hydrazine while the oxidation mixture was heated to boiling. A 2,4-dinitrophenylhydrazone formed which melted at 109–116° after two crystallizations from ethanol.

Anal. Calcd. for $C_{10}H_{12}N_4O_4$: C, 47.62; H, 4.80; N, 22.21. Found: C, 47.76; H, 4.34; N, 21.96.

A mixed melting point with authentic methyl ethyl ketone 2,4-dinitrophenylhydrazone, m.p. 109–117°, was 109–116°.

The oxidation product from a similar experiment was converted to a semicarbazone melting at 142–143° after sublimation at 130–145° (1 mm.).

Anal. Calcd. for $C_6H_{11}N_3O$: C, 46.49; H, 8.59; N, 32.54. Found: C, 46.67; H, 8.71; N, 32.49.

A mixed melting point with authentic methyl ethyl ketone semicarbazone, m.p. 142–142.5°, was 141–142.5°.

Acetylation of Glaucarubol.—A 500-mg. sample of glaucarubol was dissolved in 2 ml. of pyridine and 4 ml. of acetic anhydride with warming. The clear solution was heated on a steam-bath for 1.5 hours. Removal of the solvents gave 760 mg. of a white solid. This was dissolved in benzene and chromatographed on 20 g. of acid-washed alumina. The column was washed with benzene and ether. Elution with 150 ml. of 35% ethyl acetate in ether gave 528 mg. of oil which was dissolved in 125 ml. of hot Skellysolve C. Cooling gave a precipitate which afforded 465 mg. of long white crystals, m.p. 199–201°, $[\alpha]^{25}D$ +23° (*c* 1.0 in pyridine), on standing for two days.

Anal. Calcd. for $C_{20}H_{23}O_8(CH_3CO)_5$: C, 59.40; H, 6.31; CH_3CO, 35.5. Found: C, 59.71; H, 6.23; CH_3CO, 36.

Hydrolysis of the Acetate of Glaucarubol.—A 100-mg. sample of the acetate of glaucarubol was treated with 3 ml. of 5% alcoholic potassium hydroxide and heated under reflux for 45 minutes, during which time the acetate dissolved. The solution was allowed to stand for one day. The ethanol was removed and the residue dissolved in water. Addition of acid gave 44 mg. of white crystals, m.p. 280–285°. A mixed melting point with glaucarubol was 278–285°. A comparison of the infrared absorption curves of the hydrolysis product and glaucarubol showed identity.

Aqueous Hydrochloric Acid Hydrolysis of Glaucarubol.—A 1.000-g. sample of finely ground glaucarubol was treated with 100 ml. of 0.1 *N* hydrochloric acid and the resultant slurry heated on a steam-bath. The clear solution obtained in 30 minutes was heated for an additional 30 minutes. The resultant solution was lyophilized and the residue taken up in chloroform and chromatographed on 25 g. of acid-washed alumina. The column was washed with 80 ml. of chloroform. Elution with 110 ml. of acetone gave 568 mg. of white glassy solid. A solution of the white solid in 10 ml. of ethylene dichloride, when allowed to stand overnight, gave 277 mg. of square platelet crystals. Two crystallizations from ethylene dichloride gave 229 mg. of white platelet crystals of pure glaucanol, m.p. 229–233°, $[\alpha]^{25}D$ +147° (*c* 0.4 in methanol) and $[\alpha]^{25}D$ −65° (*c* 1.0 in 0.1 *N* sodium hydroxide).

Anal. Calcd. for $C_{16}H_{20}O_5$ (292): C, 65.73; H, 6.89. Found: C, 65.54; H, 6.54; equiv. wt., 291.

Acetylation of Glaucanol.—A solution of 44 mg. of glaucanol in 1 ml. of pyridine and 1 ml. of acetic anhydride was heated on a steam-bath for 1.5 hours. The solvents were removed and the residue taken up in benzene and chromatographed on 5 g. of acid-washed alumina. Elution with 150 ml. of benzene gave 48 mg. of a white solid. Two crystallizations from Skellysolve C gave 44 mg. of white crystals, m.p. 210–211°.

Anal. Calcd. for $C_{16}H_{17}O_5(CH_3CO)_3$ (418): C, 63.16; H, 6.26; CH_3CO, 31. Found: C, 63.53; H, 5.96; CH_3CO, 32; sapon. equiv., 104.

Acknowledgment.—The authors wish to thank Mrs. Helen Gager and Mr. F. A. Bacher for the potentiometric titrations, Miss Jean Carr and Dr. J. B. Conn for the molecular weight determinations and Mr. Robert Walker and Dr. N. R. Trenner for the infrared measurements. Acknowledgment is due to Mr. R. N. Boos and his associates for the microanalyses reported. We are grateful to Mr. B. A. Krukoff for his advice and coöperation on botanical matters.

