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Studies on Antitumor Substances. XI.$^{1)}$ Reactions of Bunte Salts with Nucleophiles

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In order to examine the relationship between the carcinostatic activities of Bunte salts and their chemical behaviours, several Bunte salts, in which some of them were difunctional, were synthesized and attempted to react with several active methylene compounds, thiols and amines. In the reactions with thiols and amines, all of the Bunte salts resulted in the formation of the corresponding disulfides or the recovery of the starting materials. But in the reaction with active methylene compounds, alkylmercapto or bisalkylmercapto derivatives and cyclic mercaptal were formed respectively.

The carcinostatic activities of alkyl thiosulfonates and the reactions with some nucleophiles have been reported by Hayashi, *et al.*$^{3-5)}$ It is considered that alkylthiosulfates (Bunte salts) might have carcinostatic activities and chemical behaviors analogous to those of alkyl thiosulfonates, because of the structural similarity. Therefore, it was first attempted to synthesize several alkylthiosulfates, in which some of them were difunctional, and to test their carcinostatic activities against mice bearing Ehrlich solid tumor. However, on the contrary, the solid tumors in treated mice were observed to be larger than those in untreated mice.$^{6)}$

$$\text{R-SSO}_3\text{Na} \qquad \text{NaO}_3\text{SS-R-SSO}_3\text{Na}$$

Alkylthiosulfates were prepared by the conventional methods as follows and then allowed to react with active methylene compounds, amines and thiols. The active methylene compounds used were as follows: malondiamide, cyanoacetamide, diethyl malonate, ethyl acetoacetate, acetyl acetone and ethyl cyanoacetate. The equivalent amounts of the alkylthiosulfates and the active methylene compounds were dissolved in absolute ethanol containing sodium ethoxide prepared from an equivalent amount of sodium and allowed to stand for several hours at room temperature.

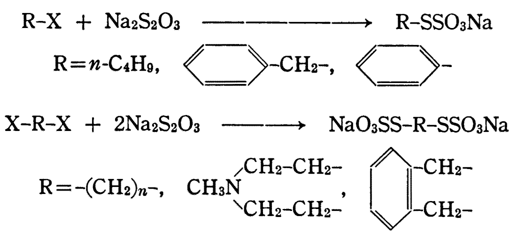

Chart 1

In the case of monofunctional sodium alkyl or aralkylthiosulfates, alkylmercapto or aralkylmercapto derivatives of active methylene compounds are expected to form. While,

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1) Part X: S. Hayashi, M. Furukawa, Y. Fujino, and H. Matsukura, *Chem. Pharm. Bull.* (Tokyo), **17**, 954, (1969).
2) Location: *a) Oe-moto-machi, Kumamoto*; *b) Yoshitomi-cho, Fukuoka*; *c) Kitahama, Higashi-ku, Osaka.*
3) S. Hayashi, H. Ueki, and J. Komiya, *Gann.*, **55**, 289 (1964).
4) S. Hayashi, M. Furukawa, J. Yamamoto, and K. Niigata, *Chem. Pharm. Bull.* (Tokyo), **15**, 1188 (1967).
5) S. Hayashi, M. Furukawa, Y. Fujino, and H. Matsukura, *Chem. Pharm. Bull.* (Tokyo), **17**, 419 (1969).
6) S. Hayashi, M, Furrukawa, and H. Ueki, Unpublished.

in the case of difunctional thiosulfates, cyclic dithio derivatives are possible to form in addition to linear dithio derivatives (Chart 2).

