Biosynthesis of Quinine and Related Alkaloids

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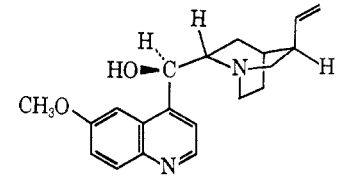
The antimalarial drug quinine (**1**) has had a fascinating history.[1] In 1820 it was isolated in a crystalline state from the bark of a Cinchona tree by Pelletier and Caventou.[2] In 1856 Perkin, having little information on the alkaloid except its molecular formula, attempted to obtain it by oxidizing allyltoluidine with potassium dichromate according to

$$\underset{\text{allyltoluidine}}{2C_{10}H_{13}N} + 3O \rightarrow \underset{\text{quinine}}{C_{20}H_{24}N_2O_2} + H_2O$$

Needless to say, this attempt was unsuccessful and yielded a dirty reddish brown precipitate. However, the results of this experiment led Perkin to investigate the oxidation of simpler amines, and from the oxidation of crude aniline (which contained some toluidine) he obtained the first synthetic dye, mauve (**2**), which was used for dyeing silk and coloring postage stamps in the 1860's.[3]



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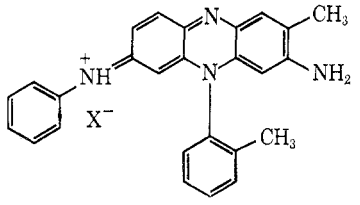


2

The gross structure of quinine was elucidated (by Rabe) in 1909[4] and its stereochemistry, as illustrated in formula **1**, in 1944.[5] It was finally synthesized by Woodward and Doering in 1944.[6]

The major commercial source of quinine is the bark of the tree *Cinchona ledgeriana*, where it occurs to the extent of 13% of the dry weight. Unlike some alkaloids quinine is apparently a metabolic end product and is deposited in the outer layer of bark where the cells are dead or dying. Commercial trees are at least 20 years old at the time of harvesting for quinine extraction.

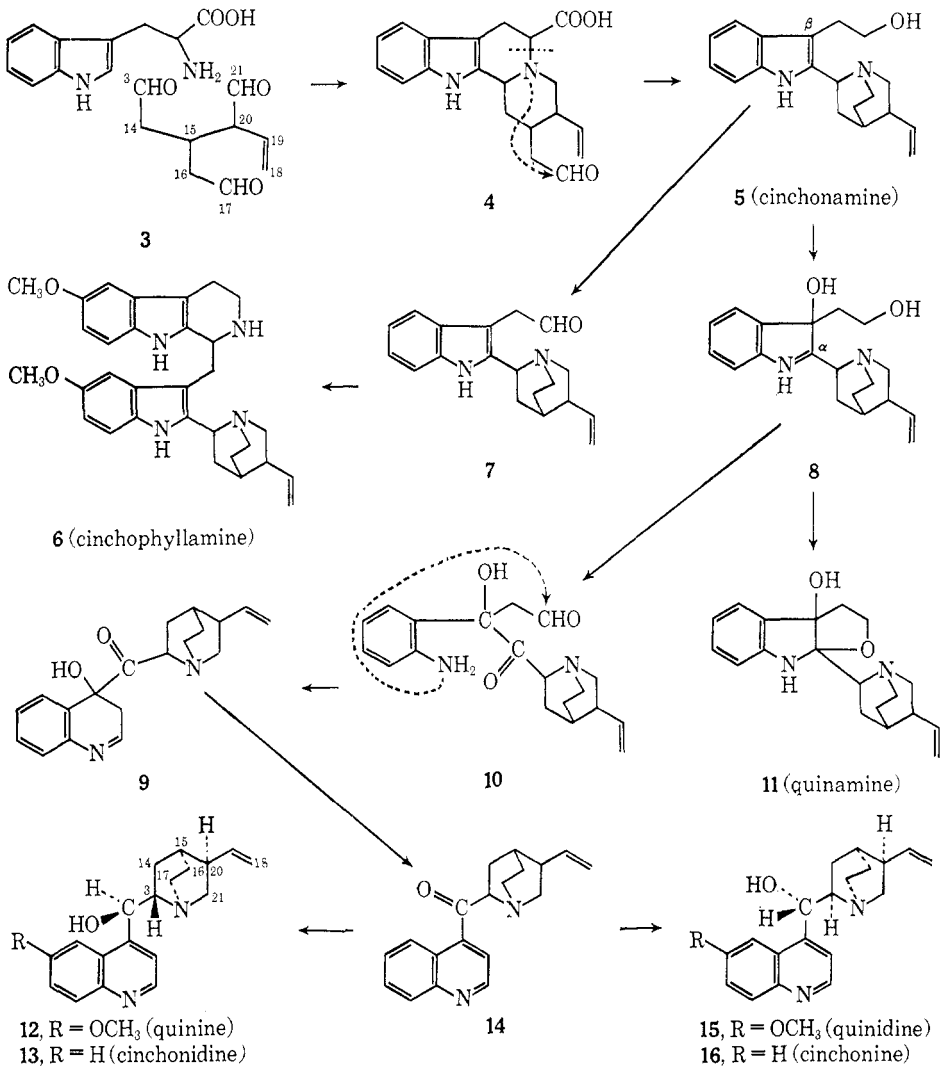
Fortunately from our point of view, quinine and other alkaloids are biosynthesized in young seedlings. This was evident from the work of de Moerloose,[7] who cultivated 1-year-old *Cinchona succirubra* plants in an atmosphere containing radioactive carbon dioxide and obtained radioactive quinine from the plants. The biogenetic relationship of the indole alkaloids cinchonamine and quinamine was suggested by Goutarel, *et al.*,[8] and a plausible biogenetic scheme based on this view, upon Turner and Woodward's[9] ideas, and on tracer work to be described is illustrated in Scheme I.

