[CONTRIBUTION FROM THE FRICK CHEMICAL LABORATORY, PRINCETON UNIVERSITY, AND THE NOYES CHEMICAL LABORATORY, UNIVERSITY OF ILLINOIS]

Studies in Purine Chemistry. New Routes to Certain 2,1,3-Triazoles, Pyrimidines and 2,1,3-Triazolo[4,5-*d*]pyrimidines[1,2]

BY EKKEHARD RICHTER AND E. C. TAYLOR[3]

RECEIVED JUNE 11, 1956

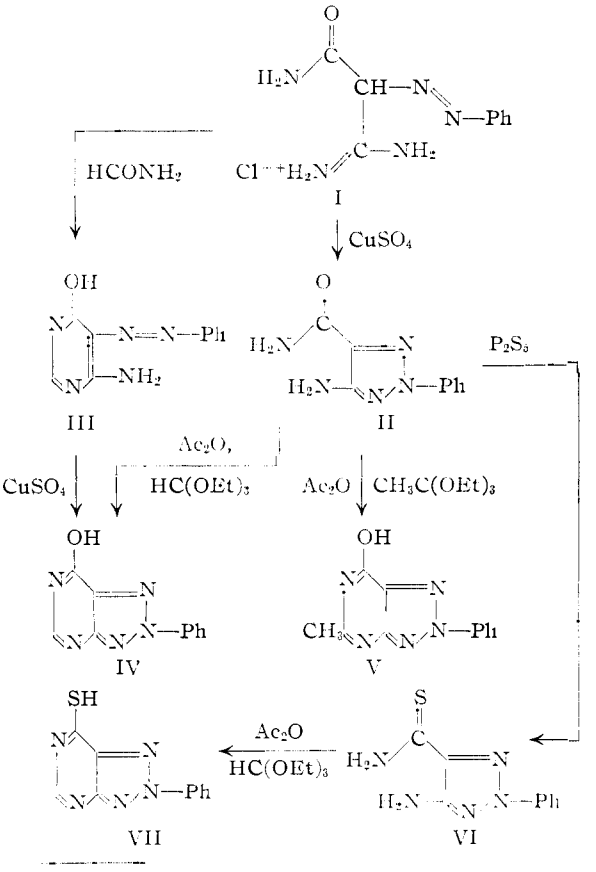
Analogs of hypoxanthine and adenine have been prepared by two independent routes from phenylazomalonamide-amidine (I) and phenylazomalondiamidine (VIII), respectively, both routes employing two successive cyclizations. Thus, compound I was cyclized with formamide to 4-hydroxy-5-phenylazo-6-aminopyrimidine (III), and oxidized with ammoniacal copper sulfate to 2-phenyl-4-amino-2,1,3-triazole-5-carboxamide (II). Oxidation of the former with copper sulfate and cyclization of the latter with ethyl orthoformate and acetic anhydride gave 2-phenyl-7-hydroxy-2,1,3-triazolo[4,5-d]-pyrimidine (IV). Similarly, VIII was converted to 2-phenyl-7-amino-2,1,3-triazolo[4,5-d]pyrimidine (X) *via* 4,6-diamino-5-phenylazopyrimidine (IX), but the alternative synthesis from 2-phenyl-4-amino-2,1,3-triazole-5-carboxamidine (XI) could not be accomplished. The analog of 6-mercaptopurine, 2-phenyl-7-mercapto-2,1,3-triazolo[4,5-d]pyrimidine (VII), was prepared by conversion of II with phosphorus pentasulfide into 2-phenyl-4-amino-2,1,3-triazole-5-thiocarboxamide (VI), which was then cyclized to VII with ethyl orthoformate and acetic anhydride. The versatility of ortho esters as cyclization reagents was further demonstrated by the synthesis of 2-phenyl-5-methyl-7-hydroxy-2,1,3-triazolo[4,5-d]-pyrimidine (V) from II and a mixture of ethyl orthoacetate and acetic anhydride. Application of the formamide cyclization procedure to malondiamide failed to yield the expected 4,6-dihydroxypyrimidine, but the action of formamide on malondiamidine led to 4-formylamino-6-aminopyrimidine. Attempted cyclization of malondiamidine with ethyl orthoformate and acetic anhydride gave *sym*-N,N′-bis-(ethoxymethylene)-malondiamidine monohydrochloride (XIII), which was converted to 4-formylamino-6-aminopyrimidine on heating with formamide.

Numerous structural analogs of the naturally occurring purines as well as derivatives of closely related heterocyclic systems have been shown to be potent inhibitors of a variety of biological systems. In addition, many of these purine antimetabolites have shown antimitotic activity, and these results have greatly stimulated recent efforts directed toward the elaboration of potential purine antagonists.[4]

In view of the demonstrated physiological activity of 8-azaguanine and related triazolopyrimidines,[4] it was disappointing to learn of the inactivity of the many derivatives of 2-phenyl-2,1,3-triazolo[4,5-d]pyrimidine prepared by Benson and co-workers.[5,6] It seemed possible, however, that the preparation of simpler derivatives of this ring system, more closely approximating the structures of the naturally occurring purines, might be worthwhile, and this communication describes the synthesis by a novel route of several new derivatives of 2,1,3-triazolo[4,5-d]pyrimidine (IV, VII and X), which may be considered to be structural analogs of hypoxanthine, 6-mercaptopurine and adenine, respectively.

