Experimental

Manometric Experiments.—As a representative example, the details of an experiment on the reaction between 100 μmoles NH_2OH and 4 μmoles GB in 0.025 *M* bicarbonate are described. Suitable variations were introduced in this standard technique as needed.

$NH_2OH \cdot HCl$ (69.5 mg., 1 mmole) was dissolved in 5 ml. of H_2O, neutralized to phenol red and diluted to 10 ml. One ml. was pipetted into a standard Warburg conical flask equipped with one side arm and containing 1.0 ml. 0.05 *M* $NaHCO_3$ solution which had previously been equilibrated for 10 minutes with a rapid stream of 5% CO_2:95% N_2. The vessel, with the side-arm left unstoppered, was attached to its manometer and gassed for 10 minutes with the above gas mixture with occasional shaking. Shortly before the end of the gassing period, 0.128 ml. (140 mg., 1 mmole) of GB was dissolved to 25 ml. in water, 25 ml. of 0.05 *M* $NaHCO_3$ was added, and 0.2 ml. of this mixture was pipetted into the side arm. Immediately the side arm was stoppered and the manometer stopcock was turned off simultaneously. The manometer was transferred to the Warburg bath at 25° where the rate of gas evolution was read at intervals according to usual procedures.

All experiments included controls for spontaneous hydrolysis of GB, decomposition of NH_2OH, etc. Experiments in phosphate buffer were run with nitrogen as the gas phase unless otherwise noted. In some experiments NaOH or HCl (2.5 *N*, 0.2 ml.) were added to the center well of the vessel with a roll of filter paper to facilitate any gas absorption.

Reactivity was evaluated from the value of $t_{0.5}$, *i.e.*, the time necessary under stated conditions for 50% of the measured reaction to take place. This "half-time" is inversely proportional to reactivity.

Determination of NH_2OH. a. Colorimetrically.—A modification of Hestrin's procedure[7] was used, employing an excess of acetylcholine and limiting amounts of NH_2OH. An aliquot of the solution to be tested, containing no more than 5 μmoles of NH_2OH was added to a test-tube or calibrated colorimeter tube containing 1.0 ml. of acetylcholine bromide (0.5 *M*, kept cold when not in use) and water to yield a total volume of 3.0 ml. Exactly 30 seconds after introduction of the sample, 1.0 ml. of 1.5 *N* NaOH was added, the contents were mixed and allowed to stand for at least one minute (standing up to 1 hour has no effect). Then 1.0 ml. of 2 *N* HCl was added, mixing was repeated (standing up to 20 minutes has no effect) and 1.0 ml. of color reagent (10% $FeCl_3 \cdot 6H_2O$ in 0.1 *N* HCl) was added from a buret with shaking. The red color, which is stable for about 15 minutes, was read in the Klett–Summerson photoelectric colorimeter with filter 54. The linear range is from 0–6 μmoles NH_2OH per sample, and the sensitivity approximately 80 Klett units per μmole.

b. Manometric.—At the time this work was in progress, Colter and Quastel[13] described the reaction: $2NH_2OH + 2MnO_2 \rightarrow N_2O + 2MnO + 3H_2O$, which permits determination of NH_2OH by the release of 0.5 mole of N_2O for each mole NH_2OH present. This reaction was carried out in phosphate buffer with N_2 in the gas phase. The side arm contained 0.2 ml. of a 10% suspension of MnO_2 in buffer. Destruction of NH_2OH was essentially complete within 15–20 minutes at 25°.

Determination of Ammonia.—This was carried out on reaction mixtures which had previously been freed of excess NH_2OH as described above. The vessel contents were filtered through Whatman #42 filter paper, diluted and analyzed by direct Nesslerization.[14]

Determination of Cholinesterase Activity.—The standard manometric procedure, with 0.015 *M* acetylcholine as substrate at 38° in bicarbonate buffer, was used. This method is sufficiently well known (*e.g.*, ref. 15) that it need not be described in detail.

Acknowledgments.—The author wishes to thank Dr. W. R. Kirner, Chemical-Biological Coördination Center, National Research Council, for the gift of certain hydroxylamine derivatives, and Mr. Reuben Proper for the synthesis of others.

