product to be identical with that prepared from formylguanidine and formamidine.[20]

2-Methylthio-*sym*-triazine (XX).—A mixture of 37.8 g. (0.17 mole) of 2-methylpseudothiourea hydriodide (XIX),[21] 14.0 g. (0.17 mole) of I and 40 cc. of acetonitrile produced a clear solution after shaking for 10 minutes at about room temperature. Shortly thereafter, crystallization of formamidine hydriodide began, and the mixture warmed appreciably. After 20 hours the mixture was filtered. The solid collected (19.4 g.) was formamidine hydriodide, m.p. 235–240° dec. (66% of theory). The filtrate was stripped of acetonitrile at reduced pressure, and the partly crystalline residue was then distilled as completely as possible. The distillate weighed 19.4 g. and had a freezing point of 31° (yield 90%). Upon redistillation a boiling point of 91° at 19 mm. was observed and a center cut taken for analysis had f.p. 32.5°.

Anal. Calcd. for $C_4H_5N_3S$: C, 37.78; H, 3.96; N, 33.05; S, 25.21. Found: C, 37.78; H, 4.08; N, 33.20; S, 25.27.

2-Methoxy-*sym*-triazine (XXI).—A mixture of 109 g. (0.98 mole) of 2-methylpseudourea hydrochloride (XXII),[22] 69 g. (0.85 mole) of I and 150 cc. of absolute ethanol was boiled for 3 hours. The solution was then distilled as completely as possible from the residue of formamidine hydrochloride and any unreacted XXII. The distillate was fractionated at reduced pressure, yielding 73.6 g., b.p. 71–73° at 21° mm., $n^{22.5}D$ 1.4936. A center-cut had a freezing point of 11°, yield 78%.

Anal. Calcd. for $C_4H_5N_3O$: C, 43.24; H, 4.54; N, 37.82. Found: C, 43.39; H, 4.75; N, 37.61.

Reaction of 2-methylpseudourea base with I under the same conditions as described for XXII gave a 10% yield of 2-amino-*sym*-triazine and no XXI was found.

Reaction of Guanidine Hydrochloride (XXIII) with I.—As a solution of 0.050 mole of I and 0.075 mole of XXIII in 10 cc. of ethanol was boiled under reflux crystallization of **2-amino-*sym*-triazine (XVIII)** occurred. The crude product (2.45 g., 50% yield) melted at approximately 200°. Recrystallization from water gave material melting at 224–227°.

2-Anilino-*sym*-triazine (XXV)[5].—Phenylguanidine carbonate[23] (8.3 g., 0.025 mole) was heated with 2.75 g. (0.034 mole) of I in 15 cc. of ethanol. A clear solution was obtained in a few minutes at reflux. Solid began to separate shortly after. The mixture was boiled under reflux for 1.5 hours, cooled and filtered. The recovered solid weighed 4.7 g., m.p. 164–167°, yield 74%. After recrystallization from ethanol the product melted at 171–173°.[24]

Anal. Calcd. for $C_9H_8N_4$: C, 62.78; H, 4.68; N, 32.54. Found: C, 62.44; H, 4.88; N, 32.57.

2-Dodecylamino-*sym*-triazine (XXVII).—Dodecylguanidine acetate[25] (9.40 g., 0.0327 mole) was treated with a solution of 1.77 g. (0.0327 mole) of sodium methoxide in 20 cc. of anhydrous ethanol to prepare the free guanidine XXVI. *sym*-Triazine (2.65 g., 0.0327 mole) was then added and the mixture was boiled under reflux for 2 hours. Filtration after cooling gave 9.0 g. of solid. Extraction of this material with 40 cc. of hot ethyl acetate left 3.0 g. This appeared to be a mixture of sodium acetate and unchanged dodecylguanidine acetate. More of the latter was also recovered from the alcoholic filtrate from the reaction mixture. From the ethyl acetate solution was recovered 4.15 g. of XXVII, m.p. 80–81° (46% yield). Recrystallization from ethyl acetate raised the m.p. to 83–84°.

Anal. Calcd. for $C_{15}H_{28}N_4$: C, 68.14; H, 10.69; N, 21.19. Found: C, 68.42; H, 10.51; N, 20.91.

When a mixture of dodecylguanidine acetate was heated with an equimolar amount of I in boiling anhydrous ethanol for 1.5 hours, little or no reaction occurred.

(20) J. P. English and J. H. Paden, U. S. Patent 2,334,162 (1943). C. Grundmann, L. Schwennicke and E. Beyer, *Chem. Ber.*, **87**, 19 (1954), also used this reaction and obtained XVIII in a maximum yield of 15%, m.p. 225–226°.

(21) H. L. Wheeler and H. F. Merriam, *Am. Chem. J.*, **29**, 478 (1903).

(22) F. Kurzer and A. Lawson, *Org. Syntheses*, **34**, 67 (1954). This procedure was modified in that commercial sodium acid cyanamide was treated directly with hydrogen chloride in methanol. The yield of XXII was 79%, m.p. 116–118°.

(23) R. Walther and W. Grieshammer, *J. prakt. Chem.*, [2] **92**, 247 (1915).

(24) Reference 5 gives m.p. 148–150° for XXV prepared by reaction of 2-phenoxy-*sym*-triazine with aniline. However, there can be no doubt of the identity of our higher-melting product in view of confirmatory infrared comparison with related products.

(25) Dodecylguanidine acetate was prepared by the procedure of J. H. Paden and A. F. McLean, U. S. Patent 2,425,341; m.p. 135–136°.

STAMFORD, CONN.

[CONTRIBUTION FROM THE DEPARTMENT OF CHEMISTRY, IOWA STATE COLLEGE]

A Stereochemical Interpretation of the Biosynthesis of Indole Alkaloids[1]

BY ERNEST WENKERT AND N. V. BRINGI

RECEIVED SEPTEMBER 4, 1958

The conversion of yohimbine and 3-epi-α-yohimbine, ajmalicine and corynantheine, cinchonamine and corynantheine to common intermediates is illustrated. The stereochemistry of all conversion products is discussed. The discovery of a unique C-15 configuration for the indole alkaloids is incorporated in a novel theory of biosynthesis of these natural products.

The study of the structure and synthesis of naturally occurring substances and their mode of biosynthesis has aroused much interest in recent years. While theories of biogenesis for whole classes of natural products, *e.g.*, for terpenoids and steroids,[2] and for alkaloids,[3] have been proposed, relatively little experimental verification has followed so far. With the advent of radiotracer techniques much headway has been made in the realm of terpenoids and steroids[4–7] and natural phenolic and enolic substances.[8,9] However, only a bare beginning has been made in the experimental determination of the biogenesis of alkaloids.[10]

Present concepts of alkaloid biogenesis are based primarily on inspection of alkaloid skeleta and their state of oxidation and represent attempts at unification of structural patterns among substances

(1) For preliminary communications of this work see (a) E. Wenkert, E. W. Robb and N. V. Bringi, THIS JOURNAL, **79**, 6570 (1957); (b) E. Wenkert and N. V. Bringi, *ibid.*, **80**, 3484 (958).

(2) *Cf.* L. Ruzicka, *Experientia*, **10**, 357 (1953).

(3) *Cf.* R. Robinson, "The Structural Relations of Natural Products," Clarendon Press, Oxford, 1955.

