7.2. The aqueous phase was concentrated under reduced pressure at room temperature to about 8 ml. and lyophilized. The residue from the lyophilization was taken up in a small volume of water, treated with carbon and Celite, and crystallized by the addition of acetone to yield 308 mg. (80%) of product melting at 252–254° dec. Two recrystallizations from acetone–water afforded pure XIII, m.p. 264–265° dec., not depressed upon admixture with the potassium salt of natural penicillin V, $[\alpha]^{28}D$ +222° (*c* 1 in water). The infrared spectra of natural and synthetic XIII were identical (in 40 peaks and shoulders in potassium bromide) and also identical with the spectrum of totally synthetic XIII made previously by another reaction sequence.[7] In microbiological assay, synthetic XIII showed 99 ± 10% of the bioactivity of natural penicillin V.

D-α-6-Phthalimidopenicillanic Acid (XIV).—To a vigorously stirred solution (*p*H 8) of 2.16 g. (10 mmoles) of D-α-XI and 1.06 g. (10 mmoles) of sodium carbonate in 15 ml. of water was added 2.19 g. (10 mmoles) of finely ground N-carboethoxyphthalimide[24] in one portion. At the end of 1.25 hours of vigorous stirring, when essentially all the solid had gone into solution, the mixture was extracted with methylene chloride. A fresh 50-ml. portion of methylene chloride was added to the aqueous phase which was acidified gradually with a total of 20 ml. (20 mmoles) of *N* hydrochloric acid, and the extraction was completed with two additional 25-ml. portions of methylene chloride. The combined organic layers were washed with two 25-ml. portions of water, dried over magnesium sulfate, and decolorized with carbon and Celite. Concentration of the methylene chloride solution with a nitrogen stream afforded 2.29 g. (66%) of colorless crystals, m.p. 145–150° dec. Two recrystallizations from acetone (at or below room temperature) gave an analytical sample of XIV, m.p. 167–170° dec., $[\alpha]^{28}D$ +278° (*c* 1 in *n*-butyl acetate). The infrared spectrum in dioxane solution (5%) had strong carbonyl maxima at 5.59, 5.65 and 5.74 μ. Compound XIV was inactive when tested by routine microbiological assay.

*Anal.* Calcd. for $C_{16}H_{14}N_2O_5S$: C, 55.49; H, 4.08; N, 8.09. Found: C, 55.41; H, 4.03; N, 8.05.

Methyl D-α-6-Phthalimidopenicillanate (XV).—The acid XIV (199 mg., 0.575 mmole) was dissolved in 2.5 ml. of dioxane and the solution treated with an excess of ethereal diazomethane. The ester was crystallized from acetone, giving 121 mg. (59%) of colorless product, m.p. 177–177.5°. An analytical sample recrystallized from the same solvent melted at 177.5–178°, $[\alpha]^{28}D$ +288° (*c* 1.1 in *n*-butyl acetate). The infrared spectrum (chloroform) of XV exhibits a broad intense band at 5.55-5.62 μ due to a combination of β-lactam absorption plus the weak phthalimide band.

*Anal.* Calcd. for $C_{17}H_{16}N_2O_5S$: C, 56.65; H, 4.48; N, 7.78. Found: C, 56.79; H, 4.73; N, 7.78.

Compound XV was compared with the racemic diastereomer, *viz.*, methyl DL-β-6-phthalimidopenicillanate (reported[25] m.p. 170–172°): the admixture melting point was 145–166°; the infrared spectra (chloroform) of the two compounds were identical in the carbonyl region (5–6 μ), but were distinctly different in the region between 7 and 15 μ.

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[CONTRIBUTION FROM THE DEPARTMENT OF CHEMISTRY OF STANFORD UNIVERSITY, STANFORD, CALIF.]

# Alkaloid Studies. XXXVI.[1] The Complete Absolute Configuration of the Diterpene Alkaloids of the *Garrya* and Atisine Groups and their Direct Correlation with the Phyllocladene-type Diterpenes[2]

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Conversion of the *Garrya* alkaloids garryfoline (I) and veatchine (II) into the 17-nor-16-ketones VIII and XXII established the absolute configuration at C-8 and C-13, since their rotatory dispersion curves were very similar to that of the 17-nor-16-ketone XI of the phyllocladene (X) series of established absolute configuration. A multistage degradation of garryfoline (I) *via* the azomethine XXVI of cuauchichicine led to the hydrocarbon XXXII, which proved to be identical with a degradation product of steviol (XXXIV) as well as with (−)-"β"-dihydrokaurene obtainable from the diterpene hydrocarbon kaurene (XXXIII). When combined with earlier rotatory dispersion evidence and conformational deductions, these results lead to the complete absolute configurational representations I and II for the principal *Garrya* alkaloids. In view of the chemical interconversion of veatchine (II) with atisine (III), the present absolute configurational assignment also applies to the *Aconitum* alkaloids related to atisine (III). It is noteworthy that these diterpene alkaloids and hence the diterpenes (−)-kaurene (XXXIII) and steviol (XXXIV) represent another group of natural products with the antipodal A/B stereochemistry as compared to the steroids or diterpenoids of the abietic acid class.

The chemical structures of the diterpene alkaloids from *Garrya* (*e.g.*, veatchine and garryfoline) and *Aconitum* (*e.g.*, atisine) species have been settled in recent years.[3] There remained two important outstanding problems—the determination of the absolute configuration of these alkaloids and their experimental interconversion with the diterpene hydrocarbons—and the present paper describes solutions[4] to both of them by a combination of chemical and optical rotatory dispersion[5] approaches.

The starting material for the present investigation was the bark of *Garrya laurifolia* Hartw. from which the alkaloid garryfoline (I) had been isolated earlier.[6] It was now found that varying amounts of veatchine (II)[7] are also present in the plant, but as these two alkaloids are known[6,8] to differ only in the stereochemistry of the C-15 hydroxyl group, they were used interchangeably for the subsequent transformations. It should be

(1) For paper XXXV see A. Sandoval, F. Walls, J. N. Shoolery, J. M. Wilson, H. Budzikiewicz and C. Djerassi, *Tetrahedron Letters*, No. 11 (1962), in press.

(2) Supported by grant No. 2G-682 from the National Heart Institute of the National Institutes of Health, U. S. Public Health Service.

(3) For reviews see: (a) K. Wiesner and Z. Valenta in L. Zechmeister's "Progress in the Chemistry of Organic Natural Products," Springer, Vienna, 1958, Vol. XVI, pp. 26–89; (b) S. W. Pelletier, *Tetrahedron*, **14**, 76 (1961).

(4) For preliminary communications see: (a) H. Vorbrueggen and C. Djerassi, *Tetrahedron Letters*, 119 (1961); (b) E. Mosettig, P. Quitt, U. Berlinger, J. A. Waters, H. Vorbrueggen and C. Djerassi, *J. Am. Chem. Soc.*, **83**, 3163 (1961); (c) C. Djerassi, P. Quitt, E. Mosettig, R. C. Cambie, P. S. Rutledge and L. H. Briggs, *ibid.*, **83**, 3720 (1961).

(5) See C. Djerassi, "Optical Rotatory Dispersion: Applications to Organic Chemistry," McGraw-Hill Book Co., Inc., New York, N. Y., 1960.

(6) C. Djerassi, C. R. Smith, A. E. Lippman, S. K. Figdor and J. Herran, *J. Am. Chem. Soc.*, **77**, 4801, 6633 (1955).

(7) J. F. Oneto, *J. Am. Pharm. Assoc.*, **35**, 204 (1946).

