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Studies on Antitumor Substances. IX.[1] Chemical Behaviors of Thiosulfonate toward Active Methylene Compound

Seigoro Hayashi, Mitsuru Furukawa, Yoko Fujino[2a] and Hayashi Matsukura[2b]

Faculty of Pharmaceutical Sciences, Kumamoto University[2a] and Dainippon Seiyaku Co., Ltd.[2b]

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The reactivities of phenyl benzenethiosulfonate, *o*-nitrophenyl benzenethiosulfonate and 1,2-bis(benzenesulfonylthio)ethane toward several active methylene compounds were examined. Consequently, bis(phenylmercapto)malondiamide obtained by the reaction of phenyl benzenethiosulfonate with malondiamide at room temperature was found to eliminate readily one of carboxamide and phenylmercapto group by warming at the temperature above 35°. On the other hand, bis(phenylmercapto)malonate was similarly eliminated one of phenylmercapto group under the same condition, without elimination of carboxylate group. 1-Benzylmercapto-1-phenylmercaptomalondiamide and 1-ethyl-1-phenylmercaptomalondiamide were eliminated one of carboxamide group, without elimination of any mercapto group. Moreover, 1,2-bis(benzenesulfonylthio)ethane was allowed to react with malondiamide and acetylacetone to give cyclic compound, ethylenedithiomalondiamide and ethylenedithioacetylacetone, respectively. With comparison of these reactions, the reaction of benzyl benzenesulfonate with cyanoacetamide was carried out to result in the benzylation of cyanoacetamide.

In order to elucidate the mechanism of the action of several thiosulfonates, having antitumor and other biological activities, their chemical behaviors have been studied by Hayashi, *et al.*[3a,b] During their investigations, it was found that benzyl arylthiosulfonate was allowed to react with active methylene compounds to introduce benzylmercapto group.[3a] While the antitumor effect of Myleran, in which the sulfonate functional group was involved, has been suggested to be attributed to the alkylating property.[4]

Thus, it was first attempted to make certain of the difference between the reactivities of thiosulfonates and sulfonates toward active methylene compounds. For the purpose of this, benzyl benzenesulfonate was submitted to react with cyanoacetamide. As anticipated, cyanoacetamide was benzylated to give the mixture of mono- and di-benzylcyanoacetamide, as shown in Chart 1. Then, in order to further extend these observations, the reactions

1) Part VIII: S. Hayashi, M. Furukawa, Y. Fujino and S. Yoshimatsu, *Chem. Pharm. Bull.* (Tokyo), **17**, 329 (1968).
2) Location: *a*) *Oe-hon-machi, Kumamoto*; *b*) *Doshomachi, Higashi-ku, Osaka.*
3) *a*) S. Hayashi, M. Furukawa, J. Yamamoto and Niigata, *Chem. Pharm. Bull.* (Tokyo), **15**, 1188 (1967); *b*) S. Hayashi, M. Furukawa, J. Yamamoto and K. Hamamura, *Chem. Pharm. Bull.* (Tokyo), **15**, 1310 (1967).
4) G.M. Timmis and R.F. Hudson, *Ann. N.Y. Acad. Sci.*, **68**, 727 (1958).

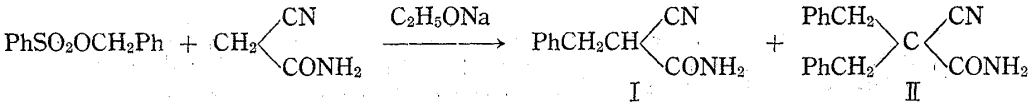

Chart 1

were attempted between aryl arylthiosulfonates, such as phenyl benzenethiosulfonate[5] and *o*-nitrophenyl benzenethiosulfonate,[6] and bifunctional thiosulfonate, 1,2-bis(benzenesulfonylthio)ethane,[7] and several active methylene compounds, such as acetylacetone, cyanoacetamide, malondiamide and ethyl malonate. The reactions were carried out in the presence of equivalent amount of sodium ethoxide in the ethanolic solution containing the equivalent amounts of the both reactants at room temperature. In the reaction of phenyl benzenethiosulfonate and *o*-nitrophenyl benzenethiosulfonate with all of these active methylene compounds except malondiamide, the corresponding derivatives, in which phenylmercapto and *o*-nitrophenylmercapto groups were introduced to the active methylene compounds, were respectively obtained. On the other hand, the equivalent amount of malondiamide reacted with phenyl benzenethiosulfonate to give three products, depending on the difference of the reaction temperature, as shown in Chart 2.