(4) *Cf.* H. Rilling, T. T. Tchen and K. Bloch, *Proc. Natl. Acad. Sci.*, **44**, 167 (1958).

(5) *Cf.* J. W. Cornforth, R. H. Cornforth, G. Popják and I. Y. Gore, *Biochem. J.*, **69**, 146 (1958).

(6) *Cf.* A. J. Birch, R. W. Rickards and H. Smith, *Proc. Chem. Soc.*, 192 (1958).

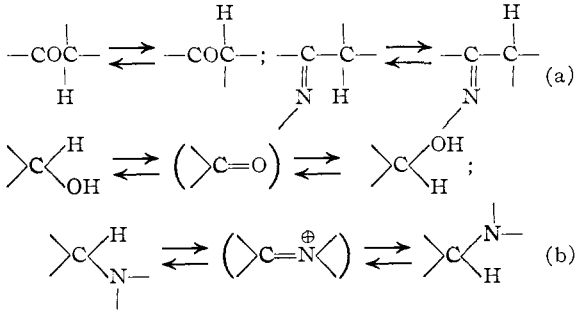
(7) *Cf.* D. Arigoni, *Experientia*, **14**, 153 (1958).

(8) *Cf.* A. J. Birch, "Biosynthetic Relations of Some Natural Phenolic and Enolic Compounds," in L. Zechmeister, "Progress in the Chemistry of Organic Natural Products," Springer–Verlag, Vienna, 1957.

(9) *Cf.* A. J. Birch, Massy-Westropp, R. W. Rickards and H. Smith, *J. Chem. Soc.*, 360 (1958).

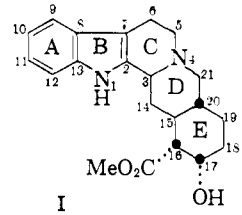
(10) *Cf.* L. Marion, *Bull. soc. chim. France*, 109 (1958).

whose skeletal features would make them appear quite unrelated. These concepts have been most useful as aids in the determination of the structure of alkaloids, and, while not yet proved to represent actual modes of biosynthesis in nature, they constitute the theoretical background for future biochemical experimentation. It appeared to us that prior to venturing into the latter, present theory should be subjected to a scrutiny of the stereochemical aspects of alkaloid structure. One probable reason for the lack of previous correlation between stereochemistry and biogenesis may reside in the fact that most alkaloids have their centers of asymmetry at sites readily equilibrated by acid–base, or oxidation–reduction processes or combinations thereof, *e.g.*

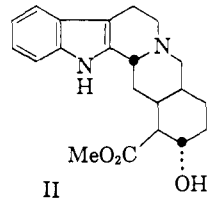


In such cases a correlation is obviously fruitless. However, the indole bases are a class of alkaloids whose stereochemical interrelationships appeared to be of biogenetic significance. Firstly, in contrast to most other classes of alkaloids, the indole class contains a wide variety of stereoisomers. Secondly, of the many asymmetric carbon atoms in most indole bases at least one is non-equilibratable. Therefore, an investigation of the stereochemical relationship of several indole bases was undertaken. The present communication describes our first attempts in the field of the yohimbine-, ajmalicine- and corynantheine-type alkaloids[11] and attempts to explain the data in terms of a new theory of biosynthesis of the indole alkaloids.

Yohimbine-type Alkaloids.—The thirteen yohimbine-type compounds of known constitution fall into two groups: the D/E *trans* and D/E *cis* systems. While all compounds in each group have been interrelated chemically,[1a,11] no chemical correlation of the two groups is yet on record. Furthermore, whereas optical data have been cited in support of an absolute configuration of the alkaloids of the D/E *trans* variety, *e.g.*, yohimbine (I),[12,13] as well as of the D/E *cis* type, *e.g.*, 3-epi-α-yohimbine (II),[14–16] no chemical substantiation has been brought forth yet. Our first goal was the correlation of the D/E *trans* and *cis* compounds.

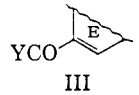


I

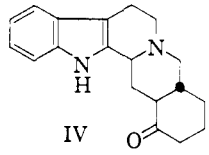


II

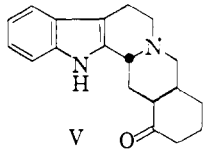
One rapid route appeared to lie in the degradation of two yohimbine isomers to triasymmetric compounds, the inversion of one or more centers of asymmetry of which would make them enantiometric or identical. The *apo* compounds (III) of yohimbine (I) and 3-epi-α-yohimbine (II) seemed most suited for this purpose, since a Curtius reaction, followed by hydrolysis, or a Schmidt reaction would be expected to lead to 16-yohimbone (IV) in the case of I and to 16-epialloyohimbone (V) in the case of II. However, the last product (V), possessing a carbonyl group adjacent to a bridgehead of a *cis*-perhydroisoquinoline system, would isomerize to the more stable *trans* product VI, an enantiomer of IV.



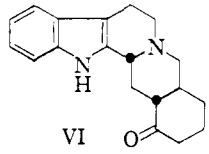
III



IV



V



VI

The Curtius route was abandoned, when it was discovered that apo-yohimbic hydrazide (III, Y = $NHNH_2$) could not be synthesized readily, crystalline material having been obtained only after long experimentation from a treatment of *apo*-yohimbine (III, Y = OMe) with 95% refluxing hydrazine.[17] However, a Schmidt reaction on apoyohimbic acid hydrochloride (III, Y = OH)[11] afforded in one step a single crystalline compound in low yield, whose analytical and spectral characteristics proved it to be 16-yohimbone (IV).[18] When the same reaction was carried out on *apo*-3-epi-α-yohimbic acid hydrochloride (III, Y = OH),[19] 16-yohimbone enantiomer (VI), as proved by identity of m.p., infrared spectra and magnitude of optical rotation, but opposite sign, was isolated. When in connection with a study of the potential chemical correlation of ajmalicine (VII) and tetrahydroalstonine (VIII)[20] their acids were exposed to a Schmidt reaction also, again the yields of products were poor. While this fact discouraged the pursuit of the Schmidt degradation of the ring

(11) For a review of their chemistry, *cf.* (a) J. E. Saxton, *Quart. Revs.*, **10**, 108 (1956); (b) R. E. Woodson, Jr., H. W. Youngken, E. Schlittler and J. A. Schneider in "Rauwolfia," Little Brown and Co., Boston, Mass., 1957, Chapter 3.

(12) W. Klyne, *Chemistry & Industry*, 1032 (1953).

(13) C. Djerassi, R. Riniker and B. Rinicker, THIS JOURNAL, **78**, 6362 (1956).

(14) P. A. Diassi, F. L. Weisenborn, C. M. Dylion and O. Wintersteiner, *ibid.*, **77**, 2028, 4687 (1955).

(15) C. F. Huebner, H. B. MacPhillamy, E. Schlittler and A. F. St. André, *Experientia*, **11**, 303 (1955).

(16) M.-M. Janot, R. Goutarel, A. LeHir, G. Tsatsas and V. Prelog, *Helv. Chim. Acta*, **38**, 1073 (1955).

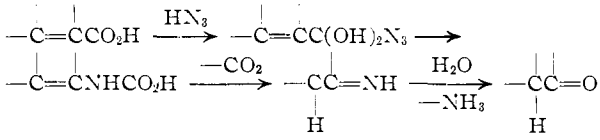
(17) This is in contrast to the relatively easy synthesis of yohimbhydrazide [C. F. Huebner, R. Lucas, H. B. MacPhillamy and H. A. Troxell, THIS JOURNAL, **77**, 469 (1955)], a procedure which could be repeated without difficulty in this Laboratory.

