[CONTRIBUTION FROM THE NOYES CHEMICAL LABORATORY, UNIVERSITY OF ILLINOIS]

Synthesis of the Thieno[3,2-b]pyrrole System

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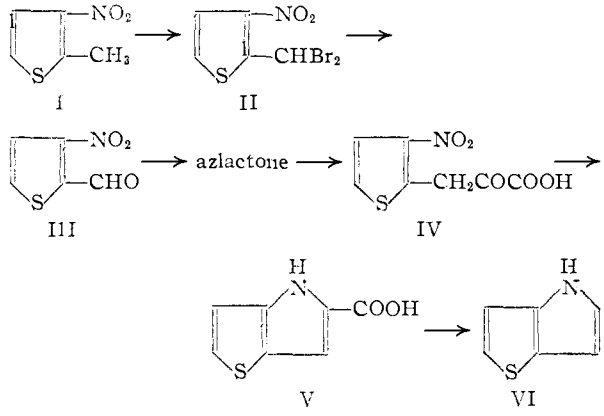
The synthesis of thieno[3,2-b]pyrrole (VI) was carried out by conversion of 3-nitro-2-methylthiophene to 3-nitro-2-thenaldehyde followed by application of the azlactone synthesis to give 3-nitro-2-thienylpyruvic acid, which was reduced to the thienopyrrolecarboxylic acid (V). Decarboxylation of V to thieno[3,2-b]pyrrole (VI) was effected by heat in an evacuated sealed tube. A similar reaction scheme proceeding from 2-nitro-3-methylthiophene was examined in order to obtain the isomeric thienopyrrole, but the final step, reduction of the pyruvic acid, was unsuccessful.

A number of thiophene analogs of physiologically active compounds have been found to be active as anti-metabolites. For example, β-3-thienylalanine inhibits the growth of the microorganisms, *S. cervisiae* and *E. coli*.[4] Attention was directed toward the preparation of a thiophene analog of indole with the hope of converting it to the corresponding tryptophan by one of the well-known methods.

The synthesis of thieno[3,2,-b]pyrrole (VI) was carried out by the modified Reissert method[5] as indicated (I → VI).

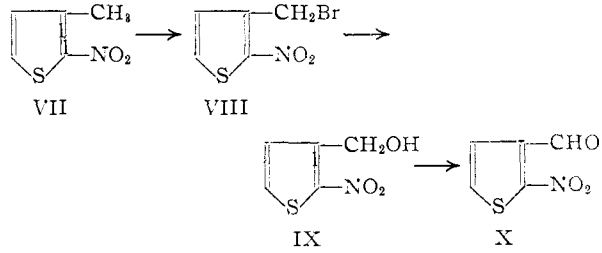


For the preparation of 3-nitro-2-methylthiophene (I) a modification of the method of Rinkes was used.[6] 2-Methylthiophene was converted to 5-methyl-2-acetylthiophene by the method of Hartough and Kosak.[7] The ketone was oxidized to 2-methyl-5-thenoic acid with alkaline hypochlorite.[8] Nitration and subsequent decarboxylation gave I.

One or two moles of N-bromosuccinimide in boiling carbon tetrachloride converted I to the mono- or dibromide II, and the latter was transformed to 3-nitro-2-thenaldehyde (III) *via* the diacetate. The pyruvic acid (IV), which was prepared by hydrolysis of the azlactone of III, was reduced to the thienopyrrolecarboxylic acid (V) by means of aqueous ammonia and ferrous sulfate.[9] This acid (V) is similar in physical appearance and melting point to indole-2-carboxylic acid, and like the latter it imparts a red-brown coloration[10] to a ferric chloride solution. It differs from indole-2-carboxylic acid in that it chars on melting rather than undergoing smooth decarboxylation. Attempted decarboxylation with mercuric acetate also failed. Decarboxylation of V was effected by heating the acid in an evacuated tube.

Thieno[3,2,-b]pyrrole is a white crystalline substance, melting at about 30°, that has an odor very similar to that of indole. When exposed to air at room temperature, it decomposes. It forms a red picrate that decomposes on standing in alcohol. The infrared spectrum (Fig. 1) is similar to that of indole in many respects.