It is suggested that tryptophan (or possibly tryptamine[10]) condenses with the nine-carbon trialdehyde **3** (the origin of this unit will be discussed later) to afford the tetrahydro-β-carboline derivative **4**. Hypothetical cleavage of the β-carboline ring and formation of the quinuclidine ring as illustrated, followed by unexceptional reductions and decarboxylation, affords cinchonamine, which is a minor alkaloid in Cinchona bark. However it is more abundant in young Cinchona plants.[11] This observation is consistent with its being a precursor of the Cinchona alkaloids which contain a quinoline ring. Recently a dimeric indole alkaloid has been isolated from the leaves of *C. ledgeriana*[12] and named cinchophyllamine (**6**). It seems probable that this alkaloid is formed by a Mannich reaction between the aldehyde **7**, which may be formed by the oxidation of cinchonamine, and tryptamine.

The stage at which the methoxy groups in cinchonophyllamine, and alkaloids such as quinine and quinidine, are introduced is currently unknown. However, hydroxylation and methylation are apparently terminal

(1) *Cf.* M. G. Kreig, "Green Medicine," Rand McNally Co., New York, N. Y., 1964.
(2) M. Pelletier and E. Caventou, *Ann. Chim. Phys.*, [2] **15**, 291, 1337 (1820).
(3) W. H. Perkin, *J. Chem. Soc.*, **14**, 230 (1862); English Patent 1984 (1856).
(4) P. Rabe, *Ann.*, **365**, 353 (1909).
(5) V. Prelog and E. Zalan, *Helv. Chim. Acta*, **27**, 535 (1944).
(6) R. B. Woodward and W. von E. Doering, *J. Am. Chem. Soc.*, **66**, 849 (1944); **67**, 860 (1945).
(7) P. de Moerloose and R. Ruyssen, *J. Pharm. Belg.*, **8**, 156 (1953); *Pharm. Tijdschr. Belg.*, **30**, 97 (1953); P. de Moerloose, *Pharm. Weekblad*, **89**, 541 (1954).
(8) R. Goutarel, M. M. Janot, V. Prelog, and W. I. Taylor, *Helv. Chim. Acta*, **33**, 150 (1950).
(9) R. B. Turner and R. B. Woodward, *Alkaloids*, **3**, 54 (1953).
(10) In some cases tryptophan and tryptamine serve equally well as precursors of the indole alkaloids; however, with some alkaloids the carboxyl group of tryptophan is apparently required for initial condensations and is lost at a later stage in the biosynthesis of the alkaloid. For example, tryptophan, but not tryptamine, is a precursor of the Ergot alkaloids [(a) R. M. Baxter, S. I. Kandel, and A. Okany, *Chem. Ind.* (London), 1453 (1961)]. Tryptamine and tryptophan were equally efficient as precursors of the *Vinca rosea* alkaloids, ajmalicine and tetrahydroalstonine; however, the incorporation of tryptamine into catharanthine and vindoline, produced by the same plant, was much less than that of tryptophan [(b) E. Leete and R. M. Bowman, unpublished work; (c) J. P. Kutney, W. J. Cretney, J. R. Hadfield, E. S. Hall, V. R. Nelson, and D. C. Wigfield, *J. Am. Chem. Soc.*, **90**, 3567 (1968)].
(11) E. Leete, unpublished observation.
(12) P. Potier, C. Kan, J. LeMan, M. M. Janot, H. Budzikiewicz, and C. Djerassi, *Bull. Soc. Chim. France*, 2309 (1966).

