$-25.8°$ (H_2O) for 1-Me-L-histidine free base isolated from the hydrolysis of anserine.

1-Methyl-L-histidine Diflavianate.—The diflavianate salt was formed by the general method of Vickery,[20] and it was recrystallized from water; m.p. 234.7° dec.

Anal. Calcd. for $C_{27}H_{23}O_{18}N_7S_2$: C, 40.65; H, 2.92; N, 12.29. Found: C, 40.45; H, 3.08; N, 12.09.

3-Methylhistidine.—The 3-Me-histidine fraction from the ion-exchange column contained 10% histidine. In the radioactive tracer experiments for which this preparation was to be used,[10b] the contamination by histidine was not deleterious, and no further purification was attempted.

1,3-Dimethyl-L-histidine (Cl^-) or 1,3,-Dimethyl-4-(β-carboxyl-β-aminoethyl)-imidazolium Chloride.—The dry, desalted fraction from the ion-exchange column that contained 1,3-$(Me)_2$-histidine was taken up in about 1.0 ml. of H_2O, treated with Norite, filtered and adjusted to about *p*H 8 with ammonia. Acetone was added to the appearance of fluffy white crystals upon cooling the mixture. The crystals were washed on the filter funnel with acetone and dried *in vacuo* at 80°. The hygroscopic salt must be stored under desiccation.

Anal. Calcd. for $C_8H_{15}O_2N_3Cl$: C, 43.50; H, 6.86. Found: C, 43.4; H, 6.7. $[\alpha]^{20}D$ $+18.1° \pm 1.6°$ ($\alpha = 0.23°$; 0.0125 g./ml. in 1 *N* HCl; 1 dm. tube).

1,3-Dimethyl-L-histidine Diflavianate.—The diflavianate was prepared by the general method of Vickery,[20] and it was recrystallized from water; m.p. 237.5° dec.

Anal. Calcd. for $C_{28}H_{25}O_{18}N_7S_2 \cdot H_2O$: C, 40.53; H, 3.28. Found: C, 40.58, H, 3.17.

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Purines. VII. The Preparation of Certain 6-Alkylamino- and 6-Dialkylaminopurines[1]

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The procedures for the preparation and isolation of high molecular weight alkylamino- and dialkylaminopurines are described. Twenty such compounds have been prepared by the methods described herein.

A great deal of interest has centered in the 6-substituted purines following the recent discovery of the biological importance of kinetin[2] and 6-mercaptopurine.[3] This activity has been divided between the development of new synthetic routes to kinetin itself and to the synthesis[4] and testing of many of its analogs.

Miller and co-workers originally prepared 6-furfurylaminopurine by the aminolysis (in a sealed tube) of 6-methylmercaptopurine[2] following the procedure of Elion and Hitchings. Since then a number of other approaches to the synthesis of kinetin have been reported. One of the most recent methods for the preparation of the 6-substituted aminopurines involves the synthesis and reduction of the corresponding 6-amidopurine with lithium aluminum hydride.

In this Laboratory an aminolytic procedure based on the use of 6-chloropurine was successfully applied to the preparation of a number of 6-substituted purines.[4] Since this procedure appeared to have a wide degree of applicability and in view of the possibility of turning up other biologically active analogs, this work has been expanded to include a number of other potentially active purine derivatives. Recently several new 6-substituted aminopurines have been reported to possess activity.[5] Since this activity may be dependent in part on fat solubilities of the compound, this Laboratory has prepared a number of high molecular weight alkylaminopurines with the side chain in the 6-position.

This was done by refluxing the chloropurine with different aliphatic amines in *n*-butyl alcohol according to the procedure described earlier.[4] However, due to solubility characteristics of the aliphatic amines, it was not possible to isolate the reaction product by the usual crystallization techniques. The lower molecular weight homologs were isolated by steam distilling the unreacted aliphatic amine from the aqueous basic solution of the reaction mixture. The course of the steam distillation could be followed by the slow progression of the insoluble aliphatic amine through the condenser. The product was then obtained by the ethereal extraction of the basic media left in the distilling flask.

As the aliphatic side chain increased in length the products became contaminated with traces of the starting amine which could not be removed by continuous distillation with steam.

Attempts to remove the unreacted amine by co-distillation at reduced pressures with diphenyl ether were quite successful but no practical way was found for isolating the product in turn from the diphenyl ether. Co-distillation under similar conditions with ethylene glycol was successfully applied to the isolation of 6-isohexylaminopurine, but the process proved to be impractical for the distillation of the higher homologs.

Finally it was observed that it was possible to remove amines as large as octadecylamine from sirupy mixtures of 6-substituted purines by the use of super-heated steam. This was the procedure which was adopted for the isolation of the higher molecular weight 6-substituted purines.

One rather interesting steric effect was noted in

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TABLE I

Isolation procedure: A, conventional steam distillation; B, modified steam distillation with flask heated 150° and passing super-heated steam through; C, crystallized from reaction mixture. Crystallizing procedure: 1, crystallized from 75–90% ethanol–water solution; 2, crystallized from 100% ethanol; 3, crystallized from water.