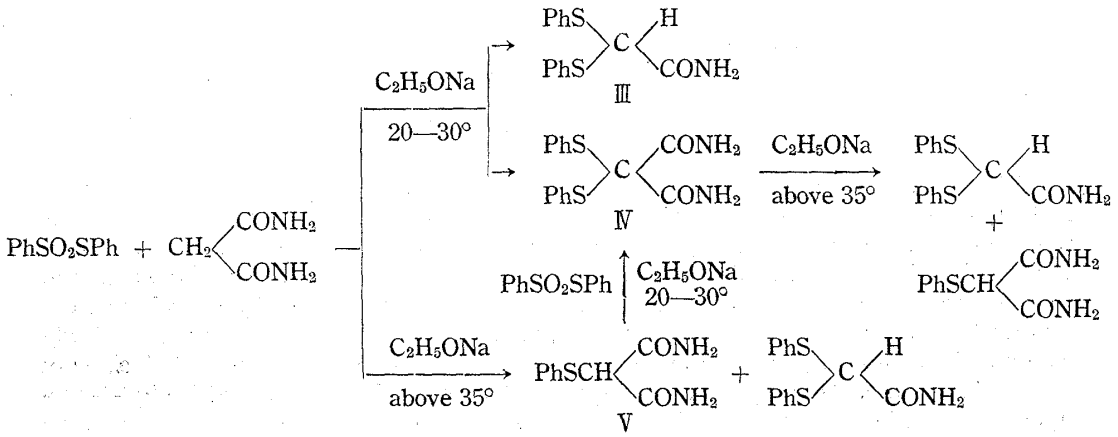

Chart 2

Namely, at room temperature bis(phenylmercapto)acetamide (III) and bis(phenylmercapto)malondiamide (IV) were obtained in 44% and 11% yields, respectively. On the other hand, when the same reaction was carried out at the temperature above 35°, phenylmercaptomalondiamide (V) and bis(phenylmercapto) acetamide (III) were formed in 56% and 21% yields, respectively. These results might suggest that one of two phenylmercapto groups induced to malondiamide at room temperature was readily eliminated by heating above 35° to give phenylmercaptomalondiamide (V). In order to ascertain this assumption, phenylmercaptomalondiamide (V) was first treated with the equimolar amount of phenyl benzenethiosulfonate at room temperature. In the result, bis(phenylmercapto)malondiamide (IV) was obtained in 75% yield, accompanying the secondary formation of bis(phenylmercapto)-acetamide (III) in 10% yield, which probably resulted by the elimination of one carboxamide group from bis(phenylmercapto)malondiamide (IV). Elimination reaction of bis(phenylmercapto)malondiamide (IV) was then confirmed to give phenylmercaptomalondiamide (V) and bis(phenylmercapto)acetamide (III) in 20% and 26% yields, respectively, by warming at the temperature above 35° under the same conditions. Similarly, the reaction between phenylmercaptomalondiamide and benzyl benzenethiosulfonate gave 1-benzylmercapto-1-

5) E. Vinkler and F. Klirenyi, *C.A.*, **49**, 2346 (1955).
6) J.D. Loudor and A. Livingston, *J. Chem. Soc.*, **1935**, 896.
7) S. Hayashi, H. Ueki, S. Harano, J. Komiya, S. Iyama, K. Harano, K. Miyata, K. Niigata and Y. Yonemura, *Chem. Pharm. Bull.* (Tokyo), **12**, 1271 (1964).

