and heated 2 hr. at 160–180° with 175 g. of polyphosphoric acid.[23] Upon decomposition of the acid and isolation of the product as previously described,[24] except that retreatment of the acid-insoluble neutral component with more polyphosphoric acid was omitted, there was obtained 26.0 g. (79% over-all) of crude 3,4-dihydroisoquinoline which distilled at 64–67° (0.60 mm.), $n^{25}D$ 1.5793. A 31% yield of picrate was previously obtained with the polyphosphoric acid treatment at a somewhat lower temperature.[24]

The 26.0 g. of 3,4-dihydroisoquinoline was heated under reflux with 5.2 g. of 5% palladium–charcoal for 12 hr. The catalyst was filtered off, washed with ether, the ether volatilized from the filtrate and the residue distilled. A 72% yield (57% over-all, 18.35 g.) of crude isoquinoline was collected at 62–67° (0.85 mm.), $n^{25}D$ 1.6174. A similar yield was reported previously using equal weights of 3,4-dihydroisoquinoline and palladium dust.[25] Purification of the 18.35 g. of crude isoquinoline was accomplished by precipitation of the picrate from benzene solution, digestion of the solid with 4 l. of butanol and liberation of the free amine with 20% aqueous sodium hydroxide followed by distillation. A 32% over-all yield (10.43 g.) of pure isoquinoline with $n^{25}D$ 1.6208 (lit.[26] $n^{25}D$ 1.6208) was collected at 57–58° (0.60 mm.).

(23) Victor Chemical Works, Chicago, Ill.

(24) H. R. Snyder and F. X. Werber, THIS JOURNAL, **72**, 2964 (1950).

(25) E. Späth, F. Berger and W. Kuntara, *Ber.*, **63**, 135 (1930).

(26) H. Frieser and N. L. Glowacki, THIS JOURNAL, **71**, 514 (1949).

COLLEGE PARK, MARYLAND

[CONTRIBUTION FROM THE DEPARTMENT OF CHEMISTRY OF WAYNE STATE UNIVERSITY]

Alkaloid Studies. XVI.[1] Alkaloids of *Rauwolfia tetraphylla* L. The Structures of Tetraphylline and Tetraphyllicine[2,3]

BY CARL DJERASSI, JACK FISHMAN, MARVIN GORMAN, J. P. KUTNEY AND S. C. PAKRASHI

RECEIVED OCTOBER 4, 1956

From the root bark of the West Indian tree *Rauwolfia tetraphylla* L. there have been isolated the following alkaloids: reserpine, ajmaline, serpentinine, ψ-yohimbine, tetraphylline and tetraphyllicine. The last two alkaloids are new and evidence is presented for the structure assignment of these substances. Tetraphylline (I) is a stereoisomer of reserpinine and isoreserpinine and must differ from these two members of the ajmalicine group in the configuration of one or more of three centers (positions 15, 19 and 20) since destruction of the asymmetric center at C-3 does not yield identical derivatives. The structure of tetraphyllicine (X) follows from its conversion to desoxyajmaline upon hydrogenation and the isolation of acetaldehyde by ozonolysis, and it represents an interesting biogenetic intermediate in this class of alkaloids. Tetraphyllicine is identical with the hydrolysis product of rauvomitine thus proving the latter's constitution as tetraphyllicine trimethoxybenzoate (XI). A possible structure for ajmalidine (XII), an alkaloid isolated in trace amounts from *R. sellowii*, is presented. Some observations on serpentinine are also recorded in the Experimental portion of this paper.

In spite of the great number of *Rauwolfia* species growing on the American continent, relatively few have been studied chemically[4] and at the time that this investigation was initiated, the alkaloid composition of only one American *Rauwolfia* species, *R. heterophylla* Roem. and Schult. (synonymous[5] with *R. hirsuta* Jacq.) had been recorded.[6,7] Through the kind coöperation of Dr. Murrell P. Morris and Mr. Bartolome Cancel of the U. S. Department of Agriculture Experiment Station in Mayaguez, Puerto Rico, a considerable quantity of root bark of the West Indian *R. tetraphylla* L.[5] was secured and this was processed for alkaloids by substantially the scheme outlined earlier by Hochstein and collaborators.[7]

Reserpine was separated by virtue of the chloroform or benzene solubility of its acetate and could be isolated in *ca.* 0.09% yield by direct crystallization. Preliminary experiments indicated that fractionation[7] of the remaining alkaloids into medium and strong bases was of no advantage in this particular case and the total, reserpine-depleted bases were separated by a combination of chromatographic and counter-current distribution techniques. As described in detail in the Experimental portion of this paper, the principal alkaloids encountered were serpentinine (0.033%)[8] and two new bases which we named tetraphylline (0.024%) and tetraphyllicine (0.023%); trace quantities of ajmaline and ψ-yohimbine also were noted. The relevant experiments leading to the structure elucidation of these two alkaloids are described below.

Elementary analysis indicated the composition $C_{22}H_{26}N_2O_4$ for tetraphylline and this was confirmed by analysis of its nitrate and perchlorate salts. The presence of one C-methyl and two methoxyl groups was demonstrated by appropriate functional group analysis and since the infrared spectrum exhibited the typical bands at 5.92 and 6.17 μ of the —C—O—C=C—$COOCH_3$ grouping,[9] the nature of all four oxygen atoms is accounted for. The ultraviolet absorption spectrum[2a] of tetraphylline is completely superimposable upon that

(1) Paper XV. C. Djerassi, J. Herran, H. N. Khastgir, B. Riniker and J. Romo, *J. Org. Chem.*, **21**, 1510 (1956).

(2) Portions of this work have been reported in two preliminary communications; (a) C. Djerassi and J. Fishman, *Chemistry & Industry*, 627 (1955); (b) C. Djerassi, M. Gorman, S. C. Pakrashi and R. B. Woodward, THIS JOURNAL, **78**, 1259 (1956).

(3) Generous fellowship support was provided by grants from Chas. Pfizer and Co. (Brooklyn, N. Y.) and from the American Heart Association.

