Polymethylene Bis-isothiuronium Salts. Antituberculous Activity

By ARTHUR C. GLASSER and RICHARD M. DOUGHTY

Several polymethylene 1-*m*-chlorophenyl- and 1-phenyl-bis-isothiuronium dihydrobromides were synthesized and examined for their *in vitro* antituberculous activity as well as their effect upon mice through aerosol inhalation studies.

NUMEROUS publications have reported upon a diversity of physiological activities for the isothiuronium type molecule. Hypoglycemic action was indicated by Kawai and co-workers (1) as an extension of their earlier work on the diguanidines (2). Deirscherl and Weingarten have shown anticoagulant as well as hypoglycemic activities (3). Fuller has reported on the germicidal activity of bis-isothioureas ranging from 4 to 16 methylene groups (4), and Umezawa has shown antifungal properties for isothiourea derivatives of 2-butyne (5). Brooks *et al.* have indicated a low level of antituberculous activity for some *N*-substituted isothioureas (6), and recent interest in the antituberculous activity of substituted thioureas (7) and related dihydrothiouracils (8) has caused the authors to examine the bis-isothiuronium class of compounds for their *in vitro* inhibition of the growth of *M. tuberculosis*. In addition, the effect of the compounds upon mice through short-term aerosol inhalation studies was observed.

Two short series of substituted polymethylene bis-isothiuronium salts, arising from the reaction of 1-*m*-chlorophenyl-2-thiourea and 1-phenyl-2-thiourea with various dibromo alkanes, were synthesized and examined for the above noted activities.

The *in vitro* determination of the antituberculous effect was carried out by a serial dilution technique as reported previously utilizing the H37Rv strain of *M. tuberculosis* var. hominis[1] grown on Dubos media with added beef serum (7). The minimum inhibitory concentrations ranged from 5 to 1.25 mg. per cent with the *m*-chlorophenyl compounds appearing to be slightly more active.

Observable effects of the compounds upon motor activity of mice and the gross pathological effects upon their lungs following exposure to an aerosol of the compounds in acetone–propylene glycol were noted. The most frequently observed effect was that of decreased motor activity, with normal lung appearance and size being noted for all mice except one of the group exposed to compound *1* where the lungs appeared to be slightly hemorrhagic.

The results of the tests and the compounds studied are summarized in Table I.

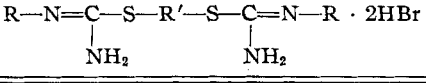
EXPERIMENTAL

Chemical

The compounds summarized in Table I were in general synthesized according to the procedure noted below.

Ethylene Bis-m-chlorophenylisothiuronium Dihydrobromide.—A solution of 9.35 Gm. (0.05 *M*) of *m*-chlorophenylthiourea prepared according to Kurzer (9) and 4.75 Gm. (0.025 *M*) dibromoethane in 125 ml. of absolute ethanol was refluxed 24 hr. on a steam bath. After cooling, ethyl ether was added to induce precipitation, and after standing overnight 11.5 Gm. of an off-white solid was filtered from the solution. The product was recrystallized from 50 ml. of absolute alcohol using ether–toluene (2:1) to induce precipitation; and yielding after

