Alkaloids of *Guatteria psilopus* Mart. Guatterine and Atherospermidine

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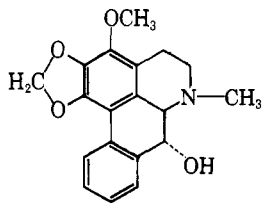
Two alkaloids, guatterine and atherospermidine, have been isolated from *Guatteria psilopus* Mart. (*Annonaceae*). These alkaloids are related to micheline and liriodenine, of which they are, respectively, the 3-methoxy derivatives. Guatterine is readily oxidized to atherospermidine, and atherospermidine can be oxidized to 1-azaanthraquinone-4-carboxylic acid. Guatterine and micheline differ in the stereochemistry of the 7-hydroxyl group.

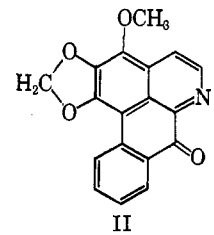
Guatteria psilopus Mart. (*Annonaceae*)[2] is rich in a number of alkaloids; thin layer chromatography of the crude bases isolated from the plant discloses about thirteen Dragendorff-staining constituents. From this mixture have been isolated two alkaloids, guatterine, $C_{19}H_{19}NO_4$, and atherospermidine, $C_{18}H_{11}NO_4$. Guatterine is a colorless base, readily soluble in dilute aqueous acid. It contains a methylenedioxy grouping, an O-methyl and an N-methyl group, and an additional oxygen atom which could not be characterized by conventional methods. The infrared spectrum showed the presence of a hydroxyl group, and that this was a nonphenolic hydroxyl group was indicated by the failure of guatterine to react with diazomethane and by the nonreactivity of the alkaloid to alkali. Attempts at acetylation and benzoylation under mild conditions failed, but acetylation under somewhat more vigorous conditions resulted in the formation of two N-acetyl compounds, both of which are phenanthrene derivatives.

The formation of the phenanthrene nucleus in the reaction of guatterine with acetic anhydride was an indication that the alkaloid was an aporphine, a conclusion for which the ultraviolet spectrum provided only uncertain evidence. Guatterine shows the characteristic maximum at about 280 mμ seen in many aporphine alkaloids, but lacks the second maximum at about 310 mμ seen in the spectra of such typical aporphine alkaloids as glaucine, dicentrine, and bulbocapnine. However, xylopine, 1,2-methylenedioxy-9-methoxynoraporphine,[3,4] has an ultraviolet spectrum that closely resembles that of guatterine.

The n.m.r. spectrum of guatterine revealed the details of its structure in such a way as to permit the formulation as I; the absolute configuration is not yet known. The n.m.r. spectrum (Figure 1) shows clearly the N-methyl and O-methyl groupings as three-proton singlets at τ 7.51 and 6.08, and the methylenedioxy grouping as a pair of one-proton peaks at 3.11 and 3.27, each with a splitting of about 2 c.p.s. (chemical shifts in τ-units). This is characteristic of the methylenedioxy grouping on the nonplanar aporphine ring system.[5] A well-defined one-proton doublet at τ 6.50–5.70 represents the hydrogen atom α to the N-methyl group, and a corresponding doublet at 5.35–5.55 is assigned to the hydrogen atom on the hydroxylated carbon atom. The large coupling constant (12 c.p.s.) of these protons indicates that they are *trans* oriented, leading to the configurational assignment shown in I.



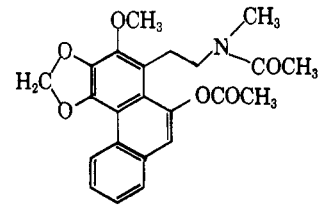
I



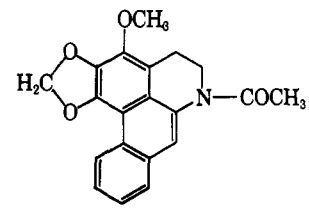
II

The four aromatic protons are present in a complex pattern that was first assigned to the four adjacent protons of an *ortho*-disubstituted benzene ring. This initial conclusion was later substantiated by direct chemical evidence, described in the sequel.