(18) This reaction has been reported independently also by R. K. Hill and K. Muench [*J. Org. Chem.*, **22**, 1276 (1957)].

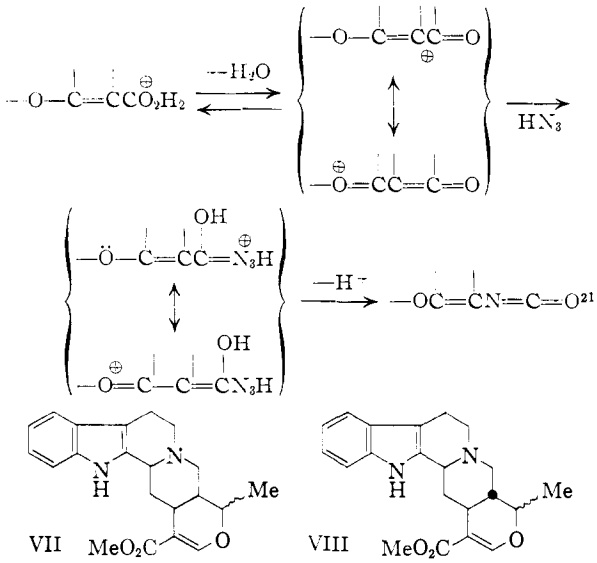
(19) C. F. Huebner and E. Schlittler, THIS JOURNAL, **79**, 250 (1957). We are most grateful to Dr. Huebner for transmitting to us the experimental details for the preparation of *apo*-3-epi-α-yohimbine.

(20) E. Wenkert and D. K. Roychaudhuri, *ibid.*, **80**, 1613 (1958).

E heterocyclic compounds beyond the isolation of the crude products, the characterization of the latter by their infrared spectra led to an interesting observation. In contrast to the ketonic Schmidt products of the above *apo* compounds, those of ajmalicine (VII) and tetrahydroalstonine (VIII) proved to be isocyanates. It would appear that the intermediate *apo* compound undergoing rearrangement must be the hydrated form of the acyl azide or its conjugate acid

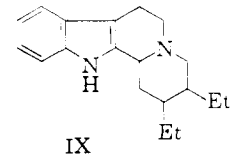


whereas the non-hydrated form must be predominant rearranging species in the more conjugated systems of VII and VIII

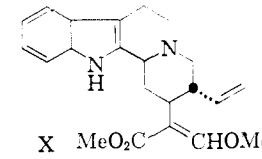


The formation of the enantiomeric ketones IV and VI from the D/E *trans* and *cis* yohimbine-type compounds, respectively, constitutes the first unambiguous chemical proof that, despite the great profusion of stereoisomerism in this alkaloid class, one asymmetric center—C-15—has a unique configuration in all these natural compounds.[22] On the basis of the aforementioned optical data[12,13] the hydrogen atom at C-15 has an α-orientation. It is immediately worthy of note that this singular characteristic of C-15 must be of utmost biosynthetic significance, since this center is the only non-equilibratable site among the five to six asymmetric carbon atoms in most alkaloids of the yohimbine type.

Ajmalicine- and Corynantheine-type Alkaloids.—The conversion of the ring E heterocyclic bases to desoxy compounds of structure IX, also derivable from 17,18-*seco* alkaloids[11] would provide a method for their correlation as well as a determination of their relationship to corynantheine (X). The more abundantly available D/E *cis* compound, ajmalicine (VII),[20,23] was therefore exposed to several procedures of degradation.

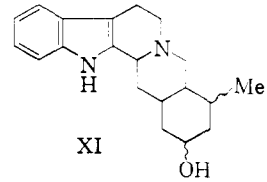


IX

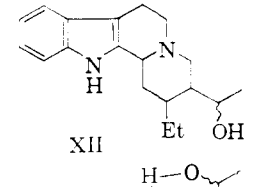


X

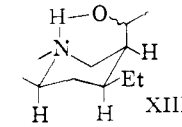
Acid-catalyzed hydration and decarboxylation of ajmalicic acid (tetrahydroserpentic acid[24]) led to ajmalicial (XI), whose spectral characteristics proved it to be a hemiacetal. When preliminary attempts at reduction of XI by ethylene thioacetal formation, followed by nickel desulfurization, proved difficult in the second step, a Wolff–Kishner reduction was employed. Desoxygenation of the resulting ajmaliciol (XII) by lithium aluminum hydride reduction of its tosylate was unsuccessful. The sulfonate ester was not easily prepared, perhaps because of the stability of the alcohol by intramolecular hydrogen bonding (*cf.* XIII) and, once formed, readily decomposed to unrecognizable products. As a consequence, the alcohol XII was oxidized but the Oppenauer procedure to 19-dihydrocorynantheone (XIV). The stereochemistry of the latter as depicted in XIV, follows from the fact that the basic conditions of the oxidation are stringent enough to equilibrate both C-3 and 15 so as to produce the most stable, *normal* configuration, regardless of the stereochemistry of the starting material. Wolff–Kishner reduction of XIV yielded dihydrocorynantheane (XV), identical in all respects with the degradation product of corynantheine (X).[25,26]



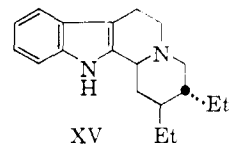
XI



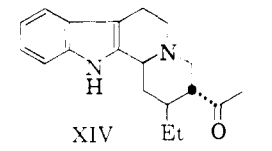
XII



XIII



XV



XIV

Because of an inadequate supply of tetrahydroalstonine (VIII) its degradation to the desoxy system IX was attempted by the above reaction processes without isolation of intermediates, but failed. Acid-induced hydration and decarboxylation of tetrahydroalstonic acid,[27] followed by

(21) For a brief discussion of the mechanism of the Schmidt reaction *cf.* J. Hine, "Physical Organic Chemistry," McGraw-Hill Book Co., Inc., New York, N. Y., 1956, p. 319.

(22) *Cf.* A. K. Bose, B. G. Chatterjee and R. S. Iyer, *Indian J. Pharm.*, **18**, 185 (1956).

(23) N. Neuss and H. E. Boaz, *J. Org. Chem.*, **22**, 1001 (1957).

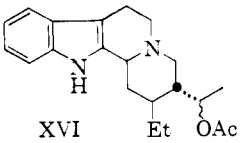
(24) F. Bader and H. Schwarz, *Helv. Chim. Acta*, **35**, 1594 (1952).

(25) M.-M. Janot and R. Goutarel, *Bull. soc. chim. France*, 588 (1951).

(26) The conversion of XIV into XV constitutes a proof of the stereochemistry of corynantheine (X) and, hence, corroborates the stereochemical findings, along synthetic lines, by E. E. van Tamelen, P. E. Aldrich and T. J. Katz, [THIS JOURNAL, **79**, 6426 (1957)].

(27) T. M. Sharp, *J. Chem. Soc.*, 1353 (1938).

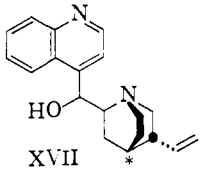
Wolff–Kishner reduction, yielded low-melting tetrahydroalstoniol, which could be characterized as its acetate XVI.



