was purified by chromatography on silica gel, using a 19:1 mixture of ethyl acetate–methanol as eluent. Kugelrohr distillation yielded the product, **11**, as a colorless oil, $n^{20}D$ 1.4691 (lit.[93] for racemic material $n^{20}D$ 1.4695), $[\alpha]^{27}D$ +42° (*c* 0.91, isooctane)). Absorption spectral and o.r.d. characteristics are collected in Table IV.

Anal. Calcd. for $C_5H_{12}OS$: C, 49.95; H, 10.06; S, 26.67. Found: C, 49.80; H, 10.27; S, 26.05.

(93) L. Bateman, J. I. Cunneen, and J. Ford, *J. Chem. Soc.*, 3056 (1956).

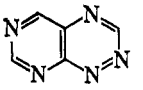
A Synthesis of Pyrimido[4,5-*e*]-*as*-triazines (6-Azapteridines)[1a,b]

Edward C. Taylor and Robert W. Morrison, Jr.[2]

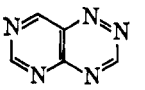
Contribution from the Frick Chemical Laboratory, Princeton University, Princeton, New Jersey. *Received October 7, 1964*

Condensation of dibromomalononitrile (as its potassium bromide complex) with aminoguanidine bicarbonate has been shown to give 3,5-diamino-6-aminocarbonyl-as-triazine (**14**), *which has been cyclized to derivatives of pyrimido[4,5-e]-as-triazine* (**2**) *by the use of appropriate one-carbon reagents. The structure of the cyclization product with diethyl carbonate, 3-amino-6,8-dioxo-5,6,7,8-tetrahydropyrimido[4,5-e]-as-triazine* (**15**), *was confirmed by an independent synthesis from alloxan and aminoguanidine to give alloxan β-guanyl hydrazone* (**16**), *followed by careful cyclization with dilute ammonium hydroxide. Attempts to prepare a derivative of the pyrimido[4,5-e]-as-triazine system by hydrogen sulfide reduction of 2-phenyl-4,6-diamino-5-phenylazopyrimidine* (**3**) *to the hydrazo stage, followed by cyclization with triethyl orthoformate, or by hydrogen sulfide reduction of the formyl derivative of* **3**, *led only to 2-phenyladenine* (**7**).

The antibiotics Toxoflavin and Fervenulin, derivatives of the pyrimido[5,4-*e*]-*as*-triazine system (**1**), have aroused considerable recent interest[3] because of their close structural similarity to the physiologically active pteridines and purines and because of the extreme toxicity of the former compound, apparently due to its participation in the formation of hydrogen peroxide.[4] The structural simplicity of these antibiotics raised the possibility that the high degree of physiological activity observed might be structurally nonspecific. The present paper describes the preparation and properties of derivatives of the isomeric pyrimido[4,5-*e*]-*as*-triazine system (**2**), which at the time this work was begun was unknown.[5]



pyrimido[5,4-*e*]-*as*-triazine
1



pyrimido[4,5-*e*]-*as*-triazine
2

H. M. Taylor, in an unpublished Ph.D. thesis,[6] described a number of unsuccessful attempts to prepare derivatives of this system by condensation of alloxan and 1,3-dimethylalloxan with semicarbazide, thiosemicarbazide, and aminoguanidine. He encountered considerable difficulty in the final cyclization reactions designed to lead to the bicyclic system because of extreme base lability of the pyrimidine ring. An alternative approach commencing with an *as*-triazine precursor likewise failed. Recently, and concurrently with the present work, Heinisch, Ozegowski, and Mühlstadt[7] reported the successful condensation of alloxan and 1-methylalloxan with S-alkylisothiosemicarbazides to give intermediates which subsequently cyclized on heating in pyridine to 3-S-alkyl derivatives of the desired pyrimido[4,5-*e*]-*as*-triazine system.