(4) These are listed by W. B. Mors, P. Zaltzman, J. J. Beereboom, S. C. Pakrashi and C. Djerassi, *Chemistry & Industry*, 173 (1956).

(5) R. E. Woodson, Jr., *North American Flora*, **29**, part 2 (1938).

(6) C. Djerassi, M. Gorman, A. L. Nussbaum and J. Reynoso, THIS JOURNAL, **76**, 4463 (1954).

(7) F. A. Hochstein, K. Murai and W. H. Boegemann, *ibid.*, **77**, 3551 (1955).

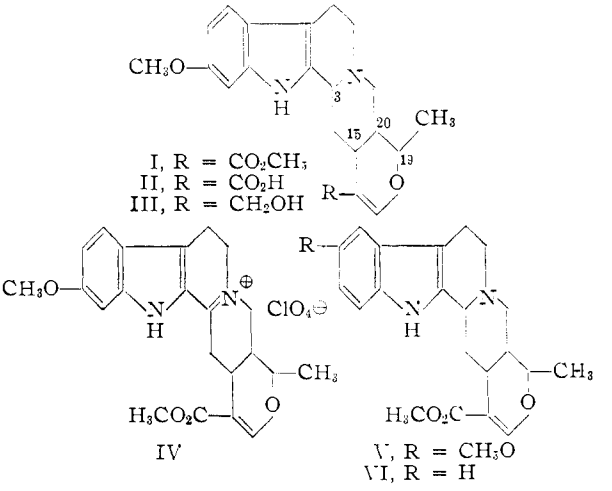
(8) *Cf.* E. Schlittler, H. U. Huber, F. E. Bader and H. Zahnd, *Helv. Chim. Acta*, **37**, 1912 (1954). For additional observations on serpentinine, see Experimental portion of present paper.

(9) *Cf.* F. E. Bader, *ibid.*, **36**, 215 (1953); M. M. Janot, R. Goutarel and J. Massonneau, *Compt. rend.*, **234**, 850 (1952).

of reserpinine (I)[10] and isoreserpinine (I)[11] and electrometric titration indicated the same order of basicity (pK'a 6.6). In addition to the two infrared bands in the 6-μ region mentioned above, tetraphylline shows a shoulder at 6.10 μ in chloroform solution which is very well resolved when the spectrum is run in Nujol mull. This band appears to be due to the methoxylated benzene ring and is also noticeable with reserpinine[10] but not with isoreserpinine[11] when all three spectra are run on the same instrument.[12] It appeared, therefore, that tetraphylline is a stereoisomer of reserpinine and isoreserpinine and should be represented by structure I, the attachment of the methoxyl group at C-11 following from ultraviolet data on model compounds.[10] Further support for the postulated ring E structure was adduced by two lines of evidence: (a) lithium aluminum hydride reduction of tetraphylline (I) furnished the corresponding alcohol, tetraphyllinol (III) with infrared bands typical of the enol ether (6.05 μ) and anisole (6.10 μ) systems. The ultraviolet absorption spectrum[2a] now exhibited a definite minimum at 252 mμ which was obscured in the parent alkaloid by superimposition of the unsaturated ester chromophore.[13] (b) Alkaline hydrolysis of tetraphylline led to an amorphous acid, tetraphyllic acid (II) from which tetraphylline (I) could be regenerated after methylation with diazomethane.

The evidence available at the present time in support of structure I for reserpinine[10] and isoreserpinine[11] is of the same circumstantial nature as outlined above for tetraphylline. In view of the fact that selenium dehydrogenation of the related alkaloid aricine (V)[11] to a methoxylated alstyrine derivative proceeded in comparatively good yield,[14] a similar dehydrogenation was carried out with tetraphylline (I) in order to effect a direct chemical proof for the methoxylated indole moiety. The dehydrogenation of tetraphylline (I) was run parallel with that of aricine (V) and while we were able to substantiate the results of the French and Swiss workers,[14] only traces of a picrate could be obtained from tetraphylline. The amounts at our disposal did not permit further characterization.

There are now available three alkaloids—tetraphylline, reserpinine and isoreserpinine—which apparently are all represented by structure I and which must, therefore, differ at one or more of the following four centers: 3, 15, 19 and 20. At least one of these, C-3, could be eliminated experimentally by taking advantage of the recently reported[15] mercuric acetate dehydrogenation of yohimbine-like alkaloids which leads to the corresponding yellow 3-dehydro derivative. While yohimbine reacts readily within 2 hours,[15] a period of 5–6 hours was necessary with tetraphylline and isoreserpinine[11,16] as well as with the related alkaloid aricine (V) which was examined at the same time.[17] In each instance, crystalline perchlorates of the corresponding 3-dehydro alkaloids were obtained and their spectral properties were in good agreement with those reported for similar chromophores.[15] 3-Dehydrotetraphylline perchlorate (IV) proved to be different from 3-dehydroisoreserpinine perchlorate[18] from which it follows that these two alkaloids must differ at one or more of the three other asymmetric centers (15, 19 and 20). Sodium borohydride reduction of 3-dehydrotetraphylline perchlorate (IV) regenerated tetraphylline (I) which would suggest[19] the 3α-orientation for this alkaloid.



The second new alkaloid isolated from *R. tetraphylla* L. was named tetraphyllicine. Considerable difficulties were encountered in determining its analytical composition and three independent de-

(10) This alkaloid has been isolated independently by various investigators and the name reserpinine is now accepted for it; A. Popelak, H. Spingler and F. Kaiser, *Naturwiss.*, **40**, 625 (1953); A. Hofmann, *Helv. Chim. Acta*, **37**, 849 (1954); F. L. Weisenborn, M. Moore and P. Diassi, *Chemistry & Industry*, 375 (1954); E. Schlittler, H. Saner and J. M. Muller, *Experientia*, **10**, 133 (1954); N. Neuss, H. E. Boaz and J. W. Forbes, THIS JOURNAL, **76**, 3234 (1954.)

