the appropriate enzyme proteins.

* Originally designated β-biotin by Kögl and ten Ham.[1] † CoA·SH = Coenzyme-A.

[1] Kögl and ten Ham, *Naturwiss.*, 1943, **31**, 208.
[2] du Vigneaud, *Science*, 1942, **96**, 454.
[3] Cf. Woolley, "A Study of Antimetabolites," Chapman and Hall, London, 1952, p. 40.
[4] (*a*) Knappe, Schlegel, and Lynen, *Biochem. Z.*, 1961, **335**, 101; Lynen, Knappe, Lorch, Jüttung, Ringelmann, and Lachance, *ibid.*, p. 123; (*b*) Knappe, Ringelmann, and Lynen, *ibid.*, p. 168.

To achieve this object we sought first to substitute small inert groups, such as methyl, on the nitrogen atoms of biotin to obtain (I; R = R′ = Me, R″ = H). The diamine (II; R = R′ = NH_2, R″ = H) appeared a likely intermediate, for it is obtainable [5] from biotin in excellent yield and has therefore the natural *cis,cis*-configuration, an important factor as stereoisomers of the vitamin have lower activities.[6] We prepared the


(I)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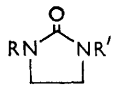
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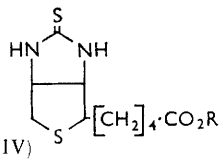
(IV)

NN′-dibenzylidene derivative (II; R = R′ = N:CHPh, R″ = Me) by the interaction of its methyl ester with benzaldehyde, but our subsequent efforts to prepare *NN*′-bis-metho-salts caused simultaneous methylation of the sulphide group which we could neither prevent nor, contrary to expectation,[7] reverse.

Model experiments for another approach had shown that *NN*′-ditoluene-*p*-sulphonylethylenediamine reacted readily with diazomethane to give the dimethyl derivative, acid hydrolysis [8,9] of which, followed by treatment with carbonyl chloride,[9] is known to give the 2-imidazolidone (III; R = R′ = Me). The compound (II; R = R′ = $NH{\cdot}SO_2{\cdot}C_6H_4Me$-*p*, R″ = H), obtainable only in erratic and usually poor yield, which was needed to reproduce the reaction sequence in the biotin series, reacted, however, neither with diazomethane nor with dimethyl sulphate under the mild conditions that had succeeded in the model series. This line was not pursued, for we had by this time reached our objective in a simpler way.

Despite reports [10] that methylation by the Eschweiler–Clarke procedure fails with compounds such as amides and ureas, in which strongly polar groups are attached to the nitrogen atom, we found in experiments with 2-imidazolidone that the 1,3-dimethyl derivative (III; R = R′ = Me) was in fact produced, albeit with much polymer arising doubtless from the intermediate bishydroxymethyl compound (III; R = R′ = $CH_2{\cdot}OH$). The parallel reaction between biotin and formaldehyde in the presence of formic acid gave 1,3-dimethylbiotin (I; R = R′ = Me, R″ = H) in moderate yield and its methyl ester (I; R = R′ = R″ = Me) was prepared from it by treatment with diazomethane.

In the absence of formic acid biotin reacted with two molecules of formaldehyde, to give the 1,3-bishydroxymethyl derivative (I; R = R′ = $CH_2{\cdot}OH$, R″ = H). An attempt to convert this into the bismethoxymethyl derivative (I; R = R′ = $CH_2{\cdot}OMe$, R″ = H) by treatment with methanolic hydrochloric acid, successful with imidazolidone models,[11] led to polymerisation.

1,3-Dibenzylbiotin [12] (I; R = R′ = CH_2Ph, R″ = H) was obtained with difficulty as an amorphous solid by hydrogenation of the above-mentioned *NN*′-dibenzylidene ester (II; R = R′ = N:CHPh, R″ = Me), followed by immediate treatment of the unstable crude intermediate (II; R = R′ = $NH{\cdot}CH_2Ph$, R″ = Me) with carbonyl chloride and hydrolysis of the resulting ester with alkali.

