opened and the contents directly analyzed by gas chromatography (F & M Model 609 flame ionization gas chromatograph, 6-ft 5% Apiezon L column). The results are summarized in Table III. The unreacted sulfonium salts did not decompose under our conditions of analysis.

**Acknowledgments.** This work was supported by a grant from the National Science Foundation. J. W. K. also gratefully acknowledges the receipt of a grant for equipment from the Research Corporation.

# Amaryllidaceae Interconversions. Partial Syntheses of [2]Benzopyrano[3,4-*c*]indoles[1,2]

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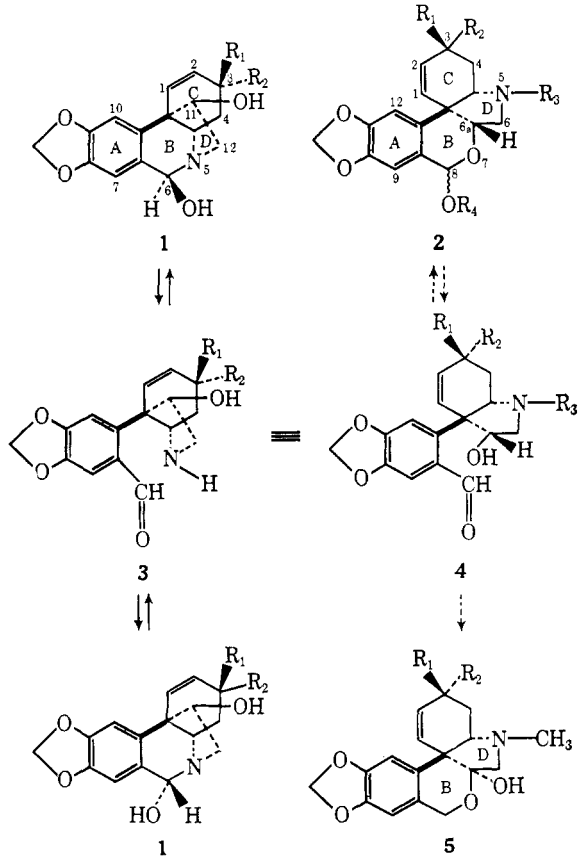
**Abstract:** Partial syntheses of pretazettine (**7a**), precriwelline (**7b**), and several related compounds are reported. Evidence is cited that haemanthidine (**1a**), 6-hydroxycrinamine (**1b**), and many derivatives of these alkaloids undergo a reversible rearrangement to the [2]benzopyrano[3,4-*c*]indole nucleus under mild conditions. Variations in temperature, solvent, or pH are often sufficient to alter the basic ring system. Biosynthetic implications of these transformations are outlined. Evidence for the $\alpha$ configuration of the $C_{6a}$-hydroxyl group of tazettine is presented.

Although the intermediate **3** and its rotational equivalent **4** ($R_3$ = H) have never been detected by spectral methods, these structures have considerable significance in the interrelationship of alkaloids related to structures **1**, **2**, and **5**. In a chemical sense the alkaloids haemanthidine (**1a**) and 6-hydroxycrinamine (**1b**) are amino alcohols formed by the ring closure of the intermediate amino aldehyde **3**. The dynamic nature of this N–$C_6$ bond formation was realized when **1a** and **1b** were shown to exist in solution as equilibrating mixtures of 6-hydroxyl epimers.[3] The epimeric forms were proposed to be interconvertible through **3**. The tendency of alkaloids of type **2** (pretazettine and precriwelline) to rearrange to the type **5** nucleus[4] (tazettine and criwelline) has been demonstrated.[5] This transformation was considered to proceed through **4** or the related alkoxide anion.

A number of examples are known for the conversion of the 6-hydroxy-5,10b-ethanophenanthridine alkaloids (**1**) to the [2]benzopyrano[3,4-*c*]indole ring systems (**2** and **5**). The first reported rearrangement of **1** to the type **5** nucleus was observed in the facile conversion of haemanthidine (**1a**) methiodide to tazettine (**5a**) upon treatment with aqueous sodium hydroxide.[7,8] Later, it was shown that N-demethyltazettine was produced when haemanthidine was treated with methanolic sodium methoxide.[9] Identical reactions were reported in the conversions of 6-hydroxycrinamine (**1b**) to criwelline (**5b**)[10] and to N-demethylcriwelline.[11] The reaction pathway for these rearrangements was studied with deuterated 6-hydroxycrinamine methiodide. It




**a**, $R_1 = OCH_3$; $R_2$ = H
**b**, $R_1$ = H; $R_2 = OCH_3$

(1) This research was supported by a grant from the National Institutes of Health (HE-7503).
(2) This work is taken from the dissertation of D. T. Bailey submitted in partial fulfillment of the requirements for the Ph.D. degree, Iowa State University, 1968.
(3) R. W. King, C. F. Murphy, and W. C. Wildman, *J. Amer. Chem. Soc.*, **87**, 1912 (1965).
(4) Compounds with the nucleus **2** have a $\beta$ (steroid convention) $C_{6a}$-hydrogen,[5] while those with nucleus **5** have a $C_{6a}$-hydroxyl that has been reported to be $\alpha$.[6]
(5) W. C. Wildman and D. T. Bailey, *J. Org. Chem.*, **33**, 3749 (1968).
(6) Y. Tsuda and S. Uyeo, *J. Chem. Soc.*, 2485 (1961).
(7) H.-G. Boit and W. Stender, *Chem. Ber.*, **89**, 161 (1956).
(8) S. Uyeo, H. M. Fales, R. J. Highet, and W. C. Wildman, *J. Amer. Chem. Soc.*, **80**, 2590 (1958).
(9) H. Irie, Y. Tsuda, and S. Uyeo, *J. Chem. Soc.*, 1446 (1959).
(10) H. M. Fales, D. H. S. Horn, and W. C. Wildman, *Chem. Ind.* (London), 1415 (1959).
(11) P. W. Jeffs, F. L. Warren, and W. G. Wright, *J. Chem. Soc.*, 1090 (1960).

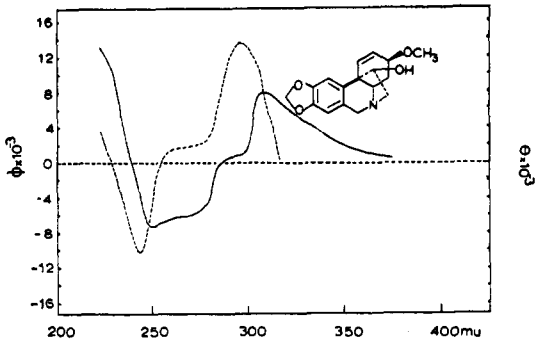



Figure 1. ORD (——) and CD (– – –) of haemanthamine (**Ia**; no $C_6$-hydroxyl group).

