[CONTRIBUTION FROM THE DEPARTMENT OF CHEMISTRY, NEW MEXICO HIGHLANDS UNIVERSITY]

Potential Purine Antagonists. I. Synthesis of Some 4,6-Substituted Pyrazolo[3,4-d]pyrimidines[1]

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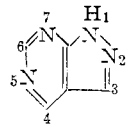
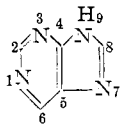
Various isomeric structural purine analogs possessing the pyrazolo[3,4-*d*]pyrimidine nucleus have been synthesized. The synthesis of this ring system has been accomplished from two pyrazole intermediates, 3-amino-4-cyanopyrazole (VI) and 3-amino-4-pyrazole carboxamide (X). The chemistry of the pyrazolo[3,4-*d*]pyrimidine ring is discussed and compared with that of purine whenever possible.

Various structural changes of the natural purines have resulted in several potent antagonists in biological systems.[2] Of special interest are the anti-tumor agents 8-aza-guanine,[3] 6-mercaptopurine,[4] 6-chloropurine[2] and 6-thioguanine.[5]

The synthesis of the pyrazolo[3,4-*d*]pyrimidine ring system was undertaken to provide new compounds isomeric with various biologically active purines in the hope that new anti-tumor agents might be discovered. In addition it was felt that a study of the chemistry of pyrazolo[3,4-*d*]pyridine would yield fundamental information of value in purine chemistry.



pyrazolo[3,4-*d*]pyrimidine



purine

A survey of the literature showed that prior to this work the only compound known possessing the pyrazolo[3,4-*d*]pyrimidine ring system was 1,3-diphenyl-6-methyl-4-hydroxypyrazolo[3,4-*d*]pyrimidine, synthesized by Justoni and Fusco[6] from 1,3-diphenyl-5-acetylamino-4-pyrazolecarboxamide.

Recent work by Shaw and Woolley[7,8] has drawn attention to the fact that adenine, hypoxanthine and xanthine can be synthesized from various 5-aminoimidazole derivatives. The biological synthesis of hypoxanthine from 5-amino-4-imidazolecarboxamide[9] again points to a synthetic route to the purines which has been largely overlooked until recently. Following this lead from the purine series it was decided to investigate the synthesis of 3-amino-4-pyrazole carboxamide (X) as a possible intermediate for synthesis of the desired derivatives of pyrazolo[3,4-*d*]pyrimidine.

Ruhemann and Orton[10] have reported the synthesis of 4-carbethoxypyrazolone-3 from ethoxymethylene malonic ester and hydrazine. We found that when ethoxymethylenemalononitrile (II) was substituted for ethoxymethylene malonic ester in this reaction 3-amino-4-cyanopyrazole (VI) was formed in 85% yield. That cyclization had indeed taken place to give the pyrazole derivative VI was shown by treatment of VI with acetyl chloride, which resulted in the preparation of 3-acetylamino-4-cyanopyrazole.

The hydrolysis of the nitrile group of 3-amino-4-cyanopyrazole (VI) with concentrated sulfuric acid gave 3-amino-4-pyrazolecarboxamide (X) isolated as the sulfate in greater than 80% yield. This sulfate when heated with formamide gave an excellent yield of 4-hydroxypyrazolo(3,4-*d*)pyrimidine (XIV), the analog of hypoxanthine. Fusion of the sulfate salt of 3-amino-4-pyrazolecarboxamide (X) with urea gave 4,6-dihydroxypyrazolo(3,4-*d*)pyrimidine (IX) in good yield.

Treatment of 4-hydroxypyrazolo(3,4-*d*)pyrimidine (XIV) with phosphorus pentasulfide in boiling tetralin gave 4-mercaptopyrazolo(3,4-*d*)pyrimidine (XVIII) in much the same manner as for the synthesis of 6-mercaptopurine[11] from hypoxanthine. It is interesting to note that no unreacted starting material was recovered in the thiation of XIV, while approximately 37% of hypoxanthine was recovered under similar reaction conditions. This may be due to greater solubility of XIV in the hydrocarbon solvent. Methylation of XVIII with methyl iodide gave 4-methylmercaptopyrazolo(3,4-*d*)pyrimidine (XVII).

Chlorination of the 4-hydroxy derivative XIV with phosphorus oxychloride and dimethylaniline gave 4-chloropyrazolo(3,4-*d*)-pyrimidine (XV) in above 80% yield.

In contrast to 6-chloropurine[2] it was found that XV could be extracted directly from the cold aqueous acidic solution resulting when the residual phosphorus oxychloride reaction mixture was poured onto ice. The chlorine atom in 4-chloropyrazolo(3,4-*d*)pyrimidine appears to be more reactive than in 6-chloropurine since XV is rapidly hydrolyzed in boiling water. Concentrated aqueous ammonia heated on the steam-bath converts XV to 4-aminopyrazolo(3,4-*d*)pyrimidine (V), while 6-chloropurine is reported stable in boiling dilute aqueous ammonia and is unaffected by concentrated aqueous ammonia when heated at 100° for 4 hours in a sealed tube.[2]

Treatment of 4-chloropyrazolo(3,4-*d*)pyrimidine-(XV) with various primary and secondary amines,

(1) This investigation was supported by research grant C-2105 from the National Cancer Institute, of the National Institutes of Health, Public Health Service.

(2) A. Bendich, P. J. Russell, Jr., and J. J. Fox, THIS JOURNAL, **76**, 6073 (1954). See additional references listed there.

(3) G. W. Kidder, V. C. Dewey, R. E. Parks, Jr., and G. L. Woodside, *Science*, **109**, 511 (1949).

(4) G. H. Hitchings and C. P. Rhoads, *Ann. N. Y. Acad. Sci.*, **60**, 183 (1954).

(5) G. B. Elion and G. H. Hitchings, THIS JOURNAL, **77**, 1676 (1955).

(6) R. Justoni and R. Fusco, *Gazz. chim. ital.*, **68**, 66 (1938).

(7) E. Shaw and D. W. Woolley, *J. Biol. Chem.*, **181**, 89 (1949).