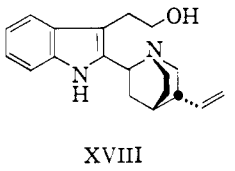
XVI

The conversion of ajmalicine (VII) into dihydrocorynantheane (XV) shows that at least one representative each of the indole alkaloids of the ring E heterocyclic and 17,18-*seco* varieties have the identical C-15 configuration. Furthermore, optical data[28] again suggest that the hydrogen atom at this center possesses an α-orientation.

Absolute Configuration.—At this point it became of interest to establish the absolute configuration of the above indole alkaloids by chemical means. A chemical correlation with the cinchona alkaloids appeared to be the most direct route, since the absolute configuration of the latter bases has been determined chemically,[29] and the carbon atom corresponding to the yohimbé C-15 [*cf.* the starred center in cinchonine (XVII)] has been shown to be substituted by an α-hydrogen. This class of alkaloids has also been considered to be in intimate biogenetic relationship with the yohimbé bases.[30] Furthermore, the recent conversion of dihydrocinchonine into dihydrocinchonamine, the dihydro derivative of the indolic cinchona base XVIII,[31] made our task relatively easy.

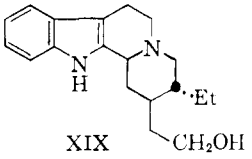


XVII

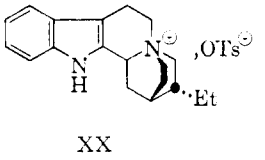


XVIII

Tosylation of dihydrocorynantheol (XIX)[32] and refluxing of the product in dimethylformamide yielded a quaternary ammonium tosylate XX. Tosylation of dihydrocinchonamine gave a quaternary chloride, which on treatment with silver tosylate afforded the identical sulfonate salt XX. These results represent the first chemical correlation of the yohimbé and cinchona alkaloids. They establish the absolute configuration of ajmalicine and corynantheine as VII and X, respectively.

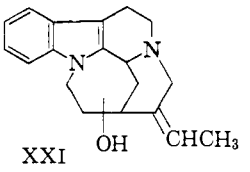


XIX

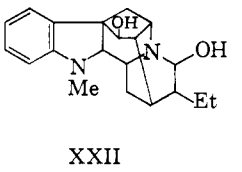


XX

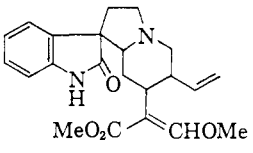
The cumulative physical and chemical evidence points to a unique C(15)-H α-configuration for all indole and phytochemically related alkaloids. Any theory attempting to portray the biosynthesis of these natural products must take in consideration this important stereochemical feature. Whereas the elucidation of the absolute configuration of alkaloids of the mavacurine (XXI), ajmaline (XXII) and rhyncophylline (XXIII) groups lies in the future, that of strychnine, the main member of the strychnos class, is as depicted in XXIV.[33] Again, its carbon analogous to the yohimbé C-15 possesses an α-hydrogen atom (*cf.* starred atom in XXIV).



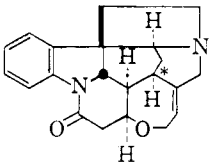
XXI



XXII

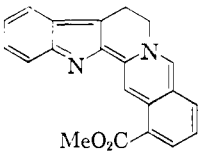


XXIII

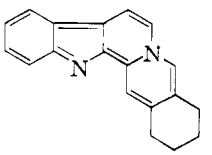


XXIV

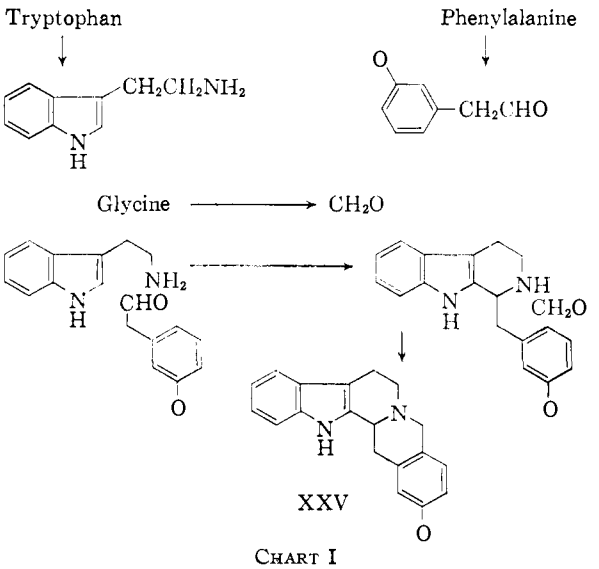
A Theory of Biosynthesis of the Indole Alkaloids.—For over two decades the pentacyclic yohimbine nucleus XXV has been considered to have arisen in nature by the interaction of the amino acids tryptophan, phenylalanine (or its phenolic derivatives) and glycine according to the Barger–Hahn scheme (*cf.* Chart I).[34] Recently



XXVII



XXVIII



XXV

CHART I

(28) M.-M. Janot, R. Goutarel, A. LeHir, G. Tsatsas and V. Prelog, *Helv. Chim. Acta*, **38**, 1073 (1955).

(29) V. Prelog and E. Zalan, *ibid.*, **27**, 545 (1944).

(30) R. Goutarel, M.-M. Janot, V. Prelog and W. I. Taylor, *ibid.*, **33**, 150 (1950).

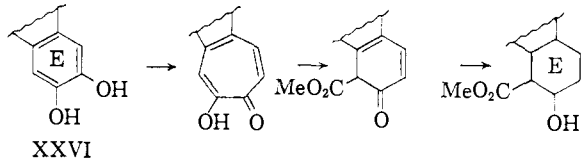
(31) E. Ochiai and M. Ishikawa, *Pharm. Bull. (Japan)*, **6**, 208 (1958).

(32) C. Vamvacas, W. v. Philipsborn, E. Schittler, H. Schmid and P. Karrer, *Helv. Chim. Acta*, **40**, 1793 (1957).

(33) A. F. Peerdeman, *Acta Cryst.*, **9**, 824 (1956).

(34) (a) G. Barger and C. Scholz, *Helv. Chim. Acta*, **16**, 1343 (1933) (b) G. Hahn and H. Werner, *Ann.*, **520**, 123 (1935).

the scheme was elaborated to account for the presence of the extraneous carbomethoxy group in ring E (*cf.* Chart II).[3] Whereas the Barger–Hahn

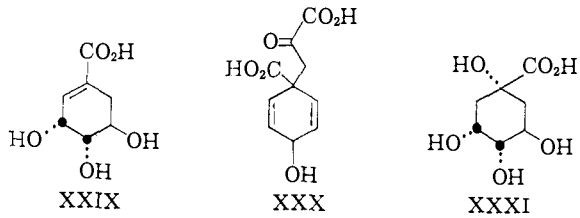


XXVI

CHART II

model has been invaluable in facilitating structure elucidations in the field of indole alkaloids, it and the Robinson extension are clearly inadequate, especially in light of our stereochemical results. Three interdependent difficulties are discernible in the model: (a) the state of oxidation of ring E, (b) the absolute configuration of C-15 and (c) the origin of the carbomethoxy group. With the exception of alstoniline (XXVII), no yohimbine-type compounds possess aromatic rings E, and, except further for sempervirine (XXVIII), all such systems have asymmetric centers at the D/E ring juncture. Furthermore, while an enzymic production of these centers by reduction at the phenol or tropolone stages is possible, its specificity at C-15 is not readily explicable. Finally, the lack of clarity regarding the expansion of phenolic ring E to a tropolone, the mechanistic unlikelihood of the contraction of the latter to a β- rather than α-oxygenated ester and the inordinate specificity of location of the ester function at C-16 in the ring contraction make the origin of the ester group very vague.