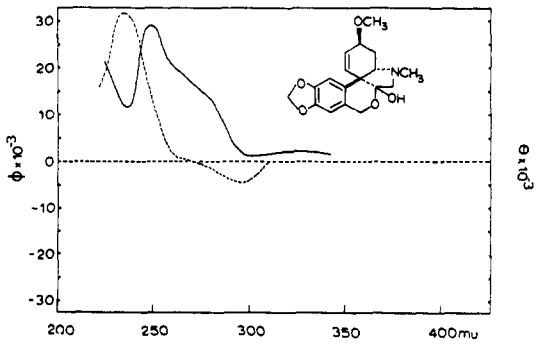



Figure 2. ORD (——) and CD (– – –) of tazettine (**5a**).

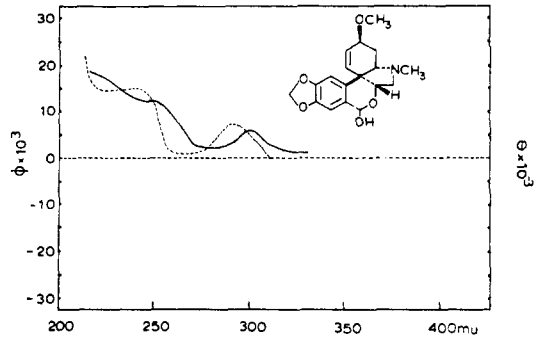



Figure 3. ORD (——) and CD (– – –) of pretazettine (**7a**).

was demonstrated that criwelline (**5b**) was formed by an intramolecular hydride shift of the $C_{11}$ proton of **1b** to the benzylic carbon atom of the product.[12]

Several examples for the conversion of **1** to compounds with the nucleus of **2** have been reported. In all cases, the stereochemistry of the hydrogen at $C_{6a}$ has not been specified. However, there is little likelihood that the $C_{11}$-hydroxyl group of the starting material has been epimerized under the reaction conditions. As a result, the configuration of the hydrogen at $C_{6a}$ in the product is assigned as shown in **2**.[5] 6-Hydroxycrinamine (**1b**) can be converted to N-nitroso-N-demethylprecriwelline (**2b**; $R_3$ = N=O, $R_4$ = H) on treatment with nitrous acid in aqueous acetic acid at room temperature.[3] The $C_3$ epimer, N-nitroso-N-demethylpretazettine (**2a**; $R_3$ = N=O, $R_4$ = H) has been formed by a similar reaction sequence from haemanthidine.[13] O-Methylprecriwelline (**2b**; $R_3$ = $CH_3$, $R_4$ = $CH_3$) was formed when 6-hydroxycrinamine was refluxed with methyl iodide in methanol.[14]

The biosynthetic conversion of the 5,10b-ethanophenanthridine alkaloids to the [2]benzopyrano[3,4-*c*]indole nucleus was demonstrated by feeding tritium-labeled alkaloids to *Sprekelia formosissima* L. It was shown that this plant converts haemanthamine (**1a**; no $C_6$-hydroxyl group) to haemanthidine (**1a**) and subsequently to tazettine (**5a**) in essentially an irreversible manner.[15]

While there is little doubt that the preceding synthetic and biosynthetic observations are basically correct, the lability of the amino alcohol, hemiacetal, and hemiketal moieties to even the mildest of isolation and identification procedures suggested that the fundamental reactions of these substances should be reexamined. Syntheses of pretazettine and precriwelline were primary objectives.

**Syntheses of Pretazettine (7a) and Precriwelline (7b).** Our previous experience with the reaction conditions necessary to convert the 6-hydroxy-5,10b-ethanophenanthridine alkaloids (**1**) to the [2]benzopyrano[3,4-*c*]-indole ring systems (**2** or **5**) indicated that treatment with all but the most dilute base (either in the course of reaction itself or in the subsequent isolation procedure) often led to type **5** structures (*cis* B–D ring fusion). These findings are consistent with the known ease of conversion of pretazettine (**7a**) to tazettine (**5a**) by base.[5] In contrast, syntheses of the type **2** compounds (with the *trans* B–D ring fusion) have, in all cases, proceeded in neutral or slightly acidic media. It appeared very possible that haemanthidine methiodide (**6a**) might rearrange to pretazettine (**7a**) under acidic conditions. To test this hypothesis, haemanthidine methiodide was dissolved in dilute hydrochloric acid (pH 4) at room temperature. The solution was made basic (pH 10) with sodium carbonate and immediately extracted with chloroform. Evaporation of the chloroform to dryness under reduced pressure gave, in almost quantitative yield, pretazettine (**7a**). By the same procedure, 6-hydroxycrinamine methiodide (**6b**) was converted to precriwelline (**7b**).

These transformations can be attributed to either the effect of the acid on the methiodides or the basic conditions required for the isolation of the product. Spectroscopic studies were undertaken to elucidate the relationship between the metho salts of **6** and the hydro salts of **7** in solution. Although the ir and nmr spectra of **6** and **7** are distinct, optical rotatory dispersion (ORD) and circular dichroism (CD) measurements provided a more sensitive method for the differentiation of the two ring systems.[16,17] These results are summarized in Figures 1–3. The spectra of haemanthamine (**1a**; no $C_6$-hydroxyl group) and haemanthamine methiodide in methanol are similar and distinctly different from those observed for tazettine. The ORD and CD of pretazettine and O-methylpre-

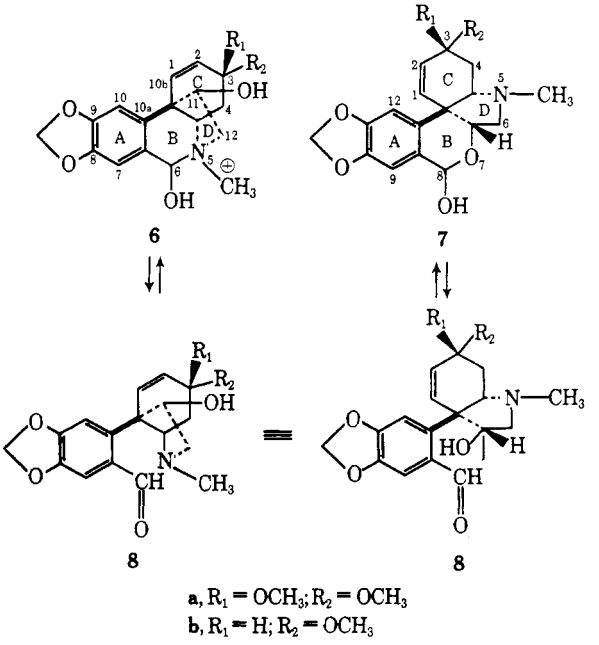
(12) C. F. Murphy and W. C. Wildman, *Tetrahedron Lett.*, 3863 (1964).
(13) S. Uyeo, H. M. Fales, R. J. Highet, and W. C. Wildman, unpublished results.
(14) C. F. Murphy and W. C. Wildman, *Tetrahedron Lett.*, 3857 (1964).
(15) H. M. Fales and W. C. Wildman, *J. Amer. Chem. Soc.*, **86**, 294 (1964).
(16) Ph.D. Dissertation, G. G. DeAngelis, Iowa State University, Ames, Iowa, 1966.
(17) K. Kuriyama, T. Iwata, M. Moriyama, K. Kotera, Y. Hamada, R. Mitsui, and K. Takeda, *J. Chem. Soc., B*, 46 (1967).