RAHWAY, NEW JERSEY

[CONTRIBUTION FROM THE CHEMICAL LABORATORY, UNIVERSITY OF NEW BRUNSWICK]

Garrya Alkaloids. III. The Skeletal Structure of the Garrya Alkaloids

BY K. WIESNER, R. ARMSTRONG, M. F. BARTLETT AND J. A. EDWARDS

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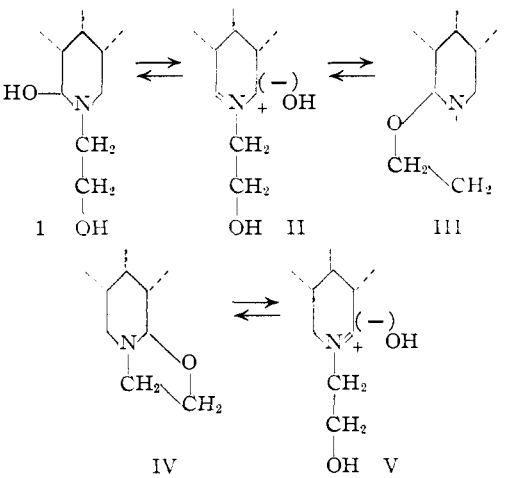
On the basis of oxidation as well as dehydrogenation experiments the diterpenoid structure VI related to phyllocladene is proposed for dihydroveatchine.

Introduction

In our previous communications on the Garrya alkaloids, garryine and veatchine,[1,2] we have shown that these two bases can be represented by the partial structures I, II and III for garryine and IV and V for veatchine.

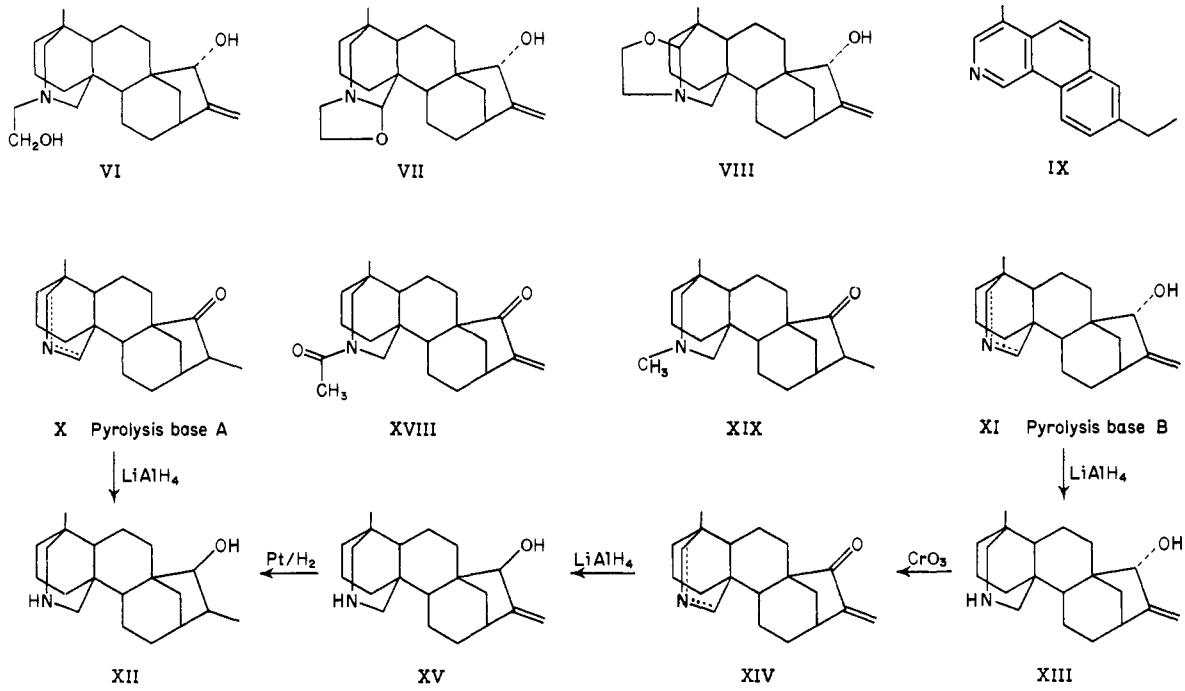
In the present communication we propose the structure VI for dihydroveatchine and structures VII and VIII for veatchine and garryine in their anhydrous form. The considerations which led us to adopt these structures were in part reported in a preliminary note[3] and were as follows: Selenium dehydrogenation at 340° gave a good yield of 1-methyl-7-ethylphenanthrene and a compound $C_{15}H_{15}N$ which was recognized as an azaphenanthrene by its ultraviolet absorption. It was at first considered to be a phenanthridine, but it is



(1) K. Wiesner, S. K. Figdor, M. F. Bartlett and D. R. Henderson, *Can. Jour. Chem.*, **30**, 608 (1952).

(2) K. Wiesner, W. I. Taylor, S. K. Figdor, M. F. Bartlett, J. R. Armstrong and J. A. Edwards, *Ber.*, **86**, 800 (1953).

(3) K. Wiesner, R. Armstrong, M. F. Bartlett and J. A. Edwards, *Chemistry and Industry*, 132 (1954).



