free of the isomethine derivative (tlc) as white prisms, mp 200–201°.

Anal. Calcd for $C_{21}H_{29}NO_3 \cdot CH_3I$: C, 54.5; H, 6.6. Found: C, 54.5; H, 6.6.

Hofmann Degradation of Neodihydrothebaine Dihydromethine Methyl Ether (XIV) Methiodide. The methiodide (2.0 g) was degraded in boiling aqueous potassium hydroxide, when trimethylamine was evolved and **2-ethyl-5,6,5′-trimethoxy-2′-vinyldiphenyl (XVI)** was isolated by ether extraction and distillation and obtained as a colorless oil, bp 190–200° (bath temperature) (0.05 mm).

Anal. Calcd for $C_{19}H_{22}O_3$: C, 76.5; H, 7.6. Found: C, 76.5; H, 7.4.

Catalytic reduction of this compound proceeded readily in ethanol over 10% palladium on charcoal, and gave 2,2′-diethyl-5,6,5′-trimethoxydiphenyl (XVII), identical in thin layer chromatographic behavior with material prepared by the reduction of 2′-ethyl-5,6,5′-trimethoxy-2-vinyldiphenyl (XVIII) described above.

Kryptothebaine (IV). The product of reaction of thebaine (31.1 g) with anhydrous magnesium iodide in ether–benzene suspension was treated with 2 *N* hydrochloric acid. A sticky insoluble mass (presumably a hydriodide) was obtained, and the aqueous and organic solutions were poured off. After the material was dissolved in methanol, the solution was diluted with ice water and cautiously basified with ammonia. A further quantity of base was obtained by adding ammonia to the aqueous acid layer separated as above. The precipitated solid was collected and recrystallized from ethanol and from aqueous 2-ethoxyethanol; the same product, kryptothebaine (IV), was obtained in each case as green-gray prisms, mp 274° (8.5 g). Recrystallization from aqueous 2-ethoxyethanol with the addition of a drop of aqueous sodium dithionite gave the enamine as pale cream prisms, mp 274°, but these rapidly became green on standing, the color darkening to almost black after several days.

Anal. Calcd for $C_{38}H_{32}N_2O_6$: C, 73.3; H, 6.75; mol wt, 622. Found: C, 73.3; H, 6.8; mol wt (Rast in camphor), 586.

Reduction of this base catalytically over 10% palladium on charcoal was sluggish and was only complete after 10 hr, and reduction with sodium borohydride was slow even in boiling 2-ethoxyethanol. The same base was obtained in each case, and unlike neodihydrothebaine (which it closely resembled in infrared absorption), it was completely insoluble in ether. No crystalline salts of this uncrystallizable base could be prepared.

Catalytic reduction of the crude product (2 g) obtained on basification of the aqueous methanolic solution of the hydriodide, however, gave, slowly, a product part of which dissolved in ether. Evaporation of the ether gave neodihydrothebaine (0.16 g) identified as its methiodide, mp 249–250° from ethanol.

Reaction of Kryptothebaine (IV) with Hydrochloric Acid. Kryptothebaine (IV), prepared as above (2 g), was boiled with 2 *N* hydrochloric acid (10 ml) for 5 min. The solution, on cooling, deposited a viscous gum (2.0 g), which crystallized from water at 0° as yellow needles, mp 234–236°, identical in melting point, mixture melting point, infrared absorption, and R_f value with thebenine (II) hydrochloride prepared in the same way from thebaine.

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The Structures of Deserpideine and Raujemidine[1]

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Abstract: The indole alkaloids deserpideine and raujemidine correspond to expressions I and Ia, respectively. The most interesting transformation of deserpideine is its hydrogenation and hydrogenolysis with Adams catalyst to yield products II, III, X, and XII.

The initial investigation of the alkaloids of *Rauwolfia nitida* Jacq. by Salkin and his group[4] led to the isolation of ajmalicine, isoreserpiline, isoreserpinine, raunitidine, raunitidine, reserpiline, and reserpinine.

We have now found that the weakly basic alkaloid fraction from certain samples of *R. nitida* yields, in addition to the known alkaloid deserpidine (II), a new crystalline alkaloid, deserpideine (I), $C_{32}H_{36}N_2O_8$, mp 149–152°, $[\alpha]^{25}D$ −133° (pyridine), whose ultraviolet spectrum is identical with its companion deserpidine. The infrared spectrum of deserpideine is also very similar to that of deserpidine, but varies slightly from the latter in the fingerprint region. In analogy with deserpidine (II), the Bohlmann bands between 3.4 and 3.6 μ were missing in deserpideine (I), indicating a *cis* C–D ring fusion.

The chemistry of deserpideine (I) initially indicated a behavior that somewhat paralleled the chemistry of deserpidine (II). The reaction of deserpideine (I) with sodium methoxide at room temperature gave the expected methyl 3,4,5-trimethoxybenzoate (IIIa) and methyl deserpideate (IV), $C_{22}H_{26}N_2O_4 \cdot H_2O$, mp 159–162°, $[\alpha]^{25}D$ +8° (pyridine). Hydrolysis of either deserpideine or methyl deserpideate with potassium hydroxide in aqueous methanol gave deserpideic acid (V), $C_{21}H_{24}N_2O_4$, mp 224–226°, $[\alpha]^{25}D$ −29° (50% methanol–water), which could be converted to deserpideic acid lactone (VI), $C_{21}H_{22}N_2O_3$, mp 159–161°, $[\alpha]^{25}D$ −58° (50% methanol–water), on treatment with acetic anhydride in pyridine. When deserpideic acid lactone was allowed to stand at 0° in sodium methoxide in methanol, methyl deserpideate (IV) was obtained. Deserpideine (I) itself could be reconstituted by treatment of methyl deserpideate with 3,4,5-trimethoxy-

(1) Preliminary communications of some of these results appeared in (a) E. Smith, R. S. Jaret, M. Shamma, and R. J. Shine, *J. Am. Chem. Soc.*, **86**, 2083 (1964); (b) M. Shamma and R. J. Shine, *Tetrahedron Letters*, 2277 (1964); and (c) E. Smith, R. S. Jaret, M. Shamma, and R. J. Shine, *Lloydia*, **27**, 440 (1964).

(2) Deceased October 23, 1965.

(3) M. S. wishes to thank the National Science Foundation for grants GP-1941 and GP-6394 in support of this research, and for a grant to the Department of Chemistry at The Pennsylvania State University for the purchase of a Varian A-60 nmr instrument and a Nuclide single-beam mass spectrometer.

(4) R. Salkin, N. Hosansky, and R. Jaret, *J. Pharm. Sci.*, 50, 1038 (1961).

benzoyl chloride. The reaction cycle was completed by converting deserpideic acid (V) to methyl deserpideate (IV). This esterification could be accomplished by either of two methods. The reaction of deserpideic acid with diazomethane proceeded poorly giving only a 25% yield of methyl deserpideate, the remainder of the product being unreacted starting material. The Fischer esterification, however, went smoothly at room temperature giving an 88% yield of methyl deserpideate.

Oxidation of deserpideine with 2 molar equiv of lead tetraacetate and reduction of the crude reaction product with borohydride gave a new compound, 3-isodeserpideine (VII), crystallized as the nitrate and the methiodide salts.

