[CONTRIBUTION FROM THE RESEARCH LABORATORIES OF G. D. SEARLE & CO.]

SYNTHESIS OF 1-MONO- AND 1,3-DI-SUBSTITUTED 6-AMINOURACILS. DIURETIC ACTIVITY[1]

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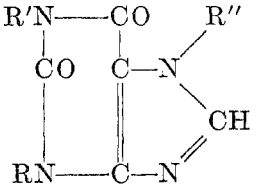
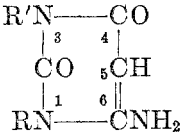
In recent years the use of the methylated xanthines theophylline and theobromine (I) in medicine, particularly as diuretics and cardiac drugs, has become general. In a search for similar products of higher therapeutic effectiveness we undertook an extensive investigation of a series of related xanthines. During the course of this study the observation was made that certain of the intermediate substituted 6-aminouracils (II) showed considerable activity as oral diuretics in experimental animals. In view of the potential usefulness of such compounds, it became of interest to study them further.





	R	R′	R″
Theophylline	CH_3	CH_3	H
Theobromine	CH_3	H	CH_3
Caffeine	CH_3	CH_3	CH_3

I

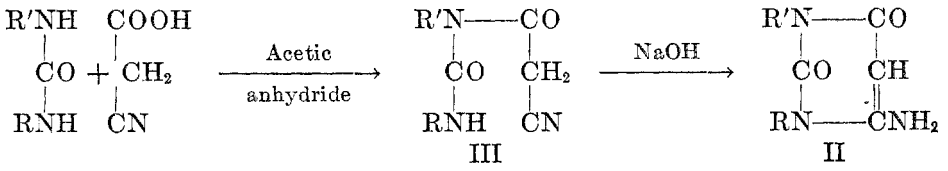
1,3-Disubstituted 6-aminouracil

II

This paper describes the preparation and properties of some new mono- and di-substituted 6-aminouracils of this type. A number of these were found to have diuretic activity, in experimental animals, equal to that shown by the xanthines. At the same time their toxicity is considerably lower.

The synthesis of the uracils was usually carried out according to the general method of Traube (1) as modified by Baum (2). The appropriate substituted ureas were condensed with cyanoacetic acid in acetic anhydride to give the intermediate cyanoacetylureas (III). These latter were obtained usually as syrups, but occasionally in crystalline form. On treatment with sodium hydroxide, ring closure took place with formation of the substituted 6-aminouracil (Method A, Equation A).

Equation A



III

II

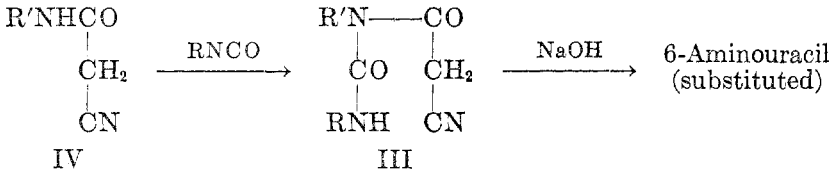
[1] Presented before the Division of Medicinal Chemistry of the American Chemical Society at the Cleveland meeting, April, 1951.

In a number of instances, the synthesis was carried out by heating an alcoholic solution of the urea with ethyl cyanoacetate, using sodium ethoxide as condensing agent (Method B). This method proved useful in the presence of free hydroxyl groups (as in the hydroxyethylureas), since it obviated the possibility of acetylation which might occur in the presence of acetic anhydride.

A third procedure (Method C) involved the alkylation, on position 3, of the appropriate 1-substituted 6-aminouracil by treatment with alkyl sulfate or iodide in alkaline solution. Although the yields were frequently rather low, this method was valuable in establishing the structures of some of the disubstituted uracils obtained by method A.

A useful method (D), employed in two cases, involved the addition of alkyl isocyanate to a substituted amide of cyanoacetic acid to yield the intermediate cyanoacetylurea, which underwent ring closure in the usual manner (Equation B).

Equation B



This procedure has thus far given relatively small yields; however it provides a means for obtaining directly the 6-aminouracils in which the substituent group on position 3 is larger than that on position 1. Compounds of that type are difficult to obtain by the usual method A (see below).

Table I lists some new disubstituted ureas obtained during the course of this work. Table II describes the new uracils, as well as several previously known, which were prepared for use in the pharmacological studies. Table III lists a number of open chain cyanoacetylureas which were isolated in crystalline form (III, Equation A and B).

Examination of Equation A suggests that the condensation of cyanoacetic acid with a substituted urea might occur at either nitrogen atom. The work of previous investigators (3, 4) leads to the conclusion, however, that with monosubstituted ureas (R' = H) the condensation occurs almost exclusively at the unsubstituted nitrogen atom, so that subsequent ring closure leads to 1-substituted 6-aminouracils. In support of this view, we have found no evidence for the simultaneous formation of 3-substituted 6-aminouracils. On this basis, structures of the monosubstituted compounds (Nos. 5 to 12 of Table II) have been assigned as indicated.