Phenylazomalonamide-amidine hydrochloride (I)[7] and phenylazomalondiamidine dihydrochloride (VIII)[8] appeared to be ideal starting materials for the preparation of these simpler 2-phenyl-2,1,3-triazolo[4,5-d]pyrimidines, for cyclization by appropriate methods should lead either to a 2,1,3-triazole or to a 5-phenylazopyrimidine, and both of these intermediates, it was expected, should be able to undergo a second cyclization to form the desired compounds. These expectations have been realized and our results are described below.

Phenylazomalonamide-amidine hydrochloride (I) was converted smoothly by oxidation with ammoniacal copper sulfate[9] to 2-phenyl-4-amino-2,1,3-triazole-5-carboxamide (II), which was then cy-



(1) This investigation was supported by research grants from the National Cancer Institute of the National Institutes of Health, Public Health Service, to Princeton University (C-2551 PET) and to the University of Illinois (C-2031 ET).

(2) Presented before the Division of Organic Chemistry at the 128th National A.C.S. Meeting, September, 1955, in Minneapolis, Minn.

(3) Frick Chemical Laboratory, Princeton University, Princeton, N. J.

(4) For excellent bibliographies of pertinent references, see (a) A. Bendich, P. J. Russell, Jr., and J. J. Fox, THIS JOURNAL, **76**, 6073 (1954); (b) J. H. Burchenal, *Federation Proc.*, **13**, 760 (1954).

(5) F. R. Benson, L. W. Hartzel and W. L. Savell, THIS JOURNAL, **72**, 1816 (1950).

(6) L. W. Hartzel and F. R. Benson, *ibid.*, **76**, 2263 (1954).

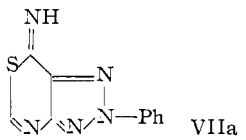
(7) E. Shaw and D. W. Woolley, *J. Biol. Chem.*, **181**, 89 (1949).

(8) E. Shaw, *ibid.*, **185**, 439 (1950).

(9) M. P. Schmidt and A. Hagenbuecker, *Ber.*, **54**, 2191 (1921).

clized with a mixture of ethyl orthoformate and acetic anhydride[10,11] to 2-phenyl-7-hydroxy-2,1,3-triazolo(4,5-d)pyrimidine (IV), the analog of hypoxanthine. Alternatively, on warming with formamide, compound I gave 4-hydroxy-5-phenylazo-6-aminopyrimidine (III), which was oxidized to IV with copper sulfate in aqueous pyridine. The former method, involving the preliminary formation of the triazole intermediate II followed by cyclization of the pyrimidine ring, was far superior to the latter, both in yield and in the quality of the product obtained. Higher ortho esters may be used in the final cyclization step, as illustrated by the synthesis of 2-phenyl-5-methyl-7-hydroxy-2,1,3-triazolo(4,5-d)pyrimidine (V) from II and a mixture of ethyl orthoacetate and acetic anhydride.

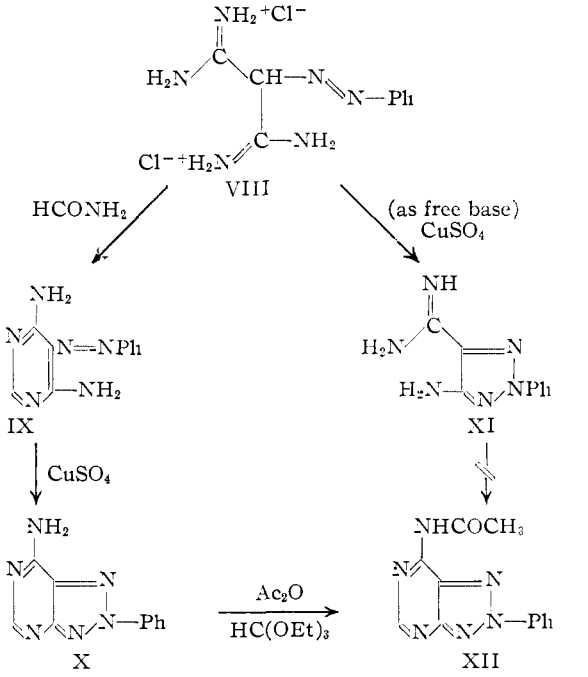
By treating II with phosphorus pentasulfide in pyridine solution, 2-phenyl-4-amino-2,1,3-triazole-5-thiocarboxamide (VI) was obtained. Cyclization of VI with ethyl orthoformate and acetic anhydride produced 2-phenyl-7-mercapto-2,1,3-triazolo-(4,5-d)pyrimidine (VII), the analog of 6-mercaptopurine. A possible alternative formulation for the ethyl orthoformate cyclization product of VI would be 2-phenyl-7-imino-2,1,3-triazolo(4,5-d)thiazine (VIIa), but this structure was readily rejected and the proposed structure VII shown to be correct, by reaction of VII with alcoholic ammonia to yield 2-phenyl-7-amino-2,1,3-triazolo (4,5-d)pyrimidine (X), which was synthesized independently by an unequivocal method (*vide infra*).