(13) J. S. Colter and J. H. Quastel, *Arch. Biochem.*, **27**, 368 (1950).

(14) The presence of NH_2OH was found to interfere with NH_3 determinations by direct Nesslerization or by microdistillation using the Conway dish technique; prior destruction of NH_2OH in the Conway dish with acetylcholine or triacetin was also unsuccessful. Kjeldahl procedures could not be applied owing to the small quantities of NH_3 involved.

(15) B. J. Jandorf and P. D. McNamara, *J. Pharmacol. Exp. Therap.*, **48**, 77 (1950).

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[CONTRIBUTION FROM DANIEL SIEFF RESEARCH INSTITUTE, WEIZMANN INSTITUTE OF SCIENCE]

Conformational Analysis of Certain Morphine Derivatives[1]

BY DOV ELAD AND DAVID GINSBURG[2]

RECEIVED DECEMBER 9, 1955

The conformations of the epimeric sets of alcohols—dihydrocodeine (I), dihydroisocodeine (II), and dihydropseudocodeine (IIIa), dihydroallopseudocodeine (IVa), are discussed in the light of previous work. The rates of saponification of the respective acetates support the formulation of I and IVa as the axial isomers and II and IIIa as the equatorial isomers.

The relative rates of saponification of dihydrocodeine (I) and dihydroisocodeine (II), of dihydropseudocodeine (IIIa) and dihydroallopseudocodeine (IVa), and of dihydrothebainol A (IIIb) and dihydrothebainol B (IVb) were studied in order to provide additional evidence for the conformations of these epimers.

Previous work has shown that conformational considerations apply equally well in heterocyclic systems as in alicyclic systems.[3–5]

Catalytic reduction of dihydrocodeinone gives dihydrocodeine,[6] whereas aluminum isopropoxide reduction yields dihydroisocodeine.[7]

Results of oxidation experiments on the epimeric alcohols I, II, IIIa and IVa[8] and Rapoport's stereochemical correlation of the various asymmetric centers of the morphine molecule,[9] indicate that I and IVa are the axial isomers while II and IIIa are the equatorial isomers.

The relative rates of saponification of the acetates of these alcohols reported in the Experimental section further support these formulations.

Two epimeric dihydrothebainols have been re-

(1) Presented at XIVth Congress of Pure and Applied Chemistry, Zurich, July, 1955.

(2) Israel Institute of Technology, Haifa, Israel. Inquiries should be addressed to this author.

(3) D. H. R. Barton, *J. Chem. Soc.*, 1027 (1953).

(4) R. E. Reeves, *Adv. in Carbohydrate Chem.*, **6**, 107 (1951); J. T. Edwards, *Chemistry and Industry*, 1102 (1955).

(5) G. Fodor and K. Nador, *J. Chem. Soc.*, 721 (1953); D. Ginsburg, *U. N. Bulletin of Narcotics*, **6**, 32 (1954); A. K. Bose, *Chemistry and Industry*, 130 (1954).

(6) L. F. Small, H. M. Fitch and W. E. Smith, THIS JOURNAL, **58**, 1458 (1936).

(7) M. M. Baizer, *et al.*, *J. Org. Chem.*, **16**, 543 (1951).

(8) H. Rapoport, *et al.*, *ibid.*, **15**, 1103 (1950).

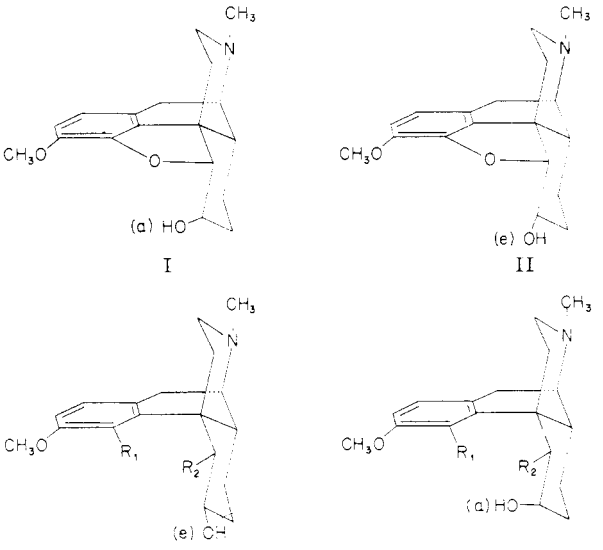
(9) H. Rapoport and J. B. Lavigne, THIS JOURNAL, **75**, 5329 (1953), in which previous references are given.

