[CONTRIBUTION FROM THE WELLCOME RESEARCH LABORATORIES]

The Formation of 6-Hydroxy- and 7-Hydroxypteridines from 4,5-Diaminopyrimidines and α-Ketoacids and Esters

BY GERTRUDE B. ELION, GEORGE H. HITCHINGS* AND PETER B. RUSSELL

The nature of the products obtained from the condensation of 2,4,5-triamino-6-hydroxypyrimidine and pyruvic acid or ethyl oxomalonate is dependent on the acidity of the reaction medium.[1,2] In general, strong acid (2 *N* sulfuric acid) favors the formation of 6-hydroxypteridines, whereas a weakly acidic medium (*p*H 5) favors 7-hydroxy isomers. The course of the reaction of 2,4,5,6-tetraaminopyrimidine with α-keto acids and esters is likewise subject to these influences, with some interesting differences.

The yields of the isomeric products obtained from 2,4,5-triamino-6-hydroxypyrimidine (I) and 2,4,5,6-tetraminopyrimidine (II) with α-keto acids and esters are given in Table I. The data for the condensations of I with pyruvic acid[2] and with ethyl oxomalonate,[1] although previously reported, are included for completeness.

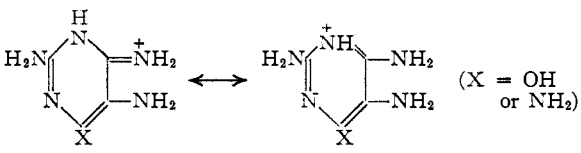
TABLE I

YIELDS OF ISOMERS AT DIFFERENT ACIDITIES

Pyrimidine	Reagent	*p*H 5 7-OH	*p*H 5 6-OH	2 *N* Sulfuric acid 7-OH	2 *N* Sulfuric acid 6-OH
I	Pyruvic acid	10	42	0	76
I	Ethyl oxalacetate	40	Trace	11	69
I	Ethyl oxomalonate	85	0	29	42
II	Pyruvic acid	37	13	3	92
II	Ethyl oxalacetate	66	0	22	49
II	Ethyl oxomalonate	87	0	90	0

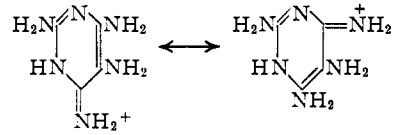
As will be seen from this table the type and amount of the isomer formed depends not only on the acidity of the medium but also on the nature of the ketonic reagents and the pyrimidines.

It is well known that 4,5-diaminopyrimidines acylate readily on the 5-position.[3,4] Forrest and Walker[5] have suggested that in acid solution 4,5-diaminopyrimidines exist as hybrids, of the type shown, making the 5-amino group the only center of nucleophilic activity. If these views are correct, one would expect the electrophilic carbon of the carbonyl group of α-keto esters to react chiefly with the 5-amino group, thereby giving rise to pteridines of the 7-hydroxy type. In weakly acid solution this is indeed the case. Moreover, in a series where the electrophilic nature of the carbonyl carbon is increased by the second radical on the carbonyl group, the yield of the 7-hydroxy isomer is likewise increased in the direction: $CH_3 < CH_2COOC_2H_5 < COOC_2H_5$. The results obtained with pyruvic acid are not strictly comparable with those obtained with α-keto esters, although qualitatively similar.



In strongly acid solution (*i. e.*, 2 *N* sulfuric acid) the 5-amino group of the pyrimidine will be protonated. This appears to reverse the direction of the reaction so as to favor the formation of the 6-hydroxy isomer. A notable exception is found in ethyl oxomalonate, which still shows a marked tendency to give the 7-hydroxy derivative.

The substitution of an amino group for the 4-hydroxy group of the pyrimidine (I) favors the formation of the 7-hydroxypteridines. In a 4,5,6-triaminopyrimidine there are symmetrical resonance possibilities involving the 4- and 6-amino groups such as those shown below.