VIIa

Corresponding routes to the adenine analog X were then investigated. Heating phenylazomalondiamidine dihydrochloride (VIII) with formamide gave 4,6-diamino-5-phenylazopyrimidine (IX) which was converted into 2-phenyl-7-amino-2,1,3-triazolo(4,5-d)pyrimidine (X) by oxidation with copper sulfate in pyridine solution. However, an alternative synthesis of X *via* a triazole intermediate could not be accomplished. Thus, oxidation of phenylazomalondiamidine dihydrochloride (VIII) with ammoniacal copper sulfate at steam-bath temperature, under conditions comparable to those employed for the conversion of I to II, gave the carboxamide II. The anticipated 2-phenyl-4-amino-2,1,3-triazole-5-carboxamidine (XI) had apparently been hydrolyzed under these conditions. Numerous other attempts to obtain XI from VIII and copper sulfate using pyridine as solvent were unsuccessful. XI was finally obtained, although in low yield, by the use of phenylazomalondiamidine (VIII) as the free base and milder oxidation conditions.

It was anticipated that the acetyl derivative of X (XII), rather than X, would be the cyclization product of XI with acetic anhydride and ethyl orthoformate, since X itself, when treated with these reagents, gave 2-phenyl-7-acetylamino-2,1,3-triazolo(4,5-d)pyrimidine (XII). However, all attempts to carry out this conversion were unsuccessful.



The conversions I → III and VIII → IX, followed by reduction of the phenylazo group to an amino group, represent new and straight-forward routes to 4-hydroxy-5,6-diaminopyrimidine and 4,5,6-triaminopyrimidine, respectively. IX has been prepared previously by the condensation of phenylazomalononitrile with formamidine hydrochloride[12] but the present synthesis appears to be more convenient and gives a product of higher purity. Unexpectedly, phenylazomalondiamide could not be cyclized with formamide to 4,6-dihydroxy-5-phenylazopyrimidine; no reaction took place on heating for 11 hours at 150–160° or at 180°, while heating at 195–200° gave only small amounts of a dark solid which had evidently undergone considerable decomposition.

An attempt to extend the formamide cyclization procedure to simpler derivatives of malonic acid was then made. Malondiamide was heated with formamide, but instead of the expected reaction product, 4,6-dihydroxypyrimidine,[13] a white, crystalline refractory solid (m.p. > 400°) was obtained which could not be characterized.[13a] Malondiamidine dihydrochloride gave 4-formylamino-6-aminopyrimidine in 28.2% yield. Although it could be readily hydrolyzed to 4,6-diaminopyrimidine by boiling with 0.1 *N* hydrochloric acid, this synthesis appears to be inferior to the previously published procedure.[14]

(10) A. Albert, D. J. Brown and G. Cheeseman, *J. Chem. Soc.*, 474 (1951).

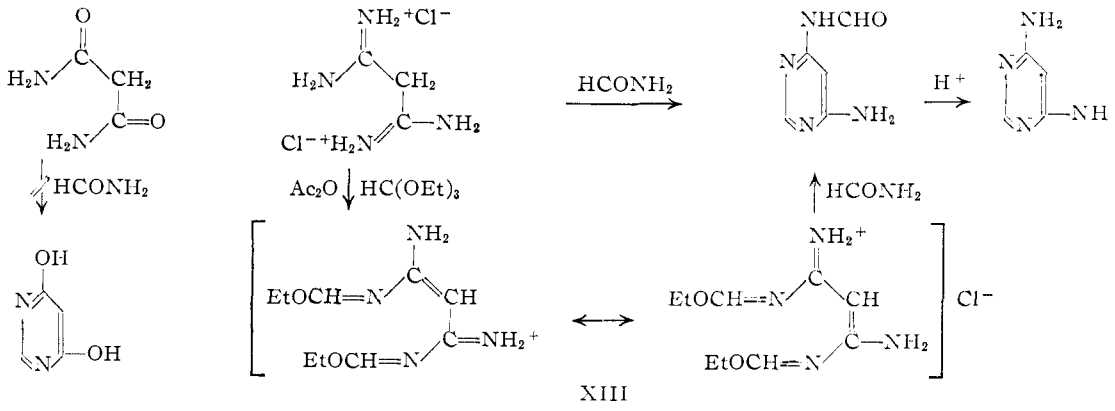
(11) E. C. Taylor, Jr., J. A. Carbon and D. R. Hoff, THIS JOURNAL, **75**, 1904 (1953).

