Synthesis and Antifungal Activity of Some Amide and Urea Derivatives of Bromal and Dichloroacetaldehyde

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Seventeen amide and urea derivatives of bromal and dichloroacetaldehyde and one brominated derivative of butyl acrylamide were synthesized and their physical constants determined. These compounds were subjected to tests for antifungal activity toward *Trichophyton rubrum, Microsporum gypsum,* and *Aspergillus niger.* All but one of the bromal compounds studied showed fungus-inhibiting properties; of the dichloroacetaldehyde derivatives, however, only the acrylamide and methacrylamide derivatives indicated activity. Total halogen content and terminal halogen groups appeared to be factors in enhancing the antifungal activity.

THE SYNTHESIS of the compounds reported in this paper was prompted by the fact that many of our newer therapeutic agents on the market today as well as many of our older drugs are urea, urethan, carbamate, or simple amide derivatives. Many pharmacological activities could be suggested for these compounds if structural similarities are considered. Many of the presently used tranquilizers, for instance, are amide, urea, or carbamate derivatives; examples are 2-ethyl-3-methylvaleramide, 3,4,5-trimethoxycinnamide, and ectylurea. Several urea derivatives, including the sulfonyl ureas, are used as hypoglycemic agents for the treatment of diabetes mellitus. Still others, such as the nitrosoureas, urethan, the phosphoramides, and certain carbamates, have found a place in cancer chemotherapy.

The potential of the amide and urea derivatives of bromal and dichloroacetaldehyde as sedative-hypnotic drugs also exists. Byrum and LaRocca (1) reported the sedative-hypnotic acitivity of chloral and trichlorobutyraldehyde amides, and Chapman *et al.* (2) have shown that certain acetamide and benzamide derivatives possess hypnotic activity. Triacetamide and 2-ethyl-3-methylvaleryl urea are presently being marketed as sedative and hypnotic drugs, respectively.

Studies have also shown the potentialities of certain substituted amides as antifungal agents. Leonard and Blackford (3) made a study of the α-bromoacetamides and reported remarkable levels of fungus inhibition. Such compounds as butylphenylsalicylamide and substituted carbanilides are presently being used in antifungal foot preparations. Antifungal activity has also been demonstrated for certain amide derivatives of dichloroacetaldehyde and bromal (4).

The purpose of this paper is to report the synthesis of some amide and urea derivatives of bromal and dichloroacetaldehyde and to give the results of antifungal studies carried out in this investigation. Additional pharmacological studies, including results of anticancer, antispasmodic, anticonvulsant, and tranquilization tests, will be reported in another paper.

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EXPERIMENTAL

Materials.—The intermediates used in this investigation were obtained through ordinary commercial sources. The amides, ureas, and bromal were purchased from Eastman Kodak Co. Westvaco Chemical Co. supplied the dichloroacetaldehyde.