Scheme I: Tentative Biosynthetic Scheme for the Cinchona Alkaloids



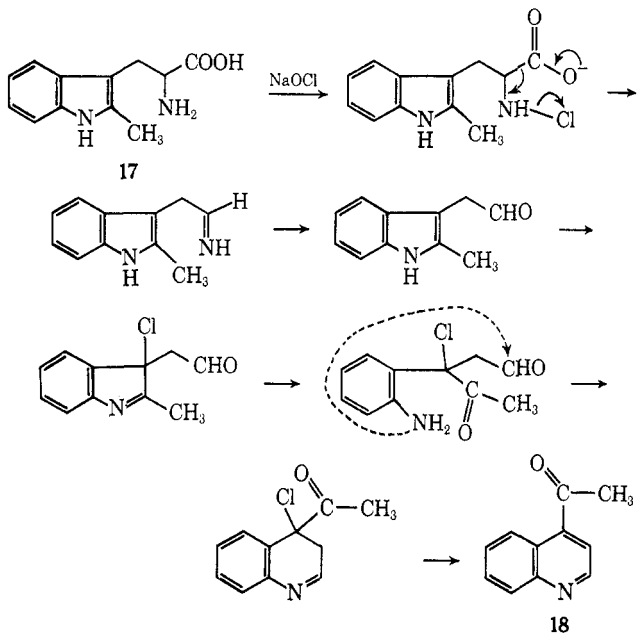
steps in the formation of the indole alkaloids vindoline and resperpine.[13,14]

Quinamine (**11**) is another minor alkaloid in Cinchona plants, and its formation can be rationalized as follows. Electrophilic attack by OH^+ at the β position of the indole nucleus of cinchonamine would afford the 3-hydroxyindolenine derivative **8**. Cyclization of the primary alcohol group at the α position yields quinamine. Witkop[15] has actually achieved the conversion of cinchonamine to quinamine *in vitro* by oxidation with peracetic acid, the hydroxyindolenine **8** being a probable intermediate. The indolenine **8** is also considered to be the source of the Cinchona alkaloids, which contain a quinoline nucleus. Hydrolysis of the C=N bond and oxidation of the primary alcohol group could possibly afford the intermediate **10**. Cyclization of the primary amino group with the aldehyde group would yield compound **9**, the quinoline nucleus in **14** then being formed by dehydration. Reduction of the ketone affords cinchonidine (**13**) and cinchonine (**16**) which are epimeric at this newly formed asymmetric carbon and also at C-3. Quinine and quinidine are the corresponding methoxylated alkaloids. van Tamelen and Haarstad[16] have obtained 4-acetylquinoline (**18**) from 2-methyltryptophan (**17**) in 20% yield by treatment with sodium hypochlorite. The mechanism of this remarkable transformation is considered to be analogous to the formation of quinine and is illustrated in Scheme II.

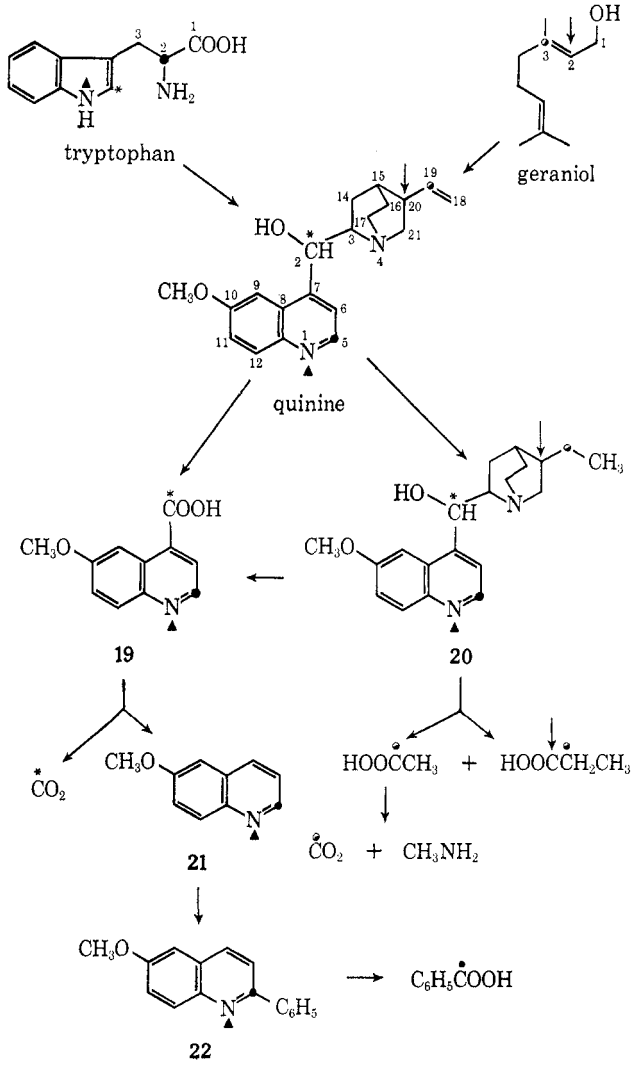
The first evidence in favor of this biosynthetic scheme for quinine was obtained by feeding DL-tryptophan-2-^{14}C to *C. succirubra* plants by means of cotton wicks inserted in the stems of the plants.[17] The plants were allowed to metabolize the radioactive tryptophan for 6 weeks, and were then harvested. Extraction of the plants yielded radioactive cinchonamine and quinine. The quinine was degraded according to Scheme III. The quinine is numbered in an unconventional manner in order to illustrate its biogenetic relationship to indole alkaloids of the Corynanthe type,