(12) J. Baddiley, B. Lythgoe and A. R. Todd, *J. Chem. Soc.*, 386 (1943).

(13) R. Hull, *ibid.*, 2214 (1951).

(13a) NOTE ADDED IN PROOF.—It has been reported recently (D. J. Brown, *J. Chem. Soc.*, 2312 (1956)) that the condensation of malondiamide with formamide in ethanol solution in the presence of sodium ethoxide gives 4,6-dihydroxypyrimidine in good yield.

(14) G. W. Kenner, B. Lythgoe, A. R. Todd and A. Topham, *ibid.*, 574 (1943).



XIII

It was thought that a mixture of ethyl orthoformate and acetic anhydride might effect the same transformation since these reagents have been shown to accomplish the cyclization of certain β-aminoamides (II and VI). However, the action of an ethyl orthoformate–acetic anhydride mixture on malondiamidine dihydrochloride led only to the formation of *sym*-N,N′-bis-(ethoxymethylene)-malondiamidine monohydrochloride (XIII). Unchanged starting material was recovered in the absence of acetic anhydride. The formation of a monohydrochloride is readily understandable, since a highly resonance-stabilized cation is formed by the addition of a single proton to *sym*-N,N′-bis-(ethoxymethylene)-malondiamidine. XIII was converted to 4-formylamino-6-aminopyrimidine by heating with formamide under conditions similar to those employed for the preparation of this pyrimidine from malondiamidine dihydrochloride.

No significant physiological activity has been found for any of the compounds prepared in this study. Compound VII, designed as an analog of 6-mercaptopurine, was inactive in inhibiting the growth of Sarcoma 180 in mice.

Acknowledgment.—The authors are indebted to the Parke, Davis and Company for carrying out the physiological screening of these compounds.

Experimental[15]

Phenylazomalonamide-amidine Hydrochloride (I).—This compound was prepared according to the method of Shaw and Woolley.[7] It has been pointed out more recently[16] that maximum yields are obtained by maintaining the *p*H of the coupling mixture at 4–5 for at least 2–3 hours after initiation of the coupling. We have found, however, that in large scale preparations of this compound it is essential to stir vigorously throughout the reaction in order to obtain maximum yields, and that in the absence of stirring, even with strict control of the *p*H, partial decomposition takes place. Vigorous stirring is absolutely necessary in the preparation of phenylazomalondiamidine[8,17] and phenylazomalondiamide.

(15) All melting points are uncorrected. We are indebted for the microanalyses to Dr. Joseph Alicino of Metuchen, N. J., and to Mrs. Lucy Chang, Mrs. Esther Fett and Mr. Joseph Nemeth of the University of Illinois.

(16) L. H. Smith, Jr., and P. Yates, THIS JOURNAL, **76**, 6080 (1954).

(17) It is of interest that both phenylazomalonamide-amidine and phenylazomalondiamidine can be isolated and characterized as the free bases. Phenylazomalonamide-amidine melts at 191° dec. and may be recrystallized from ethanol in the form of bright yellow crystals (Calcd. for $C_9H_{11}ON_5$: C, 52.67; H, 5.40; N, 34.13. Found: C, 52.73; H, 5.42; N, 33.83). Phenylazomalondiamidine melts at 181° dec. and may also be obtained as a bright yellow crystalline solid upon recrystallization from ethanol (Calcd. for $C_9H_{12}N_6$: C, 52.92; H, 5.92; N, 41.15. Found: C, 52.78; H, 5.86; N, 40.95).

2-Phenyl-4-amino-2,1,3-triazole-5-carboxamide (II).—To a suspension of 14.22 g. of crude phenylazomalonamide-amidine hydrochloride in 500 ml. of 50% aqueous alcohol at room temperature was added a solution of 50 g. of copper sulfate pentahydrate in a mixture of 100 ml. of water and 200 ml. of concentrated ammonium hydroxide. An additional 100 ml. of concentrated ammonium hydroxide was added after one hour, and the reaction mixture was allowed to stand at room temperature for 24 hours. It was then heated for 3 hours on a steam-bath. The mixture was cooled thoroughly in an ice-bath and the crude 2-phenyl-4-amino-2,1,3-triazole-5-carboxamide was separated by filtration and washed with water until the washings were colorless (*ca.* 600 ml.); yield 11.31 g. (94.6%). Two recrystallizations from 95% ethanol with the use of decolorizing charcoal gave 7.61 g. (63.6%) of colorless, soft needles, m.p. 165–167°. The melting point was raised to 171° by further recrystallization from ethanol followed by sublimation (140–150° (0.01 mm.)).

Anal. Calcd. for $C_9H_9ON_5$: C, 53.20; H, 4.46; N, 34.47. Found: C, 53.28; H, 4.24; N, 34.49.