tazettine in methanol are similar and differ significantly from those observed for the other two ring systems. The ORD and CD spectra of pretazettine hydrochloride in methanol were almost identical with those of haemanthamine in the same solvent. This suggests that pretazettine in methanol possesses the hemiacetal structure 7a. However, the hydrochloride of pretazettine in this solvent exists in the 5,10b-ethanophenanthridine nucleus as the metho salt of haemanthidine (**6a**).[18] This structure for pretazettine hydrochloride was supported by nmr data. Pretazettine hydrochloride in $D_2O$ and haemanthidine methiodide in $D_2O$ were found to give identical nmr spectra.

When a methanolic solution of pretazettine (**7a**) was acidified (pH 4) with dilute hydrochloric acid, the alkaloid rearranged to the 5,10b-ethanophenanthridine ring system (**6a**). The original tertiary base was completely reformed when the acidic solution was made basic (pH 10) with dilute ammonium hydroxide. Pretazettine hydrochloride in aqueous solution exists in the quaternary amine form (**6a**). An aqueous solution of the quaternary salt, made basic (pH 10) with ammonium hydroxide, rearranged completely to tazettine in 1 hr. There was no indication that **7a** had been present. It appears that in aqueous solution, pretazettine preferred to remain as the more soluble quaternary amine (**6a**). The recovery of pretazettine, as the tertiary amine, by chloroform extraction from a basic aqueous solution is due, not only to the basic conditions, but also to the solubility of the tertiary form of the amine in chloroform.[20] The partial syntheses of pretazettine and precriwelline described earlier were not caused by the acidic hydrolysis, but rather by the chloroform and the dilute base used in the isolation procedure.

Independent evidence for this interconversion was found in the thin layer chromatography of pretazettine on silica gel. When the alkaloid (**7a**) was chromatographed on silica gel plates in chloroform–methanol–diethylamine (92:3:5), the compound had an $R_f$ value of approximately 0.5. Under these conditions, pretazettine has the migratory mobility typical of other compounds having one hydroxyl group. When pretazettine was chromatographed on silica gel in the same solvent, but without the diethylamine, pretazettine remained essentially at the origin. This suggests that the silica gel is sufficiently acidic to cause the rearrangement of the base to the quaternary amine form (**6a**).

Precriwelline (**7b**) undergoes this same type of interconversion. On chromatography on silica gel in nonbasic solvents, precriwelline did not migrate. Even in chloroform–methanol–diethylamine (92:3:5) the base still existed in the quaternary form (**6b**). When the diethylamine content of the solvent system was increased to 10%, precriwelline migrated to $R_f$ 0.2 indicating that the base under these conditions existed in the hemiacetal form (**7b**). When **6a** is placed in a




weakly basic methanolic solution, the amino aldehyde intermediate **8** is formed by hydrolysis of the amino alcohol. Rotation of 90° about the $C_{10a}$–$C_{10b}$ bond and some deformation of the C and D rings brings the $C_{11}$-hydroxyl group into close proximity of the aldehyde. Base-catalyzed formation of the hemiacetal gives pretazettine (**7a**). This process can also be visualized to occur in the reverse manner. The existence of two isomeric forms for pretazettine and precriwelline is due to the presence of an N-methyl group and a hydroxyl function, each capable of nucleophilic attack on the carbonyl of the intermediate **8**. Because of the lability of both the hemiketal and the $\alpha$-amino alcohol moieties, neither product is so stable as to preclude ring opening and the reformation of the other isomer upon a change in the solvent conditions.

**N-Demethylpretazettine (2a; $R_3$, $R_4$ = H).** Since haemanthidine methiodide (**6a**) can be converted to pretazettine (**7a**), it was expected that N-demethylpretazettine (**2a**; $R_3$, $R_4$ = H) might be formed by the rearrangement of haemanthidine (**1a**). Several reactions are known which would indicate the existence of N-demethylpretazettine. The Eschweiler–Clarke methylation is a well-known reaction for the alkylation of primary and secondary amines to form tertiary bases. The methylation of haemanthidine (**1a**) under these conditions has been reported to give tazettine in good yield.[21] It seems most probable that pretazettine was the true reaction product, but the strong base used in the product isolation had caused a rearrangement to tazettine. To test this hypothesis, haemanthidine was resubmitted to the Eschweiler–Clarke conditions. When the reaction was completed, the product was recovered using a milder basic isolation procedure than had been used in the original work. Pretazettine was the only product present. Since tertiary amines are not susceptible to attack under Eschweiler–Clarke conditions, pretazettine cannot, in this case, be derived from the formation and rearrangement of N-methylhaemanthidine. Therefore, a secondary amino group must have been present in sufficient concentra-

(18) A recent communication[19] reported the CD spectrum of pretazettine. This curve was identical with that obtained from pretazettine hydrochloride rather than the free base as the authors indicated.

(19) W. Döpke and P. W. Jeffs, *Tetrahedron Lett.*, 1307 (1968).

(20) The isomerization of pretazettine (**7a**) to the metho salt of haemanthidine (**6a**) is completely reversible. There is no apparent pathway by which the $C_{6a}$-hydrogen of pretazettine can be reversibly inverted in the isomerization process. This adds further proof for the $\beta$ configuration for this hydrogen in **7a**. In a recent communication,[19] pretazettine was assigned a structure which incorrectly implied an $\alpha$ hydrogen at the $C_{6a}$ position.

