TABLE II

Compound	Calculated dose, mg./kg./day	Concentration, % in diet	Response,[a] % reduction
20	400	0.267	36
	100	.067	38
	40	.027	36
	20	.013	36
	10	.007	29
	5	.0033	11
	2.5	.0017	4
1	312	.208	6
2	156	.104	4
4	100	.067	27
	10	.007	0
5	100	.067	36
6	500	.333	48
8	250	.167	31
10	500	.333	65
	100	.067	22
11	100	.067	30
15	312	.208	34
	100	.067	0
16	75	.050	6
17	219	.146	57
	10	.007	0
18	100	.067	46
	10	.007	5
22	500	.333	9
	250	.167	17
	100	.067	49
Triparanol	100	.067	49
	10	.007	27

[a] $\%\ \text{reduction} = 100\left(1 - \frac{\text{mg.\% cholesterol treated}}{\text{mg.\% cholesterol control average}}\right)$

limited. At the end of treatment (1 or 2 weeks) the mice were bled by heart puncture, the bloods were centrifuged, and the plasma separated. Cholesterol determination was done on the plasma for each mouse.

Experimental[3]

Preparation of Carbinols. Sodamide (Method A).—Sodamide was added portionwise to an excess of γ-picoline stirred and cooled in ice–water. This mixture frequently became very dark as the picolyl sodium formed. The appropriate ketone dissolved in additional γ-picoline was added very rapidly and the resulting mixture was stirred overnight. This mixture was then poured into a large excess of water and allowed to crystallize. After filtering, the products could then be recrystallized from a suitable solvent.

General Grignard Procedure (Method B).—To the Grignard solution prepared from 0.2 g.-atom of magnesium and 0.2 mole of *p*-chlorobenzyl chloride in 100 ml. of ether was slowly added a suspension of 0.19 mole of the pyridyl ketone in 300 ml. of ether with stirring. The mixture was then heated to reflux for 4 hr. with continued stirring. The reaction mixture was then decomposed by the addition of an equivalent of ammonium chloride in saturated aqueous solution. The salts were filtered and washed with more ether which was then concentrated to an oil by distilling the ether. The residual oil was distilled *in vacuo* and the product recrystallized from a suitable solvent.

Acknowledgment.—We are indebted to Dr. John Schmidt and Don Martin for the pharmacological screening and to E. F. Shelberg and his staff for analytical data. We wish to thank Dr. James Short of the Organic Chemistry Department for help and suggestions.

(3) Melting points were taken on a Hoover capillary melting point apparatus with the thermometer calibrated against melting point standards.

Hexahydropyrimidines. IV.[1] Synthesis of 2-[4-(N,N-Bis(2-chloroethyl)amino)aryl]-1,3-bis-(aralkyl)hexahydropyrimidines as Antitumor Agents[2]

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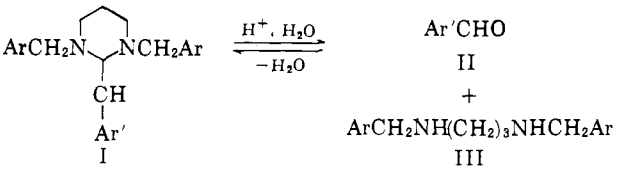
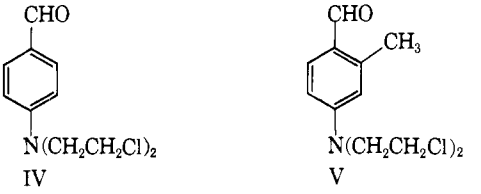
In a recent publication, the synthesis of a number of 1,2,3,-substituted hexahydropyrimidines as potential antitumor agents was reported.[1] It was suggested that these compounds I may be thought of as potential aldehydes, since they can hydrolyze under mild acid conditions to liberate the free aldehyde II.