The reaction of biotin methyl ester with acetic anhydride gave a mono-*N*-acetyl derivative, considered to be (I; R = Ac, R′ = H, R″ = Me), because substitution is more

[5] Hofmann, Melville, and du Vigneaud, *J. Biol. Chem.*, 1941, **141**, 207.
[6] du Vigneaud, *Chem. Eng. News*, 1945, **23**, 623.
[7] Krollpfeiffer, Schneider, and Wissner, *Annalen*, 1950, **566**, 139; Krollpfeiffer and Hahn, *Chem. Ber.*, 1953, **86**, 1049.
[8] Meisenheimer, *Annalen*, 1924, **438**, 217.
[9] Boon, *J.*, 1947, 307.
[10] Moore, *Org. Reactions*, 1949, **5**, 301.
[11] Cf. Hoover and Vaala, U.S.P. 2,373,136.
[12] Cf. Goldberg and Sternbach, U.S.P. 2,489,235.

likely to have occurred at the less hindered side.[4b,13] When sodium acetate was added to the reaction mixture, the 1,3-diacetyl derivative (I; R = R′ = Ac, R″ = Me) resulted.

Treatment of biotin with nitrous acid under conditions analogous to those[14] which gave 1,3-dinitroso-2-imidazolidone (III; R = R′ = NO) from 2-imidazolidone yielded only the mononitroso-derivative, presumably (I; R = NO, R′ = R″ = H). Reduction of the product with zinc dust and sulphuric acid, followed by treatment with benzaldehyde, gave the benzylideneamino-compound (I; R = N:CHPh, R′ = R″ = H). In the model series used to elaborate this procedure 1-benzylideneamino-2-imidazolidone (III; R = N:CHPh, R′ = H) was synthesised.

To obtain the thione analogue (IV; R = H) of biotin, which we expected to differ significantly from the vitamin in its reactivity with carbon dioxide, we treated the diamino-acid (II; R = R′ = NH_2, R″ = H) with carbon disulphide in methanol, to give the thiocarbamic acid internal salt (II; R = NH·CS_2^-, R″ = $^+NH_3$). This, after acidification with hydrochloric acid, slowly lost hydrogen sulphide on being heated and formed the ester (IV; R = Me) from which the free acid (IV; R = H) was obtained on alkaline hydrolysis.

None of our derivatives in tests kindly conducted by Mr. A. W. Rule showed either bactericidal or bacteriostatic activity against *Staphylococcus aureus*, *Escherichia coli*, *Pseudomonas aeruginosa*, *Streptococcus pyogenes*, *Lactobacillus arabinosus*, *Saccharomyces cerevisiae*, or *Candida albicans*, growing in media containing graded amounts of biotin. The compounds were also examined for vitamin-like activity in biotin-free media. 1,3-Dimethylbiotin alone supported growth of *L. arabinosus*, its activity being about 90% of that of biotin (or equal to it on a molar basis). The last result is explicable if the bacillus is able to demethylate the derivative and thus regenerate the vitamin.

Mention is made finally of the isolation of a new member of the biotin family, here designated γ-biotin. When a commercial sample of crystalline biotin was hydrolysed with aqueous barium hydroxide, to give the diamino-acid (II; R = R′ = NH_2, R″ = H), 1% by weight of a crystalline substance was isolated from the reaction mixture. Its empirical formula $C_{16}H_{20}N_2O_3S$ differs from that of biotin by C_6H_4. The substance, which was soluble in aqueous sodium hydrogen carbonate and reprecipitated by dilute acid, showed an activity of about 60% of that of biotin (79% on a molar basis) when tested on *L. arabinosus* in biotin-free media. Its infrared spectrum differed from that of biotin in having bands at 725 and 697 $cm.^{-1}$ (monosubstituted benzene ring), reduced intensity of the band at 3333 $cm.^{-1}$ (NH stretching), and a shift of 56 $cm.^{-1}$ to lower frequency of the imidazolidone-carbonyl stretching band (here at 1639 $cm.^{-1}$). All these facts, together with the compound's relative resistance to hydrolysis (for it is only a minor constituent of the starting material), are consistent with its formulation as an *N*-phenylbiotin.

