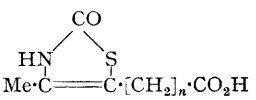
612. *Thiazole Analogues of Dethiobiotin.*

By G. Swain.

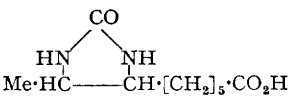
4-*Methyl*-5-ω-*carboxy*-n-*amylthiazol*-2-*one*, a compound structurally related to dethiobiotin, has been prepared by (*a*) reaction between potassium thiocyanate and 6-chloro-7-keto-octane-1-carboxylic acid and (*b*) a Friedel–Crafts reaction between 4-methylthiazol-2-one and ω-carbethoxyvaleryl chloride, followed by hydrolysis and reduction of the product. The synthesis of a number of related thiazoles is also described.

The synthesis of the compounds now reported was undertaken in search of antibacterial substances deriving activity from ability to antagonise the utilisation of biotin, essential for the growth of many micro-organisms. Interest was centred principally on 4-methyl-5-ω-carboxyalkylthiazol-2-ones (I), which show resemblance in molecular structure to dethiobiotin (II), a compound possessing growth-promoting properties for yeast, yet effectively inhibiting the growth *in vitro* of *Lactobacillus casei* by a competitive antagonism of biotin (cf. Dittmar, Melville, and du Vigneaud, *Science*, 1944, **99**, 203; Lilly and Leonian, *ibid.*, p. 205; Dittmar and du Vigneaud, *ibid.*, 1944, **100**, 129).

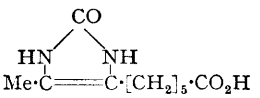
The unsaturated precursor (III) of dethiobiotin, more closely similar to (I) than is (II), was known to possess weak antibiotin activity in the *Lactobacillus casei* growth test (observation by Dr. Madinaveitia in these laboratories).



(I.)



(II.)



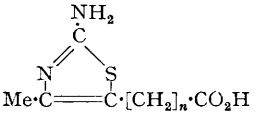
(III.)

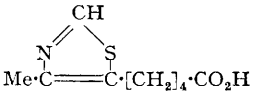
The acids (VI; $n = 4, 5$), obtained by chlorination and hydrolysis of the substituted acetoacetic esters (IV; R = CN or CO_2Et), reacted with potassium thiocyanate in weakly alkaline solution to give the thiazolones (I; $n = 4, 5$) directly, a method employed by Tscherniac (*J.*, 1919, **115**, 1071) for the preparation of 4-methylthiazol-2-one from chloroacetone. The yields in the final stage, however, were not good.

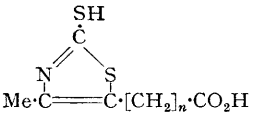
Reaction of (VI; $n = 3, 4, 5$) with thiourea and ammonium dithiocarbamate gave the corresponding 2-amino- and 2-mercapto-thiazoles respectively. Initial attempts to prepare (I) from the 2-mercaptothiazoles (IX; $n = 3, 4, 5$) by reaction with monochloroacetic acid in a manner analogous to the conversion of 2-mercaptopyrimidines into the corresponding 2-hydroxypyrimidines (Wheeler and Liddle, *Amer. Chem. J.*, 1908, **40**, 547) led only to formation of the carboxymethylthio-thiazoles (X; $n = 3, 4, 5$), which were resistant to hydrolysis by concentrated hydrochloric acid. Similarly unsuccessful was the attempted formation of 2-chloro-4-methyl-5-ω-carboxy-*n*-butylthiazole. Diazotisation of (VII; $n = 4$) and treatment

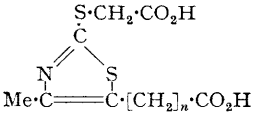
with copper–hydrochloric acid (cf. Erlenmeyer, Buchmann, and Schenkel, *Helv. Chim. Acta*, 1944, **27**, 1432) yielded 4-*methyl*-5-ω-*carboxy*-n-*butylthiazole* (VIII).