(11) A. Stoll, A. Hofmann and R. Brunner, *Helv. Chim. Acta*, **38**, 270 (1955).

(12) These three comparison curves were run at the Squibb Institute for Medical Research through the kind coöperation of Dr. P. A. Diassi.

(13) A similar observation already has been made with related alkaloids possessing the same type of heterocyclic E ring: M. W. Klohs, M. D. Draper, F. Keller and W. Malesh, *Chemistry & Industry*, 1264 (1954); R. Robinson and A. F. Thomas, *J. Chem. Soc.*, 3479 (1954).

(14) R. Goutarel, M. M. Janot, A. LeHir, H. Corrodi and V. Prelog, *Helv. Chim. Acta*, **37**, 1805 (1954).

(15) F. L. Weisenborn and P. A. Diassi, THIS JOURNAL, **78**, 2022 (1956); E. Wenkert and D. K. Roychaudhuri, *J. Org. Chem.*, **21**, 1315 (1956). We are indebted to Dr. Wenkert for an advance copy of his manuscript.

(16) We are indebted to Dr. A. Hofmann (Sandoz, Basle, Switzerland) for a sample of isoreserpinine nitrate which was used for this reaction.

(17) Wenkert and Roychaudhuri (ref. 15) were the first to demonstrate the stability of the heterocyclic E ring toward mercuric acetate by preparing 3-dehydroajmalicine perchlorate. Since three stereoisomers of ajmalicine (VI) are known (tetrahydroalstonine, mayumbine and akuammigine—for leading references see Robinson and Thomas (ref. 13)), it would be interesting to determine the course of the mercuric acetate dehydrogenation in each case. The tetradehydro derivatives of ajmalicine and tetrahydroalstonine occur in nature (serpentine and alstonine) and these two alkaloids, therefore, cannot just be C-3 epimers but must differ at one or more of the other asymmetric centers.

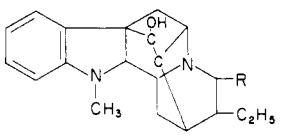
(18) This substance is identical with 3-dehydroreserpinine perchlorate prepared by Dr. P. A. Diassi (Squibb Institute for Medical Research). Reserpinine and isoreserpinine differ, therefore, only at C-3 and Dr. Diassi (private communication) has shown that reserpinine possesses the more stable conformation.

(19) Some of the complicating factors involved in assigning configurations at C-3 have already been outlined by Wenkert (ref. 15), but in this instance molecular rotation difference (alkaloid − 3-dehydro perchlorate) calculations also support this assignment.

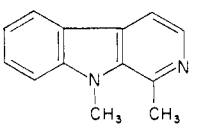
terminations out of seven indicated the empirical formula $C_{20}H_{26}N_2$[2a] while the remaining ones were in better agreement with $C_{20}H_{24}N_2O$.[2b] In our first preliminary note[2a] on this subject, the latter values were ignored since they were assumed to be due to incomplete combustion. Only when direct oxygen analyses were obtained[2b] did it become clear that tetraphyllicine should be represented by the empirical formula $C_{20}H_{24}N_2O$ and we have no obvious explanation why high carbon values should have been observed in different analytical laboratories. The ultraviolet absorption spectrum[2a] of tetraphyllicine was identical with that of ajmaline (VII)[20,21] and its basicity (pK'a 8.5) was of the same order of magnitude. Functional group analysis of tetraphyllicine demonstrated the presence of one C-methyl and one N-methyl group and its infrared spectrum showed some similarity to that of ajmaline (VII) ($C_{20}H_{26}N_2O_2$). This resemblance became quite striking when the infrared spectrum of tetraphyllicine was compared with that of desoxyajmaline (VIII) ($C_{20}H_{26}N_2O$)[20,22] and the rotatory dispersion curves (700–320 mμ) of the two bases were very similar (as are their melting points) except for the fact that the values for tetraphyllicine are consistently higher by well over 100° between 375–320 mμ. Tetraphyllicine contains only one active hydrogen atom and since it can be transformed into an O-acetate (from which tetraphyllicine can be regenerated upon saponification), both nitrogen atoms must be tertiary as in ajmaline (VII). This apparent structural similarity was strengthened greatly by the course of the selenium dehydrogenation of tetraphyllicine which yielded *ind*-N-methylharman (IX), one of the characteristic degradation products of ajmaline (VII).[20]

The great resemblance of desoxyajmaline (VIII) and tetraphyllicine coupled with the fact that their empirical formulas differed only by two hydrogen atoms suggested that tetraphyllicine was a dehydrodesoxyajmaline and this was shown to be indeed the case by catalytic hydrogenation of tetraphyllicine which yielded desoxyajmaline (VIII). On the basis of structure VIII[21] for desoxyajmaline, only two positions (vinyl or ethylidene) appeared to be consistent with the general properties of tetraphyllicine and a definite decision could be reached by ozonolysis which gave nearly 55% of acetaldehyde. Coupled with the extensive degradative and synthetic evidence[20,21] for structure VII for ajmaline, tetraphyllicine must then be represented by expression X.[2b]

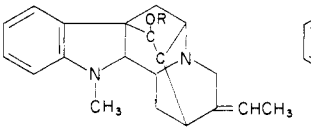
In the light of current views[21,23] on the biogenetic relationships existing among the various indole and indoline alkaloids of the yohimbine type, the isolation of tetraphyllicine (X) is quite significant.



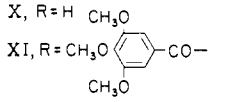
VII, R = OH
VIII, R = H



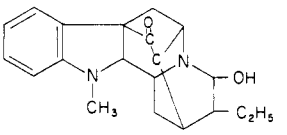
IX



X, R = H



XI, R = CH_3O



XII

The double bond[24] is located in precisely the spot to be expected for a precursor of the heterocyclic E ring of the ajmalicine (δ-yohimbine) group of alkaloids (*e.g.*, I, V, VI). It should be noted that in each plant in which tetraphyllicine (X) has been isolated[25–27] it is accompanied by one or more members of the ajmalicine–serpentine series.

