Synthesis of Potential Antineoplastic Agents. X. Preparation and Reactions of Aldehydes Related to Benzaldehyde Mustard[1-3]

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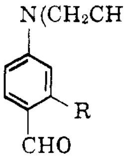
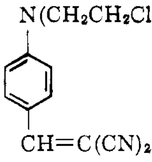
The preparation of a series of benzaldehyde mustards with various substituents on the benzene ring is reported. These aldehydes were used to prepare derivatives related to active antineoplastic agents and the activities compared. A number of other reactions in this area are reported.

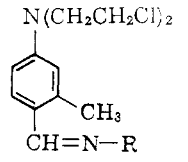
In the past we have reported the use of the readily available[4,5] *p*-[bis(2-chloroethyl)amino]benzaldehyde (benzaldehyde mustard) (I) in the synthesis of a variety of compounds for screening as potential antineoplastic agents.[6-11] In most of these studies we also prepared compounds from the related 4-[bis(2-chloroethyl)amino]-*o*-tolualdehyde (II).[7] This program has lead to the discovery of a number of compounds with outstanding activity. *p*-[Bis(2-chloroethyl)amino]benzylidenemalononitrile (III),[6] which was the most active of the products formed from I in the Knoevenagel reaction,[6,8] is currently undergoing clinical trial.[12] It might be noted that the related product from II and malononitrile had nearly the same activity[13] as III, indicating little effect by the ring substituent in this series. Three Schiff bases (IV) derived from II are also considered worthy of clinical trial.[14] It is of interest to note that in the Schiff base series[7,10] the compounds prepared from II were consistently more active than those from I.



I, R = H
II, R = CH_3



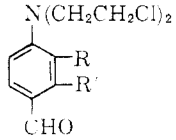
III



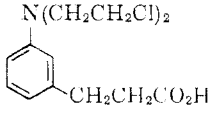
IV

R = *p*-FC_6H_4,
o-$C_6H_5C_6H_4$,
or cyclopentyl

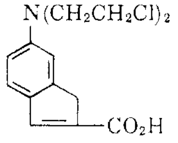
In view of the activity of these derivatives of I and II and in the differences in activity of Schiff bases derived from I and II, it was decided to alter the substituents on the benzaldehyde ring to see if an even more active agent could be obtained. The aldehydes (V) shown in Table I were prepared for this study. The appropriate anilines were hydroxyethylated with ethylene oxide[15,16] and these products converted to the aldehyde mustards (V) by reaction with phosphorus oxychloride and dimethylformamide in two steps[4] or, more conveniently, in one step.[5] The aldehydes (V) with R = H, R′ = CF_3 and with R = OC_2H_5, R′ = H were essentially inactive and nontoxic at doses of 640 mg./kg. The other aldehydes all possessed some activity, but with the exception of V (R = OCH_3, R′ = H) none approached II in activity or range of activity. As anticipated[5,11] the 2,4-dinitrophenylhydrazones of these aldehydes were inactive and nontoxic at doses up to 640 mg./kg. Reaction of 3-[*m*-(N,N-bis(2-chloroethyl))aminophenyl]propionic acid[17] (VI) with dimethylformamide–phosphorus oxychloride led to the formation of VII which did not show any outstanding activity. The expected aldehyde was in all probability an intermediate in the formation of VII.



V



VI



VII

As shown in Table II, two of these new aldehydes were treated with malononitrile to give compounds related to III. Neither of these compounds showed appreciable activity despite the fact that the aldehyde V (R = OCH_3, R′ = H) was one of the best of the new aldehydes.

Table II also lists the new Schiff bases prepared. None of the Schiff bases prepared from *p*-fluoroaniline, 2-aminobiphenyl, or cyclopentylamine were as active over as wide a range as those (IV) previously prepared. While it did not have the range of activity of IV (R = *p*-FC_6H_4), it might be noted that the compound from *p*-fluoroaniline and V (R = OC_2H_5, R′ = H) was at least as active over a shorter range. This and other observations indicate no correlation between the activity of the aldehyde and the activity of the aldehyde derivatives. None of the Schiff bases prepared from new amines had anything to offer when compared with

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(1) Part IX: F. D. Popp, S. Roth, and J. Kirby, *J. Med. Chem.*, **6**, 83 (1963).

