anol containing approximately 2 mg. of sodium methoxide. After refluxing 12 hr., the solution was neutralized and concentrated to a small volume. A solid product remained, which, after being recrystallized from ethanol, melted at 139.5–140.5° (34% yield). The analysis and infrared absorption spectrum (pyridinium ring[14] and nitrile absorption) indicated the substance to be the 1:2 (picoline methiodide: acrylonitrile) condensation product.

Anal. Calcd. for $C_{13}H_{16}N_2I$: C, 45.73; H, 4.73. Found: C, 46.15; H, 4.78.

Addition of α-Picolyl Sodium to 1-Cyanocyclohexene.—To an ether suspension of sodium amide freshly prepared from 2.3 g. of sodium and liquid ammonia there was added dropwise 10.2 g. of 1-cyanocyclohexene dissolved in 20 ml. of dry ether. The addition was carried out over a period of 1 hr. in a system protected from atmospheric moisture. After being refluxed for 18 hr., the mixture was cooled to room temperature, poured into an ice–water mixture and neutralized with dilute hydrochloric acid. Ether extraction, followed by a sodium bicarbonate wash, drying and removal of ether yielded a residue, the fractionation of which gave a forerun of recovered α-picoline and 1.0 g. of product, b.p. 150–162° (0.4 mm.), $n^{25}D$ 1.5100. The boiling point range and analysis indicated a mixture of isomers, which was not separated but which appeared to consist of 1:2 (picoline:1-cyanocyclohexene) condensation products.

Anal. Calcd. for $C_{20}H_{25}N_3$: C, 78.13; H, 8.20. Found: C, 78.26; H, 8.20.

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(14) Mr. John Baran of this Laboratory has shown that the N-alkyl pyridinium ring exhibits bands at 6.18, 6.36 and 6.61 μ in the infrared, whereas the pyridine system absorbs at 6.29, 6.38 and 6.78 μ.

[CONTRIBUTION FROM THE DEPARTMENT OF CHEMISTRY, UNIVERSITY OF WISCONSIN]

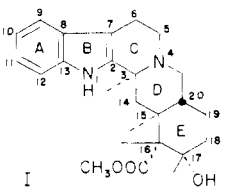
The Stereospecific Synthesis of *dl*-Yohimbane. Stereochemistry of Yohimbine[1]

BY EUGENE E. VAN TAMELEN, MAURICE SHAMMA AND PAUL ALDRICH

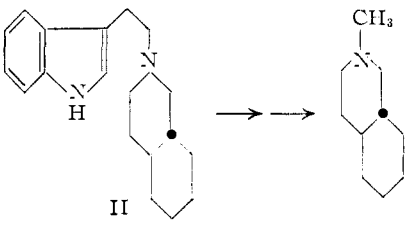
RECEIVED APRIL 26, 1956

Proceeding from *dl-trans*-2-hydrindanone, the stereospecific synthesis of *dl*-yohimbane establishes the D/E ring juncture of the parent natural product yohimbine (I),[2] as *trans*.

The stereochemical relationships expressed in the formula I for yohimbine are based[3] upon (1) certain eliminations and epimerizations bearing configurational significance which the alkaloid or closely related substances undergo,[3a–c] and (2) the Hofmann degradation of dihydro*chano*desoxyyohimbol (II) to *d*-N-methyl-*trans*-decahydroisoquinoline, a result which was taken[3d] to indicate a *trans* D/E ring juncture for yohimbine itself. The base II results from the catalytic hydrogenation of *chano*desoxyyohimbol, an unusual product obtained by heating yohimbic acid at 280° *in vacuo* with either thallous oxide or carbonate.[3d] Now the position of the double bond in *chano*desoxyyohimbol remains uncertain; and it is clear that its termination at C_{15} (or C_{20})—a distinct possibility in view of the drastic treatment required for its production—invalidates any configurational deductions which can be made regarding the *chano* base and therefore the D/E juncture of yohimbine, since the stereochemical outcome of the reduction to II would not be clear. Because of the ambiguities[4] involved in assigning, on the basis of rela-



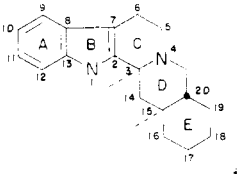
I



II

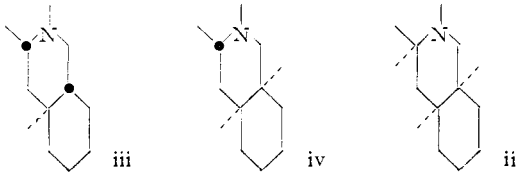
(1) First reported in a Communication to the Editor, THIS JOURNAL, **76**, 950 (1954).