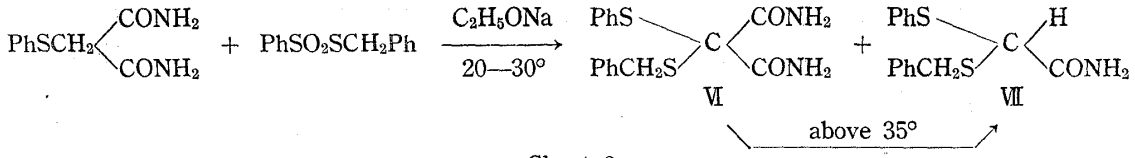

Chart 3

phenylmercaptomalondiamide (VI) and 1-benzylmercapto-1-phenylmercaptoacetamide (VII), according to Chart 3. Different from bis(phenylmercapto)malondiamide (IV), the elimination reaction of 1-benzylmercapto-1-phenylmercaptomalondiamide (VI) under the same condition, however, did not afford the anticipated compound which resulted from the elimination of either phenylmercapto or benzylmercapto group, but only 1-benzylmercapto-1-phenylmercaptoacetamide (VII) which lost one of the carboxamide group was obtained in good yield. The elimination of 1-ethyl-1-phenylmercaptomalondiamide (VIII), which was prepared by the treatment of phenyl benzenethiosulfonate with ethylmalondiamide, was analogously carried out to afford 1-phenylmercaptobutamide (IX), without any elimination of ethyl and phenylmercapto groups, as shown in Chart 4. From these results, it might be considered that

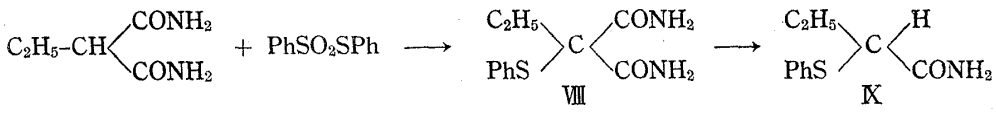

Chart 4

the carboxamide group could be more easily eliminated than the substituted mercapto group or the alkyl group.

The reaction of phenyl benzenethiosulfonate with diethyl malonate also afforded similarly diethyl bis(phenylmercapto)malonate (X) at room temperature, while diethyl phenylmercaptomalonate (XI) at the temperature above 35°, according to Chart 5. However, no reaction

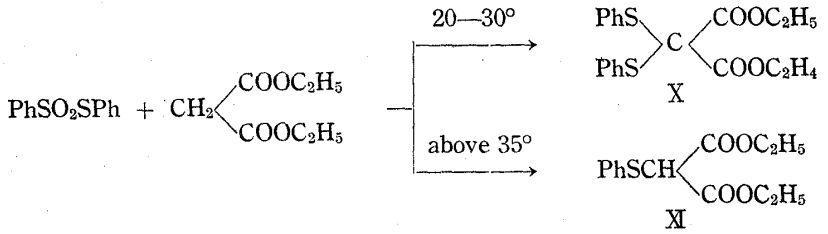

Chart 5

product eliminated the carboxylate group was obtained in this reaction. On the other hand, the reaction of phenyl benzenethiosulfonate with acetophenone gave rise to bis(phenylmercapto)acetophenone at room temperature, while diphenyldisulfide at the temperature above 35°, and phenylmercaptoacetophenone anticipated was not obtained in the course of the reaction.

In the reaction between bifunctional thiosulfonate and active methylene compound, there should be two possible courses of the reaction, for example, the formation of the cyclic compound

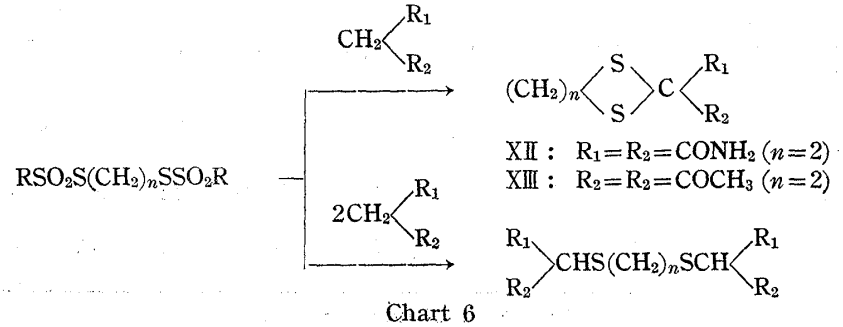

Chart 6

by the condensation of the reactants and the formation of bissubstituted mercapto compound by the reaction of one mole of the thiosulfonate with two moles of the active methylene compound according to Chart 6.