The action of acetic anhydride upon guatterine led to two compounds, one of which could not be prepared in later experiments. The acetate normally produced (m.p. 190–192°) was clearly a phenanthrene derivative from its characteristic ultraviolet absorption spectrum. Its n.m.r. spectrum was readily interpretable in accord with structure III; the methylenedioxy grouping appeared now (in contrast to the original aporphine) as a sharp singlet at τ 3.84.[5] Signals for the methoxy group (5.91), and the O-acetyl group (7.53) were three-proton singlets, and the $-N(CH_3)(COCH_3)$ grouping appeared as the characteristic (for this structural feature) three-proton doublets at 6.98–7.23 (NCH_3) and 7.98–8.03 ($NCOCH_3$). The total proton integration between τ 2.0 and 10.0 accounted for 22 protons (for $C_{23}H_{23}NO_6$), the extra aromatic proton being found as a one-proton multiplet signal centered at 1.0. The location of the acetoxy grouping at position 10, as shown in III, is not established, but, since an initial loss of the elements of water during the reaction is likely (*cf.* IV), it is probable that the compound has the structure shown and is not the 9-acetoxy compound.



III



IV

The second acetylation product (m.p. 222–223°), isolated in a single experiment, has an ultraviolet spectrum that shows that it is also a phenanthrene derivative.

(1) Presented in summary form at the Third International Symposium on the Chemistry of Natural Products, International Union of Pure and Applied Chemistry, Kyoto, Japan, April 1964. In the abstracts of papers of this Symposium atherospermidine is called psilopine.

(2) The authors are grateful to Drs. R. F. Raffauf and G. E. Ullyot, Smith, Kline and French Laboratories, and to Dr. O. Ribiero, for providing the plant material used in this study

(3) J. Schmutz, *Helv. Chim. Acta*, **42**, 335 (1959).

(4) Numbering according to "The Ring Index," American Chemical Society, Washington, D. C., 1959, p. 704; see ref. 15.

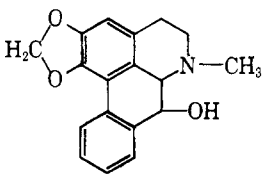
(5) S. Goodwin, J. N. Shoolery, and L. F. Johnson, *Proc. Chem. Soc.*, 306 (1958).

Its n.m.r. spectrum (τ-values) is remarkably simple, showing sharp singlets for the methylenedioxy (3.79, 2H), methoxy (5.92, 3H), and $NCOCH_3$ (substantiated by the infrared spectrum) (7.75, 3H) groupings. Five aromatic protons in the region of 2.28–2.57 and the four protons of the $-CH_2CH_2-$ grouping complete the spectrum and lead to the structure IV.

Final proof of the structure I for guatterine rests upon the establishment of atherospermidine as II. Atherospermidine is a golden yellow compound that dissolves in mineral acid to a deep red solution. This behavior is the same as that observed for liriodenine (desmethoxy II),[6-9] and the ultraviolet–visible absorption spectra of atherospermidine and liriodenine and their salts show considerable similarity. Atherospermidine is readily formed from guatterine by oxidation, and the oxidation of atherospermidine with chromic acid yields 1-azaanthraquinone-4-carboxylic acid. The formation of the azaanthraquinone establishes the location of the methylenedioxy and methoxy groups as 1,2,3- with respect to each other. The placement of these groups as in I and II is based upon the n.m.r. spectra of guatterine and its derivatives. The methoxyl group always appears at τ 5.98–6.08. Vernengo[10] has pointed out that methoxyl groups at positions 1 and 11 in a number of aporphines appear in n.m.r. spectra at τ 6.28–6.45, while those in positions 2, 3, 9, and 10 appear at 6.07–6.20. The methoxyl group in guatterine and in atherospermidine is thus placed at 3 rather than at 1.

Atherospermidine has been isolated earlier from *Atherosperma moschatum* Labill., but was not recognized identical with our alkaloid until the direct comparison of our material with an authentic specimen of atherospermidine.[11,12]

The stereochemistry of the hydroxyl group in guatterine, shown by the τ-value of 12 c.p.s. to be as in I, appears to be opposite to that of the hydroxyl group in micheline (ushinsunine, V).[8] The n.m.r. spectrum of



V

micheline[13] shows the relevant protons (CHO and CHN) as one-proton signals with a coupling constant of about 3 c.p.s., an indication that the dihedral angle is small. A model of micheline shows that this condition is met by the *cis* disposition of the hydrogen atoms.

(6) M. A. Buchanan and E. E. Dickey, *J. Org. Chem.*, **25**, 1389 (1960).

(7) W. I. Taylor, *Tetrahedron*, **14**, 42 (1961).

(8) S. S. Yang, W. Y. Huang, L. C. Lin, and P. Y. Yeh, *Chemistry* (Taipei), 144 (1961).

(9) T. H. Yang, *J. Pharm. Soc. Japan*, **82**, 798 (1962).