(8) K. Wiesner and J. A. Edwards, *Experientia*, **11**, 255 (1955); J. A. Edwards, Ph.D Thesis, University of New Brunswick, 1955 (cited on p. 41 of ref. 3a).

emphasized at this stage that any stereochemical conclusions reached with the *Garrya* alkaloids garryfoline (I) and veatchine (II) are directly applicable to atisine (III), a prototype of the *Aconitum* alkaloids, as these two groups of bases have been interrelated[9] through a common intermediate, as well as to all the other diterpenoid alkaloids with which an experimental connection has been achieved.[3]

Attention first was directed toward the hitherto undefined absolute configuration of the C/D ring juncture of these alkaloids. For this purpose, a mixture of garryfoline (I) and veatchine (II) was reduced with lithium aluminum hydride and directly acetylated since the crystalline F-dihydrogarryfoline diacetate (V) could be separated readily from the oily[10] C-15 epimer, F-dihydroveatchine diacetate. For further characterization, the diacetate V was hydrogenated[11] catalytically to the crystalline tetrahydrogarryfoline diacetate (VI). Lemieux–Johnson oxidation[12] of the terminal olefinic bond of F-dihydrogarryfoline diacetate (V) under carefully controlled conditions led in excellent yield to the ketol acetate VII, which was reduced with calcium in liquid ammonia[13] to the important 17-nor-16-oxo-15-deoxy-F-dihydrogarryfoline acetate (VIII). This ketone exhibited a positive Cotton effect of very similar amplitude to that observed[14] for the 17-nor-16-ketone XI from phyllocladene (X). Since the complete absolute configuration of phyllocladene (X) has been established[15] by a combination of chemical and optical rotatory dispersion arguments[16] and since the configuration at C-9 should not affect[17] the sign of the Cotton effect, the absolute configuration at C-8 and C-13 of the *Garrya* alkaloids (and hence of C-8 and C-12 of atisine (III)) can now be considered as established. Additional rotatory dispersion measurements with 15-ketones cited below corroborate this conclusion.

Next, there was attempted the elucidation of the C-9 stereochemistry by an approach which had been used earlier in the cafestol series.[18] For this purpose, veatchine (II) was transformed by the procedure of Dvornik and Edwards[19] into the diacetate chloride XVIII,[9] thence by internal Hofmann elimination to the azomethine acetate XIX[9] and finally by lithium aluminum hydride reduction and then acetylation to the acetoxy amide XX. Lemieux–Johnson oxidation[12] provided the ketol acetate amide XXI, which was reduced with calcium in liquid ammonia, as described above for the related garryfoline derivative VII, to the 17-nor-16-ketone XXII. As was to be expected its optical rotatory dispersion Cotton effect was very similar to that of the ketone of VIII derived from garryfoline (I). The availability of the two C-15 epimeric ketol acetates VII and XXI afforded additional useful rotatory dispersion evidence. Introduction of acetoxy groups adjacent to the carbonyl group of cyclopentanones generally does not affect the sign of the Cotton effect,[20] but does produce some wave length shifts in the position of the extrema depending upon the orientation of the acetoxy substituent. The Cotton effects of both ketol acetates VII and XXI exhibited the same sign (positive) as did the acetoxy-free analogs VIII and XXII. Of particular interest is the observation that the first extremum of the Cotton effect of the veatchine ketol acetate XXI occurred at 342 mμ as compared to 327.5 mμ for the ketol acetate VII of the garryfoline series. This points toward a more axial character for the acetoxy substituent in the veatchine series and such a conclusion is in complete concordance with the earlier deductions[6] from chemical studies in the garryfoline (I) and cuauchichicine series which lead to the C-15 stereochemistry shown in I.

Peracid oxidation of the 17-nor-16-ketone XXII readily afforded a lactone, to which is assigned structure XXIII by analogy to the identical re-

(9) S. W. Pelletier, *J. Am. Chem. Soc.*, **82**, 2398 (1960).

(10) K. Wiesner, W. I. Taylor, S. K. Figdor, M. F. Bartlett, J. R. Armstrong and J. A. Edwards, *Ber.*, **86**, 800 (1953).

(11) The β-configuration (steroid notation) is assigned to the methyl group on the assumption that hydrogenation proceeds from the less hindered α-side.

(12) R. Pappo, D. S. Allen, R. U. Lemieux and W. S. Johnson, *J. Org. Chem.*, **21**, 478 (1956). For a similar application in the atisine series see ref. 19.

(13) J. H. Chapman, J. Elks, G. H. Phillips and L. J. Wyman, *J. Chem. Soc.*, 4344 (1956); see also J. S. Mills, H. J. Ringold and C. Djerassi, *J. Am. Chem. Soc.*, **80**, 6118 (1958).

(14) C. Djerassi, R. Riniker and B. Riniker, *ibid.*, **78**, 6362 (1956).

(15) L. H. Briggs, B. F. Cain and R. C. Cambie, *Tetrahedron Letters*, **8**, 17 (1959); P. K. Grant and R. Hodges, *Tetrahedron*, **8**, 261 (1960).

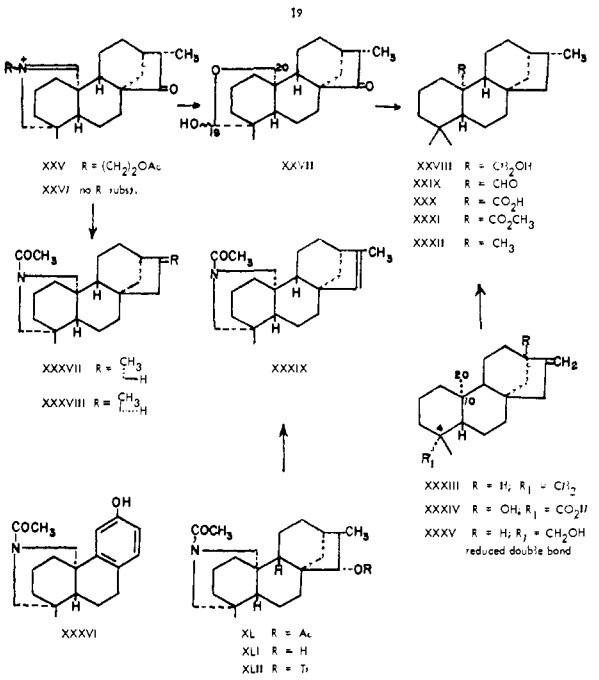
(16) See C. Djerassi, M. Cais and L. A. Mitscher, *J. Am. Chem. Soc.*, **81**, 2386 (1959).

(17) J. F. Grove, J. MacMillan, T. P. C. Mulholland and W. B. Turner, *J. Chem. Soc.*, 3049 (1960).

(18) R. A. Finnegan and C. Djerassi, *J. Am. Chem. Soc.*, **82**, 4342 (1960).

(19) D. Dvornik and O. E. Edwards, *Can. J. Chem.*, **35**, 860 (1957).

(20) C. Djerassi, O. Halpern, V. Halpern, O. Schindler and C. Tamm, *Helv. Chim. Acta*, **41**, 250 (1958). For an exception to this generalization see C. Djerassi, J. Fishman and T. Nambara, *Experientia*, **17**, 565 (1961).




action in a related cafestol derivative[18] and the usual course of other Baeyer–Villiger oxidations.[21] It was intended to open the lactone ring and to submit the hydroxy acid to successive methylation and oxidation. Application of the octant rule[22] to the resulting keto ester (analogous to XVII) would have yielded valuable information about the configuration of the C-9 center. However, the lactone XXIII proved remarkably stable to alkali and fairly vigorous conditions were required to open it. Moreover, acidification, even under the most careful conditions, led to relactonization so that the further steps could not be performed. The stability of the lactone ring was demonstrated further by treatment with lithium aluminum hydride, which caused first reduction of the amide grouping and only afforded the glycol XXIV on more extensive heating. In order to check this unusual behavior of the veatchine lactone XXIII, the 17-nor-16-ketone XI was prepared in 86% yield by Lemieux–Johnson oxidation[12] of phyllocladene (X) and then subjected to Baeyer–Villiger oxidation,[21] but in this instance the lactone XVI behaved in the anticipated manner and after alkaline opening, methylation and oxidation provided the keto ester XVII, with the expected[16] positive Cotton effect. The only explanation that can be offered for the difference in behavior between the lactones XXIII and XVI is that in the veatchine series (XXIII) the interaction between C-20 and the methylene groups at C-14 and C-12 produces a conformational distortion in ring C, which manifests itself in the reactivity of ring D substituents. As a further example of this altered reactivity, there may be cited the ready oxidation of the phyllocladene 16-ketone XI with selenium dioxide in acetic acid to the 15,16-diketone XV, while the veatchine 16-ketone XXII was recovered essentially unchanged under identical conditions.

