Synthesis and Preliminary Pharmacological Evaluation of Alkylpiperazine Esters

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Twenty-nine alkylpiperazine esters have been synthesized and tested for the action on the CNS and for anti-inflammatory, analgesic, antipyretic, antispasmodic, and antimicrobic activities. Many of the compounds tested, in particular XV, display analgesic, anti-inflammatory, and antipyretic properties.

MANY ALKYLPIPERAZINE esters, mainly acrylates, have been synthesized and investigated chiefly for their anti-inflammatory, antispasmodic, and antimicrobic activities. This research was suggested by the known anti-inflammatory activity of β-4-biphenylylacrylic acid (1, 2), and of other acrylic acids (3), by the autonomotropic properties exerted by amino-alcohol esters (4), and by the antimicrobic properties observed for various acrylic acids (5–9) and quaternary ammonium salts of their basic esters (10). The activity on the CNS was also tested together with the analgesic and antipyretic activities.

The compounds dealt with in the present paper are mainly symmetrical dialkylpiperazine bis-acrylates and asymmetrical alkylpiperazine mono-acrylates. Only X, XI, and XXIX are esters of saturated aliphatic acids. They have been synthesized for the purpose of determining the influence produced by the disappearance of the double olefine bond on the pharmacological properties studied.

The synthesis methods used are the general ones for esters; *i.e.*, the reaction of acid chlorides with alcohols, or the reaction of acids or their sodium salts with chloro-derivatives. In the latter case, the use of isopropyl alcohol, in which the sodium salts of the acids are soluble to a certain extent, is particularly advantageous. I and II were isolated from the reaction mixture directly as the hydrochlorides.

The new esters are colorless crystalline solids, except for XVIII, which is a very viscous oil. Their properties are given in Tables I and II. The hydrochlorides of the esters are sparingly soluble in water, except for those in which an atom of nonpiperazine nitrogen is also present.

EXPERIMENTAL

Chemistry.—Melting points were taken on a Townson-Mercer melting point apparatus and are corrected.

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Intermediates.—The references for the synthesis of the required acrylic acids have already been reported by the authors in another paper (3). 3-(3-Pyridyl)-propionic acid was prepared according to Merz and Stolte (11), and 2-(4-biphenylyl)-butyric acid according to Cavallini and Massarani (12).

The intermediate piperazines, *i.e.*, 1,4-bis-(1-methyl-2-chloroethyl)-piperazine (b. p. 150–152°/15 mm.), 1-(4-chlorophenyl)-4-(2-chloroethyl)-piperazine dihydrochloride (m. p. 201–203° dec.), 1-benzyl-4-(2-chloroethyl)-piperazine dihydrochloride (m. p. 304–308° dec.), 1-(4-methoxyphenyl)-4-(2-chloroethyl)-piperazine dihydrochloride (m. p. 220–222° dec.), and 1-(4-tolyl)-4-(2-chloroethyl)-piperazine dihydrochloride (m. p. 221–223° dec.) were prepared according to the general methods for the preparation of chloroamines described by Wilson and Tishler (13). 1-(4-Fluorophenyl)-4-(2-chloroethyl)-piperazine dihydrochloride was prepared according to Janssen (14) and 1-(2-chloroethyl)-piperazine dihydrochloride according to Hromatka and Engel (15).

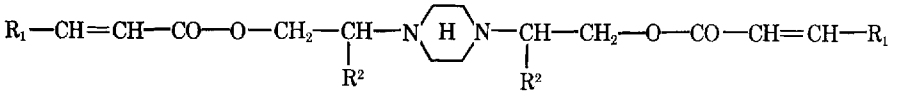
Esters

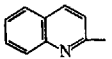
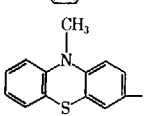
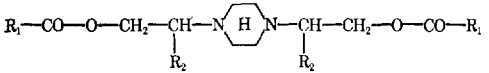
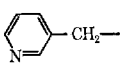
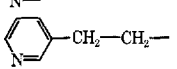
1,4-bis[2-(Crotonyloxy)ethyl]-piperazine Dihydrochloride (I).—*Method A.*—A mixture of 48.9 Gm. of α-crotonic acid, 64 Gm. of 1,4-bis (2-chloroethyl)-piperazine (13), 45 Gm. of anhydrous potassium carbonate, and 1300 ml. of anhydrous benzene are refluxed for 16 hr., with stirring. After cooling to room temperature, potassium chloride and the excess potassium carbonate are removed by filtration and the benzene solution thoroughly washed, first with dilute sodium carbonate solution, and then with water. Finally, after drying over sodium sulfate, hydrogen chloride is bubbled through. The filtered product, washed with anhydrous ether and dried under vacuum at 50°, weighs 71.7 Gm. After crystallization from ethanol, it melts at 221.5–223.5° dec.

