[CONTRIBUTION FROM THE LEDERLE LABORATORIES DIVISION, AMERICAN CYANAMID COMPANY]

PUROMYCIN.[1] SYNTHETIC STUDIES. I. SYNTHESIS OF 6-DIMETHYLAMINOPURINE, AN HYDROLYTIC FRAGMENT

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Alcoholysis of puromycin, $C_{22}H_{29}N_7O_5$, has been shown to give three cleavage products (2), (a) an amphoteric water-soluble moiety, $C_7H_9N_5$, (b) an aminopentose shown to be either 3-aminoribose or 3-aminoxylose, and (c) the ester of the known *p*-methoxy-L-phenylalanine. The acidic properties of the $C_7H_9N_5$ moiety, unusual for an oxygen-free compound, along with two N-methyls could only be explained by an aminopurine or an aminotriazolopyridine bearing the two N-methyls and the amine on the six-membered ring. Spectroscopic data led to the proposal of 6-dimethylaminopurine as the most logical structure (2). That the $C_7H_9N_5$ moiety was indeed 6-dimethylaminopurine has now been verified by synthesis by the Traube approach (3) as described in this communication. Fragment (b) has been identified as 3-amino-D-ribose by synthesis as described in the accompanying paper III of this series. Further synthesis of model compounds demonstrated that the pentose was attached to the 9-position of the 6-dimethylaminopurine (paper II).[2]

Condensation of thiourea with ethyl cyanoacetate according to Traube (3) gave the pyrimidol (I) in 92–95% yield. Monomethylation of the sodium salt of I to the 2-methylthiopyrimidine (II) with methyl iodide as described by Johnson and Johns (4) proceeded in 82% yield. The same yield could be obtained by substitution of methyl sulfate for methyl iodide. For preparative purposes it was not necessary to isolate I, but the sodium salt of I formed in the condensation could be methylated with methyl sulfate to give II in 82% yield over-all. Treatment of the pyrimidol (II) with phosphorus oxychloride by the procedure of Johnson and Johns (4) gave low yields of the chloropyrimidine (III) although they record 40–45%. It has now been found that addition of dimethylaniline (5) increased the yield to 60–62%. Replacement of the chloro group with aqueous dimethylamine[3] at 125° gave 94% of the diamine, VI.

2-Methylmercapto-4-amino-6-dimethylaminopyrimidine (VI) was smoothly nitrosated in 10% acetic acid to the 5-nitrosopyrimidine (V) in 95% yield. Reduction of V with sodium hydrosulfite to the triamine (IV), followed by

[1] The isolation and biological activity of this antibiotic have been described by Porter, Hewitt, et al. (1). In some earlier papers, the antibiotic "puromycin" has been referred to by the trade marked name "Achromycin" [*Cf.* Waller, *et al.*, *J. Am. Chem. Soc.*, **75**, 2025 (1953) and Baker and Schaub, *J. Am. Chem. Soc.*, **75**, 3864 (1953)]. This trade name has since been reassigned to a non-related antibiotic, "tetracycline".

[2] These three papers were part of a seminar presented at the Fifth Summer Seminar on Natural Products at the University of New Brunswick, Fredericton, N. B., Canada during the week of August 18, 1953.

[3] Similar reactions of III with aniline (4) and methylamine (6) have been described.