TABLE I.—SUBSTITUTED POLYMETHYLENE BIS-ISOTHIURONIUM SALTS



Compd.	R	R′	M.p., °C.[a]	Yield, %	R.S.[d]	Formula	Anal., N[e] Calcd.	Found	Min. Inhib. Concn., mg. %	Motor Activity[f]
1	*m*-ClC_6H_4—	(—CH_2—)$_2$	235–236	73	A/ET	$C_{16}H_{18}Cl_2Br_2N_4S_2$	9.98	9.83	5.0	−4/4
2	*m*-ClC_6H_4—	—$CH_2(CH_3)CH$—	138–139[b]	68	A	$C_{17}H_{18}Cl_2N_4S_2$	13.57	13.21	1.25	−3/4
3	*m*-ClC_6H_4—	(—CH_2—)$_4$	190–191	57	A/ET	$C_{18}H_{22}Cl_2Br_2N_4S_2$	9.53	9.40	2.5	=4/4
4	C_6H_5—	(—CH_2—)$_2$	215–216[c]	54	A/ET	$C_{16}H_{20}Br_2N_4S_2$	11.39	11.21	5.0	=4/4
5	C_6H_5—	(—CH_2—)$_3$	176–177[c]	62	A/E	$C_{17}H_{22}Br_2N_4S_2$	11.07	10.95	2.5	−2/4
6	C_6H_5—	—$CH_2(CH_3)CH$—	131–132[b]	50	A/E	$C_{17}H_{20}N_4S_2$	16.36	16.46	2.5	−2/4
7	C_6H_5—	(—CH_2—)$_4$	213–214[c]	65	A/ET	$C_{18}H_{24}Br_2N_4S_2$	10.71	10.63	5.0	=3/4
8	C_6H_5—	(—CH_2—)$_5$	188–189[c]	55	A/ET	$C_{19}H_{26}Br_2N_4S_2$	10.50	10.31	5.0	‡4/4

[a] Fisher-Johns block, corrected. [b] As free base. [c] Grogan, C. H., Rice, L. M., and Sullivan, M. X., *J. Org. Chem.*, **18**, 728(1953). Reported m.p.: C_2, 214–215°; C_3, 179–180°; C_4, 216–217°; C_5, 191–192°. [d] Recrystallization solvents: A, ethyl alcohol; E, ethyl ether; ET, ether–toluene (2:1). [e] Microanalysis by Dr. Kurt Eder, Geneva, Switzerland. [f] (−)decreased; (+) increased; visually observed activity based on four mice, 15 min. after a 20 min. exposure to an aerosol of 10 mg./ml. in 1:1 acetone–propylene glycol. Double values indicate a relatively greater activity.

Received February 24, 1965, from the Department of Pharmaceutical Chemistry, College of Pharmacy, University of Kentucky, Lexington.
Accepted for publication April 22, 1965.

[1] Standard Culture Department, National Tuberculosis Association, Trudeau Laboratory, Saranac Lake, N. Y.

drying *in vacuo*, 10.3 Gm. (73%) of white crystalline solid, melting at 235–236°.

Anal.—Calcd. for $C_{16}H_{18}Br_2Cl_2N_4S_2$: N, 9.98. Found: N, 9.83.

Biological

The methods used for the antituberculous testing have been noted previously and are described in detail in a previous communication (7).

Mice in groups of four, contained in individual cages, were exposed in a closed chamber for 20 min. to an aerosol of the compound being studied. The aerosols were prepared from a solution of 10 mg./ml. of the compound in 1:1 acetone–propylene glycol, and the estimated exposure concentration was 0.5 mg./L. at 20 p.s.i.

Following exposure, the mice were removed and observed for abnormal activities at periods of 5 min. for a total of 20 min. In the absence of continued effects, the mice were sacrificed 24 hr. after completion of the observation period, and the lungs examined for gross pathological changes such as edema or hemorrhage. The most frequently noticed effect was a decreased motor activity ranging from two out of four mice for compounds *5* and *6* to four out of four mice for compounds *1*, *3*, and *4*. One exception to this pattern was seen with compound *8*, where four out of four mice showed increased motor activity. Normal lung size and appearance were noted for all mice except one of the group exposed to compound *1*. In this case the lung appeared slightly hemorrhagic.