With mixed disubstituted ureas (R differs from R'), the cyanoacetic acid condensation could likewise occur at either nitrogen atom. Ring closure would then yield two isomeric disubstituted aminouracils. In carrying out such syntheses, we have obtained evidence that the condensation occurs predominantly, but not exclusively, at the nitrogen to which the smaller substituent group is

attached. The cyanoacetic acid appears to be directed mainly to that nitrogen at which steric hindrance is at a minimum.

A detailed study of this reaction was made on the uracils obtained, by method A, from two mixed disubstituted ureas, namely, N-ethyl-N′-*n*-propylurea (V) and N-ethyl-N′-allylurea (VI). These were of particular interest pharmacologically. By a long fractional recrystallization of the crude ring closure reaction product obtained from V, we were able to separate out the two isomeric uracils in pure form: the predominant isomer, 1-*n*-propyl-3-ethyl-6-aminouracil in 60% yield and the reverse isomer, 1-ethyl-3-*n*-propyl-6-aminouracil in 5% yield. The residual 35% could not be separated further. Both of these isomers were synthesized also by the independent method C. (Compounds 24 and 36 of

TABLE I

MIXED DISUBSTITUTED UREAS R′NHCONHR

R′	R	M.P.,[a] °C.	RS[e]	FORMULA	ANALYSES NITROGEN	
					Calc'd	Found
H	Methallyl	117–119	A	$C_5H_{10}N_2O$	24.56	24.40
Methyl	*n*-Propyl	65–66	B	$C_5H_{12}N_2O$	24.12	24.00
Methyl	Isopropyl	94–96[b]	C	$C_5H_{12}N_2O$	24.12	23.70
Ethyl	*n*-Propyl	79–80	D	$C_6H_{14}N_2O$	21.52	21.45
Ethyl	Isopropyl	158–159	E	$C_6H_{14}N_2O$	21.52	21.30
Ethyl	*n*-Hexyl	57–58	D	$C_9H_{20}N_2O$	16.27	16.21
Ethyl	β-Hydroxyethyl	56–57[b]	F	$C_5H_{12}N_2O_2$	21.20	20.54
Ethyl	γ-Hydroxypropyl	35–37	F	$C_6H_{14}N_2O_2$	19.17	19.14
Ethyl	β-Hydroxypropyl	—[c]	—	$C_6H_{14}N_2O_2$	19.17	18.79
Ethyl	Cyclohexyl	113–115	E	$C_9H_{18}N_2O$	16.46	16.31
Ethyl	γ-Phenylpropyl	47–49[d]	—	$C_{12}H_{18}N_2O$	13.58	13.54

[a] Uncorrected. [b] Slightly hygroscopic. [c] Obtained as a syrup which was not further purified. [d] No suitable recrystallizing solvent was found; the melting point and analytical data were that of the crude material. [e] Recrystallization solvents: A, water; B, ethyl acetate-benzene; C, EtOAc-ether; D, hexane; E, EtOAc; F, benzene-ethanol.

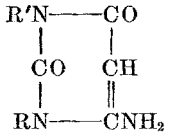
Table II). By preparing mixtures of these pure isomers in various ratios, it was possible to set up a mixture melting point curve (Fig. 1). By reference to this curve it was established that the crude reaction product obtained from N-ethyl-N′-*n*-propylurea by method A under our conditions contains approximately 80% of the predominant aminouracil and 20% of the reverse isomer.

A similar study was made of the uracils obtained from N-ethyl-N′-allylurea. In this case we were not able to separate out either isomer in pure form by fractional crystallization. However by reference to a melting point curve (Fig. 1) obtained from the pure isomers synthesized by methods C and D (Nos. 26 and 39, Table II), it was established that here also the isomeric uracils are formed approximately in the ratio of 80 to 20, the predominant isomer being 1-allyl-3-ethyl-6-aminouracil.