The available test data for those hexahydropyrimidines indicated no significant activity when screened in the carcinoma 755, sarcoma 180, and leukemia 1210 systems. However, the 2-substituted 1,3-bis(*p*-methoxybenzyl)hexahydropyrimidines prepared from benzaldehyde, 2,4-dichlorobenzaldehyde, and *o*-ethoxybenzaldehyde displayed reproducible activity in a tissue culture screen. These results suggest that possibly a more potent cytotoxic aldehyde is necessary for *in vivo* antitumor activity. To this end, hexahydropyrimidines have been prepared from benzaldehyde nitrogen mustard IV and *o*-tolualdehyde nitrogen mustard V.



Due to the potential alkylating action of the nitrogen mustard grouping, the derivatives of these aldehydes might show selective antitumor activity if they are released preferentially at the tumor site, unless, of course, the nitrogen mustard grouping is capable of more rapid alkylation from the hexahydropyrimidine transport molecule.

The hexahydropyrimidines reported in Table I were synthesized by condensing the aldehydes IV or V with secondary 1,3-diamines of the general structure III in a refluxing solution of ethanol or acetonitrile. The latter solvent was found to be much preferred in two of the syntheses. The diamines III were prepared by reduction of the corresponding di-Schiff base VI.

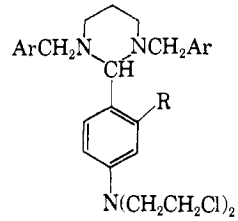
(1) J. H. Billman and J. L. Meisenheimer, part III: *J. Med. Chem.*, **6**, 682 (1963).

(2) This investigation was supported by a Public Health Service Fellowship (GF-13,650) from the Division of General Medical Sciences, National Institutes of Health, Public Health Service.

(3) National Institutes of Health Fellow, 1961–1963. Taken from the Ph.D. thesis of J. L. M., Indiana University, 1963.

TABLE I

2-[4-(N,N-BIS(2-CHLOROETHYL)AMINO)ARYL]-1,3-BIS(ARALKYL)HEXAHYDROPYRIMIDINES



No.	Ar	R	Yield, % (pure)	M.p., °C. (cor.)	Nitrogen, %, Calcd.	Nitrogen, %, Found
VIII	*p*-Methoxyphenyl	H	43.3	107–107.5	7.74	7.60
IX	*p*-Chlorophenyl[a]	H	39.2	145–146	7.63	7.65
X	2,4-Dichlorophenyl[a]	H	82.3	138–139	6.77	6.67
XI	*p*-Methoxyphenyl	CH_3	47.1	93–94	7.55	7.41
XII	*p*-Chlorophenyl	CH_3	41.8	130–131.5	7.43	7.56
XIII	2,4-Dichlorophenyl[b]	CH_3	44.2	125–126	6.63	6.65
XIV	3,4-Dichlorophenyl	CH_3	58.3	114.5–115.5	6.63	6.71

[a] Reaction solvent was acetonitrile. [b] Sensitive to light. [c] Microanalyses were performed by Miss Joanna Dickey of Indiana University, Midwest Microlab, Indianapolis, Indiana, and Alfred Bernhardt Microanalytical Laboratories, Mülheim, Germany.

$$ArCH{=}N(CH_2)_3N{=}CHAr \xrightarrow[NaBH_4]{H_2\text{–}PtO_2 \text{ or}} ArCH_2NH(CH_2)_3NHCH_2Ar$$

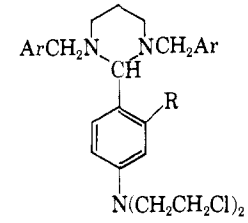
VI III

a, Ar = *p*-methoxyphenyl
b, Ar = *p*-chlorophenyl
c, Ar = 2,4-dichlorophenyl
d, Ar = 3,4-dichlorophenyl

A low-pressure hydrogenation of compound VIa using platinum oxide catalyst furnished N,N′-bis(*p*-methoxybenzyl)-1,3-diaminopropane IIIa.[1] The other diamines were obtained by reduction of the corresponding di-Schiff base with sodium borohydride.[4] The di-Schiff bases were prepared by treating the appropriate aromatic aldehyde with 1,3-diaminopropane in a molar ratio of 2:1. For example, compound VIb was obtained from *p*-chlorobenzaldehyde and 1,3-diaminopropane.