(10) M. J. Vernengo, *Experientia*, **19**, 294 (1963).

(11) I. R. C. Bick, personal communication. Atherospermidine was first isolated by I. R. C. Bick, P. S. Clezy, and W. D. Crow [*Australian J. Chem.*, **9**, 111 (1956)]. Since the present paper was first submitted, the structures of atherospermidine and spermatheridine (liriodenine) have been described by I. R. C. Bick and C. K. Douglas [*Tetrahedron Letters*, **No. 25**, 1629 (1964)]. It is of interest to note that liriodenine is also a constituent of *A. moschatum*. These alkaloids are now recognized in three plant families: *Magnoliaceae*, *Monimiaceae*, and *Annonaceae*.

(12) We are grateful to Dr. Bick for the specimen of atherospermidine used for the comparison.

(13) We are grateful to Professor Yang for the specimen of micheline used for this measurement.

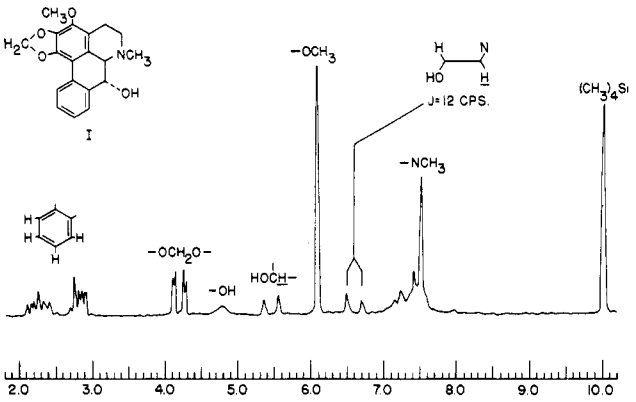


Figure 1.—N.m.r. of guatterine (in deuteriochloroform).

Experimental

Ultraviolet spectra were measured in 95% ethanol with a Cary Model 14 spectrophotometer. Infrared spectra were measured in chloroform solution or in potassium bromide disks, using a Perkin-Elmer Model 21 spectrophotometer. Nuclear magnetic resonance spectra were measured in deuteriochloroform with a Varian A-60 instrument with tetramethylsilane as an internal standard.

Isolation of Guatterine.—Dried and ground leaves and stems of *Guatteria psilopus* Mart. (1 kg.) were extracted with several portions of methanol in a Soxhlet apparatus. The methanol extracts were concentrated to 1 l. and added with stirring to 2 l. of 0.5 *N* sulfuric acid. After 24 hr. at 5°, the aqueous solution was decanted from a tarry residue, and the tar was re-extracted in the same way with methanol–sulfuric acid. The acidic aqueous extract was washed with ether and then made alkaline with ammonium hydroxide. Repeated ether extraction was followed by concentration of the ether extracts to a small volume. Addition of acetone and cooling to 5° overnight resulted in the separation of crude guatterine, which was purified by recrystallization from aqueous acetone. The yield of colorless needles of the alkaloid was 2.1 g. It melted at 146–148° and had $[\alpha]^{20}D$ −57.1° ($CHCl_3$).

Anal. Calcd. for $C_{19}H_{19}NO_4$: C, 70.14; H, 5.89; N, 4.31; OCH_3, 9.55. Found: C, 70.44, 70.05, 69.58; H, 6.17, 5.97, 6.22; N, 4.76, 4.15; OCH_3, 9.53, 9.67.

Guatterine gave a positive test for the methylenedioxy grouping. The fourth oxygen was found not to be phenolic, for the compound was unaffected by alkali and was recovered unchanged after treatment with diazomethane. Further, on paper chromatograms, after exposure to iodine vapor, spots of guatterine were stable indefinitely, while phenolic alkaloids gave spots that under similar treatment soon turned green (for example, bulbocapnine, apomorphine).

The ultraviolet absorption spectrum of guatterine showed λ_{max} 242 mμ (log ε 4.27) and 281 (4.26); λ_{min} 231 mμ (log ε 4.21) and 256 (3.86). The ultraviolet spectrum was unaltered in acid solution, and the spectrum of the methiodide was qualitatively very similar to that of the base, differing only in the accentuation of a shoulder appearing at about 320 mμ in the spectrum of guatterine.

The infrared spectrum of guatterine showed the presence of a hydroxyl group at 3400 $cm.^{-1}$, but no other clearly distinctive features were apparent. The proton magnetic resonance spectrum is described in the introductory discussion.