Two additional mixed disubstituted ureas, namely, N-methyl-N′-*n*-propyl-

TABLE II

MONO- AND DI-SUBSTITUTED 6-AMINOURACILS



COMPOUND	R′	R	METHOD	M.P., [a] °C.	RECRYST. [g] SOLVENT	FORMULA	ANALYSIS [l] NITROGEN	
							Calc'd	Found
1[b]	H	H	A, B	310–312	Water	$C_4H_5N_3O_2$	—	—
2[b]	H	Methyl	A, B	306–307	Water	$C_5H_7N_3O_2$	—	—
3[c]	H	Ethyl	A, B	288–290	Water	$C_6H_9N_3O_2$	—	—
4[d]	H	β-Hydroxyethyl	B	261–262	Water	$C_6H_9N_3O_3$	—	—
5	H	*n*-Propyl	A	273–275	EtOH-50%	$C_7H_{11}N_3O_2$	24.85	24.68
6	H	Allyl	A	273–274	EtOH-50%	$C_7H_9N_3O_2$	25.14	25.20
7	H	*n*-Butyl	A	266–267	EtOH-50%	$C_8H_{13}N_3O_2$	22.94	22.92
8	H	Isobutyl	A	271–273	EtOH-50%	$C_8H_{13}N_3O_2$	22.94	22.81
9	H	Benzyl	A	285–286	EtOH-50%	$C_{11}H_{11}N_3O_2$	19.35	19.15
10	H	γ-Methoxypropyl	A	205–207	Water	$C_8H_{13}N_3O_3$	21.09	21.07
11	H	β-Dimethylaminoethyl	A	260–261	Water	$C_8H_{14}N_4O_2$	28.28	28.20
12	H	Methallyl	A	266–268	EtOH-50%	$C_8H_{11}N_3O_2$	23.19	23.01
13[b]	Methyl	Methyl	A	305–307	Water	$C_6H_9N_3O_2$	—	—
14	Methyl	Ethyl	A, C	232–233	Water	$C_7H_{11}N_3O_2$	24.85	24.74
15	Methyl	*n*-Propyl	A, C	165–167	Water	$C_8H_{13}N_3O_2$	22.94	22.97
16[b]	Methyl	Isopropyl	A	210–212	Water	$C_8H_{13}N_3O_2$	22.94	23.30
17	Methyl	*n*-Butyl	C	136–138	EtOH-20%	$C_9H_{15}N_3O_2$	21.30	21.26
18	Methyl	Isobutyl	C	173–175	EtOH-20%	$C_9H_{15}N_3O_2$	21.30	21.14
19	Methyl	Allyl	C	143–144	EtOAc	$C_8H_{11}N_3O_2$	23.19	23.01
20	Methyl	Methallyl	C	145–146	Water	$C_9H_{13}N_3O_2$	21.53	21.60
21	Methyl	β-Hydroxyethyl	B, C	216–217	Water	$C_7H_{11}N_3O_3$	22.69	22.70
22[e]	Ethyl	Methyl	C	208–209	Water	$C_7H_{11}N_3O_2$	—	—

23[f]	Ethyl	Ethyl	A	198–199	Water	$C_8H_{13}N_3O_2$	22.94	22.52
24	Ethyl	*n*-Propyl	A, C	169–170	EtOAc	$C_9H_{15}N_3O_2$	21.30	21.31
25[k]	Ethyl	Isopropyl	A	200–201	Methanol	$C_9H_{15}N_3O_2$	21.30	21.09
26[h]	Ethyl	Allyl	A, C	143–144	EtOAc-Ether	$C_9H_{13}N_3O_2$	21.53	21.71
27[k]	Ethyl	Methallyl	A	157–159	EtOH-10%	$C_{10}H_{15}N_3O_2$	20.10	20.14
28	Ethyl	*n*-Butyl	A, C	146–147	EtOAc	$C_{10}H_{17}N_3O_2$	19.89	19.81
29[k]	Ethyl	*n*-Hexyl	A	161–163	EtOH-40%	$C_{12}H_{21}N_3O_2$	17.56	17.56
30[k]	Ethyl	β-Hydroxyethyl	A, B	180–181	Water	$C_8H_{13}N_3O_3$	21.09	20.95
31[k]	Ethyl	β-Hydroxypropyl	A	167–170	Water	$C_9H_{15}N_3O_3$	19.71	19.55
32[k]	Ethyl	γ-Hydroxypropyl	A	170–174	Water	$C_9H_{15}N_3O_3$	19.71	19.58
33[k]	Ethyl	Cyclohexyl	A[i]	176–178	EtOAc	$C_{12}H_{19}N_3O_2$	17.72	17.50
34[k]	Ethyl	γ-Phenylpropyl	A	141–143	EtOH-40%	$C_{15}H_{19}N_3O_2$	15.38	15.25
35	*n*-Propyl	Methyl	C	160–161	EtOAc	$C_8H_{13}N_3O_2$	22.94	22.89
36	*n*-Propyl	Ethyl	A, C	146–147	EtOAc	$C_9H_{15}N_3O_2$	21.30	21.28
37	*n*-Propyl	*n*-Propyl	A	136–138	EtOH-20%	$C_{10}H_{17}N_3O_2$	19.89	19.97
38	*n*-Propyl	Allyl	C	117–118	Water	$C_{10}H_{15}N_3O_2$	20.10	20.06
39	Allyl	Ethyl	D	143–144	Water	$C_9H_{13}N_3O_2$	21.53	21.31
40[k]	Allyl	Isobutyl	A	92–97	EtOH-50%	$C_{11}H_{17}N_3O_2$	18.82	18.93
41[k]	Allyl	Phenyl	A	190–194	EtOH-50%	$C_{13}H_{13}N_3O_2$	17.27	17.25
42[k]	Allyl	Benzyl	A	218–220	EtOH-50%	$C_{14}H_{15}N_3O_2$	16.33	16.18
43	*n*-Butyl	Ethyl	D	135–136	EtOH-20%	$C_{10}H_{17}N_3O_2$	19.89	19.79
44	*n*-Butyl	Allyl	C	95–97	EtOH-30%	$C_{11}H_{17}N_3O_2$	18.82	18.83
45	*n*-Butyl	*n*-Butyl	A	105–108	EtOH-60%	$C_{12}H_{21}N_3O_2$	17.56	17.86
46[k]	γ-Methoxypropyl	Phenyl	A	75–76	EtOH-50%	$C_{14}H_{17}N_3O_3$	15.26	15.09
47	Benzyl	Benzyl	A	122–125	EtOH-50%	$C_{18}H_{17}N_3O_2$	13.67	13.13