A similar method was examined for the preparation of the isomeric acid. The key steps leading to the aldehyde X are



In contrast to the reactivity of 3-nitro-2-methylthiophene with N-bromosuccinimide, the isomeric compound VII is unaffected by this reagent in boiling carbon tetrachloride.[11] However, it was found that reaction could be made to occur by substituting ethylene dibromide for the lower-boiling solvent.

It was possible to convert the bromide VIII directly to the aldehyde X by oxidation with selenium dioxide, but the results were not consistent.[12] A more reliable method involved oxidation of the alcohol IX by N-bromosuccinimide in a method similar to that of Grob and Schmid.[13]

Conversion of the aldehyde X to the corresponding pyruvic acid was carried out by the method outlined above. Reduction of the pyruvic acid gave a non-crystalline product which underwent decomposition on attempted purification.

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(5) J. R. Johnson, R. B. Hasbrouck, J. D. Dutcher and W. F. Bruce, *ibid.*, **67**, 423 (1945).

(6) I. Rinkes, *Rec. trav. chim.*, **51**, 1134 (1932).

(7) H. Hartough and A. Kosak, THIS JOURNAL, **69**, 3093 (1947).

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(11) F. Pilgrim, Ph.D. Thesis, University of Illinois, Urbana, Ill., 1949.

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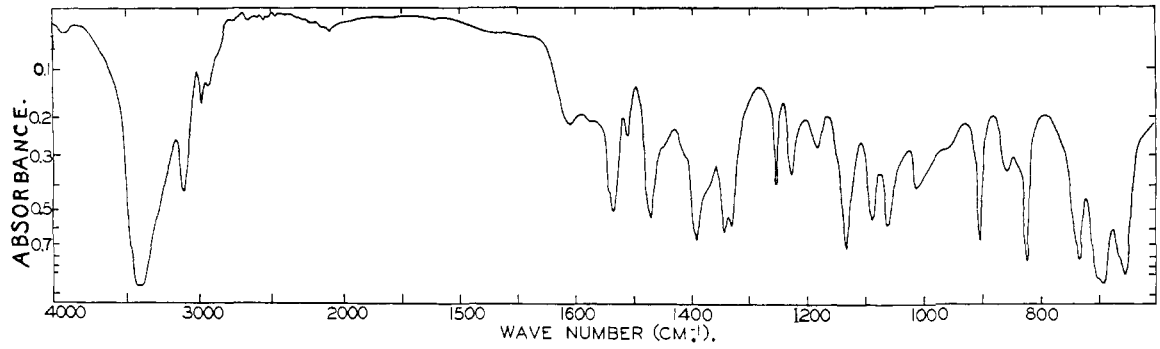

Fig. 1.

Experimental

2-Methyl-5-thenoic Acid.—This acid was prepared by acylation of 2-methylthiophene followed by a haloform reaction according to the procedures described by Hartough, *et al.*[7,8]

2-Methyl-3-nitro-5-thenoic Acid.—The following procedure is a convenient modification of the method of Rinkes.[6] Fuming nitric acid (300 g., sp. gr. 1.5) was added slowly to 700 ml. of acetic anhydride contained in a 2-l. round-bottomed flask fitted with a Hershberg stirrer and cooled by means of a Dry Ice–acetone mixture. The resulting solution was cooled to −12°, and 210 g. of finely powdered 2-methyl-5-thenoic acid was added, with stirring, in portions of 1–2 g., during the course of 45 minutes. A pale pink solid separated; after addition of the acid, the mixture was stirred for ten minutes, during which time the temperature fell to −20°. The crystalline solid was filtered and triturated with 400 ml. of water and 100 g. of ice. When the ice had melted, the pink solid was collected and washed three times with water; the wet product was recrystallized from 95% ethanol. Large orange-pink crystals (126.5 g., 45.7%), m.p., 178–180°, separated on standing. A sample was sublimed at 160° (0.8 mm.) to yield white crystals, m.p. 180–181° (lit.[6] 181°).

A by-product, 2-methyl-3,5-dinitrothiophene, can be isolated by pouring the acetic anhydride filtrate onto 2 kg. of ice and 2 l. of water, filtering the sticky red solid which precipitates and recrystallizing it from ethanol and then from high-boiling petroleum ether to give tan colored crystals (8–15 g.) melting at 97–99° (lit.[6] 99–100°).