4-Amino-5-phenylazo-6-hydroxypyrimidine (III).—A mixture of 7.30 g. of phenylazomalonamide-amidine hydrochloride and 50 ml. of formamide in a 100-ml. flask was placed in an oil-bath preheated to 150°. During the next 15 minutes, the temperature of the bath was raised slowly to 170° and then allowed to fall to 150° during the following 15 minutes. The reaction mixture was then heated for one hour, during which time the temperature of the oil-bath was allowed to fall to 110°. The deep red reaction solution was poured into 600 ml. of water and the mixture allowed to stand overnight to assure complete separation of the orange-brown solid. This solid was collected by filtration, washed thoroughly with water and dried over phosphorus pentoxide to give 4.09 g. (63%) of crude product, m.p. 223–225° dec., with preliminary softening below the decomposition point. The product was recrystallized twice from ethanol and finally from dimethylformamide to give orange crystals, m.p. 244–246° dec.

Anal. Calcd. for $C_{10}H_9ON_5$: C, 55.81; H, 4.21; N, 32.55. Found: C, 55.83; H, 4.49; N, 32.75.

2-Phenyl-7-hydroxy-2,1,3-triazolo(4,5-d)pyrimidine (IV). **Method A.**—A mixture of 4.38 g. of 2-phenyl-4-amino-2,1,3-triazole-5-carboxamide (m.p. 165–167°), 40 ml. of acetic anhydride and 40 ml. of ethyl orthoformate was heated under reflux for 4 hours and then evaporated to near dryness under reduced pressure. The residue was boiled for a few minutes with 100 ml. of absolute ethanol to destroy residual acetic anhydride and the solution was again evaporated to dryness. One recrystallization of the crude product from 95% ethanol gave 3.10 g. (67.5%) of almost colorless crystals, m.p. 270–273°; further recrystallization with the use of decolorizing charcoal yielded 2.54 g. of colorless crystals which were sublimed (250° (0.01 mm.)) for analysis, m.p. 281°. The compound exhibited ultraviolet absorption maxima in absolute ethanol at 240, 300 and 307 mμ (log ε 4.93, 5.16 and 5.16).

Anal. Calcd. for $C_{10}H_7ON_5$: C, 56.34; H, 3.31; N, 32.85. Found: C, 56.41; H, 3.23; N, 32.57.

Method B.—To a solution of 2.89 g. of 4-amino-5-phenylazo-6-hydroxypyrimidine in 50 ml. of pyridine was added a solution of 14 g. of copper sulfate pentahydrate in 40 ml. of

water, and the resulting mixture was heated on a steam-bath for 24 hours. By the end of this time the reaction mixture had almost evaporated to dryness. The residue was stirred with 700 ml. of water and the mixture set aside at 5° overnight. The solid which had separated was collected by filtration, washed with water until the washings were colorless, placed in a Soxhlet cup and extracted with 200 ml. of absolute ethanol for 20 hours. The yellow extract was then treated with charcoal, concentrated to 30 ml. and stored at 5° overnight to yield 0.52 g. (18.2%) of yellow crystals, m.p. *ca.* 250°. Purification by twice dissolving in dilute ammonium hydroxide, treating with charcoal and precipitating with hydrochloric acid gave 0.38 g. of an almost colorless solid which was recrystallized from 95% ethanol to give colorless crystals, m.p. 274–275° (with preliminary softening). Comparison of the infrared spectra of the products prepared by method A and method B showed them to be identical.

2-Phenyl-5-methyl-7-hydroxy-2,1,3-triazolo(4,5-d)pyrimidine (V).—A mixture of 1.85 g. of 2-phenyl-4-amino-2,1,3-triazole-5-carboxamide, 50 ml. of ethyl orthoacetate and 50 ml. of acetic anhydride was heated under reflux for 23.5 hours, the solvents removed by evaporation under reduced pressure and the residual sirup dissolved in 50 ml. of 95% ethanol. The solution was again evaporated under reduced pressure and the residual sirup digested with another 50-ml. portion of 95% ethanol to induce crystallization. The resulting mixture was chilled at 5° overnight and filtered to give 0.74 g. of colorless crystals, m.p. 155°. An additional 0.51 g. of light yellow product (m.p. 137°) was obtained from the filtrate to give a total yield of 1.25 g. (55%). Four recrystallizations from 95% ethanol with the use of charcoal gave small colorless crystals, m.p. 167°. The compound exhibited an ultraviolet absorption maximum in ethanol solution at 301 mμ (log ε 5.05).

Anal. Calcd. for $C_{11}H_9ON_5 \cdot {}^1/_2C_2H_5OH$: C, 57.59; H, 4.83; N, 27.99. Found: C, 57.38; H, 5.19; N, 28.12.