Recently, there has been announced[28] the isolation of a new ester alkaloid—rauvomitine ($C_{30}H_{34}N_2O_5$)—from *R. vomitoria* Afz. and its hydrolysis to trimethoxybenzoic acid and a new base, $C_{20}H_{24}N_2O$. The physical constants of this hydrolysis base were practically identical with those reported[2a] for tetraphyllicine (X) and the presumable identity of the two bases was supported by the observation that while no tetraphyllicine could be isolated from the reserpine-depleted alkaloids of *R. vomitoria* Afz.,[29] tetraphyllicine could be obtained very readily if this crude fraction was first saponified.[30] Subsequently, an authentic sample[31] of the hydrolysis base[28a] of rauvomitine was secured and direct comparison demonstrated its complete identity with tetraphyllicine (X). Rauvomitine is, therefore, tetraphyllicine trimethoxybenzoate and possesses structure XI.

In an earlier investigation[25] of the alkaloids of *Rauwolfia sellowii*, there was isolated in minute quantity a new alkaloid ($C_{20}H_{24}N_2O_2$) which was named ajmalidine. Attention was called to its apparent close connection with ajmaline and it was noted that the alkaloid possessed a carbonyl function which from its infrared frequency was assumed to form part of a five-membered ring. In the light

(20) For earlier references see: F. A. L. Anet, D. Chakravarti, R. Robinson and E. Schlittler, *J. Chem. Soc.*, 1242 (1954); R. Robinson, *Chemistry & Industry*, 285 (1955); F. C. Finch, J. D. Hobson, R. Robinson and E. Schlittler, *ibid.*, 653 (1955); A. Chatterjee and S. Bose *Experientia*, **9**, 254 (1953).

(21) R. B. Woodward, *Angew. Chem.*, **68**, 13 (1956). Structure VII for ajmaline was proposed in this article.

(22) We are indebted to Prof. R. B. Woodward (Harvard University) for an authentic specimen.

(23) R. Robinson, "The Structural Relations of Natural Products," Oxford University Press, London, 1955.

(24) The curare alkaloid mavacurin (H. Bickel, H. Schmid and P. Karrer, *Helv. Chim. Acta*, **38**, 649 (1955)) bears a striking structural resemblance to tetraphyllicine and also contains such an ethylidene function.

(25) S. C. Pakrashi, C. Djerassi, R. Wasicky and N. Neuss, THIS JOURNAL, **77**, 6687 (1955).

(26) Additional plants in which tetraphyllicine has been encountered will be reported shortly.

(27) Serpinine, isolated by S. Bose (*Naturwiss.*, **42**, 71 (1955)) in trace amounts from *R. serpentina* Benth. may be identical with tetraphyllicine.

(28) (a) E. Haack, A. Popelak and H. Springler, *Naturwiss.*, **42**, 627 (1955); (b) J. Poisson, R. Goutarel and M. M. Janot, *Compt. rend.*, **241**, 1840 (1955).

(29) We are grateful to Dr. F. A. Hochstein (Chas. Pfizer and Co., Brooklyn, N. Y.) for providing us with this alkaloid fraction.

(30) This observation was first made by Poisson, Goutarel and Janot (ref. 28b).

(31) We are indebted to Dr. E. Haack (C. F. Boehringer, Mannheim-Waldhof) for this gift.

of the newest ajmaline structure VII[21] it appears quite possible that ajmalidine should be represented by XII.[2b]

Experimental[32]

Isolation of Alkaloids from *Rauwolfia tetraphylla* L.—In the original small-scale extraction[2a] separation[7] into weak, medium and strong bases was carried out followed by chromatography. It was noted that the alkaloid composition of this plant necessitated only separation into benzene (or chloroform)-soluble acetates and into the remaining alkaloids and this scheme was followed in the larger extraction.[33]

The air-dried *R. tetraphylla* root bark (30 kg.) was ground to pass a 20-mesh screen in a hammer mill. The ground root was then extracted for 6 hours with 160 l. of boiling methanol and the solvent was decanted. A second extraction with 120 l. of methanol for 8 hours was followed by filtration and the cake was washed with 64 l. of fresh methanol. The combined extracts and washings were concentrated *in vacuo* to 10 l., 34 l. of 10% aqueous acetic acid was added and some insoluble tar was removed by filtration through Supercel. The filtrate was extracted with three 50-l. portions of benzene and the combined benzene extracts were washed with an excess of 5% ammonium hydroxide and evaporated to dryness *in vacuo*. The residue (116 g.) was dissolved in 300 cc. of methanol and seeded with reserpine. After several hours, 29 g. of crude **reserpine** was separated by filtration and recrystallized to yield 26 g. of pure material. By processing the mother liquors, an over-all yield of 0.09% (all yields based on dry root) was realized.

The acid aqueous solution from the original benzene extraction was adjusted to *p*H 11.4 with sodium hydroxide and extracted with four 24-l. portions of chloroform. The combined chloroform extracts were concentrated to dryness *in vacuo* and the residue (613 g.) was refluxed with benzene for 6 hours yielding 300 g. of benzene-soluble alkaloids which were used in the further purification schemes.

A 58-g. portion was chromatographed on 1.5 kg. of ethyl acetate—washed alumina (activity II–III) and eluted with benzene, benzene–chloroform, chloroform and chloroform–methanol mixtures. A total of 95 fractions (500–1000 cc. each) was collected. Elution with 9:1 benzene–chloroform (fractions 22–34) followed by crystallization from methanol yielded 0.024% of **tetraphylline**. Crystallization of fractions 35–70 (8:2 and 7:3 benzene–chloroform) furnished 0.023% of tetraphyllicine. Rechromatography of the tetraphyllicine mother liquors and crystallization from acetone containing a small amount of methanol afforded *ca.* 0.0005% of **ψ-yohimbine**, m.p. 269–272°, which proved to be identical with an authentic specimen (m.p. 273–275°) of this alkaloid in the Lilly collection by mixture melting point, infrared and X-ray pattern comparisons.