Biological Results.—Although these hexahydropyrimidines are relatively bulky, data from their tests against Walker 256 carcinoma[5] indicate that these compounds may be useful as transport molecules for the nitrogen mustard moiety, and they are relatively nontoxic. The degree of antitumor activity appears related to the electron donating ability of the substituents on N-1 and -3.

The general structure–activity relationships can be drawn from the activity or inactivity of the compounds reported. The hexahydropyrimidines derived from *o*-tolualdehyde nitrogen mustard IV were found to furnish somewhat greater tumor inhibition than the related hexahydropyrimidine synthesized from benzaldehyde nitrogen mustard. For example, 2-[4-(N, N-bis(2-chloroethyl)amino)-*o*-tolyl]-1,3-bis(*p*-methoxybenzyl)hexahydropyrimidine (XI, Table I) gave cures[6] of Walker 256 carcinoma for all animals at doses of 100 mg./kg. with no animal deaths. The similar 2-[4-(N,N-bis(2-chloroethyl)amino)phenyl]-1,3-bis(*p*-methoxybenzyl)hexahydropyrimidine VIII gave 93% inhibition at this dosage.



VIIa, R = CH_3
b, R = H

VII

a, R = CH_3
b, R = H

A wide range of activity was obtained by varying the substituents Ar in the VIIa structure from *p*-methoxyphenyl (100% inhibition at 100 mg./kg.) to *p*-chlorophenyl (71% inhibition at 100 mg./kg.) to 2,4-dichlorophenyl (22% inhibition at 100 mg./kg.). These substituents established the same relative order of activity in the VIIb series. The markedly increased antitumor activity thus appears to be related to electron donors on the benzyl group, even though this ring is well removed from the nitrogen mustard group. In accordance with these findings, we are preparing other heterocyclic ring systems with various other substituents which will vary the electron density about the 1,3-nitrogen atoms.

There remains to be answered the question as to the nature of the nitrogen mustard carrier at the time of alkylation. This might be the molecules listed in Table I in their entirety, but could presumably be the aldehydes IV or V. These aldehydes could be released from the hexahydropyrimidine structure upon mild acidic or enzymatic hydrolysis. A more electron-releasing group on Ar of the compound in Table I will increase both the alkylating activity of the nitrogen mustard group as well as facilitate acidic hydrolysis to the free aldehyde II and diamine III. Therefore, no conclusion as to the mode of action can be drawn at this time, although certain of the hexahydropyrimidines being synthesized will furnish data regarding this problem.

Experimental

The preparation of N,N′-bis(*p*-methoxybenzyl)-1,3-diaminopropane (IIIa) and N,N′-bis(*p*-chlorobenzyl)-1,3-diaminopropane (IIIb) has been described in a previous publication.[1] The benz-

(4) J. H. Billman and A. C. Diesing, *J. Org. Chem.*, **22**, 1068 (1957).
(5) Screening data were obtained by the Cancer Chemotherapy National Service Center, Bethesda, Maryland.
(6) Complete inhibition in a 12-day test period.

aldehyde nitrogen mustard IV and *o*-tolualdehyde nitrogen mustard V were obtained commercially.[7] All melting points are corrected.

N,N'-Bis(2,4-dichlorobenzylidene)-1,3-diaminopropane (VIc).—A 44.9-g. (0.606 mole) portion of 1,3-diaminopropane was added to a refluxing solution of 216.0 g. (1.23 moles) of 2,4-dichlorobenzaldehyde in 400 ml. of absolute ethanol. The addition took 20 min. and a white oil separated from the solution during this period. The mixture was refluxed for 1 additional hr., then 150 ml. of absolute ethanol was added to dissolve the oil and the solution was poured into a beaker. As the mixture cooled, a white oil again separated. However, this crystallized as the solution approached room temperature. There was obtained 208.5 g. (89.1%) of white solid, m.p. 103–104.5°. This material was recrystallized from absolute ethanol to give 206.0 g. (87.6%) of crystals, m.p. 104.0–104.5°.