(21) W. C. Wildman, *Chem. Ind.* (London), 123 (1956).

tion to allow the methylation to occur. Secondary amines react with nitrous acid to give N-nitroso derivatives, but tertiary amines provide only the corresponding hydronitrite salts. 6-Hydroxycrinamine reacts with nitrous acid to give **2b** ($R_3$ = N=O, $R_4$ = H).[3]

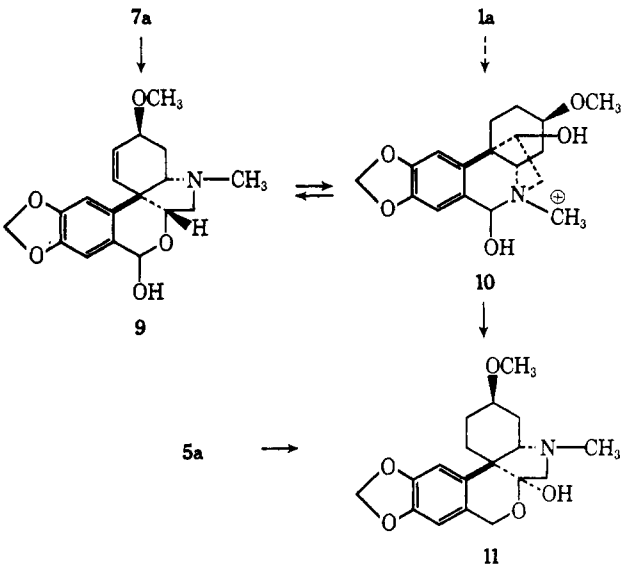
These two reactions indicate the presence of a secondary amine, but not its actual nature. The amine might be either the amino aldehyde **3** (where the amino group and the aldehyde remain closely associated), the hydroxy aldehyde **4** (R = H) (where the C–D ring portion of the intermediate may rotate freely), or the hemiacetal **2** ($R_3$, $R_4$ = H). Evidence has been found which indicates the hemiacetal form must be present in acidic media. When haemanthidine was refluxed in acidic methanol, the acetal, O-methyl-N-demethylpretazettine (**2a**; $R_3$ = H, $R_4$ = $CH_3$), was obtained. The ir spectrum of this product was very similar to that of O-methylpretazettine except for the additional N–H stretching band (3385 $cm^{-1}$). The nmr spectrum and mass spectrum are consistent with this assignment. The structure of O-methyl-N-demethylpretazettine was proven by conversion to the previously known O-methylpretazettine[5] (**2a**; $R_3$, $R_4$ = $CH_3$) upon treatment with methyl iodide. In a similar manner, O-methyl-N-demethylprecriwelline (**2b**; $R_3$ = H, $R_4$ = $CH_3$) has been formed from 6-hydroxycrinamine (**1b**).

Because of the evidence that haemanthidine can be converted to **2a** ($R_3$, $R_4$ = H), a study of the ORD and CD spectra of **1a** under various pH conditions was undertaken in an attempt to observe this transformation. Our results indicate that there is no difference between the spectra of haemanthidine and haemanthamine (which cannot isomerize) between pH 2 and 12 in methanol. This demonstrates that haemanthidine must exist predominantly with the 5,10b-ethanophenanthridine nucleus under these conditions. Therefore, N-demethylpretazettine is formed from haemanthidine by a reversible process but only has a short lifetime.

### Reduced Strain Compounds

The *trans* B–D ring fusion introduces considerable strain into the [2]benzopyrano[3,4-*c*]indole nucleus and it was of interest to study the rearrangements of less strained compounds having the same general structure. A more flexible skeleton can be achieved either by removal of the $C_1$–$C_2$ unsaturation or by conversion of the *trans* B–D ring fusion to the *cis* form.

Catalytic hydrogenation of pretazettine in glacial acetic acid afforded a single product. Ir and nmr[22] spectra in chloroform were consistent with the expected structure **9**. A substance with identical spectra was isolated when dihydrohaemanthidine methiodide was dissolved with warming in dilute hydrochloric acid (pH 4), the solution made basic (pH 10) with ammonium hydroxide, extracted with chloroform and concentrated. The product was soluble in chloroform, carbon tetrachloride, and methyl and ethyl alcohol. However, when the compound was dissolved in methanol and the solvent removed by evaporation, the residue was no longer soluble in carbon tetrachloride or chloroform. It now gave nmr spectra in $CD_3OD$ and $D_2O$ that indicated the compound existed in the 5,10b-ethanophenanthridine nucleus (**10**) as the metho salt of dihydrohaemanthidine. Consistent with this structural assignment, the compound dissolved easily in water at room temperature. Upon warming this aqueous solution to approximately 40°, compound **10** reverted to **9**, which is insoluble in water. This rearrangement could be reversed by cooling the solution to room temperature. The tertiary amine **9** could be regenerated by dissolving the quaternary form **10** in water, making the solution basic (pH 10) with ammonium hydroxide. Chloroform extraction afforded **9**. To further study the dihydro series, dihydrohaemanthidine was prepared.[23] As was observed in the case of




haemanthidine, dihydrohaemanthidine appeared to be stable in the 5,10b-ethanophenanthridine nucleus in methanol in the pH range 2–12.

The double bond does not seem to play a significant role in the conversion of either haemanthidine methiodide or pretazettine to tazettine.[5,12] A similar hydride shift mechanism was expected to convert dihydropretazettine to dihydrotazettine. This rearrangement does occur, but it requires much more forceful conditions in terms of base strength, temperature, and time. The product (**11**) was identical with that obtained by the catalytic reduction of tazettine.

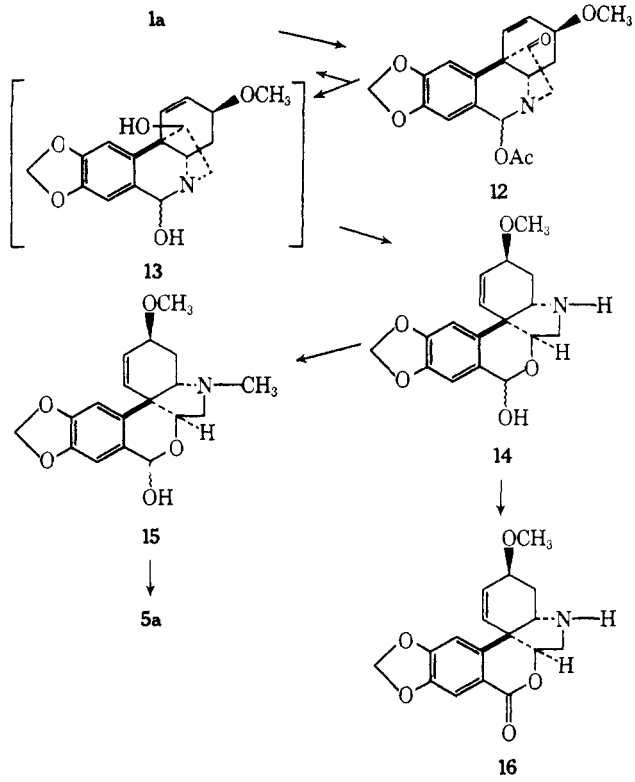
Synthesis of the pretazettine nucleus with the inverted (*cis*) B–D ring fusion (**15**) requires molecular modification of **1a**. The configuration of the $C_{11}$-hydroxyl group of **1** determines the configuration of the B–D ring fusion in the rearranged product. 11-Epihaemanthidine (**13**) would serve for entry into the *cis* B–D [2]benzopyrano[3,4-*c*]indole series. In order to prepare **13**, haemanthidine was oxidized by acetic anhydride and dimethyl sulfoxide. 6-Acetyl-11-oxohaemanthidine (**12**) was the sole product. The ir spectrum of this compound showed a broad carbonyl absorption (1751 $cm^{-1}$). The nmr spectrum indicated an acetate