(2) A portion of this material was presented before the Division of Medicinal Chemistry at the 145th National Meeting of the American Chemical Society, New York, N. Y., Sept., 1963.

(3) This work was supported in part by research grants from the American Cancer Society (T 177B) and from the National Cancer Institute, U.S.P.H.S. (CA 06606-01).

(4) R. C. Elderfield, I. S. Covey, J. B. Geiduschek, W. L. Meyer, A. B. Ross, and J. H. Ross, *J. Org. Chem.*, **23**, 1749 (1958).

(5) R. H. Wiley and G. Irick, *ibid.*, **26**, 593 (1961).

(6) F. D. Popp, *J. Chem. Soc.*, 5271 (1960).

(7) F. D. Popp, *J. Org. Chem.*, **26**, 1566 (1961).

(8) F. D. Popp, *ibid.*, **26**, 3019 (1961).

(9) F. D. Popp, *ibid.*, **26**, 3020 (1961).

(10) F. D. Popp and W. Kirsch, *ibid.*, **26**, 3858 (1961).

(11) F. D. Popp, *J. Med. Pharm. Chem.*, **5**, 627 (1962).

(12) L. Rane, F. D. Popp, M. L. Stevens, D. Kirkpatrick, and J. Colsky, *Proc. Am. Assoc. Cancer Res.*, **3**, 351 (1962).

(13) Unless otherwise specified all activities referred to in this paper are for studies against an established Dunning leukemia in Fisher rats and have been supplied by Dr. Leo Rane of the University of Miami Medical School.

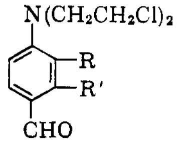
(14) L. Rane and W. Dunning, private communications.

(15) M. Freifelder and G. R. Stone, *J. Org. Chem.*, **26**, 1477 (1961).

(16) The bis(2-hydroxyethyl)anilines were later purchased from Frinton Laboratories. We thank Dr. Freifelder of Abbott Laboratories for a generous sample of one of these bis(2-hydroxyethyl)anilines.

(17) We wish to thank Dr. R. B. Ross, C.C.N.S.C., for a generous sample of this material.

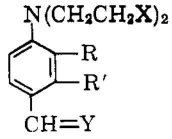
TABLE I
BENZALDEHYDE MUSTARDS



				% calcd.			% found			2,4-Dinitrophenylhydrazone							
											% calcd.			% found			
R	R'	Yield, %	M.p., °C.	C	H	N	C	H	N	M.p., °C.	C	H	N	C	H	N	
OC_2H_5	H	75	63–64	53.80	5.91	4.83	53.86	6.04	4.99	212–213	48.52	4.50	14.89	48.79	4.55	14.78	
OCH_3	H	79	35–36	52.19	5.48	5.07	52.07	5.32	5.25	204–205	47.38	4.20	15.35	47.65	4.31	15.27	
CH_3	H[a]	84	Oil	55.40	5.81	5.39	55.80	6.17	5.61	198–199	49.10	4.35	15.91	49.29	4.53	15.80	
H	Cl	b								200–202			15.20			14.99	
H	F	94	85–87	50.01	4.58	5.30	49.71	4.50	5.16	224–226	45.96	3.63	15.77	45.65	3.67	15.92	
H	NO_2[c]	94	111–112	45.38	4.16	9.62	45.73	4.46	9.75	208–210	43.54	3.42		43.09	3.62		
H	CF_3	81	Oil	45.88	3.85	4.46	46.14	4.14	4.74	134–136	43.74	3.26	14.17	43.73	3.08	13.95	
d,e		85	Oil	60.82	5.10	4.73	60.77	4.92	4.90	214–215	52.95	4.02	14.70	52.67	4.01	15.02	

[a] *Anal.* Calcd.: Cl, 23.94. Found: Cl, 24.02. [b] Purchased from Frinton Laboratories. [c] *Anal.* Calcd.: Cl, 24.36. Found: Cl 24.60. [d] 2-N,N-Bis(chloroethyl)amino-1-naphthaldehyde. [e] *Anal.* Calcd.: Cl, 23.94. Found: Cl, 24.02.