Configuration of secondary hydroxy groups as in garryine is represented as - - -OH.

now necessary to revise this view. Dr. Hughes and Mr. Nathan at the Gates and Crellin Laboratories, California Institute of Technology, have kindly performed an X-ray diffraction analysis of the azaphenanthrene and have made it probable that irrespective of the position of the nitrogen the substitution is 1-methyl-7-ethyl. Our previous isolation of 1,2,3,4-benzenetetracarboxylic acid by permanganate oxidation of the azaphenanthrene in conjunction with the probable substitution of this compound, and biogenetic considerations, prompted us to propose[3] the structure IX for the azaphenanthrene, an assumption which we have recently confirmed by synthesis.[4]

At this stage we do not yet wish to enter into the arguments which of the structures VII and VIII should be assigned to garryine and which to veatchine. However, for convenience the formula VII will be used to represent veatchine. There is some reason to believe that this assignment will prove to be correct.

The pyrolysis bases A and B described previously may now be represented by the structures X and XI. The location of the Δ^1-piperideine double bond is still left open. Reduction of X and XI with lithium aluminum hydride gives tetrahydrobase A and dihydrobase B. We may now represent these compounds by XII and XIII. Compound XIII has been converted into dihydroveatchine VI by treatment with 2-chloroethanol. On the other hand, compound XII on treatment with the same reagent does not give tetrahydroveatchine but a product isomeric with it. This has been explained by assuming that the secondary hydroxy group formed by hydride reduction of the keto group in X has the opposite configuration as compared to the secondary hydroxy group in the original alkaloids. However, as the identity of the skeletal structure of X and XI is essential for many arguments presented in the sequel, an experimental connection was established in the following manner.

Compound XIII was oxidized by chromic acid in pyridine. This gave a compound $C_{20}H_{27}ON$ represented by the formula XIV. The ultraviolet spectrum of this compound showed a maximum at 236 mμ (log ϵ 4) indicating an α,β-unsaturated ketone. The infrared spectrum showed no bands in the OH and NH region, but had two strong bands in the carbonyl region. One of them at 1650 cm.$^{-1}$ is identical with the C=N peak of the bases X and XI. The other at 1730 cm.$^{-1}$ is the band of the ketonic carbonyl in the five-membered ring in conjugation with the exocyclic double bond.[5] Lithium aluminum hydride reduction of XIV gave a tetrahydroproduct XV, $C_{20}H_{31}ON$, which showed only end absorption in the ultraviolet, no carbonyl but a strong OH band in the infrared spectrum. This product proved to be isomeric but not identical with compound XIII. This had been expected since it was already assumed that hydride reduction of the keto group in X produces a secondary alcohol with the opposite configuration of the hydroxy group, as compared to the configuration of this group in the original alkaloids. Hydrogenation of XV with platinum oxide in acetic acid gave compound XII, identified by mixed melting point and infrared spectrum. This sequence thus establishes a direct experimental connection between compound X and dihydroveatchine and demon-

(4) M. F. Bartlett and K. Wiesner, *Chemistry and Industry*, in press.

(5) Careful measurements of the carbonyl maximum in the infrared spectra of various derivatives of veatchine possessing an unconjugated keto group in the five-membered ring gave values ranging between 1738 and 1743 cm.$^{-1}$. In view of the smallness of the shift between the frequency of the conjugated and unconjugated ketone, independent evidence of the relative positions of the secondary hydroxy group and of the exocyclic methylene group was considered necessary and will be presented later in this paper.

strates the presence of the unchanged garrya skeleton in base X.

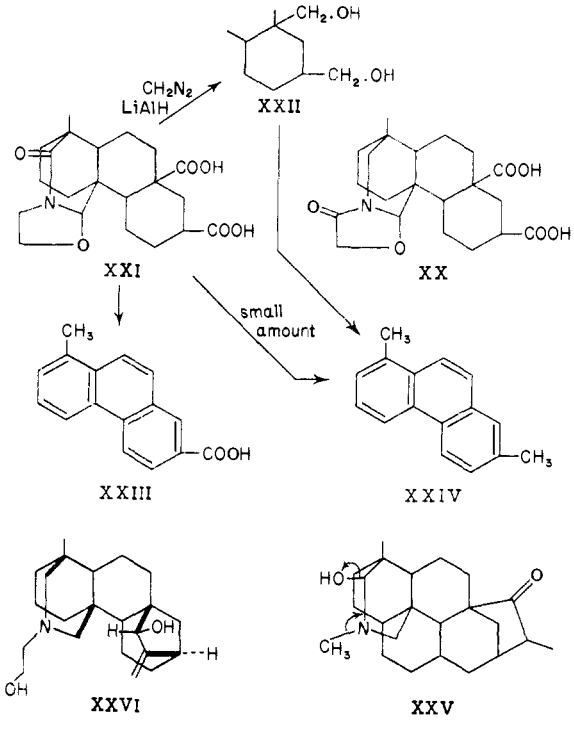
The oxidation of compound XII with chromic acid in pyridine gave a base $C_{20}H_{31}ON$ (XVI) which in the infrared spectrum showed an OH band, a >C=N— (at 1650 cm.$^{-1}$) and no carbonyl. We conclude from these data that the action of chromic acid consisted in the abstraction of two hydrogen atoms from the secondary piperidine base to yield a Δ^1-piperideine(tetrahydropyridine), a course of oxidation similar to the biochemical oxidation of proline and presumably pipecolic acid.