On the other hand, if the methanolysis of deserpideine with sodium methoxide were allowed to proceed in refluxing methanol, a considerable amount of a new compound was obtained. This material, mp 174–176°, analyzes for $C_{21}H_{20}N_2O_2 \cdot 0.5C_2H_5OH$, has a very high dextrorotation, $[\alpha]^{25}D$ +288° (pyridine), and an infrared spectrum exhibiting only one carbonyl band which appears at 5.83 μ. The nmr spectrum shows one methoxyl singlet at δ 3.88 due to three protons, and the mass spectrum has a molecular ion peak at *m/e* 332. From these data, structure VIII was considered for this new compound.

To ascertain this structural assignment, our highly dextrorotatory compound, presumed to be VIII, was converted to the alcohol IX by means of lithium aluminum hydride and compared with authentic IX that we had synthesized by a route developed by Elderfield and Fischer.[5] Comparison of the two alcohols (IX) as their hydrobromide salts showed the two salts to be identical. They both had identical infrared spectra taken as KBr pellets and they showed the same characteristics on melting. Paper chromatography R_f values in a number of solvent systems further showed the identity of the two materials. The structure of the highly dextrorotatory compound was thus shown to be 11-demethoxytetrahydroalstoniline (VIII), and its formation has no analogy in the reserpine series.

Significant information about the stereochemistry of the new alkaloid could be derived from a study of its rate of quaternization with methyl iodide. Rates of methiodide formation had previously proven to be a useful tool in the study of the stereochemistry of the yohimbinoid alkaloids.[6] These rates were determined in acetonitrile solution using pseudo-first-order kinetics, a large excess of methyl iodide being present. The pseudo-first-order rate constants for deserpideine and its derivatives and a variety of other indole alkaloids are given in Table I. In the yohimbine series, such a very fast rate as that exhibited by deserpideine (I) and its derivatives IV and VI is associated with the pseudo-configuration, but in the present case a more specific statement is desired; namely, that in deserpideine and its derivatives the basic nitrogen is unhindered.

A second piece of data of great utility was supplied by mass spectrometry. Since deserpideine free base is rather unstable, the mass spectra were run on its more stable crystalline derivatives, methyl deserpideate (IV) and deserpideic acid lactone (VI). The molecular ion peaks of methyl deserpideate and the lactone, at *m/e* 382 ($C_{22}H_{26}N_2O_4$) and *m/e* 350 ($C_{21}H_{22}N_2O_3$), respectively, were two mass units less than for the corresponding compounds in the deserpidine series. These results immediately indicated the presence of either an unsaturation or an additional ring in compounds of the deserpideine series, and further confirmed the assignment of the $C_{32}H_{36}N_2O_8$ formulation to deserpideine, which is two mass units less than that for deserpidine. The choice between an unsaturation and an extra ring was decided by the study of a third piece of data, the nmr spectra. The spectra of deserpideine and its derivatives show an absorption at δ 5.55 corresponding to one proton. This absorption is in the region of vinylic absorption, so that deserpideine possesses a trisubstituted double bond that is not conjugated to the chromophoric system. The most likely positions for such a double bond would be C-14(15) or C-19(20).

Table I. Pseudo-First-Order Rates of Methiodide Formation

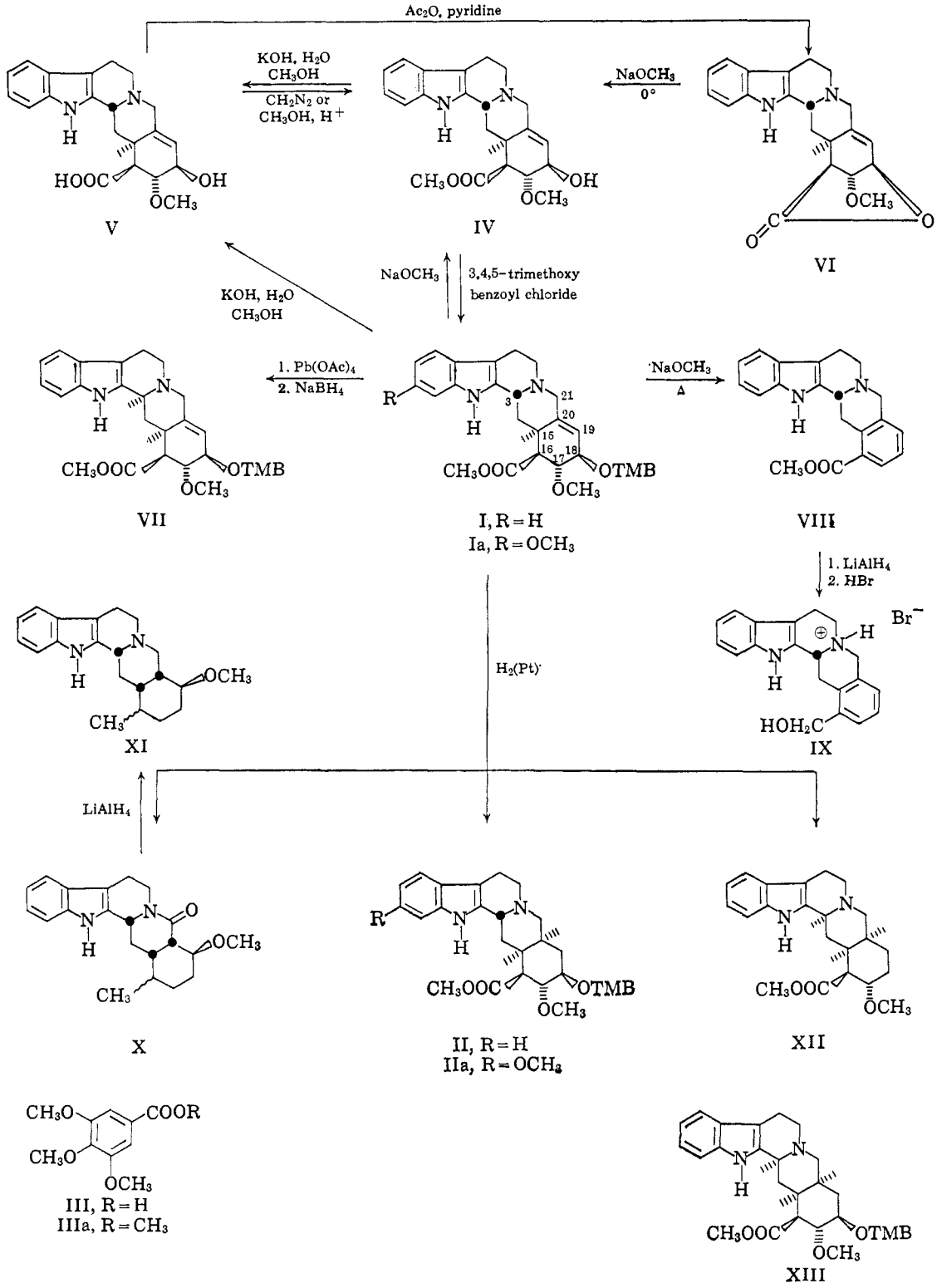
Alkaloids and derivatives	$k \times 10^4$, sec^{-1}	Stereochemistry
Deserpideine (I)	300	...
Methyl deserpideate (IV)	350	...
Deserpideic acid lactone (VI)	220	...
3-Isodeserpideine (VII)	31	...
Deserpidine (II)	7.2	Epiallo
16-Methyl-19-methoxyalloyohimbane (XI)	2.5	Allo
α-Yohimbine methyl ether (XII)	1.1	Allo
3-Isodeserpidine	1.0	Allo
Yohimbine	48.7	Normal

In an attempt to reduce this isolated double bond, deserpideine was hydrogenated using Adams catalyst in ethanol at room temperature. The alkaloid did take up hydrogen, but only slowly. If the reaction were allowed to proceed for 12 hr, a complex mixture of products ensued.

