[CONTRIBUTION FROM THE CHEMO-MEDICAL RESEARCH INSTITUTE AND THE GEORGETOWN UNIVERSITY MEDICAL CENTER[1]]

DIISOTHIURONIUM DIHYDROHALIDE SALTS

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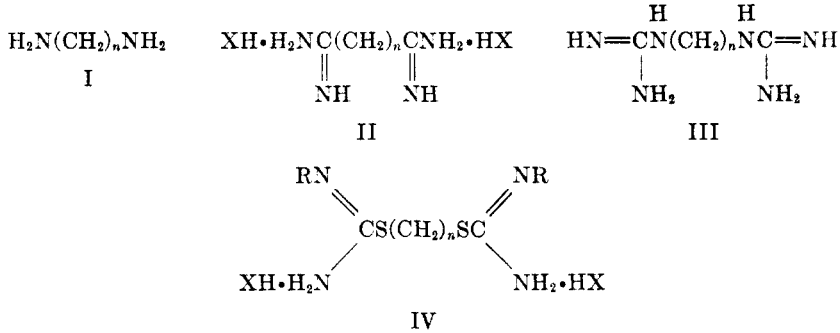
Received December 23, 1952

During the course of the investigation of literally thousands of compounds, mixtures, extracts, etc., in the perpetual search for possible chemotherapeutic agents for neoplasms, certain polymethylene compounds with *alpha-omega* polar groups were found to be naturally occurring inhibitory substances for certain types of tumors (1, 2). Boyland (1) concluded, after a study of a number of naturally occurring and synthetic compounds of this type, that certain polymethylene compounds with acidic or basic end groups were able to inhibit the growth of tumors.

An extended investigation covering aliphatic diamines (I), diamidines (II), diguanidines (III), as well as a large number of aromatic bases, was carried out (3, 4). Many of these substances were found to have inhibitory properties toward certain types of tumors. Because of the close similarity of structure to the general type polymethylene *alpha-omega* dipolar compound, and in particular to the diamidines and diguanidines, the diisothiuronium salts (IV) appeared to be a promising group of compounds to investigate.



The general activity of *alpha-omega* polymethylene type compounds (or polymethylene chains in which one or more carbon atoms has been replaced by nitrogen, sulfur, or oxygen) has been further demonstrated by Hendry, *et al.* (5) who have demonstrated that varying degrees of inhibition of tumors are provided by *alpha-omega* dienes and diepoxides as well as compounds having different polar terminal groups; such as, *alpha*-epoxide and *omega*-hydroxy, halo, alkalene, and ester groups. The analogy may also be extended to include the mustards and nitrogen mustards. The central chain need not be aliphatic or substituted aliphatic; but may be acyclic or heterocyclic alkyl or aryl.

[1] Supported in part by a grant from the National Cancer Institute.

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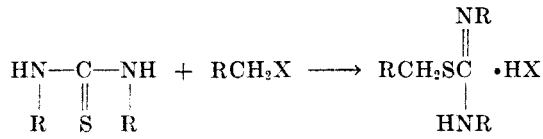
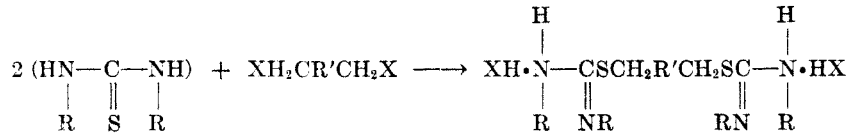
In a comprehensive survey of monofunctional and polyfunctional compounds Hendry, *et al.* (6) found that the majority of monofunctional compounds were inactive and that activity was encountered largely in polyfunctional compounds. In the absence of specific mechanisms of action, such as the alkylation of amino groups postulated for urethans, the general activity of polyfunctional (polypolar) compounds may be tentatively explained on the basis of multipoint attachment to nucleoprotiens or proteins shielding or inhibiting the activity of essential active sites on these molecules. Thus, Boyland's hypothesis of the activity of *alpha-omega* acidic or basic groups must be extended, in view of accumulated data, to include bispolar or polypolar compounds in general irrespective of the acidic or basic nature of the "end groups."