By acetylation and mild alkaline hydrolysis of compound XIII its N-acetyl derivative was prepared (XVII). By oxidation of this compound with chromic acid in pyridine a product, $C_{22}H_{31}NO_2$ (XVIII), was obtained with the same ultraviolet absorption spectrum as base XIV. The infrared spectrum of this amide showed no hydroxyl band and peaks for the conjugated five-membered ring ketone (1730 cm.$^{-1}$) and the amide carbonyl (1638 cm.$^{-1}$).

We have prepared also **N-methyl dihydrobase A (XIX)**. This compound has as the only unsaturation the carbonyl group in the five-membered ring and it was considered to be the most suitable derivative for the determination of the infrared frequency of this grouping. It was obtained by preparing the methiodide of base A (X) and reducing it with sodium borohydride. This reagent[6] reduces only the quaternary Schiff base grouping and leaves the carbonyl group in the five-membered ring untouched. The infrared spectrum of the resulting product $C_{21}H_{33}ON$ showed a single carbonyl maximum at 1740 cm.$^{-1}$ in agreement with an unconjugated keto group in a five-membered ring.

Further insight into the structure and location of the five-membered ring which carries the secondary hydroxy group and the exocyclic double bond was gained by more vigorous oxidation of veatchine by permanganate. Countercurrent distribution of the acidic products gave two beautifully crystalline lactam dicarboxylic acids A and B, $C_{21}H_{29}O_6N$, differing precisely in the same way as the two oxoveatchines described in our previous papers. They obviously are derived by opening the ring carrying the secondary hydroxy group, with loss of the carbon of the exocyclic methylene group. We may formulate them (keeping in mind the convention respecting the structure of the oxide ring in veatchine and garryine) by structures XX and XXI. The free acids were difficult to analyze, but the empirical formulas were established by precisely checking analyses of the crystalline monoesters and the oily diesters, both of which could be sublimed in high vacuum. In agreement with structures XX and XXI the acids A and B gave, with diazomethane, dimethyl esters which by alkaline saponification gave monomethyl esters. The carbomethoxy group in these monoesters was resistant to further hydrolysis. The monoesters differed strongly in melting point. Infrared spectra of both diesters showed an ester carbonyl at 1726 cm.$^{-1}$. The lactam carbonyl bands were in diester A at 1705 cm.$^{-1}$ and diester B at 1650 cm.$^{-1}$.

(6) B. Witkop and J. B. Patrick, THIS JOURNAL, **75**, 4474 (1953).



No hydroxy band was present in the infrared spectrum of either compound. Acids A and B gave on refluxing with acetic anhydride the anhydrides A and B. These two compounds had the following peaks in the carbonyl region of the infrared spectrum: A, 1800, 1762, 1700 cm.$^{-1}$; B, 1800, 1775, 1640 cm.$^{-1}$ The last wave numbers belong to the lactam carbonyl, for A in a five-membered ring, for B in a six-membered ring. The first two wave numbers in both A and B are the two peaks of a substituted glutaric anhydride.[7] It can be seen that the findings discussed above confirm both the size of the carbocyclic ring which has been opened as well as the relative position of the exocyclic double bond and the secondary hydroxy group. The hindered nature of one of the two carboxy groups is also in agreement with the structure proposed.

The orientation of the five-membered carbocyclic ring with respect to the rest of the molecule follows from dehydrogenation experiments performed on the pure crystalline acid B (XXI) and on its reduction product. The dimethyl ester of acid B was reduced with lithium aluminum hydride and the resulting triol (XXII) was dehydrogenated with selenium at 340°. A good yield of pimanthrene, identified by mixed melting point of the trinitrobenzene complex and by ultraviolet and infrared spectra of the free hydrocarbon in comparison with an authentic specimen, was obtained. Dehydrogenation of the acid B in a 1-g. quantity yielded an acidic fraction which was esterified with diazomethane and purified by chromatography and one recrystallization of the ester. The infrared spectrum of this compound showed a band at 1722 cm.$^{-1}$ identical with the corresponding band in the spectrum of phenanthrene-2-carboxylic acid methyl ester. The rest of the infrared spectra of both compounds were extremely similar. The dehydro-

(7) Compare G. Stork and R. Breslow, *ibid.*, **75**, 3291 (1953).

genation ester also had the correct methoxy content for 1-methyl-7-carbomethoxyphenanthrene.

Figure 1 shows (curve 1) the ultraviolet spectrum of phenanthrene-2-carboxylic acid methyl ester and (curve 2) the spectrum of the dehydrogenation ester. The complete parallelism of both curves is in agreement with our assumption that the dehydrogenation ester is 1-methyl-7-carbomethoxyphenanthrene. Formulas XXI–XXIV represent the reactions discussed. The neutral fraction from the dehydrogenation of acid B (XXI) after purification by chromatography yielded traces of a hydrocarbon which on the basis of the similarity of the ultraviolet spectra could possibly be pimanthrene. It is obvious that, as pimanthrene is the chief dehydrogenation product of the triole XXII, the 7-methyl group of pimanthrene must originate from the reduction of one carboxy group. If now indeed pimanthrene is also formed in small quantity from the acid itself, its formation may be explained by reduction of the carboxy group in the form of the anhydride, by the reducing medium of the selenium dehydrogenation. Such cases already have been described.[8] We have reported previously[1] the dehydrogenation of a large amount of an impure and poorly characterized oxogarryinedicarboxylic acid. This gave a small yield of precisely identified pimanthrene. The formation of pimanthrene in this case must now also be interpreted in the manner just described.

