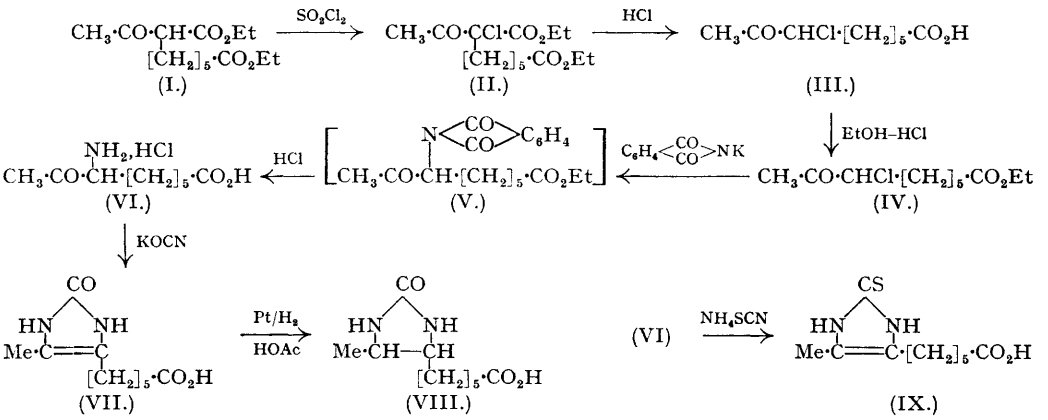
312. *A Synthesis of* DL-*Dethiobiotin and Some Related Compounds.*

By G. SWAIN.

An alternative synthesis of 6-amino-octan-7-one-1-carboxylic acid hydrochloride (VI) affords a modified route to DL-dethiobiotin (VIII). The synthesis of DL-5-(p-*carboxyhexahydrobenzyl*)-4-*methyliminazolid*-2-*one* (XVI), an analogue of dethiobiotin, is described.

SYNTHESES of DL-dethiobiotin (VIII) have been reported by Wood and du Vigneaud (*J. Amer. Chem. Soc.*, 1945, **67**, 210), McKennis and du Vigneaud (*ibid.*, 1946, **68**, 832), Bourquin, Schnider, and Grüssner (*Helv. Chim. Acta*, 1945, **28**, 528), and Duschinsky and Dolan (*J. Amer. Chem. Soc.*, 1945, **67**, 2079). Except the last, in which the pentamethylenecarboxylic acid side chain is introduced into a preformed iminazolone nucleus, all the syntheses proceed through formation of 6-amino-octan-7-one-1-carboxylic acid (VI), or an ester thereof, and differ in the synthetic approach to this compound.

During the course of work directed to the preparation of compounds structurally similar to dethiobiotin in the hope of obtaining biotin antagonists, it has been found that this intermediate amino-ketocarboxylic acid can be prepared through the following reaction sequence :