In view of the failure to elucidate the C-9 stereochemistry by degradation of ring D, attention was directed toward an attempted interrelation between garryfoline (I) and one of the known diterpene hydrocarbons, since no such interconversion between these two groups of natural products had as yet been achieved. The starting material for this reaction sequence was the azomethine XXVI[6,10,23] which was prepared in improved yield *via* the acetate chloride XXV by the internal Hofmann procedure.[19] Elimination of the nitrogen function was accomplished in nearly quantitative yield by treatment of the azomethine XXVI with nitrous acid[24]; the resulting hemiacetal XXVII[25] was subjected to Wolff–Kishner reduction, which effected simultaneous reduction of the 15-keto and masked 19-aldehydo functions. Oxidation of the primary alcohol XXVIII with the chromium trioxide–pyridine reagent[26] provided the aldehyde XXIX and very vigorous Wolff–Kishner reduction transformed it into the hydrocarbon XXXII. *A priori*, one might have expected[24] the isomeric hemiacetal structure[27] (hydroxyl group at C-20 rather than at C-19 as in XXVII) from the nitrous acid treatment of the azomethine XXVI, but the following facts definitely establish the correctness of XXVII. The formation of XXVII is most readily visualized as proceeding through the alternate azomethine double bond isomer, as there exists ample precedent in the chemistry of the *Garrya* alkaloids[3a] for a mobile equilibrium between these two forms. The evidence for structure XXVII for the hemiacetal is based on the extremely hindered nature of the derived aldehyde XXIX and acid XXX. Thus, the aldehyde exhibited a negative Cotton effect in methanol solution, which remained unchanged upon the addition of hydrochloric acid[28] indicating the great resistance toward acetal formation; attempts to prepare a semicarbazone or ethylenethioketal proved fruitless. Furthermore, extraordinarily vigorous conditions (see Experimental) had to be employed in the Wolff–Kishner reduction in order to eliminate the carbonyl group.

(21) For reviews see C. H. Hassall, "Organic Reactions," John Wiley and Sons, Inc., New York, N. Y., 1957, Vol. IX, chap. 3.

(22) W. Moffitt, R. B. Woodward, A. Moscowitz, W. Klyne and C. Djerassi, *J. Am. Chem. Soc.*, **83**, 4013 (1961).

(23) K. Wiesner, R. Armstrong, M. F. Bartlett and J. A. Edwards, *ibid.*, **76**, 6068 (1954).

(24) This procedure was first developed in the atisine series by O. E. Edwards and R. Howe, *Proc. Chem. Soc.*, 62 (1959), who had assigned the unrearranged hemiacetal structure (hydroxyl at C-20 rather than at C-19) to their substance and who kindly sent us details of their experimental procedure. Subsequently, J. W. Apsimon, O. E. Edwards and R. Howe, *Can. J. Chem.*, **40**, 630 (1962); also concluded that the atisine hemiacetal has the same ring E structure as our garryfoline analog XXVII (see ref. 4a).

(25) Both garryfoline derivatives with a 15-keto group—the azomethine XXVI and the hemiacetal XXVII—exhibit negative Cotton effects as do the 15-ketones XIII and XIV of the phyllocladene series (R. Henderson and R. Hodges, *Tetrahedron*, **11**, 226 (1960) and unpublished experiments by Prof. L. H. Briggs and collaborators of the University of Auckland). This represents independent evidence for the correctness of our absolute configurational assignments at C-8 and C-13.

(26) G. I. Poos, G. E. Arth, R. E. Beyler and L. H. Sarett, *J. Am. Chem. Soc.*, **75**, 422 (1953).

(27) This would not have had any bearing on the structure of the ultimate hydrocarbon XXXII, which would have been obtained from either intermediate.

(28) C. Djerassi, L. A. Mitscher and B. J. Mitscher, *J. Am. Chem. Soc.*, **81**, 947 (1959).

Chromium trioxide oxidation of the alcohol XXVIII[29] in acetic acid solution led to the acid XXX, which was characterized further as the crystalline methyl ester XXXI. Through the coöperation of Dr. W. Simon (E. T. H., Zurich),[30] the apparent dissociation constant of the acid XXX was measured ($pK^*_{MCS}$ 9.49) and compared with that found for deoxypodocarpic acid ($pK^*_{MCS}$ 8.45) and for dehydroabietic acid ($pK^*_{MCS}$ 7.92), two models of diterpenes with axial and equatorial carboxyl groups attached to C-4. The much higher $pK$ value of the acid XXX eliminates C-4 as the possible site of attachment and supports the C-10 locus, for which a minimum $pK^*_{MCS}$ of 8.91 would be calculated by Simon's method.[30]

The crystalline hydrocarbon XXXII was not identical with the two known 16-epimeric dihydrophyllocladenes (XII),[31] thus eliminating immediately for the diterpene alkaloids the 5$\alpha$,9$\alpha$,-10$\beta$-stereochemistry (steroid notation) known[15] to exist in phyllocladene (X). The hydrocarbon was, however, identical[4b] with (−)-"$\beta$"-dihydrokaurene, the minor hydrogenation product[32] of (−)-kaurene (XXXIII),[32,33] a naturally occurring diterpene hydrocarbon known to be stereoisomeric with phyllocladene (X). Furthermore, (−)-"$\beta$"-dihydrokaurene (XXXII) is identical with "stevane-B,"[34] a degradation product of steviol (XXXIV).[35] The degradation of garryfoline (I) to the hydrocarbon XXXII therefore represents the first direct tie-up with two members (XXXIII, XXXIV) of the diterpene class. This interconversion[4b] also settles the remaining uncertain feature of the steviol structure (XXXIV),[34,35] namely, the location of the carboxyl group which could be attached to C-4 or C-10. The alcohol XXXV derived from steviol (XXXIV) and related to stevane-B (XXXII) is completely different from the isomeric alcohol XXVIII, obtained from garryfoline (I) and known to be substituted at C-20. It follows, therefore, that the carboxyl group of steviol (XXXIV) is attached to C-4 and this conclusion is in complete harmony with recent $pK^*_{MCS}$ measurements.[4b]

The above described proof of absolute configuration at C-8 and C-13, the non-identity of the hydrocarbon XXXII with the dihydrophyllocladenes (XII), the extremely hindered nature of the aldehyde XXIX, coupled with the earlier summarized[3b,36,37] stereochemically relevant properties of atisine (III) and the chemically interrelated diterpene alkaloid ajaconine, are only compatible with a 5$\beta$,10$\alpha$-ring juncture combined with a 9,10-*anti* backbone. This latter feature—requiring a 9$\beta$-oriented hydrogen atom—could be established independently[4c] by considering the rotatory dispersion curves of a number of 13-keto ring D *seco*-acids (of type XVII) derived from (−)-kaurene (XXXIII) and steviol (XXXIV) in the light of the octant rule.[22] The identical absolute stereochemistry XXXIII for (−)-kaurene was also derived recently[38] from studies on some new *Gibberella* metabolites. Finally, Edwards[39] succeeded recently in the stereochemically unambiguous synthesis of the antipode of the phenol XXXVI originally obtained[40] from atisine (III), thus providing independent evidence for the 5$\beta$,10$\alpha$-absolute configuration.

In summary, it can be stated that the absolute configurations implied in stereoformulas I, II, III, XXXIII and XXXIV for garryfoline, veatchine, atisine, (−)-kaurene and steviol are now completely settled in every detail and that these natural products (and their chemically related relatives[3]—*e.g.*, garryine, atidine, ajaconine, etc.) can be added to the rapidly increasing list of terpenoids[41] with the antipodal stereochemistry of the A/B ring fusion as compared to that of the steroids. It is also pertinent to note that in all but one instance, optical rotatory dispersion measurements played an important or crucial role in establishing this unusual stereochemical feature.

In a number of transformation products, a new asymmetric center is generated at C-16 and we are outlining below the evidence on which the stereochemical assignments of the center rest in the various stereoformulas. The azomethine X-XVI is prepared by acid rearrangement of I and subsequent alkaline treatment of XXV. Therefore, it can be assumed that the methyl group occupies the more stable $\alpha$ (*exo*) orientation. Wolff–Kishner reduction of the azomethine XXVI followed by catalytic hydrogenation of the azomethine double bond and N-acetylation provided the amide XXXVII, which was not identical with the isomer XXXVIII obtained by catalytic hydrogenation of the $\Delta^{15}$-olefin XXXIX. The latter, in turn, had been prepared from the veatchine azomethine acetate (XIX) by hydrogenation and

(29) The attachment of the primary alcohol grouping to C-20 rather than to C-19 is also indicated by the observation that the alcohol XXVIII could be eluted from an alumina column with 1:1 hexane–benzene, while benzene–ether mixtures were required for the hemiacetal XXVII.

(30) P. F. Sommer, V. P. Arya and W. Simon, *Tetrahedron Letters*, **20**, 18 (1960).

(31) C. W. Brandt, *New Zealand J. Sci. Techn.*, **20**, 8B (1938). We are indebted to Prof. L. H. Briggs of Auckland University for the two authentic specimens.