From these investigations it is clear that the structure and location of the ring carrying the exocyclic methylene group is in agreement with the experimental data. There is, however, one more point in the proposed skeletal structure which requires clarification. In the tautomeric equilibrium between the different forms of either garryine or veatchine it is clear that according to our formulation the tertiary vinylamine form is not capable of actual existence. Consequently the existence of garryine and veatchine in the anhydrous forms $C_{22}H_{33}O_2N$ is made possible only by the formation of internal carbinolamine ethers. If this is the case such an anhydrous form should be impossible in a compound which, while retaining all the structural features of garryine or veatchine, has no hydroxy group capable of ether formation.

Such a compound was obtained in the following manner. The free base was liberated from the methiodide of pyrolysis base A by alkali. This compound was obviously the carbinolamine XXV. All the properties of the base after recrystallization from ether were in agreement with this structure. The compound analyzed for $C_{21}H_{33}O_2N$ after drying *in vacuo*. It did not lose water to form an anhydrous derivative even after 24 hours in high vacuum at 90°. In the infrared spectrum it showed a pronounced hydroxy band and a ketonic band at 1738 cm.$^{-1}$. Under forcing conditions (sublimation at 190°) it reverted into pyrolysis base A. The mechanism to this reaction is indicated by arrows in formula XXV. If the carbinolamine XXV is recrystallized from methanol, it forms a methyl ether $C_{22}H_{35}O_2N$. This compound has the correct methoxy content and no hydroxy band in the infrared spectrum. It has a carbonyl band at 1743 cm.$^{-1}$. Finally we should like to state that formula XXVI may well be a correct expression for the stereochemistry of dihydroveatchine.

(8) A. Windaus and W. Thiele, *Ann.*, **521**, 160 (1935).

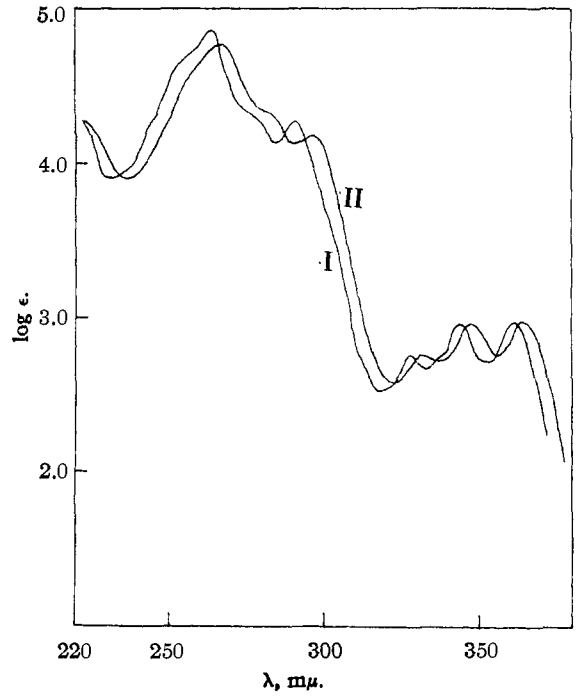


Fig. 1.—Ultraviolet spectra in alcohol: I, phenanthrene-2-carboxylic methyl ester; II, ester obtained by dehydrogenation of acid B.

The discussion of the stereochemistry at this point would be premature and it will be presented when conclusive evidence is available. Also experiments toward decision as to which of the two available structures belongs to garryine and which to veatchine are in progress.

Acknowledgment.—One of us (K.W.) wishes to express his gratitude to the National Research Council, Ottawa, for the support of these investigations over the period of the last three years. We further thank Professor F. J. Toole for his continued support and interest in our work. We wish to thank Mr. Henry Hellmers of the U. S. Department of Agriculture, Glendora, California, for his effort in collecting the plant material. The infrared spectra were determined by Dr. R. L. Bohon, The Anderson Physical Laboratory, Champaign, Illinois. The analyses were performed by Dr. S. M. Nagy, Department of Chemistry, Massachusetts Institute of Technology, Cambridge, Mass., and Dr. R. Dietrich, Mikroanalytisches Laboratorium, Zurich.

Experimental Part

Oxidation of XII with Chromic Acid in Pyridine.—Chromic acid (0.83 g.) was added to 15 ml. of pyridine at 20°. Base XII (0.83 g.) in 14 ml. of pyridine was added slowly. The resulting solution was shaken for several hours and then allowed to stand overnight. After pouring into water and extraction with benzene the crude product was chromatographed on alumina. Absolute benzene–ether (1:1) eluted 0.47 g. of crystalline compound XVI which was recrystallized from acetone. It melted at 220° and was sublimed in high vacuum for analysis. *Anal.* Calcd. for $C_{20}H_{31}NO$:

C, 79.67; H, 10.37; N, 4.65. Found: C, 79.73; H, 10.36; N, 4.65.