(13) E. Leete, *J. Am. Chem. Soc.*, **82**, 6338 (1960).
(14) A. A. Qureshi and A. I. Scott, *Chem. Commun.*, 948 (1968).
(15) B. Witkop, *J. Am. Chem. Soc.*, **72**, 2311 (1950).
(16) E. E. van Tamelen and V. B. Haarstad, *Tetrahedron Letters*, 390 (1961).
(17) N. Kowanko and E. Leete, *J. Am. Chem. Soc.*, **84**, 4919 (1962).

Scheme II: Possible Mechanism of the Formation of 4-Acetylquinoline from 2-Methyltryptophan



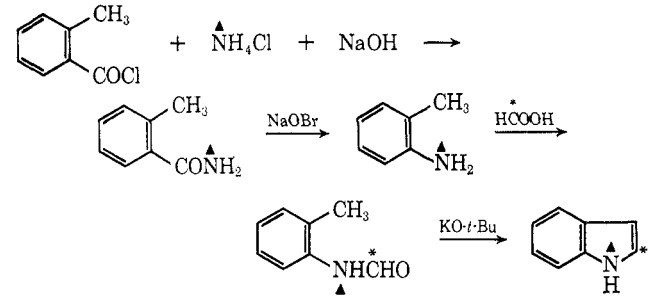
Scheme III: Degradation of the Quinine Derived from Labeled Tryptophan and Geraniol



e.g., corynantheine. Oxidation of quinine yielded quininic acid (**19**), which was decarboxylated by heating with copper chromite. The resultant 6-methoxyquinoline (**21**) was allowed to react with phenyllithium in boiling toluene, yielding 6-methoxy-2-phenylquinoline (**22**), the position of phenylation being established by an independent synthesis of authentic material.[18] Oxidation of the methiodide of this compound with potassium permanganate yielded benzoic acid which had essentially the same specific activity as the quinine, indicating that all the radioactivity of the alkaloid was located at C-5.

We have now carried out a feeding experiment with tryptophan labeled with ^{15}N on the indole nitrogen and with ^{14}C at the α carbon of the indole nucleus.[19] The enriched nitrogen and the ^{14}C were introduced into the indole nucleus by the sequence of reactions illustrated in Scheme IV. *o*-Toluyl chloride was allowed to react

Scheme IV: Synthesis of Indole-1-^{15}N,2-^{14}C



with ammonium chloride containing 89% excess ^{15}N in the presence of sodium hydroxide to yield *o*-toluamide. A Hofmann reaction on this compound afforded *o*-toluidine which was formylated with formic-^{14}C acid. The resultant formyl-*o*-toluidine was converted to indole by treatment with potassium *t*-butoxide.[20] This doubly labeled indole was then converted to tryptophan by established methods.[21]

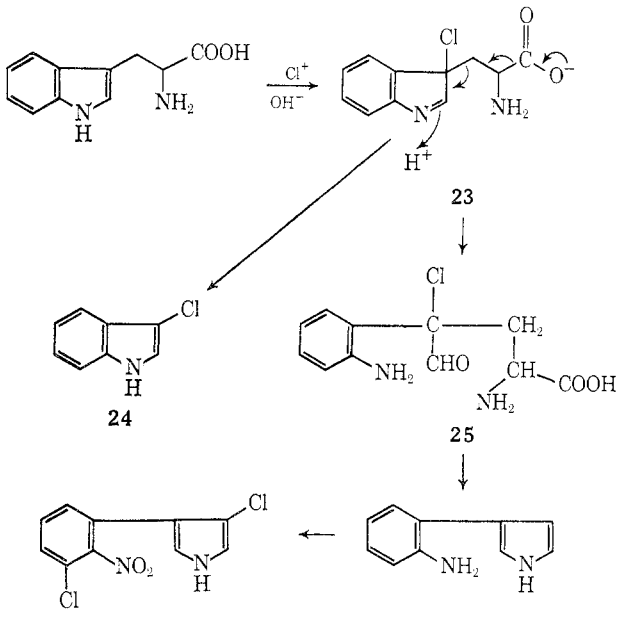
The quinine derived from this doubly labeled tryptophan was degraded as before. The ^{15}N was determined using a modification of the method of Günther, *et al.*[22] The nitrogen gas, obtained by heating the alkaloid or its degradation product (less than 0.5 mg required) in an evacuated sealed tube with calcium oxide and cupric oxide, was analyzed in a mass spectrometer. Only the nitrogen in the quinoline nucleus was enriched with ^{15}N, and all the ^{14}C was located at C-2. Furthermore, the specific incorporation of the ^{15}N and ^{14}C into these positions was identical (0.97%). These results thus provide convincing evidence in favor of the biosynthetic scheme illustrated in Scheme I.

