Studies on Thiophene Compounds. XIX. Syntheses of 2, 3, 4, 5-Tetradehydrobiotin and 2, 3, 4, 5-Tetradehydronorbiotin[1]

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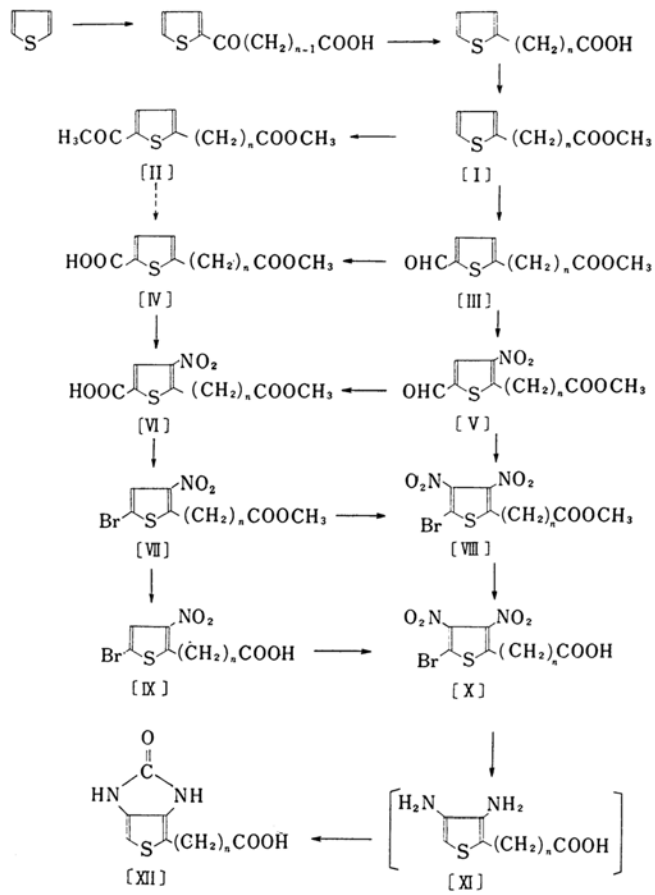
Biotin is a very important growth-promoting factor for yeast and other micro-organisms. Since Harris[2] and his co-workers first synthesized biotin, various methods have been reported by many authors. However, all of the methods proposed required many steps for the synthesis, and consequently the over-all yield was low. According to the patent[3], biotin can be prepared with the hydrogenation of 2, 3, 4, 5-tetradehydrobiotin (or so-called aromatic biotin). In the present paper are reported the syntheses of aromatic biotin and of aromatic norbiotin, which has one less methylene group in the side chain than does aromatic biotin. Both of the compounds were synthesized by starting with thiophene. The following procedures were followed.

Results and Discussion

In the preparation of the aromatic biotin and the aromatic norbiotin, it was most difficult to introduce two amino groups into the 3- and 4-positions of the thiophene nucleus. It has been known that the nitration of 2, 5-dibromo-, 2, 5-dichloro- and 2, 5-dimethyl-thiophene gives the 3, 4-dinitro-derivatives of the respective starting compounds[4-6]. An attempt was thus made to introduce a nitro group into

1) Presented at the Meeting of the Society of Organic Synthetic Chemistry (Japan), Tokyo, Oct., 1959.
2) S. A. Harris et al., *Science*, **97**, 447 (1943).
3) U. S. Pat. 2502422; *Chem. Abstr.*, **44**, 6440 (1950).
4) R. Mozingo et al., *J. Am. Chem. Soc.*, **67**, 2092 (1945).
5) W. Steinkopf et al., *Ann.*, **512**, 136 (1934).
6) W. Steinkopf et al.. ibid., **536**, 128 (1938).