This type of resonance would remove nucleophilic activity from the 4- and 6-amino groups, leaving the 5-amino group comparatively stronger as a nucleophilic center and thereby increasing the yields of the 7-hydroxypteridines. It will be noted that in the reaction of II with ethyl oxomalonate the nature of the pyrimidine and the ketonic reagent both favor the formation of the 7-hydroxy isomer to such an extent that even in 2 *N* sulfuric acid none of the 6-hydroxy isomer is formed.[6]

Since the condensation of ethyl oxalacetate with I in 2 *N* sulfuric acid had been found to produce 7-methylxanthopterin,[7] it is apparent that the condensation is accompanied by a saponification of the remaining ester group and a simultaneous decarboxylation. The intermediate acetic ester, such as is formed when *o*-phenylenediamine reacts with oxalacetic ester[7] to form a quinoxalone acetic ester, is not isolable in the reaction of I or II with oxalacetic ester either in 2 *N* sulfuric acid or at *p*H 5. Such an intermediate can be isolated, however, when I is condensed with methylacetylene dicarboxylate in alcoholic solution, a reaction

* Harvard University Ph.D. 1933.

(1) Purrmann, *Ann.*, **548**, 284 (1941).

(2) Elion and Hitchings, THIS JOURNAL, **69**, 2553 (1947).

(3) Traube, *Ber.*, **33**, 3035 (1900).

(4) Hitchings and Elion, THIS JOURNAL, **71**, 467 (1949).

(5) Forrest and Walker, *J. Chem. Soc.*, 79 (1949).

(6) While this work was in progress, the synthesis of 2,4-diamino-7-hydroxypteridine-6-carboxylic acid was reported by Steinbuch, *Helv. Chim. Acta*, **31**, 2051 (1948).

(7) Russell, Purrmann, Schmitt and Hitchings, THIS JOURNAL, **71**, 3412 (1949).

TABLE II

ULTRAVIOLET ABSORPTION SPECTRA

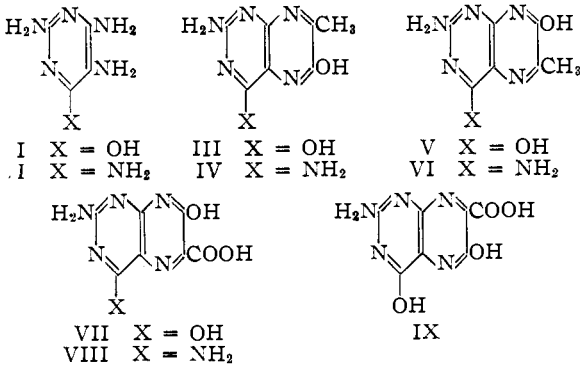
	Compound	*p*H = 1 Maxima λmμ	*p*H = 1 Maxima E_m	*p*H = 1 Minima λmμ	*p*H = 1 Minima E_m	*p*H = 11 Maxima λmμ	*p*H = 11 Maxima E_m	*p*H = 11 Minima λmμ	*p*H = 11 Minima E_m
III	7-Methylxanthopterin	230	13,600	250	8,900	252	15,800	305	960
		265	10,600	298	1,800	385	7,900		
		358	7,550						
IV	2,4-Diamino-6-hydroxy-7-methylpteridine	260[a]	7,900	295	3,400	255	13,300	305	1,760
		350	7,100			385	6,100		
V	6-Methylisoxanthopterin	290	12,000	255	2,500	253	10,400	243	9,650
		336	14,400	305	7,900	278	4,800	270	4,600
						336	14,200	292	3,500
VI	2,4-Diamino-6-methyl-7-hydroxypteridine	298	14,600	265	2,900	255	12,500	245	11,500
		333	15,000	315	12,100	280[a]	4,900		
						340	15,000	292	3,800
VII	Isoxanthopterin-6-carboxylic acid	290	11,000	245	4,800	258	12,000	248	11,000
		370	15,800	305	4,500	342	16,800	295	5,050
VIII	2,4-Diamino-7-hydroxypteridine-6-carboxylic acid	260	11,800	245	10,200	262	13,000	250	11,700
		297	7,300	285	7,000	350	15,500	298	3,100
		367	15,300	312	3,200				
IX	Xanthopterin-7-carboxylic acid	240[a]	12,800	300	1,100	255	17,000	310	780
		375	8,300			395	7,600		

[a] Inflection.

analogous to the synthesis of 2-hydroxyquinoxaline-3-acetic acid from *o*-phenylenediamine and ethylacetylene dicarboxylate.[8] This intermediate, upon saponification, decarboxylates spontaneously to give 69% of 6-methylisoxanthopterin and 6% of 7-methylxanthopterin.