(2) In the interests of rational nomenclature we wish to propose the generic term *yohimbane* for the ring system (i) and that substances



i

possessing this 3,15,20-*cis*,*trans*-pentacyclic system be named as derivatives of yohimbane. We suggest further that the system featuring the 3,15,20-all-*cis* arrangement (ii) be designated as *alloyohimbane* and that the epimers at C_3 of (i) and (ii) be termed, respectively, *epiyohimbane* (iii) and *epialloyohimbane* (iv). Finally, it seems worthwhile to continue application (*cf.* C. F. Huebner, H. B. MacPhillamy, E. Schlittler and A. F. St. André, *Experientia*, **11**, 303 (1955)) of the configurational conventions used commonly in the steroids series, *viz.*, α-, signifying a substituent placed below the plane of the fused system (dotted line); and β-, denoting a substituent attached above the plane (solid line). Formula (i) features an α-oriented C_3-hydrogen, a choice in keeping with the absolute stereochemical assignment made by Klyne, *Chem. and Ind.*, **14**, 1032 (1954).



iii iv ii

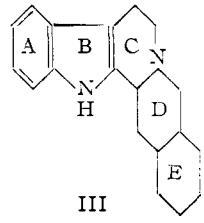
Thus yohimbine becomes, systematically, 16α-carbomethoxy-17α-hydroxyyohimbane; and reserpine, 11,17α-dimethoxy-16β-carbomethoxy-18β-(3′,4′,5′-trimethoxybenzoyloxy)-*epi*alloyohimbane.

(3) (a) G. Stork, as quoted by B. Witkop and S. Goodwin, THIS JOURNAL, **75**, 3371 (1953); (b) M.-M. Janot, R. Goutarel, A. LeHir, M. Armin and V. Prelog, *Bull. soc. chim.*, 1085 (1952); (c) R. Cookson, *Chem. and Ind.*, **15**, 337 (1953); (d) B. Witkop, THIS JOURNAL, **71**, 2559 (1949).

(4) See, for example, W. G. Dauben, R. C. Tweit and C. Manners-kantz, *ibid.*, **76**, 4420 (1954).

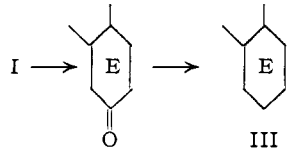
tive stabilities, configurations to substituents attached to a *cis*-bicyclo[4,4,0] system, the assigned[3] stereochemical relationships of C_3 and C_{16} to C_{15} or C_{20} in yohimbine are uncertain to a degree which parallels the probability that the D/E ring juncture is *cis*. Thus to the extent that the objections above are justified, the formulation *I* is in jeopardy in all points, with the exception of the C_{16}–C_{17} assignment.

Because of these considerations, we felt compelled to secure incontrovertible evidence regarding the *terminus a quo* in the stereochemical arguments: the nature of the D/E ring juncture in yohimbine. The degradation product yohimbane (III) appeared ideally suited for stereochemical



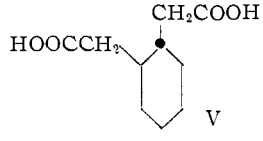
III

study in that it is derived[5] from yohimbine through operations (Oppenauer oxidation—accompanied by loss of the carbomethoxyl function—to yield



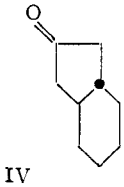
III

yohimbone, followed by Wolff–Kishner reduction) which can hardly affect asymmetric centers 15 and 20. A synthesis of III, for example, which, by its very nature, defines the stereochemical relationship of these two centers would also, therefore, establish the relationship of these same centers in the parent alkaloid. The "stereorational" synthesis which was developed (1) utilizes a starting material, *dl-trans*-2-hydrindanone (IV), the stereochemistry of which had been proved unequivocally by resolution of the diacid V from which it is pre-