Anal. Calcd. for $C_{17}H_{14}Cl_4N_2$: N, 7.22. Found: N, 7.40.

N,N'-Bis(3,4-dichlorobenzylidene)-1,3-diaminopropane (VId).—This di-Schiff base was prepared in a manner similar to VIc from 25.2 g. (0.34 mole) of 1,3-diaminopropane and 125.0 g. (0.715 mole) of 3,4-dichlorobenzaldehyde. This furnished 110.5 g. (83.7%) of crude material, m.p. 74.5–76.5°. It was recrystallized 3 times from absolute ethanol to yield 94.0 g. (71.2%) of white solid, m.p. 76.5–77.5°.

Anal. Calcd. for $C_{17}H_{14}Cl_4N_2$: N, 7.22. Found: N, 7.45.

N,N'-Bis(2,4-dichlorobenzyl)-1,3-diaminopropane (IIIc).—A mixture of 79.0 g. (0.203 mole) of N,N'-bis(2,4-dichlorobenzylidene)-1,3-diaminopropane (VIc) and 1 l. of methanol was cooled in an ice–water bath. The di-Schiff base was relatively insoluble in the cold methanol. The mixture was stirred and 23.0 g. (0.61 mole) of solid sodium borohydride were added in small portions over a 10-min. period. The mixture was warmed until there was an evolution of gas. The external heating was discontinued, although stirring was continued until 1 hr. after all of the solids had dissolved. One-half of the solvent was distilled at atmospheric pressure and when the solution had cooled to room temperature, 1 l. of a 1.2 *M* solution of sodium hydroxide was added. The mixture was shaken briefly and then divided into two approximately equal parts, each part was extracted 3 times with 75-ml. portions of diethyl ether. The ether extracts were combined and dried over sodium hydroxide pellets. The solution was filtered into a round-bottomed flask and the ether was removed with a rotary evaporator. There was obtained 70.0 g. (78.7%) of a clear, colorless oil.

Anal. Calcd. for $C_{17}H_{18}Cl_4N_2$: N, 7.15. Found: N, 7.29.

A small portion of this oil was dissolved in acetonitrile and cooled overnight. A white solid separated and was recrystallized from acetonitrile to give material which melted at 46.0–47.0°.

N,N'-Bis(3,4-dichlorobenzyl)-1,3-diaminopropane (IIId) was prepared by a procedure like that used to prepare IIIc. A 78.0-g. (0.201 mole) sample of VId was reduced using 50.0 g. (1.32 moles) of sodium borohydride. The product was 61.2 g. (77.7%) of a clear, colorless oil, $n^{25}D$ 1.5880. An analytical sample was obtained by short-path distillation at 170° (0.1 mm.).

Anal. Calcd. for $C_{17}H_{18}Cl_4N_2$: N, 7.15. Found: N, 7.32.