Name (compound)	Isolation crystallization	Yield, %	Melting point, °C.	Empirical formula	Carbon, % Calcd.	Carbon, % Found	Hydrogen, % Calcd.	Hydrogen, % Found
6-Monoalkylaminopurines								
6-(*n*-Hexylamino)-purine	A-1	49	176–178	$C_{11}H_{17}N_5$	60.3	60.2	7.76	8.00
6-Cyclohexylaminopurine	B-1	80	210–211	$C_{11}H_{15}N_5$	60.8	60.2	6.92	6.93
6-(*n*-Heptylamino)-purine	A-1	66	168.5–170	$C_{12}H_{19}N_5$	61.8	61.7	8.16	8.42
6-(1-Methylheptylamino)-purine	B-1	82	94–96	$C_{13}H_{21}N_5$	63.2	62.7	8.51	8.35
6-(*n*-Octylamino)-purine	B-1	84	165–167	$C_{13}H_{21}N_5$	63.2	62.7	8.51	8.61
6-(2-Ethylhexylamino)-purine	B-1	65	158–159	$C_{13}H_{21}N_5$	63.2	63.0	8.51	8.4
6-(*n*-Decylamino)-purine	B-2	79	164.5–165.5	$C_{15}H_{25}N_5$	65.5	65.0	9.09	9.31
6-(*n*-Dodecylamino)-purine	B-2	91	156–156.5	$C_{17}H_{29}N_5$	67.3	67.4	9.58	9.79
6-(*n*-Hexadecylamino)-purine	B-2	83	144–145	$C_{21}H_{37}N_5$	70.2	69.9	10.3	10.2
6-(*n*-Octadecylamino)-purine	B-2	95	107–108	$C_{23}H_{41}N_5$	71.4	71.4	10.6	10.6
6-Dialkylaminopurines								
6-(Di-*n*-amylamino)-purine	A-1	90	88.5–89.5	$C_{15}H_{25}N_5$	65.5	65.6	9.09	9.23
6-(Di-isoamylamino)-purine	A-1	95	114.5–115	$C_{15}H_{25}N_5$	65.5	65.2	9.09	9.21
6-(Di-*n*-hexylamino)-purine	A-1	87	95.5–96.0	$C_{17}H_{29}N_5$	67.3	67.4	9.58	9.69
6-(Di-isohexylamino)-purine	A-1	75	89–90	$C_{17}H_{29}N_5$	67.3	67.6	9.57	9.83
6-(Di-2-ethylhexylamino)-purine	B-1	75	83–83.5	$C_{21}H_{37}N_5$	70.2	70.4	10.3	10.4
6-(Di-*n*-heptylamino)-purine	B-1	80	75–76	$C_{19}H_{33}N_5$	68.9	69.1	9.97	10.1
6-(Di-*n*-octylamino)-purine	B-1	85	85–86	$C_{21}H_{37}N_5$	70.2	70.4	10.3	10.49
6-(Di-*n*-nonylamino)-purine	B-1	89	77–78	$C_{23}H_{41}N_5$	71.4	71.2	10.6	10.8
6-(Di-*n*-decylamino)-purine	B-1	87	75–76	$C_{25}H_{45}N_5$	72.2	72.0	10.8	10.9
6-Alkanolaminopurines								
6-(6-Hydroxy-*n*-hexylamino)-purine	C-3	83	175–176	$C_{11}H_{17}N_5O$	56.1	56.1	7.24	7.37

these studies. All the hindered secondary amines (diisopropylamine, di-1-methylbutylamine, di-*sec*-butylamine and dicyclohexylamine) which were treated were found to be unreactive under these conditions of amination. However, the secondary amine di-(2-ethylhexyl)-amine, on the other hand, coupled with the 6-chloropurine.

Furthermore, it is interesting to note that the primary amine 1-methylheptylamine, which might have presented a steric problem, was reactive.

Experimental

Aminolysis Procedure.—One gram of 6-chloropurine (6.4×10^{-3} mole) was suspended in 10 ml. of *n*-butyl alcohol and to this mixture was added 16×10^{-3} mole of the desired amine. The mixture was then refluxed for four hours; the course of the reaction could be followed by the disappearance of the insoluble 6-chloropurine.

Isolation Procedure A.—The reaction mixture was placed in a 500-ml. round-bottomed flask containing 100 ml. of 0.1 *N* sodium hydroxide. This flask was suspended in an oil-bath and the contents were steam distilled until 500 ml. of distillate had been collected. This amount of distillate was sufficient to remove all the unreacted amine as well as the *n*-butyl alcohol. The non-volatile residue was then extracted with ether, which upon evaporation yielded the product. The 6-substituted amine was then in turn recrystallized from one of the several solvents.

Isolation Procedure B.—To the reaction mixture was added 100 ml. of 0.1 *N* sodium hydroxide. The mixture was stirred for 30 minutes and then extracted with ether. This extract was placed in a 500-ml. flask and the ether and butanol removed by distillation. The flask was then immersed in an oil-bath maintained at 150° and then steam distilled with super-heated steam. The steam was prepared by passing ordinary steam, with all condensate removed, through a Fisher super-heater which was maintained at a temperature of 350° with a Fisher burner. Because of the elevated temperatures it was necessary to use metal to glass connections on the discharge side of the super-heater.

Sufficient steam pressure was applied to cause the sirupy product to spread out in a thin film over the bottom of the flask. The distillation was continued until no more unreacted amine could be observed collecting in the condenser. Upon cooling, the product solidified in the flask and was removed by extraction with absolute alcohol.

The results of these experiments are given in Table I.

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