initial formation of a protein–zinc–glycine complex. Only at higher concentrations of glycine does this complex dissociate to form the stable zinc diglycinate and soluble protein.

Tartrate has been found to decrease the solubility of γ-globulin in the absence of heavy metals. In the presence of zinc this effect was largely counteracted by the chelating properties of the anion. The effect of ionic strength on the solubility of a zinc–γ-globulin complex was very small at *p*H 7.4. In contrast, at *p*H values below 7, where the protein carries a positive net charge, addition of sodium chloride strongly decreased the solubility of a zinc–γ-globulin complex even in the presence of glycine.[7]

On the basis of the immunological assays performed the antibody composition of extracted γ-globulins differed from the composition of the original γ-globulin. In the case of antistreptolysin-O and influenza A protective antibody these differences did not exceed 60%. Diphtheria antitoxin was concentrated by a factor of 2 in one of the residues. The lowest specific activity was found in the supernatant of the precipitate formed in the presence of 5 m*M* zinc. A fairly high activity in fraction 1 (52% higher than in the starting material) may be related to the existence of two different fractions in diphtheria antitoxin.[18]

(18) W. J. Kuhns, *J. Exptl. Med.*, **99**, 577 (1954).

The diphtheria antitoxin activity of some fractions[12,13] was determined by injection into one rabbit only instead of two rabbits. The experimental error should therefore be higher than ±10%. However even allowing for an experimental error of 15–30% the extreme values of specific activities of the antibodies tested indicated a small but consistent fractionation.

The discrepancy between the activity of the starting material and the sum of the subfractions in the case of virus HAI antibodies might be explained on the following basis: The purification procedure might uncover combining sites on the antibody molecule which in the starting material were unavailable due to complex formation with other antibodies. There is no reason, however, why this effect should only occur in the case of the tested virus antibodies. It is more likely to be an artifact related to the presence of traces of zinc.

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BOSTON, MASS.

[CONTRIBUTION FROM THE LILLY RESEARCH LABORATORIES]

Synthesis of Cytosines

BY CALVERT W. WHITEHEAD AND JOHN J. TRAVERSO

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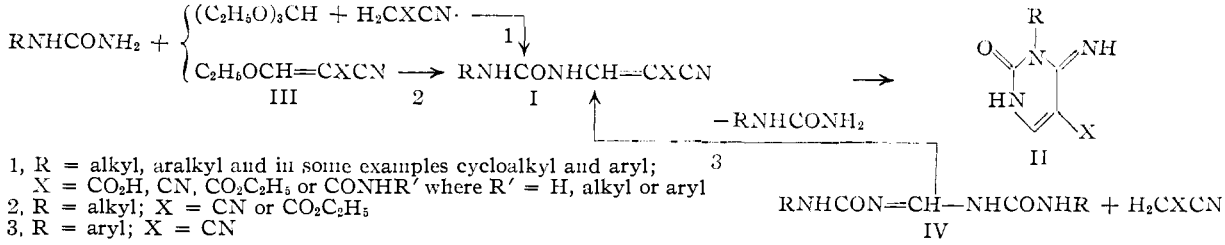
Cyclization of substituted β-ureidoacrylonitriles yielded 5-carboxy-, 5-carbethoxy-, 5-cyano- and 5-carbamylcytosines. The facility in which cyclization occurred was dependent upon the nature of substituent groups on the β-ureidoacrylonitriles. Acid hydrolysis converted 5-cyanocytosines to 5-cyanouracils and 5-carbethoxycytosines to 5-carboxycytosines. Decarboxylation of 5-carboxycytosines yielded homologs of cytosine.

Current interest in the pharmacological properties of pyrimidines motivated an investigation, in this Laboratory, of possible new methods for their synthesis. This present paper contains a description of methods that were employed in the synthesis of 5-cyano-, 5-carboxy-, 5-carbethoxy- and 5-carbamylcytosines (II, X = CN, CO_2H, $CO_2C_2H_5$ and $CONH_2$), and of their conversion to other new pyrimidines.

Synthesis of the cytosines reported here involved cyclization of an intermediate β-ureidoacrylonitrile. Ureidoacrylonitriles (I) of the type RNHCONHCH=CXCN (R = alkyl, aralkyl, cycloalkyl or aryl and X = CO_2H, $CO_2C_2H_5$, $CONH_2$ and CN) were prepared by methods previously described.[1,2]

Cyclization of the ureidoacrylonitriles (I) was effected by heating the open chain intermediate or by treating it with a basic catalyst. It was found that under these conditions ring closure occurred more readily with some derivatives than with others. When R was phenyl or substituted phenyl and X was CN, cyclization occurred readily either by heating the ureidoacrylonitrile or by treating it with sodium methylate. Alkylureidoacrylonitriles (I, R = alkyl) did not cyclize when heated and were, therefore, treated with the base in alcoholic solution. Here the ease in which the alkylureidoacrylonitrile cyclized was apparently dependent upon the nature of both groups R and X. The ring closure could not be effected when R was an alkyl group branched at the α-carbon and X was a carbethoxy group, as in the examples of β-(3-cyclohexylureido)-α-carbethoxyacrylonitrile (I, R = cyclohexyl, X = $CO_2C_2H_5$) and β-(3-isopropylureido)-α-carbethoxyacrylonitrile (I, R = isopropyl, X = $CO_2C_2H_5$). Ring closure did occur when X was a cyano group and R was an α-branched alkyl group. Thus, β-(3-cyclohexylureido)-α-cyanoacrylonitrile, β-(3-isopropylureido)-α-cyanoacrylonitrile and β-(3-*t*-butylureido)-α-cyanoacrylonitrile all yielded the corresponding cytosines. The ease with which cyclization occurred was apparently determined mostly by the nature of the particular

(1) C. W. Whitehead, THIS JOURNAL, **75**, 671 (1953).
(2) C. W. Whitehead, *ibid.*, **74**, 4267 (1952).