The identify of the isomeric pteridines reported here was established by their ultraviolet absorption spectra (Table II). The spectra of 7-methylxanthopterin (III) and its 2,4-diamino analog (IV) while almost identical at *p*H 11 are markedly different at *p*H 1. This similarity between the spectra of hydroxypyrimidines and the corresponding aminopyrimidines in alkaline solution has been reported previously.[9] While both III and IV exhibit basic characteristics, being soluble in hot dilute mineral acids, IV is the stronger base, being soluble in 2 *N* sulfuric acid even in the cold.

The spectra of 6-methylisoxanthopterin (V) and its 2,4-diamino analog (VI) are very similar at both *p*H 1 and *p*H 11, there being only a slight displacement of one band of VI toward the longer wave lengths at *p*H 1. This close resemblance is not unexpected in view of the similarity in the physical and chemical properties of these two compounds.



The identity of 2,4-diamino-7-hydroxypteridine-6-carboxylic acid (VIII) was established by the resemblance of its spectrum to that of isoxanthopterin carboxylic acid (VII) and its dissimilarity with that of xanthopterin carboxylic acid (IX).

Experimental

7-Methylxanthopterin (III). A. From Pyruvic Acid. Previously Reported.[2] B. From Ethyl Oxalacetate.—To 200 ml. of 2 *N* sulfuric acid were added 2.4 g. of 2,4,5-triamino-6-hydroxypyrimidine sulfate hydrate[10] and 4 g. of sodium ethyl oxalacetate. The mixture was refluxed for one hour. On cooling, a precipitate separated (0.20 g., 11%) which consisted mainly of 6-methylisoxanthopterin according to its ultraviolet absorption spectrum. The filtrate was adjusted to *p*H 6 with ammonium hydroxide. The brown precipitate was collected by centrifugation, washed with water, alcohol and ether and dried at 100° (1.3 g., 69%). It was identified as 7-methylxanthopterin by its spectrum.

2,4-Diamino-6-hydroxy-7-methylpteridine (IV). A. From Pyruvic Acid.—A mixture of 1.25 g. of 2,4,5,6-tetraaminopyrimidine sulfate hydrate[11] and 1 ml. of pyruvic acid in 50 ml. of 2 *N* sulfuric acid was refluxed for one hour. The red solution was chilled for one hour and a small brown precipitate (100 mg.) filtered off. The ultraviolet absorption spectrum of this precipitate indicated that it contained 30 mg. (3% yield) of isomer VI.

The filtrate was heated to 80° and the *p*H adjusted to 6 by the addition of ammonium hydroxide. After standing at 4° overnight, the orange precipitate was collected, washed with water, alcohol and ether and air-dried (0.93 g., 95%). A small sample (100 mg.) was purified for analysis by solution in 75 ml. of water containing 2 ml. of

(8) Ruhemann and Stapleton, *J. Chem. Soc.*, **77**, 239 (1900).

(9) Williams, Ruehle and Finkelstein, THIS JOURNAL, **59**, 526 (1937).

(10) Traube and Dudley, *Ber.*, **46**, 3839 (1913).

(11) Traube, *Ber.*, **37**, 4544 (1904).

2.5 *N* sodium hydroxide solution and filtration into 25 ml. of boiling water containing 2 ml. of glacial acetic acid and dried at 110°.

Anal. Calcd. for $C_7H_6ON_6 \cdot {}^1/_2H_2O$: C, 41.8; H, 4.5; N, 41.8. Found: C, 41.4; H, 4.2; N, 41.3.

B. From Ethyl Oxalacetate.—To 75 ml. of 2 *N* sulfuric acid were added 1.65 g. of 2,4,5,6-tetraaminopyrimidine sulfate hydrate and 4 g. of sodium ethyl oxalacetate. The mixture was refluxed for one and one-half hours and the deep orange solution permitted to stand overnight at room temperature. A small brown amorphous precipitate (70 mg.) was collected and found by inspection of the ultraviolet absorption spectrum to contain about 30 mg. (2% yield) of isomer IV and 30 mg. (2% yield) of isomer VI.[12] The filtrate was diluted to 200 ml. with water and the *p*H adjusted to 6 with ammonium hydroxide. The amorphous orange precipitate (0.87 g.) was collected by centrifugation and was likewise found to be a mixture of the two isomers, containing 0.61 g. (47% yield) of IV and 0.26 g. (20% yield) of VI.