(n=3 or 4)

the vacant 4-position of the thiophene nucleus of 4-(5-bromo-2-thienyl)-butyric acid, but the result was unsuccessful. Nitration under severe conditions caused an oxidative decomposition and gave merely tarry material. No nitrated product was obtained from 5-bromo-2-methylthiophene either. As has been mentioned in another paper[7] of this series, ω-(5-bromo-2-thenoyl)-fatty acid can be nitrated at the 4-position. It must, therefore, be easier to introduce a nitro group into the 4-position if an electron-releasing amino group is placed in the neighboring 3-position. However, the nitration of 3-(3-acetylamino-5-bromo-2-thenoyl)-propionic acid unexpectedly gave 3-acetylamino-5-bromo-2nitro-thiophene, which was produced as the result of the replacement of the succinoyl group by the nitro group in the 2-position. From this experimental result, it could be deduced that the 3-acetylamino group can not promote an electrophilic substitution at the 4-position. As the third method to introduce an amino group into a β-position of the thiophene nucleus, there is the amination of β-halogenothiophenes, in which method the halogene atom is activated by a nitro or a carboxyl group. According to this scheme, an amination of 3-bromo-4-nitro-2-thiophenecarboxylic acid was carried out by the method mentioned in an earlier paper[8] of this series, but no aminated product was obtained. In general, a nitro group can be introduced into the 3-position of the thiophene nucleus when the thiophene nucleus has an electron-releasing group such as chloro or alkyl group in the 2-position and an electron-withdrawing group such as formyl or acetyl group in the 5-position. It is, however, impossible to introduce an additional nitro group to the 4-position after the first nitration has been carried out. As reported in a previous paper[9] of this series, it is necessary to put an electron-releasing group in the 5-position in order to introduce the second nitro group, which has to be

7) S. Nishimura and E. Imoto, *J. Chem. Soc. Japan, Pure Chem. Sec.* (*Nippon Kagaku Zasshi*), **82**, 1413 (1961).

8) S. Nishimura, T. Kitagawa and E. Imoto, ibid., **82**, 1543 (1961).

9) S. Nishimura, A. Sakumoto and E. Imoto, ibid., **82**, 1540 (1961).

neighbored by the first nitro group at the 3-position. The formyl or acetyl group in the 5-position, which is necessary for the introduction of the first nitro group, can be converted to a bromo group by Hunsdiecker's reaction after its oxidation to the carboxylic acid. As the aromatic biotin and the aromatic norbiotin have a ω-caboxybutyl and a ω-carboxypropyl group respectively, the free terminal carboxyl group may simultaneously be replaced by a bromo group by Hunsdiecker's reaction. To avoid this complication, it is necessary to protect the carboxyl group by a method such as esterification. According to this scheme, methyl 4-(5-acety-2-thienyl)-butyrate was oxidized with sodium hypochlorite with the intention of preparing methyl 4-(5-carboxy-2-thienyl)-butyrate, but the oxidation yielded dicarboxylic acid, which was produced by the simultaneous saponification of the terminal methyl ester. Even isopropyl 4-(5-acetyl-2-thienyl)-butyrate, which is expected to resist saponification, was useless for the purpose of oxidation without saponification. This means that alkaline oxidation can not prevent simultaneous saponification. As an alternative method, methyl 4-(5-formy-2-thienyl)-butyrate, prepared by the formylation of methyl 4-(2-thienyl)-butyrate, was oxidized by chromic acid in acetic acid, and thus methyl 4-(5-carboxy-2-thienyl)-butyrate was obtained. Although methyl 4-(5-carboxy-3-nitro-2-thienyl)-butyrate was prepared with the nitration of methyl 4-(5-carboxy-2-thienyl)-butyrate, the oxidation of methyl 4-(5-acetyl-3-nitro-2-thienyl)-butyrate prepared by the nitration of methyl 4-(5-acetyl-2-thienyl)-butyrate gave a more favorable result. Similarly, methyl 5-(5-carboxy-3-nitro-2-thienyl)-valerate was obtained by the oxidation of methyl 5-(5-formyl-3-nitro-2-thienyl)-valerate in acetic acid. The carboxyl group thus obtained was converted to the bromine atom by Hunsdiecker's reaction. Then, 4-(5-bromo-3, 4-dinitro-2-thienyl)-butyric and -valeric acids were derived through the nitration of methyl ω-(5-bromo-3-nitro-2-thienyl)-butyrate and -valerate respectively, followed by the saponification of the terminal methyl ester. When ω-(5-bromo-3, 4-dinitro-2-thienyl)-butyric and -valeric acids were treated with tin and hydrochloric acid, 3, 4-dinitro groups were reduced to 3, 4-diamino groups and the 5-bromo atom was eliminated by reduction. As 3, 4-diaminothiophenes are unstable in an atmosphere of air, the reduced products were not isolated, and the double salts of the diamino-compounds with tin chloride were treated with phosgene after decomposition with an alkaline solution to obtain the aromatic biotin and the aromatic norbiotin respectively. The aromatic biotin obtained here was identical to that obtained by Cheney[10] and his co-workers by another method.