We first examined the possibility that derivatives of pyrimido[4,5-*e*]-*as*-triazine might be accessible from 4-amino-5-phenylazopyrimidine precursors (*i.e.*, **3**). Reduction of the phenylazo grouping under appropriately mild conditions might allow the hydrazo intermediate reduction stage (**4**) to be trapped by a suitable one-carbon reagent, thus leading either to a triazinopyrimidine (**5**) or to a 7-anilinopurine (**6**). The reduction of phenylazo groupings proceeds through the hydrazo stage, but cleavage of the N–N bond inevitably takes place in the presence of strong reducing agents. Isolation of the intermediate hydrazo compounds is possible, however, when hydrogen sulfide is used as the reducing agent.[8] Accordingly, 2-phenyl-4,6-diamino-5-phenylazopyrimidine (**3**) was reduced with hydrogen sulfide in dimethylformamide solution in the presence of triethyl orthoformate. Surprisingly, the product was

(1) (a) This work was supported in part by a research grant (CA-02551) to Princeton University from the National Cancer Institute, National Institutes of Health, Public Health Service. (b) A preliminary report of this work has appeared: E. C. Taylor and R. W. Morrison, Jr., *Angew. Chem.*, **76**, 342 (1964); *Angew. Chem. Intern. Ed. Engl.*, **3**, 312 (1964).

(2) N.S.F. Summer Fellow, 1961; National Institutes of Health Fellow, 1961–1964.

(3) G. D. Daves, R. K. Robins, and C. C. Cheng, *J. Am. Chem. Soc.*, **84**, 1724 (1962), and references cited therein.

(4) H. E. Latuasan and W. Berends, *Biochim. Biophys. Acta*, **52**, 502 (1961).

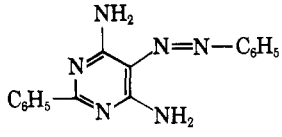
(5) E. Jeney and T. Zsolnai, *Zentr. Bakteriol. Parasitenk. Abt. I Orig.*, **177**, 220 (1960), have stated that 3-amino-6,8-dioxo-5,6,7,8-tetrahydropyrimido[4,5-*e*]-*as*-triazine hydrochloride was tested for antitubercular activity, but no reference was made to the source or properties of the compound nor was its synthesis reported.

(6) H. M. Taylor, Ph.D. Thesis, University of North Carolina (1959); University Microfilms, Inc., Ann Arbor, Mich., library card no. MIC 59-5587.

(7) L. Heinisch, W. Ozegowski, and M. Mühlstadt, *Ber.*, **97**, 5 (1964),

(8) H. Zollinger, "Azo and Diazo Chemistry," Interscience Publishers Inc., New York, N. Y., 1961, p. 300.

neither 2-phenyl-7-anilinoadenine (**6**) nor 2,6-diphenyl-1,2-dihydropyrimido[4,5-*e*]-*as*-triazine (**5**), but was shown to be 2-phenyladenine (**7**) by comparison with an authentic sample. Apparently the N–N bond had undergone reductive cleavage either before cyclization of **4** with triethyl orthoformate or after cyclization to **6** had occurred. In an attempt to avoid the former possibility, the phenylazopyrimidine (**3**) was first formylated with formic–acetic anhydride, and then treated with hydrogen sulfide in dimethylformamide solution. The product again was 2-phenyladenine (**7**).



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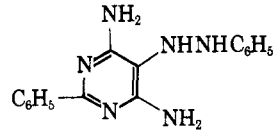

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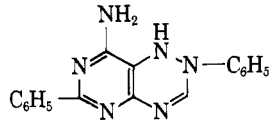

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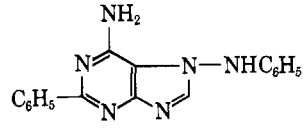

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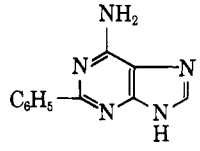
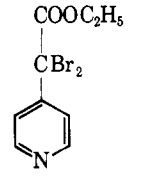