$CH_3{\cdot}CO{\cdot}CH(CO_2Et){\cdot}[CH_2]_n{\cdot}R$ (IV.) $CH_3{\cdot}CO{\cdot}C(CO_2Et)Cl{\cdot}[CH_2]_n{\cdot}R$ (V.) $CH_3{\cdot}CO{\cdot}CHCl{\cdot}[CH_2]_n{\cdot}CO_2H$ (VI.)

(VII.) 

(VIII.) 

(IX.) 

(X.) 

Of the substituted 4-methylthiazolones that with the *n*-hexoic acid side chain (I; $n = 5$) appeared to be of most interest and, in addition to the synthesis already mentioned, it has been prepared by a Friedel–Crafts reaction between 4-methylthiazol-2-one and ω-carbethoxy-*n*-valeryl chloride followed by hydrolysis and reduction of the resulting 4-*methyl*-5-ω-*carbethoxy*-n-*valerylthiazol*-2-*one*. Ochiai and Nagasawa (*Ber.*, 1939, **72**, 1470) showed that the 4-methyl group alone was insufficient to activate the thiazole nucleus for acylation with acetyl chloride to occur (at $C_{(5)}$), but that the presence of a 2-keto-group as in 4-methylthiazol-2-one rendered acylation possible, a fact which Duschinsky and Dolan (*J. Amer. Chem. Soc.*, 1945, **67**, 2079) applied to their analogous reaction of 2-keto-4-methyl-2 : 3-dihydroglyoxaline with ω-carbethoxy-*n*-valeryl chloride for the preparation of DL-dethiobiotin.

None of the compounds (I; $n = 4$, 5), (VII; $n = 3$, 4, 5), (VIII), (IX; $n = 3$, 4, 5), or (X; $n = 3$, 4, 5) inhibited growth *in vitro* of *Lactobacillus casei*, *Streptococcus pyogenes*, *Staphylococcus aureus*, *Bacterium coli*, or *Pyocyaneus pyocyanea* at a concentration of 1/1000.

Since the conclusion of this work Cook, Heilbron, and Stern (*J.*, 1948, 2031) have reported the synthesis of three 4-amino-2-mercapto-thiazoles and two derived Schiff's bases which bear some resemblance to dethiobiotin. These compounds were likewise devoid of anti-biotin activity for the growth of *L. casei* or *S. cerevisiae*.

Experimental.

(All m. p.s are uncorrected.)

Ethyl 3-*Chloro*-2-*keto*-6-*cyanohexane*-3-*carboxylate* (V; R = CN, $n = 3$).—Ethyl 2-keto-6-cyanohexane-3-carboxylate (19·7 g.) (Derick and Hess, *J. Amer. Chem. Soc.*, 1918, **40**, 548) in dry benzene (100 c.c.) was treated at 0—5° with sulphuryl chloride (15 g., 1·1 moles) added dropwise with stirring during 15 minutes. After a further 15 minutes the mixture was heated under reflux for ½ hour. Benzene was removed under reduced pressure, and the residual oil distilled off. *Ethyl* 3-*chloro*-2-*keto*-6-*cyanohexane*-1-*carboxylate* collected as a colourless oil (20·3 g., 87%), b. p. 130—134°/0·5—0·1 mm. (Found : C, 51·2; H, 5·9; N, 6·9; Cl, 16·1. $C_{10}H_{14}O_3NCl$ requires C, 51·8; H, 6·05; N, 6·05; Cl, 15·3%).