TABLE I

Alcohol	M.p., °C.	$[\alpha]^{28}D$	c	O-Acetate M.p., °C.	$[\alpha]^{28}D$	c	Solvent of crystn.	Yield, %[a] $LiAlH_4$	Yield, %[a] $NaBH_4$	Analyses, % Found: C	H	N
Dihydrocodeine	86–87	−132°	2.7	116–117	−130°	2.75	Ether	68	64	69.64	7.18	4.00[b]
Dihydroisocodeine	199–200	−136	2.55	163–164	−163	2.18	Ether	27	34	70.06	7.42	4.06[b]
Dihydro-ψ-codeine	155	− 49	2.21	121–122	− 66	2.62	Hexane	..	54	70.14	7.26	3.96[b]
Dihydroallo-ψ-codeine	75–76	−108	2.35	141	− 87	0.69	Hexane	80	44	69.76	7.42	3.88[b]
Dihydrothebainol A	142		..	187–189	− 25	1.7	.....	..	..	69.68	7.66	4.02[c]
Dihydrothebainol B	166		..	108	− 5.6	1.6	.....	..	..	69.42	7.92	3.84[c]

[a] Over-all yield of acetate based on ketone reduced. [b] In all cases calculated for $C_{20}H_{25}NO_4$: C, 69.95; H, 7.33; N, 4.08. [c] In these cases calculated for $C_{20}H_{27}NO_4$: C, 69.54; H, 7.88; N, 4.06.

ported. Dihydrothebainol A (IIIb) is obtained by electrolytic reduction of dihydrothebainone,[10] whereas dihydrothebainol B (IVb) is obtained by reduction of the ketone in the presence of a platinum catalyst and acid.[11] It has been shown that both of these epimers are oxidized with equal facility to the ketone by means of the benzophenone–potassium *t*-butoxide system and reduction of dihydrothebainone with either lithium aluminum hydride or sodium borohydride gives a mixture of the epimeric alcohols.[12]



IIIa, IVa: $R_1 = R_2 =$ —O—
IIIb, IVb: R_1 = OH; R_2 = H

Saponification of the corresponding acetates has now shown that dihydrothebainol A is the equatorial isomer and B is the axial one (Table IV).

Experimental

Melting points are uncorrected. Rotations were measured in chloroform solution.

Lithium Aluminum Hydride Reduction. General Procedure.—The ketone (500 mg.) was added to a suspension of lithium aluminum hydride (500 mg.) in dry tetrahydrofuran (50 ml.) and the mixture was refluxed for 5 hr. The solvent was removed under reduced pressure and the residue was treated with cold water. The precipitate was removed by filtration and was washed several times with chloroform. The aqueous phase was extracted with chloroform, the chloroform extracts were combined and the solvent was removed under reduced pressure. The residue was esterified and chromatographed as described below. The mixture of crude alcohols was obtained in 95% yield in the case of dihydrocodeinone reduction and 90% yield in the case of dihydropseudocodeinone reduction.

Sodium Borohydride Reduction—General Procedure.—The ketone (400 mg.) was dissolved in methanol (30 ml.) and sodium borohydride (1 g.) was added. After standing at room temperature for 1 hr. the solution was concentrated to one-half its volume under reduced pressure. Sodium hydroxide solution (10%, 20 ml.) was added and the mixture was momentarily heated to boiling.[13] After dilution with water and extraction with chloroform, the chloroform was removed under reduced pressure. The residue was esterified and chromatographed as described below. The mixtures of crude alcohols were obtained in 95–98% yield.

