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

# Biosynthesis of Indole Alkaloids. The Aspidosperma and Iboga Bases[1]

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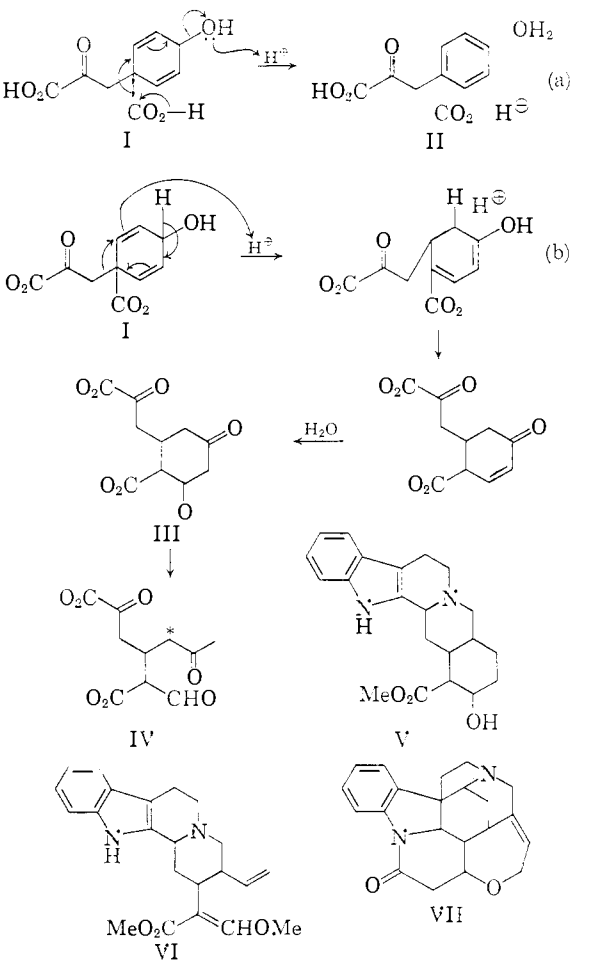
The prephenic acid hypothesis of indole alkaloid biosynthesis is brought up-to-date. An alternate scheme, a monoterpenoid hypothesis, is presented. The natural occurrence of the new structure patterns of the Aspidosperma and Iboga alkaloids and of their stereochemistry is interpreted in terms of the hypotheses presented.

An analysis of the structure types of indole alkaloids and an interpretation of their possible phytochemical relationships on the basis of available biochemical knowledge led recently to the development of a new theory of biosynthesis of the natural indole bases.[2] It suggested that the non-tryptophan or non-tryptamine part of most indole alkaloids is derived from carbohydrates *via* a pathway involving shikimic and hydrated prephenic acids and was able to account for both the skeletal and the stereochemical features of the indolic natural products. The rapid advances during the last two years in the isolation and structure determination of alkaloids of new structure type and related compounds and in the area of carbohydrate metabolism necessitate a reinspection of the biosynthetic hypothesis in light of the new findings.

**Prephenic Acid Hypothesis.**—On the assumption that the known interaction between shikimic and pyruvic acids leading to prephenic acid[3] proceeded *via* a hydrated prephenic acid, the latter had been considered to be the crucial intermediate from which most indole bases are derived and from which their absolute configuration is predetermined.[2] However, the lack of intervention of such hydrated form in the microbiological conversion of shikimic to prephenic acid[4] now suggests that prephenic acid (I) is itself the direct progenitor of many alkaloidal plant constituents. Its well-recognized decarboxylative dehydration (path a below)[4b] produces the aromatic $C_6$–$C_2$ units (II) known to be incorporated in the nuclei of benzylisoquinoline[5] and Amaryllidaceae[6] alkaloids, while its rearrangement, hydration and *retro*-aldolization in a previously described manner path (b below)[2a] affords intermediates III and IV whose formation (at their starred atoms) creates structural units readily discernible in indole alkaloids, such as yohimbine (V) and corynantheine (VI), and in indoline alkaloids, such as the Strychnos bases [*cf.* strychnine (VII)].

