[CONTRIBUTION FROM THE DEPARTMENT OF CHEMISTRY, IOWA STATE UNIVERSITY, AMES, IOWA]

General Methods of Synthesis of Indole Alkaloids. II. A Flavopereirine Synthesis[1,2]

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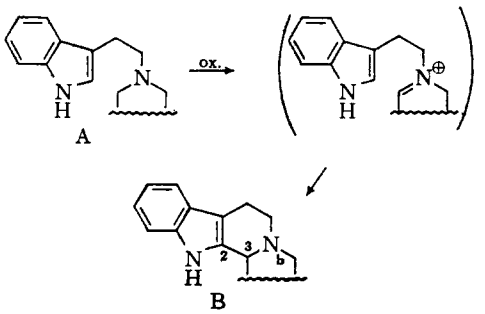
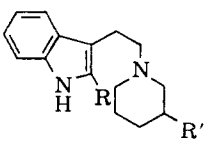
The reactions of three N-[β-(3-indolyl)-ethyl]-piperidines with mercuric acetate have been investigated. A new synthesis of the alkaloid flavopereirine has been developed.

Since tryptamines of structure A in general are readily available by synthesis, the oxidative cyclization outlined below (A → B) represents a crucial step in any synthesis of indole alkaloids of structure B from 2,3-*seco* precursors. As a recent synthesis of flavopereirine illustrates,[2] palladium can act as the dehydrogenation agent in the oxidative cyclization. We now wish to report the use of mercuric acetate as the oxidizing agent in this reaction sequence.



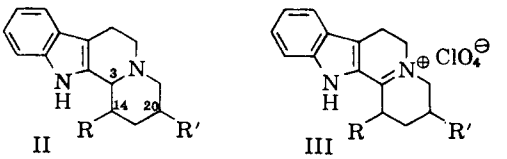
The oxidation of tertiary amines to immonium salts, especially of N-alkylpiperidines to 1-alkyl-1-piperideines, by mercuric acetate is a well-known reaction[4] which has been utilized in the area of indole alkaloids for the preparation of 3-dehydro derivatives of B.[5-7] As a consequence, it was expected that the mercuric acetate-induced conversion of A to B would lead to over-oxidized products.

Three tryptamines were used for our study. N-[β-(3-Indolyl)-ethyl]-piperidine(Ia)[8] and 1-[β-(3-indolyl)-ethyl]-3-ethylpiperidine(Ib) were prepared by lithium aluminum hydride reductions of the piperidides, obtained by the interaction of methyl 3-indolylacetate and piperidine as well as of the ester and β-ethylpiperidine, respectively.[9] N-[β-(2-Methyl-3-indolyl)-ethyl]-piperidine (Ic) was made by the reaction of piperidine with 2-methyltryptophyl bromide.[10]



Ia, R = R′ = H
b, R = H, R′ = Et
c, R = Me, R′ = H

A preliminary reaction of mercuric acetate and Ia, under conventional conditions of reaction and work-up,[4-7] yielded mostly starting material. This fact and an earlier observation of the strong retardation of the rate of mercuric acetate oxidation of N-methylpiperidine on addition of an equivalent amount of indole[11] were the first indications that fairly stable mercury complexes of the indoles or of both indoles and tertiary amines were formed in these reactions. When, as a consequence, the oxidation of Ia was carried out with a greater excess of oxidizing agent and at more elevated temperature, only a small quantity of starting material could be recovered. The major product was the tetracyclic amine IIa, while its oxidation product, isolated as the perchlorate IIIa, was present in minor quantity. The isolation also of piperidine, characterized as its N-*p*-toluenesulfonyl derivative, indicated that the oxidation of the piperidine nucleus of Ia had occurred to a small extent in an exocyclic manner and the resulting undesired immonium salt had undergone hydrolytic fragmentation on acid work-up. Optimum yields of the desired amine IIa could be obtained when the combined products of the oxidative cyclization were exposed to sodium borohydride reduction and the over-oxidized product IIIa thus was reverted to its amine precursor IIa.



a, R = R′ = H
b, R = H, R′ = Et
c, R = Et, R′ = H

The preponderant formation of IIa in preference of IIIa under exhaustive oxidation conditions appeared most likely to be a consequence of one of two factors or a combination of both: (a) the conversion of IIa into IIIa was slow and/or (b) the concentration of IIa during the oxidation was low because the stability of the mercury complexes of its precursor 1-piperideine (most probably in its enamine form) prevented the cyclization into IIa

(1) This work was first presented as part of a lecture by E. W. at the 17th National Organic Chemistry Symposium of the American Chemical Society at Bloomington, Ind., June 26–29, 1961. The authors acknowledge gratefully hereby the financial support of the work by a Public Health Service grant (MY-5815) from the U. S. Department of Health, Education and Welfare.

(2) Part I, E. Wenkert and J. Kilzer, *J. Org. Chem.*, **27**, 2283 (1962).

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(4) N. J. Leonard and W. K. Musker, *J. Am. Chem. Soc.*, **82**, 5148 (1960), and earlier papers.

(5) F. L. Weisenborn and P. A. Diassi, *ibid.*, **78**, 2022 (1956).

(6) E. Wenkert and D. K. Roychaudhuri, *J. Org. Chem.*, **21**, 1315 (1956).

(7) E. Wenkert and D. K. Roychaudhuri, *J. Am. Chem. Soc.*, **80**, 1613 (1958).

(8) R. C. Elderfield, B. Fischer and J. M. Lagowski, *J. Org. Chem.*, **22**, 1376 (1957).