Although both isomers are soluble in hot mineral acid, the 6-hydroxy isomer (IV) is appreciably more soluble in cold mineral acid than VI. Nevertheless, the separation of these isomers by fractional precipitation is difficult because of the tendency of VI to adsorb IV during precipitation. Since each of the isomers was obtained pure under other conditions, no further attempt was made to separate the isomers in this experiment.

6-Methylisoxanthopterin (V). A. From Pyruvic Acid. Previously Reported.[2] **B. From Ethyl Oxalacetate.**—Two grams of 2,4,5-triamino-6-hydroxypyrimidine was dissolved in 200 ml. of 1 *N* acetic acid and a solution of 4 g. of sodium ethyl oxalacetate in 40 ml. of 0.5 *N* acetic acid was added. The solution was heated on a steam-bath for thirty minutes, some gas being evolved. The orange-yellow precipitate was collected after cooling and dissolved in 200 ml. of 0.2 *N* sodium hydroxide solution, treated with charcoal and filtered into 250 ml. of boiling 2 *N* hydrochloric acid. The 6-methylisoxanthopterin (1.1 g., 40%) was purified once more by this procedure, giving an almost colorless powder whose spectrum was identical with an authentic sample of 6-methylisoxanthopterin.

When the acid filtrates were adjusted to *p*H 6 with sodium hydroxide solution, only a trace of 7-methylxanthopterin was obtained as a brown precipitate.

2,4-Diamino-7-hydroxy-6-methylpteridine (VI). A. From Pyruvic Acid.—To a hot solution of 10 g. of 2,4,5,6-tetraaminopyrimidine sulfite[13] in 400 ml. of boiling water was added 10 ml. of pyruvic acid and the *p*H adjusted to 5 by the addition of 44 ml. of 2 *N* sodium hydroxide solution. The mixture was heated on the steam-bath for one-half hour. A dark yellow precipitate formed gradually. After standing overnight at room temperature, the precipitate was collected, washed and dried at 100° (5.5 g.). On standing at room temperature for three weeks, the filtrate deposited another 1.75 g. of a brown precipitate.

Extraction of the first crude precipitate with 300 ml. of boiling 0.15 *N* sulfuric acid left an almost insoluble colorless residue which was obtained crystalline by solution in 120 ml. of hot 0.3 *N* sodium hydroxide solution and filtration into 500 ml. of hot 0.2 *N* sulfuric acid. This product (1.3 g., 14%) was identified as VI by its ultraviolet absorption spectrum.

The hot sulfuric acid extract was chilled for one hour and the dark yellow precipitate which formed was identified as isomer VI (1.25 g., 14%). On standing overnight at 4°, the sulfuric acid filtrate deposited another 0.55 g. (6%) of a yellow precipitate. This and the second precipitate from the reaction mixture were both found to be mixtures of the two isomers on the basis of their spectra. The total yield of isomer VI was 37%; that of IV was 13%.[12]

For analysis the colorless crystalline sulfate of VI was prepared by recrystallization from 50 parts of 2 *N* sulfuric acid and was dried at 100°.

Anal. Calcd. for $C_7H_8N_6O \cdot {}^1/_2H_2SO_4 \cdot 1{}^1/_2H_2O$: C, 31.3; H, 4.5; N, 31.3; H_2O, 10.05. Found: C, 31.2; H, 4.5; N, 31.2; H_2O (dried three hours at 140°), 10.2.

B. From Ethyl Oxalacetate.—To a solution of 12.9 g. of 2,4,5,6-tetraaminopyrimidine sulfite in 400 ml. of water and 20 ml. of 2 *N* sodium hydroxide solution was added 20 g. of sodium ethyl oxalacetate. The *p*H was brought to 5 by the addition of glacial acetic acid and the mixture heated at 90° for one-half hour. An evolution of gas was observed and a pale yellow precipitate formed gradually. At the end of the reaction the *p*H was found to have dropped to 3. The reaction mixture was chilled, filtered, and the crude precipitate purified by solution in 600 ml. of water containing 6 ml. of saturated sodium hydroxide solution and filtration into 100 ml. of hot water containing 15 ml. of glacial acetic acid. The almost colorless precipitate after filtration and drying at 110° weighed 7.4 g. (66%). Its ultraviolet absorption spectrum was identical with VI prepared from pyruvic acid.