Experimental

Methyl δ-(3,4-Diaminotetrahydro-2-thienyl)valerate Dihydrochloride.—A solution of δ-(3,4-diaminotetrahydro-2-thienyl)valeric acid (1·74 g.) in anhydrous methanol (100 ml.) was saturated with hydrogen chloride. The mixture was refluxed for 2 hr., then evaporated to about 5 ml., treated with ether (10 ml.), and cooled to 0°, to give the *diamino-ester dihydrochloride* as needles (1·93 g., 80%), m. p. 205·25—205·75° (decomp.), $[\alpha]_D^{24}$ −13° (*c* 1 in H_2O) (Found: C, 39·1; H, 7·1. $C_{10}H_{22}Cl_2N_2O_2S$ requires C, 39·3; H, 7·3%).

Methyl δ-(3,4-Dibenzylideneaminotetrahydro-2-thienyl)valerate.—Methanolic 0·59N-sodium methoxide (17 ml., 0·01 mole) was added to a solution of the foregoing ester hydrochloride (1·53 g., 0·005 mole) in methanol (25 ml.), and the pH of the mixture was adjusted until a weakly alkaline reaction (pH ~9) was obtained on moist test paper. Benzaldehyde (1·06 g., 0·01 mole) was added and the mixture was heated under reflux for 6 hr. during which a little sodium chloride separated. This was filtered off and the filtrate was evaporated under reduced pressure.

[13] Traub, *Nature*, 1956, **178**, 649; *Science*, 1959, **129**, 210.
[14] McKay, Park, and Viron, *J. Amer. Chem. Soc.*, 1950, **72**, 3659.

The oily residue was treated with methanol (5 ml.) and water (5 ml.) and, on being scratched and cooled, afforded a white granular solid (1·69 g., 83%), m. p. 73·5—74·5°. A solution of the product in ether–light petroleum (b. p. 60—80°) was cooled in acetone–solid carbon dioxide to give the *Schiff's base* as prisms, m. p. 74·5—75·5° (Found: C, 70·5, 70·25; H, 6·9, 6·7; N, 7·0. $C_{24}H_{28}N_2O_2S$ requires C, 70·55; H, 6·9; N, 6·9%).

δ-(*Tetrahydro-3,4-ditoluene*-p-*sulphonamido-2-thienyl*)*valeric Acid*.—A solution of δ-(3,4-diaminotetrahydro-2-thienyl)valeric acid sulphate [5] (see under γ-biotin) (0·316 g., 0·001 mole) in N-sodium hydroxide (6 ml., 0·006 equiv.) was added slowly with stirring to a solution of toluene-*p*-sulphonyl chloride (0·4 g., 0·0021 mole) in ether (6 ml.) at 0°. Stirring was continued for 1 hr. at 0° and then at room temperature for a further 16 hr. The ethereal layer was separated and the aqueous layer was washed with ether (2 × 6 ml.) and acidified with N-hydrochloric acid. The milky mixture was extracted with chloroform (5 × 10 ml.) which on evaporation, finally under reduced pressure, gave a white friable solid (0·346 g.). The product was dissolved in methanol (3 ml.), and the filtered solution was treated with ethyl acetate (6 ml.) and evaporated to smaller volume. On being cooled, the solution deposited the *diamide monohydrate* as needles (0·197 g., 36%), m. p. 145—148°. Repeated crystallisation from methanol–ethyl acetate yielded the pure compound, m. p. 151—153°, $[\alpha]_D^{22}$ −65° (*c* 1 in satd. aq. $NaHCO_3$) (Found: C, 50·6; H, 5·85; N, 5·2. $C_{23}H_{30}N_2O_6S_3,H_2O$ requires C, 50·8; H, 5·9; N, 5·1%).

1,3-*Dimethyl*-2-*imidazolidone*.—A mixture of 2-imidazolidone (10·75 g., 0·125 mole), 40% aqueous formaldehyde (21 ml., 0·28 mole), and 90% w/w formic acid (64 g., 1·25 mole) was heated on a steam-bath for 48 hr. and then evaporated under reduced pressure. 5N-Sodium hydroxide (40 ml.) was added and the mixture was extracted with ether (5 × 50 ml.). Evaporation of the ethereal solution gave a yellow oil (4·19 g.) which was distilled under reduced pressure. The fraction of b. p. 105—105·5°/16 mm. (3·33 g.) gave an alkaline reaction with moist indicator paper. 2N-Hydrochloric acid (5 ml.) was added and extraction with ether (5 × 10 ml.) yielded 1,3-dimethyl-2-imidazolidone as a pale yellow oil (0·53 g.). A further quantity (2·00 g.; total crude yield 18%) was obtained by extraction of the aqueous layer with chloroform. The chloroform extract was distilled under reduced pressure, to give the pure compound (1·56 g.), b. p. 106—108°/17 mm. (Found: C, 52·6; H, 8·7; N, 24·3. Calc. for $C_5H_{10}N_2O$: C, 52·6; H, 8·8; N, 24·5%).