4-*Chloro*-5-*ketohexane*-1-*carboxylic Acid* (VI; $n = 3$).—Ethyl 3-chloro-2-keto-6-cyanohexane-3-carboxylate (20 g.) was heated under reflux for 3½ hours with acetic acid (30 c.c.) and concentrated hydrochloric acid (40 c.c.). The solution was diluted with water (200 c.c.), and the precipitated oil extracted with ether. The ethereal solution was washed with water and then exhaustively extracted with sodium hydrogen carbonate solution. The combined extracts were acidified with hydrochloric acid, and the oil which separated was extracted with ether and dried (Na_2SO_4). Removal of the ether and distillation of the residual oil *in vacuo* gave 4-*chloro*-5-*ketohexane*-1-*carboxylic acid* as a colourless oil (71·1 g., 46%), b. p. 116—118°/0·08 mm. (Found : C, 46·95; H, 6·0; Cl, 19·8. $C_7H_{11}O_3Cl$ requires C, 47·1; H, 6·2; Cl, 19·9%).

Ethyl 2-*Keto*-7-*cyanoheptane*-3-*carboxylate* (IV; R = CN, $n = 4$).—Ethyl acetoacetate (72 g., 1·5 moles) was added slowly with cooling to a solution of sodium (8·5 g.) in ethyl alcohol (100 c.c.). 1-Bromo-4-cyanobutane (60 g., 1·0 mole) was added, and the mixture heated under reflux for 7 hours. Sodium bromide was filtered off and the filtrate distilled under reduced pressure. The fraction boiling up to 130° at 15 mm. was rejected and the remaining oil distilled at 0·5—1·0 mm. The fraction, b. p. 125—165°, was refractionated to give *ethyl* 2-*keto*-7-*cyanoheptane*-3-*carboxylate* (44·5 g., 57%), b. p. 126—128°/0·6—0·7 mm. (Found : C, 62·6; H, 8·05; N, 8·0. $C_{11}H_{17}O_3N$ requires C, 62·6; H, 8·06; N, 6·6%).

Ethyl 3-*Chloro*-2-*keto*-7-*cyanoheptane*-3-*carboxylate* (V; R = CN, $n = 4$).—Prepared in the same manner as was the lower homologue (V; R = CN, $n = 3$), *ethyl* 3-*chloro*-2-*keto*-7-*cyanoheptane*-3-*carboxylate* was obtained as a colourless oil (yield, 81·6%), b. p. 136—138°/0·05 mm. (Found : C, 53·15; H, 6·4; N, 6·45; Cl, 15·1. $C_{11}H_{16}O_3NCl$ requires C, 53·8; H, 6·5; N, 5·7; Cl, 14·45%).

5-*Chloro*-6-*ketoheptane*-1-*carboxylic Acid* (VI; $n = 4$).—This *acid*, prepared in the same way as the lower homologue (VI; $n = 3$), was obtained as a colourless oil (yield, 64%), b. p. 126—128°/0·05—0·06 mm. (Found : C, 50·1; H, 6·65; Cl, 17·8. $C_8H_{13}O_3Cl$ requires C, 49·9; H, 6·75; Cl, 18·4%).

4-*Methyl*-5-ω-*carboxy*-n-*butylthiazol*-2-*one* (I; $n = 4$).—5-Chloro-6-ketoheptane-1-carboxylic acid

(9·6 g.) was added dropwise during ½ hour to a well stirred solution of potassium thiocyanate (5·0 g.) and sodium hydrogen carbonate (7·3 g.) in water (150 c.c.), cooled to below 10°. After being stirred below 10° for 8 hours the mixture was kept at room temperature for 1 week. Acidification with hydrochloric acid caused separation of an oil which rapidly solidified. The solid (3·3 g.; m. p. 132—134°) was filtered off, washed, and crystallised from water. 4-*Methyl*-5-*ω*-*carboxy*-n-*butylthiazol*-2-*one* was obtained in long colourless needles (2·9 g.), m. p. 135° (Found : C, 50·4; H, 6·05; N, 6·7; S, 15·2. $C_9H_{13}O_3NS$ requires C, 50·2; H, 6·05; N, 6·5; S, 14·9%).