(32) L. H. Briggs, B. F. Cain, B. R. Davis and J. K. Wilmhurst, *Tetrahedron Letters*, **8**, 8 (1959).

(33) L. H. Briggs, B. F. Cain, R. C. Cambie and B. R. Davis, *ibid.*, **24**, 18 (1960).

(34) F. Dolder, H. Lichti, E. Mosettig and P. Quitt, *J. Am. Chem. Soc.*, **82**, 246 (1960).

(35) E. Mosettig and W. R. Nes, *J. Org. Chem.*, **20**, 884 (1955).

(36) A. J. Solo and S. W. Pelletier, *Chemistry & Industry*, 1108 (1960).

(37) D. Dvornik and O. E. Edwards, *Tetrahedron*, **14**, 54 (1961).

(38) B. E. Cross, R. H. B. Galt, J. R. Hanson and W. Klyne, *Tetrahedron Letters*, 145 (1962).

(39) O. E. Edwards, private communication. The synthesis involved the recently described (J. Apsimon and O. E. Edwards, *Proc. Chem. Soc.*, 461 (1961)) photolysis of a suitably substituted podocarpic acid azide.

(40) D. Dvornik and O. E. Edwards, *Chemistry & Industry*, 623 (1958).

(41) *Eperuic acid:* J. D. Cocker and T. G. Halsall, *J. Chem. Soc.*, 4262 (1956), and C. Djerassi and D. Marshall, *Tetrahedron*, **1**, 238 (1958); *iresin:* C. Djerassi and S. Burstein, *J. Am. Chem. Soc.*, **80**, 2593 (1958); *farnesiferol-A:* L. Caglioti, H. Naef, D. Arigoni and O. Jeger, *Helv. Chim. Acta*, **41**, 2278 (1958); *cafestol:* ref. 14 and 16; *andrographolide:* M. P. Cava and B. Weinstein, *Chemistry & Industry*, 851 (1959); *aconitine:* M. Przybylska and L. Marion, *Can. J. Chem.*, **37**, 1843 (1959); *darutigenol:* A. Diara, C. Asselineau and E. Lederer, *Bull. soc. chim. France*, 2171 (1960); *copalic acid:* T. Nakano and C. Djerassi, *J. Org. Chem.*, **26**, 167 (1961); *polyalthic acid:* K. W. Gopinath, T. R. Govindachari, P. C. Parthasarathy and N. Viswanathan, *Helv. Chim. Acta*, **44**, 1040 (1961); *manniellic acid:* J. Haeuser, R. Lombard, F. Lederer and G. Ourisson, *Tetrahedron*, **12**, 205 (1961).

acetylation to XL, partial saponification to the N-acetyl alcohol XLI, followed by tosylation (XLII) and dehydrotosylation[42] to XXXIX. Inspection of models shows that catalytic hydrogenation of the olefin in XXXIX would clearly proceed from the less hindered $\alpha$-side to generate the thermodynamically less favored $\beta$-oriented methyl isomer XXXVIII in contrast to the $\alpha$-isomer (XXXVII) produced during the Wolff–Kishner reduction. The same $\alpha$-orientation must then be assigned to the methyl group of the hydrocarbon XXXII ("$\beta$"-dihydrokaurene), which is the minor product[32] of the catalytic hydrogenation of (−)-kaurene (XXXIII). This again is reasonable, since one would expect hydrogenation of the exocyclic methylene group to proceed predominantly from the $\alpha$-side to give (−)-"$\alpha$"-dihydrokaurene possessing a $\beta$-oriented methyl group.

## Experimental[43]

**Extraction of Alkaloids.**—The earlier recorded[6] extraction scheme was modified in the following manner. The evaporated alcoholic extract (2.4 kg.) of newly collected[44] bark of *Garrya laurifolia* Hartw. was stirred with 6 l. of 10% acetic acid and 6 l. of methylene chloride until practically all of the residue had dissolved. Celite was added and stirring continued for 1 hr. After filtering the dark mixture over Celite, the layers were separated, the aqueous phase cooled to 4° and made strongly alkaline with cold 50% sodium hydroxide. The fast darkening mixture was immediately extracted three times with methylene chloride and any emulsion formed filtered over Celite. The combined methylene chloride phase was immediately extracted with 3 l. of 10% acetic acid and the organic phase discarded. The aqueous layer was again cooled, made alkaline and extracted with methylene chloride to give after drying and evaporation of the solvent 195 g. of a crystalline mixture of garryfoline (I) and veatchine (II). For the following reactions the alkaloids *per se* were not separated but rather purified at a subsequent stage as indicated below.

**F-Dihydrogarryfoline Diacetate (V).**—The crude alkaloid mixture (30.8 g.) dissolved in 150 cc. of ether was added dropwise to 12 g. of lithium aluminum hydride suspended in 900 cc. of ether. After heating under reflux for 8 hr., the excess reagent was decomposed by the addition of a saturated sodium sulfate solution, the inorganic material filtered and washed well with ether. Evaporation of the dried ether extract afforded a colorless gum (29 g.), which crystallized from acetonitrile upon seeding with F-dihydrogarryfoline (IV).[6] For the present purposes, the total crude mixture was acetylated (40 hr., room temperature) with 50 cc. of acetic anhydride and 25 cc. of pyridine. Repeated evaporation to dryness (using toluene or benzene) and crystallization from 50 cc. of methanol provided in two crops 15.1 g. of F-dihydrogarryfoline diacetate (V), m.p. 102–103°, the oily[10] F-dihydroveatchine diacetate remaining in the mother liquors. The analytical sample crystallized from methanol as colorless needles, m.p. 105–106°, $[\alpha]_D$ −83° (*c* 0.59).

*Anal.* Calcd. for $C_{26}H_{39}NO_4$: C, 72.69; H, 9.15; N, 3.26. Found: C, 72.91; H, 9.08; N, 3.32.

**Tetrahydrogarryfoline Diacetate (VI).**—Hydrogenation of 3.58 g. of F-dihydrogarryfoline diacetate (V) was effected in 125 cc. of ethanol with 330 mg. of 10% palladized charcoal catalyst over a period of 2.5 hr. Crystallization of the crude product from methanol afforded 2.63 g. of crystals, m.p. 76–83°, raised to 84–86° (2.13 g.) upon one recrystallization. The analytical specimen exhibited m.p. 87–88°, $[\alpha]_D$ −51° (*c* 0.48).

*Anal.* Calcd. for $C_{26}H_{41}NO_3$: C, 72.35; H, 9.58; N, 3.25. Found: C, 72.63; H, 9.78; N, 3.22.

**17-Nor-16-oxo-F-dihydrogarryfoline Diacetate (VII).**—F-Dihydrogarryfoline diacetate (V) (2.15 g.) was dissolved in 80% acetic acid (150 cc.), cooled to 4° and sodium paraperiodate (3.23 g.) and osmium tetroxide (0.044 g.) added. The mixture was stirred for 20 hr. at 4°, filtered, the white residue washed with acetic acid and the slightly yellowish solution evaporated at 40° (20 mm.). Water was added and the solution again evaporated to give a white crystalline residue, which was partitioned between methylene chloride and sodium bicarbonate solution. The layers were separated, the aqueous phase was extracted two additional times with methylene chloride and after drying and evaporating, a slightly yellowish crystalline residue (2.026 g.) was obtained. Recrystallization from methanol yielded 1.785 g. of long, colorless needles of the ketol acetate VII, m.p. 145–146° which was homogeneous on thin layer chromatography (benzene–ethyl acetate 4:1). One further recrystallization provided the analytical sample, m.p. 146–147°, $[\alpha]_D$ −82° (*c* 0.90), $\lambda_{max}^{CHCl_3}$ 5.70–5.75 $\mu$ (broad band) and 8.15 $\mu$; R. D. (*c* 0.11 in dioxane): $[\alpha]_{589}$ −58°, $[\alpha]_{327.5}$ +687°, $[\alpha]_{287.5}$ −1674°, $[\alpha]_{265}$ −1488°.

*Anal.* Calcd. for $C_{25}H_{37}NO_5$: C, 69.57; H, 8.64; N, 3.25; acetyl, 19.20. Found: C, 69.28; H, 8.68; N, 3.21; acetyl, 18.27.