1,3-*Dimethylbiotin*.—A mixture of biotin (1·22 g., 0·005 mole), 37% w/w aqueous formaldehyde (0·96 g., 0·012 mole), and 90% w/w aqueous formic acid (3 g., 0·06 mole) was heated on a steam-bath for 40 hr., then evaporated under reduced pressure, to give a mixture of crystals and a pale yellow gum. A solution of the product in methanol–ethyl acetate, when cooled to 0°, gave prisms (0·924 g.), m. p. 165—170°. Recrystallisation from methanol gave pure 1,3-*dimethylbiotin* (0·459 g., 34%), m. p. 172—174°, $[\alpha]_D^{23.5}$ +46° (*c* 1 in 0·1N-NaOH) (Found: C, 53·0; H, 7·0; N, 10·2. $C_{12}H_{20}N_2O_3S$ requires C, 52·9; H, 7·4; N, 10·3%).

1,3-*Dimethylbiotin Methyl Ester*.—1,3-Dimethylbiotin (0·15 g.) in methanol (3 ml.) with an excess of ethereal diazomethane gave a product which in methanol–ether, cooled in acetone–solid carbon dioxide, yielded the *ester* as elongated prisms (0·091 g., 58%), m. p. 86·5—87·5° (Found: C, 54·3, 54·6; H, 7·7, 7·8; N, 10·0. $C_{13}H_{22}N_2O_3S$ requires C, 54·5; H, 7·7; N, 9·8%).

1,3-*Bishydroxymethylbiotin*.—Paraformaldehyde (0·15 g., equivalent to 0·005 mole of CH_2O) in methanol (5 ml.) containing sodium hydroxide (0·18 mg.) was added slowly with stirring to a solution (pH ~9) of biotin (0·488 g., 0·002 mole) in methanolic 0·59N-sodium methoxide (3·4 ml., 0·002 mole) at 50°. The mixture was kept at this temperature for 1 hr., then evaporated under reduced pressure. Acidification with N-hydrochloric acid of a filtered solution of the residue in water (2 ml.) afforded the *bishydroxymethyl derivative* (0·274 g., 45%), m. p. 140·5—141·5° on rapid heating but 171—179° on slow heating, $[\alpha]_D^{21}$ +95° (*c* 1 in satd. aq. $NaHCO_3$) (Found: C, 47·6; H, 6·4; N, 9·6. $C_{12}H_{20}N_2O_5S$ requires C, 47·4; H, 6·6; N, 9·2%).

1,3-*Dibenzylbiotin*.—δ-(3,4-Dibenzylideneaminotetrahydro-2-thienyl)valeric acid (0·6 g., 0·00147 mole) in methanol (55 ml.) was hydrogenated at room temperature over 10% palladium–charcoal (3 g.) (uptake complete in ~30 min.). The catalyst was filtered off and extracted with hot methanol (2 × 30 ml.), and the combined methanolic solutions were evaporated under reduced pressure to about 20 ml. 10% Aqueous potassium carbonate (20 ml.) was added and the mixture, cooled to 0°, was stirred whilst carbonyl chloride was passed through it until it became acid to Congo Red. The potassium chloride which had separated was filtered off and washed with a little methanol, and the filtrate was extracted with chloroform (3 × 25 ml.).

The chloroform solution was dried and evaporated, to give the crude 1,3-dibenzylbiotin methyl ester as a light brown oil (0·162 g., 25%).