(18) O. Döbner, *Ann.*, **249**, 98 (1888).
(19) E. Leete and J. N. Wemple, *J. Am. Chem. Soc.*, in press.
(20) F. T. Tyson, "Organic Syntheses," Coll. Vol. III, John Wiley & Sons, Inc., New York, N. Y., 1955, p 480.
(21) H. R. Snyder and F. J. Pilgrim, *J. Am. Chem. Soc.*, **70**, 3787 (1948).
(22) H. Günther, H. G. Floss, and H. Simon, *Z. Anal. Chem.*, **218**, 401 (1966).

Pyrrolnitrin (**26**), an antifungal compound produced by a *Pseudomonas* culture, is apparently produced from tryptophan by a similar cleavage of the indole nucleus.[23] Under certain conditions of fermentation 3-chloroindole (**24**) is formed, and it was suggested by Gorman and Lively that the metabolism of tryptophan is initiated by a chloro peroxidase enzyme as illustrated in Scheme V, the first product formed being the 3-

Scheme V

Hypothetical Scheme for the Biosynthesis of Pyrrolnitrin from Tryptophan



chloroindolenine derivative **23**. A fragmentation reaction affords 3-chloroindole, while opening the indolenine ring yields **25**. Formation of the pyrrole ring, oxidation of the aromatic amino group to a nitro group, and chlorination afford pyrrolnitrin.

The origin of the nine-carbon aldehyde **3** became fairly obvious from related work on the biosynthesis of the indole alkaloids of *Vinca rosea*, which had been carried out by Scott, Arigoni, Battersby, and ourselves. Most of the indole alkaloids contain a nine- or ten-carbon unit, in addition to the tryptophan-derived portion. There has been much speculation on the origin of this unit.[24] 3,4-Dihydroxyphenylalanine, prephenic acid, acetic acid, and mevalonic acid have all been considered as precursors of this unit. In 1961, Wenkert[25] and Thomas[26] independently suggested that this nontryptophan-derived unit is formed from a cyclopentanomonoterpene (**27**) (Scheme VI) which could be formed from geraniol or its isomer nerol. The Corynanthe unit (so called because it occurs in the

(23) M. Gorman and D. H. Lively in "Antibiotics," Vol. II, D. Gottlieb and P. D. Shaw, Ed., Springer-Verlag, Berlin, 1967, p 433.

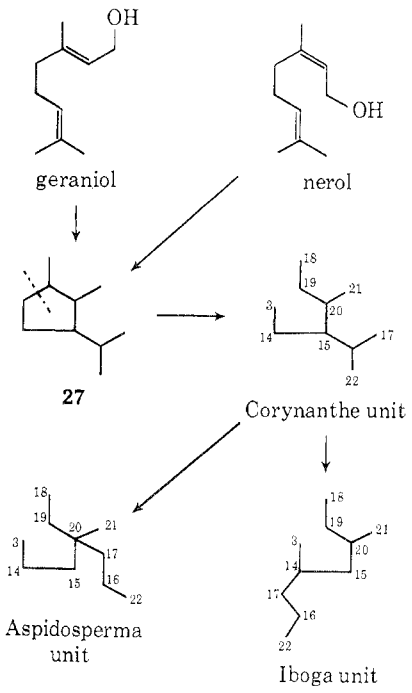
(24) For an historical account of this speculation see E. Leete in "Biogenesis of Natural Compounds," P. Bernfeld, Ed., 2nd ed, Pergamon Press, Oxford, England, 1967, Chapter 17.

(25) E. Wenkert, *J. Am. Chem. Soc.*, **84**, 98 (1962) [manuscript received Jan 28, 1961].

(26) A. F. Thomas, *Tetrahedron Letters*, 544 (1961) [manuscript received Aug 18, 1961].

Scheme VI

Schematic Representation of the "Monoterpene Hypothesis"



alkaloid corynantheine) is produced by cleavage of the cyclopentane ring at the position indicated with a dotted line. The numbering of this unit corresponds to the numbering of corynantheine to indicate the ultimate origin of each carbon atom. Wenkert[27] also put forward ingenious mechanisms for the rearrangement of the Corynanthe unit to the Aspidosperma and Iboga units which arise by migration of the isopropyl side chain at C-15. Examples of alkaloids which contain these various units are illustrated in Scheme VII.

C-22 has been lost in the formation of some alkaloids, for example, in cephaeline and ibogaine. Initial experiments which favored the monoterpene hypothesis were carried out with labeled mevalonic acid,[28-33] the precursor of terpenes. The expected pattern of labeling was found in the indole alkaloids of *Vinca rosea* and other species.