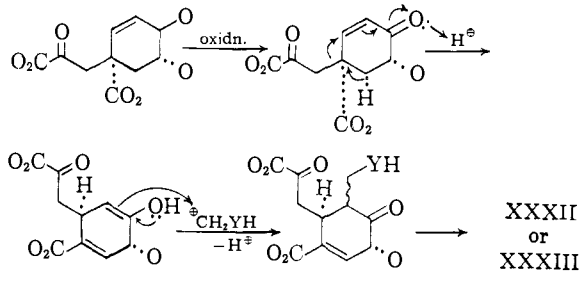
Since the deficiencies of the Barger–Hahn scheme revolve exclusively around the phenylalanine portion of the yohimbine skeleton, a substitute for the amino acid is required. More specifically, a carboxy-substituted hydroaromatic system is needed. A compound satisfying this requirement has been shown to be the biosynthetic precursor of phenylalanine in bacteria. In a most elegant series of recently published experiments Davis has demonstrated that shikimic (XXIX) and prephenic



XXIX XXX XXXI

acids (XXX) are the natural progenitors of aromatic amino acids.[35] Thus it appears that the latter could furnish the carbon atoms for ring E and part of ring D of the yohimbé bases. However, the stereomechanistic aspects of the formation of the prephenate unit from shikimate and of its mode of interaction with formaldehyde and tryptamine, or their equivalents, remain to be analyzed. The presence of quinic acid (XXXI), whose 5-dehydro derivative is a precursor of shikimic acid,[35] in the cinchona plant and the frequent attachment of 3,4,5-trimethoxybenzoyl and 3,4,5-trimethoxycinnamoyl groups[36] to the yohimbé skeleton among rauwolfia alkaloids, *e.g.*, reserpine, rescinnamine,[11] lend credence to a shikimic–prephenic acid hypothesis.

As illustrated in Chart III, the reaction between shikimate and pyruvate to yield prephenate has two potential mechanistic pathways: (a) nucleophilic displacement of phosphate from 5-phosphoshikimate by enolpyruvate at C-1, *i.e.*, an $S_{N}2'$ reaction; (b) formation of an enol ether of pyruvate with the 5-hydroxy group of shikimate, followed by intramolecular transfer of the pyruvate moiety to C-1, *i.e.*, an $S_{N}1'$ reaction.[37] Since either mechanism requires retention of configuration,[38] the pyruvate sidechain in the hydrated prephenate reaction product must have an orientation identical with that of the 5-hydroxy group in shikimate.[39] The reaction between the hydrated prephenate and the conjugate acid of formaldehyde (Y = O), formaldimine (Y = NH) or N-formaltryptamine (Y = NR) can proceed in a concerted manner, *i.e.*, by *trans* addition across the double bond, to yield a 15,20-*trans* compound (XXXII), or in a two-step process to give either a *trans* or *cis* product (XXXIII). In either event the migrating pyruvate sidechain must again retain its configuration.[40]



Compounds XXXII and XXXIII, thus derived in two simple operations from shikimic acid, fulfill all functional and stereochemical requirements as phytogenic precursors of the yohimbine-type alkaloids. Hydration–dehydration and oxidation–reduction processes, in an unpredictable sequential order, and condensation of the pyruvate sidechain into C-2 of an indole ring[3] can lead to alkaloids such as

(35) B. D. Davis in W. D. McElroy and H. B. Glass, "A Symposium on Amino Acid Metabolism," The Johns Hopkins Press, Baltimore, Md., 1955, p. 799.

(36) *Cf.* (a) R. L. Metzenberg and H. K. Mitchell, *Biochem. J.*, **68**, 168 (1958); (b) H. Shimazono, W. J. Schubert and F. F. Nord, THIS JOURNAL, **80**, 1992 (1958).

(37) Path (b), in essence a Claisen rearrangement, has been proposed already by Woodward as a possible mode of formation of prephenic acid (private communication from Professors Woodward and Davis).

(38) *Cf.* G. Stork and W. N. White, THIS JOURNAL, **78**, 4609 (1956).

(39) For the absolute configuration of shikimic acid (XXIX) *cf.* H. O. L. Fischer and G. Dangschat, *Helv. Chim. Acta*, **20**, 705 (1937).

(40) (a) An interesting biochemical analogy of this rearrangement is found in the 1,2-migration of a pyruvate unit during the formation of homogentisic acid in tyrosine metabolism (W. E. Knox, in W. D. McElroy and H. B. Glass, "A Symposium on Amino Acid Metabolism," The Johns Hopkins Press, Baltimore, Md., 1955, p. 836); (b) the introduction of a formaldehyde unit and the 1,2-migration of the pyruvate sidechain in the hydrated prephenate has been illustrated in Chart III as a single process, *i.e.*, a Prins reaction. This crucial conversion of a prephenate moiety to a potential ring E of the indole alkaloids has several variants (as is even more the case for the ensuing hydration–dehydration and oxidation–reduction steps). One such variant, perhaps more compatible with known phytochemical transformations than the Prins reaction, involves prior acid-catalyzed migration of the pyruvate substituent, followed by an aldol-Mannich type insertion of the one-carbon fragment