**17-Nor-16-oxo-15-deoxy-L-dihydrogarryfoline Acetate (VIII).**—A solution of 432 mg. of the ketol acetate VII in 20 cc. of tetrahydrofuran (redistilled from lithium aluminum hydride) was added with vigorous stirring over a period of 5 min. at −70° to 567 mg. of calcium in 60 cc. of liquid ammonia and 20 cc. of dry tetrahydrofuran. After stirring for a further 3 min., the mixture was poured on crushed ice and extracted with methylene chloride. Since t.l.c. analysis (benzene–ethyl acetate 8:1) of the total product indicated partial hydrolysis of the acetate functions, the material was acetylated at room temperature with acetic anhydride–pyridine and then chromatographed on 34 g. of neutral alumina. The first benzene eluates furnished 40 mg. of the over-reduction product **17-nor-16-acetoxy-15-deoxy-F-dihydrogarryfoline acetate (IX)**, m.p. 97–98° (from methanol), $[\alpha]_D$ −40° (*c* 0.82), $\lambda_{max}^{CHCl_3}$ 5.79 and 7.95 $\mu$.

*Anal.* Calcd. for $C_{25}H_{39}NO_4$: C, 71.89; H, 9.41. Found: C, 71.83; H, 9.58.

Further elution with benzene gave the pure **ketone VIII** (60 mg.), which exhibited after recrystallization from methanol, m.p. 69–70°, $[\alpha]_D$ −48° (*c* 0.59), $\lambda_{max}^{CHCl_3}$ 5.74 and 6.85 $\mu$; R. D. (*c* 0.12 in dioxane): $[\alpha]_{589}$ −27°, $[\alpha]_{323}$ +1525°, $[\alpha]_{312}$ +687° (shoulder), $[\alpha]_{281}$ −2070°, $[\alpha]_{260}$ −1730°.

*Anal.* Calcd. for $C_{23}H_{35}O_3N$: C, 73.95; H, 9.45. Found: C, 74.08; H, 9.48.

Elution with benzene–ether mixtures afforded 152 mg. of recovered starting material VII, m.p. 140–146°. Attempts to increase the yield of ketone VIII by increasing the amount of calcium or extending the reaction time led only to larger amounts of the over-reduction product IX.

**Azomethines XIX and XXVI from Veatchine and Cuauchichicine.**—Hydrogen chloride gas was passed at 0° into a solution of 84 g. of crude garryfoline–veatchine mixture in 500 ml. of ether and after evaporating *in vacuo*, the residue was heated on the steam-bath for 20 min. with 400 cc. of acetic anhydride and 200 cc. of pyridine, cooled and then stirred overnight with 500 cc. of chloroform. Most of the solvent was removed and after keeping at 4° for 2 days, the crystals were filtered and washed with acetone; yield 88 g., m.p. 228–230°. Recrystallization from ethanol–hexane provided 62.4 g. of colorless crystals of veatchine diacetate chloride (XVIII) and cuauchichicine acetate chloride (XXV) m.p. 244–245°, which was used directly in the next step.

To a vigorously stirred (Vibro Mixer) solution of 9.28 g. of mixed chlorides XVIII and XXV in 20 cc. of chloroform and 15 cc. of water was added 24 cc. of 40% potassium hydroxide solution and after 1 min. stirring, the layers were separated and the aqueous solution re-extracted with chloroform. The combined washed and dried chloroform extracts were evaporated slowly in an open erlenmeyer flask over a period of 1.5 hr. to give ultimately 7.75 g. of light orange gum. The combined crude product (45.5 g.) from five such experiments was dissolved in 200 cc. of methylene chloride and

(42) H. R. Nace, *J. Am. Chem. Soc.*, **81**, 5428 (1959).

(43) All melting points were taken in capillaries and are uncorrected. All rotations were measured in chloroform solution. Thin-layer chromatography (t.l.c.) was performed on silica gel G (E. Merck) plates while column chromatography was performed on E. Merck neutral alumina (activity II unless noted otherwise). We are indebted to Mr. E. Meier and Mr. J. Consul of the Stanford University Microanalytical Laboratory for all microanalyses and Mrs. Ruth Records for the optical rotatory dispersion curves.

(44) Collected by Dr. D. K. Cox (Botanical Department, Syntex, S. A., Mexico City) near Rio Hondo, Huixquilucan (State of Mexico).

diluted with 400 cc. of hexane. The methylene chloride was distilled off and the hexane solution was decanted from the resulting precipitate, which was redissolved in methylene chloride and the process repeated twice. The combined hexane solutions yielded 31 g. of soluble material which was shown by t.l.c. (ethyl acetate) to consist approximately of 60% of XIX and 20% of XXVI together with unidentified material. Chromatography on 1.5 kg. of alumina and elution with hexane–benzene and pure benzene gave 9.8 g. of veatchine acetate azomethine (XIX), 5.0 g. of cuauchichicine azomethine (XXVI) and *ca.* 10 g. of intermediate fractions consisting of mixtures of XIX and XXVI. Recrystallization from hexane provided pure **veatchine acetate azomethine (XIX)**, m.p. 119–120°, $[\alpha]_D$ −84° (*c* 0.89), $\lambda_{max}^{KBr}$ 5.80 and 6.08 μ, which proved to be identical by direct comparison with a specimen[9] furnished by Dr. S. W. Pelletier of the Rockefeller Institute.

*Anal.* Calcd. for $C_{22}H_{31}NO_2$: C, 77.37; H, 9.15; N, 4.10. Found: C, 77.02; H, 9.10; N, 4.20.

Similar recrystallization of **cuauchichicine azomethine (XXVI)** gave the analytical sample, m.p. 137–138°, $[\alpha]_D$ −114° (*c* 0.72), $\lambda_{max}^{CHCl_3}$ 5.79 and 6.07 μ; R. D. (*c* 0.095 in dioxane): $[\alpha]_{589}$ −90°, $[\alpha]_{325}$ −1150°, $[\alpha]_{302}$ −112°, $[\alpha]_{267.5}$ −1178°. This material proved to be identical with the earlier prepared[6,10] selenium pyrolysis product.

*Anal.* Calcd. for $C_{20}H_{27}NO$: C, 80.22; H, 9.76; N, 4.68. Found: C, 80.39; H, 9.32; N, 4.43.

**Transformations of Veatchine Acetate Azomethine (XIX).**—The azomethine XIX (10.57 g.) was reduced in ether solution with lithium aluminum hydride (10 g.) by heating under reflux for 5.5 hr. and then stirring at room temperature overnight. After decomposition with sodium sulfate, the resulting secondary amine (m.p. 160–167°) was directly acetylated (20 hr., 23°, acetic anhydride–pyridine) and purified by chromatography on 400 g. of alumina and elution with benzene–ether mixtures. **The O,N-diacetate XX** (9.7 g., m.p. 110–120°) was recrystallized from ether, whereupon it exhibited m.p. 125–126°, $[\alpha]_D$ −49° (*c* 1.14), $\lambda_{max}^{CHCl_3}$ 5.81 and 6.18 μ.

*Anal.* Calcd. for $C_{24}H_{35}NO_3$: C, 74.76; H, 9.15. Found: C, 74.57; H, 9.06.

The above O,N-diacetate XX (1.158 g.) was dissolved in 75 cc. of 80% acetic acid and stirred for 5 hr. with osmium tetroxide (75 mg.) until maximum darkness of the solution was reached. The solution was cooled to 4° and sodium paraperiodate (600 mg.) was added and stirring continued for 12 hr., when the second portion of periodate (600 mg.) was added, followed 12 hr. later by the third and last addition of periodate (620 mg.). After 12 hr. at 4°, the reaction mixture was stirred for 1 hr. at room temperature, filtered, the white precipitate washed with acetic acid, the filtrate evaporated at 40° (25 mm.), water was added and the evaporation repeated. The colorless residue was taken up in a mixture of methylene chloride and sodium bicarbonate solution and shaken until the evolution of carbon dioxide ceased. The phases were separated, the aqueous phase extracted two more times with methylene chloride and the combined methylene chloride solutions dried and evaporated. The crude product (1.07 g.) was recrystallized from ethyl acetate to give 0.88 g. of the **ketol acetate amide XXI**, m.p. 180–181°. The analytical specimen was obtained from the same solvent, m.p. 185–186°, $[\alpha]_D$ + 43° (*c* 0.55); $\lambda_{max}^{CHCl_3}$ 5.71, 5.76 and 6.16 μ; R. D. (*c* 0.103 in dioxane): $[\alpha]_{589}$ +19°, $[\alpha]_{342}$ +2580°, $[\alpha]_{328}$ +1510°, $[\alpha]_{295}$ −2640°, $[\alpha]_{260}$ −1470°.