2,4-Diamino-7-hydroxypterine-6-carboxylic Acid (VIII).—Nineteen and one-half grams of 2,4,5,6-tetraaminopyrimidine sulfate hydrate and 46 ml. of ethyl oxomalonate were added to 625 ml. of 2 *N* sulfuric acid and the mixture heated on the steam-bath for one hour. The starting material gradually dissolved and a new yellow precipitate formed. After standing overnight at 4° the precipitate was collected, washed with water, alcohol and ether and dried at 100° (15.2 g., 90%). The product has only a slight solubility in hot 2 *N* sodium carbonate solution, the soluble portion having the same ultraviolet absorption spectrum as the insoluble residue. The compound has no melting point and does not decarboxylate on heating to 250°.

The condensation was repeated at *p*H 5, using 5.12 g. of 2,4,5,6-tetraaminopyrimidine sulfate hydrate and 12 ml. of mesoxalic ester in 220 ml. of 0.2 *N* acetic acid containing 3.3 g. of sodium acetate. The mixture was heated for forty-five minutes on the steam-bath. The yield was 3.85 g. (87%), the product having the same ultraviolet absorption spectrum as the one prepared in 2 *N* sulfuric acid.

Anal. Calcd. for $C_7H_6N_6O_3$: C, 37.8; H, 2.7; N, 37.8. Found: C, 37.7; H, 2.8; N, 37.8.

Condensation of 2,4,5-Triamino-6-hydroxypyrimidine with Methyl Acetylene Dicarboxylate.—A mixture of 2 g. of 2,4,5-triamino-6-hydroxypyrimidine and 3 g. of methyl acetylenedicarboxylate in 50 ml. of 95% ethanol was refluxed for three hours. The solution was cooled and the yellow precipitate removed by filtration. When attempts to purify this precipitate, which was probably a mixture of isoxanthopterin-6-acetic methyl ester and xanthopterin-7-acetic methyl ester, failed, the crude material was refluxed with 100 ml. of *N* sulfuric acid until the evolution of gas, which was violent at first, ceased. After cooling, the pale yellow powder was collected, dissolved in 200 ml. of water by the addition of 20 ml. of 2 *N* sodium hydroxide solution, treated with charcoal and filtered into 100 ml. of boiling 2 *N* hydrochloric acid. The almost colorless precipitate which formed showed a spectrum identical with that of 6-methylisoxanthopterin (1.88 g., 69%).

The presence of approximately 0.15 g. (5.5%) of 7-methylxanthopterin in the sulfuric acid mother liquors was shown by the formation of 0.15 g. of pterorhodin when 0.5 g. of xanthopterin was added to the solution and the mixture was aerated at 90°.[7]

Acknowledgment.—We are indebted to Samuel W. Blackman for the microanalyses reported here.

Summary

The condensations of 2,4,5-triamino-6-hydroxypyrimidine and 2,4,5,6-tetraaminopyrimidine with

(12) For the determination of the composition of mixtures of isomers, the absorption curves of the precipitates were compared with summation curves constructed for varying percentages of the pure isomers. Yields calculated in this way are to be considered as approximations.

(13) Mallette, Taylor and Cain, THIS JOURNAL, **69**, 1814 (1947).

several α-keto esters has been investigated. The type and yield of the isomers produced were found to be influenced by the acidity of the medium, the nature of the ketonic reagent and the nature of the pyrimidine.

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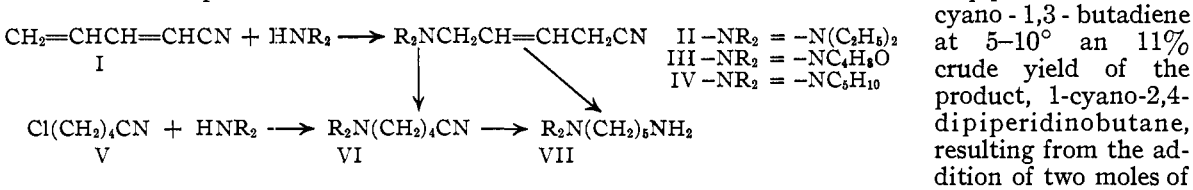
Addition Reactions of 1-Cyano-1,3-butadiene[1,2]