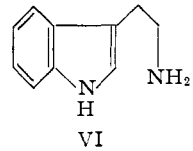


V

pared[6] and (2) involves transformations which do not affect either of the asymmetric carbon atoms present in the starting ketone. It is obvious that between tryptamine (VI) and the hydrindanone,



IV

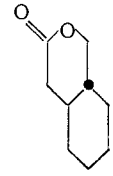


VI

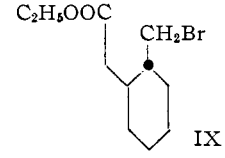
there are just the number of carbon and nitrogen atoms required for yohimbane and the problem resolves itself into the search for a method of inserting the sidechain nitrogen of VI into the ketonic ring of IV, followed by cyclization–reduction to form ring C.

trans-2-Hydrindanone was conveniently prepared in quantity, as previously described,[7] by pyrolysis of the diacid V, the nitric acid oxidation product of *trans*-2-decalone. The latter ketone, in contrast to the *cis* isomer, was not readily accessible when this research was initiated, and of the possible synthetic routes which were investigated, that involving the lithium–liquid ammonia reduction[8] of $\Delta^{1,9}$-2-octalone was followed.

Perbenzoic acid oxidation of the bicyclic ketone proceeded smoothly at room temperature and afforded in 76% yield, the lactone (VII) of *trans*-2-hydroxymethylhexahydrophenylacetic acid, which was secured, after distillation, in a crystalline state (m.p. 38.5°). In order to attain a system which would allow attachment of the tryptamine moiety, the lactone was subjected to the action of anhydrous ethanolic hydrogen bromide and thereby was converted to the ethyl ester IX of *trans*-2-bromo-



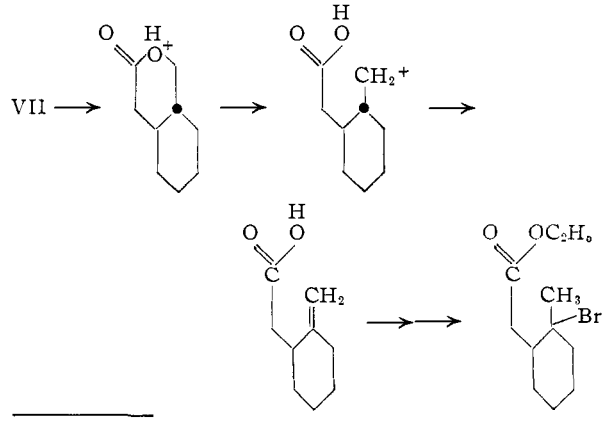
VII



IX

methylhexahydrophenylacetic acid (VIII). The desired ring-opening of VII is not readily accomplished; of the various conditions tried, only several days mechanical shaking at room temperature of the lactone and ethanol, which had been saturated with gaseous hydrogen bromide at −5,° gave material with an acceptable halogen analysis. The bromoester, a liquid boiling at 129° (14 mm.), appears to be stable on standing for some months at room temperature.

On the basis of the detailed course of peracid oxidation of ketones[9] opportunity for epimerization at the bridge carbon of III could not arise, and the intermediate VII can be safely regarded as the *trans* isomer. It is possible to visualize a mechanism for the lactone ring opening which would



(5) J. Jost, *Helv. Chim. Acta*, **32**, 1301 (1949).

(6) W. Hückel, M. Sachs, J. Yantschulewitsch and F. Nerdel, *Ann.* **518**, 155 (1935).

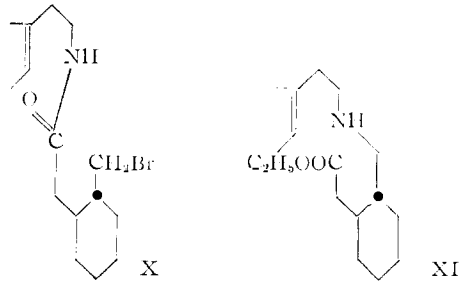
(7) R. S. Thakur, *J. Chem. Soc.*, 2147 (1932).

(8) E. E. van Tamelen and W. C. Proost, Jr., THIS JOURNAL, **76**, 3632 (1954).