4-*Methyl*-5-*ω*-*carboxy*-n-*amylthiazol*-2-*one* (I; $n = 5$).—(*a*) Prepared in a similar manner from 6-chloro-7-keto-octane-1-carboxylic acid (10·3 g.; Swain, *J.*, 1948, 1552), this *thiazolone* separated from water in colourless plates (1·2 g., 10·5%), m. p. 128—129° (Found : C, 52·25; H, 6·4; N, 6·1. $C_{10}H_{15}O_3NS$ requires C, 52·4; H, 6·55; N, 6·1%). (*b*) Bromoacetone (170 g.) was added during 1 hour to a stirred solution of potassium thiocyanate (155 g.) and sodium hydrogen carbonate (84 g.) in water (2 l.), the temperature being kept below 10°. After being stirred for a further 48 hours at room temperture the solution was decanted from separated tar and extracted with ethyl acetate (6 × 150 c.c.). The extract was dried (Na_2SO_4) and distilled. The oil (63 g.) remaining after removal of the ethyl acetate crystallised on cooling and was purified by distillation. The fraction, b. p. 120—125°/0·1 mm. (46 g., 32%), consisted of pure 4-methylthiazol-2-one, and on cooling crystallised to a pale yellow solid, m. p. 101—102° (Tscherniac, *loc. cit.*, gives m. p. 102—103°). Powdered aluminium chloride (26 g., 2 moles) was added during 10 minutes in small amounts at room temperature to a stirred mixture of 4-methylthiazol-2-one (11·5 g.), *ω*-carbethoxy-*n*-valeryl chloride (22 g., 1·2 mole) (Blaise and Koehler, *Bull. Soc. chim.*, 1910, **7**, 219), and tetrachloroethane (120 c.c.; redistilled). The mixture was stirred and heated in an oil-bath at 100—110° (bath temp.) for 5 hours, and the resulting dark brown viscous liquid was poured into ice–water (250 c.c.) and stirred for 1 hour. The tetrachloroethane was separated, washed with water, and steam-distilled. The residual oil was dissolved in ether (400 c.c.) and the ethereal solution decanted from a little insoluble tar, washed with sodium hydrogen carbonate solution, and dried (Na_2SO_4), and the ether removed. The viscous brown oil (11·0 g.) obtained was distilled and the fraction, b. p. 220—230°/0·06—0·07 mm. (5·9 g.), collected. The pale yellow, viscous oil crystallised slowly and on recrystallisation from benzene–light petroleum (b. p. 60—80°) (1 : 1) gave 4-*methyl*-5-*ω*-*carbethoxy*-n-*valerylthiazol*-2-*one* in colourless leaflets (3·3 g.), m. p. 61—62° (Found : C, 52·15; H, 6·35; N, 5·3; S, 11·9. $C_{12}H_{17}O_4NS$ requires C, 53·1; H, 6·3; N, 5·2; S, 11·8%). The 2 : 4-*dinitrophenylhydrazone* crystallised from ethyl alcohol in small red prisms, m. p. 188—190° (decomp.) with sintering at 174—176° (Found : C, 47·95; H, 4·7; N, 15·8. $C_{18}H_{21}O_7N_5S$ requires C, 47·9; H, 4·7; N, 15·5%). In a second experiment the crude oily ester (11·0 g.), obtained from the above Friedel–Crafts reaction by ether extraction, was hydrolysed without further purification by heating at 60° for 1 hour with N-sodium hydroxide solution (88 c.c.). The dark solution was extracted once with ether and then neutralised with N-hydrochloric acid. The oil which separated solidified slowly and was recrystallised from water (charcoal). 4-*Methyl*-5-*ω*-*carboxy*-n-*valerylthiazol*-2-*one* separated in small colourless prisms (2·3 g.), m. p. 154—155° (Found : C, 49·0; H, 5·4; N, 5·8; S, 12·9. $C_{10}H_{13}O_4NS$ requires C, 49·4; H, 5·35; N, 5·8; S, 13·2%).