The rather potent physiological activity of isothiuronium and diisothiuronium salts has been demonstrated in several instances. Kawai, *et al.* (7) demonstrated the hypoglycemic action of several diisothiuronium salts as well as that of diamidines and diguanidines. Fastier, *et al.* (8) showed the inhibition of aerobic glycolysis in muscle extracts by diisothiuronium salts; and Blaschko and Duthie (9) reported that certain amidines, diamidines, and diisothiuronium salts all inhibited amine oxidase activity. Recently, Bandelin and Tuschhoff (10), in a survey of long chain alkyl isothiuronium salts and N-substituted isothiuronium salts, showed their activity as germicides.

Isothiuronium salts, (isothiourea or pseudothiourea salts), are in general readily formed by heating or refluxing thiourea or a substituted thiourea and the desired alkyl halide either without solvent (10) or in a variety of solvents. Ethanol has been used in a number of cases (11, 12); *n*-butanol and isoamyl alcohol have been employed (7); as has toluene (10). Stevens (13) showed that ethyl isothiuronium hydrochloride could be formed by heating thiourea hydrochloride with ethanol. Johnson and Sprague (12) found that this method could be extended to higher aliphatic alcohols.



R = hydrogen, alkyl, aryl



R′ = polymethylene, alkylaryl, alkalene

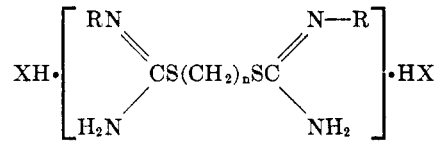
In the present work either 95 % or absolute ethanol was found to be an excellent reaction medium since the salts were generally obtained in excellent yields and in a high state of purity with little or no decomposition. When using higher-boiling media, such as solvents that boil much over 100°, more or less decomposition with the formation of ammonium halides and foul-smelling decomposition

products was observed. The degree of decomposition was dependent on the time of heating and the boiling point of the reflux medium.

Although the reaction to form either mono or diisothiuronium hydrohalide salts proceeded faster when the higher-boiling reflux media were employed, under the conditions employed herein the reaction could be set up and left unattended for long reflux periods, from 6–12 hours to several days, with little or no decomposition.

TABLE I

DIISOTHIURONIUM DIHYDROHALIDE SALTS



Simple: R = H

X	n	FORMULA	YIELD, %	M.P., °C.	ANALYSES, HALOGEN ION[d]	
					Calc'd	Found
Br	2	$C_4H_{12}Br_2N_4S_2$	91	236–237[a,b,*]	46.99	46.77
Br	3	$C_5H_{14}Br_2N_4S_2$	90	203–204[a]	45.13	45.08
Cl	4	$C_6H_{16}Cl_2N_4S_2$	76	227–227.5	25.39	25.44
Cl	5	$C_7H_{18}Cl_2N_4S_2$	84	212.5–213[c]	24.18	24.17
Br	6	$C_8H_{20}Br_2N_4S_2$	95	210–211[c]	40.34	40.18
Br	7	$C_9H_{22}Br_2N_4S_2$	87	168	38.96	38.90
Br	8	$C_{10}H_{24}Br_2N_4S_2$	94	189.5–190	37.68	37.84
Br	9	$C_{11}H_{26}Br_2N_4S_2$	89	143–144[e]	36.46	36.45
Cl	10	$C_{12}H_{28}Cl_2N_4S_2$	78	187.5–188[c]	19.52	19.42