While the rearrangement of prephenic acid (path b), a structurally symmetrical and hence optically inactive substance, might be expected to yield racemic products,[7] enzymic intervention in the first step, in a manner not unlike the enzyme activity in the reactions of citric acid in the tricarboxylic acid cycle,[8] must be responsible for the specific absolute configuration in the natural products.




A most striking recent development in plant chemistry was the discovery of several non-alkaloidal (in most cases not even nitrogeneous) glycosides all of whose structures are based on the "*seco*-pre-

(1) This work was presented at the International Symposium on the Chemistry of Natural Products, under the auspices of the Section of Organic Chemistry of the International Union of Pure and Applied Chemistry, Melbourne, Canberra and Sydney, Australia, August 15–25, 1960.

(2) (a) E. Wenkert and N. V. Bringi, *J. Am. Chem. Soc.*, **81**, 1474, 6535 (1959); (b) E. Wenkert, *Experientia*, **15**, 165 (1959).

(3) B. D. Davis, *Adv. in Enzymol.*, **16**, 247 (1955).

(4) (a) J. G. Levin and D. B. Sprinson, *Biochem. and Biophys. Research Comm.*, **3**, 157 (1960); (b) D. B. Sprinson, *Adv. Carbohyd. Chem.*, **15**, 235 (1960).

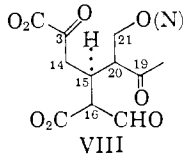
(5) E. Leete, *Chemistry & Industry*, 977 (1958); *J. Am. Chem. Soc.*, **81**, 3948 (1959); A. R. Battersby and B. J. T. Harper, *Chemistry & Industry*, 364 (1958); A. R. Battersby and R. Binks, *Proc. Chem. Soc.*, 287, 360 (1960).

(6) A. R. Battersby, R. Binks and W. C. Wildman, *Proc. Chem. Soc.*, 410 (1960).

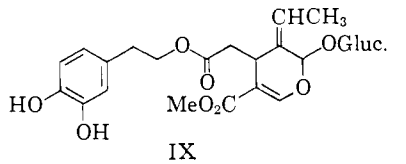
(7) This constitutes one of two valid explanations for the origin of *d,l*-mitraphylline [G. M. Badger, J. W. Cook and P. A. Ongley, *J. Chem. Soc.*, 867 (1950)] and *d,l*-akuammicine ($\psi$-akuammicine) [P. N. Edwards and G. F. Smith, *Proc. Chem. Soc.*, 215 (1960)]. The alternative explanation is presented below.

(8) A. G. Ogston, *Nature*, **162**, 4129 (1948); V. R. Potter and C Heidelberger, *ibid.*, **164**, 180 (1948); V. Lorber, M. F. Utter, H. Rudney and M. Cook, *J. Biol. Chem.*, **185**, 689 (1950).

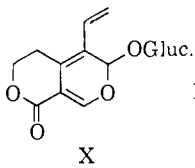
phenate-formaldehyde" (SPF) unit (VIII) which, as indicated above, is the precursor of many indole alkaloids, *e.g.*, VI and VII. Oleuropeine (IX),[9] gentiopicrin (X),[10] bakankosin (XI)[11] and swertiamarin (XII)[12] are neutral plant constituents with a common SPF nucleus differing from each other only in state of oxidation and in type and number of prosthetic groups. It thus appears that *the SPF moiety is an important metabolic cell constituent of higher plants whose interaction with a variety of simple organic substances, e.g., amino acids and carbohydrates, is responsible for the formation of a wide spectrum of superficially unrelated plant products.*