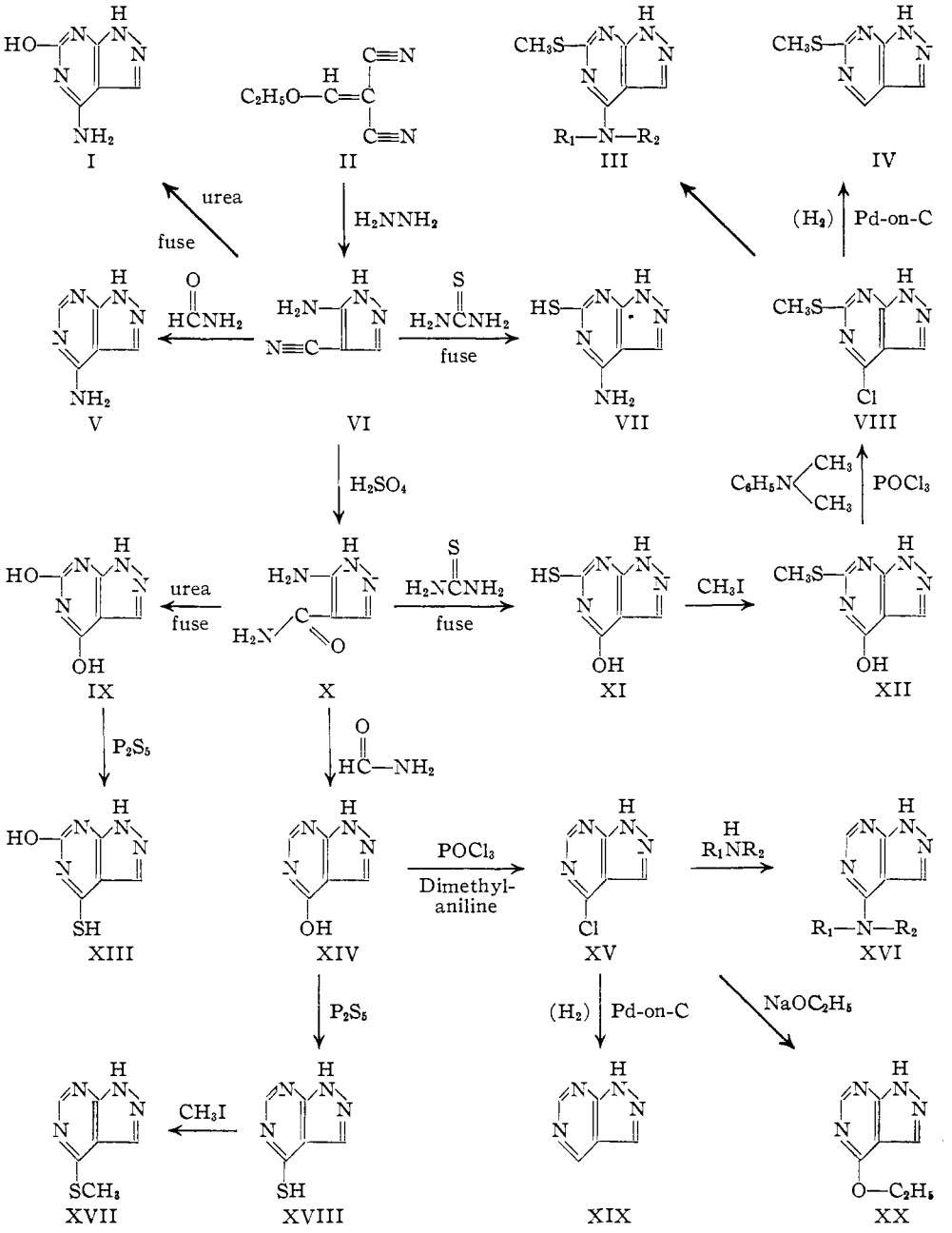
(8) E. Shaw and D. W. Woolley, *ibid.*, **185**, 439 (1950).

(9) M. P. Schulman, *et al.*, *Federation Proc.*, **9**, 225 (1950).

(10) S. Ruhemann and K. J. P. Orton, *J. Chem. Soc.*, **67**, 1008 (1895).

(11) G. B. Elion, E. Burgi and G. H. Hitchings, THIS JOURNAL, **74**, 413 (1952).

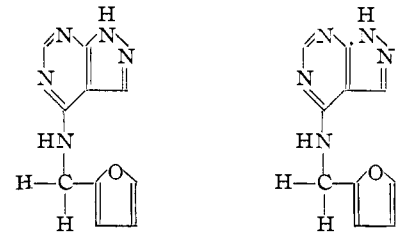
REACTION SCHEME



in aqueous or alcoholic solution heated on the steam-bath, resulted in nucleophilic displacement of the chlorine atom to yield the substituted amino derivatives listed in Table I.

Furfurylamine and XV gave 4-furfurylaminopyrazolo(3,4-*d*)pyrimidine which is an isomeric structural analog of "kinetin," a recently isolated cell division factor.[12,13]

4-Chloropyrazolo(3,4-*d*)pyrimidine (XV) treated with sodium ethoxide gave 4-ethoxypyrazolo(3,4-*d*)pyrimidine (XX). Dechlorination of XV by catalytic hydrogenation with palladium-on-charcoal catalyst yielded the parent compound pyrazolo(3,4-*d*)pyrimidine (XIX) in 76% yield. The melting point of XIX was found to be 213–214° as compared to 215–216°[14] reported for purine. The



kinetin

4-furfurylaminopyrazolo-(3,4-*d*)pyrimidine

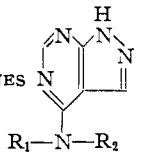
(12) C. O. Miller, F. Skoog, M. H. Von Saltza and F. M. Strong, THIS JOURNAL, **77**, 1392 (1955).

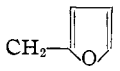
(13) C. O. Miller, F. Skoog, F. S. Okumura, M. H. Von Saltza and F. M. Strong, *ibid.*, **77**, 2662 (1955).

(14) A. G. Beaman, *ibid.*, **76**, 5635 (1954); A. Albert and D. J. Brown (see footnote 15) report m.p. 212–213° for purine.

TABLE I

4-SUBSTITUTED-AMINOPYRAZOLO(3,4-*d*)PYRIMIDINES



R_1	R_2	M.p., °C.	Calculated C	Calculated H	Calculated N	Found C	Found H	Found N	Method	Yield, %	Recrystn. solvent[b]
H	CH_3[a]	227–228			47.0			46.8	1	83	A
CH_3	CH_3	233–234	51.6	5.5	42.9	51.6	5.3	42.6	1	61	D
C_2H_5	C_2H_5	196–197	56.5	6.8		56.5	6.8		1	85	B
H	$HC(CH_3)_2$	253–254	54.2	6.2	39.6	54.4	6.1	39.7	1	70	A
H	C_6H_5	263–264	62.6	4.3	33.1	62.5	4.3	33.4	2	55	C
H	$CH_2C_6H_5$	215–217	64.0	4.9	31.1	63.8	5.0	31.1	2	78	B
H	C_2H_5	259–260			43.0			43.4	1	77	B
H		223–225	55.8	4.2	32.6	55.7	4.4	33.0	2	82	C
CH_3	C_6H_5	234–236	63.8	5.3	31.0	64.1	5.3	31.4	2	63	C
H	*n*-C_4H_9	205–206	56.7	6.4	36.9	56.4	6.7	36.9	2	75	B
H	*o*-$CH_3C_6H_5$	260–261			31.0			31.3	2	85	C