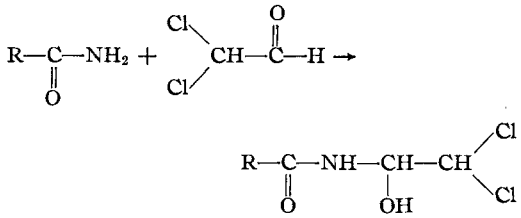
TABLE I.—DERIVATIVES OF BROMAL AND DICHLOROACETALDEHYDE

No.	Compound
1	Urea, 1-(2,2,2-tribromo-1-hydroxyethyl)-
2	Carbamic acid, (2,2,2-tribromo-1-hydroxyl)-, ethyl ester
3	Acrylamide, *N*-(2,2,2-tribromo-1-hydroxyethyl)-
4	Acrylamide, 2-methyl-*N*-(2,2,2-tribromo-1-hydroxyethyl)-
5	Urea, 1-(2,2,2-tribromo-1-hydroxyethyl)-3-*tert*-butyl-
6	Hexanamide, *N*-(2,2,2-tribromo-1-hydroxyethyl)-
7	Ethane, 1,1-bis (*N'*-methylureido)-2,2,2-tribromo-
8	Urea, 1-(2,2,2-tribromo-1-hydroxyethyl)-3-*p*-phenethyl-
9	Propionamide, 2,3-dibromo-*N*-(2,2,2-tribromo-1-hydroxyethyl)-
10	Propionamide, 2,3-dibromo-2-methyl-*N*-(2,2,2-tribromo-1-hydroxyethyl)-
11	Acrylamide, *N*-(2,2-dichloro-1-hydroxyethyl)-
12	Acrylamide, 2-methyl-*N*-(2,2-dichloro-1-hydroxyethyl)-
13	Ethane, 1,1-bis (*N'*-methylureido)-2,2-dichloro-
14	Carbamic acid, (2,2-dichloroethylidene) di-, diethyl ester
15	Urea, 1-(2,2-dichloro-1-hydroxyethyl)-3-allyl-
16	Propionamide, 2,3-dibromo-*N*-(2,2-dichloro-1-hydroxyethyl)-
17	Propionamide, 2,3-dibromo-2-methyl-*N*-(2,2-dichloro-1-hydroxyethyl)-
18	Propionamide, 2,3-dibromo-*N-tert*-butyl-

Synthesis and Purification.—The derivatives of bromal and dichloroacetaldehyde listed in Table I have been synthesized and their physical constants determined. (The compound numbers assigned correspond to those used in the tables that follow.)

The compounds were prepared according to procedures previously reported for the preparation of amides of dichloroacetaldehyde (5) and bromal (6). These procedures involved the condensation of the appropriate aldehyde with the various amides and were modified for the present investigation only in regard to temperatures of reaction and in mole/ratio of reactants. For the preparation of all compounds reported in this paper the condensation temperature was 37°, and equimolar portions of reactants were used in all cases. The procedures worked as well for the preparation of the urea and urethan derivatives of bromal and dichloroacetaldehyde as for the amide derivatives. The one compound reported that is not a bromal or dichloroacetaldehyde derivative, the 2,3-dibromo-*N-tert*-butylpropionamide, was prepared by brominating the appropriate unsaturated amide. (See the procedure for bromination of acrylamide and methacrylamide compounds below.) The general reaction equations are as follows:

Type *A*



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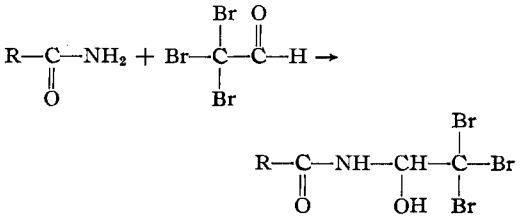

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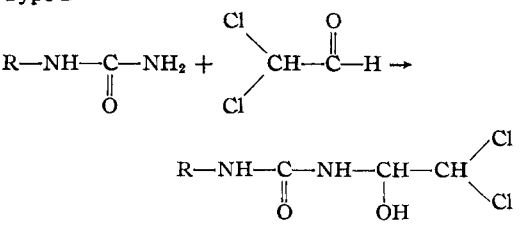

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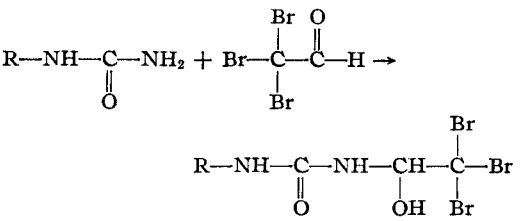
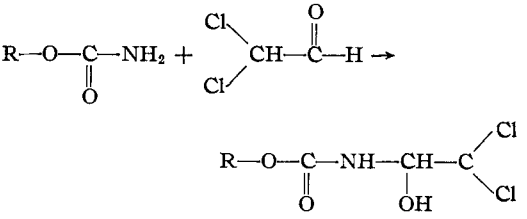