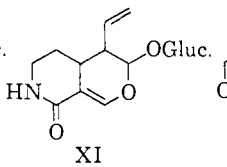
VIII



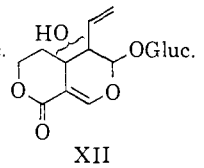
IX



X

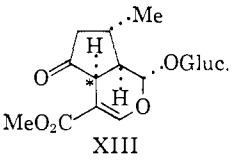


XI

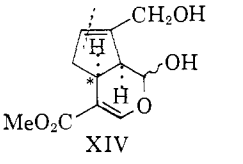


XII

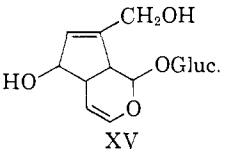
**Monoterpenoid Hypothesis.**—The recent advances in the characterization of SPF-based plant glucosides (IX–XII) have been accompanied by an equally rapid development in the structure determination of cyclopentane monoterpenic glucosides. These plant products, *e.g.* verbenalin (XIII),[11] genipin (XIV),[13] aucubin (XV)[14] and asperuloside (XVI),[15] appear to have their structures based on the monoterpene unit (XVII), whose many representatives in lower states of oxidation have been known for some time.[16] Comparison of the skeletal features of compounds XIII–XVI with IX–XII and the non-indole portion of VI and VII reveals three striking similarities. Firstly, both the substances based on the SPF unit and the terpenic compounds possess $C_{10}$ skeleta.[17] Secondly, cleavage or formation of the cyclopentane ring in the terpenic systems at a specific bond [*vide* dotted line in genipin (XIV)] by any of a variety of chemical processes makes the bare skeleta of these substances identical with those of the SPF type. Finally, the absolute configuration of the terpenic compounds [*cf.* starred atoms in verbenalin (XIII) and genipin (XIV)] is the same as that of the majority of indole alkaloids (*cf.* C(15) in VIII).[2] While this extraordinary similarity may be merely fortuitous, the possibility of the cyclopentanomonoterpenes being derived from prephenic acid or the indole alkaloids from acetic and mevalonic acids cannot be excluded at this time.[18]



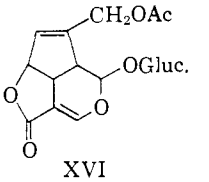
XIII



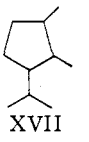
XIV



XV

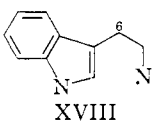


XVI

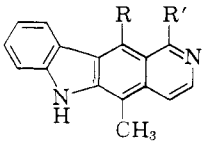


XVII

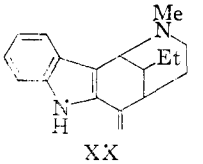
**Aspidosperma and Iboga Alkaloids.**—Among the most significant, recent advances in the chemistry of indole alkaloids has been the structure determination of bases with skeletal features distinctly different from the structure patterns of the alkaloids of the yohimbine (V)–corynantheine (VI) and Strychnos (VII) types. While the latter substances represent the biosynthetic consequence of combinations of tryptamine (XVIII) and SPF (VIII) units, no tryptamine (XVIII) moiety is appar-



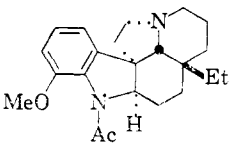
XVIII



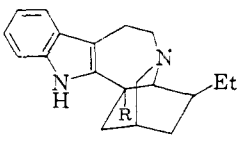
XIXa, R = H, R′ = $CH_3$
b, R = $CH_3$, R′ = H



XX



XXI



XXIIa, R = H
b, R = $CO_2Me$

(9) L. Panizzi, M. L. Scarpati and G. Oriente, *Gazz. chim. ital.*, **90**, 1449 (1960).

(10) L. Canonica, F. Pelizzoni, P. Manitto and G. Jommi, *Tetrahedron Letters*, **No. 24**, 7 (1960).

(11) G. Büchi and R. E. Manning, *ibid.*, **No. 26**, 5 (1960).

(12) T. Kubota and Y. Tomita, *ibid.*, **No. 5**, 176 (1961).

(13) C. Djerassi, T. Nakano, A. N. James, L. H. Zalkow, E. J. Eisenbraun and J. N. Shoolery, *J. Org. Chem.*, **26**, 1192 (1961).

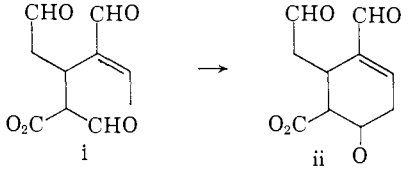
(14) W. Haegele, F. Kaplan and H. Schmid, *Tetrahedron Letters*, **No. 3**, 110 (1961).