A thin layer chromatogram of the reduction product showed at least ten spots. The crude hydrogenation mixture was, therefore, separated into an acidic, a basic, and a neutral fraction by classical extraction techniques. A crystalline compound was obtained in 68% yield from the acidic fraction which was easily characterized as 3,4,5-trimethoxybenzoic acid (III). The identification of this material strongly indicates the position of the double bond to be at C-19(20), so that hydrogenolysis of the allylic 3,4,5-trimethoxybenzoyl group can then occur.

Following removal of the acidic and neutral components, crystallization of the basic fraction was possible, and a white crystalline compound was obtained. This compound was shown to be identical with authentic deserpidine (II) in a number of ways. The two compounds had the same infrared spectra in chloroform and in acetonitrile solution, and their nmr spectra were also identical. Thin layer chromatograms in a number of solvent systems always resulted in the two compounds having the same R_f values. A methiodide rate study showed the synthetic material to have a rate of 7.2×10^{-4} sec^{-1}, characteristic of the expected epiallo configuration. Finally, the melting points (224–226°) were the same and a mixture melting point was undepressed. As a precautionary measure, rotations were determined and found to be the same, $[\alpha]^{25}D$ −123° in chloroform. This shows that the deserpidine isolated from the hydrogenation reaction has the same ab-

(5) R. Elderfield and B. Fischer, *J. Org. Chem.*, 23, 949 (1958).
(6) M. Shamma and J. Moss, *J. Am. Chem. Soc.*, 83, 5038 (1961); M. Shamma and J. Richey, *ibid.*, 85, 2507 (1963).



solute configuration as natural deserpidine. Thus, the identity of the two compounds was clearly established, and this transformation proves the stereochemistry of deserpideine to be as indicated in I.

The neutral fraction readily crystallized into a colorless compound which contained no carbonyl band between 5 and 6 μ, but possessed a very intense absorption at 6.13 μ. The loss of basicity of the nitrogen and the disappearance of the ester carbonyl bands accompanied by the presence of the 6.13-μ peak clearly point to allylic hydrogenolysis of the C-21(N-4) bond followed by intramolecular ester–amide conversion to form lactam X.[7] Further evidence for the structural assignment for the neutral compound is seen in the nmr and mass spectra of the material. The mass spectrum shows a molecular ion peak at *m/e* 338 which is the correct molecular weight calculated for 16-methyl-19-methoxy-21-oxoyohimbane (X). The nmr spectrum shows only one methoxyl absorption at δ 3.25 and shows a C–CH_3 doublet at δ 1.03. The absence of a vinylic absorption and the presence of the C–CH_3 doublet clearly indicate reduction of the double bond

(7) F. L. Weisenborn and H. E. Applegate, *J. Am. Chem. Soc.*, **78**, 2021 (1956).

originally present in I. The stereochemistry of the asymmetric centers at C-3, C-15, C-19, and C-20 in lactam X must be as indicated from our knowledge of the stereochemistry of deserpideine. Upon reduction of lactam X with lithium aluminum hydride, 16-methyl-19-methoxyalloyohimbane (XI) was obtained which showed a molecular ion peak at *m/e* 324 in accordance with the formula $C_{21}H_{28}N_2O$. The infrared spectrum showed no carbonyl absorption and specifically the band at 6.13 μ had disappeared. The rate of methiodide formation for compound XI was found to be 2.5×10^{-4} sec^{-1} which is a slow rate strongly characteristic of an allo configuration.

After removing the crystalline deserpidine, the basic fraction of the hydrogenation product still contained several minor components as monitored by thin layer chromatography, and it was found possible to separate one additional basic component. The infrared spectrum of this compound showed the absence of the band at 6.3 μ, characteristic of the 3,4,5-trimethoxybenzoyl group, and the presence of Bohlmann bands at 3.4–3.6 μ. It was, therefore, assumed that this compound had lost the 3,4,5-trimethoxybenzoyl group *via* hydrogenolysis, and that the proton at C-3 had epimerized to the α configuration. A rate of methiodide formation was needed to decide from which side of the molecule hydrogenation had occurred, and a choice had to be made between the normal and the allo configuration for the new base. The rate was found to be extremely slow, 1.1×10^{-4} sec^{-1}, indicating an allo configuration and α hydrogenation, so that the compound had to be the known α-yohimbine methyl ether (XII).[8] Indeed, when the two compounds were compared, they were found to be identical. The isolation of this product further supports the assignment of stereochemistry of the asymmetric centers at C-15, C-16, and C-17 in deserpideine (I). The isomerization of C-3 in this reaction is not surprising since it is known that reserpine is converted to 3-isoreserpine under these reducing conditions.[9]

Catalytic hydrogenation of 3-isodeserpideine (VII) under the same conditions as for deserpideine also led to a complex mixture of products. Extraction of the acidic material gave a 65% yield of 3,4,5-trimethoxybenzoic acid, the same yield as obtained from the hydrogenation of deserpideine. Addition of methanol to the amorphous material obtained after extraction of the acidic material caused it to crystallize. The crystalline material, obtained in a 28% yield, was shown to be a α-yohimbine methyl ether (XII) by comparison with an authentic sample. The yield of this compound is significantly greater than in the hydrogenation of deserpideine which gave a 12% yield, and this would be expected since the hydrogen at C-3 is already in the α configuration in 3-isodeserpideine (VII). A small amount of 3-isodeserpidine (XIII) also could be obtained from the hydrogenation.

Hydrogenation of methyl deserpideate (IV) with Adams catalyst led to a complex mixture of products. This reaction mixture was separated into a neutral lactam fraction and a basic fraction. Preparative thin layer chromatography of the basic fraction yielded pure methyl deserpidate (XIV) which was shown to be identical with authentic material.

These data give compelling evidence that deserpideine must have structure I and is, therefore, 19-dehydrodeserpidine. The position of the double bond is demonstrated by the results of the hydrogenation experiments, the rate studies, and the formation of 11-demethoxytetrahydroalstoniline (VIII). All asymmetric centers were shown to have the same stereochemistry as deserpidine.

Application of Hudson's lactone rule[10] to the deserpideine series to determine the absolute configuration led to ambiguous results. When the rotations were measured in methanol, the change in the molecular rotation from deserpideic acid, $[M]^{25}D$ $-107°$, to deserpideic acid lactone, $[M]^{25}D$ $-206°$, was found to be $-99°$, indicating that the hydroxyl group at C-18 is α. This configuration is opposite to that assigned on the basis of the isolation of (−)-deserpidine from the hydrogenation reaction. However, when the rotations were measured in pyridine, the change in molecular rotation was found to be $+73.3°$, indicating a β configuration for the C-18 hydroxyl. Thus, the absolute configuration that may be assigned by use of Hudson's lactone rule is shown to be solvent dependent in the present case. This finding along with other known exceptions[11-14] shows that assignments of absolute configuration on the basis of Hudson's lactone rule should be done with extreme caution.