[a] Analysis run on sample dried 24 hours at 135°. Analysis on sample dried at room temperature, calcd. for $C_6H_7N_5 \cdot 2H_2O$: C, 38.9; H, 5.95. Found: C, 39.1; H, 6.05. [b] Recrystallization solvents: A, water; B, ethanol–water; C, ethanol; D, benzene–ethanol.

ultraviolet spectrum of XIX was found to be almost identical with that of purine. Pyrazolo(3,4-*d*)pyrimidine shows a λ_{max} of 261 mμ at *p*H of 1 as compared to λ_{max} of 260 mμ at *p*H of 1 for purine.[12] However, in contrast to purine which is reported to be soluble in one part in two parts of water at 20 degrees[15] it was found that 200 parts of water were needed at 30 degrees to dissolve one part of XIX. Treatment of 4-chloropyrazolo-(3,4-*d*)pyrimidine-(XV) with thiourea in boiling alcohol provided another route to the 4-mercapto derivative XVIII.

An attempt to chlorinate 4,6-dihydroxypyrazolo-(3,4-*d*)pyrimidine with dimethylaniline and phosphorus oxychloride yielded a compound $C_{12}H_{10}N_4Cl$ which is probably 4-methylanilino-6-chloropyrazolo(3,4-*d*)pyrimidine instead of the desired derivative, 4,6-dichloropyrazolo(3,4-*d*)pyrimidine. The formation of this compound is similar to the preparation of 2,8-dichloro-6-diethylaminopurine from uric acid, phosphorus oxychloride and triethylamine.[16] A side reaction which introduces a methylanilino group under similar conditions has been noted in the pyrimidine series.[17,18]

Thiation of 4,6-dihydroxypyrazolo(3,4-*d*)pyrimidine (IX) with phosphorus pentasulfide in pyridine gave 4-mercapto-6-hydroxypyrazolo(3,4-*d*)pyrimidine (XII). A similar reaction[12] recently has been reported for xanthine. The structure XIII was assigned the reaction product since it was found to be different from the isomeric 4-hydroxy-6-mercaptopyrazolo(3,4-*d*)pyrimidine (XI) synthesized by fusing 3-amino-4-pyrazolecarboxamide (X) and thiourea. The presence of a 4-mercapto group was quite evident from the ultraviolet absorption spectra of XIII which showed a peak at 330 mμ at *p*H of 11. Absorption in this region is characteristic of 6-mercaptopurine derivatives.[12]

(15) A. Albert and D. J. Brown, *J. Chem. Soc.*, 2060 (1954).

(16) R. K. Robins and B. E. Christensen, THIS JOURNAL, **74**, 3624 (1952).

(17) F. E. King, T. J. King and P. C. Spensley, *J. Chem. Soc.*, 1247 (1947).

(18) J. R. Marshall and J. Walker, *ibid.*, 1016 (1951).

Treatment of 4-hydroxy-6-mercaptopyrazolo-(3,4-*d*)pyrimidine (XI) with phosphorus pentasulfide in pyridine yielded 4,6-dimercaptopyrazolo-(3,4-*d*)pyrimidine XI and methyl iodide and sodium hydroxide at 5° gave 4-hydroxy-6-methylmercaptopyrazolo(3,4-*d*)pyrimidine (XII). Chlorination of XII with phosphorus oxychloride in the presence of dimethylaniline yielded above 70% of 4-chloro-6-methylmercaptopyrazolo(3,4-*d*)pyrimidine (VIII). Treatment of VIII with various primary and secondary amines resulted in the preparation of the 4-substituted-amino-6-methylmercaptopyrazolo(3,4-*d*)pyrimidines listed in Table II. Heating VIII with sodium methoxide in methanol gave 4-methoxy-6-methylmercaptopyrazolo(3,4-*d*)-pyrimidine. Dechlorination of VIII with palladium-on-charcoal catalyst and hydrogen at room temperature gave 6-methylmercaptopyrazolo(3,4-*d*)pyrimidine (IV).

4-Amino-6-hydroxypyrazolo(3,4-*d*)pyrimidine-(I), the isoguanine analog, was synthesized by urea fusion of 3-amino-4-cyanopyrazole (VI). Thiourea fusion with VI gave 4-amino-6-mercaptopyrazolo(3,4-*d*)pyrimidine (VII).

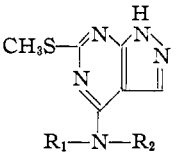
A new and rather novel synthesis of the fused pyrimidine ring was discovered when 3-amino-4-cyanopyrazole (VI) was heated with boiling formamide. Upon treating the reaction product with boiling dilute hydrochloric acid and adjusting the *p*H of the solution to 8 with ammonium hydroxide, 4-aminopyrazolo(3,4-*d*)pyrimidine (V), the adenine analog, was isolated in approximately 60% yield. The structure of the product thus obtained was established by the fact that synthesis of V from 4-chloropyrazolo(3,4-*d*)pyrimidine and aqueous or alcoholic ammonia gave an identical product.

The guanine analog, 4-hydroxy-6-aminopyrazolo-(3,4-*d*)pyrimidine, could not be made from XII with alcoholic or aqueous ammonia heated in a bomb to 150 or 200°.

A comparison of the general solubility of the pyrazolo(3,4-*d*)pyrimidines and the corresponding

TABLE II

6-METHYLMERCAPTO-4-SUBSTITUTED-AMINOPYRAZOLO(3,4-*d*)PYRIMIDINES



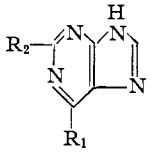
R_1	R_2	M.p., °C.	Calculated C	Calculated H	Calculated N	Found C	Found H	Found N	Method	Yield, %	Recrystn. solvent[a]
H	H	>300			38.7			38.5	1	58	A
CH_3	CH_3	263–265	45.8	4.3	33.5	45.5	5.1	33.4	1	45	B
H	NH_2	>300	36.7	4.1	42.9	36.6	3.9	42.4	1	30	A
H	$-(CH_2)_2N(C_2H_5)_2$	130–132	51.4	7.2	30.0	51.0	6.9	29.6	2	35	C

[a] Recrystallization solvents: A, water; B, ethanol–water; C, ethanol.

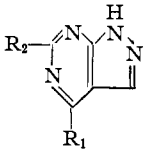
purine derivatives (see Table III) reveals that in general the pyrazolo(3,4-*d*)pyrimidines are more insoluble in boiling water than the purines.