3-Nitro-2-methylthiophene.—The following is a necessary modification of Rinkes' procedure.[6] A 1-l. round-bottomed flask containing a mixture of 163 g. of the nitro acid, 290 g. of quinoline and 6 g. of copper powder was fitted with a thermometer suspended into the mixture. A length of rubber tubing leading to an aspirator was loosely inserted into one of the necks of the flask to draw off vapors of water and quinoline. The flask was heated slowly with an electrical heating mantle at such a rate that the internal temperature rose approximately ten degrees every five minutes. The mass became fluid at about 85–95° and, after 55 minutes, the temperature had risen to 210°. At this point the mixture was cooled under tap water, and a solution of 600 ml. of water and 250 ml. of 85% phosphoric acid was added. The mixture was steam distilled; the distillate (5 l.) was acidified with a little dilute hydrochloric acid (1:1), cooled in an ice-bath, and the crude nitro compound (74.5 g., 59.7%) was collected. Recrystallization from low-boiling petroleum ether gave 64 g. of nearly white crystals, m.p. 44–46°. From the filtrate there was obtained an additional 5.5 g. of pure product making the total yield 69.5 g. (55.5%). It was possible to decarboxylate the acid by the mercuric acetate method, but the yield was lower (46%).[14]

3-Nitro-2-thenyl Bromide.—A solution of 14 g. of 3-nitro-2-methylthiophene (m.p. 44–46°) dissolved in 100 ml. of carbon tetrachloride was dried by distillation of a little of the solvent. There was then added, all at once, 17.3 g. of N-bromosuccinimide (recrystallized from water, dried over sulfuric acid) and 0.05 g. of benzoyl peroxide, and the mixture was refluxed for 24 hr. The mixture was cooled in an ice-bath and the crystalline succinimide was separated. The filtrate was evaporated on the steam-bath to an orange, slightly lachrymatory, thick liquid which solidified on cooling in an ice-bath. This was triturated with low-boiling petroleum ether and washed out of the flask with the aid of this solvent to give 20 g. (92%) of sticky orange solid. The crude bromide was recrystallized from about 400 ml. of low-boiling petroleum ether, which deposited 10 g. (m.p. 57–60°) of light yellow needles. An additional 1.1 g. of pure product was obtained from the filtrate so that the total yield was 11.1 g. (51.2%). The analytical sample melted at 60–62°.

Anal. Calcd. for $C_5H_4O_2NSBr$: C, 27.04; H, 1.82; N, 6.31. Found: C, 27.20; H, 2.08; N, 6.39.

3-Nitro-2-thenyl Acetate.—The acetolysis of 3-nitro-2-thenyl bromide was carried out by the method of Shriner, Fuson and Curtin.[15] For analysis the ester was recrystallized once from ethanol and twice from low-boiling petroleum ether, from which it separated in the form of slender white needles melting at 58.5–60.5°.

Anal. Calcd. for $C_7H_7O_4NS$: C, 41.80; H, 3.51; N, 6.96. Found: C, 42.08; H, 3.58; N, 6.92.

3-Nitro-2-thenal Bromide.—A mixture of 1.1 g. of 3-nitro-2-methylthiophene, 2.8 g. of N-bromosuccinimide, 0.01 g. of benzoyl peroxide and 25 ml. of dry carbon tetrachloride was refluxed for 20 hr. The solution was cooled in an ice-bath, the succinimide removed by filtration and the filtrate evaporated on the steam-bath to a thick reddish liquid (1.8 g., 75.8%) which solidified on cooling. For analysis it was recrystallized three times from low-boiling petroleum ether, which deposited the dibromide in the form of faintly yellow triangular plates melting at 72.8–74°.

Anal. Calcd. for $C_5H_3NO_2Br_2S$: C, 19.95; H, 1.01; N, 4.65. Found: C, 19.77; H, 1.20; N, 4.42.

3-Nitro-2-thenaldehyde.—A mixture of 16.5 g. of 3-nitro-2-methylthiophene, 42 g. of N-bromosuccinimide (recrystallized from water, dried over sulfuric acid), 0.15 g. of benzoyl peroxide and 200 ml. of dry carbon tetrachloride was refluxed for 66 hr., cooled in an ice-bath and filtered to remove the succinimide along with some unreacted N-bromosuccinimide.