7

We next examined the possibility that the desired pyrimido[4,5-*e*]-*as*-triazine system might be accessible by annulation of a pyrimidine ring to a preformed *as*-triazine. Extensive studies in this laboratory[9–11] have demonstrated the versatility of *o*-aminonitriles as intermediates for the construction of fused pyrimidine systems, and thus our first objective was the preparation of a 5-amino-6-cyano-*as*-triazine. Holland[12] has recently shown that ethyl 4-pyridyldibromoacetate (**8**) reacts with thiosemicarbazide to give the thiosemicarbazone **9** which may be cyclized to the *as*-triazine **10**. An analogous reaction between dibromomalononitrile (available as its potassium bromide complex)[13] and



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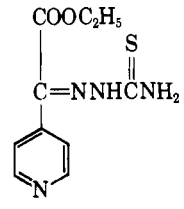

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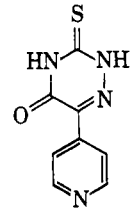
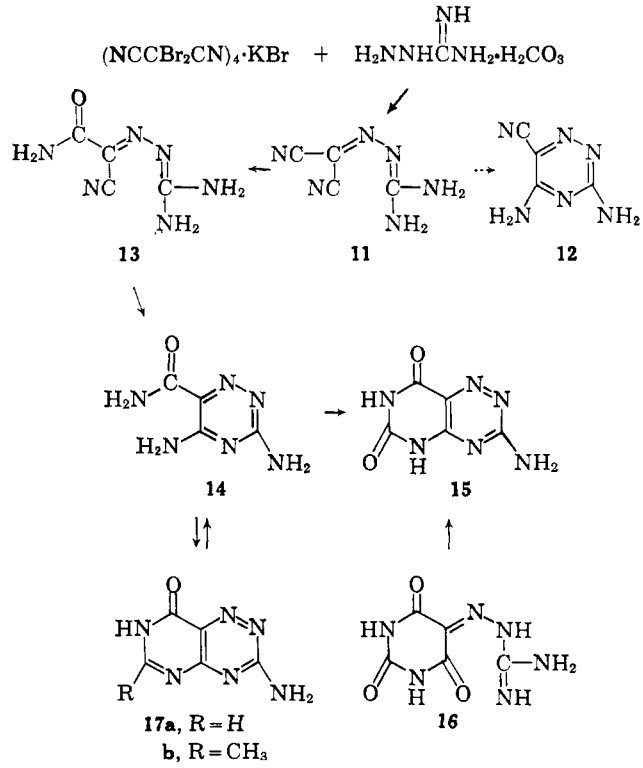


10

aminoguanidine should lead to the desired 3,5-diamino-6-cyano-*as*-triazine precursor **12.**

Accordingly, the dibromomalononitrile–potassium bromide complex was allowed to react with aminoguanidine bicarbonate in ethanol solution at 5–10°, but instead of the expected product **12,** an extremely labile compound was isolated which showed (infrared) the presence of both nitrile (2215 cm.$^{-1}$) and amide (1705 cm.$^{-1}$) groupings. This material could not be purified, for all attempts to dissolve it in warm solvents such as water, dimethylformamide, or 2-ethoxyethanol resulted in a rapid subsequent reaction to give a new compound in quantitative yield. The infrared spectrum of this new material indicated the continued presence of the amide grouping (1695 cm.$^{-1}$) but the absence of the nitrile grouping. Its n.m.r. spectrum showed the absence of C–H protons. Microanalysis indicated the empirical formula $C_4H_6N_6O$, corresponding to the expected aminonitrile **12** plus one molecule of water. The product was thus apparently the *o*-aminocarboxamide **14.** A comparison of infrared spectra showed that **14** was identical with the compound prepared by Taylor by condensation of diethyl oxomalonate with thiosemicarbazide, followed by chlorination with phosphorus oxychloride and then amination.[6] It would appear that the dibromomalononitrile–potassium bromide complex reacts with aminoguanidine to give **11,** carbon dioxide, water, and hydrogen bromide, and that the dinitrile **11** is subsequently hydrolyzed to **13,** the first isolable, if unstable, intermediate. This subsequently undergoes intramolecular cyclization upon warming in solution to **14.**