*Anal.* Calcd. for $C_{23}H_{33}NO_4$: C, 71.29; H, 8.58; N, 3.61. Found: C, 71.37; H, 8.74; N, 3.40.

The above ketol acetate amide XXI (4.0 g.) was reduced with 2.33 g. of calcium and 600 cc. of liquid ammonia in 250 cc. of dry tetrahydrofuran (18 min. addition time, 5 min. additional stirring) as described above for the ketol acetate VII from garryfoline. The infrared spectrum of the crude product (3.0 g.) showed partial loss of the amide function and the material, therefore, was acetylated at room temperature for 2 hr. with acetic anhydride and pyridine before submitting it to chromatography on 150 g. of alumina. Benzene and benzene–ether (1:1) eluted 0.8 g. of oily material, which according to infrared analysis and t.l.c. represented over-reduction product (analogous to IX). Increasing amounts of ether eluted 1.77 g. (m.p. 160–162°) of crystalline **17-nor-16-ketone XXII**, the analytical sample of which crystallized from hexane; m.p. 163–164°, $[\alpha]_D$ −24° (*c* 0.83), $\lambda_{max}^{CHCl_3}$ 5.79 and 6.19 μ; R. D. (*c* 0.16 in dioxane): $[\alpha]_{589}$ −38°, $[\alpha]_{322.5}$ +1996°, $[\alpha]_{315}$ +1075° (shoulder), $[\alpha]_{280}$ −2018°, $[\alpha]_{250}$ −1037°.

*Anal.* Calcd. for $C_{21}H_{31}NO_2$: C, 76.55; H, 9.48; N, 4.25. Found: C, 76.27; H, 9.56; N, 4.43.

Conversion of the 17-nor-16-ketone XXII to the **lactone XXIII** was accomplished by oxidation with trifluoroperacetic acid.[45] For this purpose, 10.5 cc. of freshly distilled trifluoroacetic anhydride was dissolved in 50 cc. of dry methylene chloride and 2.1 cc. of 90% hydrogen peroxide was added to the ice-cold solution. After stirring for 1 hr. with exclusion of moisture, the solution was added slowly over a period of 20 min. to an ice-cold mixture of 2.19 g. of the ketone XXII, 30 g. of anhydrous disodium hydrogen phosphate and 200 cc. of methylene chloride. The mixture was permitted to warm to room temperature, then heated under reflux for 4 hr. and finally left at room temperature overnight. The product (2.38 g.) was isolated with methylene chloride and chromatographed on 120 g. of alumina (activity IV). Elution with benzene–ether mixtures and recrystallization from methylene chloride–cyclohexane gave 1.34 g. of the **lactone XXIII**, m.p. 170–171°, $[\alpha]_D$ −58°, $\lambda_{max}^{KBr}$ 5.83 and 6.11 μ.

*Anal.* Calcd. for $C_{21}H_{31}NO_3$: C, 73.00; H, 9.05; N, 4.05. Found: C, 73.11; H, 9.11; N, 4.08.

Heating for 12 hr. with 2% methanolic potassium hydroxide solution was required to open the lactone ring and even very careful acidification resulted in immediate relactonization.

The above lactone XXIII (373 mg.) was heated under reflux for 18 hr. with 1.6 g. of lithium aluminum hydride in 30 cc. of tetrahydrofuran and after processing in the usual manner and recrystallizing from cyclohexane, there was obtained 224 mg. of the **N-ethyl glycol XXIV**, m.p. 177–179°. Evaporation of the mother liquors and infrared examination showed the complete absence of the amide band but the existence of a moderate lactone peak. Consequently, the mother liquor material was reduced for an additional 36 hr. with lithium aluminum hydride affording a total yield of 309 mg. of glycol XXIV, the analytical specimen (no infrared carbonyl absorption) of which showed m.p. 181–182°, $[\alpha]_D$ (*c* 1.31) after recrystallization from benzene–cyclohexane.

*Anal.* Calcd. for $C_{21}H_{37}NO_2$: C, 75.17; H, 11.12. Found: C, 74.77; H, 10.89.

**Transformations of Phyllocladene (X).**—A solution of 816 mg. of phyllocladene (X), kindly provided by Dr. R. Hodges of the University of Glasgow, in 50 cc. of dioxane, 15 cc. of water and 10 cc. of acetic acid was stirred for 10 min. with 12 mg. of osmium tetroxide and to the rapidly darkening solution was added in portions 1.35 g. of sodium metaperiodate over a period of 30 min. After stirring at room temperature for 22 hr., the reaction mixture was processed in the usual manner to provide after chromatography and elution with benzene 711 mg. of the **17-nor-16-ketone XI**,[46] m.p. 100–101°. A sample (608 mg.) of this ketone was oxidized with trifluoroperacetic acid[45] exactly as described above for the veatchine ketone XXII to afford 629 mg. of **lactone XVI**,[47] m.p. 144–148°, which was practically homogeneous by t.l.c. (benzene–ethyl acetate 3:1). Two recrystallizations from methanol led to the analytical sample, m.p. 153–154°, $[\alpha]_D$ −6° (*c* 1.26), $\lambda_{max}^{CHCl_3}$ 5.85 μ.

*Anal.* Calcd. for $C_{19}H_{30}O_2$: C, 78.57; H, 10.41. Found: C, 78.51; H, 10.33.

The lactone ring was opened by heating under reflux for 11 hr. 125 mg. of the lactone XVI with 20 cc. of methanol and 630 mg. of potassium hydroxide. Dilution with water, ether extraction (discarded), gradual addition over a 90-min. period of 11 cc. of 2 *N* sulfuric acid at 0° and extraction with methylene chloride gave 132 mg. of gummy hydroxy acid, which was methylated with ethereal diazomethane (20 min.) and the hydroxy methyl ester (142 mg.) in 15 cc. of pyridine oxidized at 0° by adding it gradually to 97 mg. of chromium trioxide mixed with 10 cc. of pyridine. After stirring at room temperature for 17 hr., the crude **keto ester XVII**[46] (115

(45) W. D. Emmons and G. B. Lucas, *J. Am. Chem. Soc.*, **77**, 2287 (1955).

(46) W. Bottomley, A. R. H. Cole and D. E. White, *J. Chem. Soc.*, 2624 (1955).

(47) This substance has also been synthesized in the laboratory of Prof. L. H. Briggs (private communication).

mg., m.p. 110–145°) was recrystallized from methanol to afford 40 mg. of the pure substance, m.p. 175–176°, $[\alpha]_D$ +18° (*c* 1.45), $\lambda_{max}^{CHCl_3}$ 5.80 μ; R. D. (*c* 0.06 in methanol): $[\alpha]_{589}$ +24°, $[\alpha]_{313}$ +1045°, $[\alpha]_{271}$ −1250°, $[\alpha]_{250}$ −885°. The mother liquors (65 mg.) consisted of *ca.* 20% keto ester XVII and 60–80% of lactone XVI as determined by t.l.c. (benzene–ethyl acetate 2:1).

*Anal.* Calcd. for $C_{20}H_{32}O_3$: C, 74.96; H, 10.06. Found: C, 75.05; H, 9.89.

For the preparation of the **17-nor-15,16-diketone XV**, a solution of 137 mg. of the ketone XI in 8 cc. of acetic acid was heated under reflux for 40 hr. with 60 mg. of freshly sublimed selenium dioxide. The product was extracted with methylene dichloride and purified by chromatography on 18 g. of alumina, elution with benzene–ether (1:1) and recrystallization from hexane, whereupon 60 mg. of the yellow diketone XV was obtained, m.p. 213–214°, $[\alpha]_D$ +282° (*c* 0.63), $\lambda_{max}^{CHCl_3}$ 5.72 and 5.78 μ; R. D.[48] (*c* 0.051 in isoöctane) $[\alpha]_{589}$ +282°, $[\alpha]_{514}$ +1630°, $[\alpha]_{452}$ −1380°, $[\alpha]_{378}$ −830°, $[\alpha]_{358}$ − 1000° (shoulder), $[\alpha]_{335}$ − 1260°, $[\alpha]_{327}$ − 1160°, $[\alpha]_{32\text{-}}$ − 1430°, $[\alpha]_{311}$ − 1030°, $[\alpha]_{303}$ −1170°, $[\alpha]_{300}$ −730°, $[\alpha]_{288}$ −760°, $[\alpha]_{268}$ −100°.