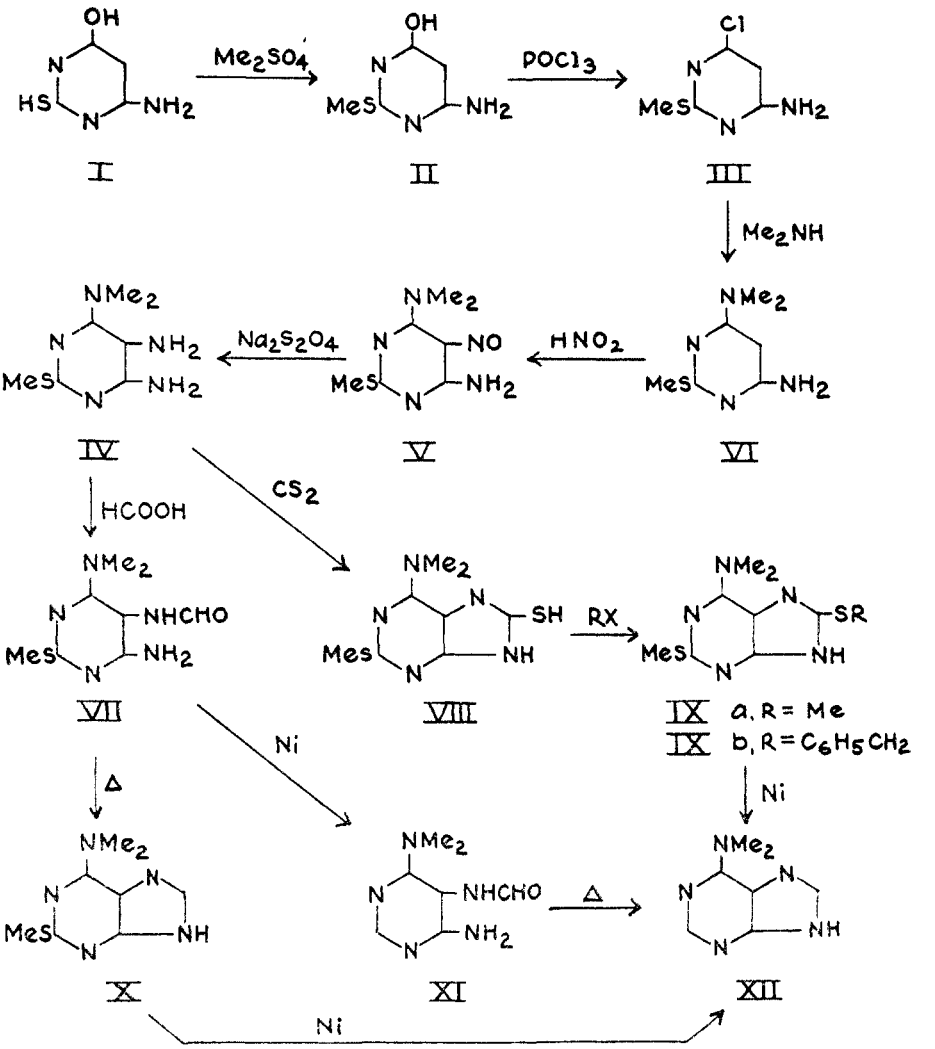

CHART I

formylation gave the 5-formamidopyrimidine (VII) in 76% over-all yield for the two steps. Reductive formylation of V directly to VII with zinc and formic acid, although more rapid, was less efficient (50% yield). Ring closure of VII to 2-methylmercapto-6-dimethylaminopurine (X) was best done on a small scale by short fusion at 250° (99% yield), although boiling quinoline, formamide, or dilute alcoholic sodium hydroxide could also be employed. The latter reagent was most efficient on a large scale. Desulfurization of X with Raney nickel (7) in 1 *N* sodium hydroxide at 100° afforded the final product, 6-dimethylaminopurine (XII) in 43% yield.[4] This compound was identical in all respects with the $C_7H_9N_5$ moiety from puromycin (2).

[4] Soon after the completion of this synthesis, Elion, Burgi, and Hitchings (8) described this compound prepared by a different route.

Two other approaches for the conversion of the triamine (IV) to 6-dimethylaminopurine (XII) were investigated, one of which gave superior over-all yields to the one described above. The triamine (IV) reacted rapidly with carbon disulfide in pyridine[5] to give 91–95% yields of the 8-mercaptopurine (VIII). Methylation of the sodium salt of VIII formed 90% of the 8-methylmercaptopurine (IXa), even though the 7- or 9-positions could potentially be alkylated. Similarly, benzylation of the sodium salt of VIII gave the 8-benzylmercaptopurine (IXb). Desulfurization of VIII, IXa, or IXb with Raney nickel gave 6-dimethylaminopurine (XII) in 16, 17, and 0% yields, respectively. The desulfurization of IXa to XII proves that methylation of the 8-mercaptopurine (VIII) took place on the mercapto group and not on the 7- or 9-position.

Since desulfurization of the four 2-methylmercaptopurines gave low yields and poor material balances, it was considered probable that the acidic NH group of the purine was irreversibly co-ordinating with the nickel.[6] To avoid this difficulty the non-acidic 5-formamidopyrimidine (VII) was desulfurized in 69% yield to XI which smoothly ring closed to 6-dimethylaminopurine (XII) in near quantitative yield. This last method is the one of choice since it gives the best over-all yields and avoids the difficult isolation of XII from an aqueous solution.