13 **11** **12**

14 **15**

17a, R = H
b, R = CH_3

16

Confirmation of the structure assigned to **14** was obtained by treatment with diethyl carbonate in ethanolic sodium ethoxide solution to give 3-amino-6,8-dioxo-5,6,7,8-tetrahydropyrimido[4,5-*e*]-*as*-triazine (**15**), which was then prepared independently and unambiguously by the following method. Alloxan was condensed with aminoguanidine bicarbonate in glacial acetic acid solution to give alloxan β-guanyl hydrazone (**16**) which was cyclized with dilute ammonium hydroxide to **15,** identical in every respect with the product obtained from the diethyl carbonate cyclization above. It is interesting to note that Taylor[6] had

(9) E. Richter, J. E. Loeffler, and E. C. Taylor, *J. Am. Chem. Soc.*, **82** 3144 (1960).
(10) E. C. Taylor and P. E. Loeffler, *ibid.*, **82,** 3147 (1960).
(11) E. C. Taylor and R. V. Ravindranathan, *J. Org. Chem.*, **27,** 2622 (1962).
(12) A. Holland, *J. Chem. Soc.*, 3260 (1962).
(13) R. A. Carboni, *Org. Syn.*, **39,** 64 (1959).

likewise prepared **16** with the intention of cyclizing it to **15,** but had observed under his attempted cyclization conditions (aqueous sodium hydroxide or concentrated ammonium hydroxide) only a variety of cleavage products. It should also be pointed out that **15** showed no tendency toward hydration, and thus cannot be assumed to be identical with the compound described as 3-amino-6,8-dioxo-5,6,7,8-tetrahydropyrimido[4,5-*e*]-*as*-triazine prepared by Heinisch, Ozegowski, and Mühlstadt,[7] and isolable only as its hydrate.

Condensation of **14** with formamide in ethanolic sodium ethoxide solution[14] gave 3-amino-8-oxo-7,8-dihydropyrimido[4,5-*e*]-*as*-triazine (**17a**), and an analogous condensation with acetamide or ethyl acetate gave the corresponding 6-methyl derivative (**17b**). Since **17a** was readily prepared in anhydrous form and was yellow, it was clearly not identical with the white product obtained by a different route[6] and claimed to be the hydrate of **17a**.

The hydrolytic instability of 3-amino-8-oxo-7,8-dihydropyrimido[4,5-*e*]-*as*-triazine (**17a**) was remarkable. Boiling for a few seconds in dilute ammonium hydroxide was sufficient to reconvert it to 3,5-diamino-6-aminocarbonyl-*as*-triazine (**14**). The 6-methyl derivative **17b** was considerably more stable, for it survived purification by suspension in boiling water, dissolution with ammonium hydroxide, and reprecipitation with acetic acid. Its increased stability is in agreement with the observations of Albert[15] that the steric bulk of a methyl group can prevent covalent hydration (the initial step in hydrolytic ring cleavage).

Experimental[16]

2-Phenyladenine (**7**). *Method A.* Hydrogen sulfide was bubbled into a refluxing solution of 2.00 g. of 2-phenyl-4,6-diamino-5-phenylazopyrimidine, 25 ml. of dimethylformamide, and 5 ml. of triethyl orthoformate for 6 hr. When the reaction mixture had cooled, 175 ml. of carbon disulfide was added and the mixture was chilled overnight. An amorphous white product was isolated, yield 0.35 g. (24%), m.p. 321–322°. A comparison of its infrared spectrum with that of an authentic sample of 2-phenyladenine[17] showed the two naterials to be identical.

