centrifuging; yield 993 mg. One recrystallization from aqueous methanol, followed by recrystallization from hot water, gave orange-brown needles which sintered at 225° and melted with decomposition at 240–250° (micro-block).

Anal. Calcd. for $C_{36}H_{48}N_{12}O_{16}S_2 \cdot 2H_2O$: C, 47.57; H, 5.77. Found: (sample dried at 56° *in vacuo*) C, 47.25; H, 5.60. Calcd. for $C_{36}H_{48}N_{12}O_{16}S_2$: C, 49.53; H, 5.54. Found: (sample dried at 100° *in vacuo*) C, 49.31; H, 5.30.

Streptidine Di-*d*-camphorsulfonate.—To a solution of 1.05 g. of streptidine dihydrochloride in 3 cc. of methanol was added a solution of 1.6 g. of sodium *d*-camphorsulfonate in 3 cc. of methanol. On standing at 25° overnight, sodium chloride had separated. It was filtered and the filtrate was concentrated to effect more complete separation of sodium chloride. After filtering again, the filtrate containing streptidine di-*d*-camphorsulfonate was diluted to 6.5 cc. with methanol and treated with 10 cc. of absolute ethanol. A voluminous precipitate of fine white needles separated at once. The crystals were filtered and dried; weight, 1.49 g. After recrystallization from methanol and then from 1:1 methanol–ethanol, there was obtained 0.90 g. of needles, melting at about 185–190° (micro-block) with some bubbling, resolidifying at about 220–230°, and finally melting with decomposition at about 285–292°. The rotation was $[\alpha]^{25}D$ +13.5° (*c*, 4.83% in water) after drying at 100° *in vacuo*.

Anal. Calcd. for $C_{28}H_{50}N_6O_{12}S_2 \cdot H_2O$: C, 45.15; H, 7.04; N, 11.28. Found: (sample dried at 56° *in vacuo*) C, 45.42; H, 7.10; N, 11.41. Calcd. for $C_{28}H_{50}N_6O_{12}S_2$: C, 46.27; H, 6.93. Found: (sample dried at 100° *in vacuo*) C, 46.25; H, 6.52.

Octaacetylstreptidine.—A mixture of 1.01 g. of streptidine dihydrochloride, 494 mg. of fused sodium acetate and 20 cc. of acetic anhydride was refluxed gently for one hour. The solution was then concentrated to dryness *in vacuo*, water was added, and an insoluble crystalline product separated; yield 1.52 g. The product was recrystallized once from chloroform–petroleum ether and once from chloroform–ether; yield 1.22 g.; m. p. 260–262° (micro-block).

Anal. Calcd. for $C_{34}H_{34}N_6O_{12}$: C, 48.15; H, 5.73; N, 14.04; acetyl, 57.52. Found: C, 48.20; H, 5.73; N, 13.75; acetyl, 57.0.

The acetyl determination was carried out by saponification with hot 1 *N* sodium hydroxide in 50% methanol for two hours prior to distillation of acetic acid. A cryoscopic molecular weight determination on octaacetylstreptidine carried out in dioxane as solvent gave a value of 540 (calcd. 598.6). The accuracy of this determination is estimated to be of the order of ±10%. An attempted ebullioscopic determination of the molecular weight using methanol as solvent gave no significant results, since the octaacetylstreptidine underwent methanolysis. The methanolysis involved partial deacetylation, which indicates that a portion of the acetyl groups are represented by O-acetyl.

Streptidine Dihydroiodide.—A mixture of 304 mg. of octaacetylstreptidine, 150 mg. of red phosphorus, 576 mg. of iodine and 5 cc. of hydriodic acid (sp. g., 1.7) was heated in a bomb tube for seven hours at 160–190°. The solution was diluted with water, filtered and concentrated to dryness *in vacuo*. The residue was dissolved in methanol, filtered and mixed with about two volumes of ether whereupon white crystals of streptidine dihydroiodide separated. The salt was recrystallized once from methanol–ether; yield 48 mg. It was converted to a picrate, m. p. 284–285°. The melting point of a mixture of this picrate with streptidine dipicrate was 284–285°.

Acknowledgment.—The authors wish to acknowledge the coöperation of Dr. N. R. Trenner and Messrs. R. P. Buhs and F. A. Bacher who determined infrared and ultraviolet absorption spectra, of Dr. J. B. Conn and Mr. W. A. Bastedo, Jr., who carried out the molecular weight determinations, and of Mr. R. N. Boos and his associates for the microanalyses.