2-Phenyl-4-amino-2,1,3-triazole-5-thiocarboxamide (VI).—A mixture of 7.61 g. of 2-phenyl-4-amino-2,1,3-triazole-5-carboxamide, 12 g. of phosphorus pentasulfide and 150 ml. of anhydrous pyridine was heated under reflux for one hour, cooled and poured into 1500 ml. of water. The resulting mixture was allowed to stand with occasional stirring for several hours and the solid which had separated was collected by filtration and washed with a little cold water. Two recrystallizations from 95% ethanol gave 6.43 g. (78.5%) of orange-yellow crystals, m.p. 185–187°. The material was purified for analysis by an additional six recrystallizations from ethanol followed by sublimation (160° (0.01 mm.)) to give lemon-yellow crystals, m.p. 193°.

Anal. Calcd. for $C_9H_9SN_5$: C, 49.30; H, 4.14; N, 31.94. Found: C, 49.63; H, 4.09; N, 32.00.

2-Phenyl-7-mercapto-2,1,3-triazolo(4,5-d)pyrimidine (VII).—A mixture of 3.55 g. of 2-phenyl-4-amino-2,1,3-triazole-5-thiocarboxide (m.p. 185–187°), 40 ml. of acetic anhydride and 40 ml. of ethyl orthoformate was heated under reflux for 4 hours and then concentrated almost to dryness under reduced pressure. The residue was boiled for a few minutes with 100 ml. of absolute ethanol to destroy residual acetic anhydride and the solution again evaporated to dryness under reduced pressure. The residue was dissolved in a mixture of 60 ml. of dimethylformamide and 10 ml. of water, which was digested with decolorizing charcoal (steam-bath) and filtered. Dilution with water of the yellow filtrate followed by long chilling caused the separation of a yellow solid which was collected by filtration, washed with water and dried over phosphorus pentoxide to give 2.38 g. (64%) of 2-phenyl-7-mercapto-2,1,3-triazolo(4,5-d)pyrimidine, m.p. 323°. The product was obtained in the form of beautiful yellow crystals by further recrystallization from aqueous dimethylformamide followed by sublimation (250° (0.01 mm.)), but the melting point remained unchanged. The compound exhibited ultraviolet absorption maxima in absolute ethanol at 255, 268, 366 and 374 mμ (log ε 5.03, 5.03, 5.11 and 5.13).

Anal. Calcd. for $C_{10}H_7SN_5$: C, 52.39; H, 3.08; N, 30.55. Found: C, 52.53; H, 3.25; N, 30.71.

4,6-Diamino-5-phenylazopyrimidine (IX).—A solution of 7.25 g. of phenylazomalondiamidine dihydrochloride in 100 ml. of formamide was heated in an oil-bath at 150° for 1 hour. The cooled reaction mixture was poured into 500 ml. of ice-water and, after 2 hours standing, the resulting suspension was filtered and the collected yellow solid washed well with water. After drying over phosphorus pentoxide, the crude 4,6-diamino-5-phenylazopyrimidine weighed 3.95 g. (70.5%), m.p. 295°. Recrystallization from aqueous dimethylformamide gave well-formed yellow crystals, m.p. 302°.

Anal. Calcd. for $C_{10}H_{10}N_6$: C, 56.06; H, 4.71; N, 39.23. Found: C, 55.76; H, 4.68; N, 39.29.

2-Phenyl-7-amino-2,1,3-triazolo(4,5-d)pyrimidine (X). Method A.—A mixture of 2.61 g. of 4,6-diamino-5-phenylazopyrimidine, 9 g. of copper sulfate pentahydrate, 30 ml. of water and 60 ml. of pyridine was heated in an oil-bath under reflux for 3 hours with continuous stirring, and was then poured into 500 ml. of ice-water. After 1 hour, the yellow precipitate was collected by filtration, washed with 50 ml. of water and recrystallized from a mixture of 200 ml. of dimethylformamide and 30 ml. of water. The light yellow product, after drying over phosphorus pentoxide, weighed 1.26 g. (48.7%), m.p. 338°. The product was obtained as a pale yellow crystalline solid upon repeated recrystallization from aqueous dimethylformamide, m.p. 340°. The compound exhibited ultraviolet absorption maxima in absolute ethanol solution at 286 and 320 mμ (log ε 5.11 and 5.12).

Anal. Calcd. for $C_{10}H_8N_6$: C, 56.59; H, 3.80; N, 39.61. Found: C, 56.74; H, 3.68; N, 39.83.

Method B.—A mixture of 300 mg. of 2-phenyl-7-mercapto-2,1,3-triazolo(4,5-d)pyrimidine (VII) and 30 ml. of absolute ethanol saturated with dry ammonia was sealed in a glass tube and heated for 6 hours at 170°. The reaction mixture was evaporated to dryness and twice sublimed at 250° (0.05 mm.) to give pure 2-phenyl-7-amino-2,1,3-triazolo-(4,5-d)pyrimidine, m.p. 338°. It was shown to be identical with an authentic sample as prepared by method A above by comparison of ultraviolet absorption spectra and by a mixed melting point determination.