1,4-bis{2-[3-(1-Naphthyl)acrylyloxy]ethyl}-piperazine Dihydrochloride (II).—*Method B.*—A 8.71-Gm. quantity of 1,4-bis(2-hydroxyethyl)-piperazine (4) is added to a solution of 21.66 Gm. of 3-(1-naphthyl)-acrylyl chloride (10) in 100 ml. of anhydrous benzene. The reaction mixture is refluxed for 2 hr., and, after standing overnight at room temperature, the solid precipitate is filtered off and dried at 50° under vacuum. The product (17.9 Gm.) is crystallized from ethanol and gives colorless crystals, m.p. 269–271° dec.

1,4-bis{2-[3-(3-Pyridyl)acrylyloxy]ethyl}-piperazine (IV).—*Method C.*—A 77-Gm. quantity of sodium 3-(3-pyridyl)-acrylate (3) and 43 Gm. of 1,4-

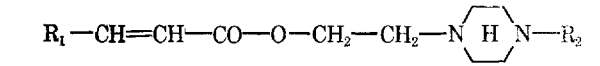
TABLE I.—SYMMETRICAL DIALKYLPIPERAZINE BIS-ESTERS

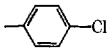
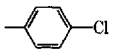
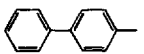
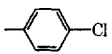
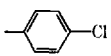
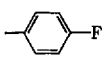
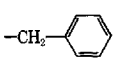
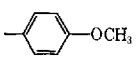
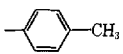
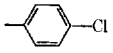
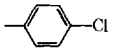
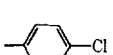
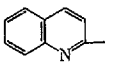
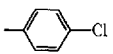


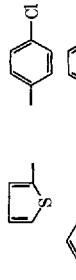
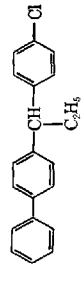
Compd.	R_1	R_2	Method	Reflux, hr.	Yield,[a] %	M.p., °C.	Recrystn. Solvent	Formula	Anal., % Calcd.	Anal., % Found
I	CH_3	H	*A* *B*	16	66 72	221.5–223.5[b]	Ethanol	$C_{16}H_{26}N_2O_4 \cdot 2HCl$	C, 50.13 H, 7.36 N, 7.30	C, 50.41 H, 7.27 N, 7.21
II		H	*B*	2	59	269–271[b]	Ethanol	$C_{34}H_{34}N_2O_4 \cdot 2HCl$	C, 67.21 H, 5.97 N, 4.61	C, 67.28 H, 5.81 N, 4.54
III		H	*C*	48	81	130.5–131.5	Benzene–cyclohexane	$C_{24}H_{28}N_4O_4$	C, 66.03 H, 6.47 N, 12.84	C, 65.91 H, 6.49 N, 12.79
IV		H	*C*	15	72	123–124	Isopropanol	$C_{24}H_{28}N_4O_4$	C, 66.03 H, 6.47 N, 12.84	C, 65.98 H, 6.55 N, 12.66
V		H	*C*	60	75	135–136.5	Benzene–cyclohexane	$C_{24}H_{28}N_4O_4$	C, 66.03 H, 6.47 N, 12.84	C, 66.11 H, 6.46 N, 12.91
VI		H	*C*	48	69	165–166.5	Benzene–cyclohexane	$C_{32}H_{32}N_4O_4$	C, 71.62 H, 6.01 N, 10.44	C, 71.80 H, 6.06 N, 10.28
VII		H	*C*	48	49	116–117	Benzene–cyclohexane	$C_{32}H_{32}N_4O_4$	C, 71.62 H, 6.01 N, 10.44	C, 71.66 H, 6.07 N, 10.40
VIII		H	*C*	60	60	178–180	Ethanol–benzene	$C_{40}H_{40}N_4S_2O_4$	C, 68.15 H, 5.72 N, 7.95	C, 68.30 H, 5.80 N, 7.88
IX		CH_3	*C*	60	39	149–150	Benzene–cyclohexane	$C_{28}H_{32}N_4O_4$	C, 67.22 H, 6.94 N, 12.06	C, 67.16 H, 6.88 N, 12.01
										