Acknowledgement. The authors are indebted to Mr. Louis Brancone and staff for the microanalyses.

EXPERIMENTAL

2-Methylmercapto-4-amino-6-pyrimidol (II). To a solution of 18.4 g. of sodium methoxide in 300 cc. of absolute alcohol was added 33.2 cc. of ethyl cyanoacetate and 25.6 g. of thiourea. The mixture was refluxed and stirred for 2 hours. After the addition of 60 cc. of water, the mixture was treated dropwise with 29 cc. of methyl sulfate at such a rate that the solution refluxed gently (five minutes). After being refluxed and stirred for an additional ten minutes, the mixture was cooled in an ice-bath. The product was collected and washed with water; yield 34.7 g., m.p. 261–262° dec. Concentration of the filtrate to about one-half *in vacuo* gave an additional 5.5 g. (total 82%) of product, m.p. 260–263° dec.

Johnson and Johns (4) record m.p. 267° dec.

2-Methylmercapto-4-amino-6-chloropyrimidine (III). To a mixture of 40 g. of II and 16 cc. of dimethylaniline was added cautiously 200 cc. of phosphorus oxychloride. The solution was refluxed on a heating mantle for 8 hours, then concentrated to a syrup *in vacuo*. The warm syrup was poured into about 350 cc. of water and was stirred until solution was complete. The orange solution, decanted from a little insoluble gum, was treated with concentrated ammonium hydroxide with cooling until a definite excess was present (about 500 cc.). The mixture was heated on the steam-bath for one hour, then concentrated *in vacuo* to about one-third and cooled. The solid was collected, washed with water, and ground in a mortar with 25 cc. of 1 *N* sodium hydroxide to remove starting material. The product was removed and washed with water; yield, 18 g., m.p. 125–128°. The original mother liquor was further concentrated and the product was leached with alkali to give an additional 9.6 g. (total 62%), m.p. 126–127°. Acidification of the alkaline leachings gave 4% of starting material.

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[5] This type of cyclization to a purine has been described by Cook and Smith (9).

[6] The 7- and 9-alkyl derivatives of 1,8-bis-methylmercapto-6-dimethylaminopurine, where there was no NH function, were smoothly desulfurized in 50–75% yields in alcohol as described in the accompanying paper.

Longer or shorter reflux periods gave lower yields. Johnson and Johns (4) record m.p. 132° and yields of 40–45% without the use of dimethylaniline.

2-Methylmercapto-4-amino-6-dimethylaminopyrimidine (VI). A mixture of 12 g. of III and 30 cc. of 25% aqueous dimethylamine was heated in a steel bomb at 125° for 4 hours. The product was collected from the cooled mixture by filtration and washed with water; yield, 11.8 g. (94%), m.p. 156–160°. Recrystallization of a sample from water gave white crystals, m.p. 162–164°.

Anal. Calc'd for $C_7H_{12}N_4S$: C, 45.7; H, 6.55; N, 30.4.
Found: C, 45.7; H, 6.62; N, 30.0.

At the end of 3 hours reaction, there was still some starting material present. The *monohydrochloride* was obtained in quantitative yield, m.p. 279° dec. by solution of 100 mg. of the base in absolute alcohol and addition of 1 cc. of saturated absolute alcoholic hydrogen chloride. Recrystallization in the same manner gave white crystals, m.p. 282° dec.

Anal. Calc'd for $C_7H_{12}N_4S \cdot HCl$: C, 38.0; H, 5.92; N, 25.3; Cl, 16.0.
Found: C, 38.1; H, 6.15; N, 25.0; Cl, 16.0.

2-Methylmercapto-4-amino-5-nitroso-6-dimethylaminopyrimidine (V). A mixture of 11.8 g. of VI and 230 cc. of 10% acetic acid was heated to 90° and was filtered from a trace of insoluble material. The solution was cooled in an ice-bath to 3° and a solution of 5.45 g. of sodium nitrite in 11 cc. of water was added portionwise. After one hour at 0° the blue nitroso compound was collected on a filter, washed with water, and dried on the steam-bath; yield, 12.9 g. (94%), m.p. 213–214° dec. Recrystallization from absolute alcohol gave blue crystals, m.p. 219–220° dec.