Type *C*



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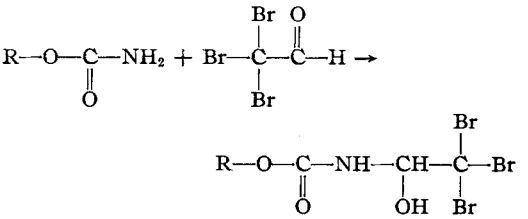


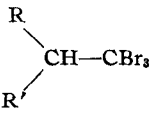
The bromination of the acrylamide and methacrylamide compounds was brought about by the addition of elemental bromine to the double bond of the unsaturated amide after its condensation with bromal or dichloroaldehyde. The unsaturated amide product was dissolved in an ethanol–chloroform mixture in a flask and placed on a heater stirrer. An equimolar portion of bromine was added dropwise. This mixture was refluxed for 2 to 3 hr. and then partially evaporated with air. The product was purified by recrystallization from carbon tetrachloride.

Analytical Methods.—Chlorine and bromine analyses were carried out using a Parr bomb apparatus, and nitrogen determinations were by the Kjeldahl procedure. The analytical data for the 18 compounds are given in Tables II and III.

Procedure for Antifungal Studies.—A study of the fungus-inhibiting properties of the compounds was carried out by a modification of a method used by Bateman (7), Vincent (8), and Leonard and Blackford (3). This procedure consists of comparing the growth rates of the test fungus upon nutrient agar containing a known concentration of the compound to be tested with that of a control, identically treated, but containing none of the test compound. All compounds were tested in triplicate for each concentration.

Preparation of Culture Medium.—The culture medium contained neopeptone (3 Gm.), beef extract (5 Gm.), nutrient agar (6 Gm.), potassium acid phosphate (3 Gm.), and water (300 ml.) with a small amount of antifoam 60 added to prevent foaming. A glass-enclosed magnetic stirrer bar was added to a flat-bottomed boiling flask containing the medium and the product then steam sterilized for 20 min. at 15 lb. of pressure. When the flask was removed from the autoclave, it was immediately placed on a magnetic stirrer heater. The test compound which had previously been suspended in 10 ml. of a sterile 1% cellulose gum solution was now added to the flask containing the medium. Constant stirring was maintained as the temperature of the hot plate was gradually lowered. This allowed a point of consistency to be reached whereby the culture medium could be poured into plates, yet keeping the test compound in suspension. The plates used were previously chilled sterile 9-cm. Petri dishes.

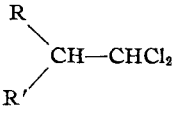
TABLE II.—DERIVATIVES OF BROMAL



No.	Compd. Condensed With	R	R′	M.p., °C.[a]	% Yield	Anal., % Calcd.	Found
1	Urea	—NHCONH$_2$	—OH	137–139	36	Br, 70.40 N, 8.21	Br, 69.80 N, 7.92
2	Urethan	—NHCOOCH$_2$CH$_3$	—OH	135	41	Br, 64.90 N, 3.78	Br, 64.80 N, 3.86
3	Acrylamide	—NHCOCH=CH$_2$	—OH	174	67	Br, 68.20 N, 3.98	Br, 67.50 N, 4.24
4	Methacrylamide	—NHCOC(CH$_3$)=CH$_2$	—OH	138	57	Br, 65.50 N, 3.83	Br, 64.50 N, 3.89
5	*tert*-Butylurea	—NHCONH$_2$C(CH$_3$)$_3$	—OH	164–165	29	Br, 60.50 N, 7.05	Br, 59.70 N, 7.00
6	Hexanamide	—NHCOCH$_2$(CH$_2$)$_3$CH$_3$	—OH	144–145	49	Br, 60.50 N, 3.54	Br, 59.20 N, 3.72
7	Methylurea[b]	—NHCONHCH$_3$	—NHCONHCH$_3$	190–192	21	Br, 58.39 N, 13.38	Br, 57.60 N, 13.45
8	*p*-Phenethylurea	—NHCONH—C$_6$H$_4$—OCH$_2$CH$_3$	—OH	149–153[c]	39	Br, 52.04 N, 6.10	Br, 50.20 N, 5.90
9	Acrylamide[d]	—NHCOCH(Br)CH$_2$Br	—OH	125–129	17	Br, 78.10 N, 2.74	Br, 76.10 N, 3.15
10	Methacrylamide[d]	—NHCOC(CH$_3$)BrCH$_2$Br	—OH	113–115	60	Br, 75.88 N, 2.66	Br, 76.16 N, 2.91