Oxidation of XIII with Chromic Acid in Pyridine.—Base XIII (1.7 g.) in 17 ml. of pyridine was oxidized in the same way as above by 3 g. of chromic acid in 30 ml. of pyridine. By chromatography on alumina, benzene–ether (1:1) eluted 0.50 g. of crystalline material XIV. The compound melted after several recrystallizations from acetone at 157–158°. It was sublimed in high vacuum for analysis. *Anal.* Calcd. for $C_{20}H_{27}NO$: C, 80.76; H, 9.15; N, 4.71; 1(C)-CH_3, 5.04. Found: C, 80.44; H, 9.17; N, 4.71; (C)-CH_3, 1.68.

Base XV by Lithium Aluminum Hydride Reduction of XIV.—Base XIV (0.22 g.) was reduced in the standard way with an excess of lithium aluminum hydride in ether. The crude product was obtained in quantitative yield and was recrystallized five times from ether to m.p. 181°. It was sublimed *in vacuo* for analysis. *Anal.* Calcd. for $C_{20}H_{31}NO$: C, 79.67; H, 10.37; N, 4.65. Found: C, 79.57; H, 10.41; N, 4.70.

Hydrogenation of Base XV to XII.—The hydrogenation was performed with Adams catalyst in glacial acetic acid; an uptake of 1 mole of hydrogen was observed. The product was found identical with tetrahydrobase A (XII) by melting point (202°), mixed melting point and infrared spectrum. A Kuhn–Roth determination on tetrahydrobase A was performed. Calcd. for $C_{20}H_{33}NO$: 2(C)-CH_3, 9.90. Found: (C)-CH_3, 6.70.

N-Acetyl Dihydrobase B (XVII).—Dihydrobase B XIII (0.25 g.) was acetylated at room temperature in 5 ml. of absolute pyridine with 3 ml. of acetic anhydride. The product was saponified by standing with 10 ml. of 50% ethanol containing 0.1 g. of sodium hydroxide. A quantitative yield of the crude material was obtained which was recrystallized from ether–petroleum ether to a melting point 148°. *Anal.* Calcd. for $C_{22}H_{33}NO_2$: C, 76.92; H, 9.68; N, 4.08. Found: C, 76.82; H, 9.65; N, 4.18.

Compound XVIII by Oxidation of Compound XVII.—Compound XVII (0.24 g.) was oxidized in the way already described with 0.24 g. of chromic acid in 9 ml. of pyridine. The crude product was chromatographed on alumina; benzene–ether eluted 0.13 g. of crystalline material. This was recrystallized to a melting point of 167–168° from ether and sublimed in high vacuum for analysis. *Anal.* Calcd. for $C_{22}H_{31}NO_2$: C, 77.38; H, 9.15; N, 4.10. Found: C, 77.37; H, 9.02; N, 4.09.

Compound XIX by the Reduction of the Methiodide of X.—The methiodide of X (500 mg.) was dissolved in 40 ml. of methanol and a solution of 60 mg. of sodium borohydride in 10 ml. of methanol was added with stirring. After standing for several hours water was added to the mixture and the alcohol was evaporated *in vacuo*. The aqueous solution was extracted with chloroform. After drying the chloroform extract over sodium sulfate and distilling off the chloroform, the crude product (400 mg.) was recrystallized from methanol to a melting point of 144–145°. *Anal.* Calcd. for $C_{21}H_{33}ON$: C, 79.90; H, 10.54; N, 4.43. Found: C, 79.61; H, 10.40; N, 4.75.

The Carbinolamine XXV.—The methiodide of X was dissolved in water and a large excess of sodium hydroxide was added. The precipitated base was extracted with alcohol-free chloroform and recrystallized from absolute ether to a melting point of 117–119°. For analysis one sample was dried at 90°, another at room temperature in high vacuum. The infrared spectra of both samples were identical. *Anal.* Calcd. for $C_{21}H_{33}O_2N$: C, 76.07; H, 10.03. Found: C, 75.86; H, 9.81.

Carbinolamine Methyl Ether.—The compound XXV was recrystallized from methanol to a constant melting point of 110–111°. Samples were prepared by drying in high vacuum at 90° or sublimation. The compound had no hydroxy band in the infrared and produced a depression of melting point when mixed with the previous product. *Anal.* Calcd. for $C_{22}H_{35}O_2N$: C, 76.47; H, 10.21; N, 4.11; OCH_3, 8.94; (N)-CH_3, 4.34. Found: C, 76.15; H, 10.09; N, 4.28; OCH_3, 9.18; (N)-CH_3, 5.65.