Atherospermidine.—After removal of guatterine by ether extraction, as described above, the residual ammoniacal solution was extracted thoroughly with chloroform. The green-fluorescing, deep yellow chloroform solution was concentrated to a small volume and chromatographed on neutral alumina (activity III). A mixture of benzene–chloroform (1:1) eluted the alkaloid as a yellow-orange band. After crystallization from chloroform, atherospermidine formed deep yellow needles, m.p. 283–285°.

Its ultraviolet spectrum showed λ_{max} 247 mμ (log ε 4.39), 281 (4.53), 316 sh (3.80), 383 (3.71), and 440 (3.92). Its red solution in 1% aqueous hydrochloric acid showed λ_{max} 263 mμ (log ε 4.46), 283 (4.36), 410 (3.78), and 505 (3.58).

Anal. Calcd. for $C_{18}H_{11}NO_4$: C, 70.81; H, 3.63; N, 4.59. Found: C, 70.91, 70.64; H, 4.36, 3.71; N, 4.74.

Atherospermidine Oxime.—A solution of 99 mg. of atherospermidine and 164 mg. of hydroxylamine hydrochloride in 8 ml. of

pyridine was heated under reflux for 30 min. The mixture was poured into 2 *N* acetic acid and the yellow precipitate was collected and recrystallized from 1-butanol. The oxime (67 mg.) melted at 247–250° dec., and had λ_{max} 280 mμ (log ε 4.47), 376 sh (3.78), and 410 (3.92).

Anal. Calcd. for $C_{18}H_{12}N_2O_4$: C, 67.50; H, 3.78; N, 8.75. Found: C, 67.32, 67.48; H, 4.12, 3.90; N, 8.86.

Oxidation of Guatterine to Atherospermidine.—A mixture of 412 mg. of colorless guatterine and 416 mg. of chromium trioxide in 6 ml. of pyridine was allowed to stand for 18 hr. Ethanol (1.5 ml.) and pyridine (2 ml.) were added and the mixture was stirred and diluted with water (60 ml.). The aqueous solution was extracted repeatedly with chloroform until the extracts were no longer fluorescent. The extract was dried over sodium sulfate and evaporated, and the yellow-brown residue was recrystallized from chloroform to yield 122 mg. of golden yellow needles of atherospermidine, identical with the compound isolated from the plant extracts.

Acetylation of Guatterine. A. Acetate III.—A mixture of 200 mg. of guatterine, 200 mg. of anhydrous sodium acetate, and 2.5 ml. of acetic anhydride was heated under reflux for 90 min. After the addition of 10 ml. of water and heating for a further 30 min., the solution was cooled, made basic with ammonia and extracted with chloroform. The dried chloroform solution was evaporated and the residue was crystallized from acetone–water. The colorless needles melted at 190–192°.

Anal. Calcd. for $C_{23}H_{23}NO_6$: C, 67.46; H, 5.66; N, 3.42. Found: C, 67.42; H, 5.84; N, 3.20.

The infrared spectrum showed characteristic absorption at 1750 (O-acetyl) and 1645 cm.$^{-1}$ (N-acetyl). The ultraviolet spectrum had λ_{max} 252 mμ (log ε 4.57), 284 sh (4.31), 324 (3.87), and 364 (3.10).

B. Acetate IV.—In another experiment, a mixture of 300 mg. of guatterine, 320 mg. of sodium acetate, and 3.5 ml. of acetic anhydride was refluxed for 2 hr. The excess acetic anhydride was removed by heating under vacuum and the residue was taken up in ethyl acetate. The filtered solution was evaporated to dryness and the residue was dissolved in acetone. After treatment with charcoal the solution was diluted with water and allowed to stand. The product (83 mg.) separated as tiny white needles, m.p. 222–223°.

Anal. Calcd. for $C_{20}H_{17}NO_4$: C, 71.63; H, 5.11. Found: C, 71.15; H, 5.85.

The ultraviolet spectrum showed λ_{max} 262 mμ (log ε 4.85), 286 (4.58), 320 (4.08), 330 (4.08), and 368 (3.23). The infrared spectrum showed a peak at 1663 cm.$^{-1}$ (N-acetyl).

Repeated attempts to reproduce the preparation of the acetate (m.p. 223°) were fruitless; the 192° acetate was always the product isolated. Although the carbon–hydrogen analysis was imperfect (the single analytical result agrees with $C_{20}H_{19}NO_4$—calcd.: C, 71.21; H, 5.68), the integration of the n.m.r. spectrum shows the required 17 protons, all of them accounted for in the interpretation of the spectrum based upon the structure IV.