Experimental

4-(5-Bromo-2-thienyl)-butyric Acid.—Into a solution of 4-(2-thienyl)-butyric acid[11] (6 g.) in 50% aqueous acetic acid (65 ml.) was slowly stirred, drop by drop, a solution of bromine (1.85 ml.) in 50% aqueous acetic acid (47 ml.) at $-8\sim-10$°C over a period of 2 hr. After the addition was completed, the reaction mixture was stirred at the same temperature. The crystals which were precipitated from the reaction mixture by the addition of water (100 ml.) were collected by filtration, washed with water and recrystallized from methanol-water. The yield of 4-(5-bromo-2-thienyl)-butyric acid (m. p. 58$\sim$59.5°C) was 56% after recrystallization.

Found: Br, 31.45; Neut. equiv., 250. Calcd. for $C_8H_9O_2BrS$: Br, 32.08%; Neut. equiv., 249.

Nitration of 4-(5-Bromo-2-thienyl)-butyric Acid. — Into a solution of 4-(5-bromo-2-thienyl)-butyric acid (2.5 g.) in acetic anhydride (10 ml.) was slowly stirred mixture of concentrated nitric acid (2 ml.), acetic anhydride (10 ml.) and acetic acid (10 ml.) at $-5\sim-10$°C. The reaction mixture was stirred for 30 min. at the same temperature after the additon and then poured into ice-water. An oily product was separated and solidified after the mixture had been left to stand for several days. The solid collected, m. p. 56$\sim$58°C after recrystallization from methanol-water, did not show a depression of the melting point on mixing with the starting compound. A nitration of 4-(5-bromo-2-thienyl)-butyric acid with a mixed acid consisting of sulfuric acid and nitric acid at 0$\sim$5°C also gave merely a brown tarry matter.

Nitration of 2-Bromo-5-methylthiophene. — A nitration of 2-bromo-5-methylthiophene[12] with a mixed acid consisting of concentrated nitric acid and concentrated sulfuric acid merely gave a brown tarry matter; no crystalline products were separated.

Nitration of 4-Acetylamino-2-bromo-5-thenoyl-propionic Acid.—4-Acetylamino-2-bromo-5-thenoyl-propionic acid[13] (0.32 g.) was stirred into sulfuric acid (2 ml.) which had been greatly cooled by the addition of pieces of dry ice. Into the above viscous solution was stirred dropwise a mixed acid consisting of concentrated nitric acid (0.17 ml.) and concentrated sulfuric acid (0.1 ml.) at a temperature below -20°C. The reaction temperature was kept constant by the frequent addition of small pieces of dry-ice. After adding the mixed acid, the reaction mixture was stirred for one hour and then diluted with iced water. The precipitates were collected by filtration, washed with water and recrystallized from methanol. The pale yellow needles obtained melted at 174$\sim$175°C, produced no 2,4-dinitrophenylhydrazone, and must be 4-acetylamino-2-bromo-5-nitrothiophene.

10) L. C. Cheney and J. R. Piening, *J. Am. Chem. Soc.*, **67**, 731 (1945).
11) L. F. Fieser and R. G. Kennelly, ibid., **57**, 1611 (1935).
12) K. Dittmer, ibid., **71**, 1201 (1949).
13) R. Motoyama, *J. Chem. Soc. Japan, Pure Chem. Sec.* (*Nippon Kagaku Zasshi*), **78**, 794 (1957).