TABLE III

COMPARISON OF SOLUBILITY OF CERTAIN ANALOGOUS PURINES AND PYRAZOLO(3,4-*d*)PYRIMIDINES IN AQUEOUS SOLUTION AT 100°



Purine



Pyrazolo(3,4-*d*)pyrimidine

R_1	R_2	Solubility in H_2O at 100°, 1 part sol./*x* parts H_2O: *x* for purine[15]	*x* for pyrazolo(3,4-*d*)pyrimidine
OH	H	70	190
OH	OH	400	800
NH_2	H	40	1000
$HNCH_3$	H	50	10
CH_3NCH_3	H	15	10
SH	H	180	500
H	SCH_3	150	350
SCH_3	H	20	300
H	H	2[a]	200[b]

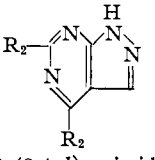
[a] At 20°. [b] At 30°.

It is interesting to note that although 4-aminopyrazolo(3,4-*d*)pyrimidine (V) is approximately 1/25th as soluble in boiling water as adenine, 4-methylaminopyrazolo(3,4-*d*)pyrimidine is five times as soluble as the corresponding purine isomer in aqueous solution at 100°. This would seem to indicate some type of intermolecular hydrogen bonding of V in the crystal lattice which is uncommonly high and in some way is concerned with the 4-amino group and the two nitrogens of the pyrazole ring. Methylation of the 4-amino group would seem to destroy the ability of the molecule to arrange itself in such a favorable pattern for intermolecular hydrogen bonding. Thus 4-methylaminopyrazolo(3,4-*d*)pyrimidine is strongly attracted by water molecules which compete favorably for the sites of hydrogen bonding. This postulate receives experimental support from the fact that 4-methylaminopyrazolo(3,4-*d*)pyrimidine crystallizes from an aqueous solution in the form of a dihydrate which loses the last mole of water only under rather drastic conditions.

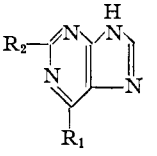
The ultraviolet absorption spectra of the pyrazolo(3,4-*d*)pyrimidines are remarkably similar to those of the corresponding purine derivatives. Table IV lists the absorption maxima of a number of derivatives of both ring systems.

TABLE IV

ULTRAVIOLET ABSORPTION SPECTRA OF CERTAIN PURINES AND THE CORRESPONDING PYRAZOLO(3,4-*d*)PYRIMIDINES



Pyrazolo(3,4-*d*)pyrimidine



Purine

R_1	R_2	*p*H	λmax pyrazolo-(3,4-*d*)-pyrimidine	*p*H	λmax purine deriv.
OH	OH	11	242, 267	10	240, 277[a]
OH	H	1	250	0.75	248[a]
		11	261	10.35	258[a]
SH	H	1	321	1	327[11]
		11	315	11	312[11]
NH_2	H	1	258	2.1	262[a]
		11	263	12.0	267[a]
Cl	H	1	261	5.23	265[2]
NH_2	CH_3S	1	235, 275	1	246, 284[b]
SCH_3	H	1	297	1	295[11]
		11	250, 286	11	293[11]
$HN-CH_3$	H	1	265	1	267[11]
		11	271	11	272[11]
$C_2H_5NC_2H_5$	H	1	273	1	276[11]
		11	287	11	282[11]
$HN-NH_2$	H	1	264	1	267[11]
SH	OH	11	260, 330	10.4	244, 340[14]
CH_3NCH_3	H	1	268	2.0	267[a]
		11	284	11	281[11]
H	H	1	261	1	260[14]
		11	268	10.4	271[14]
H $N-C_6H_5$	H	1	275	1	285[11]
		11	290	11	295[11]
H	SCH_3	11	254, 301	11.6	240 300–302[a]
OH	SH	1	248, 287	1	295[b]

[a] S. F. Mason, *J. Chem. Soc.*, 2073 (1954). [b] R. K. Robins, K. J. Dille, C. H. Willits and B. E. Christensen, THIS JOURNAL, **75**, 265 (1953).

In preliminary anti-tumor screening tests carried out at the Southern Research Institute, 4-aminopyrazolo(3,4-*d*)pyrimidine, the adenine analog, has shown a definite inhibitory action against Adenocarcinoma 755 in mice. The results of further biological testing will be reported elsewhere.

Experimental[19]

Preparation of 3-Amino-4-cyanopyrazole (VI).—Ethoxymethylenemalononitrile,[20] 150 g., was carefully added in small portions to 100 g. of 85% hydrazine hydrate. After approximately one-half of the ethoxymethylenemalononitrile had been added, the reaction mixture was cooled slightly in cold water and the remaining ethoxymethylenemalononitrile added at such a rate that the contents of the flask boiled gently upon the addition of each 3 to 5 g. portion. After all the ethoxymethylenemalononitrile had been added, the reaction flask was heated for one hour on the steam-bath. To the solidified mass was added 100 ml. of water and the solution set aside in the refrigerator overnight. The mushy solution was then filtered and the solid washed with 50 ml. of cold water and sucked as dry as possible. The crude product was dried in a vacuum desiccator over calcium chloride. Ordinary air drying was accompanied by considerable discoloration of the product. The crude yield of tan colored material was 97 g., m.p. 169–170°, λ_{max} at *p*H of 11, 240 mμ. Recrystallization from water raised the m.p. to 174–175°. *Anal.* Calcd. for $C_4H_4N_4$: C, 44.5; H, 3.7; N, 51.8. Found: C, 44.8; H, 3.6; N, 51.2.

Preparation of 3-Acetylamino-4-cyanopyrazole.—To 100 ml. of acetyl chloride was added 0.5 g. of 3-amino-4-cyanopyrazole (VI). The solution was refluxed for 8 hours and then evaporated to dryness on the steam-bath. The residue was dissolved in 25 ml. of boiling water, the solution neutralized with ammonium hydroxide and allowed to cool. The yield of product was 0.4 g., m.p. 218–220°. Recrystallization from water raised the m.p. to 221–222°. *Anal.* Calcd. for $C_6H_6N_4O$: N, 37.3; C, 48.0; H, 4.0. Found: N, 37.3; C, 47.9; H, 4.3.

Preparation of 3-Amino-4-pyrazolecarboxamide (X).—Concentrated sulfuric acid, 170 ml., was cooled to 20° and 50 g. of finely powdered 3-amino-4-cyanopyrazole (VI) was added with stirring so that the temperature did not rise above 40°. The addition of VI took about one-half hour. The solution was stirred at room temperature for one hour more to allow complete solution to take place. The sulfuric acid solution was then poured with stirring into a mixture of 500 ml. of water and 250 g. of ice and the solution set aside overnight in the refrigerator. The solution was then filtered and the product washed free of excess sulfuric acid. After drying in the oven at 110° the yield of sulfate salt of 3-amino-4-pyrazolecarboxamide was 67.0 g., m.p. 217–220°. Recrystallization of a small sample from water for analytical purposes gave m.p. 222–225°. For analysis the sample was dried in the oven at 130° for 5 hours. *Anal.* Calcd. for $C_4H_6N_4O \cdot {}^1/_2H_2SO_4$: C, 27.4; H, 4.0. Found: C, 27.4; H, 4.36.