* With decomposition. [a] Mathias, *Bol. fac. filosof. ciênc. e letras, Univ. São Paulo, Quim.*, No. **1**, 75 (1942); C_2, m.p. 225–227°; C_3 m.p. 199–200°. [b] *Org. Syntheses*, **30**, 35 (1950) gives m.p. 225–227°. [c] Reference (7). C_5, m.p. 210°; C_6, m.p. 230–231°; C_{10}, m.p. 186°. [d] All analyses were performed in triplicate (on the salts dried to constant weight at 110°) by titration with 0.05 *N* $AgNO_3$ using dichlorofluoroscein as an indicator. [e] This salt consistently came out as a hemihydrate on precipitations from absolute ethanol with anhydrous ether. When heated for 6 hours in a drying pistol (toluene reflux, P_2O_5 desiccant) it gave the m.p. reported. The hemihydrate softens between 92 and 97°.

By the general method employing ethanol as a reaction medium all of the polymethylene unsubstituted diisothiuronium salts through C^{10} were prepared. These are listed in Table I. A representative group of di (mono-N-alkyl) substituted isothiuronium hydrobromides was prepared and is listed in Table II. Several di (mono-N-aryl) substituted isothiuronium hydrobromides were prepared and are shown in Table III. Several miscellaneous types with variations in R and R′ were prepared and are listed in Table IV.

Under the synthetic conditions employed in this investigation, primary chlorides, bromides, and iodides reacted readily with thiourea. An increased reflux time or smaller volume of ethanol for identical reflux times was required to com-

plete the reaction when employing chlorides. With the mono N-alkyl or aryl substituted thioureas, the chlorides reacted slowly when the N substituent was alkyl and gave little or no reaction when the N substituent was aryl. Bromides reacted readily with mono N-alkyl or aryl substituted thioureas. In the case of N,N′-dialkyl or aryl substituted thioureas, chlorides gave little or none of the

TABLE II

DI-N-ALKYLISOTHIURONIUM DIHYDROBROMIDES

R	n	FORMULA	YIELD, %	M. P., °C.	ANALYSES, HALOGEN ION	
					Calc'd	Found
Methyl	2	$C_6H_{16}Br_2N_4S_2$	90.2	179.5–180	43.41	43.59
Methyl	3	$C_7H_{18}Br_2N_4S_2$	87	183–184	41.82	41.71
Methyl	4	$C_8H_{20}Br_2N_4S_2$	82	233–234*	40.34	40.33
Methyl	5	$C_9H_{22}Br_2N_4S_2$	94	177.5	38.96	38.93
Methyl	6	$C_{10}H_{24}Br_2N_4S_2$	82	176[a]	37.67	37.74
Allyl	2	$C_{10}H_{20}Br_2N_4S_2$	94	161.5	38.03	38.10
Allyl	3	$C_{11}H_{22}Br_2N_4S_2$	95	—[b]	36.80	36.51
Allyl	4	$C_{12}H_{24}Br_2N_4S_2$	93	129–130	35.65	35.55
Allyl	5	$C_{13}H_{26}Br_2N_4S_2$	92	117–117.5	34.57	34.62
Allyl	6	$C_{14}H_{28}Br_2N_4S_2$	93	111–112	33.55	33.36
Allyl	10	$C_{18}H_{36}Br_2N_4S_2$	92	—[c]	29.52	29.61
Butyl	2	$C_{12}H_{28}Br_2N_4S_2$	93	145–146	35.33	35.09
Butyl	3	$C_{13}H_{30}Br_2N_4S_2$	90	—[d]	34.27	33.84
Butyl	4	$C_{14}H_{32}Br_2N_4S_2$	83	123–124	33.27	33.18
Butyl	5	$C_{15}H_{34}Br_2N_4S_2$	94	—[e]	32.33	32.34
Butyl	6	$C_{16}H_{36}Br_2N_4S_2$	96	119.8–120.4	31.44	31.35
Butyl	10	$C_{20}H_{44}Br_2N_4S_2$	92	—[f]	28.31	28.28
Decyl	2	$C_{24}H_{52}Br_2N_4S_2$	90	200–201	25.75	25.92
Decyl	4	$C_{26}H_{56}Br_2N_4S_2$	88	87–90[g]	24.63	24.67