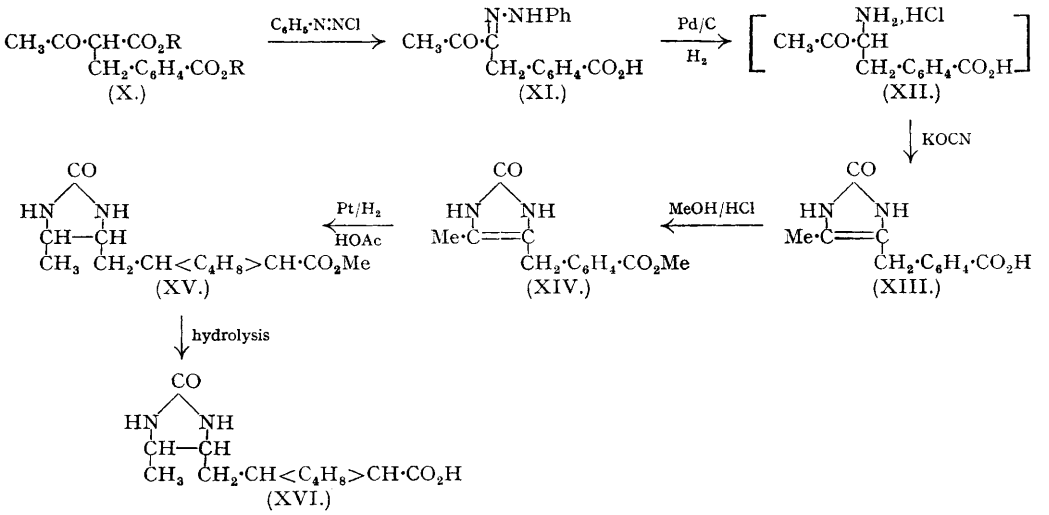
Although it was not possible to isolate the phthalimido-compound (V), yet hydrolysis of the crude reaction product gave (VI), which was readily converted into 4-methyl-5-(ω-carboxy-*n*-pentyl)iminazol-2-one (VII) and the corresponding *thione* (IX) by treatment with potassium cyanate and ammonium thiocyanate, respectively. Hydrogenation of (VII) in acetic acid solution with Adams's platinum catalyst at room temperature and pressure yielded DL-dethiobiotin (VIII) identical with an authentic specimen. Only one isomer was obtained, probably that with the *cis*-configuration at C_4–C_5, since the method of hydrogenation employed is known to favour *cis*-addition to $-C{=}C-$ linkages (cf. Duschinsky and Dolan, *loc. cit.*).

The synthesis of DL-5-(p-*carboxyhexahydrobenzyl*)-4-*methyliminazol*-2-*one* (XVI), a compound closely related to dethiobiotin, but bearing a *cyclo*alkylenecarboxylic acid side chain in place of the *n*-pentylcarboxylic acid side chain present in dethiobiotin, was commenced by a route similar to that described above. Difficulties in the initial stages, however, led to the abandonment of this synthesis in favour of one corresponding to that described by Bourquin, Schnider, and Grüssner (*loc. cit.*) for dethiobiotin, involving the following stages (all substituents in the benzene ring being in the *p*-position) (see p. 1553).

The final hydrogenation with Adams's platinum catalyst in acetic acid solution at room temperature and pressure resulted in absorption of 4 moles of hydrogen and gave rise to a product which was apparently homogeneous. The method of hydrogenation would here again favour the *cis*-configuration in both ring systems.

The compounds (VII), (VIII), (IX), and (XVI) were tested by Dr. J. Madinaveitia for growth-inhibitory activity against *Lactobacillus casei* " in vitro " ; the last two had no inhibitory effect at a concentration of 1/1000. The inhibitory action of DL-dethiobiotin (VIII) (cf. Dittmar, Melville, and du Vigneaud, *Science*, 1944, **99**, 203; Lilly and Leonian, *ibid.*, p. 205; Dittmar and du Vigneaud, *ibid.*, 1944, **100**, 129) was confirmed, and its precursor (VII) shown to possess a

feeble inhibitory action. The inhibition produced by both (VII) and (VIII) was completely reversed by added biotin.



Experimental.

(All melting points uncorrected.)

Ethyl α-Chloro-α-acetylsuberate (II).—Ethyl α-acetylsuberate (27·2 g.; Bourquin, Schnider, and Grüssner, *loc. cit.*) in dry benzene (100 c.c.) was cooled to below 5°, and sulphuryl chloride (15 g., redistilled) added dropwise with stirring during 15 minutes. After a further 15 minutes the temperature was raised slowly to the refluxing point and the mixture maintained under reflux for 15 minutes. Benzene was removed under reduced pressure, and the remaining oil distilled. The fraction, b. p. 134—136°/0·03—0·04 mm. (27·6 g.; 90%), consisted of pure *ethyl α-chloro-α-acetylsuberate* (Found: C, 54·6; H, 7·0; Cl, 12·2. $C_{14}H_{23}O_5Cl$ requires C, 54·8; H, 7·5; Cl, 11·6%).