*Anal.* Calcd. for $C_{19}H_{28}O_2$: C, 79.12; H, 9.79. Found: C, 78.95; H, 9.67.

When the veatchine ketone XXII was exposed to the same conditions, infrared examination of the oily product showed the presence of only trace amounts of the diketone.

**Conversion of Cuauchichicine Azomethine (XXVI) to (−)-"β"-Dihydrokaurene (XXXII).**—A mixture of 20 cc. of acetic acid and 50 cc. of peroxide-free dioxane was added over a period of 2.5 hr. at room temperature in a current of nitrogen to a solution of 3.0 g. of cuauchichicine azomethine (XXVI), sodium nitrite (7.0 g.) and anhydrous sodium acetate (2.5 g.) in 400 cc. of dioxane and 200 cc. of water. After 22 hr. stirring, the solution was evaporated to dryness, the residue partitioned between ether and water, and the washed and dried ether solution evaporated to furnish 3.13 g. of crystals (m.p. 178–192°), which were recrystallized from benzene to afford 2.6 g. of the **hemiketal XXVII**, m.p. 200–203°. Chromatography of the mother liquors provided an additional 280 mg. of identical purity, thus raising the yield to over 90%. The analytical specimen was recrystallized again from benzene and exhibited m.p. 203–205°, $[\alpha]_D$ −128° (*c*, 1.13), $\lambda_{max}^{CHCl_3}$ 5.80 μ; R. D. (*c* 0.102 in dioxane): $[\alpha]_{589}$ −94°, $[\alpha]_{322.5}$ −1494°, $[\alpha]_{290}$ +368°, $[\alpha]_{270}$ −63°.

*Anal.* Calcd. for $C_{20}H_{30}O_3$: C, 75.43; H, 9.50; O, 15.07. Found: C, 75.77; H, 9.54; O, 14.89.

Using the identical conditions, veatchine acetate azomethine (XIX) was transformed into an analogous hemiacetal, which was recrystallized from ethanol, m.p. 156–157°, $[\alpha]_D$ −63° (*c* 0.89).

*Anal.* Calcd. for $C_{22}H_{32}O_4$: C, 73.30; H, 8.95; O, 17.75. Found: C, 73.54; H. 8.48; O, 17.79.

Wolff–Kishner reduction of the cuauchichicine hemiacetal XXVII was performed by Huang-Minlon's modification[49] and involved initial refluxing for 3.5 hr. of 2.68 g. of hemiacetal XXVII with 10 cc. of 95% hydrazine in 50 cc. of diethylene glycol and 15 cc. of 1-butanol. Potassium hydroxide (3.6 g.) was added and solvent distilled off over a period of 3 hr. until the inside temperature had reached 210°, whereupon refluxing was continued for 14 hr. Dilution with water, extraction with ether and purification by chromatography on 130 g. of alumina afforded in the hexane–benzene (1:1) eluates 1.23 g. of the primary **alcohol XXVIII** (20-hydroxy-"β"-dihydrokaurene), m.p. 80–85°. The analytical sample was recrystallized from acetonitrile; m.p. 89.5–90°, $[\alpha]_D$ −47° (*c* 1.09).

*Anal.* Calcd. for $C_{20}H_{34}O$: C, 82.69; H, 11.80; O, 5.51. Found: C, 82.95; H, 11.93; O, 5.49.

The above alcohol XXVIII (101 mg., m.p. 89–90°) in 10 cc. of pyridine was added over a period of 4 hr. to a slurry of 105 mg. of chromium trioxide in 10 cc. of pyridine and then stirred for an additional 14 hr., all operations being conducted in a current of nitrogen. Dilution with water, extraction with ether and chromatography on 30 g. of alumina gave in the hexane eluates 70 mg. of **aldehyde XXIX**, m.p. 75–83°. This material was very sensitive to oxidation and recrystallization from methanol gradually raised the melting point until it reached that (m.p. 213–215°) of the acid XXX. Consequently, the aldehyde was only characterized by its infrared spectrum ($\lambda_{max}^{CHCl_3}$ 3.60 and 5.88 μ) and rotatory dispersion curve (*c* 0.105 in methanol; unchanged upon addition of concd. hydrochloric acid[23]): $[\alpha]_{589}$ −71°, $[\alpha]_{320}$ −818°, $[\alpha]_{277.5}$ +218°, $[\alpha]_{270}$ +135°, and was then subjected directly to Wolff–Kishner reduction. The aldehyde was recovered unchanged on attempted semicarbazone (5 hr. refluxing) or ethylene thioketal (boron trifluoride procedure[50] for 2 hr.) formation, while the corresponding steviol (XXXIV) derivative with the aldehyde function attached to C-4 reacted readily.[34,35]

The crystalline aldehyde XXIX (62 mg.) was heated under reflux for 18 hr. with 10 cc. of 95% hydrazine, 50 cc. of ethylene glycol and 1.75 g. of potassium hydroxide, the mixture was then concentrated by distillation (4 hr.) until the inside temperature had reached 217° and refluxing was continued for 3 days. After processing in the usual way, chromatography on 30 g. of acidic alumina (activity I) gave on elution with hexane 14 mg. of hydrocarbon, while ether removed 41 mg. of crude unreacted aldehyde XXIX, the infrared spectrum of which showed some hydrazone band at 6.1 μ. The hydrocarbon was crystallized from acetonitrile to furnish 6.4 mg. of long needles of (−)-"β"-**dihydrokaurene (XXXII)**, m.p. 51–52°, $[\alpha]_D$ −43° (*c* 1.85), which gave a 15° depression upon admixture with "β"-dihydrophyllocladene (XII).[31] Identity with "stevane-B,"[34] kindly provided by Dr. E. Mosettig (National Institutes of Health) and earlier[34] identified with (−)-"β"-dihydrokaurene (XXXII),[32] was established by mixture melting point determination, infrared comparison and gas phase chromatography (SE-30 column, 175° using a Wilkens Instrument Co., Hy-Fl, model 600).

*Anal.* Calcd. for $C_{20}H_{34}$: C, 87.51; H, 12.49. Found (on 1.2 mg.): C, 87.9; H, 12.3.

A specimen (110 mg.) of the alcohol XXVIII (m.p. 81–83°) in 15 cc. of acetic acid was oxidized over a period of 1 hr. at 14° by gradually adding 0.5 cc. of a solution of chromium trioxide (5.1 g.) in water (15 cc.) and sulfuric acid (4.5 cc.). Extraction with ether, evaporation to dryness, extraction with boiling hexane and sublimation of the soluble material afforded a small amount of oil distilling at 160–180° (0.3 mm.), then 100 mg. of long needles (m.p. 194–204°) of the **acid XXX**, which came over at 165° (0.05 mm.). Recrystallization from methanol provided the analytically pure acid, m.p. 213–215°, $[\alpha]_D$ −40° (*c* 1.4), $pK^*_{mcs}$ 9.49 ± 0.07.[30]

*Anal.* Calcd. for $C_{20}H_{32}O_2$: C, 78.89; H, 10.59. Found: C, 78.72; H, 10.54.

A small sample of the acid was transformed with diazomethane into the methyl ester XXXI, which was recrystallized from methanol; m.p. 80–81°.

**Conversion of Cuauchichicine Azomethine (XXVI) to the Amide XXXVII.**—A solution of 271 mg. of the azomethine XXVI in diethylene glycol (40 cc.) and 95% hydrazine (5 cc.) was heated under reflux for 16 hr., potassium hydroxide (0.87 g.) added and the mixture distilled (2 hr.) until the inside temperature had risen to 210°. After heating for 10 hr. at that temperature, dilution with water, ether extraction, chromatography on 25 g. of alumina and benzene elution, there was obtained 69 mg. of crystals, the infrared spectrum of which showed the disappearance of the carbonyl group and the presence of the azomethine double bond. The substance was hydrogenated without further purification at room temperature in 25 cc. of acetic acid with 49 mg. of platinum oxide catalyst and the resulting product acetylated with acetic anhydride and pyridine (2 hr., steam-bath). Chromatography on 25 g. of alumina and benzene–ether (8:2) elution provided 68 mg. of the **amide XXXVII**, m.p. 111–113°, the analytical sample of which crystallized from hexane; m.p. 114.5–115°, $[\alpha]_D$ −85° (*c* 0.76).

*Anal.* Calcd. for $C_{22}H_{35}NO$: C, 80.19; H, 10.71. Found: C, 80.37; H, 10.43.