1, R = alkyl, aralkyl and in some examples cycloalkyl and aryl; X = CO_2H, CN, $CO_2C_2H_5$ or CONHR′ where R′ = H, alkyl or aryl
2, R = alkyl; X = CN or $CO_2C_2H_5$
3, R = aryl; X = CN

group X when R was not branched at the α-carbon. When the ureidoacrylonitriles having R groups not branched on the α-carbon are listed beginning with the most readily cyclized derivative followed by a less readily cyclized derivative, the groups represented by X fall in the approximate order, CN, $CO_2C_2H_5$, $CONH_2$, CO_2H, CONH-alkyl and CONH-aryl.

Since the conversion of I to II occurred without a change in their empirical formulas, changes in physical properties, melting points, solubilities and infrared absorption were the criteria for determining whether the cytosine (II) had been formed. In general the cytosines were higher melting and less soluble in organic solvents than the intermediate ureidoacrylonitriles. A comparison was made of the infrared absorption spectra of the ureidoacrylonitriles with the spectra of the cytosines. This showed the infrared spectra of the cyclized products were similar and consistent with the spectra of known cytosines and different from the spectra of the open chain ureidoacrylonitriles.

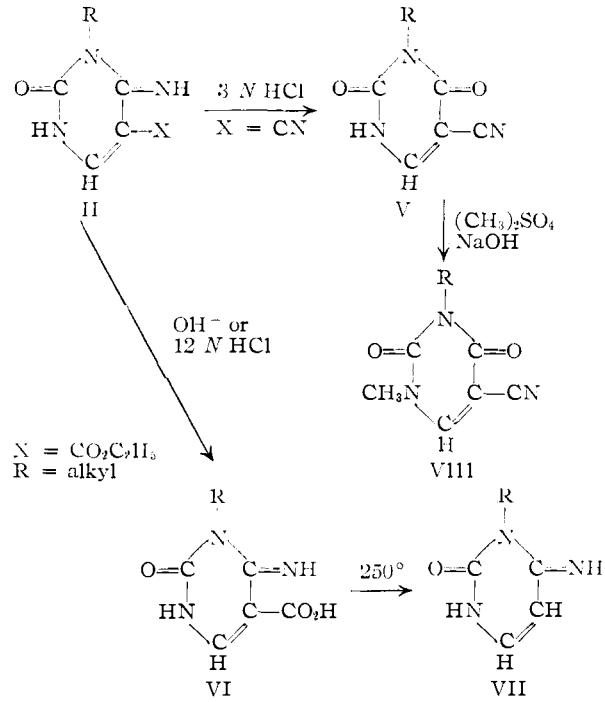
The 5-cyanocytosines (II, X = CN) were readily hydrolyzed with refluxing 3 *N* hydrochloric acid to yield 5-cyanouracils (V). In contrast to this 5-carbethoxycytosines (II, X = $CO_2C_2H_5$) remained unchanged when treated with 3 *N* hydrochloric acid under identical conditions. Furthermore, the 5-carbethoxycytosines were not hydrolyzed to the expected 5-carboxy- or 5-carbethoxyuracils by refluxing 12 *N* hydrochloric acid. In this case the 12 *N* acid hydrolyzed the ester groups to yield 5-carboxycytosines (VI). The difference in behavior of the 4-imino group of the 5-carbethoxycytosines and the 4-imino group of the 5-cyanocytosines toward acid hydrolysis was obviously due to a dissimilarity in the nature or effect of the carbethoxy and cyano groups. Since cyano and carbethoxy groups both have similar electromeric effects, other factors such as steric hindrance or hydrogen bonding may be responsible for the difference in response toward hydrolysis. Alkaline hydrolysis of 5-carbethoxycytosines also yielded 5-carboxycytosines.

Carboxycytosines (VI) were decarboxylated to yield homologs of cytosine (VII). Alkyl 5-cyanouracils were methylated with dimethyl sulfate to obtain 3-alkyl-1-methyl-5-cyanouracils (VIII).

The reactions of triethyl orthoformate, active methylene compounds and N-alkyl- or N-cycloalkylureas (eq. 1) yielded the expected β-(3-alkylureido)-acrylonitriles upon standing or after being refluxed for several hours. When N-arylureas were treated in this manner the products were not β-(3-arylureido)-acrylonitriles but N-arylurethans.[3]

(3) The reaction of arylureas with orthoesters to yield carbamates is presented in another paper.

When the N-arylureas were treated with triethyl orthoformate and malononitrile in excess acetic anhydride under mild conditions, β-(3-arylureido)-α-cyanoacrylonitriles were obtained. Under more vigorous conditions cyclization occurred and 3-aryl-5-cyanocytosines were obtained rather than the intermediate arylureidoacrylonitriles. This was also the case when malononitrile was added to 1,3-bis-(phenylcarbamyl)-formamidine (IV), the intermediate β-(3-phenylureido)-α-cyanoacrylonitrile cyclized in the refluxing solvent to yield 3-phenyl-5-cyanocytosine. In the reactions of ethyl ethoxymethylenecyanoacetate (III, X = $CO_2C_2H_5$) and ethoxymethylenemalononitrile (III, X = CN) with N-alkylureas to yield the β-(3-alkylureido)-acrylonitriles, it was expedient to avoid isolation of the ureidoacrylonitriles. A basic catalyst was added to the reaction mixture to cyclize the intermediate and the cytosines were obtained. The intermediate I was isolated in those cases where cyclization could not take place. When ethyl ethoxymethylenecyanoacetate was heated with 1,3-dimethylurea, 1,3-dimethyl-5-carbethoxycytosine resulted and the ureidoacrylonitrile was not isolated.