Three grams of the above sulfate salt was dissolved in 50 ml. of water and the solution adjusted to *p*H of 9 with ammonium hydroxide. After three days the solution was filtered to yield 1.1 g. of 3-amino-4-pyrazole carboxamide (X), m.p. 186–188°, λ_{max} *p*H of 11, 252 mμ. Recrystallization from water raised the m.p. to 187–189°. *Anal.* Calcd. for $C_4H_6N_4O$: C, 38.1; H, 4.76; N, 44.4. Found: C, 38.4; H, 4.55; N, 44.1.

Preparation of 4-hydroxypyrazolo(3,4-*d*)pyrimidine (XIV).—Seventy-five grams of the sulfate salt of 3-amino-4-pyrazolecarboxamide (X) and 200 ml. of C.P. formamide were heated at 180–190° for 45 minutes. The cooled solution was diluted with one liter of cold water and filtered to yield 48.0 g. of XIV. An analytical sample was obtained by recrystallization of the crude product from water. *Anal.* Calcd. for $C_5H_4N_4O$: C, 44.1; H, 2.94; N, 41.2. Found: C, 44.3; H, 3.0; N, 41.1.

Preparation of 4,6-Dihydroxypyrazolo(3,4-*d*)pyrimidine (IX).—One hundred grams of the sulfate salt of 3-amino-4-pyrazolecarboxamide (X) and 200 g. of urea were heated together at 160° for 20 minutes. The clear solution went mushy and heating was continued for another 20 minutes at 190° until the mushy melt became too solid to stir. The resulting solid was dissolved in hot dilute sodium hydroxide and the boiling basic solution was then carefully acidified with acetic acid. The solution was allowed to stand approximately ten minutes and was then filtered. The crude yield of 4,6-dihydroxypyrazolo(3,4-*d*)pyrimidine (IX) was 74 g. Ultraviolet absorption spectra showed this material to be 90 to 95% pure. Further purification was accomplished by reprecipitation from a hot basic solution with acetic acid. A small amount was recrystallized from a large volume of water and dried at 130° for analysis. *Anal.* Calcd. for $C_5H_4N_4O_2$: C, 39.5; H, 2.63; N, 36.85. Found: C, 39.2; H, 2.75; N, 36.73.

Preparation of 4-Amino-6-mercaptopyrazolo(3,4-*d*)pyrimidine (VII).—Thirty-five grams of 3-amino-4-cyanopyrazole (VI) and 70 g. of thiourea were heated at 180° for 30 minutes until the clear solution became mushy. Heating was then continued at 200° for an additional ten minutes. The cooled melt was dissolved in hot dilute sodium hydroxide. The hot solution was treated with charcoal and filtered and the boiling filtrate carefully acidified with glacial acetic acid. The product, 4-amino-6-mercaptopyrazolo(3,4-*d*)pyrimidine (VII), was filtered almost immediately from the hot solution to yield 26.0 g. of light tan material. A small sample was reprecipitated from a hot basic solution with acetic acid and dried at 130° for analysis; λ_{max} *p*H of 11 is 250, 293 mμ. *Anal.* Calcd. for $C_5H_5N_5S$: C, 35.9; H, 2.99; N, 41.9. Found: C, 36.0; H, 3.16; N, 41.5.

Preparation of 4-Hydroxy-6-mercaptopyrazolo(3,4-*d*)pyrimidine (XI).—Fifty grams of the sulfate salt of X was fused with 100 g. of thiourea in the same manner as in the preparation of 4,6-dihydroxypyrazolo(3,4-*d*)pyrimidine (IX). The product was isolated as in the preparation of IX to yield 24.0 g. Reprecipitation of a small sample from hot dilute sodium hydroxide gave an analytical sample. *Anal.* Calcd. for $C_5H_4N_4OS$: C, 35.7; H, 2.38; N, 33.3. Found: C, 35.9; H, 2.39; N, 32.9.

Preparation of 4-Chloropyrazolo(3,4-*d*)pyrimidine (XV).—Ten grams of dry 4-hydroxypyrazolo(3,4-*d*)pyrimidine (XIV) was added to 300 ml. of phosphorus oxychloride. To this mixture was added 30 ml. of dimethylaniline, and the solution was refluxed for 1.5 hours. The excess phosphorus oxychloride was removed under reduced pressure using a water-bath as a source of heat. The sirupy residue was poured with vigorous stirring onto a mixture of approximately 400 g. of crushed ice and 100 ml. of water. After ten minutes the aqueous solution was extracted with ether, 6 × 300-ml. portions. The ethereal solution was then washed twice with a 200-ml. portion of cold water each time and then dried overnight over anhydrous sodium sulfate.

Distillation of the ethereal solution left 9.8 g. of almost colorless product, dec. 130°. It was necessary to remove the last 20 or 30 ml. of ether by a warm stream of air to prevent the occurrence of discoloration and decomposition of the product. A crude sample of 4-chloropyrazolo(3,4-*d*)pyrimidine (XV) was recrystallized from benzene to give a decomposition range of 130–135°. *Anal.* Calcd. for $C_5H_3N_4Cl$: C, 38.9; H, 1.95; N, 36.2. Found: C, 38.7; H, 2.16; N, 36.3.

Extraction of the acidic aqueous solution with a liquid–liquid continuous extractor resulted in considerable hydrolysis of the product. Best results were obtained using large volumes of ether and rapidly extracting the cold aqueous solution.

Preparation of 4-Mercaptopyrazolo(3,4-*d*)pyrimidine (XVIII). Method 1.—Ten grams of 4-hydroxypyrazolo(3,4-*d*)pyrimidine (XIV) was finely powdered and intimately mixed with 50 g. of phosphorus pentasulfide. This mixture was added, a small portion at a time, with vigorous stirring, to 300 ml. of tetralin heated to 165°. Addition was completed in one-half hour, during which time the temperature was allowed to rise slowly to 185°. The reaction mixture was then heated at 190–195° for four hours with continued stirring. The cooled solution was filtered and the solid material washed with a small amount of petroleum ether and allowed to dry at room temperature. This crude solid was added carefully to one liter of water at 80°. The solution was then boiled for ten minutes and enough potassium hydroxide was added to effect complete solution. The solution was stirred with charcoal and filtered. Acidification of the hot filtrate with acetic acid yielded a light tan precipitate, yield 10.1 g. Ultraviolet absorption spectra showed this product to be above 95% pure. Recrystallization from a 30% ethanol solution yielded white crystals, m.p. >360°. The sample dried at 130° for analysis.

Anal. Calcd. for $C_5H_4N_4S$: C, 39.5; H, 2.63; N, 36.9. Found: C, 39.6; H, 2.78; N, 36.8.