2-Phenyl-4-amino-2,1,3-triazole-5-carboxamidine (XI).—To a solution of 4.53 g. of phenylazomalondiamidine (free base)[17] in 100 ml. of warm pyridine was added a solution of 10 g. of copper sulfate pentahydrate in 20 ml. of water. The mixture was warmed gently on a steam-bath for 18 hours and then poured into 1000 ml. of ice-water. After standing at 0° for 2 days, the mixture was filtered and the collected dark-brown solid washed with water until the washings were colorless and then extracted with three 150-ml. portions of boiling absolute ethanol. All the aqueous filtrates above were taken to dryness and the residue extracted with 150 ml. of boiling absolute ethanol. The combined ethanol extracts were treated with charcoal and concentrated to about 30 ml. After the addition of 100 ml. of water and cooling at 0° overnight, light red needles were collected by filtration, washed with water and dried over phosphorus pentoxide; yield 1.05 g. (23.4%), m.p. 145°. The product was recrystallized 4 times from aqueous ethanol with the addition of charcoal and once more from absolute ethanol to give fine colorless needles, m.p. 168°.

Anal. Calcd. for $C_9H_{10}N_6$: C, 53.45; H, 4.98; N, 41.56. Found: C, 53.44; H, 4.70; N, 41.46.

2-Phenyl-7-acetylamino-2,1,3-triazolo(4,5-d)pyrimidine (XII).—A mixture of 0.23 g. of 2-phenyl-7-amino-2,1,3-triazolo(4,5-d)pyrimidine (X), 10 ml. of ethyl orthoformate and 10 ml. of acetic anhydride was heated under reflux for 3 hours. Excess solvent was removed *in vacuo* and the residue was dissolved in 35 ml. of hot absolute ethanol, decolorized with charcoal and chilled overnight. The pale green crystals were collected by filtration and dried over phosphorus pentoxide; yield 0.15 g. (54.4%), m.p. 205°. The product was twice recrystallized from absolute ethanol with the addition of charcoal, but the pale green coloration persisted, m.p. 209°. The compound exhibited ultraviolet absorption maxima in absolute ethanol at 231.5 and 316 mμ (log ε 5.00 and 5.18).

Anal. Calcd. for $C_{12}H_{10}ON_6$: C, 56.68; H, 3.96; N, 33.06. Found: C, 56.74; H, 3.89; N, 33.33.

4-Formylamino-6-aminopyrimidine.—A mixture of 21.03 g. of malondiamidine dihydrochloride and 50 ml. of formamide was heated at 170–175° in an oil-bath for 2.5 hours, then cooled at 0–5° for 1 hour and filtered. The solid so obtained was washed with absolute ethanol and dried over phosphorus pentoxide in a vacuum desiccator for 50 hours to give 12.18 g. of a light yellow product, m.p. *ca.* 200° dec. An additional

0.10 g. was obtained by concentration of the mother liquor. The crude product was digested at 50° with 100 ml. of water and then filtered, washed with absolute ethanol and again dried over phosphorus pentoxide to give 4.47 g. (28.2%), m.p. 265° dec. Repeated recrystallization from absolute ethanol with the use of charcoal yielded colorless crystals, m.p. 285° dec.

Anal. Calcd. for $C_5H_6ON_4$: C, 43.47; H, 4.38; N, 40.56. Found: C, 43.32; H, 4.69; N, 40.74.

The compound exhibited an absorption maximum in the ultraviolet (in 0.1 *N* hydrochloric acid) at 265 mμ. After boiling for two hours in 0.1 *N* hydrochloric acid, hydrolysis of the formyl group was complete, and the resulting solution exhibited an absorption maximum at 263.5 mμ, identical with the maximum exhibited by an authentic sample of 4,6-diaminopyrimidine.

Phenylazomalondiamide.—A solution of 18.6 g. of aniline in 120 ml. of 6 *N* hydrochloric acid was diazotized at 5° by the addition of a solution of 13.8 g. of sodium nitrite in 50 ml. of water. After five minutes, the benzenediazonium chloride solution was poured with vigorous stirring into a mixture of 20.42 g. of malondiamide in 200 ml. of water in an ice–salt-bath. With continued stirring, 95 ml. of 5 *N* ammonium hydroxide was added slowly. The reaction mixture, which was then at *p*H 6, was maintained at *p*H 5–6 by the addition of a sodium acetate solution. Stirring was continued for an additional 30 minutes (total stirring time was 45 minutes). The mixture was then removed from the ice-bath and set aside at room temperature for 2.5 hours, and then stored in the refrigerator overnight. The solid product was collected by filtration, washed with a little cold water and dried over phosphorus pentoxide to give 24.7 g. An additional 4.7 g. of product was obtained from the filtrate to give a total yield of 29.4 g. (71.3%), m.p. 220° dec., with preliminary softening. The crude light-brown product was recrystallized from 95% ethanol with the use of charcoal to give soft, yellow-orange needles, m.p. 241° dec. The compound can better be recrystallized from aqueous dimethylformamide or aqueous formamide as bright yellow crystals.