(15) J. Grimshaw, *Chemistry & Industry*, 403 (1961).

(16) G. W. K. Cavill, *Rev. Pure Appl. Chem.*, **10**, 169 (1960).

(17) The pyruvate carboxyl of the SPF unit rarely appears in the natural products and hence is discounted in discussions of the SPF system. Decarboxylaton of α-keto acids or their masked counterparts has ample biochemical backing.

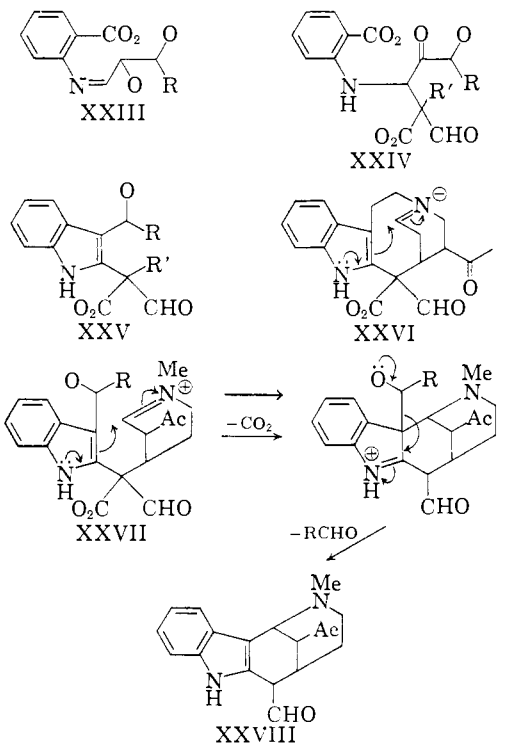
(18) Should the monoterpenoid hypothesis apply to the indole alkaloids, yohimbine-like (V) bases (ii) would appear at the very end rather than beginning of biosynthetic development.



i ii

ent in the Aspidosperma bases olivacine (XIXa),[19] ellipticine (XIXb)[20] and uleine (XX)[21] and no immediately obvious relationship appears to exist between the SPF unit and aspidospermine (XXI) and the Iboga bases ibogamine (XXIIa)[22] and coronaridine (XXIIb).[23] However, it can be shown that slight chemical modifications of the fundamental building blocks VIII and XVIII or their progenitors and their interaction with each other both at other than customarily assumed sites and at various stages of biosynthetic development lead to all of the novel alkaloid structure patterns.

Initial interaction of the indole-containing unit (XVIII) and the SPF (VIII) system, or its equivalent, has been considered traditionally to take place at the latter's C-3 and/or C-21 positions. On the assumption that the tryptamine (XVIII) unit in natural products of higher plants is derived by a sequence of steps similar to that in microorganisms leading to tryptophan[24] and that it (XVIII) or its precursors may interact with any of the reactive sites of the SPF (VIII) moiety, the following possibly pathway becomes clear. $\alpha$-Oxidation of glycosylideneanthranilic acid (XXIII),




a tryptophan progenitor,[24] and Mannich condensation of the product with SPF (VIII) (at C-16) yields a complex (XXIV) whose metabolism in a conventional manner[24] would be expected to produce an $\alpha$-alkyl-$\beta$-glycosylindole (XXV). If no reaction takes place between the $\alpha$- and $\beta$-substituents in XXV until after the $\beta$-glycosyl group is extruded and replaced by an ethanamino side-chain, in the same way in which tryptophan itself is formed,[24] compound XXVI is produced which, among other roles (*vide infra*), may play the part of the immediate precursor (*cf.* arrows in XXVI) of the Strychnos bases (*e.g.* VII).[25] If, however, the $\alpha$-substituent, *i.e.* the SPF half of intermediate XXV, does interact (through its active C-19 or 21) with the indolic $\beta$-position and extrusion (*via retro*-aldolization) of the $\beta$-glycosyl function follows the cyclization, the Aspidosperma skeleta of olivacine (XIXa), ellipticine (XIXb) and uleine (XX) are the consequence; *e.g.* XXVII → XXVIII, the possible genesis of the uleine skeleton.