The next obvious step was to test an actual monoterpene as the precursor of the ubiquitous nine- or ten-carbon unit, and four research groups[34-37] independently prepared labeled geraniol and administered it to *Vinca*

(27) E. Wenkert and B. Wickberg, *J. Am. Chem. Soc.*, **87**, 1580, 5810 (1965).

(28) T. Money, I. G. Wright, F. McCapra, and A. I. Scott, *Proc. Natl. Acad. Sci. U. S.*, **53**, 901 (1965).

(29) F. McCapra, T. Money, A. I. Scott, and I. G. Wright, *Chem. Commun.*, 537 (1967).

(30) T. Money, I. G. Wright, F. McCapra, A. I. Scott, and E. S. Hall, *J. Am. Chem. Soc.*, **90**, 4144 (1968).

(31) H. Goeggel and D. Arigoni, *Chem. Commun.*, 538 (1965).

(32) A. R. Battersby, R. T. Brown, R. S. Kapil, A. O. Plunkett, and J. B. Taylor, *ibid.*, 46 (1966).

(33) A. R. Battersby, R. T. Brown, R. S. Kapil, J. A. Knight, J. A. Martin, and A. O. Plunkett, *ibid.*, 888 (1966).

(34) P. Loew, H. Goeggel, and D. Arigoni, *ibid.*, 347 (1966).

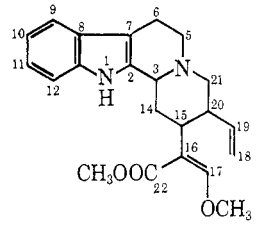
(35) A. R. Battersby, R. T. Brown, J. A. Knight, J. A. Martin, and A. O. Plunkett, *ibid.*, 346 (1966).

(36) E. S. Hall, F. McCapra, T. Money, K. Fukumoto, T. R. Hanson, B. S. Mootoo, G. T. Philips, and A. I. Scott, *ibid.*, 348 (1966).

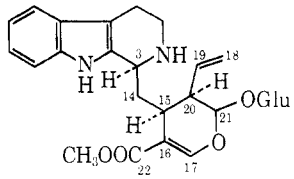
(37) E. Leete and S. Ueda, *Tetrahedron Letters*, 4915 (1966).