Method 2.—Two grams of 4-chloropyrazolo(3,4-*d*)pyrimidine (XV) and 2 g. of thiourea were added to 100 ml. of

(19) All melting points are uncorrected and were taken on a Fisher-Johns melting point block unless otherwise stated.

(20) W. Huber, THIS JOURNAL, **65**, 2224 (1943).

absolute ethanol and the solution refluxed for two hours. The solution was cooled and filtered. The crude 4-mercaptopyrazolo(3,4-*d*)pyrimidine (XVIII) was recrystallized from ethanol–water to give 1.6 g. of white crystals. The ultraviolet absorption spectrum of this compound was found identical to that of XVIII prepared by method 1.

Preparation of 4-Aminopyrazolo(3,4-*d*)pyrimidine (V). Method 1.—Three grams of 4-chloropyrazolo(3,4-*d*)pyrimidine (XV) was dissolved in 150 ml. of concentrated ammonium hydroxide. The solution was evaporated on the steam-bath to 50 ml. Then 200 ml. more of concentrated ammonium hydroxide was added and the solution evaporated on the steam-bath to 150 ml. This solution was boiled with a little charcoal and filtered. The cooled filtrate (*p*H of 11) yielded 0.85 g. of colorless needles. *Anal.* Calcd. for $C_5H_5N_5$: C, 44.4; H, 3.70; N, 51.9. Found: C, 44.2; H, 3.58; N, 51.6.

Method 2.—Thirty grams of 3-amino-4-cyanopyrazole (VI) was added to 60 ml. of C.P. formamide, and the solution was vigorously boiled for 30 minutes. The cooled solution was diluted with 100 ml. of cold water and filtered. The crude light tan product was suspended in 400 ml. of hot water and 50 ml. of concd. HCl was added and the solution gently boiled for 15 minutes with a small amount of decolorizing carbon. The hot filtrate was adjusted to *p*H of 8 with concentrated ammonium hydroxide. This solution was allowed to cool to room temperature and then filtered. The product was washed with distilled water to give 21.0 g. of colorless 4-aminopyrazolo(3,4-*d*)pyrimidine (V). This product was further purified for analysis by reprecipitation with acetic acid from a hot strongly basic solution. *Anal.* Calcd. for $C_5H_5N_5$: C, 44.4; H, 3.70; N, 51.9. Found: C, 44.0; H, 4.0; N, 51.6.

Method 3.—4-Aminopyrazolo(3,4-*d*)pyrimidine (V) was also prepared from XV, 5.0 g., and alcoholic ammonia heated in a bomb at 100° for 12 hours. The alcoholic solution was then filtered and the crude precipitate dissolved in dilute hydrochloric acid and purified as in method 2. The yield of V was 3.9 g. The products obtained by methods 1, 2 and 3 were judged to be identical on the basis of identical ultraviolet absorption curves at *p*H of 1 and 11.

Reaction of 4,6-Dihydroxypyrazolo(3,4-*d*)pyrimidine (IX) with Phosphorus Oxychloride and Dimethylaniline.—Ten grams of 4,6-dihydroxypyrazolo(3,4-*d*)pyrimidine and 30 ml. of dimethylaniline were added to 250 ml. of phosphorus oxychloride. The solution was refluxed for one hour and the excess phosphorus oxychloride removed under reduced pressure and the residual red sirup poured onto crushed ice. The resulting aqueous solution was extracted with ether, 3 × 200 ml., and the ethereal solution was washed with water and dried over anhydrous sodium sulfate. Distillation of the ether left 4.5 g. of an orange colored product. This crude product was recrystallized from xylene to yield 2.4 g. of light yellow needles, m.p. 225–227° dec. *Anal.* Calcd. for $C_{12}H_{10}N_4Cl$: C, 55.5; H, 3.9. Found: C, 55.8; H, 3.8.

Preparation of 4-Amino-6-hydroxypyrazolo(3,4-*d*)pyrimidine (I).—Ten grams of 3-amino-4-cyanopyrazole (VI) was heated with 20 g. of urea at 180–200° for 20 minutes until the clear melt became solid. The cooled solid was then dissolved in 2 *N* sodium hydroxide and the solution boiled gently for 10 minutes with charcoal. The boiling filtrate was acidified carefully with glacial acetic acid and the solution filtered hot to yield 11.5 g. of light tan product. This product was purified by dissolving a small sample in boiling dilute sodium hydroxide and precipitating I from the hot solution with acetic acid. This treatment was repeated to give an analytically pure sample; λ_{max} *p*H of 11, 270 mμ; at *p*H of 1, 250 mμ. *Anal.* Calcd. for $C_5H_5N_5O$: C, 39.8; H, 3.3; N, 46.4. Found: C, 39.5; H, 3.3; N, 45.9.

Preparation of 4-Hydroxy-6-methylmercaptopyrazolo(3,4-*d*)pyrimidine (XII).—Fourteen grams of 4-hydroxy-6-mercaptopyrazolo(3,4-*d*)pyrimidine was dissolved in a solution of 10 g. of sodium hydroxide in 300 ml. of water. The solution was cooled to 5° and shaken with 12 g. of methyl iodide. After 10 minutes the solution was charcoaled, filtered and acidified with acetic acid to yield 12.0 g. of crude product. Recrystallization of a small portion from glacial acetic acid gave an analytically pure sample; λ_{max} at *p*H of 1, 263 mμ; λ_{max} at *p*H of 11, 235, 269 mμ. *Anal.* Calcd. for $C_6H_6N_4SO$: N, 30.8. Found: N, 30.9.

Preparation of 4-Chloro-6-methylmercaptopyrazolo(3,4-*d*)pyrimidine (VIII).—To 400 ml. of phosphorus oxychloride and 30 ml. of dimethylaniline was added 22.0 g. of crude 4-hydroxy-6-methylmercaptopyrazolo(3,4-*d*)pyrimidine (XII). The solution was refluxed for 30 minutes at which time all the solid had dissolved. The product was isolated in the same manner as that employed for 4-chloropyrazolo(3,4-*d*)pyrimidine (XV). Sixteen grams of light yellow colored solid m.p. 171–174° dec., was isolated from the ethereal extract. Recrystallization from *n*-heptane raised the m.p. to 178–179° dec. *Anal.* Calcd. for $C_6H_5N_4ClS$: C, 35.9; H, 2.49; N, 27.9. Found: C, 36.4; H, 2.44; N, 27.7.