Anal. Calc'd for $C_7H_{11}N_5OS$: C, 39.4; H, 5.20; N, 32.9.
Found: C, 39.5; H, 5.35; N, 32.6.

2-Methylmercapto-4,5-diamino-6-dimethylaminopyrimidine (IV). The still moist nitrosopyrimidine (V), prepared as above from 25 g. of VI, was suspended in 580 cc. of water warmed to 50°. Then 58 g. of sodium hydrosulfite was added to the stirred mixture in three portions over 5–10 minutes. Some gas was evolved, the temperature rose to 56°, and the solid became lighter in color. After the addition of 20 g. more of sodium hydrosulfite over a period of 10 minutes, the temperature was kept at 70° for 10 minutes more. The mixture was cooled in an ice-bath, filtered, and the solid was washed with water; yield, 23.9 g. (89%), m.p. 148–150°. Recrystallization of a sample from alcohol gave white crystals, m.p. 154–155°.

Anal. Calc'd for $C_7H_{13}N_5S$: C, 42.2; H, 6.58; N, 35.2.
Found: C, 42.3; H, 6.68; N, 35.1.

If the intermediate nitroso compound was dried, it was difficult to resuspend in water. Reduction was much slower, required more sodium hydrosulfite, and both the yield and quality of product were somewhat lowered.

2-Methylmercapto-4-amino-5-formamido-6-dimethylaminopyrimidine (VII). (*A*). A solution of 3.4 g. of IV in 34 cc. of 90% formic acid was heated on the steam-bath for 1 hour, then concentrated to a thin syrup *in vacuo*. The residue was dissolved in warm water, made basic with ammonia water, and cooled; yield, 3.3 g. (85%), m.p. 221–222° (gas). A sample formed white crystals from alcohol, m.p. 225–226° (gas), resolidifying by 250° and remelting at 280° dec., the m.p. of X.

Anal. Calc'd for $C_8H_{13}N_5OS$: C, 42.3; H, 5.77; N, 30.9.
Found: C, 42.6; H, 5.82; N, 30.5.

(*B*). To a solution of 500 mg. of V in 5 cc. of 90% formic acid heated on the steam-bath was added zinc dust in small portions until the solution became colorless. The mixture was heated for 30 minutes more, then filtered from zinc formate. The filtrate was concentrated to a thin syrup *in vacuo*, dissolved in water, and poured into excess ammonia water; yield, 265 mg. (50%), m.p. 221–222° (gas), resolidifying at 235° and remelting at 283–285° dec. A mixture with preparation *A* gave no depression.

When the reaction time was increased to 1 hour, the yield dropped to 38%.

2-Methylmercapto-6-dimethylaminopurine (X). (*A*). A solution of 500 mg. of VII in 5 cc. of formamide was refluxed for 50 minutes, cooled, and diluted with water to give 330 mg.

(72%) of insoluble solid, m.p. 278–282° dec. Recrystallization from 50% aqueous Methyl Cellosolve[7] with the aid of Norit gave white crystals, m.p. 284° (slight dec.).

Anal. Calc'd for $C_8H_{11}N_5S$: C, 45.8; H, 5.31; N, 33.5.

Found: C, 46.1; H, 5.42; N, 32.7, 32.9, 32.6.

(*B*). When boiling quinoline for 10 minutes was used as a solvent for ring closure, the product was isolated by the addition of 4 volumes of benzene; yield, 65% ,m.p. 278–280° dec.

(*C*). When 2.50 g. of VII was placed in a bath at 250° for 5 minutes, fusion took place with the evolution of water and the melt resolidified; yield, 2.3 g. (99%), m.p. 276–280° dec. This was the most satisfactory procedure up to about 5 g. of material, but above this quantity the yield and quality became poorer due to resolidification before reaction was complete.

The best procedure for large scale work was *D*.

(*D*). A solution of 2.48 g. of VII in 30 cc. of alcohol and 10 cc. of 5% sodium hydroxide was refluxed for 15 minutes, acidified with 7 cc. of acetic acid, and cooled. The product was collected and washed with dilute alcohol; yield, 1.30 g. (70%), m.p. 275–277° dec. By dilution of the mother liquor with water and concentration was recovered 0.25 g. (10%) of starting material, m.p. 210–215° dec.

