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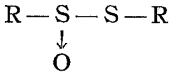
165. Tetsuji Kametani,[*2] Keiichiro Fukumoto,[*2] Yuriko Takayanagi,[*2] Takashi Teshigawara,[*3] and Osamu Umezawa[*3] : Anti-cancer Agents. IV.[*1] Desulfurization by Hydrazine Hydrate. (1). The Reaction of *o*-, *m*-, and *p*-Nitrobenzyl Disulfide with Hydrazine Hydrate.

(*Pharmaceutical Institute, School of Medicine, Tohoku University,*[*2]
and Kowa Chemical Laboratories[*3])

In previous papers[1,2),*1] it was shown that three kinds of alkyl thiolsulfinates, isobutyl, *tert*-butyl, and amyl thiolsulfinates, were found to be effective against Ehrlich ascites tumor by the screening test of various thiolsulfinates synthesized to date.



$R-\underset{\overset{\downarrow}{O}}{S}-S-R$

(I) R = isobutyl
(II) R = *tert*-butyl
(III) R = amyl

These compounds are almost all unstable to heat and very irritating to the skin and eyes, that they are difficult to be used as an anti-cancer agent. Among them, only dibenzyl thiolsulfinate came as stable crystals and attempts were made to synthesize various kinds of dibenzyl thiolsulfinate derivatives with various functional groups in the phenyl ring such as nitro, amino, etc.

The synthesis of *p*-nitrobenzyl thiolsulfinate by oxidation of its corresponding disulfide was examined, but it was not oxidized and the starting material was recovered. This was thought to be due to the influence of nitro group and the oxidation of *p*-aminobenzyl disulfide after reduction of *p*-nitrobenzyl disulfide to it with hydrazine hydrate according to the method of Möhlau[3)] was tried. Contrary to the expectation, however, nitro group was not reduced by hydrazine hydrate, but desulfurization occurred.

First, *o*-, *m*-, and *p*-nitrobenzyl disulfide, as the starting material, were prepared by the route shown in Chart 1. In the case of *para*-isomer, benzyl chloride was nitrated as usual[4)] and *p*-nitrobenzylthiuronium chloride was prepared by treatment with thiourea in ethanol.[5)] It was hydrolyzed as usual with 10% sodium hydroxide solution, but a large amount of resinous substance formed. Therefore, its thiuronium salt was hydrolyzed at room temperature with 10% sodium carbonate solution in ethanol by which *p*-nitrobenzyl disulfide was obtained very easily in an excellent yield. In this case, pyridine was also successfully used in place of 10% sodium carbonate solution.

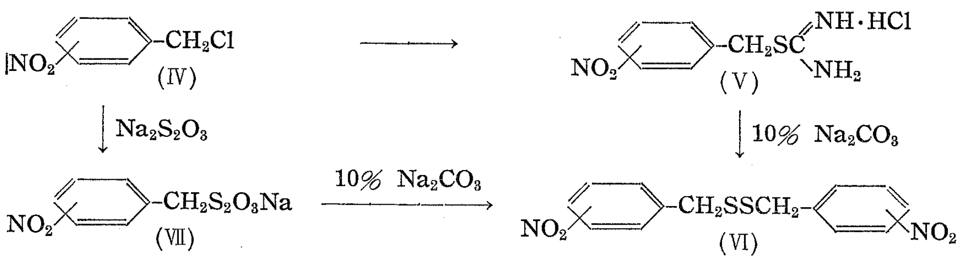
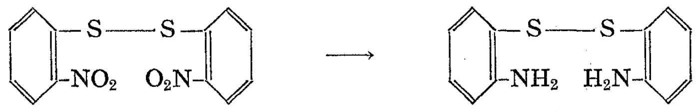


Chart 1.

[*1] Part III. T. Kametani, K. Fukumoto, O. Umezawa : Yakugaku Kenkyu, **31**, 132(1959).
[*2] Kita-4-bancho, Sendai (亀谷哲治, 福本圭一郎, 高柳百合子).
[*3] Honcho, Chuo-ku, Tokyo (勅使河原 巍, 梅沢 修).
1) T. Kametani, K. Fukumoto, O. Umezawa : Yakugaku Kenkyu, **31**, 60(1959).
2) *Idem* : *Ibid.*, **31**, 125(1959).
3) R. Möhlau, H. Beyschlag, H. Köhres : Ber., **45**, 133(1912).
4) F. J. Alway : J. Am. Chem. Soc., **24**, 1062(1902).
5) H. Kofod : Org. Syntheses, **35**, 66(1955).