Found: C, 26.65; H, 2.09; N, 10.11. Calcd. for $C_6H_5O_3BrN_2S$: C, 27.1; H, 1.89; N, 10.05%.

Oxidation of Methyl 4-(5-Acetyl-2-thienyl)-butyrate with Sodium Hypobromite. — A solution of sodium hypobromite was prepared by stirring 5 ml. of bromine into a solution of sodium hydroxide (10 g.) in water (15 ml.) and ice (40 g.) below 0°C. Methyl 4-(5-acetyl-2-thienyl)-butyrate (4 g.) was added to the solution of sodium hypobromite prepaped above and stirred for 5 hr. at 5~10°C. An aqueous solution of sodium bisulfite was added under ice-cooling to the reaction mixture to decompose an excess of sodium hypobromite. After removing the bromoform and carbon tetrabromide by an extraction with ether, the alkaline aqueous layer was acidified. The colorlesss precipitates (1.6 g.) were collected by filtration, washed with water and recrystallized from water, m. p. 192~194°C. The neutralization equivalent of the crystals was 110, which coincided with the calculated value (107) for 4-(5-carboxy-2-thienyl-butyric acid. The mixed melting of the crystals with the authentic sample, which had been synthesized by the oxidation of 4-(5-acetyl-2-thienyl)-butyric acid (m. p. 193~194.5°C), showed no depression.

Isopropyl 4-(2-Thienyl)-butyrate.—A solution of 50 g. of 4-(2-thienyl)-butyric acid in 80 ml. of ether was refluxed, together with 30 ml. of thionyl chloride and few drops of pyridine, for 4 hr. Ether and an excess of thionyl chloride were distilled off in vacuo. The residue was dissolved in a solution of dimethylaniline (40 g.) and isopropyl alcohol (20 g.) and refluxed for 4 hr. After cooling, the reaction mixture was poured into diluted hydrochloric acid and extracted with ether. The ethereal extract was washed successively once each with diluted hydrochloric acid and with an aqueous solution of sodium bicarbonate, and then dried over anhydrous sodium sulfate. After distilling off the ether, the distillation under reduced pressure gave 52 g. of isopropyl 4-(2-thienyl)-butyrate, b. p. 140~149°C.

Found: C, 61.88; H, 7.38. Calcd. for $C_{11}H_{16}O_2S$: C, 62.26; H, 7.55%.

Isopropyl 4-(2-Acetyl-5-thienyl)-butyrate.—Twenty grams of isopropyl 4-(2-thienyl)-butyrate were heated and stirred with acetic anhydride (11 ml.) and phosphoric acid (0.8 ml.) for 4 hr. at 100~110°C. After cooling, the reaction mixture was poured into water, neutralized with powdered sodium carbonate, and extracted with ether. The ether portion was washed with water and dried over anhydrous sodium sulfate. After distilling off the ether, the distillation under reduced pressure gave 20.5 g. of isopropyl 4-(2-acetyl-5-thienyl)-butyrate (b. p. 185~188°C/3.5 mmHg), which solidified after cooling with dry-ice. After the recrystallization from petroleum ether, crystals of m. p. 15~16.5°C were obtained.

Found: C, 61.38; H, 7.10. Calcd. for $C_{13}H_{18}O_3S$: C, 61.42; H, 7.08%.

Oxidation of Isopropyl 4-(2-Acetyl-5-thienyl)-butyrate with Sodium Hypobromite.—To a solution of sodium hypobromite, which had been prepared from sodium hdydroxide (20 g.), water (7 ml.) and bromine (2.4 ml.) by the method similar to that mentioned in the case of (II (n=3)), a solution of isopropyl 4-(2-acetyl-5-thienyl)-butyrate (4 g.) in dioxane (6 ml.) was added and stirred below 10°C for 3 hr. After treating the reaction mixture by a usual method, 1.7 g. of crude acid (m. p. 186~193°C) were obtained and recrystallized from water, m. p. 192~194°C. The mixed melting point with an authentic sample of 4-(2-carboxy-5-thienyl)-butyric acid showed no depression.