The filtrate was evaporated on the steam-bath *in vacuo*, and to the remaining oil were added 120 ml. of ethanol, 150 ml. of water and 25 g. of sodium acetate. The resulting mixture was refluxed for 1.5 hr., 50 ml. of hydrochloric acid (sp. gr. 1.18) was added, and the refluxing was continued for 0.75 hr. The solution was then treated with Darco, the filtrate diluted with 1 l. of saturated sodium chloride solution and the resulting mixture extracted with four 50-ml. portions of ether. The ether solution was dried over magnesium sulfate and the solvent removed by distillation. The residue was distilled from a Claisen flask and 6.3 g. (34.8%) of yellow liquid, which partially solidified in the receiver, was collected at 113–127° (2–3 mm.). The aldehyde became reddish brown on standing and therefore was analyzed as the **semicarbazone** which forms, on recrystallization from dimethylformamide, small yellow crystals melting at 247–248° dec.

(14) H. Hartough, "Thiophene and Its Derivatives," Interscience Publishers, Inc., New York, N. Y., 1952, p. 373.

(15) R. Shriner, R. Fuson and D. Curtin, "The Systematic Identification of Organic Compounds," 4th ed., John Wiley and Sons, Inc., New York, N. Y., 1956, p. 200.

Anal. Calcd. for $C_6H_6O_3N_4S$: C, 33.64; H, 2.82; N, 26.16. Found: C, 33.92; H, 2.70; N, 26.40.

2-Methyl-4-(3-nitro-2-thenal)-5-oxazolone.—A mixture of 6.3 g. of 3-nitro-2-thenaldehyde, 4.74 g. of aceturic acid, 0.2 g. of sodium acetate and 13.7 ml. of acetic anhydride was heated for 1 hr. on the steam-bath, whereupon the mixture became red, and bright red crystals separated. After cooling at 5–10° for about 5 hr., the crystals were filtered, washed twice with small portions of acetic acid and once with water. The air-dried product (6.2 g., 64.7%, m.p. 182–185°) was pure enough for further use. For analysis the azlactone was recrystallized three times from methyl ethyl ketone, which deposited yellow-orange needles melting at 185–186°.

Anal. Calcd. for $C_9H_6N_2O_4S$: C, 45.38; H, 2.54; N, 11.76. Found: C, 45.80; H, 2.73; N, 11.67.

When an attempt was made to recrystallize the azlactone (2 g.) from 95% ethanol the solid went into the boiling solution slowly but did not separate on cooling. After evaporation of the alcohol at room temperature, 1.3 g. of reddish brown crystals were deposited, m.p. 147–150°. This compound, in contrast to the azlactone, is easily soluble in hot ethanol and was recrystallized for analysis from this solvent, which deposited a mass of lemon-yellow needles melting at 150.5–152°. Presumably this is ethyl α-acetamino-β-(2-nitro-3-thienyl)-acrylate, which was formed by opening of the azlactone ring by ethanol.

Anal. Calcd. for $C_{11}H_{12}O_5N_2S$: C, 46.48; H, 4.26; N, 9.86. Found: C, 46,81; H, 4.16; N, 10.00.

3-Nitro-2-thienylpyruvic Acid.—A mixture of 6.1 g. of 2-methyl-4-(3-nitro-2-thenal)-5-oxazolone (m.p. 182–185°) and 170 ml. of 1 *N* hydrochloric acid was refluxed for 2 hr. The yellow-orange solution was treated with Darco, filtered and allowed to cool in the ice-chest overnight. The yellow solid which separated (4.5 g., 82.1%) was recrystallized from acetic acid, which deposited 3.5 g. of tiny yellow crystals, m.p. 195–197° dec. From the filtrates, diluted with water, there was obtained an additional 0.3 g. of the pure acid. The total yield was 3.8 g. (69%). The analytical sample, prepared exactly as described above from a different batch of the azlactone, was recrystallized twice from acetic acid, which deposited tiny yellow needles, m.p. 191.5° dec. It is not known why the product obtained above (m.p. 195–197°) melted at a higher temperature than the analytical sample.

Anal. Calcd. for $C_7H_5O_5NS$: C, 39.07; H, 2.34; N, 6.51. Found: C, 39.43; H, 2.14; N, 6.62.