Method B. A mixture of 35.8 g. of 2-phenyl-4,6-diamino-5-phenylazopyrimidine and 200 ml. of formic–acetic anhydride was heated on the steam bath for 15 min. and then allowed to stand at room temperature for 3 hr. Filtration gave 40.0 g. of an orange solid, m.p. 262–263°. A new peak in the infrared spectrum was observed at 1705 cm.$^{-1}$. Then 15.0 g. of this crude material was dissolved in 100 ml. of dimethylformamide and refluxed for 7 hr. while a stream of hydrogen sulfide was bubbled into the refluxing mixture. The solution was cooled, 400 ml. of carbon disulfide was added, and the solution was chilled overnight to give 6.01 g. (62%) of an off-white solid, m.p. 319–321°. Several recrystallizations from pyridine raised the melting point to 324.5–325.5°. A comparison of infrared spectra indicated that this product was 2-phenyladenine.

Initial Reaction between Dibromomalononitrile–Potassium Bromide Complex and Aminoguanidine Bicarcarbonate. Method A. To a 600-ml. beaker containing 300 ml. of ethanol and 5 ml. of water was added 50.7 g. (0.050 mole, 0.200 equiv.) of dibromomalononitrile–potassium bromide complex[13] and the mixture was placed in an ice bath on the magnetic stirrer. When the temperature reached 5–10°, about one-half of 27.2 g. (0.200 mole) of aminoguanidine bicarbonate was added. After effervescence began, the remaining aminoguanidine bicarbonate was added at a rate such that the temperature was maintained at 5–10°. Stirring was continued for 2 hr. and then the mixture was allowed to stir at room temperature for an additional 1.5 hr. The white precipitate was filtered off and the filtrate was evaporated *in vacuo*. The residue was taken up and washed well with acetone. This pale yellow solid was then suspended in 90 ml. of water on the magnetic stirrer and 6 *N* sodium hydroxide was added until the pH was 7. Stirring was continued for about 10 min. The yellow product was collected and washed with water and then with warm acetone until the filtrates were nearly colorless, yield 9.60 g. (31%), m.p. >350°, $\lambda_{max}^{H_2O}$ 238 and 333 mμ. This product showed infrared bands for both nitrile (2215 cm.$^{-1}$) and amide (1705 cm.$^{-1}$) functions. An analytical sample could not be prepared, for the product reacted further whenever it dissolved in hot solvents (water, dimethylformamide, or 2-ethoxyethanol). The reaction in water gave the cleanest product (see below).

Method B. A mixture of 10.14 g. (0.010 mole) of dibromomalononitrile–potassium bromide complex and 60 ml. of absolute ethanol was stirred for 10 min. The potassium bromide was filtered off and 5.44 g. (0.040 mole) of aminoguanidine bicarbonate was added. Stirring was continued for 1 hr. after effervescence began and the mixture was then refluxed for 20 min. The reaction mixture was evaporated *in vacuo* and the residue was washed well with acetone. The solid was suspended in 30 ml. of water in an ice bath, and the pH was adjusted to 7 with 28% ammonium hydroxide. The product was collected and washed with water and acetone, yield 1.56 g. (25%). It was identical in every respect with the product prepared above by method A.

3,5-Diamino-6-aminocarbonyl-as-triazine (**14**). A suspension of 3.27 g. of the initial adduct between dibromomalononitrile–potassium bromide complex and aminoguanidine bicarbonate in 100 ml. of water was refluxed for 6 hr. and the reaction mixture was allowed to cool slowly. Filtration gave 3.26 g. (100%) of fine, off-white needles, m.p. >350°. An analytical sample was prepared by suspending the solid in boiling water, adding 1 *N* hydrochloric acid dropwise to effect solution, and then adding ammonium hydroxide with stirring until the pH was 7. The product was obtained as very fine, off-white needles: m.p. >350°; $\lambda_{max}^{H_2O}$ 213 mμ (ε 30,400), 242 (12,200), and 317 (7400).

Anal. Calcd. for $C_4H_6N_6O$: C, 31.17; H, 3.93;

(14) R. Hull, *J. Chem. Soc.*, 2214 (1951).

(15) A. Albert and G. B. Barlin, *ibid.* 5156 (1963), and references cited therein.