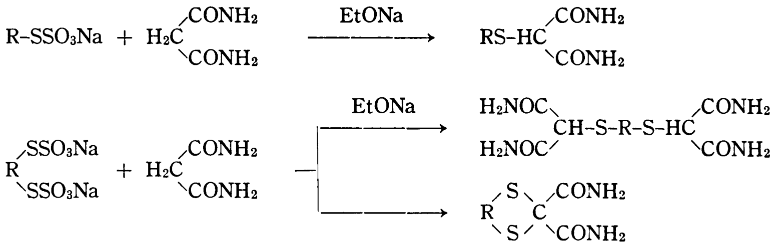


Chart 2

The thiosulfates employed in the reaction were sodium *n*-butyl-thiosulfate, sodium benzylthiosulfate, disodium ethylenebisthiosulfate, disodium trimethylenebisthiosulfate and disodium *o*-xylenedithiosulfate. These thiosulfates were unsuccessful to react with strong active methylene compounds, diethyl malonate, ethyl acetoacetate, ethyl cyanoacetate and acetylacetone and the materials were recovered, though sodium benzylthiosulfate exclusively reacted with diethyl malonate and ethyl acetoacetate to give diethyl benzylthiomalonate and ethyl α-benzylthioacetoacetate in low yields, respectively. However, the reaction with weak active methylene compounds, malondiamide and cyanoacetamide was successful to give the expected compounds, which the corresponding alkylmercapto group was introduced to the active methylene compounds, except the reaction of sodium benzylthiosulfate with malondiamide resulting in failure. Cyclic dithio derivatives were formed by the reaction of the difunctional thiosulfates, disodium ethylenebisthiosulfate and disodium trimethylenebisthiosulfate, with malondiamide or cyanoacetamide, without formation of any expected linear dithio derivatives. On the other hand, disodium *o*-xylenedithiosulfate reacted with malondiamide to give the cyclic dithio compound and with cyanoacetamide to give the linear dithio compound (Chart 3). As described in the previous paper, in the reaction of phenyl benzenethiosulfonate with malondiamide, the ready elimination of one carboximade group from initially formed bis(phenylmercapto)malondiamide was observed. Analogously, the reaction of difunctional thiosulfates, such as disodium trimethylenebisthiosulfate and disodium

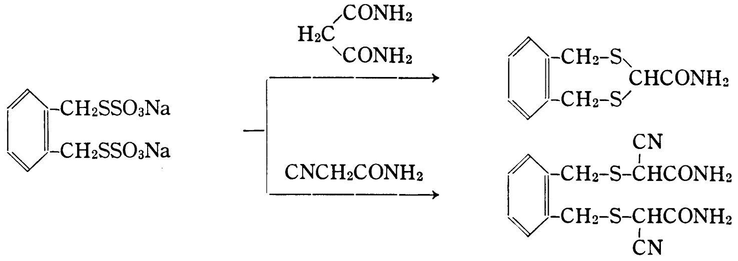


Chart 3

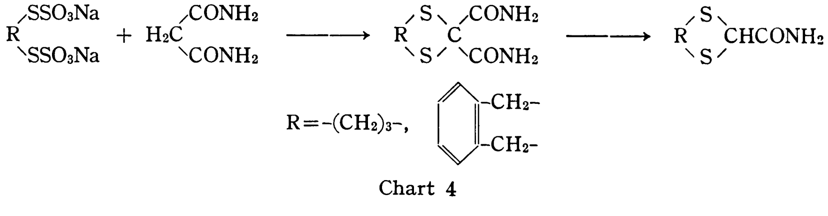
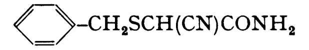
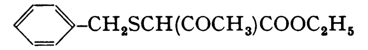
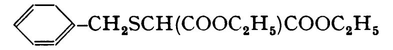
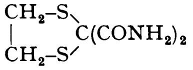
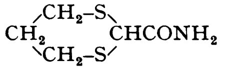
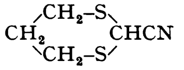
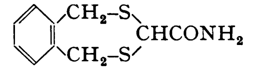
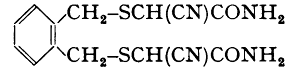


Chart 4

o-xylenedithiosulfate, with malondiamide resulted in the formation of cyclic dithioacetamides which probably formed through the elimination of one of the carboxamide group (Chart 4), which was confirmed with infrared (IR) and Mass Spectra.

The compounds obtained were summarized in Table I.