Methyl 4-(5-Formyl-2-thienyl)-butyrate (III (n=3)). — A solution of methyl 4-(2-thienyl)-butyrate (4.3 g.) in dimethylformamide (2.5 g.) was slowly combined with oxyphosphorus chloride (2.5 g.) and kept for 2 hr. at 110°C while frequency being shaken. The reaction mixture was poured into water, neutralized with sodium carbonate and extracted with ether. The ethereal extract was washed with water and dried over anhydrous sodium sulfate. After distilling off the ether, the residue was distilled under reduced pressure to obtain methyl 4-(5-formyl-2-thienyl)-butyrate in a yield of 67.5% (3.5 g.), b. p. 172~175°C.

Found: C, 62.18; H, 7.50. Calcd. for $C_{10}H_{12}O_3S$: C, 62.26; H, 7.55%.

Methyl 4-(5-Carboxy-2-thienyl)-butyrate (IV (n=3)).—A mixture of methyl 4-(5-formyl-2-thienyl)-butyrate (III (n=3)) (1.2 g.), acetic acid (10 ml.) and sodium bichromate (1.5 g.) was stirred at 60~70°C for 7 hr. After cooling, cold water and 5 g. of sodium carbonate were added to the reaction mixture to precipitate green crystals. The crystals were extracted with ether. The ether extract was was washed with water and treated with a saturated aqueous solution of sodium bicarbonate. The aqueous portion was acidified with hydrochloric acid, and the colorless precipitates obtained were colleted by filtration and washed with water, m. p. 79.5~81°C. This acid was methyl 4-(5-carboxy-2-thienyl)-butyrate, as is shown in the following analytical results.

Found: Neut. equiv., 226; Sapon. equiv., 112. Calcd. for $C_{10}H_{12}O_4S$: Neut. equiv., 228; Sapon. equiv., 114.

Methyl 4-(5-Formyl-3-nitro-2-thienyl)-butyrate (V (n=3)).—Methyl 4-(5-formyl-2-thienyl)-butyrate (6 g.) was dissolved in ice-cooled concentrated sulfuric acid (30 ml.). Into this solution was slowly stirred the mixed acid consisting of concentrated nitric acid (8.7 ml.) and concentrated sulfuric acid (30 ml.), while the reaction temperature was kept below −5°C by means of the frequent addition of small pieces of dry ice. After this addition, the reaction mixture was stirred for 15 min. at the same temperature and then poured into ice water to afford the precipitations of crude methyl 4-(5-formyl-3-nitro-2-thienyl)-butyrate (7.2 g.). The precipitates obtained were recrystallized from ligroin, m. p. 70.5~72.5°C. Yield, 82%.

Found: N, 5.48. Calcd. for $C_{10}H_{11}O_5NS$: N, 5.44%.

2,4-Dinitrophenylhydrazone: m. p. 202~203.5°C (recrystallized from methanol).

Found: N, 10.49. Calcd. for $C_{16}H_{15}O_8N_3S$: N, 10.27%.

Methyl 4-(5-Carboxy-3-nitro-2-thienyl)-butyrate (VI (n=3)).—A mixture of methyl 4-(5-formyl-3-nitro-2-thienyl)-butyrate (7.5 g.), acetic acid (60 ml.) and sodium bichromate (10 g.) was stirred at 60~

70°C for 6.5 hr. and then poured into iced water to precipitate pale green crystals. After adding 30 g. of sodium carbonate slowly under ice-cooling, the reaction mixture was extracted with ether, and the ether portion was washed with water and shaken with a cold aqueous saturated solution of sodium bicarbonate. Acidification of the alkaline solution afforded a precipitation of methyl 4-(5-carboxy-3-nitro-2-thienyl)-butyrate, m. p. 95~96.5°C. Yield, 4.45 g. (56%).

Found: N, 5.23; Neut. equiv., 276. Calcd. for $C_{10}H_{11}O_6NS$: N, 5.13%; Neut. equiv., 273.