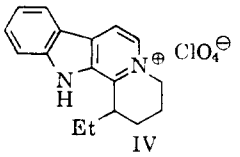
(9) *Cf.* M. S. Fish, N. M. Johnson and E. C. Horning, *J. Am. Chem. Soc.*, **78**, 3668 (1956).

(10) T. Hoshino and K. Shimodaira, *Ann.*, **520**, 19 (1935).

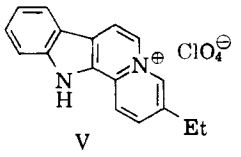
(11) E. C. Blossey, unpublished observation.

from taking place prior to their decomposition with hydrogen sulfide. Both factors seemed to be operative, since it was shown that mercuric acetate oxidation of IIa into IIIa in a separate experiment under standard conditions[6,7] was incomplete and that a reductive decomposition of the mercury complexes in a reaction mixture from the oxidation of Ia prior to work-up of the mixture with hydrogen sulfide and hydrochloric acid led to an increased recovery of starting material Ia. Whereas the sodium borohydride-induced decomposition of the complexes was incomplete, there was isolated over five times the amount of starting material obtained in a parallel experiment under normal reaction work-up.

Mercuric acetate oxidation of the β-ethylpiperidine derivative Ib gave a mixture of products from which five substances—starting material Ib, two stereoisomeric octahydroflavopereirines IIb and two stereoisomeric octahydroisoflavopereirines IIc—as well as a mixture of hexahydroflavopereirine (IIIb) and hexahydroisoflavopereirine (IIIc) could be isolated. The two isomers IIb were known compounds and readily interconvertible. Mercuric acetate oxidation of the amorphous isomer IIb[12] yielded IIIb,[13] whose sodium borohydride reduction afforded the crystalline isomer IIb.[14] Both substances IIc could be oxidized by mercuric acetate to the 3-dehydro derivative IIIc, whose identity has been established by synthesis[15] by a route analogous to the recorded synthesis of IIIb.[13] Sodium borohydride reduction as well as catalytic hydrogenation of IIIc transformed it to one isomer (IIc). Dehydrogenation of this isomer with palladium-black and maleic acid[7] or of its hydrochloride with palladium–charcoal at 275° for ten minutes[2] yielded tetrahydroisoflavopereirine (IV). The latter was also the product of dehydrogenation of IIIc with palladium-black and maleic acid. A reduction of the 3-dehydro derivative IIIc with zinc and acetic acid[7] yielded a mixture of stereoisomers (IIc).



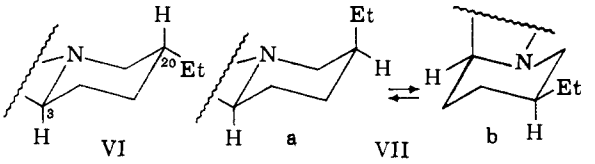
IV



V

As in the case of the oxidation of Ia (*vide supra*), optimum yields of amine products (IIb and c) could be obtained from the oxidation of Ib when the combined products of the oxidative cyclization were exposed to sodium borohydride reduction. The isolation of comparable quantities of IIb and IIc indicated that the direction of oxidation of the unsymmetrical piperidine occurred in a fairly random manner. It further was of interest that the cyclization of the intermediate 1-piperideines had taken place unstereospecifically. A previous report on a cyclization leading to IIb claimed the isolation of only one (the amorphous) isomer.[12]

Whereas the two octahydroflavopereirines (IIb) are known compounds, their stereochemistry has not been discussed in the past. Since their mode of formation does not reflect rigorously their configuration, recourse was taken to an inspection of their proton magnetic resonance spectra. The crystalline isomer[14] exhibited a p.m.r. spectrum whose lack of any signal downfield from the saturated hydrogen region (no peaks below δ *ca.* 3.2) is characteristic of an axial C(3)–H function, while the spectrum of the amorphous isomer[12] revealed a multiplet at δ 3.62–3.94 (centered at *ca.* 3.78 p.p.m.) suggestive of a C(3)–H group of a conformation somewhere between an axial and equatorial state (4.46 p.p.m. is the position of C(3)–H equatorial with respect to the piperidine ring D[16–18]). These data support a 3,20-*trans* configuration, represented conformationally by VI, for the crystalline isomer, and a *cis* configuration for the amorphous substance. It is noteworthy that the latter assumes a conformation somewhere between the two classical forms VIIa and b, thereby presumably distributing the unfavorable non-bonded interactions of the axial ethyl (VIIa) or indole (VIIb) functions over the entire ring system.



The infrared spectra of both isomers revealed the presence of 3.56 and 3.62 μ bands of medium intensity.[7,19] These bands have been suggested recently to reflect an axial C(3)–H configuration.[20] While the absorption peaks of the spectrum of isomer VII are of slightly lower intensity than those of VI, both compounds would have been considered to possess axial C(3) hydrogens on the basis of a rigidly applied generalization[20] and in the absence of the n.m.r. data. It thus is clear that the n.m.r. spectrum is more sensitive than the infrared spectrum to conformational changes in nonrigid quinolizidine systems.

It is of interest that the product of catalytic hydrogenation of flavopereirine (V) is the *trans* isomer VI.[14] Apparently, desorption of one or more compounds of intermediate oxidation state and re-adsorption on their opposite molecular faces must intervene in the reduction process. The formation of a *trans* compound in a hydrogenation of a quinolizine nucleus is reminiscent of the production of epialloyohimbane alongside expected alloyohimbane on hydrogenation of sempervirine.[7,21]

(12) J. Thesing and W. Festag, *Experientia*, **15**, 127 (1959).