REFERENCES

(1) Kawai, S., *et al.*, *Sci. Papers Inst. Phys. Chem. Res. Tokyo*, **16**, (No. 306–309), 24(1931); through *Chem. Abstr.*, **25**, 5665(1931).
(2) Kumagai, T., *et al.*, *Sci. Papers Inst. Phys. Chem. Res. Tokyo*, **9**, 271(1928); through *Chem. Abstr.*, **23**, 2425(1929).
(3) Deirscherl, W., and Weingarten, F. W., *Arzneimittel-Forsch.*, **3**, 545(1953).
(4) Fuller, A. T., *Biochem. J.*, **36**, 548(1942).
(5) Umezawa, S., Uchida, I., and Osada, K., *Proc. Fujihara Mem. Fac. Eng. Keio Univ. Tokyo*, **12**, 12(1959); *Chem. Abstr.*, **54**, 19479(1960).
(6) Brooks, J. D., *et al.*, *J. Chem. Soc.*, **1950**, 452.
(7) Glasser, A. C., and Doughty, R. M., *J. Pharm. Sci.*, **51**, 1031(1962).
(8) Glasser, A. C., and Doughty, R. M., unpublished data.
(9) Kurzer, F., *Org. Syn.*, **31**, 21(1951).

Synthesis and Pharmacological Screening of Aminoalkyl-hydrazines

By TIBERIO BRUZZESE, SILVANO CASADIO, GERMANO COPPI, and ERNESTA MARAZZI-UBERTI

Some aminoalkyl-hydrazines have been prepared by acid hydrolysis of the corresponding sydnones and submitted to pharmacological screening. Some of the compounds displayed a moderate protective action against restraint ulcer in the rat.

THE INTERESTING pharmacological properties shown by several hydrazines (1–4) and the availability of 3-aminoalkyl-sydnones (5) as intermediates, led us to the preparation of some aminoalkyl-hydrazines for pharmacological screening.

Two of these compounds (I and V, Table I) have been recently obtained by Elslager *et al.* (4) through reaction of the appropriate aminoalkyl chloride with excess hydrazine and studied only as central nervous system stimulants and antibacterial compounds. By contrast, the method used in the present work was essentially that of Fugger *et al.* (6), consisting of acid hydrolysis of the corresponding sydnones. Concentrated hydrochloric acid was used for the hydrolysis; an essential factor in obtaining high yields is that the temperature should not exceed 50–70°. Under these conditions the required aminoalkyl-hydrazine could be isolated directly from the reaction mixture as the hydrochloride. The optimal reaction conditions are summarized in Table I.

An example of the method is described under *Experimental*.

Received March 19, 1965, from the Research Laboratories, Istituto De Angeli, Milan, Italy.
Accepted for publication May 3, 1965.
The authors thank Dr. G. Sekules for microanalyses.

EXPERIMENTAL

Melting points were determined on a Townson-Mercer melting point apparatus and are corrected

(2-Morpholinoethyl)-hydrazine Dihydrochloride (IV).—A solution of 25 Gm. of 3-(2-morpholinoethyl)-4-methyl-sydnone hydrochloride in 45 ml. of concentrated hydrochloric acid was cautiously heated at 50° for 1 hr., when carbon dioxide was freely given off. The solution was washed with 20 ml. of ether and evaporated to dryness *in vacuo* at approximately 40°. The residue was triturated with a little ethanol, giving 16.6 Gm. of product. The product was crystallized from 95% ethanol, giving colorless crystals, m.p. 163–164° dec.

SCREENING RESULTS

The acute toxicity, behavioral effect, and the analgesic, IMAO, anti-inflammatory, antipyretic, diuretic, hypoglycemic, anti-ulcer, antidepressive, and hypotensive actions were evaluated, along with the coronary dilating activity and the action on isolated vessels as well as the *in vitro* antifibrillar, antibacterial, antiamebic, and antitrichomonas actions. The methods described in a previous paper (5) were used. The compounds were administered by intraperitoneal injection in the form of aqueous solution in all the *in vivo* tests, except for the hypoglycemic and diuretic tests where they were given orally. The highest dose which did not cause death of the animal or an obvious toxic symptomatology was used for each experiment.

The results of the activity tests considered most interesting are given in Table II. Properties common to all the compounds are a low toxicity, moderate antipyretic action, and a mild CNS excita-