[a] Melting points taken on Fisher-Johns apparatus, uncorrected. [b] Two molecules of methylurea react with one molecule of bromal under conditions of procedure. [c] Decomposes at this temperature. [d] Brominated after condensation with bromal.

TABLE III.—DERIVATIVES OF DICHLOROACETALDEHYDE



No.	Compd. Condensed With	R	R′	M.p., °C.[a]	% Yield	Anal., % Calcd.	Found
11	Acrylamide	—NHCOCH=CH$_2$	—OH	129	71	Br, ... Cl, 38.60 N, 7.61	Br, ... Cl, 38.00 N, 7.56
12	Methacrylamide	—NHCOC(CH$_3$)=CH$_2$	—OH	118–119	57	Br, ... Cl, 35.80 N, 7.07	Br, ... Cl, 35.30 N, 7.10
13	Methylurea[b]	—NHCONHCH$_3$	—NHCONHCH$_3$	194–197	34	Br, ... Cl, 29.20 N, 23.05	Br, ... Cl, 27.83 N, 21.70
14	Urethan[b]	—NHCOOCH$_2$CH$_3$	—NHCOOCH$_2$CH$_3$	98	24	Br, ... Cl, 26.00 N, 10.27	Br, ... Cl, 24.80 N, 10.26
15	Allylurea	—NHCONHCH$_2$CH=CH$_2$	—OH	175–176	43	Br, ... Cl, 24.10 N, 18.30	Br, ... Cl, 23.70 N, 18.67
16	Acrylamide[c]	—NHCOCH(Br)CH$_2$Br	—OH	109–110	43	Br, 67.15[e] Cl, ... N, 4.07	Br, 67.26[e] Cl, ... N, 4.14
17	Methacrylamide[c]	—NHCOC(CH$_3$)BrCH$_2$Br	—OH	103–104	47	Br, 64.50[e] Cl, ... N, 3.92	Br, 62.90[e] Cl, ... N, 4.88
18	[d]	...	...	185–187	28	Br, 55.70 Cl, ... N, 4.87	Br, 55.04 Cl, ... N, 4.88

[a] Melting points taken on Fisher-Johns apparatus, uncorrected. [b] Two molecules react with one molecule of dichloroacetaldehyde under conditions of procedure. [c] Brominated after condensation with dichloroacetaldehyde. [d] Not a derivative of dichloroacetaldehyde, but included in table for convenience. [e] Assay is for total halogen.

Inoculation.—The organisms used in making the inoculations had been grown on agar slants of the previously mentioned medium for 7 days at 30°. Separate spore suspensions of each of the three test organisms were prepared by washing the agar slants with 5-ml. portions of normal saline. Inoculations were made with sterile cotton-tipped swabs with a diameter of 5 mm.; the inoculations were made into the center of the Petri dishes containing the medium and test substance.

Growth Measurement.—The plates, incubated at 37°, were observed daily and measurements of growth taken. The values were then plotted against time and the growth rate determined as the slope of the best straight line through the points. Inhibition was then calculated according to the method of LaRocca *et al.* (9) as follows:

$$I = [(C - T)/C] \times 100\%$$

where I = percentage inhibition, C = growth rate of control in mm./hr., and T = growth rate of toxic in mm./hr.