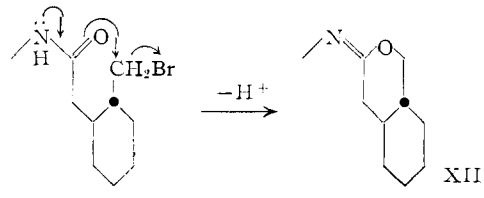
(9) (a) W. von E. Doering and E. Dorfman, *ibid.*, **75**, 5595 (1953); (b) S. L. Friess, *ibid.*, **71**, 2571 (1949).

involve a disturbance at the γ-position. However, such a circumstance was not realized since, contrary to fact, a yohimbane *skeleton* could not then result from the operations described below.

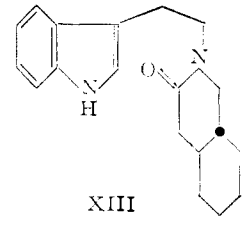
Introduction of the indolic system was accomplished through the monoalkylation of tryptamine by the bromoester, a reaction which was carried out by refluxing with potassium iodide in ethanolic solution in the presence of potassium carbonate. Either attack by the primary amino group at the ester function, yielding a *bromoamide* X, or attack at the bromomethyl group, yielding an *aminoester*



XI, might appear reasonable as an initial operation. On the basis of comparison with similar cases,[10] however, the bromoamide might be anticipated to cyclize by displacement of halogen by oxygen,

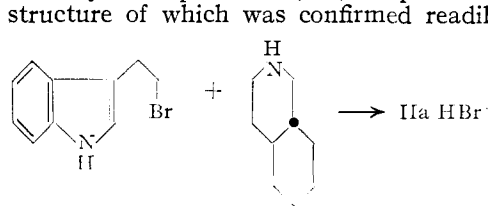


affording an iminolactone XII. Since the *lactam* XIII is the product which results from the alkyla-



tion, we believe that the process must follow largely the pattern IX → XI; at the same time, the presence of the iminolactone in the reaction product has not been excluded. The lactam, a high-melting (244–245°) solid which crystallized nicely from hot ethanol, was non-basic, stable to aqueous mineral acid and exhibited a strong absorption band at 6.20 μ, which corresponded well with the carbonyl band displayed by N-methyl-2-piperidone.

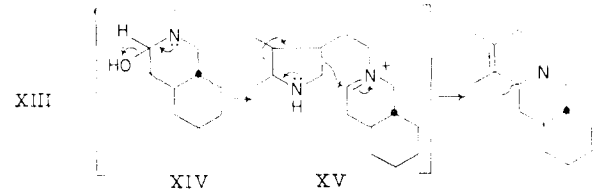
Reduction of the lactam XIII with lithium aluminum hydride afforded *dl*-N-(β-3′-indolylethyl)-*trans*-decahydroisoquinoline (IIa), m.p. 150–151°, the structure of which was confirmed readily by



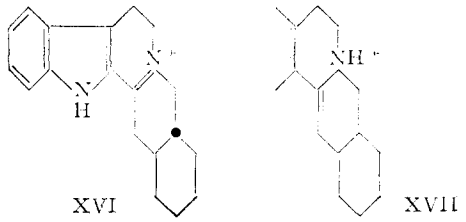
(10) H. L. Goering, THIS JOURNAL, **73**, 1737 (1951).

comparison with the base obtained by the treatment of *dl-trans*-decahydroisoquinoline with β-3′-indolylethyl bromide. The immediate product of the alkylation was a crystalline hydrobromide, m.p. 240–242°, the base derived from which did not depress the melting point of the reduction product secured from the lactam. The racemate IIa corresponds in structure to that derived[3d] for dihydro*chano*desoxyyohimbol (II), although a comparison between the two substances was not made.

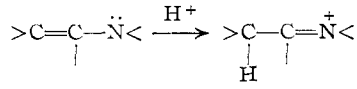
With the structure and stereochemistry of the intermediate lactam secure, attempts to effect ring closure and reduction to *dl*-yohimbane were made. After several abortive trials at *partial* lithium aluminum hydride reduction, which was expected[11]



to lead to the alkanolamine XIV and then to *dl*-yohimbane (or the C_3-epimer) *via* the imine cation XV, we turned to the Bischler–Napieralsky type ring closure. On occasion, refluxing phosphorus oxychloride–benzene served to convert XIII, after careful crystallization, to an unstable *yellow substance*, m.p. 196–198°, the analysis of which suggests its formulation as the dichlorophosphate of *dl*-$\Delta^{3(4)}$-dehydroyohimbane (XVI).[12]