2-[4-(N,N-Bis(2-chloroethyl)amino)aryl]-1,3-bis(aralkyl)-hexahydropyrimidines (Table I).—The synthesis of 2-[4-(N,N-bis(2-chloroethyl)amino)-*o*-tolyl]-1,3-bis(*p*-chlorobenzyl)-hexahydropyrimidine (XII) was typical of these compounds. A 5.20-g. (0.02 mole) sample of *o*-tolualdehyde nitrogen mustard (V) was dissolved in 25 ml. of warm absolute ethanol. To this solution was added 6.47 g. (0.02 mole) of N,N'-bis(*p*-chlorobenzyl)-1,3-diaminopropane, also in 25 ml. of absolute ethanol. The mixture was refluxed for 15 min., then 10 ml. of solvent was allowed to distil at atmospheric pressure over another 15-min. period. The solution was allowed to cool slowly to room temperature and then stored overnight in a refrigerator. This effected the separation of a light yellow solid. There was 5.3 g. of this material, m.p. 103–130°. This was recrystallized from 7.5 ml. of acetonitrile to furnish 4.05 g., m.p. 123–131°. The filtrate from the reaction mixture was reduced to approximately 1/3 of the original volume. Subsequent cooling of this solution yielded an additional 2.62 g. of white solid which melted at 129–131°. This was combined with the 4.05 g. of solid obtained from the initial crop of product and recrystallized twice from acetonitrile. This gave 4.73 g. (41.8%) of crystals, m.p. 130.0–131.5°.

Anal. Calcd. for $C_{29}H_{33}Cl_4N_3$: N, 7.43. Found: N, 7.56.

(7) Frinton Laboratories, South Vineland, N. J.

Acknowledgment is due Drs. H. W. Bond, R. B. Ross, and J. Leiter of the Cancer Chemotherapy National Service Center for their cooperation in obtaining the screening data. We wish to thank the Union Carbide Chemicals Company for the 1,3-diaminopropane which they supplied.

7-Aminodesacetoxycephalosporanic Acid and Its Derivatives

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Smith Kline and French Laboratories, Philadelphia, Pennsylvania

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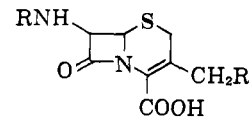
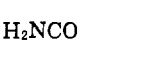
Catalytic hydrogenation of the antibiotic cephalosporin C (Ia)[1] gave a product which we have identified as the desacetoxy derivative IIa.[2] This new antibiotic material was not isolated, but its structure was established by hydrolysis to 7-aminodesacetoxycephalosporanic acid (IIb). Although IIb had no appreciable antibacterial action, three derivatives (IId, IIe, and IIf) prepared from it showed modest activities. Since the completion of this work, Morin, *et al.*,[3] have reported briefly the hydrogenation of a cephalosporin derivative; the structure given for their product is in accord with our findings.



I R' = CH_3COO
II R' = H

a, R = $H_2NCH(CH_2)_3CO$ (COOH)
b, R = H
c, R = $C_6H_3(NO_2)_2NHCH(CH_2)_3CO$ (COOH)
d, R = $C_6H_5CH_2CO$
e, R = 
f, R = H_2NCO

The hydrogenation of Ia was carried out at low pressure using a large quantity of palladium catalyst. Paper electrophoresis showed that in addition to unchanged starting material there was present a new antibacterial substance (IIa) which gave a purple color with ninhydrin and had a strong ultraviolet absorption. The reaction mixture was treated with 2,4-dinitrofluorobenzene to give a mixture of Ic and IIc, and the latter, after partial purification, was hydrolyzed with acid to afford a small yield of IIb, isolated by ion-exchange chromatography. The dinitrophenyl group did not influence the hydrolytic cleavage of the side chain, but its introduction facilitated the manipulation of the intermediates and the isolation of the product.[4]

(1) E. P. Abraham and G. G. F. Newton, *Biochem. J.*, **79**, 377 (1961).

(2) (a) E. P. Abraham and G. G. F. Newton, *ibid.*, **62**, 658 (1956), reported that cephalosporin C absorbed hydrogen in the presence of a catalyst, but they did not characterize the product; (b) according to the nomenclature recommended in ref. 5, the hydrogenation product is a derivative of 7-amino-3-methyl-Δ^3-cephem-4-carboxylic acid (IIb).

(3) R. B. Morin, B. G. Jackson, R. A. Mueller, E. R. Lavagnino, W. B. Scanlon, and S. L. Andrews, *J. Am. Chem. Soc.*, **85**, 1896 (1963).

(4) The hydrolysis and isolation procedures were similar to those described for the preparation of Ib in Belgian Patent 593,777 (1959).