RESULTS

The inhibition data from the growth measurements are shown in Tables IV and V. Of the bromal derivatives tested, all except the hexanamide derivative showed high antifungal activity in the con-

TABLE IV.—ANTIFUNGAL ACTIVITY OF BROMAL DERIVATIVES

Compd.[a]	Inhibition % Upon *T. rubrum*		Inhibition % Upon *M. gypseum*		Inhibition % Upon *Aspergillus* sp.	
	$.25 \times 10^{-2}$ M/L.	$.50 \times 10^{-2}$ M/L.	$.25 \times 10^{-2}$ M/L.	$.50 \times 10^{-2}$ M/L.	$.25 \times 10^{-2}$ M/L.	$.50 \times 10^{-2}$ M/L.
1	100	100	100	100	100	100
2	100	100	73.7	100	100	100
3	100	100	100	100	100	100
4	100	100	100	100	100	100
5	100	100	100	100	100	100
6	12.5	25	7.7	30	7.1	21
7	100	100	100	100	100	100
8	100	100	100	100	100	100
9	100	100	100	100	100	100
10	100	100	100	100	100	100

[a] Compound numbers in Tables IV and V correspond to those given in Tables II and III of this paper.

TABLE V.—ANTIFUNGAL ACTIVITY OF DICHLOROACETALDEHYDE DERIVATIVES

Compd.	Inhibiton % Upon *T. rubrum*		Inhibiton % Upon *M. gypseum*		Inhibition % Upon *Aspergillus* sp.	
	$.25 \times 10^{-2}$ M/L.	$.50 \times 10^{-2}$ M/L.	$.25 \times 10^{-2}$ M/L.	$.50 \times 10^{-2}$ M/L.	$.25 \times 10^{-2}$ M/L.	$.50 \times 10^{-2}$ M/L.
11	22.2	100	9.4	20.8	8.7	21.7
12	9.3	32.6	2.4	11.9	25.4	27.1
13	43.9	65.8	28.6	54.3	52.7	70.6
14	0	0	33.3	50	12	36
15	33.3	41.6	20	28.9	10	20
16	100	100	100	100	100	100
17	33.3	100	100	100	84.4	100
18[a]	50	62.5	13.6	30	20	40

[a] Compound not a derivative of dichloroacetaldehyde but included in table for convenience.

centrations used in the tests. In contrast, of the dichloroacetaldehyde derivatives tested, only the brominated acrylamide and methacrylamide derivatives showed any appreciable antifungal activity toward all three organisms. The acrylamide derivative of dichloroacetaldehyde exhibited activity toward the *Trichophyton* organism when tested in the higher concentrations.

It can be noted that the compounds with the highest antifungal activity were those containing the greatest amount of halogen in the molecule. For example, it was found that in most cases when a bromal derivative of an amide or urea possessed activity, the corresponding dichloroacetaldehyde derivative was inactive. It can be pointed out too that those dichloroacetaldehyde compounds showing activity contain halogen on both terminal groups of the molecule. This coincides with previous findings (4).

REFERENCES

(1) Byrum, W. R., and LaRocca, J. P., *J. Am. Pharm. Assoc., Sci. Ed.*, **41**, 100(1952).
(2) Chapman, M. V. A., *et al.*, *J. Pharm. Pharmacol.*, **9**, 20 (1957).
(3) Leonard, J. M., and Blackford, V. L., *J. Bacteriol.*, **57**, 339(1949).
(4) Easterly, W. D., Jr., and Dusenberry, J. E., *J. Pharm. Sci.*, **50**, 42(1961).
(5) Easterly, W. D., Jr., and LaRocca, J. P., *J. Am. Pharm. Assoc., Sci. Ed.*, **43**, 59(1954).
(6) Easterly, W. D., Jr., and Rosa, V. R., *ibid.*, **48**, 388 (1959).
(7) Bateman, E., *U. S. Dept. Agr. Tech. Bull.*, **346**, 1 (1953).
(8) Vincent, J. M., *J. Soc. Chem. Ind.*, **66**, 149(1947).
(9) LaRocca, J. P., Leonard, J. M., and Weaver, W. E., *J. Org. Chem.*, **16**, 47(1951).