The structurally more complex Aspidosperma and Iboga systems (XXI and XXII) must arise from a rearranged SPF unit. The crucial rearrangement can best be visualized to proceed *via* a *retro*-Michael reaction, hence a cleavage of the SPF (VIII) moiety into formyl acetate (XXIX) and fragment XXX,[26] appropriate oxidation–reduction processes and a Michael condensation. In this connection it is noteworthy that the Michael reaction is not new to indole biochemistry. The microbiological synthesis of tryptophan is known to involve a pyridoxal-aided Michael process,[24] while the biosynthesis of the indole alkaloid gramine (XXXII) probably revolves around a pyridoxal-induced *retro*-Michael degradation of tryptophan (XXXI → XXXII).[27]

Were fragment XXX (or iii) to interact with a tryptamine moiety (XVIII), or, alternatively, were a conventional tryptamine–SPF complex, *e.g.* one (XXXIII) normally leading to corynantheine-type

(19) (a) G. B. Marini-Bettolo and J. Schmutz, *Helv. Chim. Acta*, **42**, 2146 (1959); (b) J. Schmutz and H. Wittwer, *ibid.*, **43**, 793 (1960); (c) E. Wenkert and K. G. Dave, *J. Am. Chem. Soc.*, **84**, 94 (1962).

(20) R. B. Woodward, G. A. Iacobucci and F. A. Hochstein, *ibid.*, **81**, 4434 (1959).

(21) G. Büchi and E. W. Warnhoff, *ibid.*, **81**, 4433 (1959).

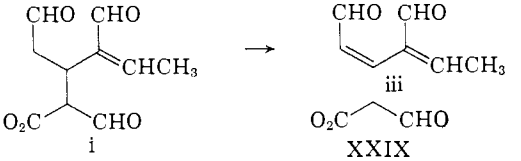
(22) M. F. Bartlett, D. F. Dickel and W. I. Taylor, *ibid.*, **80**, 126 (1958).

(23) M. Gorman, N. Neuss, N. J. Cone and J. A. Deyrup, *ibid.*, **82**, 1142 (1960).

(24) For a recent review of the biosynthesis of this amino acid, *cf.* C. H. Doy, *Rev. Pure Appl. Chem.*, **10**, 185 (1960).

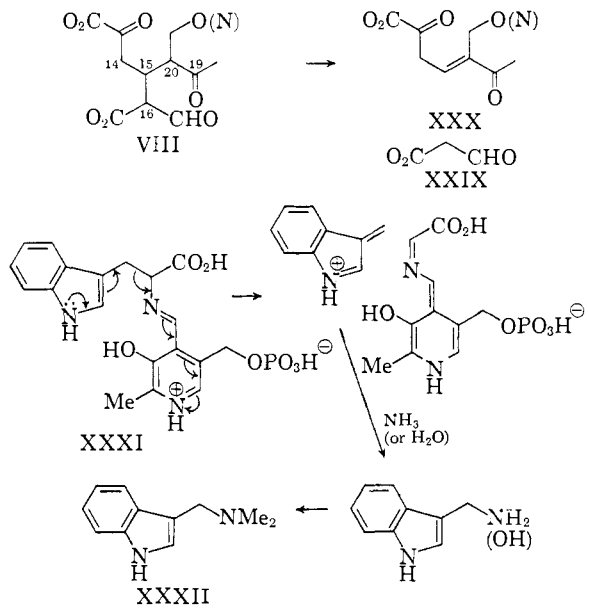
(25) This represents only one of three possible routes of Strychnos biosynthesis. The more conventional two paths involve a reaction of SPF (VIII) (at C-3) and a tryptamine (XVIII) system at the $\beta$-carbon of the indole nucleus [*cf.* E. E. van Tamelen, L. J. Dolly and R. G. Lawton, *Tetrahedron Letters*, No. **19**, 30 (1960)] or a similar condensation at the indolic $\alpha$-carbon with subsequent rearrangement [*cf.* J Harley-Mason and W. R. Waterfield, *Chemistry & Industry*, 1477 (1960)]. Unfortunately it is not possible to distinguish between the three routes at this time.