(13) A. LeHir, M.-M. Janot and D. van Stolk, *Bull. soc. chim. France*, 551 (1958).

(14) N. A. Hughes and H. Rapoport, *J. Am. Chem. Soc.*, **80**, 1604 (1958).

(15) B. Wickberg, unpublished data.

(16) W. E. Rosen and J. N. Shoolery, *J. Am. Chem. Soc.*, **83**, 4816 (1961).

(17) E. Wenkert, B. Wickberg and C. L. Leicht, *ibid.*, **83**, 5037 (1961).

(18) E. Wenkert, B. Wickberg and C. L. Leicht, *Tetrahedron Letters*, **No. 22**, 822 (1961).

(19) E. Wenkert and D. K. Roychaudhuri, *J. Am. Chem. Soc.*, **78**, 6417 (1956).

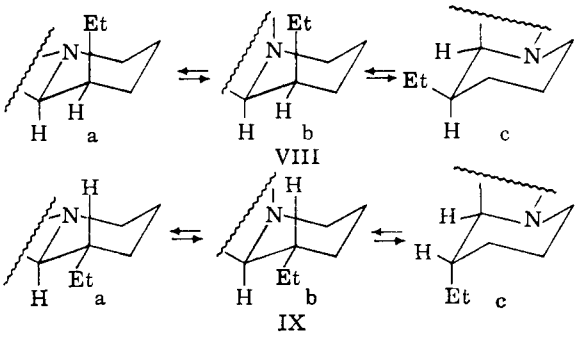
(20) W. E. Rosen, *Tetrahedron Letters*, **No. 14**, 481 (1961).

(21) E. Wenkert and L. H. Liu, *Experientia*, **11**, 302 (1955).

Similarly, the exclusive formation of VI is reminiscent of the exclusive production of yohimbane from 3-dehydroyohimbane.[7] In both instances only the more stable isomer is formed.

While, as in the case of the octahydroflavopereirines (IIb), the stereochemistry of the epimeric octahydroisoflavopereirines (IIc) was investigated with the aid of p.m.r. and infrared spectroscopy, caution needed to be exercised in the interpretation of the results. The p.m.r. spectrum of the substance melting at 116–119° revealed a one-proton doublet ($J \leq 2.3$ c.p.s.) at 3.33 p.p.m., while that of the isomer melting at 90–93° exhibited a similar signal at 3.63 p.p.m. ($J = 5.4$ c.p.s.). These signals were absent in the spectra of C(3)–D derivatives of the two isomers, obtained from a reduction of IIIc with zinc in deuterioacetic acid.[22] Perhaps most importantly, the indole NH singlet of the 116° isomer appeared at 7.48 p.p.m., distinctly upfield from the concentration-independent 7.65–7.80 p.p.m. region characteristic of the 90° isomer, yohimbane, compounds IIa, VI and VII and all other tetrahydrocarbolines of type B (*vide supra*) without polar or sterically interfering functions in proximity of Na. The infrared spectrum of the 116° compound showed two high-wave length bands (*vide supra*) in the region of the CH stretching vibration, whereas the 90° isomer revealed merely a weak 3.62 μ band. Deuteration of the compounds left the spectrum of the latter unchanged but decreased the intensity of one (3.62 μ) of the two bands in the spectrum of the former.

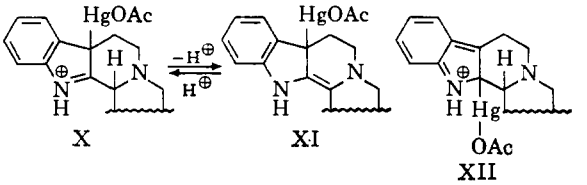
If it be assumed that the anomalous chemical shift of the NH group in the 116° isomer is caused by out-of-plane bending of the hydrogen due to steric compression by the neighboring ethyl group, structure IXa would be the most likely one to represent this substance. However, the resulting 3,14-diaxial hydrogen arrangement would require a much larger coupling constant than is observed. Models of all possible conformers of the *cis* and *trans* isomers of octahydroisoflavopereirine, VIIIa–c, IXa–c and their boat forms, indicate that only VIIIa shows the buttressing *peri* effect[23] and has the 3,14-environment necessary to conform with the remainder of the spectral data. On this basis the 116° isomer is the *cis* compound whose conformation is VIIIa, while the 90° compound is the *trans* substance with a conformation somewhere between IXb and IXc. However these conclusions can be considered as only tentative at this time.



Exposure of the hydrochloride of crystalline IIb to palladium–charcoal at 275° for ten minutes[2] yielded flavopereirine, isolated as its perchlorate V. This constitutes a short, new synthesis of this alkaloid of *Geissopermum vellosii* and *laeve*.[2,12,13,24–26]

Attempted mercuric acetate oxidation of Ic yielded no recognizable product other than some starting material. There were indications that oxidation had occurred at the site of the α-methyl group of the indole and that the reaction intermediates had polymerized.