* With decomposition. [a] Separates consistently as a hydrate, softening point 78°. Drying for 6 hours in a drying pistol (toluene reflux, P_2O_5 desiccant) gave the melting point reported.[b–f] These compounds separated as oils which solidified to glasses after prolonged drying over P_2O_5 *in vacuo*. Softening and melting points were taken while in sealed capillaries and also while immersed under anhydrous xylene. All were hygroscopic. [b] Softening point 74°; m.p., 96–97°. [c] Softening point 41–43°; m.p., 61°. [d] Softening point 65°; m.p., 92–94°. [e] Softening point 55°; m.p., 84–86°. [f] Softening point 52°; m.p. 69°. [g] Softening point as obtained from original reaction mixture and after recrystallization from absolute methanol-ether and chloroform-ether; it melts indefinitely. The substance is not a hydrate or an alcoholate as further shown by nitrogen analysis. *Anal.* Calc'd for $C_{26}H_{56}Br_2N_4S_2$: N, 8.63; Found: N, 8.61.

desired product after several days of refluxing. Bromides reacted slowly under identical conditions. Iodides reacted readily with all classes of thioureas investigated; the reaction in all cases was completed within a few minutes to two hours. In several instances where unsubstituted thiourea was involved, the lower dibromides and diiodides reacted violently and required cooling during the initial part of the reaction.

Absolute reagent methanol was also found to be a convenient and an excellent

TABLE III

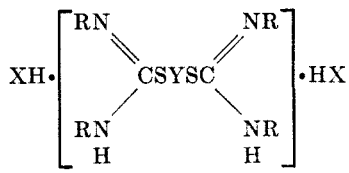
DI-N-ARYLISOTHIURONIUM DIHYDROBROMIDES

R	n	FORMULA	YIELD, %	M.P., °C.	ANALYSES, HALOGEN ION	
					Calc'd	Found
Phenyl	2	$C_{16}H_{20}Br_2N_4S_2$	81	214–215[b]	32.47	32.63
Phenyl	3	$C_{17}H_{22}Br_2N_4S_2$	81	179–180	31.57	31.29
Phenyl	4	$C_{18}H_{24}Br_2N_4S_2$	83	216–217	30.72	30.74
Phenyl	5	$C_{19}H_{26}Br_2N_4S_2$	81	191–192	29.91	29.93
Phenyl	6	$C_{20}H_{28}Br_2N_4S_2$	82	199–200	29.15	29.17
Phenyl	10	$C_{24}H_{36}Br_2N_4S_2$	87	132–133[c]	26.43	26.30
o-Methoxyphenyl	2	$C_{18}H_{24}Br_2N_4O_2S_2$	72	236–238*	28.94	28.81
o-Methoxyphenyl	3	$C_{19}H_{26}Br_2N_4O_2S_2$	79	208–210[a]	28.22	28.16
α-Naphthyl	2	$C_{24}H_{24}Br_2N_4S_2$	66	298–300*	26.98	26.86
α-Naphthyl	3	$C_{25}H_{26}Br_2N_4S_2$	65	287*	26.35	26.28

* With decomposition. [a] Separates as a monohydrate, m.p. 193–194°. The reported value is after several recrystallizations from absolute ethanol and four-hours drying in drying pistol. [b] Bertram, *Ber.*, **25**, 48 (1892); m.p. 214°. [c] The material as originally obtained and after recrystallization from absolute methanol-ether softened at 97–100°. After two additional recrystallizations from chloroform-ether the material melted at 132–133°. *Anal.* Calc'd for $C_{24}H_{36}Br_2N_4S_2$: N, 9.26; Found: N, 9.30.