(16) We are indebted for the microanalyses to the Spang Microanalytical Laboratory, Ann Arbor, Mich., and to the Schwarzkopf Microanalytical Laboratory, Woodside, N. Y. All melting points were determined on a Thomas–Hoover silicone oil bath and are uncorrected. The molecular weight determination was carried out by the Crobaugh Microanalytical Laboratories, Charleston, W. Va. All infrared spectra were determined by the Nujol mull technique.

(17) W. Traube and L. Herrman, *Ber.*, **37**, 2267 (1904).

N, 54.52; mol. wt., 154. Found: C, 31.28; H, 3.99; N, 54.59, 54.44; mol. wt., 160 (osmometric in dimethyl sulfoxide).

3-Amino-6,8-dioxo-5,6,7,8-tetrahydropyrimido[4,5-e]-as-triazine (**15**). *Method A.* To a solution of ethanolic sodium ethoxide (prepared from 0.58 g. (0.025 g.-atom) of sodium and 50 ml. of absolute ethanol) was added 0.77 g. (0.0050 mole) of 3,5-diamino-6-aminocarbonyl-*as*-triazine and 2 ml. of diethyl carbonate. The mixture was heated under reflux for 3.5 days and then was filtered. The solid was washed with ethanol and was then taken up in 75 ml. of water and filtered to remove insoluble material. The filtrate was adjusted to pH 5 with hydrochloric acid and the off-white precipitate was collected. Then, the water-insoluble residue above was extracted with aqueous ammonium hydroxide and the extract was acidified. The precipitate was collected and combined with the former acid-insoluble precipitate. The base-insoluble residue consisted of 0.15 g. of unreacted 3,5-diamino-6-aminocarbonyl-*as*-triazine. The combined yield of the acid-insoluble product was 0.30 g. (33%), m.p. >350°. An analytical sample was prepared by suspending the solid in boiling water which had been adjusted to pH 10 with ammonium hydroxide. The pH was maintained at 8–10 while the solid slowly dissolved. The solution was filtered while hot, the pH was adjusted to 6 with glacial acetic acid, and the flask was plunged into an ice–water bath and cooled as rapidly as possible. An ivory solid was obtained which melted above 350°; $\lambda_{max}^{H_2O}$ 215 mμ (ε 32,600), 244 (13,600), and 329 (8300).

Anal. Calcd. for $C_5H_4N_6O_2$: C, 33.34; H, 2.24; N, 46.66. Found: C, 33.06; H, 2.45; N, 46.75.

Method B. A mixture of 8.00 g. (0.050 mole) of alloxan hydrate, 6.85 g. (0.050 mole) of aminoguanidine bicarbonate, and 175 ml. of glacial acetic acid was refluxed with stirring for 1 hr. The mixture was then transferred to a 1-l. round-bottomed flask and was evaporated to dryness *in vacuo*. Seven hundred milliliters of water was added along with the necessary amount of 28% ammonium hydroxide to adjust the pH to 9–10. The mixture was heated to reflux and an orange-yellow precipitate[18] soon formed and then slowly dissolved. Refluxing was continued for 20 hr. The pH was adjusted to 6 with acetic acid and the flask was rapidly cooled to 0°. Filtration gave 4.21 g., m.p. >350°. The filtrate was then concentrated *in vacuo* to a volume of 160 ml. and chilled, whereupon 0.15 g. of additional product was isolated. Further concentration of the filtrate to 35 ml. followed by chilling gave only a trace of the same material.

A suspension of 2.00 g. of the crude product in 200 ml. of boiling water was adjusted to pH 10 with 28% ammonium hydroxide. The pH was maintained at 8–10 while the product slowly dissolved. The solution was filtered while hot, the pH was adjusted to 6 with glacial acetic acid, and then the flask was plunged into an ice–water bath and cooled as rapidly as possible. After chilling, the flask was placed in the refrigerator overnight. The recrystallized material was collected and washed with water, yield 1.39 g. (34% based on starting material), m.p. >350°. This product was identical in every respect with the product prepared above by method A.