In view of the intervention of mercury complexes in the above oxidation processes, involving specifically the indole ring, it is possible that similar intermediates play a role in the conversion of amines II into 3-dehydro derivatives III by mercuric acetate. Whereas heretofore such a transformation has been envisaged to proceed *via* a N_b-mercury complex only and has been assigned a specific stereochemical path,[5] some, on this basis unexplicable, results have appeared.[6,7] While an exact portrayal of the mechanistic route of the oxidation thus is not feasible at this time, the possibility of initial formation of complexes X and XI or XII and their subsequent fragmentation into mercury and 3-dehydro acetates may not be overlooked. In this connection it is of interest that complex XII has been postulated recently as the intermediate responsible for an unusual mercuric acetate-induced 2,3-bond cleavage reaction.[15]



Experimental

General Procedures.—Melting points were determined on a Kofler micro-hot-stage and are corrected. Solvents were removed *in vacuo* or under nitrogen. All reactions were carried out under nitrogen. In our use of paper chromatography circular filter papers (Whatman No. 2, 18.5 cm.) with a 6-mm. hole at the center were spotted with the samples 13 mm. from the center, generously sprayed with an aqueous buffer solution (buffer A: 30 ml. of acetic acid and 0.2 g. of potassium acetate per 100 ml.; buffer B: 15 ml. of acetic acid and 25 g. of potassium acetate per 100 ml.) and developed with benzene saturated with the stationary phase. The eluant was fed through a filter paper wick inserted through the center hole and two 14-cm. Petri dishes were used as a chromatographic chamber. After brief drying the components were located by spraying the papers with an iodoplatinate solution.[27] Cellulose columns were packed wet with the aid of a plunger, using a slurry of Whatman cellulose powder–buffer (3 g. of cellulose for each ml. of buffer) in benzene. The slurry was allowed to equilibrate for a few hours before packing. Alumina used for chromatography was obtained from Gebr. Giulini G.m.b.H.

(22) The splitting patterns of the spectra of the protic compounds and the peak disappearance in the spectra of their deuterium derivatives constitute the first experimental proof of the suggestion[16-18] that the p.m.r. signals under considerations are representative of the C(3)–H function.

(23) *Cf.* E. Wenkert and B. G. Jackson, *J. Am. Chem. Soc.*, **80**, 211 (1958).

(24) K. B. Prasad and G. A. Swan, *J. Chem. Soc.*, 2024 (1958).

(25) H. Kaneko, *J. Pharm. Soc. Japan*, 1374 (1960).

(26) Y. Ban and M. Seo, *Tetrahedron*, **16**, 5 (1961).

(27) R. Munier and M. Macheboeuf, *Bull. soc. chim. biol.*, **31**, 1144 (1949).

Ludwigshafen/Rhein, Germany, and was adjusted to about activity III by adding 6% water.

The p.m.r. spectra were obtained with *ca.* 20% deuteriochloroform solutions on a Varian model HR 60 spectrometer at 60 mc./sec. with tetramethylsilane as internal standard. The position of major peaks was determined by the audiofrequency sideband technique, that of minor peaks by linear interpolation.

N-[β-(3-Indolyl)-ethyl]-piperidine (Ia).—Methyl 3-indolylacetate (2.50 g.) in anhydrous piperidine (12 ml.) was refluxed for 24 hr. After concentration the non-volatile material was dissolved in chloroform, the solution washed with dilute hydrochloric acid and aqueous sodium bicarbonate, dried over potassium carbonate and concentrated to give crude 3-indolylacetopiperidide as a light yellow oil (2.6 g.). This was reduced with lithium aluminum hydride as described by Elderfield, *et al.*,[8] with slight modification.

A solution of the crude piperidide (2.6 g.) in tetrahydrofuran (80 ml.) was added to lithium aluminum hydride (2.5 g.) in ethyl ether (200 ml.) and the mixture refluxed for 5 hr. After decomposition of the excess hydride with moist sodium sulfate and filtration, the filtrate was extracted with dilute hydrochloric acid and water. The combined extracts were basified with ammonium hydroxide, extracted with ether and the ether solution dried and concentrated to give a crystalline residue. Crystallization of the latter from aqueous ethanol gave pure N-[β-(3-indolyl)-ethyl]-piperidine (1.94 g.), m.p. 152–153.5° (lit.[8] m.p. 151–152°).

1-[β-(3-Indolyl)-ethyl]-3-ethylpiperidine (Ib).—Heating a mixture of methyl 3-indolylacetate (3.4 g.) and 3-ethylpiperidine (2.4 g.) at 150° for 8 hr. gave crude amorphous 3-ethyl piperidide of 3-indolylacetic acid (2.5 g.). The latter was reduced with lithium aluminum hydride in the manner described above. However, since the hydrochloride of Ib could not be extracted very effectively with water from a chloroform solution, the crude reaction product was freed from non-basic impurities by treating its solution in 10% aqueous acetic acid with Norit. The crystalline material, obtained on adding ammonium hydroxide to the filtrate, was crystallized from aqueous methanol giving pure 1-[β-(3-indolyl)-ethyl]-3-ethylpiperidine (Ib) (1.64 g.), m.p. 113–115°.

Anal. Calcd. for $C_{17}H_{24}N_2$: C, 79.64; H, 9.44; N, 10.93. Found: C, 79.50; H, 9.28; N, 10.94.

N-[β-(2-Methyl-3-indolyl)-ethyl]-piperidine.—Following a procedure described previously,[10] 2-methyltryptophol (1.00 g.) and phosphorus tribromide (1.67 g.) were allowed to react in dry ether (100 ml.) for 4 hr. at room temperature. The ether solution was then decanted from insoluble material, rapidly washed with ice-cold aqueous sodium bicarbonate solution, briefly dried over potassium carbonate and finally concentrated at a low temperature to give an almost colorless crystalline residue of 2-methyltryptophyl bromide (0.80 g.), m.p. 63.5–66°. (This compound was described earlier as an oil.[10]) Because of the instability of the compound no attempts were made to purify it further.