11-Demethoxytetrahydroalstoniline (VIII) has one asymmetric center and the compound is dextrorotatory, so that the contribution of C-3 to the assymmetry of the molecule must be dextrorotatory. The method of rotational differences had previously been applied to the C-3 hydrogen in the reserpine series.[15] It was found that when the hydrogen at C-3 was α, this center made a large negative contribution to the molecular rotation. In the case of 11-demethoxytetrahydroalstoniline, the asymmetric center at C-3 makes a large positive contribution to the molecule rotation, and therefore the C-3 proton should be β. This is in agreement with the absolute configuration presently assigned to deserpideine on the basis of the isolation of (−)-deserpidine from the hydrogenation mixture.

It is fitting at this point to turn our attention to the alkaloid raujemidine obtained from *R. canescens* in 1956. This base had been reported to give an elemental analysis nearly identical with that of reserpine, and upon treatment with sodium methoxide yielded methyl 3,4,5-trimethoxybenzoate.[16] A rate of methiodide formation was, therefore, determined for raujemidine and was found to be 3.0×10^{-2} sec^{-1}. This very fast rate indicated that the molecule possesses an unhindered basic nitrogen. The nmr spectrum of raujemidine was close to that of reserpine, but it possessed an additional vinylic absorption at δ 5.50. The relationship between this alkaloid and deserpideine seemed obvious; it appeared

(8) A. Popelak and G. Lettenbauer, *Arch. Pharm.*, **295**, 427 (1962).
(9) R. Lucas, M. Kuehne, M. Ceglowski, R. Dziemian, and H. MacPhillamy, *J. Am. Chem. Soc.*, **81**, 1928 (1959); F. L. Weisenborn, *ibid.*, **79**, 4818 (1957).
(10) W. Klyne, *Chem. Ind.* (London), 1198 (1954).
(11) H. Fritz, *Angew. Chem. Intern. Ed. Engl.*, **1**, 513 (1962).
(12) J. Clark-Lewis and P. Mortimer, *J. Chem. Soc.*, 4268 (1961).
(13) G. Fodor and F. Soti, *Tetrahedron Letters*, 1917 (1964).
(14) R. Lemieux and M. Hoffer, *Can. J. Chem.*, **39**, 110 (1961).
(15) C. Huebner, H. MacPhillamy, E. Schlittler, and A. St. André, *Experientia*, **11**, 303 (1955).
(16) P. Ulshafer, M. Pandow, and R. Nugent, *J. Org. Chem.*, **21**, 923 (1956).

that raujemidine was 19-dehydroreserpine (Ia), so that raujemidine stands to reserpine in the same relationship that deserpideine is related to deserpidine.

To test this hypothesis, raujemidine was hydrogenated under the same conditions as deserpideine. It was found that the two compounds behaved similarly in the presence of platinum and hydrogen. Raujemidine hydrogenated slowly and gave a complex mixture of products. The 3,4,5-trimethoxybenzoic acid (III) was extracted from the product and was shown to be identical with authentic material. This settled the position of the double bond at C-19. An infrared spectrum of the complex residue showed an increased absorption at 6.13 μ due to the formation of a lactam. The residue was purified by preparative scale thin layer chromatography and the band that possessed the same R_f value as reserpine (IIa) was examined in detail. This compound was eluted from the silica gel and crystallized from methanol. The crystalline compound was shown to be identical with authentic reserpine by comparing physical properties. They both had identical infrared spectra in chloroform and acetonitrile. The nmr spectra were practically superimposable, the melting points were identical, and a mixture melting point was undepressed. The R_f values in a number of solvent systems were identical.

The structure of raujemidine was therefore settled to be Ia, with a molecular formula $C_{33}H_{38}N_2O_9$. Raujemidine is the second member of the 19-dehydroyohimbinoid series to be characterized. Further investigation with raujemidine was precluded by its instability, and also by lack of sample. Very recently, Arndt and Djerassi isolated 19-dehydroyohimbine from *Aspidosperma pyricollum* which represents the third member of the rare 19-dehydroyohimbinoid alkaloids.[17]

Experimental Section

Standard Experimental Procedures. All microanalyses were performed by Midwest Microlab, Inc., Indianapolis, Ind., and Schwarzkopf Microanalytical Laboratory, Woodside, N. J. The melting points were taken either on a Nalge melting point apparatus or in a sealed capillary and are uncorrected. The nmr spectra were measured on a Varian Associates A-60 spectrometer using microcells purchased from Varian Associates, with deuteriochloroform as the solvent and tetramethylsilane as the internal standard. The mass spectra of a few derivatives were graciously determined in the laboratories of Professor Carl Djerassi or Professor Klaus Biemann. The remainder of the mass spectra were obtained on a Nuclide 12-90-G1.1 mass spectrometer. Adsorbosil-1, purchased from Applied Science Laboratories of State College, Pa., was used to prepare the thin layer chromatography plates used in this study. The spots were visualized by placing the plates in iodine vapors or by shining an ultraviolet light.

Deserpideine (I). The milled root of *R. nitida*, originally collected on the island of Hispaniola, was moistened with saturated potassium carbonate solution and extracted with benzene. The benzene extract was shaken with 0.5 *M* phosphoric acid. The acid solution was extracted at pH 2 with chloroform, and the chloroform layer washed with potassium carbonate solution. Evaporation of the solvent left the weakly basic alkaloid fraction behind.

The alkaloids were precipitated from ethanol solution as their nitrate salts. The crude nitrate salts were placed in chloroform and washed with aqueous sodium carbonate solution. The chloroform layer was then separated and the solvent evaporated.

The residue was taken up in methanol, and the reserpine which crystallized out was removed by filtration. After concentration to dryness, the mother liquors were taken up in ethyl acetate to give a mixture of deserpidine and deserpideine. Crystallization of the crude hydrochloride salts from acetone gave deserpideine hydrochloride, which after recrystallization from methanol melted at 277° dec, $[\alpha]^{25}D$ −98.5° (*c* 0.5, chloroform).

Anal. Calcd for $C_{32}H_{37}ClN_2O_8$: C, 62.68; H, 6.09; Cl, 5.78; N, 4.57; O, 20.87. Found: C, 62.49; H, 6.23; Cl, 5.76; N, 4.56; O, 20.81.

The free base was liberated from a chloroform solution of the hydrochloride with sodium carbonate and crystallized from ether, mp 149–152° dec, $[\alpha]^{25}D$ −133° (*c* 1, pyridine), $[\alpha]^{25}D$ −108° (chloroform).

Anal. Calcd for $C_{32}H_{36}N_2O_8$: C, 66.64; H, 6.29; N, 4.86; O, 22.20; $5OCH_3$, 26.87. Found: C, 66.62; H, 6.60; N, 4.91; O, 22.30; OCH_3, 26.30.