Other Method. — 1) Into a solution of methyl 4-(5-formyl-3-nitro-2-thieny)-butyrate (4 g.) in acetone (200 ml.) was stirred potassium permanganate (4.5 g.) in small portions at 0~5°C. After the addition was completed, stirring was continued at the same temperature for two hours. To the reaction mixture was added 10 ml. of methanol, and it was again stirred for ten minutes. Acetone and methanol were distilled off under reduced pressure, and to the residue was added a cold aqueous saturated solution of sodium bicarbonate. The precipitates were removed by filtration, and the filtrate was acidified with hydrochloric acid under ice-cooling to afford precipitation of crystal (m. p. 92~94°C). The mixed melting point of the crystals with the 4-(5-carboxy-3-nitro-2-thienyl)-butyrate obtained above showed no depresssion. The yield was 55%.

2) A mixed acid consisting of 1.4 ml. of concentrated nitric acid and 5 ml. of concentrated sulfuric acid was slowly stirred at −5~0°C into a soluion of methyl 4-(5-carboxy-2-thienyl)-butyrate (1 g.) in concentrated sulfuric acid (200 ml.). After this addition, the reaction mixture was stirred for 15 min. at the same temperature and then poured into iced water to cause the precipitation of viscous matter. The viscous matter which solidified after standing was dissolved in ether. The ethereal solution was extracted with a cold aqueous saturated solution of sodium bicarbonate; then the alkaline solution was acidified with hydrochloric acid to precipitate crystals of m. p. 92~95°C. The precipitates showed no depression of the melting point on mixing the methyl 4-(5-carboxy-3-nitro-2-thienyl)-butyrate obtained above. The yield was 33%.

Methyl 5-(2-Thienyl)-valerate (I (n=4)). — A solution of 5-(2-thienyl)-valeric acid[14] (100 g.) in 500 ml. of methanol was saturated with dry hydrochloric acid and refluxed for 7 hr. After distilling off the methanol, the residue was poured into iced water and then extracted with ether. The ether extract was washed with water and dried over anhydrous sodium sulfate. After distilling off the ether, the residue was distilled under reduced pressure to give methyl 5-(2-thienyl)-valerate in a yield of 85.5% (92 g.), b. p. 139~143°C/13 mmHg.

Found: C, 66.02; H, 7.81. Calcd. for $C_{10}H_{14}O_2S$: C, 66.66; H, 7.77%.

Methyl 5-(5-Formyl-2-thienyl)-valerate (III (n=4)).—A formylation of methyl 5-(2-thienyl)-valerate similar to that of methyl 4-(2-thienyl)-butyrate gave methyl 5-(5-formyl-2-thienyl)-valerate in a yield of 79%, b. p. 182~186°C/6 mmHg, m. p. 63~64°C.

Found: C, 58.16; H, 6.15. Calcd. for $C_{11}H_{14}O_3S$: C, 58.41; H, 6.19%.

Methyl 5-(5-Formyl-3-nitro-2-thienyl)-valerate (V (n=4)).—A nitration of methyl 5-(5-formyl-2-thienyl)-valerate similar to that of methyl 5-(5-formyl-2-thienyl)-butyrate gave methyl 5-(5-formyl-3-nitro-2-thienyl)-valerate as an oily product in a yield of 91.7%.

Methyl 5-(5-Carboxy-3-nitro-2-thienyl)-valerate (VI (n=4)).—This compound was obtained with an oxidation of methyl 5-(5-formyl-3-nitro-2-thienyl)-valerate with sodium bichromate in a yield of 59%, or with potassium permanganate in a yield of 76%, when the treatment was similar to that of methyl 4-(5-formyl-3-nitro-2-thienyl)-butyrate. The melting point of the crystals obtained was 37~38.5°C.

Found: N, 4.84; Neut. equiv., 288. Calcd. for $C_{11}H_{13}O_6NS$: N, 4.88%; Neut. equiv., 287.