Reduction. 4-Methyl-5-*ω*-carboxy-*n*-valerylthiazol-2-one (2·4 g.) was heated under reflux for 4½ hours with 2N-hydrochloric acid (120 c.c.) and granulated zinc (10 g.) [previously amalgamated by treatment with mercuric chloride solution (50 c.c.; 3%) for 1 hour]. Further additions of concentrated hydrochloric acid (10 c.c.) were made after 1½ hours and 2½ hours. The hot solution was filtered and on cooling deposited 4-methyl-5-*ω*-carboxy-*n*-amylthiazol-2-one (0·7 g.; m. p. 128°). When recrystallised from aqueous ethyl alcohol this separated in colourless plates, m. p. 128—129°, identical with the product obtained, as already described, from 6-chloro-7-keto-octane-1-carboxylic acid and potassium thiocyanate.

2-*Amino*-4-*methyl*-5-*ω*-*carboxy*-n-*propylthiazole* (VII; $n = 3$).—4-Chloro-5-ketohexane-1-carboxylic acid (6·4 g.) dissolved in ethyl alcohol (6 c.c.) was added to a solution of thiourea (2·5 g.) in water (10 c.c.) and heated on the steam-bath for 2 hours. The solution was diluted with water (75 c.c.) and made alkaline with ammonia; a small amount of oil which separated was removed by extraction with ether, and the clear filtrate concentrated under reduced pressure. The solid which separated (3·4 g.) was filtered off and recrystallised from hot water. 2-*Amino*-4-*methyl*-5-*ω*-*carboxy*-n-*propylthiazole* was obtained in light buff needles (2·7 g., 38%), m. p. 200—202° (decomp.) with sintering at 180°. The crystals became opaque when dried at 100° (Found : C, 48·25; H, 6·1; N, 13·9; S, 16·4. $C_8H_{12}O_2N_2S$ requires C, 48·0; H, 6·0; N, 14·0; S, 16·0%). 2-*Amino*-4-*methyl*-5-*ω*-*carboxy*-n-*butylthiazole* (VII; $n = 4$) was prepared in a similar manner from 5-chloro-6-ketoheptane-1-carboxylic acid (9·7 g.) and thiourea (2·5 g.) and separated from 50% ethyl alcohol in small cream needles, m. p. 207—208° (Found : C, 49·9; H, 6·65; N, 12·75; S, 14·9. $C_9H_{14}O_2N_2S$ requires C, 50·5; H, 6·5; N, 13·1; S, 14·95%). 2-*Amino*-4-*methyl*-5-*ω*-*carboxy*-n-*amylthiazole* (VII; $n = 5$), obtained from 6-chloro-7-keto-octane-1-carboxylic acid (7·0 g.; Swain, *loc. cit.*) and thiourea (2·5 g.), separated from water in colourless needles (2·7 g.), m. p. 183—185°, with sintering at 175° (Found : C, 51·65; H, 7·05; N, 11·9; S, 14·4. $C_{10}H_{16}O_2N_2S$ requires C, 52·6; H, 7·0; N, 12·3; S, 14·0%).

4-*Methyl*-5-*ω*-*carboxy*-n-*butylthiazole* (VIII).—2-Amino-4-methyl-5-*ω*-carboxy-*n*-butylthiazole (4·0 g.) dissolved in phosphoric acid (25 c.c.; *d* 1·7) was cooled to −10° and nitric acid (6 c.c.; *d* 1·4) was added. Sodium nitrite solution (2·5 g. in 5 c.c. water) was added, through a capilliary below the surface, during 15 minutes at −5° with stirring. After being stirred for 1 hour the diazo-solution was added to a mixture of Gattermann copper (prepared from 12 g. of copper sulphate and 2·4 g. of zinc dust) and hydrochloric acid (70 c.c.; *d* 1·18). After the solution had been stirred for 1½ hours water (300 c.c.) was added followed by anhydrous sodium carbonate (95 g.). Insoluble copper compounds were filtered off and the filtrate acidified with acetic acid. The slightly oily precipitate so obtained was extracted with ether, the ether solution was dried, the solvent was removed by distillation, and the oily solid residue (4·3 g.) was crystallised from aqueous alcohol. 4-*Methyl*-5-*ω*-*carboxy*-n-*butylthiazole* separated in colourless prisms (0·9 g.), m. p. 108—109° (Found : C, 54·3; H, 6·45; N, 6·2. $C_9H_{13}O_2NS$ requires C, 54·3; H, 6·5; N, 7·0%).