Esterification—General Procedure.—The alcohol (50 mg.) was dissolved in pyridine (1 ml.) and treated with acetic anhydride (0.5 ml.). The mixture was left at room temperature overnight. Water (1 ml.) was added and the solvents removed under reduced pressure. The residue was treated with sodium bicarbonate solution and extracted with ether. Removal of the ether left a residue which was dissolved in benzene and chromatographed over alumina (Eimer and Amend, adsorption grade). Benzene–chloroform and chloroform failed to elute any substance in each case, but material came off the column first with methanol (1.5%)–chloroform and finally methanol (2%)–chloroform. Dihydroisocodeine acetate, was eluted before dihydrocodeine acetate. Dihydropseudocodeine acetate was eluted before dihydroallopseudocodeine acetate.

The pertinent data regarding these compounds are given in Table I. The acetates obtained after elution were in all cases identical with those obtained by acetylation of authentic specimens of the alcohols supplied by Dr. L. F. Small.

TABLE II

RATES OF HYDROLYSIS OF ACETATES OF DIHYDROCODEINE AND DIHYDROISOCODEINE

Time, min.	Hydrolysis, % Dihydroiso-	Dihydro-
6	4	4
36	34	13
66	45	28
120	55	39
240	67	52
480	73	60
1440	88	72

TABLE III

RATES OF HYDROLYSIS OF ACETATES OF DIHYDROPSEUDOCODEINE AND DIHYDROALLOPSEUDOCODEINE

Time, min.	Hydrolysis, % Dihydropseudo-	Dihydroallopseudo-
9	21	0
39	31	0
60	66	0
150	90	0

TABLE IV

RATES OF HYDROLYSIS OF ACETATES OF DIHYDROTHEBAINOLS A AND B

Time, min.	Hydrolysis, % A	B
30	43	6
90	49	18
210	56	31
450	..	36

(10) E. Speyer and S. Siebert, *Ber.*, **54**, 1519 (1921).
(11) A. Skita, *et al.*, *ibid.*, **54**, 1560 (1921).
(12) D. Elad and D. Ginsburg, *J. Chem. Soc.*, 3052 (1954).
(13) *Cf.* M. Gates, THIS JOURNAL, **75**, 4310 (1953).

When acetylation of authentic samples of dihydrocodeine and dihydroisocodeine is carried out as described above but the sodium bicarbonate treatment is omitted, the N,O-diacetates are obtained.

Dihydrocodeine N,O-diacetate, m.p. 106–108° (from ether). *Anal.* Calcd. for $C_{22}H_{29}NO_6$: C, 65.49; H, 7.25; N, 3.47. Found: C, 65.43; H, 7.21; N, 3.59.

Dihydroisocodeine N,O-diacetate, m.p. 162–163° (from ether). *Anal.* Found: C, 65.61; H, 7.39; N, 3.49.

Both give m.p. depressions when admixed with the O-acetates, m.p. 116–117° and 163–164°, respectively. Titration of each with sodium methoxide,[14] showed the presence of exactly one mole of acetic acid bound to nitrogen.

Saponification of Acetates—General Procedure.—The ester (30 mg.) was dissolved in ethanol (1–3 ml.) and the solution was made up to a volume of 10 ml. with 0.01 *N* sodium hydroxide solution. Aliquots of 1 ml. each were titrated from time to time with 0.01 *N* hydrochloric acid (phenolphthalein indicator).

Acknowledgment.—We thank Dr. L. F. Small for samples of all the codeine derivatives which made it possible for us to carry out this work.

REHOVOT, ISRAEL

(14) J. S. Fritz and N. M. Lisicki, *Anal. Chem.*, **23**, 589 (1951).

[CONTRIBUTION FROM THE NUTRITION AND PHYSIOLOGY SECTION, RESEARCH DIVISION, AMERICAN CYANAMID CO., LEDERLE LABORATORIES]

Syntheses of 6-Substituted Purines

BY MILON W. BULLOCK, JOHN J. HAND AND E. L. R. STOKSTAD

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Several purine derivatives which are related to kinetin (6-furfurylaminopurine) have been prepared by condensing 6-chloropurine with amines. Analogs in which the 6-amino group was replaced by a sulfur atom have been prepared by treating 6-chloropurine with sodium mercaptides. The ultraviolet absorption spectra of the purine derivatives have been determined in 0.1 *N* hydrochloric acid, 0.1 *N* sodium hydroxide and in a neutral solution. Methods for the convenient preparation of some of the intermediates are presented. A modification of the conventional method of lithium aluminum hydride reductions in which only enough water is added to cause the separation of the salts as a solid phase has simplified this method of reducing nitriles. A method for preparing 2-mercaptomethyltetrahydrothiophene directly from tetrahydrofurfuryl alcohol was investigated.