**Conversion of Veatchine Acetate Azomethine (XIX) to the Amide XXXVIII.**—Platinum oxide catalyst (196 mg.) in acetic acid (35 cc.) was prereduced and the azomethine XIX of veatchine acetate (2.282 g., m.p. 119–120°) added and hydrogenated. After 4 hr. the hydrogen consumption was

(48) For rotatory dispersion of other non-enolizable diketones see H. P. Gervais and A. Rassat, *Bull. soc. chim. France*, 743 (1961).

(49) Huang-Minlon, *J. Am. Chem. Soc.*, **68**, 2487 (1946).

(50) L. F. Fieser, *ibid.*, **76**, 1945 (1954).

constant (368 cc.), the catalyst was filtered, washed with acetic acid and the filtrate evaporated under reduced pressure. The oily residue was heated with acetic anhydride (40 cc.) and pyridine (20 cc.) for 5 hr. on the steam-bath. Evaporation *in vacuo* with addition of toluene (repeated 5 times) yielded the **diacetate XL**, which was partially saponified with 0.324 g. of sodium hydroxide in methanol (60 cc.) by heating under reflux for 4 hr. and keeping for 10 hr. at room temperature. The methanol was removed, the residue was partitioned between ether and water and the yellowish aqueous phase extracted with ether. Evaporation of the washed and dried ether extract yielded a colorless gum (2.06 g.), the infrared spectrum of which exhibited a strong amide band at 6.15 $\mu$ and only a very small acetate band. A portion (1.9 g.) of the **hydroxy amide XLI** was left at room temperature for 4 days with 7.58 g. of *p*-toluenesulfonyl chloride and 125 cc. of dry pyridine and after heating for 5 hr. on the steam-bath, water was added cautiously and the product extracted with ether. Evaporation of the washed and dried ether solution yielded the tosylate XLII as a gum ($\lambda_{max}^{CHCl_3}$ 7.35 and 8.5 $\mu$), which was dissolved in 200 cc. of dimethyl sulfoxide,[42] 20 cc. of pyridine and 100 cc. of benzene and distilled very slowly over a period of 48 hr. Dilution with water, ether extraction and chromatography on 175 g. of neutral alumina gave in the benzene–ether (3:1 and 1:1) fractions 0.96 g. of crystals (m.p. 136–140°) of the **olefin XXXIX**. Recrystallization from hexane or ethyl acetate furnished the analytical specimen, m.p. 144–145°, $[\alpha]_D$ −45° (*c* 1.10), $\lambda_{max}^{CHCl_3}$ 6.1 and 12.2 $\mu$ (trisubstituted double bond), n.m.r. signal at 306 c.p.s. (deuteriochloroform with added tetramethylsilane as internal standard) due to the single olefinic proton.

*Anal.* Calcd. for $C_{22}H_{35}NO$: C, 80.19; H, 10.71. Found: C, 80.37; H, 10.43.

Catalytic hydrogenation of 47 mg. of the olefin XXXIX in 10 cc. of acetic acid and 70 mg. of 10% palladized charcoal catalyst gave, after recrystallization from hexane, the saturated amide **XXXVIII**, m.p. 141–142°, $[\alpha]_D$ −55° (*c* 0.63). The melting point was not depressed upon admixture with its precursor XXXIX, but the infrared spectrum showed the complete disappearance of the 12.2 $\mu$ band; furthermore, gas phase chromatography (SE-30 column, 268°) readily separated a mixture of XXXIX, XXXVIII and XXXVII.

*Anal.* Calcd. for $C_{22}H_{35}NO$: C, 80.19; H, 10.71. Found: C, 80.37; H, 10.54.

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# Biosynthesis of Ergot Alkaloids: Incorporation of Mevalonic Acid into Ergosine[1]

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The degradation of radioactive ergosine, isolated from a culture of *Claviceps purpurea*, PRL 1578, fed DL-mevalonic acid-2-$C^{14}$, revealed that almost all the activity was localized in the carboxyl carbon (C-17) of ergosine. This constitutes strong evidence that the C-2 of mevalonic acid becomes the C-17 of lysergic acid. The small percentage of radioactivity recovered from the C-7 was considered to indicate a possible randomization of the C-2 of mevalonate. The manner in which this may take place has been discussed.

Studies on the biosynthesis of ergot alkaloids in the saprophytic culture of *Claviceps purpurea* have revealed that the indole nucleus and carbons 4, 5, nitrogen 6 of ergoline originate from tryptophan[2] without prior conversion to the general metabolites, 5-hydroxytryptophan,[2d,e] tryptamine[3] or kynurenine[3] and that the carboxyl group is lost during the incorporation.[2a] Preliminary experiments[3] have shown that both formate and the S-methyl group of methionine are incorporated into clavine alkaloids. The larger percentage incorporation of the latter suggests that the N-methyl group of the clavine alkaloids arises from methionine *via* transmethylation. Evidence has been presented that carbons 7, 8, 9, 10[4] and the substituent at the carbon 8 of ergoline arise from mevalonic acid (3,5-dihydroxy-3-methylpentanoic acid) as was suggested earlier by Birch[5a] and Mothes.[5b]

If it is assumed that prior or subsequent to decarboxylation, mevalonic acid is incorporated as a unit,[4a,b] the $C^{14}$ from DL-mevalonic acid-2-$C^{14}$ should appear[6] at C-17 ($I \xrightarrow{a} II$) or at C-7 ($I \xrightarrow{b} II$) of the ergot alkaloids. The distribution of the 2-$C^{14}$ of mevalonate between C-7 and C-17 might also occur to some degree. Degradation[4a] of agroclavine and elymoclavine isolated from mevalonic acid lactone-2-$C^{14}$ fed cultures has indicated that about 30% of the radioactivity was present at the C-17 in agroclavine and about 90% in the case of elymoclavine. This would appear to suggest that different biosynthetic pathways exist for the two alkaloids which is unlikely in light of their closely related structures.[4d]

In the present work a partial degradation was devised whereby the C-7 and the C-17 of lysergic acid derived from ergosine were isolated individually as carbon dioxide. The radioactive ergosine (II) obtained from *Claviceps purpurea* PRL 1578 fed with DL-mevalonic acid-2-$C^{14}$ was degraded by the sequence shown in Fig. 1. The first step was the conversion of ergosine (II) into D-lysergic acid

(1) A preliminary report of part of this work has appeared in *Tetrahedron Letters*, **No. 17**, 595 (1961). This investigation was supported in part by a grant from the National Research Council of Canada.

(2) (a) D. Groger, H. J. Wendt, K. Mothes and F. Weygand, *Z. Naturforsch.*, **14b**, 335 (1959); (b) W. A. Taber and L. G. Vining, *Chemistry & Industry*, 1218 (1959); (c) H. Plieninger, R. Fischer, W. Lwowski, A. Brack, H. Kobel and A. Hofmann, *Angew. Chem.*, **71**, 383 (1959); (d) R. M. Baxter, S. I. Kandel and A. Okany, *Chemistry & Industry*, 266 (1960); (e) H. Plieninger, R. Fischer, G. Keilich and H. D. Orth, *Liebigs Ann.*, **642**, 214 (1961).

(3) R. M. Baxter, S. I. Kandel and A. Okany, *Chemistry & Industry*, 1453 (1961).

(4) (a) A. J. Birch, D. J. McLoughlin and H. Smith, *Tetrahedron Letters*, **No. 7**, 1 (1960); (b) D. Groger, K. Mothes, H. Simon, H. G. Floss and F. Weygand, *Z. Naturforsch.*, **15b**, 141 (1960); (c) E. H. Taylor and E. Ramstad, *Nature*, **188**, 494 (1960); (d) subsequent to the preparation of our manuscript, a paper has been published reporting the finding of 78% of the incorporated radioactivity from mevalonic lactone-2-$C^{14}$ at C-17 in agroclavine (S. Bhattacharji, *et al.*, *J. Chem. Soc.*, 421 (1962).

(5) (a) A. J. Birch in Ciba Foundation Symposium on "Amino Acids and Peptides with Antimetabolic Activity," G. E. W. Westenholme and C. M. O'Connor, Editors, J. and A. Churchill Ltd., London, 1958, p. 247; (b) K. Mothes, F. Weygand, D. Groger and H. Grisebach, *Z. Naturforsch.*, **13b**, 41 (1958).

(6) The carbon atom linked to C-8 of ergoline is denoted as C-18 according to Birch, *et al.*[4a]