(22) The nmr of dihydropretazettine in $CDCl_3$ indicated that the base exists in solution as a mixture of benzylic hydroxyl epimers. The spectrum showed two minor peaks which corresponded to part of the $C_9$-aromatic hydrogen and the $C_8$-benzylic hydrogen of **15**. A more complete discussion of this phenomenon has been reported for the 5,10b-ethanophenanthridine ring system.[3]

(23) H.-G. Boit, *Chem. Ber.*, 87, 1339 (1954).

group had been introduced and that no free hydroxyl groups were present. Hydrolysis of **12** afforded 11-oxohaemanthidine. The ir spectrum indicated one ketone carbonyl (1765 $cm^{-1}$) and one hydroxyl group (3600 $cm^{-1}$). From our earlier experience with 11-oxohaemanthamine,[24] we expected the formation of haemanthidine (**1a**) and 11-epihaemanthidine (**19**) upon reduction of **18** with lithium aluminum hydride. Haemanthidine was isolated from the reduction in approximately 10% yield. The major product defied crystallization but was pure by thin-layer chromatographic criteria in several solvent systems. The ir spectrum of the compound was considerably different from that of haemanthidine but indicated the presence of the anticipated methylenedioxy, hydroxyl, and aliphatic methoxyl groups. The nmr spectrum showed two large (6.69 and 6.79 ppm) and two small (6.75 and 6.90 ppm) aromatic proton singlets, and one large (5.87 ppm) and one small (5.57 ppm) singlet corresponding to the benzylic proton. Our original interpretation of this spectrum was in terms of the desired 11-epihaemanthidine (**19**). The observed complex signals could be attributed to the compound's existence in solution as a mixture of 6-hydroxyl epimers.[3] This compound was treated with methyl iodide, followed by evaporation to dryness and solution in dilute (pH 4) hydrochloric acid. Extraction with chloroform after the aqueous solution had been made basic (pH 10) with potassium carbonate afforded 6a-epipretazettine (**21**) as the sole product. The ir and nmr[25] spectra of **15** were almost identical with those of the compound assigned initially the structure **19**. The ORD and CD spectra of 6a-epipretazettine and its pregenitor are almost identical and are superimposable with the spectra of tazettine (Figure 2).[26] These data suggest that both compounds possess the same ring system and that this system is not the 5,10b-ethanophenanthridine as originally anticipated. Although 11-epihaemanthidine (**13**) must have been the initial product from the lithium aluminum hydride reduction of 6-acetyl-11-oxohaemanthidine (**12**), the compound in solution underwent a facile isomerization to N-demethyl-6a-epipretazettine (**14**). The complex aromatic and benzylic absorptions in the nmr spectrum of N-demethyl-6a-epipretazettine must be due either to a small concentration of the 11-epihaemanthidine isomer (**13**) or to the compound's existence in solution as a mixture of $C_8$-hydroxyl group epimers (as **15** was described) or a combination of both effects. The ORD and CD of **14** in various solvents and pH conditions indicate that the change from the 5,10b-ethanophenanthridine nucleus was not reversible. Further evidence that 11-epihaemanthidine (**13**) exists as N-demethyl-6a-epipretazettine (**14**) was found in the isolation of only one oxidation product upon treatment of the base with manganese dioxide. The product was identified as 6a-epi-N-demethyl-3-epimacronine (**16**). No trace of 11-epi-6-oxohaemanthamine was detected.

To investigate the rearrangement of pretazettine to tazettine in more detail, an attempt was made to rearrange 6a-epipretazettine (**15**) by base. Compound **15** is comparable with pretazettine except that the strain inherent in the *trans* B–D ring is reduced. The rearrangement to tazettine was readily carried out, but stronger base and a longer reaction time were required.




## Discussion

From these experimental results, it is possible to delineate some of the factors which influence the facile rearrangements of compounds of types **1**, **2**, and **5**. The epimerization of the $C_6$-hydroxyl group of **1** and the evidence for the existence of N-demethylpretazettine indicate the ease with which the N–$C_6$ bond is broken to form the amino aldehyde intermediate **3**. Hemiacetal (**2**; $R_3$, $R_4$ = H) formation between the $C_{11}$-hydroxyl group and the aldehyde, must involve rotation about the $C_{10a}$–$C_{10b}$ bond of **3** and subsequent deformation of the C and D rings. For these alterations to occur, the intermediate must have a considerable lifetime. However, the favorable attack of the secondary amine on the carbonyl of **3** to reform the B ring tends to shorten the existence of this intermediate. The formation of the hemiacetal is not favored and it can only be isolated when stabilized in some manner.

(24) H. M. Fales and W. C. Wildman, *J. Amer. Chem. Soc.*, **82**, 197 (1960).

(25) Haemanthidine (**1a**) and several related alcohol amines have been shown to exist in solution as a mixture of benzylic hydroxyl epimers.[3] This was demonstrated by the two chemical shifts of the $C_6$-benzylic proton as well as the $C_7$-aromatic proton. The nmr spectrum (at 60 MHz) of 6a-epipretazettine (**14**) shows two singlets at 6.89 and 6.72 ppm which were attributed to the two aromatic protons. Integration of these peaks indicated that they accounted for 1.2 and 0.8 protons, respectively. Expansion of these peaks (at 100 MHz) revealed that the 6.89-ppm peak contained a large and a small singlet. The minor singlet and the singlet at 6.72 ppm (both assigned to the $C_9$-aromatic proton) as well as the large and small singlets associated with the $C_6$-benzylic proton, provides evidence for the epimeric character of the benzylic hydroxyl group of this compound.

(26) Tazettine (**5a**) has been proposed to contain a *cis* B–D ring fusion on the basis of spectral characteristics of certain degradation products of the base.[6] The results of this study have been challenged.[27] By our method of synthesis, 6a-epipretazettine (**15**) must have a *cis* B–D ring fusion. Although pretazettine and 6a-epipretazettine differ only in the stereochemistry of the B–D ring fusion, the ORD and CD spectra make these two ring fusions easily distinguishable. The similarity between the ORD and CD spectra of tazettine and 6a-epipretazettine lends strong support to the *cis* B–D ring fusion for tazettine.

(27) G. A. Morrison, *Fortschr. Chem. Org. Naturstoffe*, **25**, 269 (1967).