Anal. Calcd. for $C_9H_{10}O_2N_4$: C, 52.42; H, 4.89; N, 27.17. Found: C, 52.41; H, 4.83; N, 26.94.

***sym*-N,N′-Bis-(ethoxymethylene)-malondiamidine Monohydrochloride (XIII).**—A mixture of 5.80 g. of malondiamidine dihydrochloride, 30 ml. of ethyl orthoformate and 20 ml. of acetic anhydride was heated under reflux for five hours and the bulk of the solvents was removed by distillation under reduced pressure. The residue was twice digested with 50 ml. of 95% ethanol for a few minutes and the solution was evaporated to dryness. The resulting residue was digested with 50 ml. of boiling 95% ethanol and the mixture stored overnight in an ice-bath to give 4.65 g. (55.8%) of almost colorless crystals, m.p. 215° dec. Three recrystallizations from water with the addition of charcoal gave colorless crystals, m.p. 281° dec., with preliminary darkening above 250°.

Anal. Calcd. for $C_9H_{17}O_2N_4Cl$: C, 43.46; H, 6.89; N, 22.53. Found: C, 43.83; H, 6.73; N, 22.41.

Conversion of XIII to 4-Formylamino-6-aminopyrimidine.—A mixture of 8.65 g. of XIII and 30 ml. of formamide was placed in an oil-bath preheated to 175–180° and the reaction was allowed to proceed at this temperature for 3.5 hours. The mixture was then cooled, diluted with 100 ml. of absolute ethanol and chilled for one hour. The precipitated solid was collected by filtration, washed with absolute ethanol and dried over phosphorus pentoxide, yield 1.3 g. of a brownish-yellow product, m.p. *ca.* 245° dec. The mother liquor yielded an additional 1.18 g. of pale yellow product, m.p. 248° dec., while the second mother liquor yielded 0.41 g. of colorless product, m.p. 266° dec.; total yield 2.89 g. (60.2%). The infrared spectrum of this compound was identical with that given by authentic 4-formylamino-6-aminopyrimidine.

PRINCETON, NEW JERSEY

[CONTRIBUTION FROM THE NUTRITION AND PHYSIOLOGY SECTION, RESEARCH DIVISION, AMERICAN CYANAMID CO., LEDERLE LABORATORIES]

Syntheses of Some Substituted Indole-3-acetic Acids[1]

BY MILON W. BULLOCK AND JOHN J. HAND

RECEIVED JUNE 20, 1956

A series of ethyl 2-methylindole-3-acetates having substituents in the benzene ring have been prepared by a Fischer indole ring closure of the corresponding ethyl levulinate substituted phenylhydrazones. Methods of effecting the ring closure have been compared. The substituted indole-3-acetic acids were prepared by saponification of the esters.

The biological activities of 2-methylindole-3-acetic acid and a few substituted compounds have been compared.[2,3] The activities of the substituted compounds were superior to 2-methylindole-3-acetic acid for certain phytological applications. We have prepared a number of new 2-methylindole-3-acetic acids for testing.

The substituted ethyl levulinate phenylhydrazones which were required as intermediates were prepared by the method of Stevens and Higginbotham.[4] The products were all unstable in air although they could be stored for long periods of time in nitrogen. The yields, melting points and elemental analyses of these intermediates are summarized in Table I.

The ethyl 2-methylindole-3-acetates were prepared by a Fischer indole cyclization of the ethyl levulinate phenylhydrazones. The cyclization could be effected by refluxing the phenylhydrazone with a solution of sulfuric acid in ethanol, fusing with zinc chloride, and by refluxing a mixture of the phenylhydrazone and zinc chloride in xylene. Ethanolic sulfuric acid has been found to be an excellent cyclization catalyst for the preparation of ethyl 2-methylindole-3-acetate from ethyl levulinate phenylhydrazone[5]; however, when this method was employed for the cyclization of the substituted phenylhydrazone, the indole was generally contaminated with unreacted phenylhydrazone. Fusion with anhydrous zinc chloride as described by Stevens and Higginbotham[4] was effective but the procedure was found to be somewhat laborious. Refluxing a mixture of the substituted phenylhydrazone and zinc chloride in xylene has been found to be both effective and convenient. The 2-methylindole-3-acetates prepared are summarized in Table II. The letters refer to the general procedure detailed in the experimental section.

(1) Presented in part at the 129th Meeting of the American Chemical Society, Dallas, Texas, April 9, 1956.

(2) O. L. Hoffman, S. W. Fox and M. W. Bullock, *J. Biol. Chem.*, **196**, 437 (1952).

(3) F. J. Stevens and S. W. Fox, THIS JOURNAL, **70**, 2263 (1948).

(4) F. J. Stevens and D. H. Higginbotham, *ibid.*, **76**, 2206 (1954).

(5) M. W. Bullock and S. W. Fox, *ibid.*, **73**, 5155 (1951).