From the most polar fractions (71–85) there was obtained after crystallization from methanol *ca.* 0.033% of **serpentinine**; identity with authentic material[8] (kindly provided by Dr. C. F. Huebner, Ciba Pharmaceutical Products, Inc.) was established by mixture melting point (rather unreliable because of decomposition at 265–270°), infrared and ultraviolet[2a] spectral comparison.

Repeated chromatography of the mother liquors of the above alkaloids did not lead to any additional crystalline material and they were, therefore, subjected to a rough counter-current distribution in 10 funnels using 100 cc. each of chloroform (stationary phase) and citrate–phosphate buffer of *p*H 4.2. After rejecting the first and last fractions, a second distribution was carried out at *p*H 5 in 10 fractions and from funnel 9 there was isolated after crystallization from petroleum ether–ether–methanol and recrystallization from methanol 0.0008% of **ajmaline**, m.p. 156–159°, identified by mixture melting point and infrared comparison.

Serpentinine.—In view of the fact that the structure of this alkaloid has not yet been established and since there exists even some uncertainty about its empirical formula,[8] some of our results are given below and are in reasonable agreement with the composition $C_{21}H_{22}N_2O_3$, which is also favored by Schlittler and collaborators.[8]

One analytical sample (analysis C) was secured from chromatographed material which had been recrystallized five times from methanol. The other two analyses (A and B) were carried out with serpentinine which had been chromatographed and which was then subjected to a 21-stage counter-current distribution between chloroform and citrate–phosphate buffer of *p*H 5.6 (distribution coefficient 1.1), the material from tubes 7–11 being used for recrystallization from methanol. In addition to the melting point (m.p. *ca.* 265–270° with dec.), the infrared and ultraviolet spectra of the two samples were identical and the substance behaved like a homogeneous substance upon paper chromatography. A comparison of the infrared spectra of serpentine and serpentinine shows a very strong band at 2.95 μ for the latter. Serpentine has a shoulder at 6.2 μ and a band at 6.3 μ which are absent in serpentinine. The remaining important bands in the 5–7 μ region are identical in both alkaloids.

The ultraviolet spectrum (see also ref. 8) showed the following maxima in *ethanol:* 227 mμ (log ε 4.37), 257 (4.17), 281 (3.59), 294 (3.59), 308 (3.75), 373 (3.79); dilute ethanolic *potassium hydroxide:* 222 mμ (log ε 4.41), 285 (4.38), 330 (3.34) and 420 (3.21). For comparison, there are also given the values for serpentine (*ethanol:* 252 (4.49), 308 (4.30), 370 (3.61); *potassium hydroxide:* 285 (4.62), 3.30 (3.82), 420 (3.43)) and it will be noted that there are marked differences in the spectra measured in ethanol solution. In addition to the maxima typical of the anhydronium system found in serpentine, serpentinine also shows maxima at 227, 281 and 294 mμ characteristic of indoles. The possibility exists, therefore, that serpentinine is either a double molecule containing both indole and anhydronium chromophores or else that it is a hitherto unseparated mixture of an indole and an anhydronium base. It should be noted that in contrast to serpentine (*pK'*a 10.8), serpentinine exhibits *two pK'*a values (66% dimethylformamide solution) at 6.0 and 10.6 consistent with the presence of two basic systems of the type mentioned above.

Anal. Calcd. for $C_{21}H_{22}N_2O_3$: C, 71.98; H, 6.33; N, 8.00; O, 13.70. $C_{21}H_{22}N_2O_3 \cdot H_2O$: C, 68.46; H, 6.57; N, 7.60; O, 17.37; OCH_3, 8.69; H_2O, 4.89. Found (all analyses by G. Maciak, W. Brown, G. Beckman and C. Hunter): *A* (dried 4 hr. at 25° *in vacuo*, then allowed to come to equilibrium in air and stored over calcium chloride): C, 68.27, 67.74; H, 7.10, 6.95; N, 7.39. *B* (dried 24 hr. at 25° *in vacuo* and analyzed directly): C, 71.73; H, 6.61; N, 8.18; O, 13.58; loss on drying (H_2O), 4.78. *C* (dried at room temperature *without* vacuum): C, 68.66, 68.75; H, 6.93, 7.22; N, 7.54; O, 16.44; OCH_3, 9.65.

The resistance of serpentinine to catalytic hydrogenation (in contrast to serpentine) already has been commented upon,[8] but we have now observed that just like serpentine (which yields crystalline ajmalicine) serpentinine is readily reduced with sodium borohydride at room temperature to a colorless product, apparently homogeneous by paper chromatography, and showing an ultraviolet absorption spectrum virtually indistinguishable from that of ajmalicine (VI). The infrared spectrum of the product was also very similar to that of ajmalicine but all attempts at crystallization failed. A quantitative sodium borohydride reduction, kindly performed by Mr. G. Maciak, showed that both serpentine and serpentinine consumed two equivalents of reagent.

Tetraphylline (I).—The analytical sample was obtained by repeated recrystallization from methanol whereupon it crystallized as colorless, small plates, m.p. 220–223° (sometimes a double m.p. 125° and 223° was observed), $[\alpha]^{28}D$ −76° ($CHCl_3$), −35° (pyridine), *pK'*a 6.6; $\lambda_{max}^{CHCl_3}$ 2.87, 5.92, 6.10 (shoulder), 6.17 μ; λ_{max}^{nujol} 2.90, 5.96, 6.14 and 6.22 μ; ultraviolet absorption spectrum reproduced in ref. 2a: λ_{max}^{EtOH} 229 mμ (log ε 4.65) and 298 mμ (log ε 3.81), λ_{min}^{EtOH} 283 mμ (log ε 3.68)

Anal. Calcd. for $C_{22}H_{26}N_2O_4$: C, 69.09; H, 6.85; N,

(32) Melting points were determined on the Kofler block. We are indebted to Mrs. Dolores Phillips for the ultraviolet and infrared spectral measurements. The electrometric titrations were obtained by Dr. H. E. Boaz through the coöperation of Dr. N. Neuss (Eli Lilly and Co.). The microanalyses are due to Mr. Joseph F. Alicino (Metuchen, N. J.), Dr. A. Bernhardt (Mülheim, Germany), Mr. T. Toolan (Chas. Pfizer and Co., Brooklyn, N. Y.), Spang Microanalytical Laboratory (Plymouth, Mich.), and the microanalytical staff of Eli Lilly and Co., (G. Maciak, W. Brown, G. Beckman and C. Hunter).