X		H	*C*	48	45	69–71	Benzene–cyclohexane	$C_{22}H_{28}N_4O_4$	C, 64.06 H, 6.84 N, 13.58	C, 63.91 H, 6.82 N, 13.37
XI		H	*C*	48	89	[c]	...	$C_{24}H_{32}N_4O_4$	C, 65.43 H, 7.32 N, 12.72	C, 65.29 H, 7.33 N, 12.64

[a] Crude product. [b] The compound melts with decomposition. [c] Liquid product, which decomposes on heating.

TABLE II.—ASYMMETRICAL ALKYLPIPERAZINE MONO-ESTERS



Compd.	R_1	R_2	Method	Reflux, hr.	Yield,[a] %	M.p., or B.p., °C., mm.	Recrystn. Solvent	Formula	Anal., % Calcd.	Found
XII			*D*	48	72	125–126.5	Isopropanol	$C_{21}H_{23}ClN_2O_2$	C, 68.00 H, 6.25 Cl, 9.56 N, 7.55	C, 68.16 H, 6.21 Cl, 9.67 N, 7.44
XIII			*D*	48	80	150–155	Isopropano	$C_{21}H_{23}ClN_2O_3$	C, 65.20 H, 5.99 Cl, 9.16 N, 7.24	C, 65.33 H, 5.83 Cl, 9.23 N, 7.24
XIV			*D*	48	65	174.5–176	Ethylacetate	$C_{27}H_{27}ClN_2O_2$	C, 72.55 H, 6.08 Cl, 7.93 N, 6.26	C, 72.51 H, 6.11 Cl, 7.98 N, 6.19
XV			*D*	48	81	110–110.5	Isopropanol	$C_{20}H_{22}ClN_3O_2$	C, 64.59 H, 5.96 Cl, 9.53 N, 11.30	C, 64.54 H, 5.96 Cl, 9.55 N, 11.31
XVI			*D*	48	79	107–108	Ethanol	$C_{20}H_{22}FN_3O_2$	C, 67.58 H, 6.24 N, 11.82	C, 67.79 H, 6.20 N, 11.80
XVII		H	*D*	48	49	240–245[b]	...	$C_{14}H_{19}N_3O_2.3HCl$	C, 45.35 H, 5.98 Cl, 28.69 N, 11.33	C, 45.33 H, 6.01 Cl, 28.71 N, 11.16
XVIII			*D*	48	51	165–170 (0.05)	...	$C_{21}H_{25}N_3O_2$	C, 71.77 H, 7.17 N, 11.96	C, 71.71 H, 7.10 N, 12.03
XIX			*D*	48	66	88–89	Hexane–benzene	$C_{21}H_{25}N_3O_3$	C, 68.64 H, 6.86 N, 11.44	C, 68.60 H, 6.94 N, 11.29
XX			*D*	42	82	132–133	Isopropanol	$C_{21}H_{25}N_3O_2$	C, 71.77 H, 7.17 N, 11.96	C, 71.75 H, 7.18 N, 12.11
XXI			*D*	48	83	116–117	Benzene–cyclohexane	$C_{20}H_{22}ClN_3O_2$	C, 64.59 H, 5.96 Cl, 9.53 N, 11.30	C, 64.66 H, 5.94 Cl, 9.48 N, 11.28
XXII			*D*	48	69	116–116.5	Isopropanol	$C_{20}H_{22}ClN_3O_2$	C, 64.59 H, 5.96 Cl, 9.53 N, 11.30	C, 64.66 H, 5.88 Cl, 9.51 N, 11.16
XXIII			*D*	48	85	106.5–107.5	Isopropanol	$C_{25}H_{25}ClN_2O_2$	C, 71.33 H, 5.98 Cl, 8.42 N, 6.65	C, 71.59 H, 6.09 Cl, 8.44 N, 6.60
XXIV			*D*	48	89	140–141.5	Ethylacetate	$C_{24}H_{24}ClN_3O_2$	C, 68.31 H, 5.73 Cl, 8.40 N, 9.96	C, 69.01 H, 5.66 Cl, 8.32 N, 10.02