The product was shaken overnight with N-sodium hydroxide solution (2 ml.), and the resulting solution was filtered and washed with ether (3 × 2 ml.). On acidification with 2N-hydrochloric acid (1 ml.) it gave a buff solid (0·065 g., 10% overall yield), m. p. 44—48°, which could not be crystallised. The *acid*, washed with water and dried (over P_2O_5), had $\nu_{max.}$ (homogeneous) 1700 (imidazolone-CO) and 755 and 700 cm.$^{-1}$ (monosubstituted benzene) (Found: N, 6·5. $C_{24}H_{28}N_2O_3S$ requires N, 6·6%).

1-*Acetylbiotin Methyl Ester*.—A mixture of biotin methyl ester (0·388 g.) and acetic anhydride (5 ml.) was heated under reflux for 2 hr. Most of the acetic anhydride was removed under reduced pressure, the residue was treated with water (6 ml.), and an excess of sodium hydrogen carbonate was added. The mixture was extracted with chloroform (5 × 10 ml.) and evaporation of the dried extract left an oil which was triturated with a little ether to give a white solid (0·269 g., 60%), m. p. 83—85°. Recrystallisation from ether–light petroleum (b. p. 60—80°) afforded the pure *acetyl derivative* (0·203 g., 45%), m. p. 85—85·5° (Found: C, 52·3; H, 6·8; N, 9·1. $C_{13}H_{20}N_2O_4S$ requires C, 52·0; H, 6·7; N, 9·3%).

1,3-*Diacetylbiotin Methyl Ester*.—Biotin methyl ester (0·39 g.), sodium acetate (1 g.), and acetic anhydride (7·5 ml.) were heated under reflux for 2 hr. The mixture was then poured into water (50 ml.) to give the crude product as a solid (0·34 g., 69%), m. p. 69—70°. Crystallisation from ether–light petroleum (b. p. 60—80°) gave the *diacetyl derivative* (0·218 g., 44%), m. p. 70—71° (Found: C, 52·6; H, 6·5; N, 8·1. $C_{15}H_{22}N_2O_5S$ requires C, 52·6; H, 6·5; N, 8·2%).

1-*Nitrosobiotin*.—A stirred suspension of biotin (0·122 g., 0·0005 mole) in 2N-hydrochloric acid (2 ml., 0·004 equiv.) was treated slowly at 0° with a solution of sodium nitrite (0·276 g., 0·004 mole) in water (1 ml.). Then stirring was continued for a further 2·5 hr., water (1 ml.) being added to ensure complete suspension of the solid. The product was collected, washed with water, and recrystallised from aqueous ethanol to give 1-*nitrosobiotin* as pale yellow prisms (0·091 g., 67%), m. p. 131·5° (decomp.) (Found: C, 44·05; H, 5·7; N, 15·25, 15·1. $C_{10}H_{15}N_3O_4S$ requires C, 43·9; H, 5·5; N, 15·4%).

1-*Benzylideneaminobiotin*.—A stirred suspension of 1-nitrosobiotin (0·273 g., 0·001 mole) in 2N-sulphuric acid (2·75 ml., 0·0055 equivalent) was cooled in ice during gradual addition of powdered zinc (0·164 g., 0·005 equiv.). Stirring was then continued at 0° for 30 min. and then at room temperature for 1 hr. The mixture was filtered and treated with benzaldehyde (0·106 g., 0·001 mole) in ethanol (1 ml.). On being cooled to 0° it deposited crystals (0·285 g., 82%), m. p. 187·5—190°, that recrystallised from methanol–benzene to give the pure *benzylideneamino-derivative* (0·18, g., 52%) m. p. 188·5—191·5° (Found: C, 58·55; H, 6·1; N, 12·3. $C_{17}H_{21}N_3O_3S$ requires C, 58·8; H, 6·1; N, 12·1%).