(33) The extraction up to the reserpine-depletion stage was carried out in the pilot plant of Chas. Pfizer and Co., Brooklyn, N. Y. We should like to express our appreciation for this generous assistance.

7.33; C-CH_3, 3.93; methoxyl, 8.01; mol. wt., 382. Found: C, 69.48; H, 6.63; N, 7.10; C-CH_3, 3.48; methoxyl, 15.89; N-CH_3, O; mol. wt. (potentiometric titration), 387.

Tetraphylline nitrate was prepared by adding a hot solution of 30 mg. of the alkaloid in 10 cc. of methanol to 3 drops of nitric acid in 1 cc. of methanol. The precipitated salt was recrystallized from methanol as light yellow needles, m.p. 264–266° (preheated block).

Anal. Calcd. for $C_{22}H_{26}N_2O_4 \cdot HNO_3$: C, 59.31; H, 6.11; N, 9.43. Found: C, 59.52; H, 6.20; N, 9.42.

The perchlorate was crystallized when an acetic acid solution of the alkaloid was treated with a saturated aqueous solution of potassium perchlorate. After recrystallization from methanol it exhibited m.p. 273–275°, $[\alpha]D$ −33° (methanol).

Anal. Calcd. for $C_{22}H_{26}N_2O_4 \cdot HClO_4$: C, 54.71; H, 5.60; N, 5.80. Found: C, 55.12; H, 5.95; N, 5.66.

Tetraphyllinol (III).—A mixture of 100 mg. of tetraphylline, 200 mg. of lithium aluminum hydride and 40 cc. of ether was heated under reflux for 3 hours, the excess reagent was decomposed with moist ether and the solution filtered and evaporated to dryness. Recrystallization from methanol–ether gave glistening, rectangular plates, m.p. 212–216° (with previous softening) which proved to be rather unstable and apparently retained solvent tenaciously; $\lambda_{max}^{CHCl_3}$ 6.05 and 6.10 μ; ultraviolet absorption spectrum reproduced in ref. 2a: λ_{max}^{EtOH} 228 mμ (log ϵ 4.77), 270 mμ (log ϵ 3.82), 298 mμ (log ϵ 3.90); λ_{min}^{EtOH} 252 mμ (log ϵ 3.70) and 282 mμ (log ϵ 3.75).

Anal. Calcd. for $C_{21}H_{26}N_2O_3 \cdot CH_3OH$: C, 68.37; H, 7.82; methoxyl, 16.03. Found: C, 68.97; H, 8.04; methoxyl, 14.93.

Saponification and Remethylation of Tetraphylline (I).—Tetraphylline (150 mg.) was saponified exactly as described[11] for aricine (V) and the crude tetraphyllic acid (II), which was obtained as an amorphous powder, was methylated in methanol solution with diazomethane. Recrystallization afforded 40 mg. of tetraphylline, m.p. 218–220°, undepressed upon admixture with the original alkaloid; identity was confirmed further by infrared spectral comparison.

Selenium Dehydrogenation of Tetraphylline (I).—The dehydrogenation of 880 mg. of tetraphylline with 600 mg. of black selenium at 300° for 5 minutes was carried out exactly as reported[14] for aricine (V). The crude mixture was ground with sand and extracted for 20 hours in a Soxhlet apparatus with ether and then with benzene. Attempts to prepare crystalline picrates from the solvent-extracted oils proved fruitless and these were regenerated by passage over Amberlite IRA-400 (basic) and distilled at 150–160° and 0.005 mm. The distillate was again treated with picric acid and 8.5 mg. of bright yellow picrate was obtained from methanol, m.p. 245–247° dec. This substance was probably the expected methoxylated alstyrine although it was probably not completely homogeneous as had already been noticed earlier[14] of aricine (V).

Anal. Calcd. for $C_{25}H_{25}N_5O_8$: C, 57.36; H, 5.81; N, 13.38. Calcd. for $C_{26}H_{27}N_5O_8$: C, 58.09; H, 5.06; N, 13.03. Found: C, 58.22; H, 4.80; N, 12.87.

The unexpected low yield, which precluded further degradation of the dehydrogenation product, was not due to any obvious experimental differences since we had no difficulty in duplicating the yield of crystalline picrate reported[14] in the dehydrogenation of aricine.

3-Dehydrotetraphylline Perchlorate (IV).—The procedure was that of Wenkert and Roychaudhuri[15] and under those conditions complete reaction was observed in 2 hours with the model yohimbine. On the other hand, tetraphylline (400 mg.) and mercuric acetate (1.78 g.) in 20 cc. of 10% acetic acid had to be heated for 6 hours at 90° since no mercurous acetate precipitation was noted after two hours. The yield was not increased when the reaction time was extended to 22 hours. The solution was filtered, heated to boiling, saturated with hydrogen sulfide, concd. hydrochloric acid was added and the precipitate was filtered after initial heating to coagulate it. The filtrate was treated with a saturated solution of potassium perchlorate and the bright yellow crystals were filtered and recrystallized from methanol; yield 220 mg. (43%), m.p. 273–275°, $[\alpha]D$ +172.5° (methanol); λ_{max}^{EtOH} 218 mμ (log ϵ 4.62) and 390 mμ (log ϵ 4.53), inflection at 235 mμ (log ϵ 4.39).

Anal. Calcd. for $C_{22}H_{25}ClN_2O_8$: C, 54.95; H, 5.24; N, 5.83; Cl, 7.38. Found: C, 55.37; H, 5.52; N, 5.46; Cl, 6.88.