Summary

Acid hydrolysis of streptomycin has yielded an optically inactive hydroxylated base, streptidine.

Streptidine has been characterized by the following crystalline salts: dipicrate, sulfate, carbonate, dihydrochloride, dihydroiodide, dihelianthate, di-*d*-camphorsulfonate and chloroplatinate. Streptidine appears to contain one or more hydroxyl groups, but no primary amino, carboxy, methoxy or carbonyl groups. It formed an octaacetyl derivative.

Streptidine has the molecular formula $C_8H_{18}N_6O_4$.

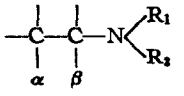
RAHWAY, NEW JERSEY RECEIVED OCTOBER 11, 1945

[CONTRIBUTION FROM THE PURDUE RESEARCH FOUNDATION AND THE DEPARTMENT OF CHEMISTRY OF PURDUE UNIVERSITY]

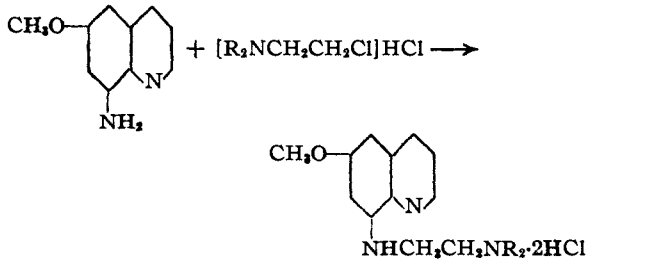
Derivatives of 6-Methoxy-8-aminoquinoline[1,2]

BY G. BRYANT BACHMAN AND H. HARRY SZMANT[3]

A number of derivatives of 6-methoxy-8-aminoquinoline containing the grouping



attached to the 8-amino nitrogen have been prepared by reactions similar to the following



It has been our purpose to study the variations in chemotherapeutic activity associated with changes in the R groups and with changes in substituent alkyl groups on the α and β carbons.

(1) Read before the Organic Section at the New York City meeting of the American Chemical Society, September, 1944.

(2) From the Ph.D. dissertation of H. Harry Szmant, Purdue University, Lafayette, Indiana.

(3) Present address: Monsanto Chemical Company, Dayton, Ohio.

Condensations between 6-methoxy-8-aminoquinoline and dialkylaminoalkyl chloride hydrochlorides usually proceeded satisfactorily at 60° in absolute ethanol. Higher condensation temperatures were unfavorable to good yields. Recrystallizations of the hydrochlorides obtained directly from the reaction mixtures gave better yields and purer products than low pressure distillations of the free bases. The properties and analyses of the salts obtained are indicated in Table I. The first compound listed was hydrolyzed with hydrobromic acid to the corresponding 6-hydroxy-8-aminoquinoline (next in list). The compounds of class X are diquinolyl amines formed by condensation of 6-methoxy-8-aminoquinoline with substituted 2-chloroquinolines. Conditions for this type of reaction were similar to those already described except that higher temperatures were required.

Pharmacological test data for these compounds will be reported elsewhere. It may be noted that significant differences in activity and toxicity accompanied the alterations in structure studied.

Acknowledgment.—We wish to express our thanks for financial support of this research provided by Eli Lilly and Company and for certain