A solution of 2-methyltryptophyl bromide (0.80 g.) and piperidine (4.0 ml.) in anhydrous methanol (60 ml.) was kept at room temperature for 24 hr. After concentration the residue was dissolved in chloroform, the solution washed with aqueous potassium carbonate solution, dried and concentrated to give an oily residue that eventually crystallized. Chromatography of the crude product on alumina and elution with benzene yielded crystalline material which on sublimation and crystallization from *n*-hexane gave N-[β-(2-methyl-3-indolyl)-ethyl]-piperidine (0.64 g.), m.p. 102.5–103.5°.

Anal. Calcd. for $C_{16}H_{22}N_2$: C, 79.29; H, 9.15; N, 11.56. Found: C, 79.60; H, 9.22; N, 11.29.

Mercuric Acetate Oxidation of Ia.—A solution of Ia (1.00 g., 4.4 mmoles) and mercuric acetate (14.0 g., 44 mmoles) in 5% aqueous acetic acid (50 ml.) was kept on a steam-bath for 1 hour and then was treated with hydrogen sulfide for 1 hour at the same temperature. After filtration of the mixture through Celite and careful washing of the filter cake with aqueous acetic acid the combined filtrates were concentrated to a small volume and diluted with 50% aqueous ethanol (100 ml.). The *p*H was brought to about 6 with sodium bicarbonate, sodium borohydride was added in excess and the mixture left at room temperature overnight. Following acidification with acetic acid and concentration to a smaller volume (50 ml.), leaving a small amount of tar undissolved, the neutral products were extracted with benzene. However, the extract gave only a small, unidentified residue (16 mg.) upon concentration.

The acidic aqueous solution was made alkaline with potassium carbonate giving a crystalline precipitate (fraction A) which was filtered and washed with a little water. The filtrate and washings were combined, the *p*H was adjusted to 11 with sodium hydroxide, and the resulting solution distilled at about 100-mm. pressure until 30 ml. of a distillate with a strong smell of piperidine had been collected (fraction B). Fraction A was added to the still residue and the mixture extracted with chloroform. The extract was washed with water, dried over potassium carbonate, filtered through alumina (*ca.* 3 ml.) and concentrated to give a crystalline residue. Crystallization from benzene–cyclohexane (4:1) yielded IIa (435 mg.), identified by m.p. 152–155°, mixed m.p. and Nujol infrared spectrum.[2] The material recovered from the mother liquor was chromatographed on an alumina column (13 × 160 mm.). Elution with benzene–*n*-hexane (3:1) yielded more of IIa (190 mg.), m.p. 153–155° (from aqueous ethanol), while continued elution with benzene and benzene–chloroform (9:1) furnished a fraction (67 mg.) which after crystallization from aqueous ethanol was identified as starting material by m.p. 152–153.5°, mixed m.p. and Nujol infrared spectrum.

The alkaline distillate (fraction B) obtained above was neutralized with hydrochloric acid, concentrated to a small volume (3 ml.), made alkaline with sodium hydroxide (350 mg.) and then heated with *p*-toluenesulfonyl chloride on a steam-bath for 10 minutes. After cooling and the disappearance of the acid chloride odor, the crystalline precipitate was collected, washed with water and crystallized from aqueous ethanol, giving N-*p*-toluenesulfonyl piperidide (114 mg.), m.p. 99.5–101°, undepressed on admixture with an authentic sample. Infrared spectra in Nujol confirmed the identity.

In a subsequent experiment, Ia (200 mg.) was oxidized under analogous conditions and the product obtained after the hydrogen sulfide treatment examined by paper chromatography using buffer B. Compounds Ia, IIa as well as IIIa (in acetate form) were indicated. The presence of IIIa to the extent of about 30 mg. (as perchlorate) was also suggested by a peak at 353 mμ in the ultraviolet spectrum of the mixture. The aqueous solution was concentrated and the residue made anhydrous by repeated evaporation with benzene containing a trace of acetic acid. The remaining sirup was extracted repeatedly with small volumes of benzene containing 0.5% of acetic acid, each extract being added to the top of a cellulose column (13 × 160 mm., buffer B). Elution with benzene gave a mixture in the first fractions containing Ia (20 mg.) and IIa (100 mg.) as shown by the subsequent alumina chromatography of the mixed bases in the manner described above. Continued elution with benzene removed a yellow material traveling as a wide band on the column. Evaporation of the solvent, precipitation with sodium perchlorate in aqueous acetic acid and crystallization of the resulting solid from methanol yielded the perchlorate of IIIa (26 mg.), identified by its m.p. 220–224° and infrared spectrum in Nujol.

It may be of significance that in another run, where the oxidation time was 2.5 hr. but other reaction conditions identical with the preceding experiment, the yields were of the same order: Ia (10 mg.), IIa (93 mg.) and IIIa (33 mg. as estimated by the ultraviolet absorption at 353 mμ).

In order to obtain information about the possible occurrence of complexed precursors of IIa in these oxidations, Ia (200 mg.) was oxidized with mercuric acetate (2.8 g.) in 5% acetic acid (10 ml.) for 2.5 hr. at 100° (*p*H of the mixture was 4.6). After cooling, the *p*H was brought to 6.0 by careful addition of aqueous sodium hydroxide, the mixture was diluted with methanol (15 ml.) and sodium borohydride was then added in small portions until present in large excess. After standing at room temperature overnight the black suspension was made slightly acidic (*p*H *ca.* 4) with hydrochloric acid and then filtered through Celite. The filtrate was made alkaline with potassium carbonate and extracted with chloroform. Concentration of the extract yielded a partly crystalline residue (150 mg.). The filter cake was suspended in 0.1 *M* hydrochloric acid (10 ml.) and the suspension treated with hydrogen sulfide at 100° for 30 minutes. Filtration followed by the usual workup yielded a second lot of chloroform-extractable bases (108 mg.).