TABLE II
ALDEHYDE DERIVATIVES



						% calcd.			% found	
X	R	R'	YH_2	Yield, %	M.p., °C.	C	H	N	C	H	N
I	H	H	Malononitrile	79	197–198	35.24	2.75	8.81	35.19	2.62	8.57
OMs[a]	H	H	Malononitrile	83	130–131	46.47	4.63	10.16	46.63	4.66	10.38
Cl	OCH_3	H	Malononitrile[b]	85	98–99	55.57	4.66	12.96	55.73	4.79	12.89
Cl	H	Cl	Malononitrile	71	147–148	51.16	3.68	12.79	51.33	3.81	12.75
I	H	CH_3	Malononitrile	77	203–204	36.68	3.08	8.56	36.39	3.33	8.66
Cl	H	H	*p*-Fluoroaniline	81	78–79	60.19	5.05	8.26	60.31	5.06	8.21
Cl	H	H	Fluorenone hydrazone	96	141–142	68.25	5.01	9.95	67.98	5.26	9.79
I	H	H	Fluorenone hydrazone	99	173–174	47.62	3.50	6.94	47.34	3.51	7.06
I	H	H	*p*-Fluoroaniline	74	104–105	39.10	3.28	5.37	38.81	3.56	5.43
Cl	CH_3	H	*p*-Fluoroaniline	87	111–113			7.93			8.31
Cl	OC_2H_5	H	*p*-Fluoroaniline	82	95–96	59.53	5.52	7.31	59.66	5.60	7.29
Cl	OCH_3	H	*p*-Fluoroaniline	70	169–171			7.59			7.64
Cl	H	Cl	*p*-Fluoroaniline	72	105–106	54.64	4.32	7.50	54.91	4.57	7.28
Cl	H	Cl	Fluorenone hydrazone	99	185–186	63.10	4.41	9.20	62.90	4.82	8.92
Cl	H	Cl	*o*-Aminobiphenyl	66	92–93	63.97	4.90	6.49	64.17	5.29	6.31
Cl	H	Cl	Cyclopentylamine	54	106–108	55.26	6.09	8.06	55.34	5.69	8.13
Cl	H	F	*p*-Fluoroaniline	67	88–89	57.15	4.52	7.84	56.84	4.64	7.81
Cl	H	F	Cyclopentylamine	79	82–83	58.01	6.39	8.46	57.73	6.36	8.55
Cl	H	CF_3	*p*-Fluoroaniline	77	Oil			6.88			6.58
I	H	CH_3	*p*-Fluoroaniline	92	149–150	40.32	3.57	5.23	39.98	3.26	4.98
I	H	CH_3	Cyclopentylamine	80	98–99	40.02	4.74	5.49	39.77	5.10	5.38
Cl	H	CH_3	Fluorenone hydrazone	95	171–172	68.81	5.31	9.63	68.81	5.26	9.76
Cl	H	CH_3	N-Benzoyl-L-tyrosine hydrazide[c]	76	188–189	62.11	5.58	10.35	61.99	5.64	10.15
Cl	H	CH_3	2,4,6-Trifluoroaniline	66	116–117	55.54	4.40	7.20	55.83	5.00	6.96
Cl	H	CH_3	*o*-Fluoroaniline	90	112–114	61.19	5.42	7.93	60.83	5.34	8.02
Cl	H	CH_3	*p*-Fluorobenzylamine	83	57–59	62.13	5.77	7.63	61.87	5.58	7.48
Cl	H	CH_3	4-Fluoro-3-methylaniline	75	90–91	62.13	5.77	7.63	61.83	5.81	7.76
Cl	H	CH_3	4-Fluoro-2-methylaniline	76	101–102	62.13	5.77	7.63	61.84	5.46	7.52
Cl	H	CH_3	2-Amino-4'-nitrobiphenyl	75	125–126	63.16	5.08	9.21	62.97	5.14	9.31
Cl	H	CH_3	1-Aminoindane	89	111–113	67.20	6.44	7.47	67.16	6.32	7.41
Cl	H	CH_3	1-Aminopyrene	98	166–167	73.70	5.27	6.10	73.32	5.25	6.02
Cl	H	CH_3	N-Amino-1,2,3,4-tetrahydroisoquinoline	73	104–106	64.61	6.46		64.59	6.58	