TABLE I

N-SUBSTITUTED 6-METHOXY-8-AMINOQUINOLINES CH_3O—(quinoline)—NH—C—C—NR_1R_2

Class	Class type	No.	R_1	R_2
I	—NH—CH_2—CH_2—NR_1R_2	1	—$CH_2CH(CH_3)_2$	—$CH_2CH(CH_3)_2$
		2	—$CH_2CH(CH_3)_2$[a]	—$CH_2CH(CH_3)_2$
		3	—$CH_2CH(CH_3)_2$	—$CH[CH(CH_3)_2]_2$
		4	—$CH_2CH(CH_3)_2$	—$CH(CH_3)CH(CH_3)_2$
		5	—$CH_2CH_2OCH_2CH_2$—	
		6	—H	—$CH_2C(CH_3)_2CH_2N(CH_3)_2$
		7	—$CH(CH_3)_2$	—CH_2CH$CH_2CH_2CH_2$O
		8	—$CH_2CH(CH_3)CH_2CH_3$	—CH_2CH$CH_2CH_2CH_2$O
		9	—$CH_2CH_2CH_3$	—CH_2CH$CH_2CH_2CH_2$O
		10	—$CH(CH_3)CH_2CH_3$	—CH_2CH$CH_2CH_2CH_2$O
		11	—$COCH_2CH_2CH(CH_3)$—	
II	—NH—$CH(CH_3)CH_2NR_1R_2$	12	—$CH_2CH_2N(C_6H_5)CH_2CH_2$—	
III	—NH—$CH_2CH(CH_3)NR_1R_2$	13	—$CH_2CH(CH_3)_2$	—$CH_2CH(CH_3)_2$
IV	—NH—$CH(CH_3)CH(CH_3)NR_1R_2$	14	—$(CH_2)_5CH_3$	—$(CH_2)_5CH_3$
		15	—$CH_2CH(CH_3)_2$	—$CH_2CH(CH_3)_2$
V[d]	—NH—$C(CH_3)_2CH_2NR_1R_2$			
VI	—NH—$CH_2C(CH_3)_2NR_1R_2$	16	—$(CH_3)_2CH_3$	—$CH_2CH(CH_2CH_3)_2$
		17	—H	—$(CH_2)_2CH_3$
		18	—H	—$(CH_2)_3CH_3$
		19	—CH_2CH_3	—$(CH_2)_4CH_3$
		20	—CH_2CH_3	—$(CH_2)_2CH_3$
		21	—$CH_2CH=CH_2$	—$CH_2CH=CH_2$
		22	—H	—$CH_2C_6H_5$
		23	—H	—$(CH_2)_4CH_3$
		24	—CH_2CH_3	—$(CH_2)_5CH_3$
		25	—CH_3	—$(CH_2)_2CH_3$
		26	—$(CH_2)_2CH_3$	—$CH_2CH=CH_2$
		27	—H	—$(CH_2)_3N(CH_2CH_2CH_3)_2$
		28	—H	—$CH_2CH=CH_2$
VII	—NH—$CH(C_2H_5)CH_2NR_1R_2$	29	—CH_2CH_3	—CH_2CH_3
		30	—$(CH_2)_2CH_3$	—$(CH_2)_2CH_3$
		31	—$(CH_2)_3CH_3$	—$(CH_2)_3CH_3$
		32	—CH_2CH_3	—$(CH_2)_3CH_3$
		33	—$CH_2CH(CH_3)_2$	—$CH_2CH(CH_3)_2$
VIII	—NH—$CH_2CH(C_2H_5)NR_1R_2$	34	—$CH_2CH=CH_2$	—$CH_2CH=CH_2$
IX	—NH—$CH(CH_3)C(CH_3)_2NR_1R_2$	35	—CH_3	—$(CH_2)_2CH_3$
X	—NH-2-quinolyl	36	6-methoxy-2-quinolyl-	
		37	6-chloro-2-quinolyl-	

TABLE I (*Concluded*)