Thieno(3,2-b)pyrrole-2-carboxylic Acid (V).—A solution of 30 g. of ferrous sulfate heptahydrate in 33.5 ml. of warm water was added in one portion to the deep violet-red solution of 3.5 g. of 3-nitro-2-thienylpyruvic acid (m.p. 195–197°) in 33.5 ml. of water and 23.5 ml. of concentrated aqueous ammonia (sp. gr. 0.90). The black mixture was warmed on the steam-bath for 0.5 hr. with occasional shaking. The mixture was filtered and the residue on the filter extracted with 20 ml. of hot dilute aqueous ammonia. The resulting brown solution was treated with Darco and evaporated on the steam-bath *in vacuo* until the ammonium salt began to separate. The mixture was cooled, acidified with hydrochloric acid (sp. gr. 1.18) and the brownish powdery solid collected (1.3 g., m.p. 193–195° dec., yield 48%). Recrystallization from ethanol–water (1:1) gave 0.9 g. (33.3%) of brown needles, m.p. 197–198° dec. After three recrystallizations from ethanol–water (1:1), the acid separated in the form of small white needles, m.p. 193–194° dec., which darkened slightly on standing.

Anal. Calcd. for $C_7H_5O_2NS$: C, 50.31; H, 3.02; N, 8.38. Found: C, 50,58; H, 3.18; N, 8.85, 8.52.

Since thieno(3,2-b)pyrrole-2-carboxylic acid undergoes extensive charring on melting, it could not be decarboxylated by heating above the melting point. On heating at 155–160° (0.5–1.5 mm.), the acid merely sublimes. On shaking with a mixture of 10% ferric chloride solution and ethanol (1:1), the acid develops a reddish brown color. The acid dissolves in concentrated sulfuric acid to give a bright yellow-orange solution which develops a red color on standing.

Chromatography of Thieno(3,2-b)pyrrole-2-carboxylic Acid.—Silicic acid (9.0 g.) was ground with 5 ml. of water in a mortar, slurried with 40 ml. of chloroform and packed in a 50-ml. buret. The column was washed with 200 ml. of chloroform that had been previously saturated with water. Thieno(3,2-b)pyrrole-2-carboxylic acid (32.3 mg., m.p. 193–194°) was ground with 0.5 g. of silicic acid and 0.5 ml. of water, and this material was added to the column. The column was eluted with chloroform saturated with water; fractions of 10 ml. size were collected. These were titrated with dilute sodium hydroxide. The thieno(3,2-b)-pyrrole-2-carboxylic acid was collected in fractions number 3 to 7. The product was isolated by acidification of the basic extracts of the chloroform fractions; m.p. 203–204°, yield 31.2 mg.

Decarboxylation of Thieno(3,2-b)pyrrole-2-carboxylic Acid.—Chromatographically pure V (178.2 mg., 1.07 mmoles) was sealed in a reaction tube that was equipped with a break-seal side arm. The reaction tube was completely immersed in an oil-bath at 210–220° for 30 minutes. A clear liquid condensed on the walls of the tube when it cooled. The break-seal side arm was attached to a high vacuum line and the carbon dioxide was distilled to a calibrated manometer from a −90° trap. The amount of carbon dioxide found was 1.07 mmoles (100%). The vapor pressure was measured at −110° and found to be 29 mm. (carbon dioxide has a vapor pressure of 26 mm. at −110°). The reaction tube was washed out with carbon disulfide, and the extracted material was sublimed at 10^{-3} mm. onto a cold finger cooled with a Dry Ice–acetone mixture. A total of 45.0 mg. (36.6%) of a white product was obtained. The material had an odor very similar to that of indole and melted between 30 and 35°. The infrared spectrum (Fig. 1), determined as a smear, was consistent with the proposed structure (VI). The product decomposes at room temperature when exposed to the air for a few minutes. A solution of picric acid in ethanol was added to the material and a red precipitate resulted. The picrate decomposes to an amorphous black powder on standing overnight in ethanol.

A control experiment was carried out with 1 mmole of indole-2-carboxylic acid. A 100% yield of carbon dioxide and a 60% yield of indole were realized.

Attempted Mannich Reaction with Thieno(3,2-b)pyrrole.—Glacial acetic acid (60 mg., 1.0 mmole) was added to 46 mg. (0.5 mmole) of piperidine followed by 50 mg. of formalin. Thieno(3,2-b)pyrrole (45 mg., 0.366 mmole) was added, and the solution was allowed to stand for 5 hr. The solution was washed into a centrifuge tube, and 2 ml. of 2.0 *N* sodium hydroxide was added. A very small amount of oil resulted. No crystalline product could be isolated.