TABLE IV

DIISOTHIURONIUM DIHYDROHALIDE SALTS-MIXED TYPES



Y	R	X	FORMULA	YIELD, %	M.P., °C.	ANALYSES HALOGEN ION	
						Calc'd	Found
Butene-2[b]	H	Cl	$C_6H_{14}Cl_2N_4S_2$	53	210–211*	25.58	25.56
Butane	Isopropyl	I	$C_{18}H_{40}I_2N_4S_2$	94	224–225*	40.25	40.24
Butane	Ethyl	I	$C_{14}H_{32}I_2N_4S_2$	92	137–138	44.19	44.22
Butane	Butyl	I	$C_{22}H_{48}I_2N_4S_2$	88	—[a]	36.94	36.82
p-Xylylene	H	Br	$C_{10}H_{16}Br_2N_4S_2$	78	241–242*	38.40	38.42

* With decomposition. [a] Viscous oil. [b]Clemence and Leffler, U. S. Patent 2,545,876, March 20, 1951.

medium for the reaction of several iodides with thiourea and N-substituted thioureas.

The majority of the diisothiuronium salts reported herein have been or are being screened at the National Cancer Institute for activity against Sarcoma 37 in mice. The general aspects of the method employed in this screening procedure have been published (14). The results of these tests at the time of this writing

are summarized in Table V. A detailed report of the biological tests and pharmacological properties of these compounds will be published separately at a later date.

TABLE V

SUMMARY OF ACTIVITY OF DIISOTHIURONIUM SALTS AGAINST SARCOMA 37 IN MICE[a]

TYPE OF COMPOUND EVALUATED[b]	NUMBER OF COMPOUNDS OF THIS TYPE TESTED	NUMBER OF COMPOUNDS PRODUCING TUMOR DAMAGE[c]	NUMBER OF COMPOUNDS NOT PRODUCING TUMOR DAMAGE[c]	NUMBER OF COMPOUNDS OF QUESTIONABLE ACTIVITY[c]
R = H	9	8	1	0
R = Alkyl	17	11	4	2
R = Aryl	8	1	7	0

[a] Each compound was evaluated against Sarcoma 37 in mice during its rapidly growing stage. The screening procedure employs initially 8 animals for each test. These are injected with a single dose of the compound near the maximum tolerated dosage level. [b] Refer to general structural formula at beginning of Table I. [c] Tumor damage isevaluated approximately 24 hours after the injection and is based on both gross and histological examination.

EXPERIMENTAL

General procedure for preparation of simple diisothiuroniums, R = H. A good grade of thiourea, m.p. 175–176°, was employed throughout. All dihalides used in this series and the other series reported were purified by fractionation and collected over a 1°-range when distilled at atmospheric pressure or a 2–3°-range when distilled under reduced pressure.

The reactions were carried out in refluxing ethanol in amounts varying from 2 to 5 cc. of ethanol per gram of reactants. The preparations involving dibromides were refluxed for 6–8 hours and those involving dichlorides for 8–10 hours in the lesser amount of solvent. In the majority of cases it was possible to recover nearly quantitative yields of the product in an impure state by removing most of the solvent *in vacuo*. The yields reported in Table I were obtained by allowing the product to crystallize (or inducing crystallization with ether) from the reaction medium. The products obtained in this way were of a relatively high state of purity as shown by the facts that halogen ion determination on the original material (except those which proved to be hydrates) yielded values within 0.1 to 0.4% of theoretical; and the melting points changed little on recrystallization. A detailed procedure may be illustrated by the preparation of the octane member.