Derivative	M. p., °C.	N, % Calcd.	N, % Found		Cl, % Calcd.	Cl, % Found
2HCl	168–170	10.48	10.50	10.70	17.65	17.5
2HBr	213–217	8.87	8.86	8.78	33.45	33.29
2HCl	174–176	9.48	9.60		16.0	16.0
2HCl	118–119	10.11	10.00	9.80	17.05	16.9
$2HCl\cdot2H_2O$	218–219	10.62	10.71	10.76	18.45	18.58
$3HCl\cdot3H_2O$	234–235	11.36	11.68	11.59	21.54	21.59
2HCl	180	10.11	9.87	9.76	17.03	16.86
2HCl	215–217	9.47	9.27	9.22	15.97	15.99
2HCl	236	10.11	10.39	10.41	17.00	17.01
2HCl	230	10.69	10.42	10.34	9.02	8.91
HCl	231	11.30	11.15	11.07	19.03	19.01
$H_2PtCl_6\cdot8H_2O$	205	[b]	[b]	[b]	22.90	22.98
2HCl	235	10.11	10.23	10.34	17.02	17.00
Base[c,g]	Oil	10.16	10.33	10.41	...	...
HCl	234	10.69	10.58	10.67	9.02	8.96
2HCl	154–155	9.48	9.68		15.96	15.80
2HCl	206–207	11.67	11.56	11.55	19.65	19.60
2HCl	216–219	12.47	12.30	12.26	10.53	10.36
HCl	220	11.10	10.91	10.91	9.35	9.10
HCl	226–229	11.98	11.93	11.95	10.09	10.24
2HCl	232–233	10.55	10.59	10.72	17.78	17.74
HCl	235	11.32	11.23	11.53	9.55	9.35
2HCl	230	10.83	10.82	10.62	18.23	18.01
HCl	237	10.69	10.47	10.82	9.02	8.92
2HCl	232	11.23	10.93	10.87	18.93	18.85
HCl	234	11.58	11.50	11.71	9.77	9.93
2HCl	235	12.21	12.28	12.30	15.43	15.87
2HCl	229	11.73	11.63	11.59	19.76	19.80
HCl	230–231	12.48	12.41	12.34	10.51	10.35
HCl	224–225	11.08	10.90	10.88	9.35	9.15
$H_2PtCl_6\cdot6H_2O$[c]	...	[e]	[e]	[e]	24.81	24.87
$H_2PtCl_6\cdot6H_2O$[c]	...	[f]	[f]	[f]	25.12	24.87
2HCl	237	9.77	9.75	9.78	16.47	16.80
HCl·	233	11.63	11.65	11.70	9.82	9.49
HCl	231	11.50	11.50	11.27	9.72	9.64
HCl	236	11.45	11.58	11.64	9.66	9.90
2HCl	282	10.29	10.48	10.65	17.33	17.48

[a] Derivative of 6-hydroxy-8-aminoquinoline. [b] Pt, calcd., 20.98; found, 21.04, 21.24. [c] The hydrochlorides of these products were not obtained crystalline. [d] Could not be prepared. The dialkylaminoalkyl chloride, being a tertiary halide, dehydrohalogenated instead of condensing. [e] Pt, calcd., 22.72; found, 22.95. [f] Pt, calcd., 23.02; found, 23.10. [g] The free base boiled at 200–205° at 0.3 mm.

chemicals supplied by the Commercial Solvents Corporation.

Experimental

Preparation of Intermediates.—The 6-methoxy-8-aminoquinoline was prepared in four steps from *p*-anisidine with over-all yields of 45%.[4] The chloroamine hydrochlorides were prepared from the corresponding aminoalcohol hydrochlorides by reaction with thionyl chloride in dry chloroform at reflux temperatures. After destruction of the excess thionyl chloride with methanol the volatile materials were removed under vacuum and the residual chloroamine hydrochlorides used without further purification.

Condensations with 6-Methoxy-8-aminoquinoline.—The condensations with chloroamine hydrochlorides were accomplished successfully in several different ways, but the most satisfactory method consisted in heating approximately equimolecular amounts of the reactants together in an alcoholic solvent such as butanol. Usually, the lowest temperature causing reasonably complete condensation within two or three days gave the best results. With the aliphatic chloroamines 60° was satisfactory, but with the chloroquinolines 140° was necessary. Yields were quite variable depending on the chloroamine used. The product hydrochlorides crystallized from the cooled reaction mixture. Often crystallization was slow and in some cases no crystalline hydrochloride was obtained. In such cases the free base was isolated, distilled under high vacuum, and analyzed as such or as its chloroplatinate. All products had a yellow or orange color.

(4) *Cf.* Rohrmann and Shonle, THIS JOURNAL, **66**, 1641 (1944).

Summary

New derivatives of 6-methoxy-8-aminoquinoline have been prepared. Most of these contain the dialkylaminoethyl side chain variously substituted. Two are substituted diquinolyl amines.

LAFAYETTE, INDIANA RECEIVED JULY 31, 1945

[CONTRIBUTION FROM THE WESTERN REGIONAL RESEARCH LABORATORY[1]]

Reaction of Formaldehyde with Proteins. II. Participation of the Guanidyl Groups and Evidence of Crosslinking

BY HEINZ FRAENKEL-CONRAT AND HAROLD S. OLCOTT

The reaction of formaldehyde with amino acids and proteins has received renewed attention and both scientific and practical application in recent years. In contrast to older aldehyde tanning procedures, elevated temperatures and acid reaction media have been found suitable for the hardening of casein fibers.[2] Such conditions have been shown to favor the reaction of the primary amide groups of proteins with formaldehyde.[3] It has long been recognized that primary amino groups are involved in this reaction. With the use of protamine as a model substance, evidence has now been obtained that guanidyl groups bind formaldehyde in neutral and acid solutions at 70°, a reaction which at room temperature has been described as proceeding readily only in the alkaline *p*H range.[4]