To reveal these possibilities, the reactions of 1,2-bis(benzenesulfonylthio)ethane and 1,3-bis(*p*-toluenesulfonylthio)propane with various active methylene compounds, such as malondiamide, acetylacetone, ethyl acetoacetate and diethyl malonate, were carried out. In the results, 1,2-bis(benzenesulfonylthio)ethane reacted with malondiamide and acetylacetone to afford the corresponding cyclic compounds, ethylenedithiomalondiamide (XII) and ethylenedithioacetylacetone (XIII), respectively, but not with the other active methylene compounds. In these cases, about 30% of materials and a small amount of oily products were merely isolated.

Table I

Product	Yield (%)	mp or bp (°C)	Formular	Analysis (%) Calcd. C	H	N	Found C	H	N
$PhSCH<^{CN}_{CONH_2}$	57	151	$C_9H_8ON_2S$	56.23	4.20	14.57	56.25	4.14	14.59
$(PhS)_2C<^{CONH_2}_{CONH_2}$	44	209—210	$C_{15}H_{14}O_2N_2S_2$	56.59	4.43	8.80	57.06	4.61	9.05
$PhSCH<^{CONH_2}_{CONH_2}$	56	234—235	$C_9H_{10}O_2N_2S$	51.41	4.80	13.32	51.32	4.83	13.25
$(PhS)_2C<^{H}_{CONH_2}$	21	154	$C_{14}H_{13}ONS$	61.11	4.76	5.09	61.26	4.70	5.45
$PhCH_2S>C<^{CONH_2}_{CONH_2}$ (PhS)	81	192—194	$C_{16}H_{16}O_2N_2S_2$	57.81	4.86	8.43	58.05	4.76	8.00
$PhCH_2S>C<^{H}_{CONH_2}$ (PhS)	11	147—150	$C_{15}H_{15}ONS$	62.25	5.22	4.84	62.19	5.16	4.98
$PhS>C<^{CONH_2}_{CONH_2}$ (Et)	96	191—192	$C_{11}H_{14}O_2N_2S$	55.44	5.92	11.75	55.18	5.94	11.43
$PhS>C<^{H}_{CONH_2}$ (Et)	51	94—95	$C_{10}H_{13}ONS$	61.49	6.71	7.17	61.30	6.71	6.88
$PhSCH<^{COCH_3}_{COCH_3}$	52	94—95/2	$C_{11}H_{12}O_2S$	63.42	5.84	—	63.28	5.92	—
$(PhS)_2C<^{COOEt}_{COOEt}$	27	107—109/2	$C_{19}H_{20}O_4S_2$	60.62	5.36	—	60.96	5.56	—
$PhSCH<^{COOEt}_{COOEt}$	29	132—134/2	$C_{13}H_{16}O_4S$	58.61	5.99	—	58.19	6.02	—
$(PhS)_2C<^{H}_{COPh}$	21	97—98	$C_{20}H_{16}OS_2$	71.39	4.80	—	71.19	4.84	—
*o-$NO_2C_6H_4SCH<^{COCH_3}_{COCH_3}$	75	136—137	$C_{11}H_{12}O_4NS$	51.95	4.76	5.51	—	—	—
o-$NO_2C_6H_4SCH<^{COOEt}_{COOEt}$	68	32— 33.5	$C_{13}H_{15}O_6NS$	49.83	4.83	4.47	49.79	4.82	4.39
o-$NO_2C_6H_4SCH<^{COOH}_{COOEt}$	97	71—72	$C_{12}H_{13}O_5NS$	50.88	4.62	4.94	51.00	4.53	4.88
$(o$-$NO_2C_6H_4S)_2C<^{H}_{CONH_2}$	65	196—198	$C_{14}H_{11}O_5N_3S_2$	46.02	3.04	11.50	45.96	2.99	11.08
CH_2–S / CH_2–S $>C<^{CONH_2}_{CONH_2}$	31	221—222	$C_5H_8O_2N_2S_2$	31.28	4.20	14.59	31.31	4.40	14.82
CH_2–S / CH_2–S $>C<^{COCH_3}_{COCH_3}$	27	104/2	$C_7H_{10}O_2S_2$	44.19	5.27	—	44.70	5.33	—
*$(PhCH_2)_2C<^{CN}_{CONH_2}$	28	164—165	$C_{17}H_{16}ON_2$	77.31	6.11	10.60	—	—	—
*$PhCH_2CH<^{CN}_{CONH_2}$	16	128—129	$C_{10}H_{10}ON_2$	65.20	8.76	15.21	—	—	—

A sign of (*) shows known compounds.