3-Amino-8-oxo-7,8-dihydropyrimido[4,5-e]-as-triazine (**17a**). To a solution of ethanolic sodium ethoxide (prepared from 1.16 g. (0.050 g.-atom) of sodium and 100 ml. of ethanol) was added 1.54 g. (0.010 mole) of 3,5-diamino-6-aminocarbonyl-*as*-triazine and 4 ml. of formamide. The mixture was stirred under reflux for 3 hr. and then filtered while hot. After washing with ethanol, the yellow precipitate was dissolved in ice water at 0–5°, filtered, and acidified with acetic acid. The product was collected while maintaining as low a temperature as possible by addition of crushed ice, yield 1.44 g. (88%), m.p. >350°. An analytical sample was prepared by vacuum sublimation: m.p. >350°; λ_{max}^{EtOH} 219 mμ (ε 14,100), 246 (21,800), and 336 (6000).

Anal. Calcd. for $C_5H_4N_6O$: C, 36.59; H, 2.46; N, 51.21. Found: C, 36.68; H, 2.50; N, 50.92.

This product was extremely labile to hydrolysis; boiling a few seconds in dilute ammonium hydroxide reconverted it to 3,5-diamino-6-aminocarbonyl-*as*-triazine.

3-Amino-6-methyl-8-oxo-7,8-dihydropyrimido[4,5-e]-as-triazine (**17b**). *Method A.* To a solution of ethanolic sodium ethoxide (prepared from 1.16 g. (0.050 g.-atom) of sodium and 100 ml. of absolute ethanol) was added 1.54 g. (0.010 mole) of 3,5-diamino-6-aminocarbonyl-*as*-triazine and 8 ml. of ethyl acetate. The mixture was stirred under reflux for 24 hr. and was then allowed to cool. The gelatinous precipitate was collected by centrifugation, washed with ethanol, dissolved in water, and filtered, and the filtrate was acidified with acetic acid. The pale yellow product was collected and washed with water and acetone to give 1.40 g. (79%), m.p. >350°. An analytical sample was prepared by suspension in boiling water, addition of 28% ammonium hydroxide to effect solution, and then acidification with acetic acid. The hot mixture was then cooled rapidly to 0° and the product was collected: m.p. >350°; $\lambda_{max}^{H_2O}$ 207 mμ (ε 17,100), 242 (21,200), and 343 (6830).

Anal. Calcd. for $C_6H_6N_6O$: C, 40.44; H, 3.40; N, 47.17. Found: C, 40.72; H, 3.40; N, 47.01.

Method B. To a solution of ethanolic sodium ethoxide (prepared from 0.58 g. (0.025 g.-atom) of sodium and 50 ml. of absolute ethanol) was added 0.77 g. (0.0050 mole) of 3,5-diamino-6-aminocarbonyl-*as*-triazine and 0.85 g. (0.019 mole) of acetamide. The mixture was refluxed for 3 days, allowed to stand at room temperature for 24 hr., and filtered. The collected solid was taken up in 60 ml. of cold water, a trace of unreacted 3,5-diamino-6-aminocarbonyl-*as*-triazine was filtered off, and the pH of the filtrate was adjusted to 5 with 1 *N* hydrochloric acid. The yellow product was collected and washed with water, yield 0.55 g. (62%), m.p. >350°. This compound was shown by comparison of infrared and ultraviolet spectra to be identical with the product obtained by method A.

(18) A small amount of this orange-yellow precipitate was prepared for analysis as the picrate salt which was recrystallized from methanol, m.p. 257.5–258.5° dec. (decomposed without melting under slow heating); λ_{max}^{EtOH} 235 mμ (ε 18,800), 298 (18,000), and 357 (22,000). *Anal.* Calcd. for $C_{11}H_9N_9O_{10}$: C, 30.92; H, 2.13; N, 29.50. Found: C, 30.66; H, 2.45; N, 29.48.