Table I

No.	Compound	mp or bp	Apprearance	Yield (%)
I	n-$C_4H_9SCH(CONH_2)_2$	195—195.5	needles	19.7
II	n-$C_4H_9SCH(CN)CONH_2$	136—136.5	plates	46.4
III	 -$CH_2SCH(CN)CONH_2$	169—170	plates	22.9
IV	 -$CH_2SCH(COCH_3)COOC_2H_5$	140—142/7mm	liquid	50.8
V	 -$CH_2SCH(COOC_2H_5)COOC_2H_5$	45— 46	prisms	17.1
VI	 $CH_2\text{-}S\text{-}C(CONH_2)_2\text{-}S\text{-}CH_2$	239—240	prisms	25.0
VII	 $CH_2(CH_2\text{-}S)_2CHCONH_2$	171	needles	24.4
VIII	 $CH_2(CH_2\text{-}S)_2CHCN$	91	needles	55.2
IX		219—220	needles	20.3
X		164—165	prisms	52.4

It has been known that thiosulfonate reacted with amine to give the corresponding sulfenamide.[7,8] Thus, sodium benzylthiosulfate was attempted to react with amines. Equivalent amounts of sodium benzylthiosulfate and amines such as piperidine, morpholine, pyrrolidine, piperazine, ethanolamine, benzylamine, dimethylamine and diethylamine were heated in benzene under reflux, respectively. However, in every case, dibenzyldisulfide was obtained, no trace of any benzylsulfenamide expected being isolated.

The reaction of sodium alkylthiosulfates with thiols is expected to give asymmetric disulfides. Thus, sodium benzylthiosulfate, disodium ethylenebisthiosulfate or trimethylenebisthiosulfate and isopropylthiol or thiophenol were allowed to react in absolute ethanol at room temperature in the presence of sodium ethoxide in nitrogen atmosphere, respectively. In the case of sodium benzylthiosulfate and isopropylthiol or thiophenol, the formation of dibenzyldisulfide and diphenyldisulfide was respectively observed, but in another cases, the materials were recovered. In all cases, the expected asymmetric disulfide was not obtained.

Experimental

n-Buthylmercaptomalondiamide (I)——To a solution of 0.92 g (0.04 mole) of sodium in 300 ml of anhyd. EtOH was added 4.08 g (0.04 mole) of malondiamide and 7.68 g (0.04 mole) of sodium n-butylthiosulfate. The solution was allowed to stand for 13 hr at room temperature with stirring. After completion of the reaction, the filtrate was concentrated in vacuum and diluted with H_2O. White precipitates deposited were collected and recystallized from anhyd. EtOH to give 1.5 g (19.7%) of needles melting at 195—195.5°. *Anal.* Calcd. for $C_7H_{14}O_2N_2S$: C, 44.19; H, 7.42; N, 14.75. Found: C, 44.47; H, 7.53; N, 14.63. IR ν_{max}^{KBr} cm^{-1}: 3350, 3180, 1660 ($CONH_2$).

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7) B.G. Boldyrev and S.A. Kolesnikova, *Zh. Obsch. Khim.*, **35**, 198 (1965) [*C.A.*, **62**, 13076e (1965)].

8) J.E. Dunbar and J.H. Rogers, *J. Org. Chem.*, 31, 2842 (1965); *Tetrahedron Letters*, **1965**, 4291.

n-Butylmercaptocyanoacetamide (II)——A mixture of 2.72 g (0.04 mole) of sodium ethoxide, 4.52 g (0.06 mole) of cyanoacetamide and 7.68 g (0.04 mole) of sodium *n*-butylthiosulfate was treated similarly to the compound described above. After completion of the reaction, the filtrate was evaporated in vacuum, diluted with H_2O and acidified with HCl. Resulted white precipitates were filtered and recrystallized from anhyd. EtOH to give 3.2 g (46.4%) of plates melting at 136—136.5°. *Anal.* Calcd. for $C_7H_{12}ON_2S$: C, 48.81; H, 7.02; N, 16.27. Found: C, 48.87; H, 6.90; N, 15.78. IR ν_{max}^{KBr} cm^{-1}: 2279 (CN), 3375, 3187, 1669 ($CONH_2$).