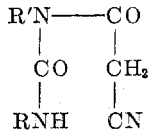
[a] For the anhydrous uracils; uncorrected. [b] Baum (2); also Ruttink, *Rec. trav. chim.*, **65**, 751 (1946). [c] Biltz and Peukert, *Ber.*, **58**, 2190 (1925). [d] Nathan and Bogert, *J. Am. Chem. Soc.*, **63**, 2567 (1941). [e] Conrad, German Patent 177, 768. [f] Scarlat, *Bull. Soc. Sci. Bucarest*, **13**, 155 (1904). [g] Many of these compounds, particularly the disubstituted, separated from water or aqueous alcohol as monohydrates melting over a broad range (85–105°). They were dehydrated by drying for 24 hours at 80°. In some cases, it was necessary to recrystallize the dehydrated material from an anhydrous solvent, such as ethyl acetate, to reach a sharp melting point. [h] Structure established also by hydrogen reduction to compound 24, using platinum catalyst. [i] Isolated in low yield (5%) by fractional recrystallization, from ethyl acetate, of the product of the ring-closure reaction. [k] Prepared from the mixed disubstituted urea according to method A, and purified by repeated recrystallization. As is indicated in the text, this material may consist of a mixture of two isomers. However it is probable that the predominant constituent has the assigned structure, and that the reverse isomer, if present, occurs in only relatively small amount. [l] Carbon and hydrogen analyses were also made on about a dozen of these compounds. In each case the values agreed well with theory.

and N-ethyl-N′-*n*-butyl-, yielded mixtures of isomeric uracils from which the predominant isomer could be separated in pure form by repeated recrystallization. In each case the larger substituent group was located in position 1. (Nos. 15 and 28, Table II). Several other mixed ureas were converted into the corresponding aminouracils, but no detailed study of isomer formation was made (Footnote k, Table II).

It is highly probable that any mixed, disubstituted urea, when converted to the 6-aminouracil according to method A, will yield a mixture of isomers; that the ratio of the amounts of the two isomers formed will depend upon the relative size of the substituent groups of the urea molecule; and that the predominant aminouracil will be the one having the larger substituent group in position 1.

TABLE III

SUBSTITUTED CYANOACETYLUREAS



R′	R	M.P.,[a] °C.	FORMULA	ANALYSES NITROGEN	
				Calc'd	Found
H	*n*-Propyl	169–170	$C_7H_{11}N_3O_2$	24.84	24.82
H	Isopropyl	145–146	$C_7H_{11}N_3O_2$	24.84	24.86
H	*n*-Butyl	152–154[b]	$C_8H_{13}N_3O_2$	22.94	22.88
H	Allyl	142–143	$C_7H_9N_3O_2$	25.14	25.06
H	Methallyl	143–145	$C_8H_{11}N_3O_2$	23.19	23.11
H	γ-Methoxypropyl	130–133	$C_8H_{13}N_3O_3$	21.09	20.79
Allyl	Ethyl	84–86[c]	$C_9H_{13}N_3O_2$	21.53	21.40
Ethyl	Isopropyl	73–75	$C_9H_{15}N_3O_2$	21.30	21.10
Ethyl	Cyclohexyl	110–112[b]	$C_{12}H_{19}N_3O_2$	17.72	17.70

[a] Uncorrected. The compounds were recrystallized from water except as noted. [b] Recrystallized from 25% aqueous ethanol. [c] Isolated as an intermediate in the preparation of 1-ethyl-3-allyl-6-aminouracil (No. 39, Table II) by method D.

Our work confirms the observation of Baum (2) that the rate of ring closure of cyanoacetylureas (Equation A) is much greater for the disubstituted, than for the monosubstituted derivatives (R′ = H). The latter require the application of heat, while the former ring-close exothermically, and also in considerably better yields. An exception to this rule is presented by N-allyl-N′-cyanoacetylurea, in which the activating effect of the allyl group induces vigorous ring closure, with excellent yield of aminouracil.

Also of interest is the observation that monosubstituted cyanoacetylureas (III, R′ = H) in which the R substituent contains an α-branched carbon chain (*e.g.* isopropyl, cyclohexyl, *sec*-butyl) do not ring-close under the conditions used in this study. Likewise, when both R and R′ substituents of disubstituted

cyanoacetylureas contain such α-branched chains, no ring closure takes place. However, when R is α-branched (isopropyl or cyclohexyl) and R′ is a normal alkyl group such as ethyl, ring closure occurs normally, although the yield of