Vigorous Permanganate Oxidation of Veatchine.—Veatchine (10 g.) was dissolved in 700 ml. of acetone. Powdered permanganate was then added to the stirred solution over a period of two hours. The stirring was continued for 5 hours and after this time the mixture was filtered. The filtered off manganese dioxide was suspended in 200 ml. of water and 200 ml. of chloroform, cooled with ice and sulfur dioxide was bubbled into the mixture until the precipitate dissolved. The aqueous layer was repeatedly extracted with chloroform. This extract was then combined with the acetone soluble material and the whole amount was separated into a basic (1.21 g.), a neutral (1.40 g.) and an acidic (4.27 g.) portion. The acids (3.491 g.) were subjected to countercurrent distribution in an automatic-transfer apparatus with 50 funnels. The solvent system was chloroform and phosphate buffer *p*H 5.91 (50 ml. of each phase). Two peaks were distinctly developed in the countercurrent distribution. They were not of the theoretical shape, undoubtedly because of overloading of the buffer.

Oxoveatchinedicarboxylic Acid B (XXI).—The contents of the funnels 36–47 (0.925 g.) were combined and recrystallized from methanol–ethyl acetate. After 5 crystallizations the substance melted at 262–263° and was dried in high vacuum for analysis. *Anal.* Calcd. for $C_{21}H_{29}O_6N$: C, 64.43; H, 7.47; N, 3.58; neut. equiv., 195.73. Found: C, 64.19; H, 7.51; N, 3.74; neut. equiv., 204.02.

The pure acid B was dissolved in a small volume of methanol and treated for a short time with ethereal diazomethane. The oily ester was sublimed in high vacuum at 180°. *Anal.* Calcd. for $C_{23}H_{33}O_6N$: C, 65.85; H, 7.93; N, 3.34; OCH_3, 14.80. Found: C, 65.80; H, 7.90; N, 3.34; OCH_3, 14.68.

Monomethyl Ester of the Acid B.—The dimethyl ester of acid B (166 mg.) was dissolved in 10 ml. of methanol and 7 ml. of 0.25 *N* sodium hydroxide. The solution was then refluxed for 2 hours. The acidic material produced was isolated in the usual way. The yield was 146 mg. of beautiful, very insoluble crystals. The substance was recrystallized 5 times from methanol to a constant melting point of 269–270° and sublimed for analysis in high vacuum at 200°. *Anal.* Calcd. for $C_{22}H_{31}O_6N$: C, 65.16; H, 7.71; OCH_3, 7.65. Found: C, 65.20; H, 7.75; OCH_3, 8.02.

Anhydride of Acid B.—Acid B (143 mg.) was refluxed with 5 ml. of acetic anhydride for 4 hours and the solution was then evaporated to dryness *in vacuo*. The crystalline residue was sublimed in high vacuum on a cold finger. The sublimed crystals were insoluble in ether and they were consequently only boiled in suspension with absolute ether and resublimed for infrared spectrum. The melting point was *ca.* 270°.

Oxoveatchinedicarboxylic Acid A (XX).—Contents of funnels 23–30 from the countercurrent distribution were combined (548 mg.) and recrystallized from methanol–ethyl acetate. The melting point was 247–249°. The acid gave erratic analyses ascribable possibly to difficulties in drying; simultaneous anhydride formation occurred if the conditions were too vigorous. It was therefore converted in the same way as acid B into the crystalline monomethyl ester. This compound was recrystallized several times from ethyl acetate and then from ether to a constant melting point of 138° and dried in high vacuum at 90° for analysis. *Anal.* Calcd. for $C_{22}H_{31}O_6N$: C, 65.16; H, 7.71; OCH_3, 7.65. Found: C, 65.20, 65.35, 65.01; H, 7.74, 7.80, 7.93; OCH_3, 7.93.

Anhydride of Acid A.—Acid A was converted in the same way as acid B into the anhydride. This substance was more soluble in ether than the anhydride of B and it was recrystallized to a melting point of 202–205° and sublimed for analysis and infrared spectrum. *Anal.* Calcd. for $C_{21}H_{27}O_5N$: C, 67.54; H, 7.29. Found: C, 67.49; H, 7.32.

Dehydrogenation of Acid B.—Acid B (1 g.) was mixed with 2 g. of selenium and heated to 340° for 9 hours in a nitrogen atmosphere. The product was then powdered and extracted with chloroform, and the acidic fraction separated in the usual way; yield of acids was 137 mg. of white foam. This was esterified with diazomethane and filtered through a column of 5.6 g. of neutral alumina (Fisher) in benzene solution. The first fractions, dissolved in a small volume of methanol, deposited 17 mg. of crystals melting between 100 and 110°. The substance obviously needed further crystallization to reach purity. It was, however, sublimed in high vacuum for infrared and ultraviolet spectrum and for a methoxyl determination. *Anal.* Calcd. for $C_{17}H_{14}O_2$: -OCH_3, 12.40. Found: -OCH_3, 12.48.