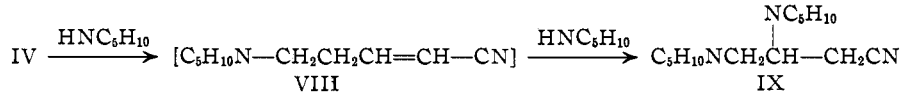
BY MILTON FRANKEL,[3,4] HARRY S. MOSHER AND FRANK C. WHITMORE[5]

Previous reports[6,7,8,9] of reactions of 1-cyano-1,3-butadiene have not included any study of the addition of amines to this substance. We have found that diethylamine, morpholine and piperidine rapidly add at zero degrees to give excellent yields of the 1-cyano-4-substituted-amino-2-butenes (II, III, IV); no catalyst other than the amine itself is required.



The actual position of the double bond in these products (II, III and IV) has not been proven but the well-established course of such additions would indicate that it is situated in the β,γ-position as represented. Attempts to rearrange IV into VIII, in which the double bond is conjugated, were unsuccessful; polymer or unchanged starting material was recovered. The maximum in the ultraviolet absorption spectrum was below 215 mμ and could not be conveniently measured. Comparison of infrared absorption spectra for I, IV and VI was indicative of an isolated double bond but could hardly be considered definitive since the geometry around the double bond is unknown. Since careful reduction with hydrogen in the presence of platinum catalyst has given the same substituted 4-amino-1-cyanobutanes (VI) as produced from 4-chloro-1-cyanobutane (V) and the corresponding secondary amine, there can be no doubt concerning the terminal position of the secondary amino group. Either the saturated or unsaturated δ-aminonitriles were reduced in good yields in the presence of Raney nickel catalyst to give the 5-substituted-amino-1-aminopentanes (VII). Were it not for the present difficulty in obtaining large amounts of the necessary 1-cyano-1,3-butadiene, this would constitute an excellent preparative method for these otherwise difficultly obtainable products.

In the reaction of excess piperidine with 1-cyano-1,3-butadiene at 5–10° an 11% crude yield of the product, 1-cyano-2,4-dipiperidinobutane, resulting from the addition of two moles of the amine, was obtained. 1-Cyano-4-piperidino-2-butene was refluxed with excess piperidine for twelve hours; a 43% yield of 1-cyano-2,4-dipiperidinobutane was isolated and the remainder of the starting material was recovered unchanged. Several attempts to increase this yield by the use of a strongly basic catalyst such as sodium methylate or Triton B were unsuccessful; yields of 10 to 20% were obtained (20 to 35% considering starting material which was recovered). In these experiments a certain amount of non-volatile polymer was always formed. It is postulated that piperidine itself is a strong enough base to catalyze the rearrangement of the 1-cyano-4-piperidino-2-butene to the conjugated 1-cyano-4-piperidino-1-butene (VIII) which then adds a second mole of the piperidine to give IX.



However, it was not possible to accomplish the addition of a second molecule of either morpholine or diethylamine to the corresponding 1-cyano-4-amino-2-butene by heating without catalyst even in a sealed tube at 120°. By the use of Triton B as a catalyst, addition occurred at room temperature with the formation of poor yields of 1-cyano-2,4-dimorpholinobutane and 1-cyano-2,4-di-(diethylamino)-butane. The necessity of a basic catalyst in these two cases constitutes circumstantial evidence that the double bond was originally present in the non-conjugated position.

(1) Taken in part from the M. S. Thesis of Milton Frankel, The Pennsylvania State College, June, 1947.

(2) Presented before the Organic Division of the American Chemical Society at the New York meeting, September 17, 1947.

(3) Parke, Davis and Co. Research Fellow 1947–1949.

(4) Present address: Aerojet Engineering Corp., Azusa, Calif.

(5) Deceased; Harvard University Ph.D. 1914.

(6) Coffman, THIS JOURNAL, **57**, 1981 (1935).

(7) Charlish, Davies and Rose, *J. Chem. Soc.*, 227–234 (1948).

(8) Bruson, U. S. Pat. 2,466,679, 1949.

(9) Snyder, Stewart and Myers, THIS JOURNAL, **71**, 1055–1056 (1949).