6-*Chloro-octan-7-one-1-carboxylic Acid* (III).—The foregoing ester (13·0 g.), glacial acetic acid (20 c.c.), hydrochloric acid (20 c.c., *d* 1·18), and water (5 c.c.) were refluxed for 3 hours, poured into water, and the oil taken up in ether. The ethereal solution was extracted several times with N-sodium carbonate solution. The combined sodium carbonate washings were acidified with hydrochloric acid, and the oil again extracted with ether, washed with water, and dried (Na_2SO_4). Removal of the ether left an oil (9·5 g.) which was distilled. 6-*Chloro-octan-7-one-1-carboxylic acid* was obtained as a colourless oil (7·2 g.; 82%), b. p. 128—134°/0·03—0·04 mm. (Found: C, 52·35; H, 7·0; Cl, 16·3. $C_9H_{15}O_3Cl$ requires C, 52·3; H, 7·3; Cl, 17·2%). The *ethyl* ester (IV), obtained by refluxing the acid (85 g., crude) for 6 hours with absolute ethyl alcohol (350 c.c.) containing dry hydrogen chloride (14 g.), was a pale yellow oil (60 g.), b. p. 116—119°/0·15—0·18 mm. (Found: C, 55·5; H, 7·65; Cl, 14·6. $C_{11}H_{19}O_3Cl$ requires C, 56·3; H, 8·1; Cl, 15·1%).

6-*Amino-octan-7-one-1-carboxylic Acid Hydrochloride* (VI).—The above ethyl ester (5·2 g.), potassium phthalimide (3·6 g.), and xylene (25 c.c.) were stirred under reflux in an oil-bath at 160—165° for 20 hours. The insoluble solid, which remained after dilution with benzene, was filtered off, and the filtrate evaporated under reduced pressure. The residual brown oil was refluxed for 12 hours with concentrated hydrochloric acid (50 c.c.). On cooling, the impure phthalic acid (2·3 g.) which separated was filtered off, and the filtrate evaporated under reduced pressure at 50°. The semi-solid residue (3·8 g.) was dissolved in water (25 c.c.) and filtered through charcoal to remove a little insoluble oil. The clear pale yellow filtrate was again evaporated under reduced pressure at 50°. The crude *hydrochloride* remained as a pale yellow, rather waxy, crystalline residue (3·0 g.; 48%) and was used without further purification for the reaction described below. Recrystallisation from *iso*propyl alcohol was effected with considerable loss to give colourless needles (0·9 g.), m. p. 123—124° (decomp.) (Found: C, 48·65; H, 8·15; N, 6·55. $C_9H_{18}O_3NCl$ requires C, 48·3; H, 8·05; N, 6·3%).

4-*Methyl-5-(ω-carboxy-*n*-pentyl)iminazol-2-one* (VII).—Potassium cyanate (2·0 g.) was stirred into a solution of the foregoing hydrochloride (3·0 g.) in water (35 c.c.). A crystalline solid separated rapidly, and after 1 hour hydrochloric acid (10 c.c., *d* 1·18) was added. After standing for 16 hours the suspended iminazolone was collected and washed with water. It was obtained in colourless needles (1·2 g.; 42%), m. p. 163—165° (decomp.) unchanged after recrystallisation from water or ethyl alcohol–ethyl acetate. (Bourquin, Schnider, and Grüssner, *loc. cit.*, give m. p. 167—169°, decomp.; Wood and du Vigneaud, *loc. cit.*, m. p. 168°, decomp.; Duschinsky and Dolan, *loc. cit.*, m. p. 160—170°.) The product slowly decomposes on storage, or on prolonged heating with solvents, becoming brown and resinous. With ferric chloride it gave a maroon and with bromine in carbon tetrachloride a purple colour (Found: C, 56·2; H, 7·1; N, 13·15. Calc. for $C_{10}H_{16}O_3N_2$: C, 56·6; H, 7·55; N, 13·2%).