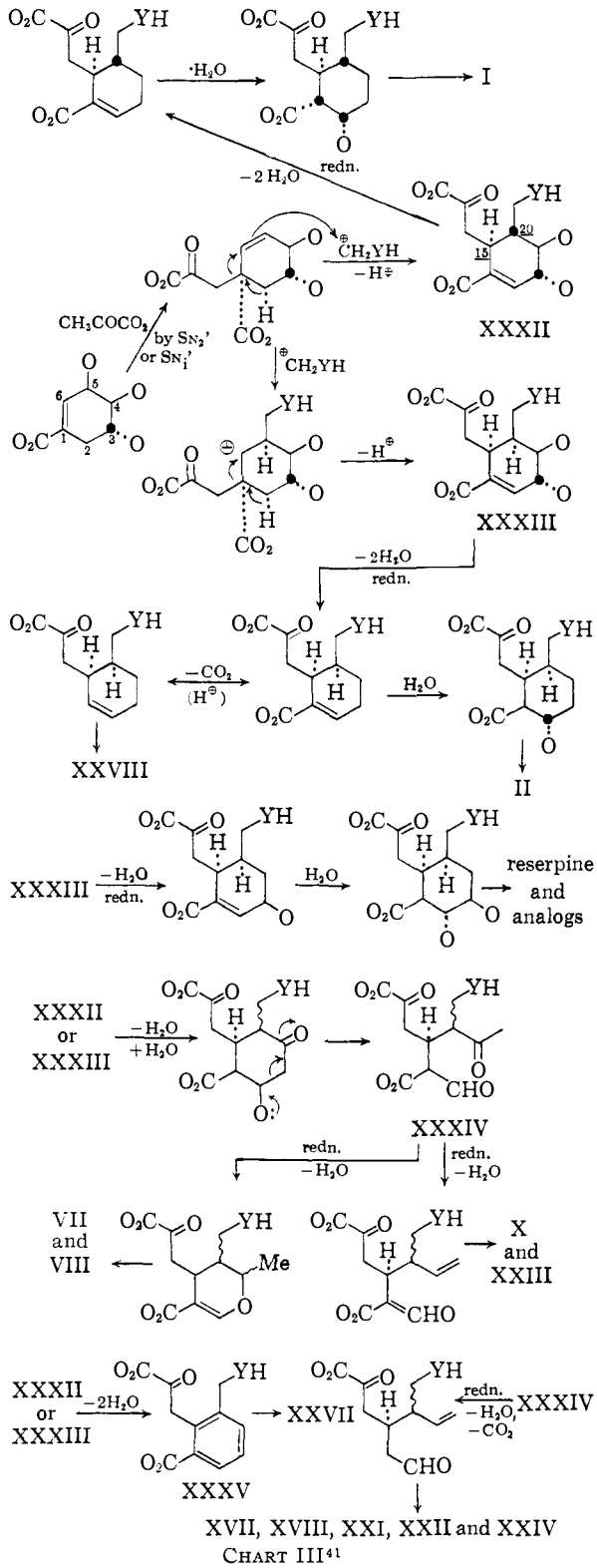
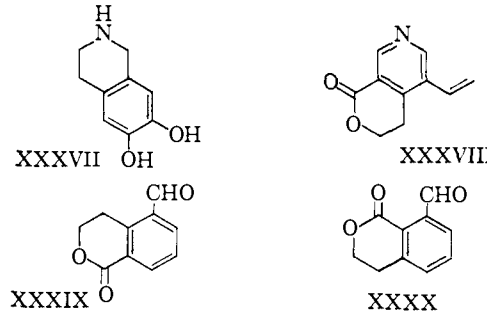


CHART III[41]

yohimbine (I), 3-epi-α-yohimbine (II), reserpine,[11] alstoniline (XXVII) and sempervirine (XXVIII) (see Chart III). Ring opening of XXXII or XXXIII, possibly by a *reto*-aldol mechanism, leads to a most crucial link (XXXIV) not only to the corynantheine- (X), rhyncophylline- (XXIII) and ajmalicine-type (VIII) systems, but also to the cinchona (XVII, XVIII) and strychnos (XXIV) bases and alkaloids of the skeleta of mavacurine (XXI), ajmaline (XXII) and emetine (XXXVI).[42,43]

Whereas the present theory necessitates the revision of all biogenetic hypotheses based on phenylalanine, these will be left for a future communication. However it is worth commenting on a recently postulated biogenetic scheme for gentianine (XXXVIII)[44] not only because the present



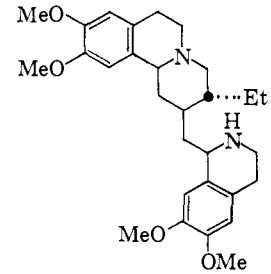
theory can accommodate its structure more readily, but also because it even permits prediction of the structure of a non-nitrogeneous bitter principle, erythrocentaurin (XXXIX or XXXX), isolated from a gentianine-bearing plant.[45]

In the previous hypothesis a "Woodward fission"[42] of XXXVII, derivable from dihydroxyphenylalanine and formaldehyde, was envisaged to precede changes in state of oxidation and the introduction of the carboxyl carbon atom.[44] The following transformations portray the origin of

(41) The second substituent on all single-bonded oxygen atoms has been omitted purposefully so as to avoid differentiation between hydroxyl groups and O-attached alkyl, acyl, phosphoryl and enzymic units.

(42) The ring cleavage of the phenylalanine portion of various alkaloids has been known as the "Woodward fission" and been accepted universally as the oxidative opening of a catechol (XXVI) ring, despite the fact that in the initial postulates [S. F. MacDonald, THIS JOURNAL, **69**, 1219 (1947); R. B. Woodward, *Nature*, **162**, 155 (1948)] hydroaromatic rings were considered to undergo cleavage; *cf.* R. B. Turner and R. B. Woodward, in R. H. F. Manske and H. L. Holmes, "The Alkaloids," Academic Press, Inc., New York, N. Y., 1953, Vol. III, p. 1; ref. 3; R. B. Woodward, *Angew. Chem.*, **68**, 13 (1956); A. J. Birch in Sir Alexander Todd, "Perspectives in Organic Chemistry," Interscience Publishers, Inc., New York, N. Y., 1956, p. 134; M.-M. Janot in Festschrift Arthur Stoll, Birkhäuser A. G., Basel, 1957, p. 499. The ring opening XXXII or XXXIII → XXXIV now supersedes the "Woodward fission."

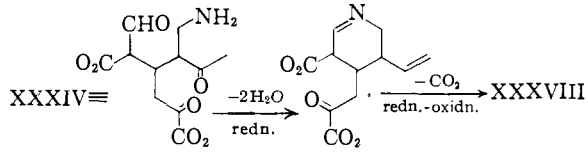
(43) For the stereochemistry of emetine *cf.* A. Brossi, A. Cohen, J. M. Osbond, Pl. A. Plattner, O. Schnider and J. C. Wikens, *Chemistry & Industry*, 491 (1958), and references contained therein.



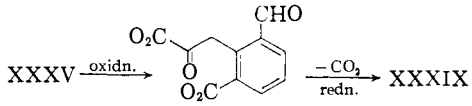
(44) T. R. Govindachari, K. Nagarajan and S. Rajappa, *Experientia*, **14**, 5 (1958).

(45) T. Kutoba and Y. Tomita, *Chemistry & Industry*, 230 (1958).

gentianine (XXXVIII) according to present views[46]



The carbocyclic precursor (XXXII or XXXIII) of XXXIV can lead *via* XXXV to erythrocentaurin



On this basis formula XXXIX can be predicted to represent the true structure of the bitter principle.

Acknowledgments.—The authors are most grateful to Professors Elderfield and Schmid and to Drs. Hochstein, Huebner, Lucas, MacPhillamy, Neuss, Raymond-Hamet, Schlittler and Sharp for their gifts of alkaloid samples, the National Institutes of Health, Public Health Service, Department of Health, Education and Welfare, for financial support (M 1301) and the Institute of Atomic Research, Ames, Iowa, for the use of a Baird infrared spectrophotometer. E. W. wishes to express his most heartfelt thanks to Professor Woodward, to his colleagues, Professors Hammond, DePuy and Chapman, and to Dr. Schlittler and his coworkers at Ciba (Summit, N. J.) for their participation in most inspiring discussions.

Experimental

Attempted Curtius Degradation of Apoyohimbine (III, Y = OMe).—The following is the only procedure that led to anything other than starting material. A mixture of 200 mg. of apoyohimbine, 1.5 ml. of 95% hydrazine and two drops of water was refluxed for 8 hr. The undissolved and unreacted apoyohimbine was filtered, the filtrate diluted with water and the resulting precipitate filtered. Its m.p. was 275–285° dec. and changed to 270° dec. on crystallization from dilute ethanol; infrared spectrum ($CHCl_3$): N–H, 2.92 (m), 3.08(s) 3.13(s)μ; C=O and C=C, 6.06(s), 6.19(s) μ.