XXV		D	60	48	100.5–101.5	Isopropanol	$C_{24}H_{29}ClN_2O_2$	C, 68.31 H, 5.73 Cl, 8.40 N, 9.96	C, 68.16 H, 5.74 Cl, 8.43 N, 9.91
XXVI		D	48	88	126–127	Isopropanol	$C_{19}H_{23}ClN_2O_3$	C, 63.24 H, 5.86 Cl, 9.82 N, 7.76	C, 64.02 H, 5.71 Cl, 9.88 N, 7.66
XXVII		D	48	89	134.5–136	Ethylacetate	$C_{19}H_{23}ClN_2SO_2$	C, 60.54 H, 5.61 Cl, 9.40 N, 7.43	C, 60.81 H, 5.72 Cl, 9.39 N, 7.42
XXVIII		D	48	87	165–167	Ethylacetate	$C_{23}H_{24}ClN_3O_2$	C, 67.38 H, 5.90 Cl, 8.65 N, 10.25	C, 67.33 H, 5.90 Cl, 8.78 N, 10.11
XXIX		D	48	82	87–88	Ethanol	$C_{28}H_{31}ClN_2O_2$	C, 72.63 H, 6.75 Cl, 7.66 N, 6.05	C, 72.80 H, 6.71 Cl, 7.49 N, 6.06

bis(2-chloroethyl)piperazine are refluxed for 15 hr. in 700 ml. of isopropanol. At the end the reaction mixture is cooled to room temperature, and the suspended solid filtered off. The mother liquor from the reaction is concentrated to a small volume, and the solid separating is filtered and added to the previously isolated precipitate. The combined solids are taken up in chloroform, rejecting the insoluble portion, and the chloroform solution, after filtration with charcoal, is evaporated under reduced pressure, to give 63.7 Gm. of product. After crystallization from isopropanol, IV melts at 123–124°.

1 - (4 - Chlorophenyl) - 4 - [2 - (cinnamoyloxy) - ethyl]-piperazine (XII).—*Method D.*—A solution of sodium ethylate, prepared from 1.87 Gm. of sodium and 45 ml. of ethanol, is added dropwise to a suspension of 14 Gm. of 1-(4-chlorophenyl)-4-(2-chloroethyl)-piperazine dihydrochloride in 70 ml. of ethanol, at 0°. The mixture is stirred for 30 min. at room temperature and then concentrated under reduced pressure, until a thick mush is obtained. A 140-ml. quantity of isopropanol and 7.16 Gm. of sodium *trans*-cinnamate are added to this, then refluxed for 48 hr. with continuous stirring. At the end the mixture is cooled to room temperature, and the suspended solid filtered. The mother liquor from the reaction is concentrated to a small volume, and the separated solid filtered and combined with the previously isolated precipitate. The combined solids are taken up in chloroform, rejecting the insoluble portion, and the chloroform solution, washed with water and filtered with charcoal, is evaporated under reduced pressure to give 11.2 Gm. of product which, after crystallization from isopropanol, melts 125–126.5°.

PHARMACOLOGICAL RESULTS

The new compounds were submitted to preliminary pharmacological screening comprising the action on the CNS (16), the anti-inflammatory (17), analgesic (18), antipyretic (19), antispasmodic (20), antibacterial and antifungal actions (10), and the acute toxicity. The highest dosage level that did not provoke an obvious toxic symptomatology in experimental animals was used for each test. In all cases the compounds were administered by intraperitoneal injection. Phenylbutazone and morphine were used as standards for comparison of the anti-inflammatory, analgesic, and antipyretic actions.

The results of the activity tests considered most interesting are reported in Tables III and IV; they show that many of the compounds have a mild general depressive action on the CNS. Several compounds (II, III, IV, V, VI, VII, VIII, IX, XVII, XVIII, XIX, XX, XXI) display significant inhibition of formalin-induced edema. As for the analgesic action, XII, XV, XVI, XXI, XXII, and XXIX greatly increase the pain threshold of mice, in particular, XV, VI, VII, IX, XVIII, and XXI show a significant antipyretic effect. All the compounds have been found to be inactive regarding antispasmodic, antibacterial, and antifungal actions. It is interesting to note that, within the sphere of the authors' investigations, the over-all pharmacological outline of the 3 esters of saturated aliphatic acids (X, XI, XXIX) does not substantially differ from that of the acrylic esters.