4-*Methyl-5-(ω-carboxy-*n*-pentyl)iminazole-2-thione* (IX).—6-Amino-octan-7-one-1-carboxylic acid hydrochloride (0·8 g., recrystallised), dissolved in water (5 c.c.) mixed with ammonium thiocyanate (0·8 g.), was evaporated to dryness on the steam-bath during ¾ hour. Water (7 c.c.) was added twice

to the residue and the mixture evaporated to dryness after each addition. 2N-Hydrochloric acid (6 c.c.) was added, and the mixture set aside for 24 hours. The solid in suspension was collected and washed with water (m. p. 183—185°, 0·55 g., 69%). Recrystallisation from water yielded the *thione* in small colourless prisms, m. p. 184°, undepressed in admixture with a specimen prepared from 6-amino-octan-7-one-1-carboxylic acid hydrochloride obtained by Bourquino, Schnider, and Grüssner's synthesis (*loc cit.*) (Found : C, 53·05; H, 6·65; N, 12·0; S, 13·9. $C_{10}H_{16}O_2N_2S$ requires C, 52·6; H, 7·0; N, 12·3; S, 14·0%).

DL-*Dethiobiotin* (VIII).—4-Methyl-5-(ω-carboxy-*n*-pentyl)iminazol-2-one (400 mg.), dissolved in acetic acid (20 c.c., distilled over chromic acid), was shaken in hydrogen at room temperature and pressure with freshly prepared Adams's platinum catalyst (400 mg.). Reduction was complete in 20 hours. The catalyst was filtered off, and the acetic acid removed under reduced pressure from a water-bath at 45°. The waxy solid residue was crystallised from hot water. DL-Dethiobiotin separated in small rosettes of prisms (250 mg.; 62·5%), m. p. 157—158°, undepressed in admixture with an authentic specimen, m. p. 159—160° (obtained from Messrs. Hofmann La Roche) (Wood and du Vigneaud, *loc. cit.*, give m. p. 157—159°; Bourquin, Schnider, and Grüssner, *loc. cit.*, m. p. 154—155° sintering at 146°, for one isomer obtained from the mother-liquors of the main product, which melted at 134—135°, clear at 145°; Duschinsky and Dolan, *loc. cit.*, give m. p. 162·5—163°) (Found : C, 55·85; H, 8·5; N, 13·0. Calc. for $C_{10}H_{18}O_3N_2$: C, 56·1; H, 8·4; N, 13·1%).

Ethyl p-*Chloromethylbenzoate.*—*p*-Chloromethylbenzoic acid (59 g.; Barkenbus and Holtzclaw, *J. Amer. Chem. Soc.*, 1925, **47**, 2191) was refluxed for 7 hours with alcoholic hydrochloric acid (600 c.c., 2·3%). Excess of alcohol was removed in a vacuum, and the ester isolated in the usual manner. It was obtained as a colourless oil (57 g.; 72·5%), b. p. 146—148°/17 mm. (Einhorn and Papastavros, *Annalen*, 1899, **310**, 205, give b. p. 260—280°).

Ethyl p-*carbethoxybenzylacetoacetate* (X ; R = Et). Acetoacetic ester (26 g., redistilled) was added to a solution of sodium (2·3 g.) in absolute ethyl alcohol (40 c.c.). Ethyl *p*-chloromethylbenzoate (20 g.) was added, and the mixture refluxed on the steam-bath for 8 hours. After cooling, it was poured into water, and the oil taken up in ether, washed with water, and dried (Na_2SO_4). Removal of the ether left an oil (38 g.) which was distilled under reduced pressure. The fraction, b. p. 146—148°/0·07 mm. (21·4 g.; 73%), consisted of pure *ethyl* p-*carbethoxybenzylacetoacetate* (Found : C, 65·95; H, 6·95. $C_{16}H_{20}O_5$ requires C, 65·76; H, 6·85%).