Schmidt Degradations.—To a stirred mixture of 400 mg. of apohimbic acid hydrochloride (III, Y = OH)[11] and 1.5 ml. of ice-cold concentrated sulfuric acid there was added 20 ml. of chloroform and, with further stirring, 100 mg. of freshly activated sodium azide in small portions. After 5 min. at room temperature the mixture was kept at 40° for 15 min., whereupon the reaction was quenched by the addition of ice, the mixture made basic with dilute ammonia and extracted with chloroform. Removal of solvent left 50 mg. (17%) of a tan crystalline material, m.p. 255–258°. Two crystallizations from aqueous methanol yielded 16-yohimbone (IV) m.p. 260–262° dec., which was purified further by high vacuum sublimation. m.p. 256° dec.; infrared spectrum ($CHCl_3$): N–H, 2.90(m) μ; C=O, 5.87(s) μ; $[\alpha]_D$ −86.1 (pyridine).

Anal. Calcd. for $C_{19}H_{22}ON_2$: C, 77.52; H, 7.53; N, 9.52. Found: C, 77.71; H, 7.28; N, 9.30.

When the identical procedure was employed on 160 mg. of *apo*-3-epi-α-yohimbic acid hydrochloride (III, Y = OH),[19] it afforded 22 mg. (17%) of crude product. Chromatography on alumina, elution with 1:4 benzene–ether, followed by crystallization from dilute methanol yielded colorless needles of 16-yohimbone enantiomer (VI), m.p. 254–256° dec., $[\alpha]_D$ 85.0° (pyridine), infrared spectrum ($CHCl_3$) superimposable on that of IV.

In view of the irreproducibility of the Bader–Schwartz procedure of the hydrolysis of tetrahydroserpentine[24] it was modified as follows. A solution of 250 mg. of ajmalicine (VII) in 20 ml. of 20% ethanolic (90%) potassium hydroxide was refluxed for 18 hr. under nitrogen. After evaporation of the solvent under vacuum, dissolution of the residue in water and acidification with hydrochloric acid, the solution was extracted with 1-butanol. Evaporation of the solvent led to 175 mg. (66%) of crude tetrahydroserpentic acid hydrochloride, m.p. 235–240° dec., 100 mg. of which was exposed to the above Schmidt procedure. The crude, amorphous product consisted of 10 mg. (20%), m.p. 140–145°; infrared spectrum ($CHCl_3$): N–H, 2.90(m) μ; N=C=O, 4.72(s) μ; no C=O peak.

Hydrolysis of 100 mg. of tetrahydroalstonine (VIII) by the above method and Schmidt reaction on the resultant acid hydrochloride (III, Y = OH)[27] yielded 17 mg. (17%) of a pale brown, amorphous product, m.p. 120–125°; infrared spectrum ($CHCl_3$): N–H, 2.90(m) μ; N=C=O, 4.72(s) μ; no C=O peak.

Ajmalicial (XI).—A solution of tetrahydroserpentic acid hydrochloride, obtained from 500 mg. of ajmalicine, 10 ml. of 2 *N* HCl and 16 ml. of water was heated on a steam-bath for 4 hr. The solution was made alkaline with sodium carbonate, and the resulting precipitate filtered. Trituration with cold ethanol gave 230 mg. (52%) of crystals. Two crystallizations from ethanol yielded colorless needles of ajmalicial (XI), m.p. 226–228°; infrared spectrum (KBr pellet): O–H, 2.80(m), 3.08(m) μ; N–H 2.91(m) μ; no C=O peak; $[\alpha]_D$ −124° (pyridine).

Anal. Calcd. for $C_{19}H_{24}O_2N_2$: C, 73.04; H, 7.74; N, 8.97. Found: C, 72.65; H, 7.85; N, 8.95.

Ajmaliciol (XII).—A mixture of 230 mg. of ajmalicial (XI), 0.6 g. of potassium hydroxide, 1.7 ml. of 95% hydrazine hydrate and 7 ml. of ethylene glycol was refluxed for 1 hr. and then heated at 200° for 4 hr. Dilution of the solution with water, extraction with chloroform and evaporation of the organic solvent yielded a glassy residue, which on trituration with cold methanol was converted to 175 mg. (80%) of crystals. Crystallization from methanol afforded colorless needles of ajmaliciol (XII), m.p. 200–201°; infrared spectrum ($CHCl_3$); N–H, 2.90(m) μ; C=C, 6.18(m) μ; $[\alpha]_D$ −25° (pyridine).

Anal. Calcd. for $C_{19}H_{26}ON_2$: C, 76.47; H, 8.78; N, 9.39. Found: C, 76.22; H, 9.12; N, 9.82.

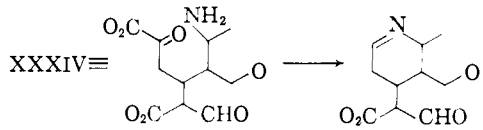
19-Dihydrocorynanthenone (XIV).—A solution of 75 mg. of ajmaliciol (XII), 0.5 g. of aluminum phenoxide, 4 ml. of cyclohexanone and 6 ml. of xylene was refluxed for 7 hr. The mixture was extracted with 2 *N* sulfuric acid, made alkaline with ammonia and extracted with chloroform. Evaporation of the solvent gave a semicrystalline substance, which on chromatography on alumina, elution with 1:4 benzene–ether and evaporation of the eluting solvent under nitrogen and *in vacuo* led to 28 mg. of colorless needles (37%). Sublimation under high vacuum, followed by crystallization from methanol, afforded 19-dihydrocorynantheone (XIV), m.p. 225–227°; infrared spectrum ($CHCl_3$): N–H, 2.90(m) μ; C=O, 5.87(s) μ; $[\alpha]_D$ 0° (pyridine), −57° ($CHCl_3$).

Anal. Calcd. for $C_{19}H_{24}ON_2$: C, 76.99; H, 8.16. Found: C, 76.98; H, 8.35.

Dihydrocorynantheane (XV).—A solution of 25 mg. of 19-dihydrocorynantheane (XIV), 0.25 ml. of hydrazine hydrate, 2 drops of glacial acetic acid and 3 ml. of ethylene glycol was refluxed for 30 min. After addition of 0.3 g. of potassium hydroxide and 0.2 ml. of hydrazine hydrate the solution was heated at 200–210° for 4 hr. It then was diluted and extracted with chloroform. Removal of the solvent, alumina chromatography of the crystalline residue, elution with 9:1 benzene–ether gave 10 mg. (42%) of colorless needles. High vacuum sublimation, followed by crystallization from dilute methanol, yielded dihydrocorynantheane (XV), m.p. 189°, mixed m.p. 188–189° with an authentic sample (m.p. 187–188°), infrared spectrum ($CHCl_3$) identical with that of an authentic sample, $[\alpha]_D$ −94° (pyridine).