Acknowledgment.—The authors thank W. L. Brown, W. Schenck, H. L. Hunter and G. M. Maciak for the microanalyses reported here and Harold E. Boaz for the infrared spectra.

Experimental

Amides of Cyanoacetic Acid (Table I).—Three moles (339.3 g.) of ethyl cyanoacetate were added to 3 moles of the appropriate amine and heated at 110° in an open flask. After 24 hours the evolution of ethanol was complete. The flask was cooled and the amide crystallized. The solid was dissolved in hot ethyl acetate, clarified with carbon and filtered. The filtrate was cooled and the product allowed to crystallize. Analytical samples were prepared by recrystallization from ethyl acetate.

TABLE I

$NCCH_2COR$

R	Yield, %	M.p., °C.	Formula	Carbon, % Calcd.	Carbon, % Found	Hydrogen, % Calcd.	Hydrogen, % Found
C_4H_8ON[a]	55	87	$C_7H_{10}N_2O_2$	54.50	54.66	6.54	6.64
$C_5H_{10}N$[b]	42	87	$C_8H_{12}N_2O$	63.15	63.08	7.96	7.79
n-$C_5H_{11}NH$	59	53	$C_8H_{14}N_2O$	62.30	62.60	9.16	9.34
$C_6H_{12}N$[c]	53	132	$C_9H_{14}N_2O$	65.10	64.79	8.50	8.65
C_7H_8ON[d]	65	130[e]	$C_{10}H_{10}N_2O_2$	63.30	63.06	5.30	5.53

[a] Morpholino. [b] Piperidino. [c] Cyclohexylamino. [d] *p*-Anisidino. [e] The reported melting point is 138°: F. B. Dains and E. L. Griffin, THIS JOURNAL, **35**, 969 (1913).

β-Ureidoacrylic Acids (Table II).—One hundred and fifty milliliters of triethyl orthoformate was added to a mixture of 0.3 mole of the appropriate urea and 31.2 g. (0.3 mole) of malonic acid or 25.5 g. (0.3 mole) of cyanoacetic acid. The mixture was stirred at room temperature for 24 hours. The excess orthoester and alcohol were removed from the solid product by filtration or by evaporation at 15 mm. pressure. The β-ureidoacrylic acid was then purified by recrystallization from methanol or by dissolving it in sodium bicarbonate solution and reprecipitating with dilute hydrochloric acid.

β-Ureido-α-carbethoxyacrylonitriles (Table III).—Mixtures of the N-alkylurea, ethyl cyanoacetate and triethylorthoformate in molar ratios of 1:1:2 ranging in quantities of 0.2 to 1.0 mole were refluxed for 12 hours. The excess orthoester and the ethanol formed during the reaction were removed on the steam-bath under reduced pressure (15 mm.). The solid product was dissolved in a minimum quantity of ethyl acetate, clarified with carbon and the solution filtered. The filtrate was cooled or diluted with petroleum ether and then cooled to yield the crystalline β-ureido-α-carbethoxyacrylonitriles.

β-Ureido-α-cyanoacrylonitriles (Table III).—Mixtures of malononitrile, the appropriate N-alkylurea and triethyl orthoformate in molar ratios of 1:1:3 ranging in quantities of 0.2 to 1.0 mole were heated at 70–100° with stirring for 1–3 hours. This excess orthoester and the ethanol were removed at 15 mm. pressure by heating on the steam-bath. The solid product was recrystallized from ethanol, a mixture of ethanol and water or from ethyl acetate. Decolorization with carbon was usually necesssary to obtain white crystalline products.

β-Ureido-α-carbamylacrylonitriles (Table III).—A mixture of cyanoacetamide, the N-alkylurea and triethyl orthoformate in molar ratios of 1:1:4 ranging in quantities of 0.2 to 1.0 mole was refluxed until the solid reactant had dissolved and the solid product had separated. This usually required 3 to 12 hours. The mixture was cooled and the solid collected. It was dissolved in a hot mixture of 90% ethanol and 10% dimethylformamide. Cold water was added to the hot solution to incipient turbidity and the product was allowed to crystallize.

β-Ureido-α-(N-substituted carbamyl)-acrylonitriles (Table III).—A mixture of triethyl orthoformate, the N-alkylurea and the N-alkylcyanoacetamide or the N-arylcyanoacetamide in molar ratios of 3:1:1 was refluxed for 2–5 hours. The solution was concentrated at 15 mm. on the steam-bath and the residue allowed to stand. After several days the product solidified and was recrystallized from a mixture of ethyl acetate and petroleum ether or from ethyl acetate alone.

5-Carbethoxycytosines (Table IV).—The following two examples illustrate the methods employed for the synthesis of carbethoxycytosines.

Method A. 3-*n*-Hexyl-5-carbethoxycytosine.—Eleven and five-tenths grams of sodium metal was added to 500 ml. of absolute ethanol. To this was added 113 g. (0.5 mole) of ethyl *n*-hexylureidomethylenecyanoacetate and the solution was refluxed for 12 hours. The alcohol was removed on the steam-bath under reduced pressure (15 mm.). The solid was dissolved in 500 ml. of ice-water and neutralized with glacial acetic acid. The precipitated 3-*n*-hexyl-5-carbethoxycytosine was recrystallized from ethanol.