Similar yields were obtained on a 25-g. scale. The reaction can also be run in the same volume of water but the rate of solution is very slow; yield, 78%, m.p. 276–278° dec.

2-Methylmercapto-6-dimethylamino-8-mercaptopurine (VIII). A solution of 2.0 g. of IV in 20 cc. of pyridine and 4 cc. of carbon disulfide was refluxed for 30 minutes on the steam-bath. Hydrogen sulfide was evolved and the product separated. After dilution with 50 cc. of water and cooling, the mixture was filtered and the product washed with water, then alcohol; yield, 2.2 g. (91%), m.p. >350°. Recrystallization from Methyl Cellosolve[7] afforded white crystals, m.p. >350°.

Anal. Calc'd for $C_8H_{11}N_5S_2$: C, 39.8; H, 4.60; N, 29.0.

Found: C, 40.1; H, 4.78; N, 29.3.

The same yield was obtained in a run of ten-fold size.

2,8-bis-Methylmercapto-6-dimethylaminopurine (IXa). To a hot solution of 1.7 g. of VIII in 7.1 cc. of 1 *N* methanolic sodium methoxide was added 0.66 cc. of methyl sulfate. A solid immediately separated with some heat of reaction. After 15 minutes, the solution was diluted with 25 cc. of water. The product was collected and washed with water, then alcohol; yield 1.6 g. (89%), m.p. 257–259°. Recrystallization from Methyl Cellosolve[7] gave white crystals, m.p. 257–259°.

Anal. Calc'd for $C_9H_{13}N_5S_2$: C, 42.4; H, 5.14; N, 27.4.

Found: C, 42.8; H, 5.38; N, 27.2.

The methylation could not be run in aqueous base since the mercaptopurine would not dissolve in the theoretical amount of 1 *N* sodium hydroxide.

2-Methylmercapto-6-dimethylamino-8-benzylmercaptopurine (IXb). To a hot solution of 700 mg. of VIII in 2.9 cc. of 1 *N* sodium methoxide and 20 cc. of methanol was added 0.27 cc. of benzyl chloride. The solution was refluxed for 10 minutes when it was neutral and some product had separated. Dilution with 50 cc. of water gave 850 mg. (97%) of insoluble product, m.p. 230–232°. Recrystallization from absolute alcohol afforded white crystals of unchanged m.p.

Anal. Calc'd for $C_{15}H_{17}N_5S_2$: C, 54.3; H, 5.15; N, 21.1.

Found: C, 54.0; H, 5.27; N, 20.8.

4-Amino-5-formamido-6-dimethylaminopyrimidine (XI). A solution of 1.00 g. of VII in 100 cc. of absolute alcohol was refluxed with 1⅓ teaspoons (about 4 g.) of desulfurizing Raney nickel (7) for 30 minutes, then filtered hot through Celite. The combined filtrate and washings were evaporated to dryness *in vacuo*; yield, 0.55 g. (69%) of white solid, m.p. 173–175° dec. Recrystallization from ethyl acetate-heptane with the aid of Norit gave white crystals, m.p. 185–187° dec.

Anal. Calc'd for $C_7H_{11}N_5O$: C, 46.4; H, 6.14; N, 38.7.

Found: C, 46.2; H, 6.31; N, 37.0, 36.8, 36.6.

[7] 2-Methoxyethanol.

6-Dimethylaminopurine (XII). (*A*). A mixture of 500 mg. of finely pulverized X and 40 cc. of 1 *N* sodium hydroxide was stirred on the steam-bath until solution was complete. Then 3 cc. of centrifuged Raney nickel (7) was added and the mixture was stirred on the steam-bath for 30 minutes. The nickel was removed by filtration through Celite and was washed with water. After acidification with acetic acid, the filtrate was evaporated to dryness *in vacuo*. The residue was dissolved in 10 cc. of water. The solution, filtered through Celite to remove some alumina, was extracted four times with equal volumes of ethyl acetate. The combined extracts, dried with magnesium sulfate, were evaporated to dryness *in vacuo*; yield, 170 mg. (43%) of white solid, m.p. 249–252°. Recrystallization from chloroform gave white crystals, m.p. 257–258°. A mixture with the $C_7H_9N_5$ moiety from puromycin (m.p. 257–258°) (2) gave no depression in m.p. The u.v. and infrared spectra of both compounds were identical.