A solution of 50 mg. of perchlorate in methanol was refluxed for 3 hours with 380 mg. of sodium borohydride, the solvent was removed *in vacuo*, water was added and the product was extracted with chloroform. Evaporation of the solvent and recrystallization from methanol furnished 23 mg. of tetraphylline (I) which was identified by mixture melting point determination and infrared comparison.

3-Dehydroaricine Perchlorate.—The mercuric acetate dehydrogenation was carried out with aricine (V) exactly as described above and yielded 57% of yellow crystals, m.p. 225–227°, $[\alpha]D$ +135° (methanol); λ_{max}^{EtOH} 310 mμ (log ϵ 3.94), 324 mμ (log ϵ 4.00) and 370 mμ (log ϵ 3.98) and shoulder at 337 mμ (log ϵ 4.03).

Anal. Calcd. for $C_{22}H_{25}ClN_2O_8$: C, 54.95; H, 5.20; N, 5.83; Cl, 7.38. Found: C, 55.44; H, 5.68; N, 5.55; Cl, 7.52.

Sodium borohydride reduction regenerated aricine in 60% yield.

3-Dehydroisoreserpinine Perchlorate.—Isoreserpinine nitrate (130 mg.)[11,16] was converted into the free base and heated in 10% acetic acid with 410 mg. of mercuric acetate for 5 hours at 90° yielding 79 mg. of yellow crystals. Recrystallization from acetone–methanol furnished the analytical sample, m.p. 297–298° dec., $[\alpha]D$ +130° (methanol); λ_{max}^{EtOH} 221 mμ (log ϵ 4.58), 332 mμ (log ϵ 4.25) and 390 mμ (log ϵ 4.35) and shoulders at 233 mμ (log ϵ 4.53) and 316 mμ (log ϵ 4.20).

Anal. Calcd. for $C_{22}H_{25}ClN_2O_8$: C, 54.95; H, 5.24; N, 5.83. Found: C, 55.21; H, 5.80; N, 5.94.

Tetraphyllicine (X).—Purification of this alkaloid is relatively easy since it is only slightly soluble in acetone and in chloroform, in contrast to the related bases found in this plant. Recrystallization from acetone furnished colorless needles, m.p. 320–322°, $[\alpha]^{28}D$ +21° (pyridine), $pK'a$ 8.5; λ_{max}^{Nujol} 6.24 and 6.80 μ with hydroxyl absorption hardly noticeable (see also ref. 28b); ultraviolet absorption spectrum reproduced in ref. 2a: λ_{max}^{EtOH} 250 mμ (log ϵ 4.07) and 294 mμ (log ϵ 3.60), λ_{min}^{EtOH} 229 mμ (log ϵ 3.75) and 262 mμ (log ϵ 3.32).

Anal. Calcd. for $C_{20}H_{24}N_2O$: C, 77.88; H, 7.84; N, 9.08; O, 5.19; -CH_3, 4.85; mol. wt., 308; active hydrogen 0.325. Found: C, 77.97, 78.23[34]; H, 7.89, 8.08; N, 8.91; O, 5.28, 5.20; C-CH_3, 3.39, 4.25; N-CH_3, 4.50; methoxyl, 0; mol. wt. (potentiometric titration), 319; active hydrogen, 0.33.

The perchlorate was prepared in ethanol solution with perchloric acid and recrystallized from the same solvent; m.p. 305–307° dec.

Anal. Calcd. for $C_{20}H_{24}N_2O \cdot HClO_4$: C, 58.75; H, 6.16; N, 6.85; Cl, 8.67. Found: C, 58.79; H, 6.26; N, 6.82; Cl, 8.79.

The methiodide was prepared by letting a benzene solution (containing some methanol) of tetraphyllicine stand with excess methyl iodide for 16 hours. Removal of the solvent and recrystallization from benzene–ethanol furnished silvery white needles (m.p. 284–285° with sintering from 265°) of the methiodide, which apparently crystallized with one mole of benzene which was not lost after drying at 56° under reduced pressure.

Anal. Calcd. for $C_{21}H_{27}IN_2O \cdot C_6H_6$: C, 61.38; H, 6.29; I, 24.04. Found: C, 62.50; H, 6.81; I, 23.62.

Miscellaneous Reactions of Tetraphyllicine.—The alkaloid was stable to lithium aluminum hydride, sodium borohydride (in contrast to ajmaline), boiling methanolic potassium hydroxide solution and toward mercuric acetate under conditions[35] where model dihydroindoles lacking an angular substituent (as is present in X) are dehydrogenated to the corresponding indole derivative. Hofmann degradation by

(34) It already has been pointed out (ref. 2b) that several carbon values were considerably higher and fell within the range 81.24–81.52. In the absence of direct oxygen determinations, this would have been compatible with $C_{20}H_{26}N_2$.

(35) T. M. Reynolds and R. Robinson, *J. Chem. Soc.*, 935 (1935).

the procedure of Weinstock and Boekelheide[36] did not lead to any recognizable products except for a small amount (*ca.* 5%) of recovered tetraphyllicine.

Selenium Dehydrogenation of Tetraphyllicine.—An intimate mixture of 300 mg. each of tetraphyllicine and black selenium was heated at 300° for 5 minutes, cooled, mixed with sand and extracted continuously for 18 hours in a Soxhlet extractor with benzene. The extract was stirred with mercury[37] for 4 hours, filtered through Celite powder and the residue after removal of the solvent was distilled at 130–140° and 0.015 mm. The yellow oil (75 mg.) which had distilled over was treated in ether solution with picric acid and the precipitate was recrystallized twice from methanol to yield 8 mg. of fine, yellow needles, m.p. 269–271° dec., undepressed upon admixture with an authentic specimen (m.p. 273–275° dec.) of *ind*-N-methylharman (IX) picrate[22,38]; the infrared spectra (potassium bromide pellet) were identical.