Method B. 3-*n*-Propyl-5-carbethoxycytosine.—Four and six-tenths grams (0.2 g. atom) of sodium metal was added to 200 ml. of absolute ethanol. After the sodium had completely reacted, 20.4 g. (0.2 mole) of N-*n*-propylurea and 33.8 g. (0.2 mole) of ethyl ethoxymethylenecyanoacetate[4] were added. The solution was allowed to stand at room temperature for 5 days. It was then concentrated on the steam-bath under reduced pressure (15 mm.). Cold water (50 ml.) was added and the solution neutralized with dilute hydrochloric acid. The 3-*n*-propyl-5-carbethoxycytosine was filtered, dried and recrystallized from alcohol.

1,3-Dimethyl-5-carbethoxycytosine.—Eight and eight-tenths grams (0.1 mole) of 1,3-dimethylurea was mixed with 16.9 g. (0.1 mole) of ethyl ethoxymethylenecyanoacetate and heated in an open flask placed in an oil-bath at 120°. After 24 hours the flask was removed and cooled. The contents were dissolved in a 50% mixture of ethyl alcohol and ethyl acetate, clarified with carbon and filtered. The filtrate was cooled in the refrigerator to yield 11 g. (36%) of 1,3-dimethyl-5-carbethoxycytosine, m.p. 178° dec.

TABLE II

β-UREIDOACRYLIC ACIDS $RCONHCH{=}CXCOOH$

R	X	Formula	M.p., °C. dec.	Yield, %	Carbon, % Calcd.	Carbon, % Found	Hydrogen, % Calcd.	Hydrogen, % Found	Nitrogen, % Calcd.	Nitrogen, % Found
H_2N	COOH	$C_5H_6N_2O_5$	200	93	34.49	34.10	3.47	3.82		
CH_3NH	CN	$C_6H_7N_3O_3$	199	29	42.60	42.40	4.17	4.54		
C_2H_5NH	CN	$C_7H_9N_3O_3$	190	28					22.94	22.29
$(CH_3)_2N$	COOH	$C_7H_{10}N_2O_5$	160–165	98					13.36	13.53
$(CH_3)_2CHNH$	CN	$C_8H_{11}N_3O_3$	189	90	48.72	48.59	5.62	5.85		
$(CH_3)_2CHNH$	COOH	$C_8H_{12}N_2O_5$	197	96					12.96	12.72
n-C_3H_7NH	COOH	$C_8H_{12}N_2O_5$	193	80					12.96	12.54
C_4H_8ON[a]	COOH	$C_9H_{12}N_2O_6$	160–170	75					11.47	11.69
n-C_4H_9NH	CN	$C_9H_{13}N_3O_3$	183	68					19.93	19.77
n-C_4H_9NH	COOH	$C_9H_{14}N_2O_5$	191	38					12.17	12.16
$HOCH_2CH(C_2H_5)NH$	COOH	$C_9H_{14}N_2O_6$	182	63					11.38	11.14
$C_5H_{10}N$[b]	CN	$C_{10}H_{13}N_3O_3$	181	58					18.83	18.49
$C_5H_{10}N$[b]	COOH	$C_{10}H_{14}N_2O_5$	155–160	23					11.57	11.72
$C_6H_{11}NH$	CN	$C_{11}H_{15}N_3O_3$	194	71					17.71	17.60
$C_6H_{11}NH$	COOH	$C_{11}H_{16}N_2O_5$	180	88					10.93	10.89
$C_6H_5CH_2NH$	COOH	$C_{12}N_{12}N_2O_5$	200	95	54.55	54.54	4.58	4.66		

[a] Morpholino. [b] Piperidino.

(4) C. C. Price, N. J. Leonard and H. F. Herbrandson, THIS JOURNAL, **68**, 1252 (1946).