Preparation of octane-1,8-diisothiuronium dihydrobromide. Into a 200-cc. round-bottom flask, equipped with a reflux condenser and a drying tube, were placed 5.5 g. (0.072 mole) of thiourea and 9.8 g. (0.036 mole) of octane-1,8 dibromide. Then 60 cc. of absolute ethanol was added and the mixture was refluxed gently, with shaking at first until the thiourea was all dissolved, for a period of 8 hours. It was cooled to room temperature and then in an ice-bath. Ether was added slowly until crystallization ensued and from time to time until very little cloudiness was produced on adding several cc. of ether at once. The crystalline material was removed with rapid suction at the aspirator and washed with two 50-cc. portions of ether. In this manner 14.2 g. of material was obtained, 92.7%, m.p. 184–185°. Two recrystallizations from absolute ethanol gave the material with m.p. 189.5–190°.

Preparation of di-N-alkylisothiuronium dihydrobromides. R = alkyl. No difficulties were encountered in the isolation or crystallization of the products except in the series where R was butyl or allyl. In these series several hygroscopic oils were obtained that could not be readily crystallized. They were all reduced to hygroscopic glasses on prolonged desiccation *in vacuo*. The following examples will illustrate the variations encountered in isolation of the products.

Preparation of butane-1,4 di-N-allylisothiuronium dihydrobromide. Butane-1,4 dibromide (5 g., 0.0232 mole) and 5.4 g. (0.0465 mole) of allylthiourea (thiosinamine), m.p. 74–75°, were refluxed for 8 hours in 50 cc. of ethanol. The reaction mixture was cooled and dry ether was added; an oil separated. A total of 150 cc. of dry ether was added to throw out the salt. After two days under refrigeration the salt solidified and partially crystallized in rosettes of fine needles. The material was very hard and was ground up in a mortar and filtered by washing onto the filter with ether. Yield, 9.6 g. (92.5%), m.p. 128–129°. Recrystallization from absolute ethanol or methanol gave the compound as long fine needles, m.p. 129–130°.

Preparation of pentane-1,5 di-N-butylisothiuronium dihydrobromide. Pentane-1,5 dibromide (5 g., 0.0217 mole) and 5.75 g. (0.0435 mole) of N-butyl thiourea, m.p. 75°, were refluxed for 8 hours in 50 cc. of absolute ethanol. On cooling and adding dry ether, an oil separated which showed no signs of crystallization or solidification after a week under refrigeration. The material was slurried with fresh anhydrous ether daily. The ether was poured off, the material was redissolved in the minimum amount of absolute methanol and was transferred to a small crystallizing dish. This was placed in a vacuum desiccator and all possible methanol removed under a vacuum. The material was stored *in vacuo* over P_2O_5 where, after about 14 days, it had solidified to a brittle hygroscopic glass. On analysis this material corresponded closely to a hemihydrate. On drying portions of the material to constant weight at 110–115°, the analysis corresponded to the anhydrous salt. There were obtained 10.1 g. of material (94%), softening point 55°, m.p. 84–86°, under anhydrous xylene (xylene distilled over sodium).

Preparation of di-N-arylisothiuronium dihydrobromides. R = Aryl. These in the N-aryl series were obtained without difficulty by the general method except that the higher members tended to be oils and required several slurries of dry ether to solidify them. Several of them were recrystallized advantageously from dry chloroform and ether.

Preparation of decane-1,10 dichloride. The synthesis of this compound was reported in reference (7) but a detailed experimental procedure was not given. Since the preparation by the action of thionyl chloride on the diglycol gave excellent results, this procedure is outlined. The decane-1,10 diol was prepared according to Organic Syntheses (15). Four runs of 0.25 mole each were made with yields varying from 54 to 72% of diol, m.p. 71.5–73.5°.