Scheme VII

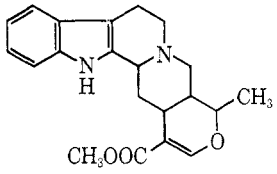
Alkaloids with the Corynanthe Unit



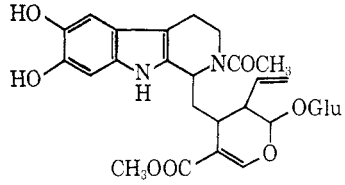
corynantheine



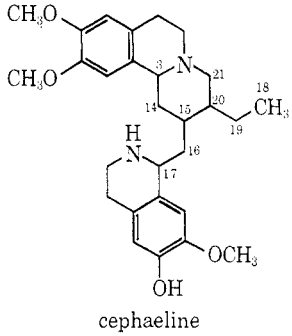
vincoside



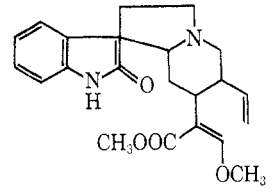
ajmalicine



ipecoside

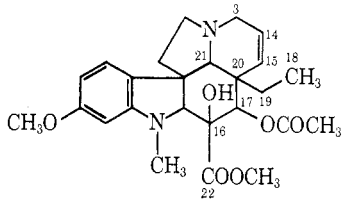


cephaeline

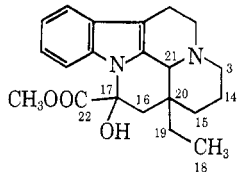


corynoxeine

Alkaloids with the Aspidosperma Unit

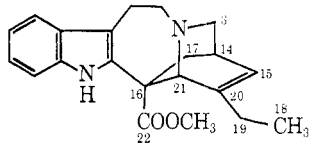


vindoline

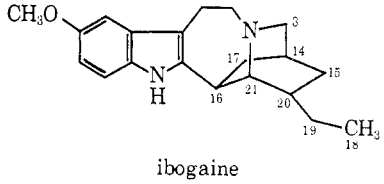


vincamine

Alkaloids with the Iboga Unit



catharanthine



ibogaine

rosea plants. Specific labeling was found in vindoline, ajmalicine, and catharanthine, in agreement with the general hypothesis depicted in Scheme VI. There was no significant difference in the incorporation of geraniol and its geometric isomer nerol.[33,38] Battersby administered geraniol, in preliminary work, as its pyrophosphate; however, geraniol emulsified in water with Tween 80 (polyoxyethylenesorbitan monooleate[39]) was efficiently incorporated into the indole alkaloids. Battersby also showed that geraniol is an efficient precursor of the nine-carbon Corynanthe unit in the isoquinoline alkaloid cephaeline.[40] Independently,[41] Battersby[33] and we[42] fed radioactive geraniol to Cinchona plants. Battersby fed geraniol-2-^{14}C to *C. ledgeriana* plants and obtained radioactive quinine (0.001% incorporation). A Kuhn–Roth oxidation on the derived dihydroquinine **20** (Scheme III) afforded radioactive propionic acid (98% of the total activity of the alkaloid) and inactive acetic acid, indicating that all the activity was located at C-20. We fed geraniol-3-^{14}C to *C. succirubra* plants and obtained radioactive quinine (0.001% incorporation), and a similar degradation indicated that all the activity was located at C-19.

So far, we have no information on the steps between geraniol and the ultimate Corynanthe unit found in the Cinchona alkaloids. However, in other species it is now clear that the naturally occurring cyclopentanomonoterpene loganin (Scheme VIII) is an important intermediate. In 1966 Battersby[43] reported that this compound, labeled with tritium on its O-methyl group, was significantly incorporated into the following *Vinca rosea* alkaloids: catharanthine, vindoline, serpentine, ajmalicine, and perivine. Degradations indicated that all the activity was located on the C-22 carbomethoxy group of these alkaloids. Loganin labeled internally with ^{14}C was obtained biosynthetically by feeding a 3:1 mixture of geraniol-2-^{14}C and nerol-2-^{14}C to *Menyanthes trifoliata* plants. The administration of this labeled loganin to *Vinca rosea* plants also resulted in specific labeling of the indole alkaloids at the expected positions.[44] Arigoni[45] obtained complementary results using loganin labeled specifically at C-8. The presence of loganin in *Vinca rosea* plants was established by radiochemical dilution,[43] and it will be of great interest to learn whether it is of widespread occurrence in the many species which produce indole alkaloids.

A possible route from geraniol (or nerol) to loganin is illustrated in Scheme VIII. Battersby[46] suggested a route *via* citronellal and iridodial, a known natural product, and a potential precursor of piperidine alkaloids such as skytanthine.[47] However, when we fed

(38) A. R. Battersby, J. C. Byrne, R. S. Kapil, J. A. Martin, T. G. Payne, D. Arigoni, and P. Loew, *Chem. Comm.*, 951 (1968).

(39) *Cf.* J. F. Parr and A. G. Norman, *Botan. Gaz.*, **126**, 86 (1965), for a discussion of the use of surfactants in plant systems.

(40) A. R. Battersby and B. Gregory, *Chem. Commun.*, 134 (1968).

(41) The author and Professor Battersby had a brief conversation at Stockholm airport in June 1966, during which time we each told the other that we had fed radioactive geraniol to Cinchona plants. Needless to say this information caused my graduate student, Jim Wemple, to work even harder to complete this piece of research.

(42) E. Leete and J. N. Wemple, *J. Am. Chem. Soc.*, **88**, 4743 (1966).

(43) A. R. Battersby, R. T. Brown, R. S. Kapil, J. A. Martin, and A. O. Plunkett, *Chem. Commun.*, 890 (1966).

(44) A. R. Battersby, R. S. Kapil, J. A. Martin, and L. Mo, *ibid.*, 133 (1968).