The recent isolation,[1] structure determination and synthesis[2] of kinetin, 6-furfurylaminopurine, the cell division factor from deoxynucleic acid, has prompted us to prepare several analogs with the hope of obtaining an antimetabolite or competitive antagonist to kinetin which would inhibit cell mitosis and possibly be useful for cancer therapy.

Miller, *et al.*,[2] synthesized kinetin by the general method of Elion, *et al.*,[3] by treating 6-methylmercaptopurine with furfurylamine. In these Laboratories we have synthesized kinetin and several analogs by a modification of the basic method of Albert and Brown.[4] We have found that condensing 6-chloropurine with at least two equivalents of the amine in the presence of a high boiling solvent such as methyl Cellosolve gives excellent yields of pure products.

Analogs in which the 6-amino group on the purine nucleus was replaced by a sulfur atom were also conveniently prepared by allowing 6-chloropurine to react with a sodium mercaptide under similar conditions. This method appears to give about the same yields as the method of Elion, Burgi and Hitchings,[3] in which the alkyl halide is condensed with the sodium salt of 6-mercaptopurine.

The melting points and analyses of the 6-substituted purine derivatives which were prepared are given in Table I. The letters in the method column refer to the general procedures illustrated in the Experimental section. The biological activities of the compounds will be published elsewhere.

The ultraviolet absorption spectra of these compounds have been determined with a Cary recording spectrophotometer in 0.1 *N* sodium hydroxide, 0.1 *N* hydrochloric acid and in water or ethanol. Many of the compounds are so insoluble in water that the determination of the absorption spectrum in this medium was impractical. The molecular extinctions are summarized in Table II.

Most of the amines which were required as intermediates in this work and which were unavailable commercially were prepared by reducing the corresponding nitrile with lithium aluminum hydride in ether. 3-Aminomethylpyridine was prepared by reducing 3-cyanopyridine catalytically since the lithium aluminum hydride reduction was unsatisfactory. N-Methylfurfurylamine was prepared by a slight modification of the method of Schwabbauer.[5] The methods of preparation, yields and physical constants of the amines are summarized in Table III.

Acknowledgments.—The authors are indebted to Mr. L. Brancone and staff for the microanalyses and to Mr. H. Lewry for the ultraviolet absorption spectra.

Experimental[6]

Since the procedures used to prepare compounds in any one series are nearly identical, only one example will be given to illustrate each procedure.

***m*-Methylbenzylamine (A).**—A solution of 100 g. (0.854 mole) of *m*-tolylnitrile in 150 ml. of anhydrous ether was added to a stirred suspension of 38 g. (1 mole) of lithium aluminum hydride in 1 liter of ether at such a rate as to maintain a vigorous reflux. The addition required 30 minutes. The suspension was refluxed an additional 30 minutes. The heating mantle was replaced by an ice-bath, and 20 ml. of water was added dropwise from a dropping funnel. Sodium hydroxide solution (20%) was then added until a granular solid second phase was present and the ether solution was

(1) C. O. Miller, F. Skoog, M. H. von Saltza and F. M. Strong, THIS JOURNAL, **77**, 1392 (1955).

(2) C. O. Miller, F. Skoog, F. S. Okumura, M. H. von Saltza and F. M. Strong, *ibid.*, **77**, 2263 (1955).

(3) G. B. Elion, E. Burgi and G. H. Hitchings, *ibid.*, **74**, 411 (1952).

(4) A. Albert and D. J. Brown, *J. Chem. Soc.*, 2060 (1954).

(5) G. Schwabbauer, *Ber.*, **35**, 410 (1902).

(6) Melting points are uncorrected.