In order to synthesize these disulfides, hydrolysis of *p*-nitrobenzyl thiosulfate with 10% sodium carbonate solution, which was prepared from *p*-nitrobenzyl chloride and sodium thiosulfate,[6] has hitherto been used. In the case of *ortho*- and *para*-isomers, objective disulfides were prepared from the corresponding thiuronium salts by the same procedure as mentioned above, and the latter compounds were also prepared from their corresponding nitrobenzyl chloride.

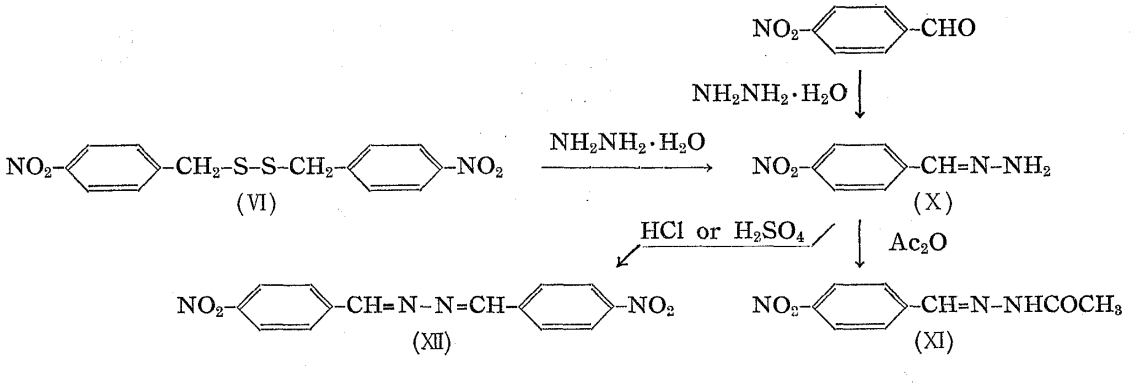
The disulfide was thought to be not oxidized by hydrogen peroxide or perbenzoic acid owing to the presence of nitro group in the phenyl ring but in the case of amino group, it was thought to be oxidized because of opposite functional group. According to this idea, the reduction of *p*-nitrobenzyl disulfide was tried with hydrazine hydrate,[3] because the following disulfide (VIII) was successfully reduced to its diamino derivative (IX) by heating with hydrazine hydrate.



(VIII) (IX)

In the present case, evolution of hydrogen disulfide and ammonia was observed during heating but the presence of sulfur in its reaction product was not examined. The yellowish-orange crystalline product of m.p. 134° so obtained, after various examinations, was recognized as *p*-nitrobenzylidenehydrazine (X). This was proved to be identical in the mixed melting point test, infrared spectrum, and other points, with authentic sample, which was prepared from *p*-nitrobenzaldehyde and hydrazine hydrate. It gave an acetyl derivative (XI) when heated with acetic anhydride, and its treatment with dil. hydrochloric or sulfuric acid solution changed it to bis(*p*-nitrobenzylidene)hydrazine (XII), i.e. *p*-nitrobenzaldehyde azine, formed via tetrazone.

In the case of *meta*- and *ortho*-isomers, desulfurization reaction was also effected and corresponding benzylidenehydrazine derivatives were obtained. Even in this case, nitro group was not reduced and this is rather an interesting fact. The mechanism of this reaction is being examined and will be reported in the near future.



Experimental[*4]

p-Nitrobenzylthiuronium Chloride—A mixture of 10 g. of *p*-nitrobenzyl chloride added to the solution of 4.5 g. of thiourea in warm EtOH (60 cc.) was refluxed for 1.5 hr. and the solution turned yellowish. The reaction mixture was concentrated under a reduced pressure, Et_2O was added, and 13.2 g. (92%) of colorless needles, m.p. 215°, was obtained. This was recrystallized from EtOH to crystals of m.p. 219~220°. *Anal.* Calcd. for $C_8H_{10}O_2N_3ClS$: C, 38.75; H, 4.02. Found : C, 38.62; H, 4.13.

[*4] All m.p.s are not corrected.

6) T. S. Price, D. F. Twiss : J. Chem. Soc., **93**, 1403(1908).

This compound was heated with NaOH solution in order to obtain the disulfide but a brownish-orange substance formed. This was not yet investigated, as this was not the disulfide.