Methyl 4-(5-Bromo-3-nitro-2-thienyl)-butyrate (VII (n=3)).—To an exactly neutralized solution of methyl 4-(5-carboxy-3-nitro-2-thienyl)-butyrate (2.5 g.) with a 1% aqueous solution of sodium hydroxide was added 1 N silver nitrate to precipitate colorless silver salt. The silver salt was collected by filtration, washed with water, dried at 100~110°C, powdered and then completely dried again over phosphorus pentoxide in a vacuum desiccator. To a refluxing suspension of the silver salt in dry carbon tetrachloride (25 ml.) was slowly added bromine (0.46 g.) in carbon tetrachloride (5 ml.). The addition required 30 min. After the addition, refluxing was continued for 1 hr. The reaction mixtue was filtered while it is hot and the solid obtained was washed twice with 140 ml. of hot carbon tetrachloride. The combined filtrate and washed liquor was washed successively with a solution of sodium bisulfite and a cold solution of sodium bicarbonate and then with water and dried over anhydrous sodium sulfate. Distilling off the carbon tetrachloride yielded a solid material, which was purified by washing with petroleum ether. The yield of methyl 4-(5-bromo-3-nitro-2-thienyl)-butyrate was 82% (2.32 g.). After recrystallization from petroleum ether, the melting point of the crystals was 49~51°C.

Found: N, 4.52. Calcd. for $C_9H_{10}O_4BrNS$: N, 4.54%.

Methyl 5-(5-Bromo-3-nitro-2-thienyl)-valerate (VII (n=4)). — Methyl 5-(5-carboxy-3-nitro-2-thienyl)-valerate (5 g.) was treated by the same procedure as described above to prepare methyl 5-(5-bromo-3-nitro-2-thienyl)-valerate (3 g.) in a yield of 53.5%. The product was an oily matter.

5-(5-Bromo-3-nitro-2-thienyl)-valeric Acid (IX (n=4)). — A solution of methyl 5-(5-bromo-3-nitro-2-thienyl)-valerate (5 g.) in acetic acid (80 ml.) and concentrated hydrochloric acid 50 ml.) was refluxed for one hour, filtered while hot by adding active charcoal, then diluted with water to precipitate crystals of 5-(5-bromo-3-nitro-2-thienyl)-valeric acid in a yield of 94% (4.5 g.), m. p. 99~102°C. The crystals were recrystallized from acetic acid-water, m. p. 102~103.5°C.

Found: N, 4.56; Neut. equiv., 307. Calcd. for $C_9H_{10}O_4BrNS$: N, 4.54%; Neut. equiv., 308.

14) P. Cagniant and A. Deluzarche, *Compt. rend.*, 222, 1301 (1946).

Methyl 4-(5-Bromo-3, 4-dinitro-2-thienyl)-butyrate (VIII (n=3)).—A mixed acid consisting of concentrated nitric acid (0.21 ml.) and concentrated sulfuric acid (1 ml.) was added slowly to a solution of methyl 4-(5-bromo-3-nitro-2-thienyl)-butyrate (1 g.) in concentrated sulfuric acid (3 ml.) at 5~10°C. After this addition, the reaction mixture was stirred for 30 min., then poured into iced water to precipitate crystals of methyl 5-(5-bromo-3,4-dinitro-2-thienyl)-butyrate. The crystals were collected by filtration and washed with water. The melting point of the crude product was 54~56.5°C, and the yield was 96% (1.1 g.). Recrystallization from ligroin raised the m. p. to 56.5~58°C.

Found: N, 7.96. Calcd. for $C_9H_9O_6BrN_2S$: N, 7.93%.

Methyl 5-(5-Bromo-3, 4-dinitro-2-thienyl)-valerate (VIII (n=4)). — Methyl 5-(5-bromo-3-nitro-2-thienyl)-valerate (5 g.) was treated similarly to the above compound to prepare methyl 5-(5-bromo-3,4-dinitro-2-thienyl)-valerate in a yield of 88% (6 g.) as a crude product, m. p. 57~60.5°C. After recrystallizing from ligroin-benzene, the melting point was 59.5~61.5°C.

Found: N, 7.59. Calcd. for $C_{10}H_{11}O_6BrN_2S$: N, 7.63%.