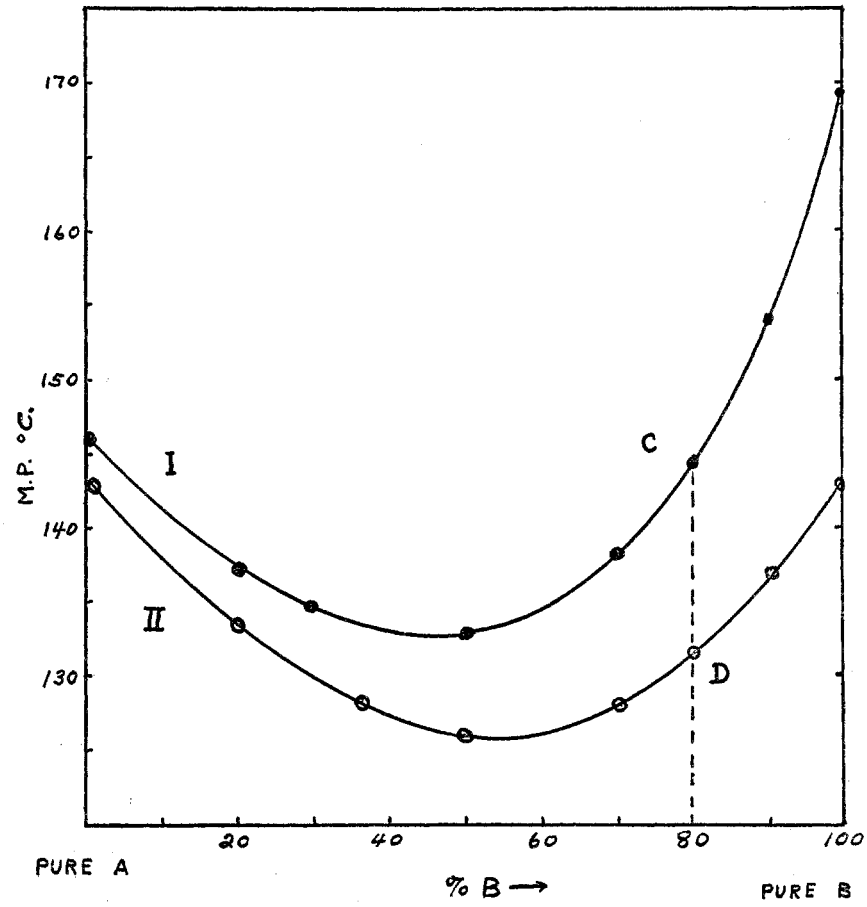


FIG. 1. MIXTURE MELTING POINT CURVES OF ISOMERIC AMINOURACILS.

Curve I: A, 1-ethyl-3-*n*-propyl-6-aminouracil, m.p. 146°; B, 1-*n*-propyl-3-ethyl-6-aminouracil, m.p. 169°; C, mixture of uracils obtained from N-ethyl-N′-*n*-propylurea by method A, m.p. 145°, indicating composition of 80% B, and 20% A.

Curve II: A, 1-ethyl-3-allyl-6-aminouracil, m.p. 143°; B, 1-allyl-3-ethyl-6-aminouracil, m.p. 143°; D, mixture of uracils obtained from N-ethyl-N′-allylurea by method A, m.p. 132°, indicating composition of 80% B, and 20% A.

aminouracil is rather low. (Nos. 25 and 33, Table II). Steric hindrance probably is involved in this behavior.

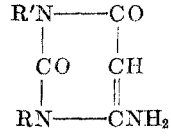
PHARMACOLOGY

Studies on the diuretic activity and toxicity of the aminouracils were carried out in our Division of Biological Research, by Drs. D. M. Green, W. E. Ham-

bourger, C. G. Van Arman, M. M. Winbury, D. L. Cook, and H. B. Freese. The compounds were screened by tests on rats, and the more active ones were further studied on dogs. The compounds were administered orally, and water and salt secretion determined under standardized conditions. Control and treatment experiments were made on successive days.

TABLE IV

DIURETIC ACTIVITY OF 1,3-DISUBSTITUTED-6-AMINOURACILS IN DOGS



COMPOUND[a]	URINE[b] EXCESS ML./KG.	T/C[c]	SODIUM[b] EXCESS MEQ./KG.	T/C[c]	EMESIS	LD/50[g] MG./KG. MOUSE
Aminophylline	6.4	2.53	1.7	2.51	0/3	600
Salyrgan[d]	11.4	3.03	2.7	14.16	0/11	118
AMINO URACILS: R′ / R						
Methyl / *n*-Propyl	7.7	2.13	1.6	3.30	0/3	1640
n-Propyl / Methyl	1.7	1.46	0.67	3.54	0/3	2000
Ethyl / *n*-Propyl	5.8	1.78	1.40	2.95	0/3	1310
n-Propyl / Ethyl	6.5	2.35	1.40	4.80	1/6	1440
Methyl / Allyl	—	1.54	—	1.63	—	1240
Ethyl / Allyl	7.59	2.38	1.73	2.83	0/3	1290
Allyl / Ethyl	4.87	1.72	0.95	2.62	0/3	1740
SC—2614[e]	6.4	2.35	1.50	3.80	5/17	1160
SC—3088[f]	8.1	2.60	1.20	3.20	0/9	1110

[a] All compounds with the exception of Salyrgan were administered orally in a dose of 100 mg./kg. Duration of each test was 5 hours. [b] Values given refer to excess excretion by treated over control animals. [c] Ratio of treated to control animals. [d] This mercurial was given intravenously in a dose of 6 mg./kg. It should be pointed out that the comparatively high activity shown by Salyrgan in comparison to the aminouracils may be accounted for in part by this intravenous administration, and subsequent more rapid action during the 5 hour test period. When a longer test period (24 hours) was used, the difference in activity was considerably less marked. [e] A mixture, as obtained by method A, of 80% 1-*n*-propyl-3-ethyl-6-aminouracil and 20% of 1-ethyl-3-*n*-propyl-6-aminouracil. See text. [f] A mixture, as obtained by method A, of 80% 1-allyl-3-ethyl-6-aminouracil and 20% of 1-ethyl-3-allyl-6-aminouracil. [g] Toxicites were determined by intragastric administration excepting for Salyrgan, which was given intraperitoneally.