In the light of the above results, the authors con-

TABLE III.—PHARMACOLOGICAL RESULTS OF SYMMETRICAL DIALKYLPIPERAZINE BIS-ESTERS

Compd.	LD_{50} (Approx.) Mouse, mmole/Kg., i.p.	mmole/ Kg., i.p.	Action on the CNS, Mouse	Analgesic Activity, Mouse: mmole/ Kg., i.p.	Analgesic Activity, Mouse: Increase of Reaction Time, %	Anti-Inflammatory Activity, Rat: mmole/ Kg., i.p.	Anti-Inflammatory Activity, Rat: Inhibition of Edema, %	Antipyretic Activity, Rat: mmole/ Kg., i.p.	Antipyretic Activity, Rat: Max. Temp. Decrease, °C.
I	0.313–0.365	0.130	Moderate muscle hypotonia, moderate ipsilateral flexor reflex decrease	0.130	29	0.130	Inact.	...	...
II	1.646–2.140	0.658	Nothing noticeable	0.658	47	0.658	53	0.658	1.7
III	0.527–0.664	0.458	Moderate CNS depression, moderate motor incoordination, muscle hypotonia	0.458	54	0.458	37	...	...
IV	2.062–2.978	0.458	Moderate pinna reflex decrease	0.458	74	0.458	40	0.458	1.9
V	1.993–2.864	0.458	Nothing noticeable	0.458	39	0.458	32	...	...
VI	1.267–1.770	0.745	Nothing noticeable	0.745	21	0.745	60	0.373	2.2
VII	0.671–0.801	0.373	Moderate CNS depression	0.373	72	0.373	50	0.373	3.2
VIII	0.312–0.440	0.284	Nothing noticeable	0.284	20	0.284	49	...	...
IX	2.045–2.691	0.861	Nothing noticeable	0.861	49	0.861	53	0.430	2.3
X	>7.758	0.970	Nothing noticeable	0.970	18	0.970	21	...	...
XI	>7.264	0.908	Nothing noticeable	0.908	39	0.908	26	...	...
Morphine[a]	...	...	...	0.0133	67	...	...	...	...
Phenylbutazone	...	...	...	...	...	0.32	18	0.32	1.6

[a] Hydrochloride.

TABLE IV.—PHARMACOLOGICAL RESULTS OF ASYMMETRICAL ALKYLPIPERAZINE MONO-ESTERS

Compd.	LD_{50} (Approx.) Mouse, mmole/Kg., i.p.	mmole/ Kg., i.p.	Action on the CNS, Mouse	Analgesic Activity, Mouse: mmole/ Kg., i.p.	Analgesic Activity, Mouse: Increase of Reaction Time, %	Anti-Inflammatory Activity, Rat: mmole/ Kg., i.p.	Anti-Inflammatory Activity, Rat: Inhibition of Edema, %	Antipyretic Activity, Rat: mmole/ Kg., i.p.	Antipyretic Activity, Rat: Max. Temp. Decrease, °C.
XII	7.550–9.707	2.157	Moderate CNS depression, muscle hypotonia	2.157	96	2.157	16	...	...
XIII	3.619–4.653	0.517	Nothing noticeable	0.517	38	0.517	Inact.	...	...
XIV	3.244–3.982	0.447	Nothing noticeable	0.447	42	0.447	Inact.	...	...
XV	0.753–0.914	0.134	Moderate CNS depression, muscle hypotonia	0.134	124	0.134	Inact.	...	...
XVI	1.069–1.322	0.281	Moderate CNS depression, muscle hypotonia, moderate ipsilateral flexor reflex decrease	0.281	97	0.281	12	...	...
XVII	0.536–0.689	0.383	Moderate CNS depression	0.383	42	0.383	35	...	...
XVIII	0.825–0.967	0.569	Moderate muscle hypotonia	0.569	74	0.569	45	0.569	3.3
XIX	0.952–1.170	0.136	Moderate CNS depression, moderate ipsilateral flexor, corneal, and pinna reflexes decrease	0.136	61	0.136	33	...	...
XX	0.936–1.195	0.071	Moderate ipsilateral flexor, corneal, and pinna reflexes decrease	0.071	69	0.071	34	...	...
XXI	1.829–2.124	0.134	Moderate CNS depression	0.134	106	0.134	41	0.134	2.4
XXII	1.398–1.748	0.067	Moderate CNS depression, moderate muscle hypotonia	0.067	94	0.067	Inact.	...	...
XXIII	6.889–7.958	0.950	Nothing noticeable	0.950	23	0.950	Inact.	...	...
XXIV	>7.584	3.792	Moderate CNS depression, muscle hypotonia	0.474	38	0.474	Inact.	...	...
XXV	0.355–0.592	0.237	Moderate CNS depression	0.237	72	0.237	Inact.	...	...
XXVI	6.097–7.760	4.434	Moderate CNS depression	0.554	28	0.554	Inact.	...	...
XXVII	5.572–7.297	4.246	Nothing noticeable	0.531	16	0.531	Inact.	...	...
XXVIII	5.245–6.709	0.976	Moderate CNS depression	0.976	37	0.976	Inact.	...	...
XXIX	>6.911	0.864	Nothing noticeable	0.864	123	0.864	Inact.	...	...
Morphine[a]	...	...	...	0.0133	67	...	...	...	...
Phenylbutazone	...	...	...	...	...	0.32	18	0.32	1.6