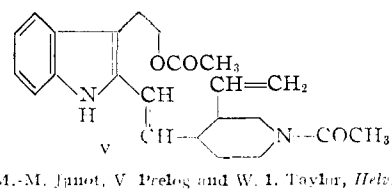
Ordinarily, however, work-up of the cyclization mixture led to the hydrochloride, m.p. 300° dec., of, presumably, the same dehydro base. We have gathered no direct evidence excluding the enamine structure XVII for the salts, although the yellow color[13] of the hydrochloride and the observation by Leonard and Gash[14] that enamines nor-



(11) *Cf.* F. Galinovsky and R. Weiser, *Experientia*, **6**, 377 (1950).

(12) The dichlorophosphate anion has been previously observed to result from dehydration–cyclization reactions brought about by phosphorus oxychloride, K. Gleu, S. Nietzche and A. Schubert, *Ber.*, **72**, 1093 (1939).

(13) Diacetylallocinchonamine (v) is reported to be colorless (R.



Goutarel, M.-M. Janot, V. Prelog and W. I. Taylor, *Helv. Chim. Acta*, **37**, 150 (1950).

(14) N. J. Leonard and V. W. Gash, THIS JOURNAL, **76**, 2781 (1954).

mally yield imine cations might be used to controvert any case made for XVII. Catalytic reduction of either the hydrochloride or the dichlorophosphate gave rise to, in high yield, *dl*-yohimbane (IIIa), with no evidence of epimer formation.[15] The free base, a colorless solid melting at 182–183°, was proved to be the desired product through infrared analysis: the spectra of IIIa and yohimbane derived from the natural source—both in either chloroform or carbon disulfide solution—were indistinguishable; by contrast *d*-alloyohimbane[16] displayed bands in the infrared region differing from those of our synthetic base.

The D/E ring juncture of yohimbine is, then, beyond question, *trans*.[17] Conformational arguments leading to the *cis–trans* (all axial) assignment of hydrogens at C_4, C_{15} and C_{16} are thereby validated and, taken together with the evidence for the axial (*cis* to carbomethoxyl) nature of the C_{17}-hydroxyl, provides unambiguously for the stereochemical representation I.

Acknowledgments.—This research was supported by a grant from the Research Committee of the Wisconsin Alumni Research Foundation. The authors are indebted to Mr. William Proost, Jr., who provided a portion of the *trans*-2-hydrindanone used in this investigation.

Experimental[18]

Lactone of *dl-trans*-2-Hydroxymethylhexahydrophenylacetic Acid (VII).—To a solution of 17.2 g. of perbenzoic acid in 245 ml. of chloroform was added 14.1 g. of *dl-trans*-hydrindan-2-one,[7] b.p. 88–89° (9–10 mm.), $n^{20}D$ 1.4756. The solution was allowed to stand in the dark at room temperature for 40 hr. At the end of this time, the chloroform was evaporated *in vacuo* at 100°, and the lactone was then distilled at 102–104° (0.1 mm.) as a colorless liquid ($n^{25}D$ 1.4905), which solidified on short standing to give crystals, m.p. 38.5°; yield 12 g. (76%).

Anal. Calcd. for $C_9H_{14}O_2$: C, 70.10; H, 9.15. Found: C, 70.35; H, 9.11.

Ethyl *dl-trans*-2-Bromomethylhexahydrophenylacetate (IX).—To 12.5 g. of hydrindanone was added 15.8 g. of perbenzoic acid in 120 ml. of chloroform, and the solution was allowed to stand at room temperature in the dark for 3 days. After evaporation of the solvent, the residue was taken up in 40 ml. of absolute ethanol, and the solution was poured into a glass tube and saturated with dry hydrogen bromide (*ca.* 70 g.) at −5°. The tube was sealed and allowed to stand overnight at room temperature. When two layers had separated, shaking was commenced and continued for 3 days. The tube then was opened, and the volatile components were removed at the water pump. The strongly acidic residue was neutralized with sodium bicarbonate; the resulting solution was extracted with ether; the ether layer was washed with saturated salt solution, dried over magnesium sulfate and finally evaporated to an oil.