TABLE III

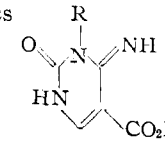
UREIDOACRYLONITRILES RNHCONHCH=CXCN

R	X	Formula	M.p., °C.	Yield, %	Carbon, % Calcd.	Carbon, % Found	Hydrogen, % Calcd.	Hydrogen, % Found
CH_3	$CO_2C_2H_5$	$C_8H_{11}N_3O_3$	156	67	48.72	48.66	5.62	5.66
$H_2C{=}CHCH_2$	$CO_2C_2H_5$	$C_{10}H_{13}N_3O_3$	157	88	53.80	53.76	5.87	5.69
C_6H_{11}	$CO_2C_2H_5$	$C_{13}H_{19}N_3O_3$	152	68	58.86	58.88	7.23	7.49
$C_6H_5CH(CH_3)$	$CO_2C_2H_5$	$C_{15}H_{17}N_3O_3$	132	75	62.70	62.55	5.96	5.92
$HOCH_2CH_2$	CN	$C_7H_8N_4O_2$	150	80			31.12[d]	30.79[d]
$CH_3OCH_2CH_2$	CN	$C_8H_{10}N_4O_2$	158	92	49.48	49.38	5.19	5.22
$C_6H_5CH_2$	CN	$C_{12}H_{10}N_4O$	153	73	63.70	64.51	4.46	5.03
$(CH_3)_2CH$	$CONH_2$	$C_8H_{12}N_4O_2$	212	68	48.97	48.98	6.17	6.16
n-C_3H_7	$CONH_2$	$C_8H_{12}N_4O_2$	234	93	48.97	48.76	6.17	6.38
Iso-C_3H_7	$CONH_2$	$C_8H_{12}N_4O_2$	212		48.97	48.98	6.17	6.16
n-C_4H_9	$CONH_2$	$C_9H_{14}N_4O_2$	235	75	51.42	51.70	6.71	6.78
CH_3	$C_6H_8O_2N$[a]	$C_{10}H_{14}N_4O_2$	194	41	50.42	50.60	5.92	6.15
$(CH_3)_2CH(CH_2)_2$	$CONH_2$	$C_{10}H_{16}N_4O_2$	228	83	53.55	53.77	7.19	7.37
C_6H_{11}	$CONH_2$	$C_{11}H_{16}N_4O_2$	242	95	55.91	56.14	6.83	6.93
CH_3	$C_6H_{10}ON$[b]	$C_{11}H_{16}N_4O_2$	159	41			23.63[d]	23.72[d]
C_2H_5	$C_5H_8O_2N$[a]	$C_{11}H_{16}N_4O_3$	143	39	52.37	52.37	6.39	6.57
$C_6H_5CH_2$	$CONH_2$	$C_{12}H_{12}N_4O_2$	235	89	59.01	58.86	4.95	5.28
n-C_7H_{15}	$CONH_2$	$C_{12}H_{20}N_4O_2$	210	69	57.11	57.01	7.99	7.83
n-C_3H_7	$CONHC_5H_{11}$	$C_{13}H_{22}N_4O_2$	174	34	58.62	58.25	8.33	8.33
$(CH_3)_2CH(CH_2)_2$	$C_5H_8O_2N$[a]	$C_{15}H_{24}N_4O_2$	169	31			19.16[d]	19.21[d]
$(CH_3)_2CH(CH_2)_2$	$CONHC_5H_{11}$	$C_{15}H_{26}N_4O_2$	136	13			19.03[d]	19.15[d]
n-C_7H_{15}	$C_5H_8O_2N$[a]	$C_{16}H_{26}N_4O_3$	122	29			17.38[d]	17.34[d]
$(CH_3)_2CH(CH_2)_2$	$C_8H_8O_2N$[c]	$C_{17}H_{22}N_4O_3$	190	35	61.80	61.74	6.71	6.30
n-C_7H_{15}	$C_8H_8O_2N$[c]	$C_{19}H_{26}N_4O_3$	160	41	63.66	63.77	7.31	7.21

[a] Carbomorpholido. [b] Carbopiperidido. [c] Carboanisidido-*p*. [d] Nitrogen values.

TABLE IV

5-CARBETHOXYCYTOSINES



R	Formula	M.p., °C.	Yield, %[a]	Carbon, % Calcd.	Carbon, % Found	Hydrogen, % Calcd.	Hydrogen, % Found
CH_3	$C_8H_{11}N_3O_3$	230	32B	48.72	48.51	5.62	5.91
C_2H_5	$C_9H_{13}N_3O_3$	201	63[b]	51.17	50.97	6.20	6.43
$HOCH_2CH_2$	$C_9H_{13}N_3O_4$	212	90B	47.57	47.32	5.77	6.01
$H_2C{=}CHCH_2$	$C_{10}H_{13}N_3O_3$	202	65	53.80	53.80	5.87	5.64
n-C_3H_7	$C_{10}H_{15}N_3O_3$	215	42B	53.32	53.10	6.71	6.64
$CH_3OCH_2CH_2$	$C_{10}H_{15}N_3O_4$	178	60	49.78	49.69	6.27	6.18
n-C_4H_9	$C_{11}H_{17}N_3O_3$	192	55	55.21	55.20	7.16	7.26
$(CH_3)_2CH(CH_2)_2$	$C_{12}H_{19}N_3O_3$	199	90	56.90	56.77	7.56	7.50
n-C_6H_{13}	$C_{13}H_{21}N_3O_3$	160	90	58.41	58.88	7.92	8.06
$C_6H_5CH_2$	$C_{14}H_{15}N_3O_3$	182	91	61.53	61.83	5.53	5.56
n-C_7H_{15}	$C_{14}H_{23}N_3O_3$	145	72	59.75	59.71	8.25	8.19

[a] B = Prepared by method B. The remainder were prepared by method A. [b] When prepared by method B, the yield was 47%.

Anal. Calcd. for $C_9H_{12}N_3O_3$: C, 51.48; H, 5.76; N, 20.10. Found: C, 51.40; H, 5.65; N, 20.31.

3-Alkyl-5-cyanocytosines. Method A.—Ten and eight-tenths grams (0.2 mole) of sodium methylate was added to 0.2 mole of the (3-alkylureido)-methylenemalononitrile[1] (Table III) dissolved in 250 ml. of methanol. The solution was allowed to stand in a stoppered flask at room temperature for 5 days. The alcohol was removed by distillation under reduced pressure (15 mm.) at room temperature. The resulting solid was dissolved in 500 ml. of cold water. Dilute acetic acid was added until the mixture was slightly acidic. The precipitated 3-alkyl-5-cyanocytosine was collected, dried on an unglazed porcelain plate and recrystallized from ethanol (Table V).

Method B.—Two-tenths mole of the appropriate N-alkylurea and 10.8 g. (0.2 mole) of powdered sodium methylate were added to 250 ml. of methanol. To this was added 24.4 g. (0.2 mole) of ethoxymethylenemalononitrile.[5] The solution was allowed to stand at room temperature in a stoppered flask for 5 days. The 3-alkyl-5-cyanocytosine (Table V) was isolated and purified as described in method A.