1-*Benzylideneamino-2-imidazolidone*.—Powdered zinc (1·635 g., 0·05 equiv.) was added during 30 min. to a stirred solution of 1-nitroso-2-imidazolidone [15] (1·15 g., 0·01 mole) in 2N-sulphuric acid (27·5 ml., 0·055 equiv.) at 0°. Then stirring was continued for 30 min. at 0° and for a further 90 min. at room temperature. The excess of zinc was filtered off and the filtrate was treated with a solution of benzaldehyde (1·06 g., 0·01 mole) in ethanol (10 ml.). On being cooled to 0° the mixture deposited needles (1·7 g., 90%), m. p. 197·5—202·5°. Recrystallisation from 95% ethanol gave the pure *benzylideneaminoimidazolidone* (1·19 g., 63%), m. p. 207—208° (Found: C, 63·5, 63·5; H, 6·0, 6·1; N, 22·05. $C_{10}H_{11}N_3O$ requires C, 63·5; H, 5·9; N, 22·2%). A further crop (0·136 g., 7%), m. p. 206—207°, was obtained on concentration of the mother-liquors.

2-*Thiobiotin Methyl Ester*.—A suspension of δ-(3,4-diaminotetrahydro-2-thienyl)valeric acid sulphate (0·632 g., 0·002 mole) in methanol (5 ml.) was treated with methanolic 3·17N-sodium methoxide (1·95 ml., 0·0062 equiv.). Carbon disulphide (5 ml.) was added and the mixture was refluxed for 2 hr. The excess of carbon disulphide was distilled off, 1·98N-hydrochloric acid (3·3 ml., 0·0065 equiv.) was added, and the mixture was again refluxed for 19 hr. Hydrogen sulphide was evolved and a white solid separated. The mixture was cooled to 0° and the crude *ester* (0·355 g., 65%), m. p. 213·5—215° (decomp.), was collected and washed with a little methanol. Recrystallisation from 95% ethanol afforded plates (0·282 g., 51%), m. p. 214·25—215·25° (decomp.) (Found: C, 47·6; H, 6·5; N, 9·9; S, 23·4. $C_{11}H_{18}N_2O_2S_2$ requires C, 48·15; H, 6·6; N, 10·2; S, 23·4%).

[15] Michels, U.S.P. 2,776,979.

2-*Thiobiotin*.—2N-Sodium hydroxide (5 ml., 0·01 equiv.) was added to the suspension of crude 2-thiobiotin methyl ester in hydrochloric acid, prepared from the diamino-acid (0·632 g., 0·002 mole) as described above, and after being shaken overnight the mixture was acidified at 0° with 2N-hydrochloric acid. The solid (0·179 g., 34%) which separated crystallised from aqueous ethanol, to give 2-thiobiotin as prisms (0·112 g., 22%), m. p. 234—235° (decomp.) (Found: C, 45·8; H, 6·0; N, 10·6. $C_{10}H_{16}N_2O_2S_2$ requires C, 46·1; H, 6·2; N, 10·8%).

γ-Biotin.—A mixture of biotin {m. p. 230° (decomp.), $[\alpha]_D^{22}$ +93° (*c* 1 in 0·1N-NaOH); 4·886 g., 0·02 mole}, barium hydroxide octahydrate (20 g., 0·0635 mole), and water (100 ml.) was heated at 150° for 21 hr., then cooled, saturated with carbon dioxide, filtered from barium carbonate, and made slightly acid to Congo Red with N-sulphuric acid; barium sulphate was filtered off. The filtrate was evaporated under reduced pressure to about 100 ml., some further solid, which separated, being removed. The filtrate was then further evaporated to about 50 ml. and treated with hot methanol (50 ml.). On being cooled to 0° the solution deposited crystals of δ-(3,4-diaminotetrahydro-2-thienyl)valeric acid sulphate (5·38 g., 85%), m. p. 247—247·5° (decomp.), $[\alpha]_D^{23}$ −15° (*c* 5 in H_2O).

The solid which had separated during concentration of the aqueous solution consisted of a mixture of small crystals of crude *γ-biotin* (0·049 g.), m. p. 176·5—179·5° (decomp.), and larger crystals of β-biotin (0·011 g.), m. p. and mixed m. p. 229—230·25° (decomp.). The γ-biotin being more readily suspended was filtered off first and the β-biotin settled on top of the filter cake with the aqueous washings from the flask. The two compounds were separated mechanically.

The γ-biotin recrystallised from aqueous methanol (charcoal) as needles, m. p. 183·25—184·25° (Found: C, 60·2; H, 6·5; N, 8·85; S, 9·9. $C_{16}H_{20}N_2O_3S$ requires C, 60·0; H, 6·3; N, 8·75; S, 10·0%).

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