By distilling the products and recycling several times the fractions analytically low in bromine content, there was obtained 10.6 g. bromoester (45% yield), analyzing for 29.1% bromine. Distillation of this material gave an analytically pure fraction, b.p. 94–96° (0.1 mm.), $n^{25}D$ 1.4882.

Anal. Calcd. for $C_{11}H_{19}BrO_2$: Br, 30.36. Found: Br, 30.2.

Lactam of *dl-trans*-N-(β-3′-Indolylethyl)-2-aminomethylhexahydrophenylacetic Acid (XIII).—Tryptamine (248 mg.), bromoester IX (284 mg.), anhydrous potassium carbonate (75 mg.) and a crystal of potassium iodide were added to 15 ml. of anhydrous ethanol, and the solution was refluxed 3 days under nitrogen. The ethanol was evaporated off at reduced pressure, water was added and the mixture was extracted several times with chloroform. The combined chloroform extracts were washed several times with 1 *N* hydrochloric acid and finally with saturated salt solution. The chloroform layer was dried over magnesium sulfate and evaporated. Crystallization of the residue from ethanol gave 217 mg. of lactam (60% yield), m.p. 239–240°. A sample was recrystallized to the constant melting point 242–243°; under the microscope the substance was observed to undergo a crystalline change at about 210–220°.

Anal. Calcd. for $C_{19}H_{24}N_2O$: C, 77.00; H, 8.16. Found: C, 77.19; H, 7.94.

Salts of *dl*-$\Delta^{3(4)}$-Dehydroyohimbane (XVI). Hydrochloride.—To 20 ml. of dry thiophene-free benzene was added 214 mg. of lactam XIII and 0.5 ml. of freshly distilled phosphorus oxychloride. The mixture was refluxed gently 3 to 4 hr. and allowed to stand overnight at room temperature; the mixture became yellow-orange in the early stages of reaction. The precipitate was removed by filtration, washed several times with benzene and dried. Several ml. of purified dioxane were added to the product, followed by sufficient absolute ethanol to effect solution. Then the excess ethanol was gently boiled off on the steam-bath until the first crystals just appeared; the solution was allowed to cool, and the crystals were filtered off, washed with benzene and dried; yield 178 mg. of bright yellow crystals, m.p. 300° dec. The product gave a rapid silver nitrate test in dilute nitric acid and a negative phosphate test[19]; it appeared to be a solvate of indeterminate constitution. A sample dried to constant weight at 173° weighed 160 mg. (70% yield).

Anal. Calcd. for $C_{19}H_{24}ClN_2$: C, 72.21; H, 7.11. Found: C, 72.71; H, 7.11.

Dichlorophosphate.—At the end of the cyclization reaction the benzene was distilled off under reduced pressure, and the crystalline reddish-orange precipitate left was dissolved in absolute ethanol and then fractionally recrystallized from the same solvent. This procedure finally yielded, on occasion, a crystalline yellow solid, m.p. 196–196°, which gave a positive phosphate test with ammonium molybdate; yield 21%. These yellow crystals slowly assumed a green color on standing.

Anal. Calcd. for $C_{19}H_{23}Cl_2N_2O_2P$: C, 55.23; H, 5.61. Found: C, 55.16; H, 5.46.

***dl*-Yohimbane (IIIa).**—$\Delta^{3(4)}$-Dehydroyohimbane hydrochloride was hydrogenated over platinum (59 mg. platinum oxide) in ethanol. Uptake of hydrogen was 105% of theory. The hydrogenation mixture was filtered, 150 mg. of powdered potassium hydroxide was added and the mixture shaken for ten minutes. The solvent was evaporated off, the residue taken up in chloroform; the solution was washed free of alkali, dried and evaporated down. Crystallization of the residue from ethanol gave 367 mg. of *dl*-yohimbane, m.p. 181.5–183.0°. Two more recrystallizations raised the melting point to 182–183°. The mother liquors were chromatographed on alumina to give an additional 29 mg. of material, which gave no mixed melting point depression with yohimbane as obtained above; total yield of product 396 mg. (91% of theory). There was