(26) (a) While this fragmentation is formulated to occur with rupture of the 15,16-bond, it alternatively could take place with 14,15-bond scission and extrusion of pyruvate. Its course undoubtedly depends largely on the position of unmasked carbonyl groups in the tryptamine–SPF complex. (b) The 19,20-enol, which must initiate the *retro*-Michael reaction, has been suggested to be responsible for the formation of the biosynthetically novel bond between C-6 (XVIII) and C-20 (VIII) in the structurally unusual alkaloid gelsemine [E. Wenkert and J. H. Hansen, *Iowa State J. Sci.*, **34**, 163 (1959)]. (c) The monoterpenoid analogy of the SPF fragmentation is represented by the change of i into formyl acetate (XXIX) and iii:




(27) All data from labeling experiments on gramine biosynthesis are consistent with this view; *cf.* A. Breccia and L. Marion, *Can. J. Chem.*, **37**, 1066 (1959); D. W. Henry and E. Leete, Abstracts of Papers of the 134th Meeting of the American Chemical Society, Chicago, Ill., Sept. 7–12, 1958, p. 47C.

(VI) compounds, to undergo a *retro*-Michael process, the formyl acetate (XXIX) unit would be lost irretrievably to the plant cell medium and a pathway to the flavopereirine (XXXIV) structure un-




covered.[28,29] However, if the cleavage reaction occurs on a system whose potential formyl acetate moiety cannot be lost because of its attachment to the tryptamine part of the tryptamine–SPF complex, *e.g.* XXVI, and the cleavage product XXXV undergoes ordinary oxidation–reduction changes, piperideines of various states of oxidation, *e.g.*, XXXVI and XXXVII, are formed whose intramolecular Michael and Mannich reactions lead to the aspidospermine(XXI)-like skeleton XXXIX and the coronaridine(XXIIb)-like nucleus XL, respectively.[29] It is noteworthy that the necessary formulation of biointermediate XXXIX predicts the likely future discovery of as yet unknown carboxylated Aspidosperma bases. Furthermore, it appears now more than coincidence that both parts of the enone chromophore of XL have been observed in Iboga alkaloids, the double bond in catharanthine[30] and the oxygen substituent in iboxygaine,[31] voacristine[32] and voacryptine.[32]

Several recent stereochemical findings especially in the field of the Aspidosperma alkaloids can be interpreted on the basis of the above biosynthetic picture. The aspidospermine(XXI)-like substances O-methylaspidocarpine[33] and pyridifoline[34] have been shown to be optical antipodes.[34] Que-

(28) *Cf.* A. R. Battersby and H. F. Hodson, *Quart. Revs.*, **14**, 77 (1960).

(29) This biosynthetic route now supersedes one previously proposed.[2b]

(30) N. Neuss and M. Gorman, *Tetrahedron Letters*, **No. 6**, 206 (1961).

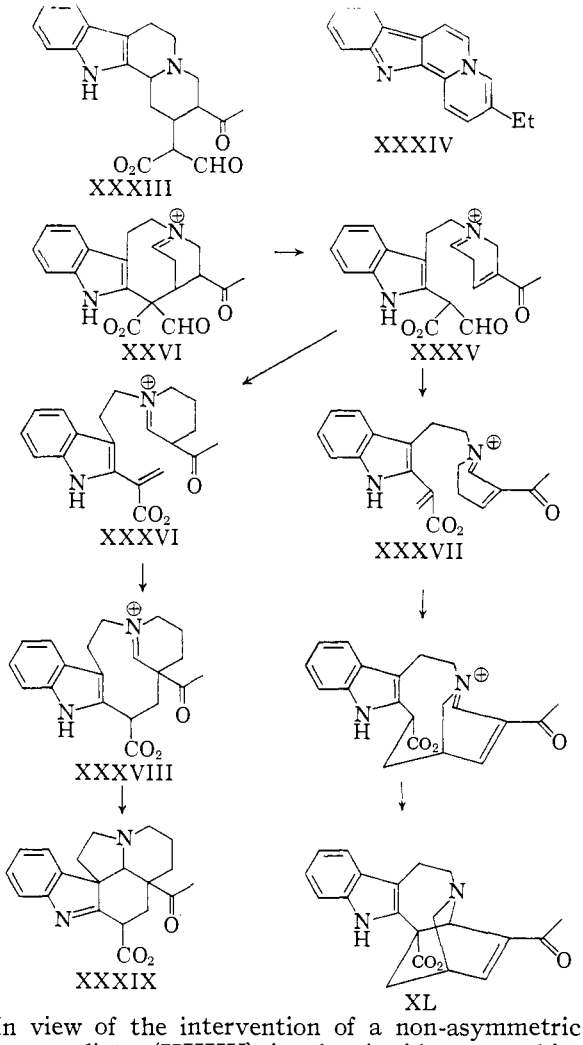
(31) R. Goutarel, F. Percheron and M.-M. Janot, *Compt. rend.*, **246**, 279 (1958).