Preparation of 4-Methylmercaptopyrazolo(3,4-*d*)pyrimidine (XVII).—Five grams of 4-mercaptopyrazolo(3,4-*d*)-pyrimidine (XVIII) was dissolved in 65 ml. of 0.8 *N* sodium hydroxide. To this solution was added 5.0 g. of methyl iodide and the mixture was shaken at 20° for 15 minutes. The solution was treated with charcoal, filtered and the filtrate acidified with acetic acid. The crude precipitate was filtered and suspended in 100 ml. of water and the *p*H adjusted to 9 with ammonium hydroxide to dissolve any unreacted starting material. The ammoniacal solution was filtered and the collected precipitate washed and recrystallized from water to give 4.1 g., m.p. 192°. A second recrystallization raised the m.p. to 193°. *Anal.* Calcd. for $C_6H_6N_4S$: C, 43.4; H, 3.6; N, 33.7. Found: C, 43.4; H, 3.2; N, 33.7.

Preparation of 4-Mercapto-6-hydroxypyrazolo(3,4-*d*)pyrimidine (XIII).—Fifteen grams of 4,6-dihydroxypyrazolo-(3,4-*d*)pyrimidine (IX) and 80 g. of phosphorus pentasulfide were added to 900 ml. of dry, reagent grade pyridine. The solution was refluxed for four hours and the excess pyridine recovered under vacuum using a steam-bath as a source of heat. To the residue was added 600 ml. of ice-water and the solution allowed to stand one-half hour at room temperature, followed by heating on the steam-bath for two hours. The solution was then placed in the refrigerator overnight. Filtration yielded 14.0 g. of crude, light tan product. This material was purified by precipitation with acetic acid from a boiling, dilute basic solution; yield 12.6 g. Recrystallization from aqueous acetic acid gave light green needles, analytically pure. *Anal.* Calcd. for $C_5H_4N_4OS$: C, 35.7; H, 2.4; N, 33.3. Found: C, 35.2; H, 2.5; N, 32.9.

Preparation of 4,6-Dimercaptopyrazolo(3,4-*d*)pyrimidine.—Fifteen grams of 4-hydroxy-6-mercaptopyrazolo(3,4-*d*)-pyrimidine (XI) and 65 g. of phosphorus pentasulfide were added to 900 ml. of dry, reagent grade pyridine. The solution was refluxed for three hours and the excess pyridine removed under vacuum. One liter of water was added to the residue and the solution heated three hours on the water-bath. The cooled solution was filtered and the crude product purified by dissolving it in hot 0.5 *N* potassium hydroxide. This solution was boiled with charcoal, filtered and the boiling filtrate carefully acidified with acetic acid to yield 12.0 g. of light green product. A second reprecipitation from a basic solution gave an analytically pure sample; λ_{max} at *p*H of 1, 265 mμ, 320 mμ. *Anal.* Calcd. for $C_5H_4N_4S_2$: C, 32.6; H, 2.18; N, 30.4. Found: C, 32.7; H, 2.22; N, 30.7.

Preparation of Pyrazolo(3,4-*d*)pyrimidine (XIX).—To a solution of 150 ml. of water and 4 ml. of concentrated ammonium hydroxide was added 4.5 g. of finely powdered 4-chloropyrazolo(3,4-*d*)pyrimidine (XV) and 1.0 g. of 10% palladium-on-charcoal. This solution was shaken on a low pressure hydrogenator at 20 lb./sq. in. until the uptake of hydrogen had ceased (approximately six hours). The solution was filtered and the charcoal residue extracted with 100 ml. of boiling water. The combined filtrates were evaporated to dryness on the steam-bath and the residue placed in a Soxhlet thimble and extracted with 200 ml. of toluene for 18 hours. The cooled toluene solution yielded 2.9 g. of white crystals, m.p. 212–213°. The sample was further purified by sublimation at 15 mm. pressure and 180–200° to yield 2.65 g. of colorless needles, m.p. 213–214°. *Anal.* Calcd. for $C_5H_4N_4$: C, 50.0; H, 3.4; N, 46.7. Found: C, 49.8; H, 3.4; N, 46.0, 48.0.

Preparation of 4-Ethoxypyrazolo(3,4-*d*)pyrimidine.—To a solution of 0.6 g. of sodium dissolved in 30 ml. of absolute ethanol was added 3.0 g. of finely pulverized 4-chloropyrazolo(3,4-*d*)pyrimidine (XV). The solution was heated on the steam-bath for one hour, diluted with 20 ml. of water and then neutralized with acetic acid. The crude product from the cooled solution was recrystallized from an ethanol and water mixture to yield 1.3 g., m.p. 162–163°. A

second recrystallization from the same solvent raised the m.p. to 168–169°; λ_{max} at *p*H of 1, 254 mμ. *Anal.* Calcd. for $C_7H_8N_4O$: C, 51.2; H, 4.9. Found: C, 50.9; H, 4.9.

Preparation of 6-Methylmercaptopyrazolo(3,4-*d*)pyrimidine (IV).—To 150 ml. of ethanol was added 10 ml. of concentrated ammonium hydroxide, 7.0 g. of 4-chloro-6-methylmercaptopyrazolo(3,4-*d*)pyrimidine (VIII) and 2.7 g. of 10% palladium-on-carbon. The solution was shaken on the low pressure hydrogenator at a hydrogen pressure of 20 lb./sq. in. for 24 hours, after which time the uptake of hydrogen had ceased. The solution was filtered and the filtrate evaporated to dryness on the steam-bath. The residue was recrystallized from an 80% ethanol–water mixture to yield 1.7 g. of white crystals, m.p. 201–204°. A second recrystallization from the same solvent raised the m.p. to 210–212°; λ_{max} at *p*H of 1, 240 and 300 mμ. *Anal.* Calcd. for $C_6H_6N_4S$: C, 43.4; H, 3.6; N, 33.7. Found: C, 43.0; H, 3.5; N, 33.1.

Preparation of 4-Methoxy-6-methylmercaptopyrazolo(3,4-*d*)pyrimidine.—To a solution of 1.0 g. of sodium in 75 ml. of absolute methanol was added 4.0 g. of 4-chloro-6-methylmercaptopyrazolo(3,4-*d*)pyrimidine (VIII). The solution was heated for four hours on the steam-bath and then neutralized with glacial acetic acid. The cooled solution yielded 3.1 g. of white needles, m.p. 190–192°. Recrystallization from a methanol and water mixture raised the m.p. to 193–194°; λ_{max} at *p*H of 1, 240 and 282 mμ. *Anal.* Calcd. for $C_7H_8N_4OS$: C, 42.9; H, 4.1; N, 28.6. Found: C, 43.0; H, 3.9; N, 29.0.

Preparation of 4-Substituted-aminopyrazolo(3,4-*d*)pyrimidines (XVI) (see Table I). General Method 1.—Five to ten grams of 4-chloropyrazolo(3,4-*d*)pyrimidine (XV) was added to 50 to 100 ml. of a 25–40% aqueous solution of the primary or secondary amine. The solution was heated for four hours on the steam-bath and then allowed to cool in the refrigerator overnight. The filtered precipitate was then washed with a little ice-water, dried and recrystallized from the solvent indicated.