The reversible rearrangement of the metho salt of haemanthidine to pretazettine differs from the haemanthidine–N-demethylpretazettine case only by the additional N-methyl group. This additional group, for steric reasons, decreases the ability of the nitrogen to attack the carbonyl group and thus reform the 5,10b-ethanophenanthridine (**6**) ring system. The N-methyl group, in effect, increases the lifetime of the intermediate. When the metho salt **6** is placed in weakly basic aqueous solution, the amino aldehyde intermediate **8** is formed by hydrolysis. There is time for rotation of 90° about the $C_{10a}$–$C_{10b}$ bond and for the necessary C and D ring bending to allow hemiacetal formation. Any group which interferes with the nucleophilic character of the nitrogen aids the formation of the **2** skeleton. It would appear in this case that the energetically unfavorable ring distortions necessary to form **7** are offset by the decreased nucleophilicity of the nitrogen.

11-Epihaemanthidine (**13**) undergoes a facile rearrangement to N-demethyl-6a-epipretazettine (**14**). In solution **13** should undergo a B-ring opening similar to that found for haemanthidine. However, once the intermediate amino aldehyde has formed, the molecule can either reclose to the strained skeleton (**13**), or more favorably, form the more flexible molecule (**14**). The configuration of the $C_{11}$-hydroxyl groups in **13** minimizes C and D ring torsional modifications required in hemiacetal formation. The proximity of the $C_{11}$-hydroxyl group to the aldehyde of the intermediate makes hydroxyl group participation more favorable and decreases the interaction of the amine with the aldehyde.

6a-Epipretazettine (**15a**) has gained the less reactive tazettine skeleton without the usual crossed-Cannizzaro reaction.[5] However, it still undergoes the base-catalyzed rearrangement to tazettine but much less readily than pretazettine. This can be rationalized if the strain of the molecule is primarily responsible for the rate of formation of the amino aldehyde intermediate. When the strain is reduced, this opening is slowed considerably, but not stopped. Both the ring opened intermediates from **15** and pretazettine can orient equally well for the hydride shift leading to tazettine.

## Biosynthesis

Previous biosynthetic studies demonstrated that *S. formosissima* is able to convert haemanthamine to haemanthidine and ultimately to tazettine.[15] While the present paper casts doubt on the natural existence of tazettine, the biosynthetic experiments remain valid. N-Demethylpretazettine, formed by the rearrangement of haemanthidine, is a promising biosynthetic intermediate. The lactonic alkaloids (*e.g.*, 3-epimacronine) can be derived by benzylic oxidation and N-methylation. Identical processes can be visualized for the $C_3$ epimers—crinamine and precriwelline.

## Experimental Section[28]

**Pretazettine (7a). a.** To a solution of 100 mg of haemanthidine in 8 ml of methanol was added 2 ml of methyl iodide. The solution was allowed to stand 2 hr at room temperature before evaporation to dryness. The resulting methiodide was dissolved in 10 ml of distilled water, previously made acidic (pH 5) with hydrochloric acid. The aqueous solution was adjusted to pH 8 with potassium carbonate and extracted four times with chloroform. The chloroform extract was evaporated under reduced pressure to give 94 mg of pretazettine. Only trace impurities were present as detected by thin layer chromatography. The synthetic material showed ir and nmr spectra as well as chromatographic characteristics identical with those of the naturally occurring alkaloid. Pretazettine hydrochloride crystallized from 95% ethanol as prisms, mp 225° (lit.[5] 224–226°).

**b. Eschweiler–Clarke Procedure.** A solution of 100 mg of haemanthidine in 0.4 ml of 88% formic acid and 0.5 ml of 37% formaldehyde was heated at 90–100° for 6 hr. The mixture was cooled, diluted to 50 ml with water, made basic (pH 8) with potassium carbonate, and extracted four times with chloroform. The extract was evaporated under reduced pressure to give 95 mg of pretazettine. Thin layer chromatography indicated that pretazettine was the only compound present.

**Pretazettine.** ORD results (MeOH) were as follows: $[\Phi]_{320}$ +4100°, $[\Phi]_{301}$ +6600° pk, $[\Phi]_{275}$ +2700° tr, $[\Phi]_{248}$ +12,100° sh, $[\Phi]_{230}$ +18,100° (last reading); CD (MeOH): $[\theta]_{290}$ +7400°, $[\theta]_{240}$ +16,000°, $[\theta]_{225}$ +26,800° (last reading).

**Pretazettine Hydrochloride.** ORD results (MeOH) were as follows: $[\Phi]_{320}$ +1000°, $[\Phi]_{302}$ +3200° pk, $[\Phi]_{280}$ −4300° sh, $[\Phi]_{255}$ −7100° tr, $[\Phi]_{230}$ +8000° (last reading); CD (MeOH): $[\theta]_{284}$ +8800°, $[\theta]_{248}$ −4300°, $[\theta]_{225}$ +8000° (last reading); nmr ($D_2O$): δ 7.08, 7.05, 6.98, 6.95 (4s, two aromatic protons), 6.53 (broad singlet, two olefinic protons), 6.08 (s, 2, methylenedioxy), 3.48 ($-OCH_3$), and 3.32 ppm ($-NCH_3$).

**Precriwelline (7b).** To a solution of 329 mg of 6-hydroxycrinamine in 20 ml of methanol was added 6 ml of methyl iodide. The solution was allowed to stand 3 hr at room temperature before evaporation to dryness. The residue was dissolved in 10 ml of distilled water previously made acidic (pH 5) with hydrochloric acid. On standing overnight, the aqueous solution yielded 240 mg of precriwelline hydrochloride as needles, mp 199–200° (lit.[5] 199–201°). The free base was recovered by adjusting the pH of an aqueous solution containing the hydrochloride to pH 8 with potassium carbonate and extracting four times with chloroform. Evaporation of this extract under reduced pressure yielded 308 mg of amorphous precriwelline. The synthetic base had ir and nmr spectra and chromatographic behavior identical with the naturally occurring compound.[5]

**N-Demethyl-O-methylpretazettine (2a; $R_3$ = H, $R_4$ = $CH_3$).** A solution of 100 mg of haemanthidine in 8 ml of methanol was acidified (pH 4) with 0.1 ml concentrated hydrochloric acid and refluxed overnight. The methanolic solution was evaporated to dryness. Distilled water (20 ml), previously adjusted to pH 10 with ammonium hydroxide, was added to the residue. The resulting aqueous solution was extracted four times with chloroform and the extract was evaporated under reduced pressure to dryness to give 104 mg of an oil. Thin layer chromatography indicated the presence of haemanthidine ($R_f$ 0.2) and N-demethyl-O-methylpretazettine ($R_f$ 0.6). Components of the mixture were separated on preparative thin-layer plates to give 28 mg of haemanthidine and 66 mg of N-demethyl-O-methylpretazettine: (amorphous) $[\alpha]^{24}D$ +82° (*c* 0.18, $CH_3OH$); $\lambda_{max}$ (95% EtOH) 242 (ε 5650) and 291 mμ (ε 4050); ir ($CHCl_3$): 1508, 1488, 1050, 942 (aromatic methylenedioxy), 2838 ($-OCH_3$), and 3382 $cm^{-1}$ (–NH); nmr ($CDCl_3$): δ 6.79 and 6.70 (2s, aromatic protons), 5.90 (s, 2, methylenedioxy), and 3.55 and 3.41 ppm (2s, $-OCH_3$).