Since the pharmacological studies are still incomplete, it is not possible to present now a definite picture of the relationship between activity and structure. 1-Monosubstituted-6-aminouracils possess little or no activity. Table IV shows some typical results obtained with several of those more active 1,3-disubstituted uracils of which the two isomeric forms were prepared and studied separately. They show activity of the same order of magnitude as the xanthine aminophylline, while their toxicity, as indicated by the LD_{50} value, is con-

siderably lower. Isomeric 6-aminouracils (*e.g.* 1-methyl-3-*n*-propyl- and 1-*n*-propyl-3-methyl-) exhibit reasonably comparable activity and toxicity.

The most promising compounds from a clinical standpoint are the mixtures of isomers designated as SC-2614 and SC-3088. (See Table IV for description.) These, as well as their constituent isomers in pure form, are now under investigation. Drs. E. V. Newman and A. A. Kattus of the School of Medicine, Johns Hopkins University, have reported (5) favorable results in the clinical use of SC-2614. However the number of their cases was limited, and more clinical work is needed to determine the usefulness of this and similar preparations as oral diuretics. Kattus and Newman (6) also have recently published extensive data on the diuretic activity of several of our aminouracils and xanthines in experimental animals.

EXPERIMENTAL

Substituted ureas. Monosubstituted ureas (R′ = H) were prepared by the action of a primary amine sulfate on potassium cyanate in aqueous solution. One mole of amine was slowly added to an equivalent amount of cold 35% sulfuric acid. To this solution, warmed to 85°, was added one mole of potassium cyanate. An exothermic reaction occurred, after which the mixture was heated for an hour on the steam-bath. The urea formed was extracted from the solid residue with warm ethanol, filtered to remove potassium sulfate, and the filtrate concentrated to dryness under reduced pressure. The crude ureas, obtained in excellent yield (95%) in this manner, were used directly for the subsequent reaction with cyanoacetic acid. Prepared in this way also were the previously known monosubstituted ureas: methyl, *n*-propyl, isobutyl, benzyl, allyl, β-hydroxyethyl, and cyclohexyl.

Disubstituted ureas having identical substituents were prepared by the action of primary amines on phosgene in the presence of aqueous sodium hydroxide. To a well-cooled solution of 2 moles of amine and 2 moles of sodium hydroxide in 300 cc. of water was gradually added, with strong stirring, a cold solution of 2.2 moles of phosgene in 500 cc. of benzene. After the addition, stirring was continued for 30 minutes longer, more sodium hydroxide solution being added, if necessary, to bring the reaction to neutral. The benzene layer was then separated, dried over sodium sulfate, and evaporated to dryness under reduced pressure, to give the nearly pure urea in almost quantitative yield. Prepared in this manner were the known ureas: diethyl, diisopropyl, dicyclohexyl, di-*n*-propyl, and dibenzyl.

Disubstituted ureas having dissimilar substituents were prepared from methyl and ethyl isocyanates (7). To an ice-cooled, stirred solution of 1.0 mole of primary amine in 3 volumes of benzene was gradually added a cooled solution of 1.0 mole of the isocyanate, also in 3 volumes of benzene. On evaporation of the solvent on the steam-bath the almost pure urea was obtained in nearly quantitative yield. Table I lists some new ureas prepared by this method; several others, not included, were used in the subsequent uracil synthesis without purification. Made in this manner also were the previously known 1-ethyl-3-methylurea and 1-butyl-3-ethylurea. Obtained from commercial sources, mainly Sharples Chemicals, Inc., were the known ureas: ethyl, isopropyl, *n*-butyl, 1,3-dimethyl, and 1,3-di-*n*-butyl.

Preparation of substituted 6-aminouracils. Method A. Illustrative of this method is the preparation of 1,3-di-*n*-propyl-6-aminouracil. A reaction mixture consisting of 360 g. (2.50 moles) of 1,3-di-*n*-propylurea, 233 g. (2.75 moles) of cyanoacetic acid, and 720 cc. of acetic anhydride was heated to 75–80° for 2 hours. A mild exothermic reaction occurred during the first stages of the heating, during which time the reaction vessel was temporarily removed from the heating bath. The solvent was then removed under reduced pressure, the external bath being held at about 80°. Water (200 cc.) was added and the distillation repeated to yield a slightly colored syrup consisting mainly of 1,3-di-*n*-propyl-1-

cyanoacetylurea. This material was ring closed by first adding 1200 cc. of water, stirring and cooling to 10°, and then rapidly running in a solution of 70% (w/v) sodium hydroxide to a permanent alkaline reaction to phenolphthalein, about 175 cc. being required. A vigorous reaction took place, the temperature rising to 60–70°, and an oil separated which solidified on cooling. The crystals were filtered off, washed with water, and twice recrystallized from 10 parts of 20% aqueous ethanol. This product, the monohydrate of the expected uracil, was dehydrated by heating at 80° for 24 hours. The resulting 1,3-di-*n*-propyl-6-aminouracil obtained in a yield of 345 g. (65% based on the starting urea), melted at 136–138°. For disubstituted 6-aminouracils with dissimilar substituent groups, essentially the same procedure was followed. However, because of the simultaneous formation of two isomeric uracils in these instances, as previously indicated, purification was usually more difficult. In general, the crude products from the ring-closure reaction were recrystallized 3 or 4 times from water, aqueous alcohol, or ethyl acetate as indicated in Table II. The yields of crude product ranged from 60 to 70% based on the starting urea.