General Method 2.—Five to ten grams of XV was added to approximately 0.15 mole of primary or secondary amine dissolved in 150 ml. of absolute ethanol. The solution was heated for four hours on the steam-bath at which time the volume had been reduced to approximately 50 ml. The solution was cooled overnight and filtered. The crude product was then recrystallized from the indicated solvents.

Preparation of 4-Substituted-amino-6-methylmercaptopyrazolo(3,4-*d*)pyrimidines (III) (see Table II).—The preparation of these compounds was carried out by treating 4-chloro-6-methylmercaptopyrazolo(3,4-*d*)pyrimidine (VIII) according to general method 1 or general method 2 for the preparation of 4-substituted-aminopyrazolo(3,4-*d*)pyrimidines (XVI).

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[CONTRIBUTION FROM THE DEPARTMENT OF CHEMISTRY OF THE UNIVERSITY OF CALIFORNIA AT LOS ANGELES]

Studies in Stereochemistry. XXV. Eclipsing Effects in the E_2 Reaction[1]

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Eclipsing effects in the E_2 reactions of the diastereomers of the 1,2-diphenyl-1-propyl-X system have been studied as a function of the leaving group [X = Cl, Br and $^+N(CH_3)_3$], of the solvent [C_2H_5OH, *n*-$C_6H_{13}CHOHCH_3$, $(CH_3)_3COH$, *n*-$C_8H_{17}OH$, $C_2H_5(CH_3)_2COH$, C_6H_6], and of the base [C_2H_5ONa, *n*-$C_6H_{13}CHOKCH_3$, $(CH_3)_3COK$, *n*-$C_8H_{17}ONa$, $C_2H_5(CH_3)_2COK$]. The ratio of rates k_{E_2} *threo*/k_{E_2} *erythro* varied from 1–11 with X = Cl, the value of the ratio increasing with increasing base strength and decreasing solvating ability of the medium. With X = Br, the rate ratio varied between 0.7 and 5.4, the solvent and base strength effects being similar to those found when X = Cl. With X = $^+N(CH_3)_3$, the rate ratio was 57 in C_2H_5OH with C_2H_5ONa as base. In $(CH_3)_3COH$ with $(CH_3)_3COK$ as base, the rate ratio was about 1, but in this case *trans*-olefin was obtained from both diastereomers. In all other cases a *trans* elimination occurred, the *threo* isomer giving *trans* and the *erythro* isomer giving *cis*-α-methylstilbene. Equilibration of the olefins with acid gave *trans*/*cis* ~50. The variation in rate ratios is interpreted in terms of the variation of the transition state from a geometry similar to that of the starting material (no groups eclipsed) to one similar to the product (four groups eclipsed).

Differences in reactivity of diastereomeric compounds have been recognized for about seventy-five years, as has the fact that *cis–trans*-olefin pairs possess different thermodynamic stability.[2] Eclipsing effects have been invoked only relatively recently to explain these phenomena and have been discussed in connection with both the relative thermodynamic stability of isomeric substances and the relative stability of isomeric transition states arising from either the same compound or diastereomerically related compounds.[3] Two distinct problems arise with respect to the magnitude of eclipsing effects in transition states, the first involving the bulk of the groups, and the second concerning the degree to which these groups are actually eclipsed.[4]

The bimolecular elimination reaction as applied to diastereomerically related starting materials pos-

(1) This work was sponsored by the Office of Ordnance Research, U. S. Army.

(2) For summary articles of the earliest literature, see: (a) P. Pfeiffer, *Z. physik. Chem.*, **48**, 40 (1904); (b) P. F. Frankland, *J. Chem. Soc.*, 654 (1912).

(3) With respect to the thermodynamic stability of isomeric olefins see: (a) G. B. Kistiakowsky, J. R. Ruhoff, H. A. Smith and W. E. Vaughan, THIS JOURNAL, **57**, 876 (1935); (b) R. B. Williams, *ibid.*, **64**, 1395 (1942); (c) D. J. Cram, *ibid.*, **71**, 3883 (1949); (d) D. Y. Curtin and B. Luberoff, Abstracts of Thirteenth National Organic Symposium of the American Chemical Society, Ann Arbor, Mich., June, 1953, p. 40; (e) R. Y. Mixer, R. F. Heck, S. Winstein and W. G. Young, THIS JOURNAL, **75**, 4094 (1953). Regarding differences in thermodynamic stability of diastereomeric 1,2-dimethylcyclopentanes, see: (f) W. Beckett, K. S. Pitzer and R. Spitzer, *ibid.*, **69**, 2488 (1947). With respect to reactivity differences in closing five-membered rings, see: (g) H. Hermans, *Z. physik. Chem.*, **113**, 338 (1924). For examples of reactivity differences in 1,2-molecular rearrangements, see: (h) D. Y. Curtin, P. I. Pollak, E. E. Harris and E. K. Meislich, THIS JOURNAL, **72**, 961 (1950), **73**, 992 (1951), and **74**, 2901, 5518, 5905 (1952); (i) D. J. Cram and F. A. Abd Elhafez, *ibid.*, **76**, 28 (1954). Examples of the phenomena as applied to acyl migrations are: (j) L. H. Welsh, *ibid.*, **69**, 128 (1947), and **71**, 3500 (1949); (k) V. Bruckner, G. Fodor, J. Kiss and C. Kovacs, *J. Chem. Soc.*, 885 (1948), and subsequent papers. Reactivity differences as applied to the 1,2-elimination reaction are: (l) W. G. Young, D. Pressman and C. D. Coryell, THIS JOURNAL, **61**, 1640 (1939); ref. 3d; (m) R. E. Lutz, D. F. Hinkley and R. H. Jordan, *ibid.*, **73**, 4649 (1951); (n) S. Winstein, E. Grunwald, K. C. Schreiber and J. Corse, *ibid.*, **74**, 1118 (1952), and quoted references; ref. 6; (o) D. Y. Curtin and D. B. Kellom, *ibid.*, **75**, 6011 (1953); (p) D. J. Cram and J. D. McCarty, *ibid.*, **76**, 5740 (1954). For an example of eclipsing effects in the reverse aldol condensation, see: (q) H. E. Zimmerman and J. English, Jr., *ibid.*, **76**, 2285, 2291, 2294 (1954). For an example of a reactivity difference in the formation of a chloronium ion, see: (r) S. Winstein and D. Seymour, *ibid.*, **68**, 121 (1946).

(4) D. Y. Curtin, *et al.*, (ref. 3d, 3o and *Record Chem. Progress*, **15**, 111 (1954)) have introduced the term "*cis* effect" in connection with their extensive correlation of differences in reactivity (or stability) of isomers with the differences in bulk of groups becoming eclipsed.