*Anal.* Calcd for $C_{18}H_{21}NO_5$: C, 65.26; H, 6.39; N, 4.23. Found: C, 65.28; H, 6.64; N, 4.39.

(28) Melting points were taken on a Kofler hot-stage apparatus and are corrected. Infrared spectra were obtained with a Beckman Model IR-12 spectrophotometer. Ultraviolet spectra were recorded on a Cary Model 14 spectrophotometer. Proton nuclear magnetic resonance spectra were obtained using Varian A-60 and HA-100 spectrometers. Optical rotations, optical rotatory dispersion, and circular dichroism spectra were determined with a modified Jasco Model 5 spectrometer. Thin layer (0.25 mm) and preparative thin layer (1.0 mm) chromatography were carried out using silica gel PF 254 + 366 (Merck). Ultraviolet light of the appropriate wavelength was used for identification. All chromatograms were developed in chloroform–methanol–diethylamine (92:3:5) unless otherwise stated. The proof of identity of two compounds was carried out, when possible, by comparison of melting points and mixture melting points and always by ir spectra and chromatographic characteristics. Elemental analyses of several compounds which could not be crystallized and were too sensitive to survive sublimation were obtained by high-resolution studies using an A.E.I. MS-9 mass spectrometer.

**N-Demethyl-O-methylprecriwelline (2b; $R_3$ = H, $R_4$ = $CH_3$).** In an analogous manner, 100 mg of 6-hydroxycrinamine was converted to a mixture of 44 mg of starting material and 48 mg of N-demethyl-O-methylprecriwelline: (amorphous) $[\alpha]^{24}D$ +193° (*c* 0.14, $CHCl_3$); $\lambda_{max}$ (95% EtOH) 243 ($\epsilon$ 6850) and 292 m$\mu$ ($\epsilon$ 4700); nmr ($CDCl_3$): $\delta$ 6.80 and 6.56 (2s, aromatic protons), 5.90 (s, 2, methylenedioxy), and 3.54 and 3.40 ppm (2s, $-OCH_3$).

*Anal.* Calcd for $C_{18}H_{21}NO_5$: C, 65.26; H, 6.39; N, 4.23. Found: C, 65.33; H, 6.55; N, 4.24.

**O-Methylpretazettine (2a; $R_3$, $R_4$ = $CH_3$).** To a solution of 50 mg of N-demethyl-O-methylpretazettine in 10 ml of methanol was added 2 ml of methyl iodide. The solution was allowed to stand at room temperature for 2 hr before evaporation to dryness under reduced pressure. The residue was dissolved in 10 ml of dilute hydrochloric acid (pH 4). The resulting solution was adjusted to pH 10 with potassium carbonate and extracted four times with chloroform. The extract, upon evaporation, gave 48 mg of O-methylpretazettine which showed ir spectra and chromatographic characteristics identical with the known compound;[5] ORD (MeOH): $[\Phi]_{320}$ +5000°, $[\Phi]_{300}$ +9500° pk, $[\Phi]_{282}$ +4000° tr, $[\Phi]_{250}$ +21,700° sh, $[\Phi]_{230}$ +24,800° (last reading); CD (MeOH): $[\theta]_{293}$ +11,200°, $[\theta]_{240}$ +28,000°, $[\theta]_{225}$ +30,500° (last reading).

**Dihydropretazettine (9). a. From Pretazettine (7a).** A solution of 300 mg of pretazettine in 10 ml of glacial acetic acid was added to a suspension of 110 mg of previously reduced Adam's catalyst in 15 ml of acetic acid and hydrogenated at room temperature and atmospheric pressure. The compound absorbed slightly more than 1 equiv of hydrogen in 2 hr. When the reduction was complete, the solvent was removed by evaporation under reduced pressure, and the resulting oil was dissolved in approximately 50 ml of water. The aqueous solution was made basic (pH 10) with ammonium hydroxide and extracted four times with chloroform. The extract yielded 280 mg of dihydropretazettine which was pure by thin-layer chromatographic criteria.

**b. From Dihydrohaemanthidine.** A solution of 90 mg of dihydrohaemanthidine in 8 ml of methanol and 2 ml of methyl iodide was allowed to stand at room temperature for 4 hr. The solution was evaporated to dryness under reduced pressure and 10 ml of dilute hydrochloric acid (pH 6) was added. The residue dissolved slowly upon warming on a steam bath. The aqueous solution was made basic (pH 10) with potassium carbonate and extracted four times with chloroform. The chloroform extract upon evaporation gave 94 mg of dihydropretazettine identical with that obtained by the reduction of pretazettine. The compound is amorphous; $[\alpha]^{24}D$ +39° (*c* 0.22, $CHCl_3$); $\lambda_{max}$ (95% EtOH) 238 ($\epsilon$ 4050) and 291 m$\mu$ ($\epsilon$ 4300); nmr ($CDCl_3$): $\delta$ 6.96 and 6.88 (2s, aromatic protons), 5.92 (s, 2, methylenedioxy), 3.40 ($-OCH_3$), and 2.45 ppm ($-NCH_3$); nmr ($D_2O$): $\delta$ 6.81 and 6.76 (2s, aromatic protons), 5.96 (s, 2, methylenedioxy), 3.41 ($-OCH_3$), and 3.03 ppm ($-NCH_3$).

*Anal.* Mass calcd for $C_{18}H_{23}NO_5$: 333.155. Found: 333.157.

**Dihydrotazettine (11).** Sufficient methanol was added to dissolve 50 mg of dihydropretazettine in 1 ml of water. This solution was made basic with 1 ml of 40% sodium hydroxide solution and refluxed 3 hr. The aqueous solution was cooled, diluted to 50 ml with water, and extracted four times with chloroform. The chloroform extract was evaporated to dryness under reduced pressure to give 42 mg of dihydrotazettine, identical in all respects with the compound prepared by the hydrogenation of tazettine. The residue crystallized as prisms from ethyl acetate, mp 164–166° (lit.[29] mp 168–169°).