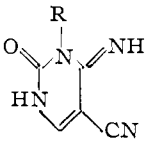
3-Aryl-5-cyanocytosines. Method C.—One-half mole of the N-arylurea was added to 148 g. (1.0 mole) of triethyl orthoformate, 153 g. (1.5 moles) of acetic anhydride and 33 g. (0.5 mole) of malononitrile in a liter round-bottomed flask. The contents of the flask were heated under reflux for 12 hours. The resulting solution was concentrated on the steam-bath and cooled. The crystalline 3-aryl-5-cyanocytosine was collected and recrystallized from a mixture of 90% ethanol and 10% dimethylformamide (Table V).

Method D.—To 250 ml. of diethyl Cellosolve was added 22 g. (0.3 mole) of malononitrile and 0.3 mole of the 1,3-bis-arylcarbamylformamidine. The mixture was refluxed for 12 hours, then concentrated at 15 mm. pressure on the steam-bath. The solid was collected and washed with 100–200 ml. of ethanol. The 3-aryl-5-cyanocytosine was crystallized from a mixture of 90% ethanol and 10% dimethylformamide.

(5) O. Diels, H. Gartner and R. Kaack, *Ber.*, **55B**, 3441 (1922).

TABLE V

5-CYANOCYTOSINES

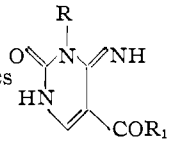


R	Formula	M.p., °C.	Yield, %[a]	Carbon, % Calcd.	Carbon, % Found	Hydrogen, % Calcd.	Hydrogen, % Found	Nitrogen, % Calcd.	Nitrogen, % Found
CH_3	$C_6H_6N_4O$	>270	56	48.00	47.89	4.03	3.88		
C_2H_5	$C_7H_8N_4O$	252	84B	51.21	51.40	4.91	5.14		
$H_2C{=}CHCH_2$	$C_8H_8N_4O$	210		54.55	54.55	4.57	4.68		
n-C_3H_7	$C_8H_{10}N_4O$	225	81					31.45	31.31
$(CH_3)_2CH$	$C_8H_{10}N_4O$	252	94	53.92	54.25	5.66			
$CH_3OCH_2CH_2$	$C_8H_{10}N_4O_2$	228	96	49.48	49.23	5.19	5.41		
n-C_4H_9	$C_9H_{12}N_4O$	208						29.15	28.81
$(CH_3)_3C$	$C_9H_{12}N_4O$	215	57					29.15	29.20
n-C_5H_{11}	$C_{10}H_{14}N_4O$	210	95 70B	58.23	58.40	6.84	7.07		
$(CH_3)_2CH(CH_2)_2$	$C_{10}H_{14}N_4O$	234	92	58.23	58.07	6.84	6.94		
C_6H_5	$C_{11}H_{18}N_4O$	275	61D	62.25	62.53	3.80	3.66	26.40	26.02
p-ClC_6H_4	$C_{11}H_7ClN_4O$	270	54C	53.56	53.51	2.85	3.07	22.72	23.03
n-C_6H_{13}	$C_{11}H_{16}N_4O$	199	94	59.98	60.17	7.32	7.91		
$C_6H_5CH_2$	$C_{12}H_{10}N_4O$	249	75	63.70	63.81	4.46	4.40		
p-$CH_3C_6H_4$	$C_{12}H_{10}N_4O$	>270	50	63.70	63.78	4.46	4.65		
n-C_7H_{15}	$C_{12}H_{18}N_4O$	200	95 95B	61.51	61.16	7.74	7.75		
$C_6H_5CH(CH_3)$	$C_{13}H_{12}N_4O$	215	82					23.32	23.49
2,6-di-$CH_3C_6H_3$	$C_{13}H_{12}N_4O$	>270	31C	64.98	65.06	5.03	5.14	23.32	23.98
n-C_8H_{17}	$C_{12}H_{20}N_4O$	201	95					22.56	22.06

[a] B, prepared by method B; C, prepared by method C; D, prepared by method D.

TABLE VI

5-CARBAMYLCYTOSINES



R	R_1	Formula	M.p., °C.	Yield, %	Carbon, % Calcd.	Carbon, % Found	Hydrogen, % Calcd.	Hydrogen, % Found	Nitrogen, % Calcd.	Nitrogen, % Found
n-C_3H_7	NH_2	$C_8H_{12}N_4O_2$	265	56	49.02	48.87	6.16	6.19		
n-C_4H_9	NH_2	$C_{10}H_{16}N_4O_2$	271	80	51.40	51.15	6.72	6.84		
CH_3	$C_4H_8ON{\cdot}H_2O$[a]	$C_{10}H_{16}N_4O_4$	241	64					21.92	22.21
C_6H_{11}	NH_2	$C_{11}H_{16}N_4O_2$	245	78	56.00	56.04	6.83	6.82		
C_2H_5	$C_4H_8ON{\cdot}H_2O$[a]	$C_{11}H_{18}N_4O_3$	244	59	48.90	48.64	6.72	6.71	20.66	20.35
$C_6H_5CH_2$	NH_2	$C_{12}H_{12}N_4O_2$	274	73	59.02	58.93	4.96	5.11		
n-C_7H_{15}	NH_2	$C_{12}H_{20}N_4O_2$	260	85	57.11	57.04	7.99	8.07		
n-C_3H_7	NHC_5H_{11}	$C_{13}H_{21}N_4O_2$	178	50					21.16	20.78
C_2H_5	NHC_6H_5	$C_{14}H_{16}N_4O_3$	264	45					19.44	19.65
$(CH_3)_2CH(CH_2)_2$	NHC_5H_{11}	$C_{15}H_{25}N_4O_2$	218	47					19.03	18.90
n-C_7H_{15}	p-$NHC_6H_4OCH_3$	$C_{19}H_{26}N_4O_3$	252	35	63.75	63.90	7.33	7.16		

[a] Morpholino (hydrated).