Benzylmercaptocyanoacetamide (III)——A mixture of 2.72 g (0.04 mole) of sodium ethoxide, 3.4 g (0.05 mole) of cyanoacetamide and 9.04 g (0.04 mole) of sodium benzylthiosulfate was treated as described above. After completion of the reaction, EtOH was distilled off and the residue was diluted with H_2O. Dibenzyldisulfide isolated was filtered off and the filtrate was acidified with HCl. White precipitates deposited were recrystallized from EtOH to give 1.9 g (22.9%) of plates melting at 169—170°. *Anal.* Calcd. for $C_{10}H_{10}ON_2S$: C, 58.32; H, 4.88; N, 13.58. Found: C, 58.63; H, 5.05; N, 13.68. IR ν_{max}^{KBr} cm^{-1}: 2270 (CN), 3360, 3175, 1670 ($CONH_2$).

Ethyl α-Benzylmercaptoacetoacetate (IV)——A solution of sodium ethoxide (from 0.6 g, 0.025 atom of sodium), 5.0 g (0.0375 mole) of ethyl acetoacetate and 8.48 g (0.0375 mole) of sodium benzylthiosulfate in 200 ml of anhyd. EtOH was refluxed with stirring for 2 hr. After filtration of the resulted precipitates and evaporation of EtOH, the residue was diluted with H_2O, followed by extraction of the separated oily product with ether. The extract was dried over Na_2SO_4 and evaporated. Distillation of the residue under reduced pressure gave 3.2 g (50.8%) of colorless liquid boiling at 142°/7 mm. *Anal.* Calcd. for $C_{13}H_{16}O_3S$: C, 61.85; H, 6.39. Found: C, 62.21; H. 6.64. IR ν_{max}^{film} cm^{-1}: 1732 ($CO_2C_2H_5$).

Diethyl Benzylmercaptomalonate (V)——A solution of sodium ethoxide (from 0.92 g, 0.04 atom of sodium), 6.4 g (0.04 mole) of diethyl malonate and 9.04 g (0.04 mole) of sodium benzylthiosulfate in 300 ml of anhyd. EtOH was heated with stirring for 10 hr under reflux. After the solution was filtered and the filtrate was evaporated, the residue was poured into H_2O and extracted with ether. The extracts were dried over Na_2SO_4 and evaporated. Distillation of the residue under reduced pressure gave 2.0 g (17.1%) of colorless liquid boiling at 175—176°/9 mm. The melting point of the prisms solidified on cooling was 45—46°. The product was confirmed to be identical with the authentic sample of diethyl benzylmercaptomalonate prepared by the another method by comparison of the IR spectra. *Anal.* Calcd. for $C_{14}H_{18}O_4S$: C, 59.54; H, 6.42. Found: C, 59.50; H, 6.11. IR ν_{max}^{film} cm^{-1}: 1745 ($CO_2C_2H_5$).

1,3-Dithiacyclopentane-2,2-biscarboxamide (VI)——A solution of sodium ethoxide (from 1.2 g, 0.05 atom of sodium), 3.9 g (0.0375 mole) of malondiamide and 7.5 g (0.025 mole) of disodium ethylenebisthiosulfate in 300 ml of anhyd. EtOH was stirred for 13 hr at room temperature. The precipitates were collected, washed with H_2O and extracted with N,N-dimethylformamide (DMF). After removal of DMF, the residue was recrystallized from hot water to give 1.2 g (25.0%) of prisms melting at 239—240°. *Anal.* Calcd. for $C_5H_8O_2S_2$: C, 31.26; H, 4.20; N, 14.57. Found: C, 30.93; H, 4.36; N, 14.37. IR ν_{max}^{KBr} cm^{-1}: 3371, 3162, 1663 ($CONH_2$). Mass Spectrum *m/e*: 192 (M^+).