Bis(*p*-nitrobenzyl) Disulfide (VI)—A solution of 2.6% Na_2CO_3 (8 cc.) was added to the solution of *p*-nitrobenzylthiuronium chloride (0.4 g.) in EtOH (5 cc.) and the mixture was allowed to stand, giving a yellowish solution. Although it became turbid, it was allowed to stand overnight, filtered, and the precipitate was washed thoroughly with 3.3% HCl, then with water until the washing became neutral. Thus, 0.1 g. (33%) of white needles, m.p. 124°, was obtained after purification from EtOH. This agreed in a mixed m.p. test with an authentic disulfide prepared according to another procedure.[6] From its mother liquor, 0.05 g. of disulfide was again obtained. Total yield, 0.15 g. (48%).

***p*-Nitrobenzylidenehydrazine (X)**—*p,p'*-Dinitrobenzyl disulfide (3.0 g.) was suspended in 50 cc. of EtOH with $NH_2NH_2 \cdot H_2O$ (3.2 g.) and the reaction mixture was refluxed for 2 hr. In this case, the mixture became reddish-black and the disulfide was dissolved in it after heating for 20 min., the evolution of gas containing sulfur being recognized by $(AcO)_2Pb$. After the evolution of gas had ceased, the reaction mixture was filtered while warm and the filtrate was allowed to stand, by which yellowish needles separated out. The crystals were collected and recrystallized from EtOH to 1.4 g. (46.7%) of yellowish needles, m.p. 132°. This substance is insoluble in NaOH or HCl and the test for N and NO_2 was positive, but that for S was negative.

Picrate: Yellowish crystals, m.p. above 263°. *Anal.* Calcd. for $C_7H_7O_2N_3$: C, 50.9; H, 4.2. Found: C, 50.27; H, 4.18.

N-Acetyl-*p*-nitrobenzylidenehydrazine (XI)—To the hydrazine (X) (0.4 g.), Ac_2O (1 cc.) was added, and the mixture was heated for 1 hr., then allowed to stand overnight at 0°. White crystals separated and this mixture was poured into ice and water. The crystals were collected, washed thoroughly with water, and recrystallized from EtOH to 0.2 g. (37%) of white scales, m.p. 194°. *Anal.* Calcd. for $C_9H_9O_3N_3 \cdot \frac{1}{3}H_2O$: C, 50.8; H, 4.53; N, 19.75. Found: C, 51.36; H, 4.03; N, 19.68.

Bis(*p*-nitrobenzylidene)hydrazine (XII)—An excess of conc. HCl was added to an EtOH solution of (X) and white amorphous precipitate formed, which was collected and washed with water. It almost agreed to m.p. 300° of bis(*p*-benzylidene)hydrazine reported in the literature.[7] Treatment of (X) with H_2SO_4 gave the same substance (XII), m.p. 298°, mentioned above, accompanying evolution of gas.

***o*-Nitrobenzylthiuronium Chloride**—To a saturated solution of thiourea (1.2 g.) in hot EtOH (15 cc.), *o*-nitrobenzyl chloride (1.6 g.) was added and the mixture was refluxed on a water bath for 2 hr., the color changing gradually from yellow to brown. The reaction mixture was concentrated to one-half the original volume and a suitable volume of Et_2O was added after cooling. The mixture was allowed to stand overnight and grayish prisms, m.p. 178~179°, were obtained after filtration. Yield, 2.0 g. (71.5%). Recrystallization from EtOH-Et_2O gave 1.6 g. (61.4%) of colorless needles, m.p. 185° (decomp.). *Anal.* Calcd. for $C_8H_{10}O_2N_3ClS$: C, 38.75; H, 4.02; N, 16.95. Found: C, 39.12; H, 4.35; N, 17.16.

***o*-Nitrobenzyl Disulfide**—The thiuronium chloride (1 g.) mentioned above was dissolved in 10 cc. of EtOH and 10% Na_2CO_3 (11 cc.) was added to it under cooling, an evolution of heat and gas being recognized. The solution turned yellowish-brown via brown color. The mixture was allowed to stand for a while at room temperature (25°) and white needles precipitated. This was allowed to stand overnight, crystals were collected, and washed, 0.65 g. (95.6%) of the disulfide, m.p. 101.5°, being obtained. This was recrystallized from EtOH to 0.6 g. (88.3%) of colorless needles, m.p. 106°.