Deserpideine Methiodide. A solution of 100 mg (0.174 mmole) of deserpideine in 1.5 ml of ethyl acetate was prepared and cooled to 0°. To this slightly yellow solution, 1.0 ml of pure methyl iodide was added. The flask was flushed with nitrogen, stoppered, and allowed to stand at 0° for 4 hr. Evaporation of the excess methyl iodide caused an insoluble residue to form. Crystallization of this residue was effected by adding three drops of methanol to the ethyl acetate mixture and heating. Filtration and recrystallization from methanol gave 112 mg (0.156 mmole, 90%) of the white crystalline methiodide. Recrystallization from methanol for an analytical sample gave a compound that melted 246–248° dec.

Anal. Calcd for $C_{33}H_{39}IN_2O_8$: C, 54.70; H, 5.43. Found: C, 54.89; H, 5.47.

Methyl Deserpideate (IV). About 100 mg of clean sodium metal was placed into 10 ml of anhydrous methanol. The solution was allowed to stand in ice for 1 hr. Then, 921 mg (1.60 mmoles) of deserpideine in 10 ml of dry methanol was added. The flask was flushed with nitrogen, stoppered, and allowed to stand in the dark at room temperature for 24 hr. The solution had acquired a yellow color. After adding 20 g of ice, the solution was adjusted to pH 6 (Hydrion paper) with dilute hydrochloric acid. The solution was then extracted with three 10-ml portions of ether to remove the methyl 3,4,5-trimethoxybenzoate. The aqueous layer was made basic by adding 5% sodium carbonate and was then extracted with three 10-ml portions of chloroform. The chloroform solution was dried over sodium sulfate and filtered, and the solvent was removed under reduced pressure. The residue that remained weighed 612 mg. This was dissolved in 2 ml of 95% ethanol, and the solution was made acidic by adding hydrochloric acid in ethanol. Crystallization proceeded very slowly. Filtration gave 400 mg (0.96 mmole, 60%) of white crystalline methyl deserpideate hydrochloride, mp 246–247° dec, λ_{max}^{EtOH} 221 and 278 mμ (log ϵ 4.59 and 3.94).

Anal. Calcd for $C_{22}H_{27}ClN_2O_4$: C, 63.07; H, 6.50; Cl, 8.46; N, 6.69; O, 15.27; $2OCH_3$, 14.97. Found: C, 63.30; H, 6.40; Cl, 8.81; N, 6.55; O, 15.45; OCH_3, 14.67.

The free base melted at 159–161° dec, $[\alpha]^{25}D$ +8.4° (*c* 1, pyridine), λ_{max}^{EtOH} 225 and 280 mμ (log ϵ 4.53 and 3.91).

Anal. Calcd for $C_{22}H_{26}N_2O_4 \cdot 0.5\ H_2O$: C, 67.50; H, 6.95; N, 7.16. Found: C, 67.38; H, 6.76; N, 7.36.

Treatment of methyl deserpideate with 3,4,5-trimethoxybenzoyl chloride in pyridine resulted in the formation of deserpideine with unchanged specific rotation and melting point.

11-Demethoxytetrahydroalstoniline (VIII). Deserpideine (2 g, 3.46 mmoles) was refluxed in 90 ml of absolute methanol and 0.2 g of sodium under nitrogen for 2 hr. The solution was acidified with glacial acetic acid and concentrated *in vacuo* to 10 ml, poured into ice water, acidified with HCl, and extracted with ether. From the ether layer, 0.7 g of 3,4,5-trimethoxybenzoic acid methyl ester was obtained. Basification with potassium carbonate and extraction with ether gave 1.2 g of crude product. Crystallization from alcoholic HCl gave 0.7 g (49%) of methyl deserpideate hydrochloride, mp 246–247° dec, $[\alpha]^{25}D$ −46° (*c* 1, methanol).

The mother liquors of the methyl ester hydrochloride crystallization after conversion to the free base were chromatographed over alumina (Merck acid washed) and eluted with benzene followed by benzene with increasing amounts of chloroform. A 50:50 mixture eluted a substance which crystallized as the hydrochloride from absolute alcohol and had a melting point of 237–239° dec, $[\alpha]^{25}D$ +249° (50% ethanol–water), λ_{max}^{EtOH} 223 and 282 mμ (log ϵ 4.70 and 4.02).

Anal. Calcd for $C_{21}H_{21}ClN_2O_2$: C, 68.38; H, 5.74; Cl, 9.61; N, 7.59; O, 8.68. Found: C, 68.37; H, 5.76; Cl, 9.32; N, 7.40; O, 8.66.

The free base VIII crystallized from ethanol, mp 174–176° dec, $[\alpha]^{25}D$ +288° (*c* 0.5, pyridine), and was susceptible to air oxidation.

Anal. Calcd for $C_{21}H_{20}N_2O_2 \cdot 0.5C_2H_5OH$: C, 74.34; H, 6.52; N, 7.88; O, 11.25; OCH, 8.73. Found: C, 74.84; H, 6.52; N, 7.70; O, 11.14; OCH_3, 9.07.

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Deserpideic Acid (V). Deserpideine hydrochloride (1.2 g, 1.96 mmoles) in 12 ml of methanol and 6 ml of 10 *N* sodium hydroxide was kept at 45° for 5 hr. The alcohol was then removed under slight vacuum, and the solution was carefully adjusted to pH 4.8 giving 3,4,5-trimethoxybenzoic acid (0.43 g). Acidification to pH 2 with concentrated HCl gave crystals of deserpideic acid hydrochloride (0.5 g, 63%). The salt was recrystallized from water, mp 282° dec, $[\alpha]^{25}D$ −32.5° (methanol). The melting point of deserpideic acid was 224–226° dec, $[\alpha]^{25}D$ −29° (50% methanol–water).

Anal. Calcd for $C_{21}H_{25}ClN_2O_4$: C, 62.30; H, 6.22; Cl, 8.76; N, 6.92. Found: C, 62.39; H, 6.69; Cl, 8.42; N, 6.84.

Deserpideic acid Lactone (VI). Deserpideic acid (2 g, 5.43 mmoles) was kept at room temperature for 24 hr in 25 ml of pyridine and 20 ml of acetic anhydride. The solution was then concentrated *in vacuo* and poured onto ice. The clear solution was made basic with sodium bicarbonate and extracted with chloroform, and the chloroform solution concentrated to incipient crystallization. Filtration and recrystallization from methanol gave deserpideic acid lactone (1.1 g, 58%), mp 159–161° dec, $[\alpha]^{25}D$ −58° (methanol).

Anal. Calcd for $C_{21}H_{22}N_2O_3$: C, 71.98; H, 6.33; N, 7.99. Found: C, 71.81; H, 6.73; N, 8.24.

Preparation of Methyl Deserpideate (IV) from Deserpideic Acid (V) and Diazomethane. A solution of diazomethane in ether was prepared by stirring 1.0 g of Du Pont's EXR-101 (70:30 N,N'-dimethylterephthalamide:white mineral oil) in 10 ml of 50% sodium hydroxide and 50 ml of ether and codistilling the diazomethane with ether. To this ethereal diazomethane solution, a solution of 340 mg (0.924 mmole) of deserpideic acid in 15 ml of ether and 45 ml of methanol was added. The yellow solution was allowed to stand at 0° overnight in a hood. After evaporating the solvent, the methyl deserpideate was removed by adding chloroform to the residue and filtering. The starting acid is insoluble in chloroform. The yield of methyl deserpideate was 87 mg (0.228 mmole, 25%).