In the preparation of monosubstituted 6-aminouracils, the ring-closure reaction in general required the application of heat as illustrated by the example of 1-*n*-butyl-6-aminouracil. A mixture of 116 g. (1.0 mole) of mono-*n*-butylurea, 94 g. (1.1 moles) of cyanoacetic acid, and 200 cc. of acetic anhydride was heated to 75–80° for two hours. On cooling, crystals of the open chain cyanoacetyl derivative began to separate. The mixture was stirred up with 500 cc. of ethyl ether and allowed to stand for 2 hours in an ice-bath. The crystals were filtered off, washed with ether, and air-dried to give 122 g. of crude 1-*n*-butyl-3-cyanoacetylurea. For ring closure, this product was suspended in a mixture of 300 cc. of water and 150 cc. of ethanol (added to increase solubility), warmed to 85°, stirred, and treated by the gradual addition of about 75 cc. of 10% aqueous sodium hydroxide. The cyanoacetyl derivative dissolved completely on addition of the alkali, and a precipitate of the aminouracil gradually separated out. The temperature was held at 85° for about 30 minutes, more alkali being added as needed to maintain a slightly alkaline reaction. The mixture was then made faintly acid with HCl, cooled, filtered, and the product washed with water. On recrystallization from 10 parts of 50% aqueous ethanol, a yield of 70 g. (38% based on the starting urea) of 1-*n*-butyl-6-aminouracil, melting at 266–267°, was obtained. The yields of other monosubstituted uracils were also rather low, ranging from 25 to 35%.

Method B. Ethyl cyanoacetate—sodium ethoxide condensation. Illustrative of this method is the preparation of 1-β-hydroxyethyl-6-aminouracil using a modified version of the procedure of Nathan and Bogert (8). To a solution of 115 g. (5 moles) of sodium in 3300 cc. of absolute ethanol were added 312 g. (3 moles) of β-hydroxyethylurea and 339 g. (3 moles) of ethyl cyanoacetate. The mixture was well stirred and refluxed for about 18 hours. The heavy precipitate of the sodium salt of the aminouracil was filtered off, washed with ethanol, and dissolved in water. Upon slightly acidifying with HCl, 1-β-hydroxyethyl-6-aminouracil precipitated. This was recrystallized from 25 parts of hot water, giving 184 g. of pure product melting at 261–262°.

Method C. Alkylation on position 3 of 1-substituted-6-aminouracils. With alkyl iodide, the preparation of 1-methyl-3-*n*-propyl-6-aminouracil was carried out as follows. A mixture of 135 g. of 1-methyl-6-aminouracil (0.96 mole), 280 cc. of 15% NaOH (1.1 moles), 480 cc. of 95% ethanol, and 204 g. of *n*-propyl iodide (1.25 moles) was refluxed for 2 hours. A precipitate of the sodium salt of the starting uracil, which formed initially, gradually redissolved. At the end of the heating period, the alcohol and excess alkyl iodide were evaporated on the steam-bath. The residual liquor was diluted to 1200 cc. with hot water, filtered from unreacted 1-methyl-6-aminouracil (40 g.), and allowed to crystallize in the cold. The crude 1-methyl-3-*n*-propyl-6-aminouracil thus obtained was recrystallized from 2 liters of hot water containing 5 g. of sodium hydroxide to hold in solution any remaining starting uracil. The product, washed with water, was dried at 80° for 24 hours to remove water of crystallization, then recrystallized from ethyl acetate; yield, 40 g., m.p. 165–167°. Compounds 22, 28, 35, 36, 38, and 44 of Table II also were made in this manner.

The use of methyl sulfate in method C is illustrated by the preparation of 1-*n*-butyl-3-

methyl-6-aminouracil. A solution of 60 g. (0.33 mole) of 1-*n*-butyl-6-aminouracil in 135 cc. of 10% sodium hydroxide and 30 cc. of ethanol was warmed to 50° and treated gradually, while stirring, with 50 cc. of methyl sulfate. A precipitate slowly formed, which was filtered off at the end of the reaction, washed with water, and recrystallized from 20% aqueous ethanol. The monohydrate thus obtained was dried at 80° for 24 hours, giving 50 g. of 1-*n*-butyl-3-methyl-6-aminouracil, melting at 136–138°. Other compounds made in this manner were Nos. 14, 18, 19, 20, 21, 24, and 26 of Table II.