1,3-Dithiacyclohexane-2-carboxamide (VIII)——A mixture of 3.4 g (0.05 mole) of sodium ethoxide, 3.8 g (0.03 mole) of malondiamide and 8.0 g (0.025 mole) of disodium trimethylenebisthiosulfate in EtOH was treated similarly to the above compound. After standing for 10 hr with stirring at room temperature, the mixture was filtered and the filtrate was evaporated under reduced pressure. The residue was poured into H_2O with stirring and the separated solid was recrystallized from EtOH to give 1.0 g (24.4 %) of needles melting at 171°. *Anal.* Calcd. for $C_5H_9ONS_2$: C, 36.87; H, 5.67; N, 8.53. Found: C, 36.84; H, 5.57; N, 8.59.

2-Cyano-1,3-dithiacyclohexane (VII)——A mixture of 3.4 g (0.05 mole) of sodium ethoxide, 2.11 g (0.025 mole) of cyanoacetamide and 8.0 g (0.025 mole) of disodium trimethylenebisthiosulfate in EtOH was treated as same as described above. Recrystallization of the produt from dil. EtOH gave 2.0 g (55.2 %) of needles melting at 91°. *Anal.* Calcd. for $C_5H_7NS_2$: C, 41.51; H, 4.73; N, 9.75. Found: C, 41.41; H, 4.87; N, 9.73.

1,3-Dithiabenzo[5,6]cycloheptane-2-carboxamide (IX)——A mixture of 3.4 g (0.05 mole) of sodium ethoxide, 2.6 g (0.025 mole) of malondiamide and 9.4 g (0.025 mole) of disodium *o*-xylenedithiosulfate in EtOH was treated as same as described above. After removal of the solvent, the residue was extracted with MeOH and recrystallized from EtOH to give 1.2 g (20.3%) of needles melting at 219—220°. *Anal.* Calcd. for $C_{10}H_{11}ONS_2$: C, 53.30; H, 4.92; N, 6.22. Found: C, 53.75; H, 5.02; N, 6.17. IR ν_{max}^{KBr} cm^{-1}: 3344, 3180, 1652 ($CONH_2$). Mass Spectrum *m/e*: 225 (M^+).

o-Xylene-α,α′-bisthiobis(cyanoacetamide) (X)——A mixture of 3.4 g (0.05 mole) of sodium ethoxide, 2.1 g (0.025 mole) of cyanoacetamide and 9.4 g (0.025 mole) of disodium *o*-xylenedithiosulfate in EtOH was treated as same as described above. After completion of the reaction, the precipitates were collected and extracted with H_2O. Acidification of the extract with HCl gave white precipitates, which was recrystallized from EtOH to give 4.4 g (52.4%) of prisms melting at 164—165°. *Anal.* Calcd. for $C_{14}H_{14}O_2N_4S_2$: C, 50.28; H, 4.22; N, 16.76. Found: C, 50.28; H, 4.30; N, 16.26. IR ν_{max}^{KBr} cm^{-1}: 2275 (CN), 3442, 3402, 3320, 3190, 1681 ($CONH_2$).

Reaction of Sodium Benzylthiosulfate with Amine——A mixture of 0.02 mole of sodium benzylthiosulfate and 0.02 mole of amine in 70 ml of benzene was heated with stirring for 3 hr under reflux. The mixture was filtered and the filtrate was concentrated. The precipitates deposited on cooling were filtered and recrystallized from EtOH to give about 50% of dibenzyldisulfide melting at 71°, which was confirmed to be identical with the authentic sample by comparison of the IR spectra.

Reaction of Sodium Benzylthiosulfate with Thiol——a) With Thiophenol: A mixture of 0.02 mole of sodium benzylthiosulfate and 0.02 mole of thiophenol in 300 ml of anhyd. EtOH containing 0.02 mole of sodium ethoxide was stirred for 24 hr at room temperature under nitrogen atmosphere. The mixture was filtered and the filtrate was concentrated. The precipitates deposited on cooling were filtered and recrystallized from EtOH to give 35% of diphenyldisulfide melting at 59°, which was confirmed to be identical with the authentic sample by comparison of the IR spectra.

b) With Isopropylthiol: A mixture of 0.02 mole of sodium benzylthiosulfate and 0.02 mole of isopropylthiol was treated as same as described above. In the result, 55% of dibenzyldisulfide, which was confirmed to be identical with the authentic sample by comparison of the IR spectra, was obtained.

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