(32) U. Renner and D. A. Prins, *Experientia*, **15**, 456 (1959); **17**, 106 (1961).

(33) S. McLean, K. Palmer and L. Marion, *Can. J. Chem.*, **38**, 1547 (1960).

(34) C. Djerassi, B. Gilbert, J. N. Shoolery, L. F. Johnson and K. Biemann, *Experientia*, **17**, 162 (1961).

brachamine, whose most likely structure is XLI[35] and whose biosynthetic development corresponds to that of intermediate XXXVIII in a lower oxidation state, has been found in both *d*- and *l*-forms.[35,36]




In view of the intervention of a non-asymmetric intermediate (XXXV) in the Aspidosperma biosynthesis, no optical relationship can exist between the alkaloids of this family and other indole bases. The appearance of some randomization of the absolute configuration of the Aspidosperma alkaloids therefore must be attributed to a lack of optical consistency in the enzymically induced Michael reaction, XXXVI → XXXVIII. Similarly, the discovery of a *d,l*-Strychnos alkaloid, $\psi$-akuammicine,[7] can be explained on the basis of the establishment of a XXVI–XXXV equilibrium prior to the complete evolution of the former (XXVI) to the Strychnos skeleton (*cf.* arrows in XXVI). Finally, the fact that all Aspidosperma bases whose relative stereochemistry has been ascertained possess the configuration depicted in XXI[33,34,37,38] is

(35) H. Kny and B. Witkop, *J. Org. Chem.*, **25**, 635 (1960).

(36) F. Walls, O. Collera and A. Sandoval, *Tetrahedron*, **2**, 173 (1958).

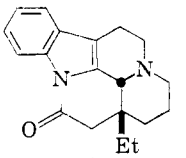
(37) (a) J. F. D. Mills and S. C. Nyburg, *Tetrahedron Letters*, **No. 11**, 1 (1959); *J. Chem. Soc.*, 1458 (1960); (b) H. Conroy, P. R. Brook and Y. Amiel, *Tetrahedron Letters*, **No. 11**, 4 (1959); (c) G. F. Smith and J. T. Wrobel, *J. Chem. Soc.*, 1463 (1960).

(38) C. Djerassi, A. A. P. G. Archer, T. George, B. Gilbert, J. N. Shoolery and L. F. Johnson, *Experientia*, **16**, 532 (1960).

in agreement with their genesis through the pathway XXXVI → XXXIX. *The Mannich-type closure of the rigid nine-membered ring in XXXVIII requires formation of a cis-perhydroisoquinoline system in XXXIX.*[39] Furthermore, the *cis-anti-cis* backbone exhibited by XXI may be the consequence of the Mannich condensation and the subsequent reduction following the path of least steric resistance.

As the above discussion indicates, the prephenic acid hypothesis (and/or the monoterpenoid hypothesis) is able to account for the structure patterns of all indole alkaloids without exception. It will be of interest to watch experimental developments in this field of alkaloid biosynthesis.