(15) Viewing the hydrogenation as proceeding by way of simultaneous, *cis* addition of hydrogen to the carbon–nitrogen double bond does not appear to account for the stereospecificity observed in the reduction, since (assuming the essential equivalence of >C=C< and >C=N⁺< as well as that of H H and H H) the approaches

>C—C< >C—N<

from the two sides of the planar imine salt XVI are sterically equivalent and since the *immediate* products of hydrogenation are, as judged by conformational analysis (*cf.* W. S. Johnson, *Experientia*, **7**, 315 (1951)), energetically equivalent. On the other hand, the concept of an independent (of N_4) attachment of hydrogen at C_3 accommodates the preference for the C_3–C_{15} *cis* product, since—assuming that at this point in the reaction N_4 is either essentially trigonal or tetrahedral with an axial hydrogen attached—the stability of such a product is higher than the C_3–C_{15} *trans* type, and consequently the transition state energy leading to IIIa would probably be sensibly lower than that involved in going to *dl*-epiyohimbane.

(16) Spectrum supplied by Dr. R. K. Hill.

(17) The synthesis of *dl*-alloyohimbane, reported by G. Stork and R. K. Hill, THIS JOURNAL, **76**, 949 (1954), and proceeding along the same lines as outlined above, serves to independently confirm the stereochemistry of yohimbine as well as that of alloyohimbine and other stereoisomers.

(18) All melting points are corrected.

(19) F. Feigl, "Spot Tests," Vol. II, 4th Ed., Elsevier Publishing Co., Inc., New York, N. Y., 1954, p. 77.

no evidence of *dl*-epiyohimbane; the remaining chromatographic fractions amounted to 67 mg. of non-crystalline, tarry residues.

Anal. Calcd. for $C_{19}H_{24}N_2$: C, 81.38; H, 8.63. Found: C, 81.71; H, 8.35.

***dl*-Yohimbane Hydrochloride.**—A 0.07-g. portion of the dichlorophosphate salt of $\Delta^{3(4)}$-dehydroyohimbane was dissolved in 30 ml. of ethanol and hydrogenated at room temperature and pressure, using 0.010 g. of platinum oxide. There was a rapid uptake of hydrogen which slowed down considerably after the first 0.5 hr.; the hydrogen absorbed corresponded to 1 mole. The catalyst was filtered off, and the ethanol was evaporated to give a residue of colorless crystals which was recrystallized from ethanol. The final product melted 276–277°.

Anal. Calcd. for $C_{19}H_{25}ClN_2$: C, 72.01; H, 7.95. Found: C, 71.52; H, 8.09.

In order to obtain the free base, a solution of 0.050 g. of *dl*-yohimbane hydrochloride in 2 cc. of ethanol was added to 0.015 g. of potassium hydroxide in ethanol. The whole was thoroughly shaken and allowed to stand for 15 minutes. The ethanol was then evaporated to dryness over a steam-bath and under a current of nitrogen. To the white residue was added 4 ml. of water, and again the mixture was thoroughly shaken for 15 minutes. The colorless, crystalline precipitate of yohimbane was then filtered off and thoroughly washed with excess water and then recrystallized from 2 cc. of ethanol.

The *dl*-N-(β-3′-Indolylethyl)-*trans*-decahydroisoquinoline (IIa). A. From *dl*-Decahydroisoquinoline and Indolylethyl Bromide.—A mixture of 8 g. of decahydroisoquinoline[3d] and 13 g. of β-3-indolylethyl bromide in 50 ml. of ethanol was refluxed for 1 hr. The mixture was then cooled in an ice-bath; the colorless precipitate of the salt which was then collected on the filter was first washed with ether and then recrystallized from ethanol and a little acetone. In this way there was obtained 14.0 g. (67%) of colorless hydrobromide crystals, m.p. 240–242°.

Anal. Calcd. for $C_{19}H_{27}BrN_2$: C, 62.80; H, 7.49. Found: C, 63.04; H, 7.57.

The free base was prepared by treating some of the above salt with aqueous sodium hydroxide. The colorless crystals of the free base melted at 150–151°, after recrystallization from ethanol.

Anal. Calcd. for $C_{19}H_{26}N_2$: C, 80.80; H, 9.28. Found: C, 81.34; H, 9.66.