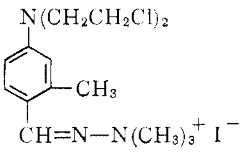
[a] OSO_2CH_3. [b] *Anal.* Calcd.: Cl, 21.87. Found: Cl, 22.00. [c] *Anal.* Calcd.: Cl, 13.09. Found: Cl, 12.91.

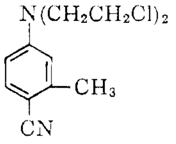
IV, although it might be noted that the activity of the bases from II and *o*-fluoroaniline, 4-fluoro-2-methylaniline, and *p*-fluorobenzylamine were essentially the same as that from II and *p*-fluoroaniline.

A few of the compounds in Table II have iodo[5] or methanesulfonate[18] groups in place of the chloro group of the mustard. None of these had anything to offer in comparison with the chloro mustards, although the activity of the methanesulfonate analog of III approached III in activity.

It has been noted earlier that addition of hypophosphorous acid to the imine linkage of the Schiff bases caused a loss of activity.[10] That this imine linkage is necessary for activity was further indicated by the reduction of the Schiff base (IV, R = p-FC_6H_4) with sodium borohydride. The resulting amine was both inactive and nontoxic.

The stability of the aromatic mustard was again demonstrated by the conversion of VIII, obtained from the corresponding aldehyde by reaction with N,N-dimethylhydrazine followed by methyl iodide, to the cyano compound IX by warming with sodium methoxide. This cyano compound was inactive and nontoxic at 480 mg./kg. while the intermediate VIII was also inactive but toxic at 320 mg./kg.


VIII


IX

Experimental[19]

Preparation of 4-[Bis(2-chloroethyl)amino]benzaldehydes.—To 22 ml. of dimethylformamide in an ice bath was slowly added 14 ml. of phosphorus oxychloride with stirring. Then 0.047 mole of the bis(2-hydroxyethyl)aniline[15,16] in 20–40 ml. of dimethylformamide was added slowly with stirring. The mixture was then heated for 1 hr. on a steam bath and poured onto ice. The product was separated by filtration or extraction with ether. Solid aldehydes were recrystallized from 95% ethanol while ether extracts of oils were washed and dried to give the aldehydes listed in Table I. The 2,4-dinitrophenylhydrazones were prepared by the usual method[20] and recrystallized from 95% ethanol or ethanol–ethyl acetate. When 20.3 g. (0.07 mole) of VI in 50 ml. of dimethylformamide was added to a mixture of 22 ml. of dimethylformamide and 12.9 g. of phosphorus oxychloride and treated as described previously, a gum was obtained which did not give a 2,4-dinitrophenylhydrazone. Trituration of this gum with ethanol gave a solid which was recrystallized from 95% ethanol to give 4.6 g. (23%) of VII, m.p. 199–200°.

Anal. Calcd. for $C_{14}H_{15}Cl_2NO_2$: C, 56.01; H, 5.04; N, 4.67; Cl, 23.62. Found: C, 55.92; H, 4.74; N, 4.77; Cl, 23.82.