***o*-Nitrobenzylidenehydrazine**—(a) By Heating with Hydrazine Hydrate: A mixture of *o*-nitrobenzyl disulfide (0.3 g.), EtOH (3 cc.), and 80% $NH_2NH_2 \cdot H_2O$ (0.3 g.) was refluxed on a water bath for about 2 hr., H_2S and NH_3 being evolved in this case. After 5 hr., the reaction mixture was allowed to stand overnight and the white amorphous substance that precipitated was extracted repeatedly with petr. ether. All the extracts were combined, dried over Na_2SO_4, and evaporated to leave yellowish, viscous oil. This gave 0.2 g. (74.6%) of yellow needles, m.p. 74°, when cooled. This was recognized to be the same as an authentic sample (m.p. 76°) by a mixed m.p. test.

This compound is easily soluble in MeOH, EtOH, Me_2CO, benzene, and $CHCl_3$, comparatively soluble in petr. ether and benzine, but sparingly soluble in water. Its treatment with 10% HCl or 10% H_2SO_4 gave an amorphous white substance of m.p. 198~201° (decomp.), which was thought to be bis(benzylidene)hydrazine derivative and was found to be identical with the m.p. reported.[7]

(b) From Benzaldehyde and Hydrazine Hydrate: While cooling, *o*-nitrobenzaldehyde (0.5 g.) was gradually added to 80% $NH_2NH_2 \cdot H_2O$ (2.5 cc.) when heat was produced and the solution became brownish. This clear, brownish solution was allowed to stand for a long time and 0.4 g. of yellowish, long needles, m.p. 76°, was obtained.

***m*-Nitrobenzylthiuronium Chloride**—A saturated hot EtOH solution of thiourea (3.2 g.) was added to *m*-nitrobenzyl chloride (7.2 g.) and, after heating on a water bath for 2.5 hr., the reaction mixture

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7) Th. Curtius, A. Lublin: Ber., **33**, 2460(1900).

was evaporated to about one-half the original volume. When cool, a suitable amount of Et_2O was added and the mixture was allowed to stand overnight. The colorless needles that precipitated were collected by filtration and purified to colorless needles, m.p. 132°, after recrystallization from $EtOH-Et_2O$. Yield, 5.2 g. (50%). *Anal.* Calcd. for $C_8H_{10}O_2N_3ClS$: C, 38.75; H, 4.02; N, 16.95. Found : C, 38.71; H, 4.29; N, 16.63.

***m*-Nitrobenzyl Disulfide**—To a deep pink solution of *m*-nitrobenzylthiuronium chloride (2 g.) dissolved in EtOH (20 cc.), 10% Na_2CO_3 (22 cc.) was added, when the mixture turned brownish and crystals beginning to separate after standing for a while. After 3.5 hr., yellowish white needles were collected by filtration, washed thoroughly with water, and dried. Thus, 1.0 g. (73.5%) of disulfide so obtained was recrystallized from EtOH to colorless needles, m.p. 104°.*5

***m*-Nitrobenzylidenehydrazine**—To a suspension of *m*-nitrobenzyl disulfide (0.5 g.) in EtOH (5 cc.), 0.5 g. of 80% $NH_2NH_2 \cdot H_2O$ was added and the mixture was heated gradually on a water bath. The disulfide began to dissolve in the reaction mixture which turned brownish. After 5 min., H_2S gas began to evolve, but the evolution of gas ceased after the reaction mixture was refluxed for 8 hr. After cool, an amorphous grayish substance was filtered off, its mother liquor was concentrated in a reduced pressure, and 0.25 g. (50%) of yellowish prisms or plates, m.p. 102.5°, was obtained. This was recrystallized from EtOH to yellowish needles, m.p. 107°. This agreed with the m.p. reported[7] and it was recognized to be identical with an authentic sample prepared from *m*-nitrobenzaldehyde and $NH_2NH_2 \cdot H_2O$ in its m.p.

In order to prepare the sample mentioned above, a mixture of *m*-nitrobenzaldehyde (0.5 g.) and $NH_2NH_2 \cdot H_2O$ (2.5 cc.) was allowed to stand at 16~21° for 10 days and yellowish prisms, m.p. 105°, were collected by filtration. Yield, almost quantitative.

Summary

When *o*-, *m*-, or *p*-nitrobenzyl disulfide was refluxed with hydrazine hydrate in ethanol, nitrobenzylidenehydrazine was found to be formed, contrary to expectation, and reduction of the nitro group was not effected. Treatment of *p*-nitrobenzylidenehydrazine with hydrochloric acid or sulfuric acid gave bis(*p*-nitrobenzylidene)hydrazine (XII) which formed N-acetyl derivative (XI) by treatment with acetic anhydride.

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*5 Price and Twiss[6] reported m.p. 103°.