The tanning action of formaldehyde on proteins has never been clearly understood. It was at one time believed that it might be due to the transformation of the original amino groups to Schiff bases ($-NH_2 + HCHO \rightarrow -N{=}CH_2 + H_2O$). More recent evidence[5] favors a simple addition reaction, leading to imino or imido methylol ($-NH-CH_2OH$) groups, but the marked decrease in hydrophilic tendency and increase in strength produced in proteins through tannage cannot readily be explained on the basis of either of these reactions. The hypothesis that has received most general acceptance is that formaldehyde sets up crosslinks by secondary condensation of the methylol groups with other reactive hydrogen atoms, similar to that which occurs in the Mannich reaction ($-NH-CH_2OH + HR \rightarrow -NH-CH_2-R + H_2O$).[6] It was not until recently that chemical evidence for a condensation reaction was obtained by Nitschmann and Hadorn,[7] who showed that there was a loss in weight, presumably water, accompanying the addition of formaldehyde to the casein molecule. While we were able to confirm this observation, it is recognized that the formation of Schiff bases would also lead to a loss of water. Further, the condensation reaction might involve the amino and peptide groups of the same lysine residue and thus might not lead to crosslinking of adjacent peptide chains.

It is apparent that intermolecular crosslinking through formaldehyde causes an increase in the average molecular weight of proteins. The decreased solubility of treated proteins may be attributed to this phenomenon. This insolubility on the other hand has probably discouraged quantitative investigations of the molecular weights of aldehyde derivatives of proteins. The protamine (salmine) used in the present study of the reactivity of guanidyl groups remained largely soluble when dilute solutions (1–3%) were treated with formaldehyde. In contrast to untreated protamine, the aldehyde derivative prepared at *p*H 6–7 was partially retained by cellulose bags upon dialysis. Osmotic pressure measurements showed that this material was of considerably greater average molecular weight than the untreated material. These observations constitute evidence for the formation of intermolecular crosslinks by formaldehyde under the experimental conditions used.

Experimental

Reaction of Protamine with Formaldehyde.—The conditions generally used were as follows: To 1 g. of protamine sulfate[8] dissolved in 80 ml. of warm water there was added 10 ml. of 3.4 *M* phosphate buffer (*p*H 7.6), or 3 *M* acetic acid, and 10 ml. of commercial 40% formaldehyde solution. The mixtures were held at 70° for four days; these conditions led to the establishment of equilibrium. The concentrations were not critical. Similar products were obtained with protamine concentrations up to 3% and with 16% formaldehyde.

The mixtures were found to be close to *p*H 6.5 or 2.8, respectively, at the end of the heating period. When the reaction proceeded in the absence of phosphate buffer, the solution dropped from neutrality to below *p*H 4. Small amounts of insoluble matter were filtered off before the reaction mixtures were analyzed. The approximate amount of formaldehyde bound by protamine at equilibrium was determined by measurements of the difference between the total and the free formaldehyde in the reaction mixture.[3] To this end an aliquot of the solution was hydrolyzed with sulfuric acid and the aldehyde distilled into

(1) Bureau of Agricultural and Industrial Chemistry, Agricultural Research Administration, U. S. Department of Agriculture. Article not copyrighted.

(2) Franz, Riederle, Fleischmann and Winkler, *J. prakt. Chem.*, **160**, 133 (1942).

(3) Fraenkel-Conrat, Cooper and Olcott, THIS JOURNAL, **67**, 950 (1945); Wormell and Kaye, *J. Soc. Chem. Ind.*, **64**, 75 (1945).

(4) Hegman, *J. Am. Leather Chem. Assoc.*, **37**, 276 (1942); Salcedo and Highberger, *ibid.*, **36**, 271 (1941).

(5) Levy and Silberman, *J. Biol. Chem.*, **118**, 783 (1937).

(6) (a) Gustavson, *Kolloid Z.*, **103**, 43 (1943); (b) Smith, Handler and Mrgudich, *J. Phys. Chem.*, **44**, 874 (1940).

(7) Nitschmann and Hadorn, *Helv. Chem. Acta*, **27**, 299 (1944).

(8) Approximately pure salmine sulfate, kindly supplied by Eli Lilly and Co.