Acetylation of Tetraphyllicine.—A mixture of 100 mg. of tetraphyllicine, 4 cc. of benzene and 2 cc. of acetic anhydride was heated under reflux for 6 hours, concentrated *in vacuo* and diluted with ice-water. After making basic with ammonia, the product was extracted with ether and then with dilute hydrochloric acid, leaving a negligible residue in the ether solution. The acid extracts were again made basic, extracted with ether and the product was chromatographed on Merck acid-washed alumina. Apparently homogeneity was indicated by its chromatographic behavior (including infrared examination of most fractions eluted with chloroform and chloroform–methanol), but the substance could not be crystallized. It was then distilled at 160–165° and 0.002 mm. whereupon a heavy, colorless oil was obtained which crystallized rapidly; it melted partially at 60°, resolidified and yielded a clear melt at 154°; $\lambda_{max}^{CHCl_3}$ 5.78 and 8.0 μ. Since it could not be recrystallized satisfactorily, the distilled material was sent for analysis and while the latter was not very satisfactory, it indicated clearly (together with the relevant infrared bands) that the substance was O-acetyltetraphyllicine.

Anal. Calcd. for $C_{22}H_{26}N_2O_2$: C, 75.40; H, 7.48; N, 7.99; acetyl, 12.28. Found: C, 75.70; H, 8.19; N, 7.22; acetyl, 10.52.

Tetraphyllicine was obtained in excellent yield when the acetate was heated with an ethereal solution of lithium aluminum hydride or with 5% methanolic potassium hydroxide.

Ozonolysis of Tetraphyllicine.—Ozone was passed for 15 minutes through an ice-cold solution of 45 mg. of tetraphyllicine in 6 cc. of 2% acetic acid containing one drop of hydrochloric acid. The solution immediately turned deep purple and the excess ozone was swept out with nitrogen gas. The resulting dark brown solution was distilled in a current of nitrogen into an ice-cold aqueous sulfuric acid–ethanol solution of *p*-nitrophenylhydrazine yielding 15 mg. (55%) of acetaldehyde *p*-nitrophenylhydrazone with m.p. 124–125°, undepressed upon admixture with authentic material. No hydrazone formed in an appropriate blank experiment.

Hydrogenation of Tetraphyllicine (X) to Desoxyajmaline (VIII).[39]—A chloroform solution of 55 mg. of tetraphyllicine was treated with 5 cc. of hydrogen chloride–saturated chloroform and the solvent was removed. The resulting hydrochloride was hydrogenated at room temperature and atmospheric pressure in 95% ethanol solution with 25 mg. of prereduced platinum oxide catalyst whereupon one equivalent of hydrogen was consumed within one hour. Filtration of the catalyst, evaporation to dryness, addition of dilute ammonium hydroxide solution and isolation with chloroform yielded a crystalline residue which was recrystallized from methanol–acetone to furnish 43 mg. of fluffy, colorless needles showing m.p. 300–305°, undepressed upon admixture with an authentic specimen of desoxyajmaline (VIII).[20–22] The infrared spectra of the two specimens in Nujol mull were identical and the rotatory dispersion curves[40] (methanol solution) agreed within the acceptable 3% range inherent in rotation measurements at such dilute concentration.

Wave length, mμ	Tetraphyllicine (X) [α] (*c* 0.12)	Hydrogenated tetraphyllicine [α] (*c* 0.14)	Desoxyajmaline (VIII) [α] (*c* 0.075)
700	+94°	+74°	+75°
650	100	89	89
589	135	112	116
550	160	130	137
500	206	169	173
450	276	224	232
400	387	324	338
375	480	397	407
350	588	478	488
340	645	513	516
335	658	526	531
332.5	667	525	539
330	671	525	540
327.5	659	520	535

(36) J. Weinstock and V. Boekelheide, THIS JOURNAL, **75**, 2546 (1953).

(37) This method for removal of selenium was described recently by J. Jacques, G. Ourisson and C. Sandris, *Bull. soc. chim.*, 1293 (1955).

(38) R. B. Woodward and W. M. McLamore, THIS JOURNAL, **71**, 379 (1949).

(39) Poisson, Goutarel and Janot (ref. 28b) have described the hydrogenation of the rauvomitine (XI) saponification base to desoxyajmaline (VIII).

(40) For experimental details see C. Djerassi, E. W. Foltz and A. E. Lippman, THIS JOURNAL, **77**, 4354 (1955), and later papers in that series. We are indebted to Mrs. Rosemarie Riniker for the current measurements.

DETROIT, MICHIGAN

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

The Synthesis of DL-Canaline, DL-Canavanine and Related Compounds[1]

BY DAVID D. NYBERG[2] AND BERT E. CHRISTENSEN

RECEIVED JULY 13, 1956

A general discussion of the most satisfactory methods of preparation of O-substituted hydroxylamines and hydroxyguanidines is given. A new and practical synthesis of DL-canaline by a five-step reaction scheme from γ-butyrolactone in 7% over-all yield is described. DL-Canavanine is prepared directly from DL-canaline. An attempt to synthesize the lower homolog of DL-canaline resulted only in the isolation of DL-serine. An interesting material believed to be a polyoxime appeared as a product in this investigation.

In 1929, Kitagawa discovered the hitherto unknown amino acid canavanine ($NH_2C(=NH)NHOCH_2CH_2CH(NH_2)COOH$)[3] in the Jack bean meal from which he was extracting urease to be used in connection with his studies of the mechanism of urea formation in pig liver.

He reported that this new amino acid upon treatment with arginase[4] would yield urea and a second

(1) Published with the approval of the Monographs Publication Committee, Oregon State College as Research Paper No. 306, School of Science, Department of Chemistry.

(2) National Science Foundation Fellow 1954–1956.

(3) M. Kitagawa and T. Tomiyama, *J. Biochem.* (*Japan*), **11**, 265 (1929).

(4) (a) M. Damodaran and K. G. A. Narayanan, *Biochem. J.*, **34**, 1449 (1940); (b) M. Kitagawa, *J. Agr. Chem. Soc. Japan*, **15**, 267 (1939); *C. A.*, **34**, 1196 (1940).