Preparation of Methyl Deserpideate (IV) from Deserpideic Acid (V) and Methanolic Hydrogen Chloride. To a solution of dry hydrogen chloride in 15 ml of anhydrous methanol 202 mg (0.544 mmole) of deserpideic acid was added. The flask was flushed with nitrogen, stoppered, and allowed to stand at room temperature in the dark for 48 hr. Th methanol was evaporated *in vacuo* giving a yellow viscous oil. This oil was extracted with 5% sodium carbonate and chloroform. The chloroform layer was dried, and the solvent was removed giving 185 mg (0.484 mmole, 88%) of an off-white solid whose infrared spectrum and nmr spectrum were identical with those of authentic methyl deserpideate. This product was recrystallized from methanol, mp 159–161° dec.

Preparation of Methyl Deserpideate (IV) from Deserpideic Acid Lactone (VI). About 25 mg of fresh sodium was placed into 3 ml of anhydrous methanol. After the sodium had reacted, the solution was cooled to 0°, and 15 mg (0.0428 mmole) of deserpideic acid lactone was added. The flask was flushed with nitrogen, stoppered, and allowed to stand in the dark at 0° for 45 hr. Dry methanolic hydrogen chloride was slowly added to bring the solution to pH 5, sodium chloride precipitating out. The solvent was removed by passing a stream of dry nitrogen over the solution. Chloroform was added to the residue, and the inorganic salts were filtered. The chloroform was evaporated under reduced pressure; the residue was taken up into 5 ml of chloroform, and the solution was washed with two 5-ml portions of 5% sodium carbonate solution. The chloroform layer was dried with anhydrous sodium sulfate and filtered and the solvent removed under reduced pressure. The product from this treatment had melting point and infrared and nmr spectra that were identical with those of authentic methyl deserpideate.

Hydrogenation of Deserpideine (I). Exactly 2.86 g (4.96 mmoles) of deserpideine was dissolved in 250 ml of 95% ethanol and added to a mixture of 565 mg of platinum oxide in 10 ml of 95% ethanol. The hydrogenation bottle was flushed with nitrogen and placed on a Parr hydrogenation apparatus and was then charged with 36 lb of hydrogen. Shaking was started and continued for 2 days. The bottle was then removed and thoroughly flushed with nitrogen. The catalyst was filtered from the solution by repeatedly passing the mixture through very retentive, quantitative filter paper (Schleicher and Schuell, Analytical Filter Paper. No. 576) until no catalyst was seen on the filter paper; four times were sufficient. The solvent was removed under reduced pressure giving 2.69 g of yellowish amorphous solid. The hydrogenation product was extracted with aqueous sodium carbonate and chloroform. The aqueous extracts were acidified by the addition of concentrated hydrochloric acid. A white crystalline precipitate formed, which was filtered from the cold solution giving 723 mg (3.41 mmoles, 69%) of a white crystalline solid, mp 168–170°. This compound was shown to be identical with authentic 3,4,5-trimethoxybenzoic acid by melting point, mixture melting point, and infrared spectrum. The chloroform extracts were dried over anhydrous sodium sulfate, filtered, and evaporated to dryness leaving 1.97 g of a tan amorphous solid. This material was then distributed between ether and 0.5 *M* phosphoric acid. The ether extracts were dried and evaporated leaving 410 mg of a yellow residue. This was the neutral fraction. Addition of ethyl acetate to this fraction caused it to crystallize. After filtration and recrystallization from methanol, 123 mg of white crystalline 16-methyl-19-methoxy-21-oxoyohimbane (X) was obtained, mp 245–247° dec, $[\alpha]^{25}D$ +142° (*c* 0.8, chloroform). An infrared spectrum showed no absorption between 5.0 and 6.0 μ. The nmr spectrum showed one methoxyl absorption at δ 3.25 and a C–CH_3 doublet at δ 1.03. The mass spectrum exhibited a molecular ion peak at *m/e* 338.

Anal. Calcd for $C_{21}H_{26}N_2O_3$: C, 74.52; H, 7.74; N, 8.28. Found: C, 74.62; H, 7.84; N, 8.55.

The acidic aqueous layer containing the basic fraction was basified with aqueous sodium carbonate to pH 8 (Hydrion paper) and extracted with ether. The ether extracts were dried over anhydrous sodium sulfate, filtered, and evaporated to dryness leaving 1.39 g of a tan amorphous solid. Addition of ethyl acetate to this material caused it to crystallize. Recrystallization from methanol gave 156 mg of white crystals, mp 224–226° dec, $[\alpha]^{25}D$ −123° (*c* 0.3, chloroform). This compound had a rate of methiodide formation of 7.2×10^{-4} sec^{-1}, characteristics of the epiallo configuration. Its melting point was the same as authentic deserpidine, and a mixture melting point was undepressed. Infrared spectra in chloroform and acetonitrile showed the compounds to be identical. The nmr spectra were superimposable. R_f values in a number of solvent systems also showed their identity: R_f 0.50, chloroform:ethanol:acetone (90:5:5); R_f 0.92, ether:methanol (80:20); R_f 0.63, ether:ethanol (95:5). Finally, both compounds had the same specific rotation, $[\alpha]^{25}D$ −123° in chloroform.

From the mother liquor of the above crystallization, 1.20 g of amorphous material was obtained. A thin layer chromatogram with chloroform:acetone:methanol (80:16:4) as the solvent system showed that the material consisted of at least nine compounds. A preparative scale, thin layer chromatogram was used to purify this mixture, but only one fraction crystallized, mp 266–267° dec, and was shown to be identical with authentic α-yohimbine methyl ether (XII). The infrared spectra were identical. Thin layer chromatograms showed the two compounds to have the same R_f values in a number of different solvent systems: R_f 0.74, chloroform:acetone:methanol (90:8:2); R_f 0.81, benzene:acetone (80:20); R_f 0.67, benzene: ether (50:50); R_f 0.42, chloroform:acetone (94:6); R_f 0.41, benzene:acetone (90:10); R_f 0.33, benzene:ether (80:20); R_f 0.53, chloroform:ether (80:20).

Preparation of 16-Methyl-19-methoxyalloyohimbane (XI). A solution of 25 mg (0.074 mmole) of 16-methyl-19-methoxy-21-oxoalloyohimbane (X) in 50 ml of anhydrous ether was added dropwise to a stirred suspension of 150 mg of lithium aluminum hydride in 50 ml of refluxing ether. After addition was completed, the stirring and refluxing were continued for 12 hr. The excess lithium aluminum hydride was decomposed by the dropwise addition of water, and the inorganic salts were filtered. The ether was evaporated leaving 21 mg (0.065 mmole, 87%) of white 16-methyl-19-methoxyalloyohimbane (XI) that could be recrystallized from either acetonitrile or methanol, mp 217–220° dec. An infrared spectrum showed the disappearance of the band at 6.13 μ, and the pseudo-first-order rate of methiodide formation was 2.5×10^{-4} sec^{-1}, indicative of an allo configuration. The mass spectrum exhibited a molecular ion peak at *m/e* 324 in accord with the molecular formulation $C_{21}H_{28}N_2O$.