The decamethylene glycol (50 g., 0.286 mole) was placed in a 3-necked reaction flask equipped with a reflux condenser, stirrer, drying tube, and dropping-funnel. Then 68.3 g. (0.573 mole) of c.p., colorless thionyl chloride was added slowly from the dropping-funnel until the initial rapid reaction subsided; the remainder then was run in rapidly. The mixture was stirred gently and heated on a hot plate just enough to cause it to simmer. The heating and stirring were continued for 8 hours. The next day a 25% excess of thionyl chloride was added and the mixture was heated gently for 2 hours. Excess thionyl chloride was decomposed with cold water followed by a saturated solution of sodium carbonate until effervescence ceased. The reaction mixture was saturated with NaCl and extracted with three 100-cc. portions of ether. The ether extracts were dried over Na_2SO_4, the ether was stripped off, and the product was distilled under reduced pressure. There was thus obtained 55.3 g. (91%), b.p. 167–170°/28 mm. Over 90% of the cut boiled between 167–168°/28 mm., d_4^{27} 0.9853.

Preparation of N-o-methoxyphenyl thiourea. Freshly distilled *o*-anisidine (24.6 g., 0.20 mole) was dissolved in 22 cc. (0.26 mole) of conc'd HCl. To this mixture was added 21.4 g. (0.22 mole) of KCNS and the mixture was heated gently to dryness on the water-bath. Heating was continued for 4 hours longer. The mixture was extracted with hot ethanol, the ethanol extract refrigerated, and the crystalline material removed. There was obtained 27 g. of a material, m.p. 153–154°, 74%, which is pure enough for most purposes. Recrystallization of the material gave 21 g. (57.5%), with m.p. 158°.

Anal. Calc'd for $C_8H_{10}N_2S$: N, 15.29. Found: N, 15.32.

Preparation of N-n-decylthiourea. *n*-Decyl isothiocyanate (20 g., 0.1 mole) was added

slowly to 100 cc. of a cooled saturated soln. of ammonia in absolute ethanol. When all the *n*-decyl isothiocyanate had been added, NH_3 was bubbled through the solution and it was allowed to warm up. Some crystalline material appeared in the mixture which redissolved on warming to 60°. The reaction mixture was warmed slowly to 60° and maintained there for 20–30 minutes. Then most of the alcohol was stripped off, giving 18 g. (83%) of material as a partly crystalline cake, m.p. 99–101°. Recrystallization from reagent methanol yielded the compound in large leaflets of a waxy consistency, m.p. 103–104°.

Anal. Calc'd for $C_{11}H_{24}N_2S$: N, 12.95. Found: N, 12.76.

Preparation of di-N,N'-isothiuronium dihydriodides. Those symmetrically disubstituted thioureas employed reacted readily with diiodides in either ethanol or methanol.

Butane-1,4 di-(N,N'-diethyl) isothiuronium dihydriodide. Butane-1,4 diiodide (6.2 g., 0.02 mole) and 5.3 g. (0.04 mole) of *s*-diethyl thiourea were refluxed gently in 50 cc. of absolute ethanol for 2 hours. On cooling and adding 2–5 cc. of dry ether the reaction mixture solidified. Yield, 10.6 g. (92%), of material with m.p. 136–138°. Recrystallization from methanol raised the m.p. to 137–138°.

Butane-1,4 di-(N,N'-diisopropyl) isothiuronium dihydriodide. s-Diisopropyl thiourea (9.6 g., 0.06 mole) and 9.3 g. (0.03 mole) of butane-1,4 diiodide were refluxed 2 hours in 50 cc. absolute methanol. On cooling the reaction mixture solidified to give 17.8 g. of material (94%), m.p. 222–223° dec. Recrystallization from methanol changed the melting point to 224–225° dec.

Butane-1,4 di-(N,N'-dibutyl) isothiuronium dihydriodide. s-Dibutyl thiourea (11.3 g., 0.06 mole) and 9.3 g. (0.03 mole) of butane-1,4 diiodide were refluxed for 2 hours in 60 cc. of absolute ethanol. Cooling, addition of ether, and refrigeration produced an oil which would not crystallize. Long desiccation *in vacuo* over P_2O_5 led to much discoloration. The compound was dried in a high vacuum for analysis after small amounts were redissolved in absolute methanol and thrown out with anhydrous ether.

WASHINGTON 7, D. C.

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