2-*Mercapto*-4-*methyl*-5-*ω*-*carboxy*-n-*propylthiazole* (IX; $n = 3$).—Ammonium dithiocarbamate (4·5 g., 1·3 moles) was added to a solution of 4-chloro-5-ketohexane-1-carboxylic acid (5·4 g.) in alcohol (50 c.c.). After 15 minutes at room temperature with occasional shaking the mixture was heated under reflux on the steam-bath for 1 hour, and most of the alcohol then distilled off. Water (30 c.c.) was added and the solution acidified with acetic acid. An oil separated and rapidly solidified. The solid (4·7 g.) was filtered off and crystallised from hot water; 2-*mercapto*-4-*methyl*-5-*ω*-*carboxy*-n-*propylthiazole* separated in long flat, yellow needles (3·5 g.), m. p. 139° (Found: C, 44·7; H, 5·5; N, 7·5; S, 29·8. $C_8H_{11}O_2NS_2$ requires C, 44·2; H, 5·1; N, 6·45; S, 29·5%). The following compounds were prepared in a similar manner: 2-*mercapto*-4-*methyl*-5-*ω*-*carboxy*-n-*butylthiazole* (IX; $n = 4$), pale yellow flat needles (from water), m. p. 171—172° (Found: C 46·6; H, 5·6; N, 6·7; S, 28·2. $C_9H_{13}O_2NS_2$ requires C, 46·8; H, 5·6; N, 6·1; S, 27·7%); and 2-*mercapto*-4-*methyl*-5-*ω*-*carboxy*-n-*amylthiazole* (IX; $n = 5$), pale yellow flat needles (from water), m. p. 135—136° (Found: C, 49·4; H, 5·8; N, 6·25; S, 26·6. $C_{10}H_{15}O_2NS_2$ requires C, 49·0; H, 6·1; N, 5·7; S, 26·1%).

2-*Carboxymethylthio*-4-*methyl*-5-*ω*-*carboxy*-n-*propylthiazole* (X; $n = 3$).—2-Mercapto-4-methyl-5-*ω*-carboxy-*n*-propylthiazole (0·8 g.), monochloroacetic acid (0·8 g.), and water (10 c.c.) were heated under reflux for 3 hours. After neutralisation of the cooled solution with ammonia an oil separated and rapidly solidified. The solid (0·85 g.) was recrystallised from water; 2-*carboxymethylthio*-4-*methyl*-5-*ω*-*carboxy*-n-*propylthiazole* separated in colourless needles, m. p. 113—114° (Found: C, 43·9; H, 4·95; N, 4·8; S, 23·6. $C_{10}H_{13}O_4NS_2$ requires C, 43·6; H, 4·7; N, 5·1; S, 23·3%). The following compounds were prepared by the same method: 2-*carboxymethylthio*-4-*methyl*-5-*ω*-*carboxy*-n-*butylthiazole* (X, $n = 4$), colourless prisms, m. p. 87—88°, from water (Found: C, 45·45; H, 5·15; N, 5·15; S, 22·0. $C_{11}H_{15}O_4NS_2$ requires C, 45·7; H, 5·2; N, 4·8; S, 22·15%); and 2-*carboxymethylthio*-4-*methyl*-5-*ω*-*carboxy*-n-*pentylthiazole* (X; $n = 5$), colourless flat needles, m. p. 89—90°, from water (Found: C, 47·55; H, 5·65; N, 5·65; S, 21·2. $C_{12}H_{17}O_4NS_2$ requires C, 47·5; H, 5·6; N, 4·6; S, 21·1%).

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