19-Acetoxydihydrocorynantheane (XVI).—Acid-catalyzed hydration and decarboxylation of the tetrahydroalstonic acid hydrochloride obtained by alkaline hydrolysis of 100

(46) The biogenetic possibility of inversion of one half of XXXIV suggests that the inversion of the other half may lead to a structure pattern to be anticipated among alkaloids of the future



mg. of tetrahydroalstonine (VIII) by the above procedure gave 45 mg. (51%) of amorphous hemiacetal. Wolff-Kishner reduction (by the method used for ajmaliciol) of 90 mg. the latter yielded a gum, which on alumina chromatography and elution with 2% methanol in ether gave 65 mg. (76%) of non-crystalline alcohol, m.p. 80–88°. A solution of 350 mg. of the latter in 7 ml. of acetic anhydride and 6 ml. of pyridine was left standing for three days at room temperature. The mixture was poured onto crushed ice, ammonia added, extracted with chloroform and concentrated. The residual dark gum was chromatographed on alumina giving 85 mg. (21%) of a pale yellow substance on 3:2 benzene–ether elution which was crystallized from methanol to colorless needles, m.p. 220–223°. Sublimation under vacuum and recrystallization from methanol produced crystalline 19-acetoxydihydrocorynantheane (XVI), m.p. 224°; infrared spectrum ($CHCl_3$): N–H, 2.87(m) μ; C=O, 5.80 (s) μ; $[\alpha]D$ −51° (chloroform).

Anal. Calcd. for $C_{21}H_{28}O_2N_2$: C, 74.08; H, 8.29; N, 8.23. Found: C, 73.88; H, 8.37; N, 8.55.

Quaternization Experiments.—A solution of 50 mg. of dihydrocorynantheol (m.p. 185–187°) (XIX) and 85 mg. of *p*-toluenesulfonyl chloride in 1 ml. of pyridine was left standing in the refrigerator for 36 hr. The precipitated, colorless crystals, 33 mg., m.p. 260°, were filtered, suspended in dilute aqueous sodium hydroxide and extracted with chloroform. Evaporation of the solvent yielded 15 mg. of a substance, m.p. 305–310° dec., which on refluxing for half an hour in 0.6 ml. of dimethylformamide and slow cooling produced glistening colorless needles of the salt XX m.p. 315–316° dec., no change in m.p. on recrystallization from dimethylformamide, $[\alpha]D$ −66° (90% methanol).

A solution of 50 mg. of dihydrocinchonamine, 85 mg. of *p*-toluenesulfonyl chloride in 1 ml. of dry pyridine was left standing in the refrigerator for 36 hr. The precipitated crystalline substance, 31 mg., m.p. 318–319° dec. was identified as a quaternary chloride by a positive halogen test, by the absence of characteristic sulfonate ester or salt absorption peaks in the infrared spectrum, and by analysis of a sample, crystallized from chloroform–methanol, m.p. 320–321° dec., whose results fitted best the molecular formula of a quaternary chloride chloroform solvate. The latter was recrystallized from dimethylformamide and dried over P_2O_5 at 2 mm. and 80° for 20 hr.

Anal. Calcd. for $C_{19}H_{25}N_2Cl$: C, 72.02; H, 7.95. Found: C, 72.43; H, 8.01.

Dissolution of 20 mg. of the chloride in 2 ml. of distilled water, addition of 17 mg. of silver *p*-toluenesulfonate, filtration of the precipitated silver chloride, basification of the filtrate with ammonia, extraction with chloroform and evaporation of the solvent gave colorless crystals, which on crystallization from dimethylformamide appeared as long needles of the salt XX, m.p. 313–315° dec., mixed m.p. 315° dec. with above salt XX, identical infrared spectrum (KBr pellet) with that of the above sample, $[\alpha]D$ −69.5° (90% methanol).

AMES, IOWA

[CONTRIBUTION FROM THE DEPARTMENT OF CHEMISTRY, THE FLORIDA STATE UNIVERSITY]

Constituents of *Helenium* Species. VII. Bitter Principles of *H. pinnatifidum* (Nutt.) Rydb., *H. vernale* Walt., *H. brevifolium* (Nutt.) A. Wood and *H. flexuosum* Raf.[1]

BY WERNER HERZ, R. B. MITRA,[2] K. RABINDRAN AND W. A. ROHDE[3]

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Several new sesquiterpene lactones have been isolated from three of the plants named in the title. *H. vernale* Walt. yielded the previously known helenalin.

The sesquiterpene lactones tenulin[4] and helenalin[5] have been isolated from a number of *Helenium* species[5a,6] which, except for *H. amarum* (Raf.)[7] and *H. autumnale*, occur in the Southwestern United States. However, there exists a subgenus, or section, *Leptopoda*, whose geographic distribution is limited to the Southeastern United States and which has not been investigated chemically. A revision of this section, which is characterized by the perennial, vernal-flowering habit and the neutral and sterile rays, has been published recently.[8] Because it was hoped that a knowledge of the main constituents might contribute to a greater understanding of the phylogenetic relationships existing in this subgenus we have undertaken its chemical examination. The present paper deals with the isolation of a number of new substances from four of the six species belonging to the section.

Extraction of *H. pinnatifidum* (Nutt.) Rydb.[8] gave in 0.08% yield a crystalline substance of formula $C_{15}H_{18}O_3$. m.p. 164–165°, which we have named pinnatifidin. The ultraviolet spectrum, λ_{max} 237.5 mμ, ϵ_{max} 13550 (in ethanol), indicated the presence of a disubstituted α,β-unsaturated ketone. This was supported by the infrared evidence (band at 1675 cm.$^{-1}$; the position of this band excludes the cyclopentenone chromophore found in helenalin and tenulin). The remaining two oxygen atoms must be ascribed to a γ-lactone group (band at 1770 cm.$^{-1}$) which is probably conjugated with a methylene group as in helenalin (high intensity at 215 mμ,[5b] band at 1.64 μ in the near infrared,[9] strong band at 1630 cm.$^{-1}$ due to the two conjugated double bonds).

H. vernale Walt.[8] which has frequently been confused with *H. pinnatifidum*, furnished as the sole crystallizable component (0.02%) a substance which was identical in all respects with helenalin. Thus these two morphologically very similar species can be differentiated by chemical means.

The crude extract of *H. brevifolium* (Nutt.) A. Wood[8] furnished, after extensive chromatography,

(1) Previous paper, W. Herz and R. B. Mitra, THIS JOURNAL, **80**, 4876 (1958).

(2) Fulbright Travel Scholar, 1957–1958.

(3) Ethyl Corporation Predoctorate fellow, 1957–1958.

(4) (a) D. H. R. Barton and P. DeMayo, *J. Chem. Soc.*, 142 (1956); (b) B. H. Brown, W. Herz and K. Rabindran, THIS JOURNAL, **78**, 4423 (1956).

(5) (a) R. Adams and W. Herz, *ibid.*, **71**, 2546, 2551, 2554 (1949); (b) G. Büchi and D. Rosenthal, *ibid.*, **78**, 3860 (1956).

(6) E. P. Clark, *ibid.*, **58**, 1982 (1936); **61**, 1836 (1939); **62**, 597 (1940).

(7) The correct designation of *H. tenuifolium* Nutt., the most common source of tenulin, appears to be *H. amarum* (Raf.)[8] and will be employed in this and subsequent papers.

(8) H. F. L. Rock, *Rhodora*, **59**, 101, 128, 168, 203 (1957).

(9) W. H. Washburn and M. S. Mahoney, THIS JOURNAL, **80**, 504 (1958). In the absence of a cyclopropane ring this band is characteristic of $=CH_2$. The methylene band usually found near 890 cm.$^{-1}$ is displaced toward higher frequencies when conjugated[10] and is difficult to identify in the sesquiterpenoid lactones which we have encountered.

(10) W. Brügel, *Angew. Chem.*, **68**, 440 (1956).