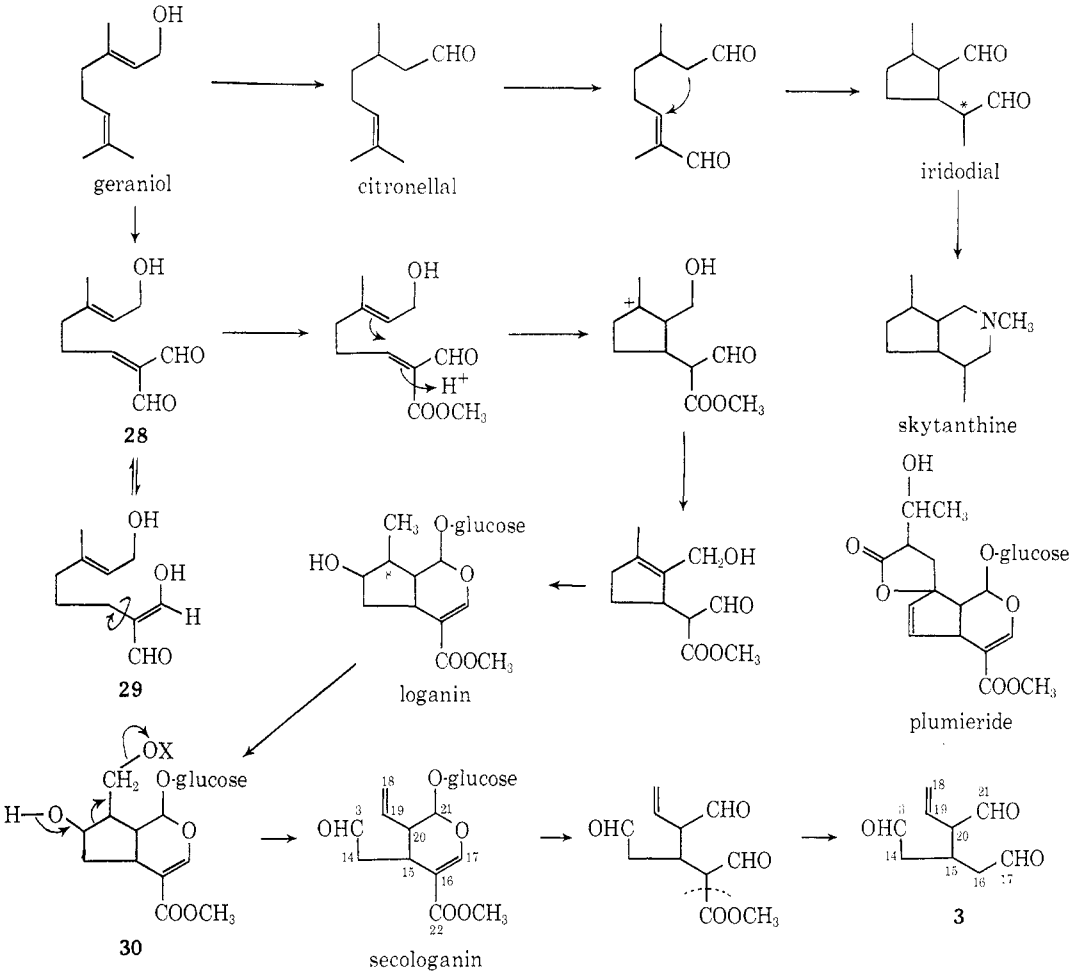
(45) P. Loew and D. Arigoni, *ibid.*, 137 (1968).

(46) A. R. Battersby, *Pure Appl. Chem.*, **14**, 117 (1967).

(47) H. Auda, H. R. Juneja, E. J. Eisenbraun, G. R. Waller, W. R. Kays, and H. H. Appel, *J. Am. Chem. Soc.*, **89**, 2476 (1967).

Scheme VIII

Hypothetical Biosynthetic Route from Geraniol to the Corynanthe Unit



iridodial, labeled at the position indicated with an asterisk, to *Vinca rosea* plants, the incorporation of activity into the alkaloids was insignificant.[48] We thus favor a route to loganin *via* the dialdehyde **28**. Enolization of this dialdehyde to **29** would make the two aldehyde carbons radiochemically equivalent. This suggestion was also made by Schmid[49] to rationalize his results on the biosynthesis of the cyclopentanomonoterpene plumieride (Scheme VIII). The pattern of labeling found in plumieride and the ubiquitous ten-carbon unit present in the indole alkaloids after feeding mevalonic acid-2-^{14}C requires that these two positions (C-17 and C-22) do become equivalent at some stage in the biosynthetic sequence.[30-33] Beyond the dialdehyde **28** the metabolic steps are quite plausible and unexceptional. It was suggested[46] that the cyclopentane ring of loganin could be cleaved at the required position by initial hydroxylation of the methyl group at C-8. The resultant hydroxyloganin (**30**, X = H) could be phosphorylated (X = PO_3H_2) and a fragmentation occur, as illustrated, to yield secologanin. A secologanin residue is present in ipecoside[40] and vincoside[50] (Scheme VII). Minor modifications of the functional groups in secologanin could afford the ten-carbon unit present in other alkaloids containing a Corynanthe unit. Loss of the glucose residue and the carboxymethyl group affords the nine-carbon aldehyde **3** which was utilized in our original scheme for the biosynthesis of quinine (Scheme I). It now seems clear that rearrangement of the Corynanthe unit to the Aspidosperma and Iboga types occurs after reaction of the former unit with tryptophan.[14,38,51]

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(48) E. Leete and R. M. Bowman, *Phytochemistry*, in press.
(49) D. A. Yeowell and H. Schmid, *Experientia*, **20**, 250 (1964).
(50) G. N. Smith (*Chem. Commun.*, 912 (1968)) isolated from *Vinca rosea* plants an alkaloid which he called strictosidine, having the structure of vincoside but with undetermined stereochemistry. More recently, Battersby and coworkers (A. R. Battersby, A. R. Burnett, and P. G. Parsons, *ibid.*, 1282 (1968)) have isolated from the same species vincoside and isovincoside (epimeric at C-3).
(51) J. P. Kutney, C. Ehret, V. R. Nelson, and D. C. Wigfield, *J. Am. Chem. Soc.*, **90**, 5929 (1968).