The oily products were not able to confirm the structure because of the difficulty of the purification due to easy decomposition during distillation. In the reaction of 1,3-bis(*p*-toluenesulfonylthio)propane with these active methylene compounds, no products anticipated were obtained even in the case of the reaction with malondiamide and acetylacetone. All of the compounds obtained in these various reactions were listed in Table 1.

Experimental

Reaction of Benzyl Benzenesulfonate with Cyanoacetamide——To a solution of EtONa prepared by dissolving 0.46 g (0.02 mole) of Na in 40 ml of abs. EtOH, 1.7 g (0.02 mole) of cyanoacetamide was added with stirring. After stirring for 10 min, 5 g (0.02 mole) of benzyl benzenesulfonate in 40 ml of abs. EtOH was added with stirring at 30—40° and EtOH was removed by evaporation *in vacuo*. To the residue was added 60 ml of H_2O. The insoluble precipitates were collected by filtration and recrystallized from ligroin–EtOH to give plate crystals of dibenzylcyanoacetamide (II). After the aqueous filtrate was allowed to stand overnight, the deposited crystals were collected and recrystallized from benzene to give prisms of benzylcyanoacetamide (I).

Reaction between Thiosulfonate and Active Methylene Compound——a) General Procedure: To a solution of EtONa prepared by dissolving 0.46 g (0.02 mole) of Na in 40 ml of abs. EtOH, 0.02 mole of active methylene compound was added with stirring at room temperature. After stirring for 10 min, a solution of 0.02 mole of thiosulfonate in 40 ml of abs. EtOH was added dropwise with stirring into the mixture at 30—40° during 30 min, and the stirring was further continued for 4 hr maintaining the temperature at 30—40°. After completion of the reaction, the solvent was removed by evaporation *in vacuo*. The resulting residue was agitated with a mixture of 80 ml of ether and 50 ml of H_2O. The etheral layer was dried over Na_2SO_4 and concentrated *in vacuo* to give a crystalline product or an oily product. The crude product was purified by recrystallization or distilation.

b) Reaction of Phenyl Benzenethiosulfonate with Cyanoacetamide: To a solution of EtONa prepared by dissolving 0.14 g of Na in 40 ml of abs. EtOH, 0.5 g of cyanoacetamide was added with stirring. After stirring for ten min, the solution of 1.5 g of phenyl benzenethiosulfonate in 40 ml of abs. EtOH was added dropwise with stirring at 30—40° to the mixture and the stirring was continued for 4 hr at this temperature. Then, the solvent was removed *in vacuo*, and the residue was extracted with a mixture of ether and H_2O (2:1). The aqueous layer was acidified with conc. HCl. The resulting precipitates were collected, washed with H_2O and recrystallized from EtOH to give phenylmercaptocyanoacetamide. From the etheral layer, a small amount of diphenyldisulfide was obtained.

c) Reaction of Phenyl Benzenethiosulfonate with Malondiamide: (1) To a solution of EtONa prepared by dissolving 0.14 g of Na in 40 ml of abs. EtOH, 0.62 g of malondiamide was added. After stirring for 30 min, a solution of 1.5 g of phenyl benzene thiosulfonate in 50 ml of abs. EtOH was added dropwise with stirring at 20—30° into the mixture during a period of 30 min. The stirring was continued for 5 hr at this temperature. The resulting precipitates were collected, washed with EtOH and recrystallized from EtOH–dimethylformamide to give 0.96 g (44%) of bis(phenylmercapto)malondiamide (IV) melting at 209—210°. The filtrate was evaporated *in vacuo* and the residue was extracted with H_2O. The insoluble precipitates were collected by filtration and recrystallized from benzene to give 0.2 g (11%) of bis(phenylmercapto) acetamide (III) melting at 154.°

ii) The reaction described above was carried out at 40°. Consequently, precipitates deposited from the reaction mixture were collected on cooling, washed with EtOH and recrystallized from EtOH–dimethylformamide to give 56% of phenylmercaptomalondiamide (V) melting at 234—235°. From the filtrate, 21% of bis(phenylmercapto)acetamide (III) was obtained.