The combined basic material (258 mg.), which in view of its weight must have contained mercurated material, was separated on an alumina column. Elution with benzene furnished a semi-solid material (42 mg.) which after crystallization from benzene–hexane and sublimation gave pure IIb (20 mg.), identified by the m.p. 153–155° and infrared spectrum in Nujol. Continued elution with benzene–chloroform (9:1) gave a crystalline solid (62 mg.) which after recrystallization from benzene–hexane and sublimation could be identified as starting material (54 mg.) by its m.p. 152–153.5°, mixed m.p. and infrared spectrum.

Hexahydrodesethylflavopereirine Perchlorate (IIIa).—Octahydrodesethylflavopereirine (IIa, 114 mg.) was treated with mercuric acetate (560 mg.) in 5% acetic acid (5 ml.) for 3 hours at 80°.[6,7] After treatment with hydrogen sulfide and filtration, excess sodium perchlorate was added to the filtrate yielding a partly crystalline precipitate (155 mg.). Its crystallization from water and then from methanol gave IIIa perchlorate (100 mg.) as yellow needles, m.p. 221.5–225° dec. after partial melting at about 212°. The high-melting modification was obtained as stout prisms on keeping a sample of the needle form suspended in hot methanol and appeared to be the more stable form even at room temperature. In view of the losses incurred in the crystallization, the methanolic mother liquor was subjected to paper chromatography (buffer B), which revealed by the presence of starting material that the oxidation was incomplete. After treatment with Norit in dilute aqueous solution, precipitation with sodium perchlorate and crystallization from methanol, IIIa perchlorate had m.p. 223–227°; ultraviolet spectrum (95% ethanol): λ_{max} 246 mμ (log ε 4.02), 353 mμ(log ε 4.36), λ_{min} 232 mμ(log ε 3.83), 277 mμ(log ε 2.48).

Anal. Calcd. for $C_{15}H_{17}O_4N_2Cl$: C, 55.47; H, 5.28; N, 8.63. Found: C, 55.61; H, 5.43; N, 8.87.

Mercuric Acetate Oxidation of Ib.—Compound Ib (400 mg.) was oxidized with mercuric acetate for 30 min. Otherwise, the reaction conditions, the workup with hydrogen sulfide and the subsequent borohydride reduction were as those described for Ia. After the excess borohydride had been destroyed with acetic acid, the solution was concentrated, made alkaline with ammonium hydroxide and extracted with chloroform. Paper chromatograms of the extract (buffer A, developed for 40 min.) revealed five components, VIII, VI, Ib, IX and VII in order of increasing mobility.

The mixed bases (375 mg.) obtained on concentration of the chloroform solution were chromatographed on an alumina column (19 × 140 mm.) and the elution started with 9:1 benzene–hexane. The eluate was examined by spot tests and paper chromatography. Elution with benzene–hexane (1:3) gave a fraction (39 mg.) which upon crystallization from hexane yielded VIII (34 mg.), m.p. 116–119°. Mixed m.p. and infrared spectra in Nujol proved it to be identical with an authentic specimen prepared from IIIc (*vide infra*). Benzene–hexane (1:2) eluted an oil (35 mg., mainly VII) which eventually crystallized from methanol, yielding VII (30 mg., probably a methanol solvate), m.p. 65–103° dec. Continued elution with the same solvent followed by benzene–hexane (1:1) gave a mixture of VI and VII (109 mg.) which was readily separated by taking advantage of the low solubility of VI in hexane and of VII in methanol. There was obtained VI (60 mg.), m.p. 160–164°, and VII (49 mg., solvate), m.p. 60–104° dec. Following a small mixed fraction which was discarded, essentially pure IX (51 mg.) was eluted by benzene–hexane (2:1). Crystallization from methanol yielded IX (46 mg., probably a methanol solvate), m.p. 60–100° dec. Recrystallization of the latter from methanol and drying *in vacuo* resulted in an oil which on crystallization from hexane gave pure IX, m.p. 90–93°. A mixed m.p. and comparison of its infrared spectrum in Nujol proved it to be identical with an authentic specimen prepared from IIIc (*vide infra*). Completing the elution with benzene, there was obtained a mixed fraction (62 mg.) containing Ib and IX. Crystallization from aqueous ethanol yielded Ib (41 mg.), identified by its m.p. 113–115°, mixed m.p. and infrared spectrum in Nujol. Upon concentration and crystallization from methanol the mother liquor gave a small crop of IX (4 mg., solvate).

Purification of compound VI by sublimation and recrystallization from aqueous methanol as well as hexane furnished hexagonal plates, m.p. 163–165° (changes into long needles at 140–145°).

Anal. Calcd. for $C_{17}H_{22}N_2$: C, 80.27; H, 8.72; N, 11.01. Found: C, 80.02; H, 8.91; N, 10.95.

All batches of VII were combined and recrystallized from methanol. On drying the crystalline material *in vacuo* there resulted a colorless oil that could not be induced to crystallize. The compound was characterized as VII by the formation of a picrate, m.p. 220–224° (from methanol) (lit.[12] m.p. 211°), and by its oxidation to IIIb (*vide infra*).