4-(5-Bromo-3, 4-dinitro-2-thienyl)-butyric Acid (X (n=3)).—A solution of methyl 4-(5-bromo-3,4-dinitro-2-trienyl)-butyrate (0.5 g.) in concentrated hydrochloric acid (15 ml.) was refluxed for 4 hr., then diluted with water after cooling to afford precipitation of crude 4-(5-bromo-3,4-dinitro-2-thienyl)-butyric acid (m. p. 134~136°C) in a yield of 87.5%. After recrystallization from 6 N-AcOH, the m. p. was 135~136.5°C.

Found: N, 8.18; Neut. equiv., 342. Calcd. for $C_8H_7O_6BrN_2S$: N, 8.26%; Neut. equiv., 339.

5-(5-Bromo-3, 4-dinitro-2-thienyl)-valeric Acid (X (n=4)).—A solution of methyl 5-(5-bromo-3,4-dinitro-2-thienyl)-valerate (2.4 g.) in acetic acid (50 ml.) and concentrated hydrochloric acid (50 ml.) was refluxed for one hour, then diluted with water after cooling to afford a precipitate of crude 5-(5-bromo-3,4-dinitro-2-thienyl)-valeric acid in a crude yield of 91% (2.1 g.). After recrystallization from 6 N-AcOH, the m. p. was 132~133°C.

Found: N, 7.90; Neut. equiv., 356. Calcd. for $C_9H_9O_6BrN_2S$: N, 7.93%; Neut. equiv., 353.

When acetic acid was not used as the solvent, tarry matter was produced as a by-product and the yield of the acid was lowered to 35%.

Another Method. — A mixed acid consisting of 0.2 ml. of concentrated nitric acid and 2 ml. of concentrated sulfuric acid was slowly stirred into a solution of 5-(5-bromo-3-nitro-2-thienyl)-valeric acid (0.9 g.) in concentrated sulfuric acid (8 ml.) at 0~5°C. After this addition, the reaction mixture was stirred for 15 min., and the reaction temperature was gradually raised to 10°C while being stirred. The reaction mixture was poured into ice-water. The crystals precipitated were collected by filtration, then washed with water. The yield of crude 5-(5-bromo-3,4-dinitro-2-thienyl)-valeric acid (m. p. 128~132°C) was 87% (0.9 g.). After recrystallization from acetic acid-water, the melting point of the crystals was 132~133°C. No depression of the melting point on mixing them with the compound prepared by the alternative method was observed.

Aromatic-Norbiotin (XII (n=3)).—A suspension of 0.4 g. 4-(5-bromo-3,4-dinitro-2-thienyl)-butyric acid in 10 ml. of concentrated hydrochloric acid was stirred, together with 0.8 g. tin in small pieces, at 25~30°C untill the tin disappeared, then evaporated to dryness in vacuo to obtain colorless tin double salt. The tin double salt was dissolved in 20 ml. of water and a solution of potassium hydroxide while bing cooled by ice; then immediately phosgen was introduced into the solution. The introduction phosgen was continued until the solution became acidic to Congo red paper. The crystals precipitated during the introduction of the phosgen were collected by filtration, washed with water and recrystallized from methanol-water to obtain curde product (m. p. 228~230°C decomp.) in a yield of 0.12 g. (45%). The melting point of the crystals was 244~245°C decomp. after recrystallization from water.

Found: N, 12.46. Calcd. for $C_9H_{10}O_3N_2S$: N, 12.93%.

The absorption maximum (λ_{max}) in a ultraviolet spectrum (solvent: ethanol): 260 mμ.

Aromatic-Biotin (XII (n=4)).—5-(5-Bromo-3,4-dinitro-2-thienyl)-valeric acid (0.4 g.) was similarly treated to obtain crude aromatic-biotin (m. p. 236~238°C decomp.) in a yield of 70% (0.2 g.). After recrystallizing them from water, the melting point of the crystals was 253~254°C decomp. (The meltint point given in the literature was 254~254.5°C decomp.). The λ_{max} (in ethanol as solvent) in ultraviolet spectrum was 260 mμ (coinciding with the value, 260 mμ given in the literature).

Found: N, 11.49. Calcd. for $C_{10}H_{12}O_3N_2S$: N, 11.67%.

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