[a] Hydrochloride.

sider that many of the compounds tested, especially XV, deserve a more detailed pharmacological study for their analgesic, anti-inflammatory, and antipyretic properties.

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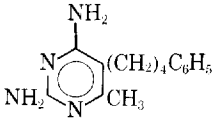
Analogs of Tetrahydrofolic Acid XXXII

Hydrophobic Bonding to Dihydrofolic Reductase IV. Inhibition by *p*-Substituted Benzoic and Benzoyl-L-glutamic Acids

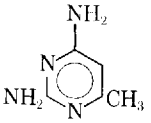
By B. R. BAKER*, THOMAS J. SCHWAN, JAROSLAV NOVOTNY, and BENG-THONG HO

A series of *p*-substituted benzoic acids and benzoyl-L-glutamic acids were synthesized and evaluated as inhibitors of dihydrofolic reductase in order to gain information on the position of the hydrophobic bonding region of the enzyme with respect to the position of the substrate, dihydrofolate, when the latter is complexed to the enzyme. Hydrophobic bonding by the *p*-substituted benzoyl-L-glutamic acids was reached 4–8 atoms from the *p*-position, thus indicating that the hydrophobic bonding region was not between the pyrimidyl and *p*-aminobenzoyl moieties of the substrate, dihydrofolate; in contrast, hydrophobic bonding with *p*-substituted benzoic acids was reached 1–4 atoms from the *p*-position, thus indicating that the *p*-substituted benzoic acids were complexed in a different region of the enzyme than the *p*-substituted benzoyl-L-glutamic acids.

THE DISCOVERY of strong hydrophobic bonding to dihydrofolic reductance with alkyl pyrimidines and 1,2-dihydro-*s*-triazines (1) has led to a major program in this laboratory on the nature, stereochemistry, and position of this hydrophobic bonding. That the aryl group of 1-aryl-1,2-dihydro-*s*-triazines and 5-arylpyrimidines of the pyrimethamine[1] type is also most probably complexed to dihydrofolic reductase by hydrophobic bonding has received strong experimental support (2). Furthermore, the 5-alkyl group of 5-alkyl-2,4-diamino-6-pyrimidines had maximum hydrophobic bonding with the 3-methylbutyl group (3); less binding was observed with 2-methylbutyl, butyl, and 1-methylbutyl, in that order. Furthermore, cyclohexyl was as good as *n*-butyl, but cyclopentyl was considerably poorer; thus, there are definite conformational requirements for alkyl groups to give maximum hydrophobic bonding (3). The magnitude of hydrophobic bonding to dihydrofolic reductase can be enormous; the phenylbutyl group of I alone had a free energy of binding of 6.0 Kcal./mole, equivalent to 80% of the total binding of the substrate, dihydrofolate. This binding by the phenylbutyl group could be calculated from the increment of 40,000 observed between I and II in their relative ability to inhibit dihydrofolic reductase.



I



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[1] Marketed as Daraprim by Burroughs Wellcome Co.