In a separate run Ib (400 mg.) was oxidized as described above, but the sodium borohydride treatment was omitted. Paper chromatography of the product indicated the presence of IIIb and IIIc (in the acetate form) as well as of the compounds isolated in the previous run. The mixture was concentrated and made anhydrous by distillation with benzene containing 0.5% of acetic acid. The residue was dissolved in 5 ml. of a thick benzene slurry of cellulose powder impregnated with buffer B. After dilution with hexane (5 ml.) the slurry was added to the top of a cellulose column (19 × 210 mm., buffer B) and packed to form a uniform layer. Benzene–hexane (1:1 and thereafter 2:1) eluted a mixture containing all of the amines. Continued elution with benzene–hexane (4:1), followed by pure benzene, yielded a mixture of the immonium salts IIIb and IIIc in the acetate form. (These acetates were hard to resolve even on paper chromatograms.) Reduction of the immonium salt mixture with sodium borohydride followed by chromatography on alumina afforded VIII (16 mg.), m.p. 116–119°, and VI (9 mg.), m.p. 162–165°.

Hexahydroflavopereirine Perchlorate (IIIb).—A solution of compound VII (20 mg., solvate) and mercuric acetate (100 mg.) in 5% aqueous acetic acid (2 ml.) was heated at 100° for 1 hr. and then treated with hydrogen sulfide as usual. Paper chromatography indicated that the reaction was complete. Precipitation with sodium perchlorate, recrystallization from acetic acid, treatment with Norit in moist acetone, concentration and recrystallization from methanol afforded the perchlorate IIIb (18 mg.), identified by m.p. 222–224.5°, mixed m.p. with an authentic sample 220–222.5° and infrared spectra in Nujol.[28]

Hexahydroisoflavopereirine Perchlorate (IIIc). (a).—When compound IX (20 mg.) was oxidized under the conditions of the preceding experiment, paper chromatograms indicated that the reaction was incomplete. Recrystallization of the crude perchlorate from acetic acid and from ethanol yielded IIIc (10 mg.), m.p. 175–177.5°, undepressed on admixture with an authentic specimen.[15] Infrared spectra of the two specimens in Nujol were identical.

(b).—A solution of compound VIII (45 mg.) and mercuric acetate (225 mg.) in 5% aqueous acetic acid (4 ml.) was heated at 80° for 3 hours. The product was worked up as in the preceding experiment, yielding the immonium salt IIIc (35 mg.), m.p. 175–177°. Mixed m.p. and infrared spectra in Nujol proved its identity with an authentic specimen.[15]

Octahydroflavopereirine VI.—To a solution of perchlorate IIIb (75 mg.) in 75% aqueous methanol (4 ml.) sodium borohydride was added in small portions until the yellow color had disappeared after a few minutes. Dilution with water produced a crystalline precipitate which was filtered, crystallized from aqueous methanol, sublimed *in vacuo* and recrystallized from benzene–hexane to give octahydroflavopereirine VI (42 mg.), m.p. 163–165° after partial melting and transformation into needles around 145°. Mixed m.p. and infrared spectra in Nujol proved its identity with the cyclization product from Ib.

Octahydroisoflavopereirines VIII and IX. (a).—Reduction of the immonium perchlorate IIIc (750 mg.)[15] by the general procedure used in the preceding experiment afforded octahydroisoflavopereirine (VIII, 480 mg.), m.p. 116–119° (from hexane), identical with the cyclization product from Ib as shown by mixed m.p. and infrared spectra. The compound appears to form different solvates with ill-defined melting points (<100°) when crystallized from aqueous methanol or aqueous ethanol.

Anal. Calcd. for $C_{17}H_{22}N_2$: C, 80.27; H, 8.72; N, 11.01. Found: C, 80.43; H, 8.97; N, 10.86.

(b).—A mixture of IIIc (30 mg.), 10% palladium–charcoal (7 mg.) and potassium acetate in ethanol (3 ml.) was hydrogenated at atmospheric pressure and room temperature.

(28) E. Wenkert, R. A. Massy-Westropp and R. G. Lewis, *J. Am. Chem. Soc.*, **84**, 3732 (1962).

The hydrogen uptake was complete in 3 min. Conventional workup afforded octahydroisoflavopereirine (13 mg.), m.p. 116–119° (from hexane).

(c).—A mixture of immonium salt IIIc (400 mg.), zinc dust (1 g.), acetic acid (5 ml.) and water (1 ml.) was refluxed for 1 hr. The initial yellow color had disappeared in 30 min. The mixture was filtered, the filtrate was concentrated and the residue basified with ammonium hydroxide and extracted with chloroform. The extractable material (290 mg.) was chromatographed on an alumina column (13 × 115 mm.). Benzene–hexane (1:2) elution afforded compound VIII (99 mg.), m.p. 116–119°, identical with the sample above. Continued elution with benzene yielded the isomer IX (112 mg., solvate from methanol). Drying and crystallization from hexane gave pure IX, m.p. 90–93°. Mixed m.p. and infrared spectra proved it to be identical with the sample prepared by cyclization of Ib.

Anal. Calcd. for $C_{17}H_{22}N_2$: C, 80.27; H, 8.72; N, 11.01. Found: C, 80.30; H, 8.67; N, 11.02.

Deuterated Octahydroisoflavopereirines VIII and IX.—The immonium perchlorate IIIc (198 mg.) was reduced with zinc dust as described in the preceding experiment. But acetic acid-*d* and deuterium oxide were substituted for acetic acid and water, respectively. The resulting amine mixture was refluxed for 20 min. in 0.1 *M* sodium *t*-butoxide in *t*-butyl alcohol (4 ml.) to ensure complete protonation at N_a, even though the infrared spectrum (chloroform) of the material prior to the base treatment did not indicate any isotope exchange during the reduction. The recovered material (148 mg.) was chromatographed on alumina to give VIII-3-*d* (53 mg.), m.p. 116–119° (from hexane), and IX-3-*d* (34 mg.), m.p. 90–93° (from hexane). Whereas no melting point depression was observed on admixture with the corresponding fully protonated compounds, the infrared spectra showed considerable differences throughout the fingerprint regions.