(39) In this connection it is of interest that eburnamonine (iv) [M. F. Bartlett and W. I. Taylor, *J. Am. Chem. Soc.*, **82**, 5941 (1960)], a Hunteria alkaloid of aspidospermine-like features, possesses the same stereochemistry (unpublished observations of Dr. B. Wickberg presented by the author at the 17th National Organic Chemistry Symposium, Bloomington, Ind., June 25–29, 1961).



iv

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[CONTRIBUTION FROM THE RESEARCH LABORATORIES OF SYNTEX, S.A., MEXICO, D.F.]

# Sapogenins. XLI.[1] A New Reaction of the Spiroketal Side Chain

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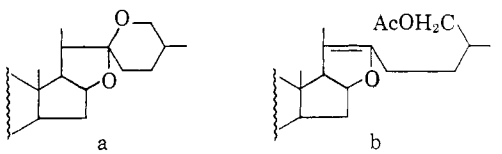
Treatment of sapogenins with acetic anhydride in the presence of boron trifluoride etherate leads to products for which 23-acetyl-$\Delta^{22}$-furostene type structures are proposed.

The Marker procedure for conversion of sapogenins to 20-keto pregnane derivatives[3] involves as the first step treatment with acetic anhydride at 200°. Under these conditions sapogenins (a) are converted to furostene derivatives (b) and in general the yields are good. Even so, numerous attempts have been made to develop reaction




conditions which would avoid the use of special autoclaves or sealed tube systems required in order to reach the 200° temperature.

The use of octanoic anhydride[4] and pyridine hydrochloride in acetic anhydride[5] have been investigated and found to be satisfactory variants of the original procedure. A study employing Lewis acids in boiling acetic anhydride has also been described,[6] although in these cases the yields of furostene were low.

Recently we noted[7] that either hecogenin ketol diacetate or 11$\beta$,12$\beta$-dihydroxytigogenin 11$\beta$,12$\beta$-acetonide acetate undergo normal furostene formation at 200° during periods ranging from 45 to 70 minutes. This stands in contrast to most procedures which employ times of from five to twenty-four hours. Additional examples of compounds capable of undergoing this rapid reaction are 11a-aza-C-homotigogenin,[8] rockogenin and tigogenin.[8]

On the basis of these observations we were led to wonder whether these reactions might not also proceed at less drastic temperatures. After ascertaining that acetic anhydride at its boiling point was without effect, even over prolonged periods of time, the use of boron trifluoride etherate was investigated.

This procedure involved treating the sapogenin in acetic anhydride at room temperature with a weight of boron trifluoride etherate equal to the steroid. Under these conditions a slightly exothermic reaction set in which rapid darkening of the mixture. If prolonged periods of time were employed, only intractable gums could be recovered. On the other hand, after ten- to twenty-minute reaction periods it was possible to isolate a crystalline product in 10 to 45% yield, either by direct crystallization or chromatography. This reaction was found to be applicable to hecogenin ketol diacetate, diosgenin, 3-desoxytigogenin and tigogenin. For the purpose of characterizing the reaction products all further work was carried out on tigogenin (I).

The substance showed unusual spectral properties. The infrared was characterized in the carbonyl region by a strong band at 5.78 $\mu$, a medium in-

(1) Paper XL, A. Bowers, E. Denot, M. B. Sánchez, F. Neumann and C. Djerassi, *J. Chem. Soc.*, 1859 (1961).

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(3) For a general description of method see L. F. Fieser and M. Fieser "Steroids," Rheinhold Publishing Corp., New York, N. Y., 1959, pp. 549–550.

(4) A. F. B. Cameron, R. M. Evans, J. C. Hamlet, J. S. Hunt, P. G. Jones and A. G. Long, *J. Chem. Soc.*, 2807 (1955).

(5) W. G. Dauben and G. J. Fonken, *J. Am. Chem. Soc.*, **76**, 4618 (1954).

(6) D. H. Gould, H. Staeudle and E. B. Hershberg, *ibid.*, **74**, 3685 (1952).

(7) J. A. Zderic, H. Carpio and C. Djerassi, *ibid.*, **82**, 446 (1960).

(8) J. A. Zderic, H. Carpio, D. Chávez Limón and A. Ruiz, *J. Org. Chem.*, **26**, 2842 (1961).