Method D. Equation B. N-*n*-Butylcyanoacetamide (IV, R′ = *n*-butyl) was prepared by allowing equimolar amounts of *n*-butylamine and ethyl cyanoacetate to stand together at room temperature for 24 hours. The crude product separating out was filtered off, washed with water, and air-dried; m.p. 71–72°. A solution of 14 g. (0.1 mole) of this N-*n*-butylcyanoacetamide and 12 g. (0.17 mole) of ethyl isocyanate in 100 cc. of toluene was refluxed for 24 hours. A precipitate, consisting of 10 g. of the starting amide, was obtained on filtering the cooled solution. The filtrate, on concentration under reduced pressure, gave a further small amount of starting amide, and finally a syrup consisting largely of the cyanoacetylurea (III, R′ = *n*-butyl). On treatment with an excess of 10% sodium hydroxide, ring closure occurred with formation of a solid product. Recrystallization of the latter from 20% aqueous ethanol gave about a gram of the expected 1-ethyl-3-*n*-butyl-6-aminouracil monohydrate, which on dehydration melted at 135–136°. (No. 43, Table II). By the same reaction, 1-ethyl-3-allyl-6-aminouracil (No. 39, Table II) was prepared in 51% yield, based on the N-allylcyanoacetamide consumed.

Isolation of crystalline cyanoacetylureas (III). In carrying out the synthesis of 6-aminouracils according to method A, the intermediate substituted cyanoacetylureas (III) were, in several instances, isolated in crystalline form. Particularly, the monosubstituted derivatives (R′ = H) tended to crystallize spontaneously either during the course of the reaction between the urea and cyanoacetic acid, or upon removal of the excess acetic anhydride. The pure compounds could be obtained by diluting the reaction mixtures with ether, filtering off the crude cyanoacetylurea, and recrystallizing to constant melting point, usually from water or aqueous ethanol. Several disubstituted cyanoacetylureas were likewise obtained in crystalline form by trituration of the original syrupy condensation products with water. In other cases, however, attempts to isolate such intermediates in crystalline form were not successful. Table III lists the compounds of this type which were obtained.

Separation of isomeric aminouracils. N-Ethyl-N′-*n*-propylurea was condensed with cyanoacetic acid and the syrupy intermediate obtained was ring closed according to method A. The resulting crude product, when dried in air, analyzed correctly for the monohydrate of an ethyl-*n*-propyl-6-aminouracil.

Anal. Calc'd for $C_9H_{15}N_3O_2 \cdot H_2O$: N, 19.53; H_2O, 8.37.
Found: N, 19.55; H_2O, 8.32.

This was dehydrated by stirring for one hour with a large excess of anhydrous ether, filtering off the partially dehydrated product, then heating at 80° for 24 hours. The anhydrous material (100 g.) was subjected to an extensive fractional crystallization using 10 to to 15 parts of ethyl acetate. In this manner, 60 g. of the predominant isomer (No. 24, Table II) and 5 g. of the reverse isomer (No. 36, Table II) were obtained in pure form. The residual mixture (35 g.) could not be separated by repeated recrystallization.

A similar fractionation was carried out on the crude product obtained from N-ethyl-N′-allylurea by method A. This also analyzed correcty for an ethyl-allyl-6-aminouracil, and its broad melting point range indicated a mixture of isomers. However, repeated recrystallization of the hydrate from water, and of the dehydrated material from ethyl acetate, failed to separate out the pure isomers.

The isomeric aminouracils involved in these studies (ethyl-*n*-propyl and ethyl-allyl) had been prepared in pure form by the independent methods C and D. A mixture melting point curve was therefore set up (Fig. 1) for each pair of isomers. Since the melting point range of such mixtures of isomers was rather broad, the melting point was arbitrarily chosen as the temperature of complete liquefaction to a milky fluid. Fairly reproducible results

could be obtained in this manner. By reference to the standard curve, it was established that in each of the above cases, the isomers were present in approximately an 80:20 ratio, the predominant isomer being the one with the larger group in position 1. This was shown by the observation that when small amounts of the pure synthetic isomers B were added to the respective isomer mixtures C or D, the melting points were raised; but when the pure reverse isomers A were mixed with C or D in small amounts, depression of the melting point occurred.

In two other instances, methyl-*n*-propyl- and ethyl-*n*-butyl-6-aminouracil, several recrystallizations from water and ethyl acetate, respectively, gave the pure predominant isomer in good yield (Nos. 15 and 28 of Table II). In each of these, likewise, the larger group occupies position 1. Several other mixed, disubstituted ureas were converted into 6-aminouracils which were then recrystallized several times from an appropriate solvent. However no detailed study was made of the homogeneity of the products.

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SUMMARY

1. A number of new 1-mono- and 1,3-di-substituted-6-aminouracils have been prepared for evaluation as diuretics.

2. The formation, from mixed disubstituted ureas, of isomeric 1,3-disubstituted-6-aminouracils in which the positions of the substituent groups are reversed, is described.

3. Several new substituted ureas and cyanoacetylureas are described.

4. The most promising compounds for use as oral diuretics are 1-*n*-propyl-3-ethyl-6-aminouracil and 1-allyl-3-ethyl-6-aminouracil. These, as well as their respective isomers in which the positions of the 1- and 3-substituent groups are reversed, show activity in experimental animals comparable to that shown by the xanthine base, aminophylline (theophylline ethylenediamine). At the same time their toxicity is considerably lower.

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