Tetrahydroisoflavopereirine Perchlorate (IV). (a).—A mixture of VIII (152 mg.), maleic acid (350 mg.) and palladium black (75 mg.) in water (10 ml.) was refluxed for 9 hr.[7] and then filtered while hot. After neutralization with sodium bicarbonate, excess sodium perchlorate was added to the filtrate. Crystallization of the precipitate from ethanol afforded tetrahydroisoflavopereirine perchlorate (167 mg.), m.p. 254–256.5°; ultraviolet spectrum (95% ethanol): λ_{max} 253 mμ(log ε 4.51), 307 mμ (log ε 4.37), 369 mμ(log ε 3.73); λ_{min} 226 mμ(log ε 4.14), 279 mμ(log ε 3.76), 327 mμ(log ε 3.20).

Anal. Calcd. for $C_{17}H_{19}O_4N_2Cl$: C, 58.20; H, 5.46; N, 7.99. Found: C, 58.16; H, 5.47; N, 7.91.

(b).—When the perchlorate IIIc (150 mg.) was dehydrogenated with maleic acid (175 mg.) and palladium black (75 mg.) following the procedure above, crystallization occurred after a few hours. After completed reaction, sodium perchlorate (100 mg.) was added to the neutralized mixture and the solid filtered off. The filter cake was triturated with 90% aqueous acetonitrile, the solution was concentrated and the crude product, obtained on cooling, crystallized from ethanol. This yielded tetrahydroisoflavopereirine perchlorate (120 mg.), m.p. 254–256°.

(c).—Dehydrogenation of compound VIII (51.5 mg.) by the method described below, precipitation with sodium perchlorate from a methanolic solution, afforded a crude product (50 mg.), m.p. 248–254°. Its ultraviolet spectrum was identical with that of compound IV except for a slightly higher absorption at 369 mμ (log ε 3.82). This would indicate that the dehydrogenation had stopped at the tetradehydro stage. Treatment with Norit in aqueous acetonitrile and repeated recrystallization from methanol yielded material identified as IV by m.p. 249–254.5°, mixed m.p. 250–255.5° and by its infrared spectrum (Nujol) which was superimposable on that of authentic material.

Flavopereirine Perchlorate (V).—Compound VI (9.5 mg.) was treated with hydrogen chloride in ethyl ether, the crude hydrochloride salt was mixed intimately with 10% palladium–charcoal (9 mg.) and the mixture heated at 275° for 10 min. The crude product was extracted with methanol. Its ultraviolet spectrum indicated that it was essentially a mixture of flavopereirine (V) and tetrahydroflavopereirine. Separation on a cellulose column as described previously[2] afforded a small quantity of a perchlorate, m.p. 221–226°, presumably the tetrahydro compound, as well as flavopereirine perchlorate (3 mg.), identified by its m.p. 326–330° (from water, stage preheated at 310°) and its infrared spectrum (KBr disk).[2,14]

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Biosynthesis of the Cinchona Alkaloids. I. The Incorporation of Tryptophan into Quinine[1]

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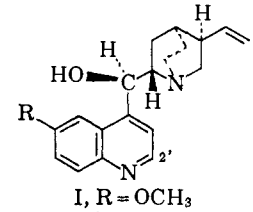
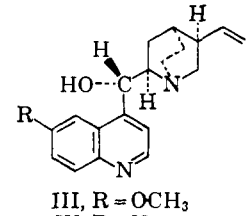
DL-Tryptophan-2-C^{14} was fed to one-year old *Cinchona succirubra* plants. Extraction of the plants six weeks later yielded radioactive quinine (0.7% incorporation) which was degraded systematically and found to have essentially all its activity located at C-2′ of its quinoline moiety. This result is in accord with a hypothesis which was suggested by Goutarel, Janot, Prelog and Taylor in 1950.

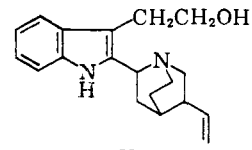
Quinine (I) is found in the bark of various *Cinchona* species,[2] and is usually accompanied by the related alkaloids cinchonidine (II), quinidine (III) and cinchonine (IV). Two of the minor alkaloids are cinchonamine (V) and quinamine (VI), and an examination of their structures led Goutarel, Janot, Prelog and Taylor[3] to suggest that all the Cinchona alkaloids have a common biogenetic origin. It was proposed that the quinoline moiety of the major alkaloids is formed from an indole having a two-carbon side chain at C-3



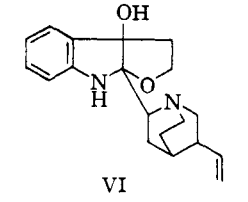
I, R = OCH_3
II, R = H



III, R = OCH_3
IV, R = H



V



VI

I, R = OCH_3
II, R = H

III, R = OCH_3
IV, R = H

(1) An account of this work was presented at the 142nd National Meeting of the American Chemical Society, Atlantic City, N. J., September 9–14, 1962. This investigation was supported by research grant MY-2662 from the National Institute of Mental Health, U. S. Public Health Service.

(2) *Cf.* J. J. Willaman and B. G. Schubert in "Alkaloid-Bearing Plants and Their Contained Alkaloids," Technical Bulletin No. 1234, U. S. Department of Agriculture, 1961, p. 189.

(3) R. Goutarel, M.-M. Janot, V. Prelog and W. I. Taylor, *Helv. Chim. Acta*, **33**, 150 (1950).