B. By Reduction of the Lactam XIII.—To a solution of 0.2 g. of lactam XIII in 50 ml. of anhydrous tetrahydrofuran was added a suspension of 0.2 g. of lithium aluminum hydride in 50 ml. of tetrahydrofuran over a period of 3 hr. The mixture was then refluxed on a steam-bath for 15 minutes. After evaporation of most of the solvent under reduced pressure, the precipitate left was taken up in methanol and filtered. Evaporation of the filtrate over a steam-bath gave yellowish crystals, m.p. 277–279°. This material was taken up in aqueous sodium hydroxide and the resulting mixture thoroughly extracted with chloroform. Evaporation of the chloroform on a steam-bath and recrystallization of the residue from methanol gave 0.04 g. (21%) of colorless crystals, m.p. 151°, which was shown, by mixed melting point determination, to be identical with the base described in A above.

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[CONTRIBUTION FROM THE DEPARTMENT OF CHEMISTRY AND RADIATION LABORATORY, UNIVERSITY OF CALIFORNIA]

Sites of Azaserine Inhibition During Photosynthesis by *Scenedesmus*[1]

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L-Azaserine has been found to have a profound effect on the reservoir sizes of many of the metabolic intermediates produced during photosynthesis by *Scenedesmus*. Marked increases in levels of glutamine and the acids of the Krebs cycle were accompanied by a corresponding depletion of the amino acid reservoirs indicating that one of the major sites of azaserine action is in reactions involving transamination. In contrast, the photosynthetic carbon cycle is virtually unaffected and the rate of formation of sucrose is increased.

The success attending the use of azaserine[3,4] as a specific inhibitor of one stage in the metabolic pathway leading to the synthesis of inosinic acid in pigeon liver prompted us to use this antibiotic in a similar attack on purine synthesis in *Scenedesmus*. However, investigation of the products resulting during photosynthesis by suspensions of these algae in the presence of azaserine showed that a more widespread interference with metabolism had occurred. The purpose of this communication is to describe the nature of these effects and to attempt to assess their importance in a general picture of the metabolic effects of azaserine.

Experimental

Experimental Procedure.—Two suspensions, each containing washed *Scenedesmus* cells (packed volume, 0.2 cc.) and KH_2PO_4 solution (0.4 cc., 3.2×10^{-6} *M*) in 21 cc. and one with added L-azaserine (4 mg.), were left for one hour in thin glass containers illuminated on each side by a 150 w. light (reflector flood) to achieve steady states with 4% CO_2 in air. Each suspension was then allowed to photosynthesize for five minutes with $NaHC^{14}O_3$ solution (0.9 cc., 360 μc.) and then flushed with air for 1 minute. The cells were then killed by pouring into boiling ethanol (88 cc.) and the resulting 80% ethanol extract was separated from insoluble material which was then reëxtracted with 20% ethanol (100 cc.). The total fixation of radioactivity was determined in each case by uniformly distributing and drying 50-μl. aliquots of the 80% ethanol suspensions plus 50 μl. of 6 *N* acetic acid on aluminum discs, and counting the radioactivity with a Scott large-window Geiger–Mueller tube. The radioactivity extracted from each suspension with 80% ethanol and with 20% ethanol was determined in a similar manner. After the combined extracts of each suspension were concentrated to 3–4 cc., aliquots calculated to contain 1×10^6 counts/min. each were applied to several washed Whatman No. 4 papers and separated first in phenol–water and then in butanol–propionic acid in the manner described by Wilson and Calvin.[5] After radioautographs of the chromatograms had been made, the various components detected on the papers were counted (Table I). Since the correction for self-absorption of radiation would be the same for each compound, no correction was applied. The results of a duplicate experiment, in which 1 mg. of azaserine was used, are also presented (Table I).

Other experiments carried out in an identical manner to those described above were: (1) a repetition of the 1-mg. azaserine experiment in which an intense photospot light was substituted for one of the reflector floods for 50 minutes

(1) The work described in this paper was sponsored by the U. S. Atomic Energy Commission.

(2) Rockefeller Research Fellow, 1955–1956.

(3) S. C. Hartman, B. Levenberg and J. M. Buchanan, THIS JOURNAL, **77**, 501 (1955).

(4) B. Levenberg and J. M. Buchanan, *ibid.*, **78**, 504 (1956).

(5) A. T. Wilson and M. Calvin, *ibid.*, **77**, 5948 (1955).