Hydrogenation of Methyl Deserpideate (IV). Platinum oxide (34 mg) in 6 ml of 95% ethanol was reduced by stirring in the presence of hydrogen for 20 min. To this mixture, 66 mg (0.173 mmole) of methyl deserpideate in 11 ml of 95% ethanol was added, and the reaction was allowed to proceed with stirring for 4 days. The mixture was flushed with nitrogen, and the catalyst was filtered (Schleicher and Schuell Filter Paper No. 576). The solvent was removed under reduced pressure leaving 60 mg of an oil. This oil was shown to consist of at least 12 components by thin layer chromatography. An infrared spectrum showed an absorption at 6.12 μ. Partial separation of the mixture was accomplished by extraction with aqueous hydrochloric acid and chloroform. The chloroform layer (neutral material) was dried and solvent removed under reduced pressure. The residue was crystallized by the addi-

tion of methanol and yielded 12 mg of white crystalline 16-methyl-18-hydroxy-19-methoxy-21-oxoalloyohimbane, mp 287–289° dec. An infrared spectrum showed no absorption between 5.0 and 6.0 μ but showed a strong amide band at 6.12 μ. The mass spectrum showed a molecular ion peak at *m/e* 354.

Anal. Calcd for $C_{21}H_{26}N_2O_3$: C, 71.16; H, 7.39. Found: C, 71.49; H, 7.76.

The aqueous hydrochloric acid fraction was basified by adding cold 5% sodium carbonate, and this was then extracted with chloroform. The chloroform extracts were dried with anhydrous sodium sulfate and filtered, and the solvent was removed under reduced pressure. This gave 45 mg of a yellow solid which was subjected to preparative scale thin layer chromatography using chloroform:methanol:acetone (60:20:20) as the solvent system. The band whose R_f value corresponded to authentic methyl deserpidate (R_f 0.49) was removed. The organic material was eluted from the silica gel by use of hot chloroform and hot methanol. This gave 20 mg of an oil which was once again subjected to preparative scale thin layer chromatography using the same solvent system, and again the proper band was removed. After elution, 10 mg of a white solid was collected. This compound gave an infrared spectrum that was identical with authentic methyl deserpidate in chloroform solution and in acetonitrile solution. The nmr spectrum was also identical with that of authentic methyl deserpidate and different from methyl deserpideate. Thin layer R_f values further established the identity of the two compounds: R_f 0.49, chloroform:methanol:acetone (60:20:20); R_f 0.46, ether:methanol (50:50).

Lithium Aluminum Hydride Reduction of 11-Demethoxytetrahydroalstoniline (VIII). 11-Demethoxytetrahydroalstoniline hydrochloride (50 mg, 0.135 mmole) was converted to the free base by extraction with aqueous sodium carbonate and ether. The ether extracts (40 ml) were dried over anhydrous sodium sulfate, filtered, and placed in a 50-ml addition tube. This solution was added dropwise to a stirred suspension of 300 mg of lithium aluminum hydride in 10 ml of ether. After addition was completed (1 hr), the mixture was stirred for 1 hr. Subsequently, 200 mg more of lithium aluminum hydride was added, and the mixture was stirred for 5 hr. The excess lithium aluminum hydride was decomposed by the dropwise addition of water. Sodium sulfate (1 g) was added; the inorganic salts were filtered, and the solvent was evaporated *in vacuo* until about half its volume was removed. The hydrobromide salt was prepared by adding methanolic hydrogen bromide to the ethereal solution of the free base. The precipitate was filtered and weighed 45 mg (0.112 mmole, 83%) after drying. Recrystallization from methanol gave yellow crystalline 1-hydroxymethyl-5,7,8,14-tetrahydro-13H-benzo[*g*]indolo[2,3-*a*]quinolizine hydrobromide (trivial name: 11-demethoxytetrahydroalstonilinol hydrobromide) that decomposed without sharp melting around 280°. Its infrared spectrum as a KBr pellet was identical with that of synthetic material.[5] Paper chromatography R_f values in three different solvent systems were the same for the two compounds: R_f 0.56, 10% acetic acid:5% sodium acetate (v/v), saturated with *n*-butyl ether; R_f 0.92, *n*-butyl alcohol saturated with water; R_f 0.88, 95:5 *n*-butyl alcohol:ethanol, saturated with water.

Anal. Calcd for $C_{20}H_{21}BrN_2O$: C, 62.34; H, 5.49. Found: C, 62.46; H, 5.62.

Preparation of 3-Isodeserpideine (VII). A solution of 576 mg (1.0 mmole) of deserpideine in 10 ml of glacial acetic acid was heated to 50° by means of an oil bath. To this stirring solution, 56 ml of 0.0792 *N* lead tetraacetate in glacial acetic acid was added dropwise over the course of 90 min. The temperature was maintained at 45–50°, and the system was kept under a nitrogen atmosphere. The resulting wine red solution was stirred at 45–50° for an additional 3 hr. The acetic acid was then removed *in vacuo*, leaving a dark red oil. This oil was dissolved in 400 ml of chloroform; 20 ml of water was added and the mixture chilled. To this mixture, cold 50% sodium hydroxide was added dropwise until the material was basic to Hydrion paper (about 10 ml was needed). The chloroform layer was separated and washed once with 20 ml of water. The chloroform layer was dried over anhydrous sodium sulfate and filtered. It was then made acidic by adding dropwise ethanolic hydrochloric acid, a color change from dark red to orange being noted. The chloroform was removed under reduced pressure leaving 581 mg (0.955 mmole, 95%) of an orange solid that resisted crystallization attempts. The infrared spectrum showed absorptions at 2.9, 3.4, 5.8, 6.1, and 6.3 μ.

The crude tetradehydrodeserpideine chloride (581 mg, 0.955 mmole) was dissolved in 25 ml of methanol and cooled in an ice bath. Sodium borohydride (676 mg) was added in small portions over the course of 15 min. After 10 min, the dark brown solution was refluxed for 5 min on a steam bath. Water (70 ml) was added, and the methanol was removed under reduced pressure. The solid was filtered and dried to constant weight (495 mg). This material was dissolved in a minimal amount of chloroform and passed through a 4-in. column of Florisil giving a yellow solution. The chloroform was evaporated leaving a yellow amorphous material that weighed 270 mg. A thin layer chromatogram of this showed it to consist of two compounds which were separated by preparative thin layer chromatography using chloroform:acetone:methanol (90:8:2) as the solvent system. The main component of the reaction was shown to be 3-isodeserpideine (230 mg, 40%). Its rate of methiodide formation was 31×10^{-4} sec^{-1} and its infrared spectrum showed the 3-iso bands at 3.4–3.6 μ.

A solution of 3-isodeserpideine was prepared by dissolving 80 mg (0.138 mmole) of pure 3-isodeserpideine (VII) in 0.5 ml of methanol. This solution was made slightly acidic by the dropwise addition of glacial acetic acid. Eight drops of a saturated ammonium nitrate in methanol solution was then added. A white precipitate formed immediately. This was filtered and recrystallized from methanol giving 73 mg (0.114 mmole, 83%) of white crystalline 3-isodeserpideine nitrate salt, mp 246–249° dec.