A control reaction was carried out with 1 mmole of indole. A 75% yield of the piperidine Mannich base of indole resulted.

2-Nitro-3-methylthiophene.—To prepare this compound (46% yield) the method of Rinkes[16] was used with certain modifications. In a typical run 100 g. of 3-methylthiophene dissolved in 200 ml. of acetic acid was added during 15–20 minutes to a nitrating mixture of 200 ml. of acetic acid and 100 g. of fuming nitric acid (sp. gr. 1.5) at −15 to −12°. If the addition was not carried out rapidly, much tar was formed.

2-Nitro-3-thenyl Bromide.—A mixture of 132 g. of 2-nitro-3-methylthiophene, 200 ml. of dry ethylene dibromide, 206 g. of N-bromosuccinimide (recrystallized from hot water and dried over sulfuric acid) and 0.8 g. of benzoyl peroxide was refluxed gently for 3 hr. During the first hour the solution had a red-brown color, and vapors of bromine escaped at the top of the condenser. The color abruptly became dark brown and bromine vapors were no longer evolved. The solution was cooled in an ice-bath, 100 ml. of carbon tetrachloride was added and the dark brown succinimide was filtered and washed with three 50-ml. portions of carbon tetrachloride. The filtrate was washed with four 200-ml. portions of water, dried over magnesium sulfate, and the solvent was distilled from a steam-bath with the aid of a water aspirator. The residue was distilled under vacuum from a 1-l. flask (a large flask was required because of excessive frothing during the later stages of the distillation) fitted with a 6-in. Vigreux column. Two fractions were collected: fraction I, 29 g., b.p. 88–110° (1.1–1.3 mm.); fraction II, 123 g., b.p. 110–147° (1.2–3.0 mm.).

Both fractions solidified on standing. The lower-boiling material was mainly unreacted 2-nitro-3-methylthiophene. The higher boiling fraction was mainly 2-nitro-3-thenyl

(16) I. Rinkes, *Rec. trav. chim.*, **52**, 1052 (1933).

bromide and corresponded to a yield of 60%. It was pure enough for further use, but if desired it could be purified by recrystallization from low-boiling petroleum ether from which it separated in the form of long white needles which melted at 63–65°.

Anal. Calcd. for $C_5H_4NO_2SBr$: C, 27.04; H, 1.82; N, 6.31. Found: C, 27.48; H, 1.80; N, 6.31.

The bromide was characterized as the pyridinium salt which was prepared by allowing the components to stand in dry ether. The salt separated from ethanol in the form of shiny leaflets, m.p. 232–233° dec.

Anal. Calcd. for $C_{10}H_9N_2O_2BrS$: C, 39.87; H, 3.00. Found: C, 40.07; H, 2.89.

2-Nitro-3-thenyl Alcohol (XXXI).—A mixture containing 161.5 g. of 2-nitro-3-thenyl bromide (crude, as prepared above), 161.5 g. of sodium formate and 1700 ml. of 50% ethanol was refluxed for a period of 2 hr., and then 200 ml. of hydrochloric acid (sp. gr. 1.18) was added and the solution refluxed for 1.5 hr. longer. The hot solution was treated with Darco and diluted with 2 l. of water. The aqueous solution was saturated with sodium chloride, and the oil was extracted with ether. The ether solution was dried over magnesium sulfate, and after removal of the solvent by distillation from a steam-bath, the residue was distilled under vacuum. Two fractions were collected: fraction I, 15 g. (12.8%), b.p. 98–114° (0.2–0.55 mm.); fraction II, 63.7 g. (55%), b.p. 129–146° (0.6–0.8 mm.).

Both fractions solidified during collection. The second fraction was sufficiently pure for conversion directly to the aldehyde in the next step, while the first fraction was purified by crystallization from methanol or 70% ethanol, from which the alcohol separated in the form of yellowish crystals, m.p. 90–92°.

Anal. Calcd. for $C_5H_5O_3NS$: C, 37.73; H, 3.17; N, 8.80. Found: C, 38.32; H, 3.12; N, 8.71.