4-[Bis(2-iodoethyl)amino]-*o*-tolualdehyde.[21]—This compound, m.p. 119–122°, was prepared in 63% yield from II by the method of Wiley.[5]

Anal. Calcd. for $C_{12}H_{15}I_2NO$: C, 32.53; H, 3.41; N, 3.16. Found: C, 32.48; H, 3.38; N, 3.18.

Condensations with Malononitrile.—The aldehydes were treated with equimolar quantities of malononitrile in dioxane with a trace of piperidine, as previously described,[6,8] and then were recrystallized from chloroform giving the compounds listed in Table II.

Schiff Bases.—Equimolar mixtures of the aldehyde and amine were treated in absolute ethanol as previously described.[7,10] The products were recrystallized from 95% ethanol to give the compounds listed in Table II.

N^4,N^4-Bis(2-chloroethyl)-N^α-(*p*-fluorophenyl)-toluene-α,4-diamine Hydrochloride.—A mixture of 5.2 g. of II (0.02 mole) and 2.22 g. (0.02 mole) of *p*-fluoroaniline in 75 ml. of absolute ethanol was heated on a steam bath for 30 min. To this mixture was added, during 5 min., 0.76 g. of sodium borohydride, and the mixture was refluxed for an additional 45 min. After the addition of an equal volume of water, the mixture was extracted with methylene chloride, and the methylene chloride concentrated *in vacuo* to give a gum which was dissolved in benzene and treated with anhydrous hydrogen chloride to give 6.6 g. (84%) of yellow solid, m.p. 144–146°, which was hygroscopic and somewhat unstable.

Anal. Calcd. for $C_{18}H_{22}Cl_3FN_2$: C, 55.18; H, 5.66; N, 7.15; Cl, 27.15. Found: C, 54.56; H, 5.94; N, 7.41; Cl, 26.67.

4-[Bis(2-chloroethyl)amino]-*o*-tolunitrile.—A mixture of 16.9 g. (0.065 mole) of II and 3.9 g. (0.065 mole) of N,N-dimethylhydrazine in 65 ml. of benzene was refluxed for 30 min., concentrated to one-half its original volume, and treated with 5 ml. of methyl iodide. After standing for 5 hr., a solid was collected and washed with ether to give 23.7 g. (82%) of solid, m.p. 168–169°. Recrystallization of a sample from 95% ethanol did not change the melting point.

Anal. Calcd. for $C_{15}H_{24}Cl_2IN_3$: C, 40.56; H, 5.45; N, 9.46. Found: C, 40.86; H, 5.63; N, 9.26.

The solid VIII (8.88 g., 0.02 mole) was added to a mixture of 1.08 g. (0.02 mole) of sodium methoxide in 45 ml. of absolute ethanol and warmed occasionally on the steam bath for a 4-hr. period. The mixture was cooled and filtered to give 4.65 g. (90%) of solid, m.p. 125–127°. Recrystallization from 95% ethanol gave material, m.p. 126.5–127°.

Anal. Calcd. for $C_{12}H_{14}Cl_2N_2$: C, 56.05; H, 5.45; N, 10.90; Cl, 27.58. Found: C, 55.82; H, 5.60; N, 10.79; Cl, 27.85.

Acknowledgment.—We wish to thank Dr. Leo Rane for many helpful discussions.

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[18] R. C. Elderfield, R. N. Prasad, and T.-K. Liao, *J. Org. Chem.*, **27**, 573 (1962).

[19] All melting points are corrected and were determined in capillaries. Analysis by Spang Microanalytical Laboratory, Ann Arbor, Mich., and Drs. Weiler and Strauss, Oxford, England.

[20] R. L. Shriner, R. C. Fuson, and D. Y. Curtin, "The Systematic Identification of Organic Compounds," 4th Ed., John Wiley and Sons, Inc., New York, N. Y., 1956, p. 219.

[21] This compound was first prepared by Mr. W. Kirsch at the University of Miami.