3-(p-Methoxyphenyl)-5-cyanocytosine.—A mixture of 16.6 g. (0.1 mole) of p-methoxyphenylurea, 29.6 g. (0.2 mole) of triethyl orthoformate, 91.6 g. (0.9 mole) of acetic anhydride and 6.6 g. (0.1 mole) of malononitrile was heated at 70–90° for one hour. The resulting solution was concentrated under diminished pressure. The resulting solid was recrystallized from ethyl acetate to yield 20.5 g. (83%) of β-[3-(p-methoxyphenyl)-ureido]-α-cyanoacrylonitrile, m.p. 149°.

Six grams (0.025 mole) of β-[3-(p-methoxyphenyl)-ureido]-α-cyanoacrylonitrile was added to 100 ml. of methanol containing 1.5 g. (0.028 mole) of $NaOCH_3$. The resulting solution was allowed to stand at room temperature. After 3 days the solution was concentrated to one-half its original volume, diluted with an equal volume of water and acidified with glacial acetic acid. The white needles were collected, yield 3.5 g. (58%), and recrystallized from a mixture of dimethylformamide and ethanol, m.p. 265° dec.

Anal. Calcd. for $C_{12}H_{10}N_4O_2$: C, 59.50; H, 4.16; N, 23.13. Found: C, 59.45; H, 4.30; N, 23.40.

5-Carbamylcytosines (Table VI).—One-tenth mole of the appropriate ureidomethylenecyanoacetamide was added to 200 ml. of ethanol containing 5.4 g. (0.1 mole) of sodium methylate and refluxed for 5 to 24 hours. The alcohol was distilled under reduced pressure and the remaining solid was dissolved in water. The solution was acidified with acetic acid. The precipitated solid was collected and dried. The 5-(N-substituted carbamyl)-cytosines were recrystallized from alcohol and the (N-unsubstituted carbamyl)-cytosines were recrystallized from dimethylformamide.

Acid Hydrolysis of 5-Cyanocytosines. 3-Cyclohexyl-5-cyanouracil.—One hundred and fifty-three grams (0.7 mole) of 3-cyclohexyl-5-cyanocytosine was dissolved in 1 liter of 3 *N* HCl. The solution was refluxed for 3 hours and cooled. 3-Cyclohexyl-5-cyanouracil separated from solution and was collected on a filter. This was recrystallized from ethanol to yield 119 g. (78%) of white crystalline product, m.p. 231°.

Anal. Calcd. for $C_{11}H_{13}N_3O_2$: C, 60.37; H, 5.98. Found: C, 60.37; H, 6.30.

3-*n*-Heptyl-5-cyanouracil.—Thirty-one grams of 3-*n*-heptyl-5-cyanocytosine was treated with 150 ml. of 3 *N* HCl as described in the above paragraph to yield 18 g. (60%) of 3-*n*-heptyl-5-cyanouracil, m.p. 156°.

Anal. Calcd. for $C_{12}H_{17}N_3O_2$: C, 61.32; H, 7.30. Found: C, 61.04; H, 7.32.

Acid Hydrolysis of 5-Carbethoxycytosines.—Twenty-five grams of 3-isoamyl-5-carbethoxycytosine was added to 300 ml. of 3 *N* HCl and refluxed for 3 hours. After this time the expected uracil had not separated and when the solution was made slightly basic with NaOH solution, 18 g. of the original cytosine was recovered. This procedure was repeated with 3-ethyl-5-carbethoxycytosine and starting material was again recovered.

Six grams of 3-isoamyl-5-carbethoxycytosine was added to 100 ml. of 12 *N* HCl and heated on the steam-bath for two hours. Upon cooling 3-isoamyl-5-carboxycytosine hydrochloride crystallized. This was recrystallized from water; yield 5.5 g. or 92%, m.p. 232.5°.

Anal. Calcd. for $C_{10}H_{16}ClN_3O_3$: C, 46.00; H, 5.74; N, 16.18. Found: C, 46.02; H, 6.20; N, 16.15.

Seven grams of 3-ethyl-5-carbethoxycytosine was added to 100 ml. of 12 *N* HCl and treated in the same manner described in the above paragraph to yield 6 g. or 85% of **3-ethyl-5-carboxycytosine hydrochloride**, m.p. 228.5–231.5° dec.

Anal. Calcd. for $C_7H_{10}ClN_3O_3$: Cl, 15.98. Found: Cl, 16.07.

5-Carboxycytosines.—Two-tenths mole of the appropriate β-ureido-α-carboxyacrylonitrile and 21.6 g. (0.4 mole) of sodium methoxide were added to 500 ml. of methanol and the solution refluxed for 12–24 hours. The alcohol was evaporated under reduced pressure (15 mm.). The solid residue was dissolved in a minimum amount of cold water. The solution was acidified with acetic acid and the 5-carboxycytosine was collected. The product was purified by recrystallization from hot water or by dissolving in $NaHCO_3$ solution and reprecipitating with acetic acid. The following carboxycytosines were prepared.

3-Methyl-5-carboxycytosine, yield 57.5%, m.p. 251° dec. *Anal.* Calcd. for $C_6H_7N_3O_3$: C, 42.60; H, 4.17. Found: C, 42.65; H. 4.22.