Dehydrogenation of the Reduced Diester B.—The dimethyl ester of acid B (1.303 g.) was reduced with an excess of lithium aluminum hydride by refluxing overnight; the product was isolated in the usual way. The yield was 1.105 g. of an oily base. This substance was mixed with 2.2 g. of powdered selenium and heated to 340° under nitrogen for 9 hours. The residue was extracted with chloroform and the

neutral fraction (495 mg.) separated from it. The benzene-soluble part of the neutral fraction (340 mg.) dissolved in a small volume of benzene was poured onto a column of 20 g. of Fisher alumina and the chromatogram developed with absolute petroleum ether. The first 10 fractions (each 50 ml.) yielded 137 mg. of colorless oil which was converted into the trinitrobenzene complex. This compound was repeatedly crystallized from ethanol to a constant melting point of 157°; the yield of the analytically pure sample was 30 mg. This melting point is 20° higher than the melting point of 1-methyl-7-ethylphenanthrene trinitrobenzolate and no depression of melting point was observed on admixture of an authentic sample of pimanthrene trinitrobenzolate which melted also at 157°. The sample was decomposed by chromatography on a small column of alumina and the crystalline hydrocarbon sublimed for infrared and ultraviolet spectrum. These were identical with the spectra of an authentic specimen of pimanthrene.

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[CONTRIBUTION FROM THE LABORATORIES OF THE SLOAN-KETTERING DIVISION OF CORNELL UNIVERSITY MEDICAL COLLEGE]

The Synthesis and Properties of 6-Chloropurine and Purine[1]

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6-Chloropurine has been prepared in good yield by the treatment of hypoxanthine with phosphorus oxychloride in the presence of dimethylaniline. The chloro atom was removed by catalytic hydrogenation in a new synthesis of purine. Purine also has been prepared by Raney nickel desulfurization of 6-mercaptopurine, the latter of which was synthesized *via* a new route directly from 6-chloropurine by reaction with thiourea. A synthesis of 4,5-diaminopyrimidine—which has been converted to purine—is described. A study of the ionization and ultraviolet absorption spectral behavior of these purines as well as of 7- and 9-methylpurine has been made. Some physical and chemical properties of these purines are given. A preliminary account of the effect of these compounds on tumor tissue is included.

Simple structural alteration of naturally occurring purines has made available a variety of purine analogs which are potent antagonists of many biological systems.[3] Outstanding examples of such purine derivatives which, *in addition*, exhibit an anti-tumor activity have resulted from the introduction of an amino group or a chlorine atom at position-2 of adenine,[4] the replacement of carbon-8 of guanine by nitrogen[5] or of carbon-2 of adenine or hypoxanthine by nitrogen[6] and by the replacement of the amino group of adenine by the mercapto group.[7]

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(2) Damon Runyon Memorial Fund Fellow, 1952-1954.

(3) (a) See, for example, R. O. Roblin, Jr., J. O. Lampen, J. P. English, Q. P. Cole and J. R. Vaughan, THIS JOURNAL, **67**, 290 (1945); (b) G. W. Kidder and V. C. Dewey, *J. Biol. Chem.*, **179**, 181 (1949); (c) G. H. Hitchings, G. B. Elion, E. A. Falco, P. B. Russell, M. B. Sherwood and H. VanderWerff, *ibid.*, **183**, 1 (1950); (d) G. H. Hitchings, G. B. Elion, E. A. Falco, P. B. Russell and H. VanderWerff, *Ann. N. Y. Acad. Sci.*, **52**, 1318 (1950); (e) G. B. Elion, G. H. Hitchings and H. VanderWerff, *J. Biol. Chem.*, **192**, 505 (1951); (f) M. Williamson, W. Jacobson and C. C. Stock, *ibid.*, **197**, 763 (1952); (g) V. L. Ryzhkov and H. K. Marchencko, *Doklady Akad. Nauk. S.S.S.R., Leningrad*, **86**, 637 (1952); (h) E. Shaw and D. W. Woolley, *J. Biol. Chem.*, **194**, 641 (1952); (i) C. Miller, *Proc. Soc. Exptl. Biol. Med.*, **83**, 561 (1953); (j) R. E. F. Matthews, *Nature*, **171**, 1065 (1953).

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An examination of the structures of a few hundred purine derivatives tested for possible effectiveness in cancer chemotherapy[8] reveals that (a) the active analogs of naturally occurring purines are those in which the new group or atom introduced is not greatly different in size from the one replaced, (b) the more active compounds seem to result from an alteration at a single site of the structure of adenine, hypoxanthine or guanine, and (c) active analogs have resulted from replacement in adenine, hypoxanthine or guanine of carbons-2 or 8 by nitrogen or by substitution at carbons-2 or 6, but not at any other position thus far tested. (Although the introduction of the ribofuranosyl group at N_9 may yield active derivatives, compounds of the nucleoside type[8b] are outside the present discussion.) The replacement of the amino group of adenine (or the hydroxyl of hypoxanthine (I)) by a chlorine or hydrogen atom would give rise, respectively, to the compounds 6-chloropurine (II) and purine (III) which embody some of the structural features listed above. This communication deals with the preparation and properties of these and related purines.

Synthetic Studies.—Neither a Sandmeyer-type reaction with adenine nor the treatment of hypoxanthine (I) with phosphorus oxychloride alone afforded the desired 6-chloropurine (II). Since the use of dimethyl- or diethylaniline greatly improves the chlorination of many hydroxypyrimidines[9-12] and uric acid[13] with phosphorus oxychloride, this reaction was applied to hypoxanthine, and 6-chloropurine (II) was obtained in good yields.[14]

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