2-Nitro-3-thenaldehyde.—In a 1-l. round-bottomed flask fitted with a reflux condenser and Hershberg stirrer were mixed 76.7 g. of crude 2-nitro-3-thenyl alcohol (prepared as given above), 48 g. of finely powdered calcium carbonate, 85.2 g. of N-bromosuccinimide (recrystallized from water, dried over sulfuric acid) and 500 ml. of carbon tetrachloride (dried by distillation). The mixture was refluxed gently with stirring. During the first hour of heating, the mixture was watched carefully, because the refluxing became vigorous enough to require removal of the source of heat momentarily. The solution became brown in color, and some bromine vapor escaped from the top of the condenser. After a total of 3.5 hr. of refluxing, the reaction flask was cooled in an ice-bath and the orange solid filtered off. The orange residue was washed on the filter with three small portions of carbon tetrachloride, and the combined yellow filtrates were dried over magnesium sulfate. After removal of the solvent *in vacuo* on the steam-bath, the residue was distilled under vacuum. Two fractions were collected: fraction I, 10.1 g. (13.4%), b.p. 84–87° (0.3 mm.); fraction II, 35.1 g. (46.5%), b.p. 91–112° (0.3–0.9 mm.).

Both fractions partially crystallized on standing. Fraction I was less pure than fraction II, although it could be used in the next step without difficulty. For analysis, a small amount of fraction II was recrystallized twice from a mixture of low-boiling petroleum ether and absolute ether (1:1), which deposited the aldehyde in the form of short yellow needles, m.p. 55.5–56.5°.

Anal. Calcd. for $C_5H_3O_3NS$: C, 38.21; H, 1.92; N, 8.91. Found: C, 38.59; H, 2.09; N, 8.92.

The semicarbazone was prepared in the usual manner. For analysis it was recrystallized from dimethylformamide, from which it separated in the form of small bright yellow needles, m.p. 265–266° dec.

Anal. Calcd. for $C_6H_6O_3N_4S$: C, 33.64; H, 2.82; N, 26.16. Found: C, 34.02; H, 2.98; N, 26.02.

2-Methyl-4-(2-nitro-3-thenal)-5-oxazolone.—This azlactone was prepared in 55.8% yield from X exactly as given for the isomeric compound except that nitromethane was used to recrystallize the product for analysis. The oxazolone separated in the form of orange plates, m.p. 196–198° dec.

Anal. Calcd. for $C_9H_6N_2O_4S$: C, 45.38; H, 2.54; N, 11.76. Found: C, 45.58; H, 2.56; N, 11.87.

2-Nitro-3-thienylpyruvic Acid.—This acid was prepared in 51.3% yield as given for the isomeric compound IV except that the heating was continued for 3 hr., since this isomer proved to be more stable toward hydrolysis. The compound separated from acetic acid in the form of bright yellow needles, m.p. 186° dec.

Anal. Calcd. for $C_7H_5O_5NS$: C, 39.07; H, 2.34; N, 6.51. Found: C, 39.36; H, 2.42; N, 6.45.

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URBANA, ILLINOIS

[CONTRIBUTION FROM THE EASTERN REGIONAL RESEARCH LABORATORY[1]]

Phosphopeptides Obtained by Partial Acid Hydrolysis of α-Casein[2]

BY N. J. HIPP, M. L. GROVES AND T. L. MCMEEKIN

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A partial acid hydrolysis of α-casein was resolved into fractions containing phosphopeptides by chromatography on Dowex 50. The impure fractions were further purified by chromatography and the electrophoretically pure subfractions were analyzed for their amino acid composition on Dowex 50. In addition to phosphoserine the following dipeptides were found: phosphoserylglutamic acid, phosphoserylalanine and phosphoserylphosphoserine. The extensive destruction of serine on complete hydrolysis of the peptides is discussed.

The physiological significance of phosphorus in casein and other phosphoproteins has been the subject of a number of recent investigations.[3] The presence of phosphorus is also advantageous in structural studies on casein since partial hydrolysis with enzymes and acid produces phosphopeptides which can be uniquely separated from the non-phosphopeptides. Numerous phosphopeptide fractions have been prepared from casein, particularly by enzymic digestion.[4] Lipmann[5] has shown that at least 50% of the casein phosphorus is present as ser-

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(2) Presented in part before the Division of Biological Chemistry, 128th Meeting of the American Chemical Society, Minneapolis, Minnesota, September 11–16, 1955.

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