3-Ethyl-5-carboxycytosine, yield 81%, m.p. 240° dec. *Anal.* Calcd. for $C_7H_9N_3O_3$: C, 45.81; H, 4.95. Found: C, 45.70; H, 5.07.

3-*n*-Butyl-5-carboxycytosine monohydrate, yield 68%, m.p. 208° dec. *Anal.* Calcd. for $C_9H_{15}N_3O_4$: C, 47.20; H, 6.59. Found: C, 47.83; H, 6.48.

3-*n*-Butyl-5-carboxycytosine, yield practically quantitative by drying the monohydrate at 140° for 2 hours, m.p. 210° dec. *Anal.* Calcd. for $C_9H_{13}N_3O_3$: C, 51.25; H, 6.21. Found: C, 51.30; H, 6.11.

3-*n*-Heptyl-5-carboxycytosine, yield 81%, m.p. 224° dec. *Anal.* Calcd. for $C_{12}H_{19}N_3O_3$: C, 56.95; H, 7.56. Found: C, 56.88; H, 7.79.

Decarboxylation of 5-Carboxycytosines. 3-Methylcytosine.—Five grams of 3-methyl-5-carboxycytosine was placed in a Pyrex test-tube and lowered into a Wood metal-bath heated at 250–255°. The carboxycytosine melted with evolution of CO_2. The mass was stirred with a glass rod for 10–15 minutes until the bubbling ceased. The contents of the test-tube were dissolved in hot water. The water solution was clarified with carbon, filtered and evaporated to a small volume. After cooling 3-methylcytosine crystallized. It was collected and recrystallized from hot water; yield 0.7 g. (19%), m.p. 260–265° dec.

Anal. Calcd. for $C_5H_7N_3O$: C, 47.95; H, 5.63. Found: C, 47.89; H, 5.65.

3-*n*-Heptylcytosine.—Ten grams of 3-*n*-heptyl-5-carboxycytosine was heated, in a small flask placed in a Wood metal bath, at 150–170°. After 15 minutes the product was dissolved in ethanol. The solution was treated with carbon, filtered and concentrated on the steam-bath. 3-*n*-Heptylcytosine crystallized and after it was dried, it melted at 169°, yield 3.5 g. (29.5%).

Anal. Calcd. for $C_{11}H_{19}N_3O$: C, 63.10; H, 9.16. Found: C, 62.80; H, 9.07.

1-Methyl-3-*n*-heptyl-5-cyanouracil.—Seventeen grams (0.074 mole) of 3-*n*-heptyl-5-cyanouracil was added to 400 ml. of water containing 3 g. (0.075 mole) of NaOH. The solution was stirred and heated at 45°. While stirring 9.3 g. (0.074 mole) of dimethyl sulfate was added dropwise. After 30 minutes the precipitated solid was collected and recrystallized from a mixture of ethanol and water; yield 16.5 g. (91.5%), m.p. 101°.

Anal. Calcd. for $C_{13}H_{19}N_3O_2$: C, 62.60; H, 7.68. Found: C, 62.81; H, 8.03.

1-Methyl-3-cyclohexyl-5-cyanouracil.—Fifty grams (0.24 mole) of 3-cyclohexyl-5-cyanouracil was treated with 9.6 g. (0.24 mole) of NaOH and 30.2 g. (0.24 mole) of dimethyl sulfate by the procedure described in the above paragraph to yield 30 g. (54.5%) of 1-methyl 3-cyclohexyl-5-cyanouracil, m.p. 175°.

Anal. Calcd. for $C_{12}H_{15}N_3O_2$: C, 61.75; H, 6.44. Found: C, 61.19; H, 6.82.

INDIANAPOLIS, INDIANA

[CONTRIBUTION FROM THE LILLY RESEARCH LABORATORIES]

Reactions of Orthoesters with Ureas. II

BY CALVERT W. WHITEHEAD AND JOHN J. TRAVERSO

RECEIVED APRIL 22, 1955

The reaction sequences that followed the action of orthoesters upon ureas were dependent upon the nature and position of substituents on the urea, the particular orthoester and the reaction conditions. Products obtained were ethoxymethylene, diethoxymethyl, and α-ethoxyethylidineureas, mono- and dicarbamylformamidines, urethans, 2-benzylidinecarbazic esters, 1,3-diarylformamidines, N-formylureas, aryltriazolones and 2-amino-1,3,4-thiadiazoles.

In a preceding paper[1] the reactions of triethyl orthoformate with alkylureas were discussed. The products of these reactions were 1,3-bis-alkylcarbamylformamidines. By varying the reactants and the reaction conditions, products other than 1,3-dicarbamylformamidines may be obtained, often in excellent yields. This paper is concerned with reactions of orthoesters and ureas to yield (1) alkoxymethylene and dialkoxymethylureas, (2) arylcarbamylformamidines, (3) urethans, (4) triazolones and (5) thiadiazoles.

(1) C. W. Whitehead, THIS JOURNAL, **75**, 671 (1953).

Although dialkoxymethylureas IV and alkoxymethyleneureas V have been proposed as intermediate products, they were not generally isolated when alkylureas were heated with orthoesters. The final products from such reactions were 1,3-dicarbamylformamidines. When, however, acetic anhydride, alkylureas and orthoesters were heated together, the compounds IV and V were obtained as major products of the reaction. Thus, 1-diethoxymethylene-3-ethylurea (IV, R = C_2H_5, R′ = R″ = H) was obtained when ethylurea was allowed to react with triethyl orthoformate II and