Oxidation of Atherospermidine. 1-Azaanthraquinone-4-carboxylic Acid.—To a solution of 176 mg. of atherospermidine in 12 ml. of 13 *N* sulfuric acid was added a solution of 204 mg. of chromium trioxide in 5 ml. of 14 *N* sulfuric acid. The mixture was allowed to stand for 16 hr. and, after dilution with 40 ml. of water, heated for 1 hr. on the steam bath. The solution was filtered and the yellow precipitate was washed with water and purified by solution in aqueous ammonia and reprecipitation with acid. There was obtained 53 mg. of pale yellow 1-azaanthraquinone-4-carboxylic acid, m.p. 327–328° dec. The compound decomposed on melting and a sublimate of golden yellow needles of 1-azaanthraquinone formed on the walls of the capillary tube.

Anal. Calcd. for $C_{14}H_7NO_4$: C, 66.41; H, 2.79; N, 5.53. Found: C, 66.44; H, 2.80; N, 5.88.

The ultraviolet spectrum showed λ_{max} 252 mμ (log ε 4.54) and 317 (3.73). The infrared spectrum (KBr) showed broad absorption in the 3350–3500-cm.$^{-1}$ region (carboxyl-OH) and peaks at 1670 and 1708 cm.$^{-1}$.

1-Azaanthraquinone.—A sample of 61 mg. of 1-azaanthraquinone-4-carboxylic acid was mixed with 60 mg. of calcium oxide and heated in a metal bath until a sublimate of yellow needles had formed on the walls of the tube. The sublimate was recrystallized from chlorobenzene, forming yellow needles, m.p. 281–283°, undepressed upon admixture with a synthetic specimen. The ultraviolet absorption spectra of the compound from atherospermidine and that prepared synthetically (see below) were identical.

1-Azaanthraquinone was prepared starting from 1-chloro-2-acetnaphthalide[14] by way of 10-chloro-1-azaanthracene.[15,16] The ultraviolet spectrum showed λ_{max} 249 mμ (log ε 4.53) and 324 (3.51). The infrared spectrum (KBr) showed peaks at 1665 and 1685 cm.$^{-1}$.

Anal. Calcd. for $C_{13}H_7NO_2$: C, 74.64; H, 3.37; N, 6.70. Found: C, 74.88; H, 3.54; N, 6.90.

(14) A. P. J. Hoogeveen, *Rec. trav. chim.*, **50**, 37 (1931).

(15) A. Etienne, *Ann. chim.* (Paris), [12] **1**, 5 (1946). Numbered here according to "The Ring Index" (A. M. Patterson, L. T. Capell, and D. F. Walker, American Chemical Society, Washington, D. C., 1959, p. 456). In the original publication the compound is named meso(9)chloroazanthracene-α.

(16) A. Etienne and M. Legrand, *Bull. soc. chim. France*, [5] **20**, 110 (1953).

Pyrolysis Studies. XIII.[1a] Kinetics of the Vapor Phase Pyrolysis of Arylethyl Methyl Carbonates. A Linear Free-Energy Relationship for *ortho* Substituents

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The absolute reaction rate constants in the pyrolysis of a series of *ortho*-, *meta*-, and *para*-substituted 1-arylethyl methyl carbonates and some *ortho*-substituted 1-arylethyl acetates have been determined. The mechanism of the pyrolysis of the carbonates is discussed. Even in the pyrolysis of the *ortho*-substituted esters, proximity effects are at a minimum for it is found that a linear free-energy relationship is obeyed between the pyrolysis of the *ortho*-substituted acetates and carbonates. Important differences are found between this relationship and the linear free-energy relationship obeyed by the *meta*- and *para*-substituted isomers.

Because of the general proximity of *ortho* substituents to the reaction center, their substituent effects have proved to be more complex than those of *meta* and *para* substituents, so that it has been found difficult in the past to correlate *ortho*-substituent effects by means of linear free-energy relationships based on *meta*- and *para*-substituent constants. This complexity is due, in part, to the fact that most of the reaction systems studied so far have involved bimolecular reactions in the condensed phase with the attendant problems, amongst others, primary steric effects and steric inhibition of solvation. However, from a study of gas phase unimolecular elimination reactions information can be obtained about *ortho*-substituent effects free from these complications. Previous papers concerned with *ortho*-substituent effects in the gas phase dealt with *ortho*-

(1) (a) Paper XII: G. G. Smith and D. V. White, *J. Org. Chem.*, **29**, 3533 (1964). (b) Postdoctoral research associate, 1963–1964.