Anal. Calcd for $C_{32}H_{37}N_3O_{11}$: C, 60.24; H, 5.83. Found: C, 60.07; H, 5.77.

Exactly 32 mg (0.055 mmole) of 3-isodeserpideine was dissolved in 1.0 ml of ethyl acetate and cooled to 0°. Then, 0.5 ml of pure methyl iodide was added, and the solution was allowed to stand at 0° for 3 days. Crystallization commenced after 6 hr. The solvent was removed under reduced pressure, and the residue was crystallized from methanol. Filtration gave 23 mg (0.032 mmole, 58%) of white crystals of 3-isodeserpideine methiodide, mp 250–253° dec. An analytical sample was prepared by recrystallizing from methanol–water to give white clusters, mp 254–255° dec.

Anal. Calcd for $C_{33}H_{39}IN_2O_8$: C, 54.70; H, 5.43. Found: C, 55.02; H, 5.64.

Hydrogenation of 3-Isodeserpideine (VII). Exactly 32 mg of platinum oxide in 4 ml of 95% ethanol was reduced to platinum black in a microhydrogenation apparatus. To this mixture 100 mg (0.173 mmole) of 3-isodeserpideine in 7 ml of 95% ethanol was added, and the reduction was allowed to proceed for 15 hr. The catalyst was removed by filtration through very retentive filter paper (Schleicher and Schuell, No. 576), and the ethanol was removed under reduced pressure. A yellow amorphous solid weighing 92 mg remained which was extracted with 5% sodium carbonate and chloroform. The aqueous layer was acidified by adding cold concentrated hydrochloric acid, and a white precipitate formed. Filtration and drying gave 16 mg (64% yield) of a white crystalline compound that was shown to be 3,4,5-trimethoxybenzoic acid by its melting point (168–170°), mixture melting point (undepressed), and infrared spectrum. The chloroform layer was then extracted with dilute hydrochloric acid, and the chloroform layer was dried and evaporated. This left 10 mg of a neutral fraction that showed a amide peak in the infrared at 6.13 μ. The aqueous hydrochloric acid layer was basified and extracted with chloroform. The chloroform layer was dried and evaporated leaving 62 mg of a yellow solid. Addition of methanol caused crystallization to occur. Filtration and drying gave 12 mg (0.033 mmole, 20%) of a white crystalline material that was shown to be α-yohimbine methyl ether (XII) by melting point and mixture melting point (266–267° dec) and thin layer R_f values.[8]

Hydrogenation of Raujemidine (Ia). Exactly 39 mg of platinum oxide in 5 ml of 95% ethanol was reduced to platinum black in a microhydrogenation apparatus. To this mixture 200 mg (0.33 mmole) of raujemidine in 12 ml of 95% ethanol was added, and the hydrogenation was allowed to proceed for 72 hr. The catalyst was removed by filtering three times through very retentive filter paper (Schleicher and Schuell, No. 576). The solvent was removed under reduced pressure leaving 190 mg of a yellow amorphous solid. This solid was extracted with 5% sodium carbonate and chloroform. The aqueous phase was acidified with concentrated hydrochloric acid leaving a white precipitate. Filtration and drying of this precipitate gave 25 mg (0.118 mmole, 38%) of a white crystalline solid that was shown to be 3,4,5-trimethoxybenzoic acid by its melting point (168–170°), mixture melting point (undepressed), and infrared spectrum. The organic phase was evaporated and gave a yellow amorphous solid. This was subjected to preparative scale thin layer chromatography using chloroform:ethanol:acetone (90:6:4) as the solvent system and the band whose R_f values corresponded to reserpine (R_f 0.60) was removed. Elution of the compound from the silica gel and recrystallization of the eluted material gave 19 mg (9% yield) of pure reserpine (IIa) as

shown by infrared and nmr spectra, melting point, and mixture melting point.

Rate Studies. The rates of methiodide formation were determined by the method used previously,[6] except for a few minor changes. Instead of using 10 mg of sample, rates were determined on 3 mg of sample in the present study. The acetonitrile that was used was carefully distilled from CaH_2 and had an observed initial resistance of greater than 1,000,000 ohms.

Biosynthesis of Methylcyclopentane Monoterpenoids. I. *Skytanthus* Alkaloids[1,2]

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Abstract: The biosynthesis of the steam-volatile *Skytanthus* alkaloids has been investigated by administering DL-mevalonate-2-^{14}C and L-methionine-methyl-^{14}C into the green stems of mature flowing *Skytanthus acutus* M. plants. Radioactivity from mevalonate-2-^{14}C was incorporated into the alkaloid. Radioactivity from DL-lysine-2-^{14}C was not incorporated, which indicates that lysine is not a precursor. Gas–liquid chromatographic analysis of the alkaloid fraction indicated that the natural oil contains a mixture of which about 90% is α-, β-, δ-, and dehydroskytanthines. The amounts of these alkaloids vary with the parts of the plant, with roots containing most. Specific activities of the alkaloids derived from mevalonate-2-^{14}C and methionine-methyl-^{14}C also vary in different parts of the plant. At least four alkaloids of unknown structure which comprise about 10% of natural *Skytanthus* oil were detected. Chemical degradations on micro quantities of alkaloid to eliminate nitrogen and to determine the amount of radioactivity located in carbons 3, 4, 7, 9, and 10 were devised. It was found that L-methionine-methyl-^{14}C was the precursor of the N-methyl group of β-skytanthine The results of biosynthesis experiments using mevalonate-2-^{14}C as a precursor provide evidence for the formation of skytanthine isomers *via:* (a) an isoprenoid pathway which involves randomization of the label between the terminal methyl carbon atoms of the monoterpene or monoterpenoid (*i.e.*, geranyl pyrophosphate) intermediate in 1.3-year-old plants, and (b) an isoprenoid pathway which does not involve randomization of the label between the monoterpene terminal methyl carbon atoms in 3-year-old plants.

Natural oil of *Skytanthus acutus* M. contains a mixture of alkaloids of the rare monoterpenoid class. At least three skytanthine isomers (Figure 1: Iα, Iβ, Iδ) and a dehydroskytanthine (II) are produced by *Skytanthus acutus* M.[4,5] *Skytanthus acutus* M. is native to the Atacama desert of Chile and owing to the characteristic shape of its seed pods is commonly referred to as "Goats-horn" by natives of the area. *Skytanthus* alkaloids have no known physiological activity, in contrast to most other methylcyclopentane monoterpenoids, most of which possess biological activity. The biosynthesis of the *Skytanthus* alkaloids is of interest because they are terpenoid alkaloids[4–9] and because they may serve as a link to higher alkaloids.[10–13]

The structures of the *Skytanthus* alkaloids suggest that the piperidine nucleus of these molecules could arise from an isoprenoid precursor. The present evidence for the route of formation of the piperidine ring in plants is based largely on studies of the alkaloid biosynthesis of anabasine[14,15] produced by tobacco, homostachydrine[16] by alfalfa, and pipecolic acid[17–20] by various plants and microorganisms. These molecules are derived from lysine. In contrast, the piperidine ring of coniine and conhydrine, which are alkaloids of hemlock, appears to be formed from a poly-β-keto acid derived from four acetate units.[21,22] Thus, if the piperidine ring of skytanthine is isoprenoid in origin, a third

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