Anal. Calc'd for $C_7H_9N_5$: C, 51.6; H, 5.57.
Found: C, 51.9; H, 5.66.

A sample of the pure purine in water was treated with an excess of saturated aqueous picric acid. The *picrate* was collected and washed with water and methanol: yellow needles, m.p. 244–245° dec. The picrate of $C_7H_9N_5$ moiety from puromycin (m.p. 244–245° dec.) (2) gave no depression in m.p. on admixture and both compounds had identical infrared spectra.

Anal. Calc'd for $C_7H_9N_5 \cdot C_6H_3N_3O_7$: C, 39.8; H, 3.09; N, 28.6.
Found: C, 40.1; H, 3.33; N, 28.4.

(*B*). A solution of 600 mg. of IXa in 60 cc. Methyl Cellosolve[7] was stirred on the steam-bath with 2 teaspoons of Raney nickel (7) for 30 minutes. The nickel was removed by filtration and washed with hot Methyl Cellosolve.[7] The combined filtrate and washings were evaporated to dryness *in vacuo*; yield, 60 mg. (16%), m.p. 240–245°, identified by mixture m.p.

Desulfurization of IXb in the same fashion gave no residue on evaporation of the filtered solution.

(*C*). A solution of 800 mg. of X in 80 cc. of Methyl Cellosolve[7] and 3.3 cc. of 1 *N* methanolic sodium methoxide was stirred on the steam-bath with 2⅔ teaspoons of Raney nickel (7) for 30 minutes. The mixture was filtered through Celite and the filtrate was evaporated to dryness *in vacuo*. The residue was dissolved in 20 cc. of 0.5 *N* hydrochloric acid. Clarified by filtration through Celite, the solution was treated with 75 cc. of 1% aqueous picric acid. The picrate was collected and washed with water; yield, 280 mg. (17%), m.p. 241–242° dec., identical with preparation *A*.

The picrate forms poorly from neutral aqueous solution, but rapidly and in good yield from 0.5 *N* hydrochloric acid.

(*D*). A solution of 200 mg. of crude XI (m.p. 173–175° dec.) in 2 cc. of quinoline was refluxed 10 minutes, cooled, and diluted with 2 cc. of benzene and 5 cc. of heptane. The product was collected and washed with benzene; yield, 150 mg. (83%), m.p. 249–252°, identical with preparation *A*.

(*E*). Direct fusion of 100 mg. of crude XI in a bath at 250–255° for 5 minutes gave 90 mg. (100%) of crude product, m.p. 235–240°. Recrystallization from absolute alcohol afforded white crystals, m.p. 252–254° in 85% recovery.

SUMMARY

An eight step synthesis of 6-dimethylaminopurine (XII) *via* the key intermediates 2-methylmercapto-4-amino-6-dimethylaminopyrimidine and 2-methylmercapto-6-dimethylaminopurine or 4-amino-5-formamido-6-dimethylaminopyrimidine has been described. The 6-dimethylaminopurine was found to be identical with the $C_7H_9N_5$ moiety obtained by alcoholysis of the antibiotic, puromycin.

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REFERENCES

(1) PORTER, HEWITT, HESSELTINE, KRUPKA, LOWERY, WALLACE, BOHONOS, AND WILLIAMS, *Antibiotics and Chemotherapy*, **2**, 409 (1952).
(2) WALLER, FRYTH, HUTCHINGS, AND WILLIAMS, *J. Am. Chem. Soc.*, **75**, 2025 (1953).
(3) TRAUBE, *Ann.*, **331**, 64 (1904).
(4) JOHNSON AND JOHNS, *Am. Chem. J.*, **34**, 175 (1905).
(5) KENNER, LYTHGOE, TODD, AND TOPHAM, *J. Chem. Soc.*, 574 (1943).
(6) BADDILEY, LYTHGOE, MCNEIL, AND TODD, *J. Chem. Soc.*, 383 (1943).
(7) MOZINGO, WOLF, HARRIS, AND FOLKERS, *J. Am. Chem. Soc.*, **65**, 1013 (1943).
(8) ELION, BURGI, AND HITCHINGS, *J. Am. Chem. Soc.*, **74**, 411 (1952).
(9) COOK AND SMITH, *J. Chem. Soc.*, 3001 (1949).