d) Reactions of Thiosulfonates with Substituted Malondiamides: i) Bis(phenylmercapto)malondiamide (IV) from Phenylmercaptomalondiamide (V): To an alcoholic solution of EtONa prepared from 0.16 g of Na and abs. EtOH, 1.5 g of phenylmercaptomalondiamide was added with stirring at 20—30°. After stirring for 20 min, a solution of 1.7 g of phenyl benzenethiosulfonate in abs. EtOH was added dropwise with stirring at 20—30° during a period of 30 min. The stirring was continued for 4 hr at this temperature and the resulting precipitates were collected by filtration, washed with EtOH and recrystallized from EtOH–DMF to give 1.7 g (75%) of bis(phenylmercapto)malondiamide. From the filtrate, 0.2 g (10%) of bis(phenylmercapto)acetamide was obtained.

ii) 1-Benzylmercapto-1-phenylmercaptomalondiamide (VI) and 1-Benzylmercapto-1-phenylmercaptoacetamide (VII): These products were obtained from the reaction between benzyl benzenethiosulfonate and phenylmercaptomalondiamide under the same condition as described above.

iii) 1-Phenylmercapto-1-ethylmalondiamide (VIII): A mixture of EtONa prepared from 0.2 g of Na, 1.3 g of ethylmalondiamide and 2.5 g of phenyl benzenethiosulfonate in EtOH were treated at 40° under the same condition. The product was recrystallized from EtOH.

e) Elimination Reaction of Disubstituted Malondiamide: i) Phenylmercaptomalondiamide (V) and Bis(phenylmercapto)acetamide (III) from Bis(phenylmercapto)malondiamide (IV): To a solution of EtONa prepared by dissolving 0.11 g of Na in 50 ml of abs. EtOH, 1.5 g of bis (phenylmercapto) malondiamide was added with stirring at 40—50°. The stirring was continued for 5 hr at this temperature and the solvent was removed under reduced pressure. The residue was extracted with H_2O, and the resulting precipitates were collected and extracted with hot EtOH. The extract gave 0.34 g (26%) of bis(phenylmercapto) acetamide on cooling. The insoluble part in hot EtOH was recrystallized from EtOH–DMF to give 0.5 g (20%).

ii) 1-Benzylmercapto-1-phenylmercaptoacetamide (VII) from 1-Benzylmercapto-1-phenylmercaptomalondiamide (VI): A suspension of 2.2 g of 1-benzylmercapto-1-phenylmercaptomalondiamide in 50 ml of abs. EtOH dissolving 0.15 g of Na was treated under the same condition as described above.

f) Reaction between Phenyl Benzenethiosulfonate and Ethyl Malonate: i) Diethyl Bis(phenylmercapto)-malonate (X): To a solution of EtONa prepared by dissolving 0.02 mole of Na in 40 ml of abs. EtOH, 0.02 mole of diethyl malonate was added with stirring at 20—30°. After stirring for 10 min, a solution of 0.02 mole of phenyl benzenethiosulfonate in 50 ml of abs. EtOH was added dropwise with stirring at 20—30° during a period of an hour. The stirring was continued for 5 hr and the solvent was evaporated under reduced pressure. The residue was extracted with ether, washed with H_2O and dried over Na_2SO_4. After the ether was removed *in vacuo*, the residual yellow oily product was purified by distillation under reduced pressure.

ii) Diethyl Phenylmercaptomalonate (XI): It was obtained by the procedure similar to that of diethyl bis(phenylmercapto)malonate at 40—50°.

g) Reaction of 1,2-Bis(benzenesulfonylthio)ethane with Malondiamide: A suspension of 1.2 g (0.01 mole) of malondiamide in 60 ml of abs. EtOH containing 0.46 g (0.02 mole) of Na was stirred at 40—50° for 20 min. To the suspension, 3.74 g (0.01 mole) of 1,2-bis(benzenesulfonylthio)ethane was added little by little with stirring at 40—45° during a period of an hour. After stirring was continued for 7 hr at 40—45°, the solvent was removed *in vacuo* and a small amount of crystalline residue was obtained. The residue was extracted with H_2O and the resulting insoluble precipitates were collected and recrystallized from H_2O.

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