1-(p-*Carboxyphenyl*)-n-*butane*-2 : 3-*dione* 2-*Phenylhydrazone* (XI).—The above ester (X ; R = Et) (21·9 g.) was added to a solution of potassium hydroxide (9·0 g. ; 2·1 equivs.) in water (300 c.c.) cooled to 0—2°. After it had been stirred at this temperature for 24 hours the solution was neutralised (litmus) by addition of N-hydrochloric acid (3·5 c.c.), and sodium acetate solution (26 g. trihydrate in 100 c.c. water) was added, followed by benzenediazonium chloride solution [aniline (7 c.c.) dissolved in 3·75 N-hydrochloric acid (60 c.c.) cooled by addition of ice (80 g.) and diazotised by running in sodium nitrite (5·5 g.) in water (25 c.c.) during 10 minutes ; stirred for a further 15 minutes before use]. A buff-coloured solid separated slowly (some frothing). After being stirred in ice for 6 hours, the mixture was allowed to attain room temperature and stirred for 12 hours. The orange-brown solid (18·0 g.) was filtered off, washed with water, and dried at 70°. It crystallised from aqueous ethyl alcohol in yellow prisms (14·1 g. ; 63·5%), m. p. 219—220° (decomp., shrinks at 190°). Further crystallisation from ethyl alcohol gave the pure *phenylhydrazone* as yellow prisms, m. p. 222—223° (decomp.) (Found : C, 69·1 ; H, 5·6 ; N, 9·55. $C_{17}H_{16}O_3N_2$ requires C, 68·9 ; H, 5·4 ; N, 9·5%).

5-(p-*Carboxybenzyl*)-4-*methyliminazol*-2-*one* (XIII).—The above phenylhydrazone (5·9 g., m. p. 219—220°) was suspended in a mixture of methyl alcohol (80 c.c.) and N-hydrochloric acid (42 c.c., 2·05 equivs.) and shaken at room temperature and pressure with palladium–charcoal (1 g., 5%) in an atmosphere of hydrogen. Absorption was complete in 12 hours. The catalyst was filtered off, and the solvent removed under reduced pressure at 30°. The solid residue was dissolved in water (60 c.c.), filtered from traces of insoluble material, and sodium acetate (5·5 g., trihydrate) added to the clear solution. Aniline was removed by extraction with ether. The aqueous solution was freed from ether by warming to 30° under reduced pressure and treated with solid potassium cyanate (2·0 g.). Solid separated slowly and after standing overnight was collected and washed with water (m. p. 330—332°, decomp., shrinks and commences to decompose at 315°) ; yield 2·5 g. (54%). Crystallisation from aqueous acetic acid (50%) afforded 5-(p-*carboxybenzyl*)-4-*methyliminazol*-2-*one* in very small colourless needles, m. p. 336—338° (decomp ; shrinks and commences to decompose at 315°) (Found : N, 11·9. $C_{12}H_{12}O_3N_2$ requires N, 12·1%). The *methyl* ester, prepared by keeping a solution in 5% methanolic hydrochloric acid for 4 days at room temperature, crystallised from methanol in small colourless needles, m. p. 256—258°, with sintering and some decomposition at 240° (Found : C, 63·05; H, 5·7; N, 11·3. $C_{13}H_{14}O_3N_2$ requires C, 63·4; H, 5·7; N, 11·4%).

DL-5-(p-*Carboxyhexahydrobenzyl*)-4-*methyliminazolid*-2-*one* (XVI).—The above methyl ester (1·0 g.) was dissolved in glacial acetic acid (20 c.c.) and shaken with hydrogen at room temperature and pressure in the presence of freshly reduced Adams's platinum catalyst (0·7 g.). Reduction was complete in 3—4 hours (absorption : 335 c.c. at N.T.P. Calc. for $4H_2$, 364 c.c.). The solution was filtered from catalyst, and the acetic acid removed under reduced pressure. The residual oil did not crystallise readily and was hydrolysed by heating at 60—70° for 1 hour with methyl alcohol (10 c.c.) and N-sodium hydroxide solution (16·2 c.c.). Acidification with N-hydrochloric acid (16·2 c.c.) caused separation of the free *acid* as an oil, which crystallised very slowly in colourless prisms (0·55 g.), m. p. 188—190° (sintering at 183—185°). Recrystallisation from water (40 c.c.) yielded nodules of small colourless prisms, m. p. 188—190° (sinter 185°, slightly opalescent melt clearing at about 210°) (Found : C, 59·6; H, 8·15; N, 12·05. $C_{12}H_{20}O_3N_2$ requires C, 60·0; H, 8·3; N, 11·7%).

IMPERIAL CHEMICAL INDUSTRIES LIMITED, RESEARCH LABORATORIES,
HEXAGON HOUSE, BLACKLEY, MANCHESTER, 9.

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