**6-Acetyl-11-oxohaemanthidine (12).** A solution of 312 mg of haemanthidine, 3 ml of dimethyl sulfoxide, and 2 ml of acetic anhydride was allowed to stand at room temperature for 10 hr. The mixture was diluted to 30 ml with water, made basic (pH 10) with ammonium hydroxide, and extracted four times with benzene. The organic layer was reextracted three times with dilute ammonium hydroxide (pH 8). The benzene solution was evaporated to dryness to provide a green oil. The color and residual dimethyl sulfoxide were removed by column chromatography using silica gel packed in chloroform. Elution with chloroform provided 301 mg of product. Although thin layer chromatography indicated only one compound was present, it remained amorphous: $[\alpha]^{24}D$ +64° (*c* 0.26, $CH_3OH$); $\lambda_{max}$ (95% EtOH) 251 m$\mu$ ($\epsilon$ 3930), 296 (4400), shoulders at 314 (2640), and 325 (1850); ir ($CHCl_3$): 1751 $cm^{-1}$ (C=O); nmr ($CDCl_3$): $\delta$ 5.93 (s, 2, methylenedioxy), 3.38 ($-OCH_3$), and 2.22 and 2.17 ppm (two portions of $-OCOCH_3$ due to the epimeric nature of the $C_6$ position[3]).

*Anal.* Calcd for $C_{19}H_{19}NO_6$: C, 63.86; H, 5.36; N, 3.92. Found: C, 63.64; H, 5.42; N, 4.04.

**11-Oxohaemanthidine.** A solution of 100 mg of 6-acetyl-11-oxohaemanthidine in 8 ml of methanol was made basic with one drop of 40% sodium hydroxide solution and allowed to stand at room temperature for 30 min. The solution was neutralized with dilute hydrochloric acid and evaporated to dryness under reduced pressure. The residue was dissolved in approximately 50 ml of water and the solution adjusted to pH 8 with ammonium hydroxide and extracted four times with chloroform. Evaporation of the organic solvent provided 88 mg of an oil. The product was purified by preparative thin layer chromatography. The major band ($R_f$ 0.5) was recovered as an amorphous material: $[\alpha]^{24}D$ +60° (*c* 0.20, $CHCl_3$); $\lambda_{max}$ (95% EtOH) 251 m$\mu$ ($\epsilon$ 3920), 295 (4600), shoulders at 314 (2740), and 326 (2000); ir ($CHCl_3$); 1765 (C=O) and 3600 $cm^{-1}$ (hydroxyl group).

*Anal.* Calcd for $C_{17}H_{17}NO_5$: 315.111. Found: 315.111.

**N-Demethyl-6a-epipretazettine (14). a. From 6-Acetyl-11-oxohaemanthidine (12).** To a solution of 301 mg of 6-acetyl-11-oxohaemanthidine in 15 ml of tetrahydrofuran was added 300 mg of lithium aluminum hydride. The reaction was allowed to proceed at room temperature for 2 hr. Excess hydride was destroyed by the addition of a few drops of water saturated with sodium sulfate, and the inorganic solids were removed by filtration. The solvent was evaporated to dryness under reduced pressure. The residue (231 mg) was shown by thin layer chromatography to be a mixture of haemanthidine and one other major compound. Preparative thin layer chromatography recovered 38 mg of haemanthidine ($R_f$ 0.3) and 182 mg of N-demethyl-6a-epipretazettine ($R_f$ 0.5).

**b. From 11-Oxohaemanthidine.** To a solution of 30 mg of 11-oxohaemanthidine in 10 ml of tetrahydrofuran was added 40 mg of lithium aluminum hydride. The reduction was allowed to proceed at room temperature for 1 hr. The reaction was quenched and the product was isolated in the same manner as reported in part a. Thin layer chromatography provided 8 mg of haemanthidine and 18 mg of N-demethyl-6a-epipretazettine: (amorphous); $[\alpha]^{24}D$ +133° (*c* 0.22, $CH_3OH$); $\lambda_{max}$ (95% EtOH) 241 ($\epsilon$ 4230) and 292 m$\mu$ ($\epsilon$ 3120); ir ($CHCl_3$): 1508, 1490, 1043, 941 (aromatic methylenedioxy), 2835 ($-OCH_3$), and 3600 $cm^{-1}$ (–OH); nmr ($CDCl_3$): $\delta$ 6.79 and 6.69 (two major aromatic singlets), 6.90 and 6.75 (two minor aromatic singlets), 5.90 (s, 2, methylenedioxy), 5.85 and 5.76 (2 parts of benzylic proton), and 3.44 ppm ($-OCH_3$); ORD (MeOH): $[\Phi]_{320}$ +2000°, $[\Phi]_{312}$ +2400° pk, $[\Phi]_{300}$ +1000° tr, $[\Phi]_{280}$ +9450° sh, $[\Phi]_{260}$ +25,200° pk, $[\Phi]_{236}$ +14,600° tr, $[\Phi]_{228}$ +18,000° (last reading); CD (MeOH): $[\theta]_{283}$ −2600°, $[\theta]_{240}$ +16,900°, $[\theta]_{225}$ +16,300° (last reading).

*Anal.* Mass calcd for $C_{17}H_{19}NO_5$: 317.126. Found: 317.125.

**6a-Epipretazettine (15).** A solution of 112 mg of 11-epihaemanthidine in 10 ml of methanol and 3 ml of methyl iodide was allowed to stand at room temperature for 2 hr. The solution was evaporated to dryness under reduced pressure, and the residue was dissolved in 6 ml of dilute (pH 5) hydrochloric acid. The aqueous solution was then adjusted to pH 10 with potassium carbonate and extracted four times with chloroform. The chloroform extract, upon evaporation, gave 90 mg of 6a-epipretazettine: (amorphous) $[\alpha]^{24}D$ +188° (*c* 0.20, $CH_3OH$); $\lambda_{max}$ (95% EtOH) 242 ($\epsilon$ 6000) and 291 m$\mu$ ($\epsilon$ 4500); nmr ($CDCl_3$): $\delta$ 6.89 and 6.72 (2s, aromatic protons),[27] 5.91 (s, 2, methylenedioxy), 5.83 and 5.75 (2 parts of benzylic proton), 3.46 ($-OCH_3$), and 2.48 ppm ($-NCH_3$); ORD (MeOH): $[\Phi]_{320}$ +4000°, $[\Phi]_{310}$ +4500° pk, $[\Phi]_{300}$ +3200° tr, $[\Phi]_{280}$ +10,800° sh, $[\Phi]_{253}$ +26,200° pk, $[\Phi]_{240}$ +16,000° tr, $[\Phi]_{225}$ +26,000° (last reading); CD (MeOH): $[\theta]_{288}$ −4000°, $[\theta]_{245}$ +24,000°, $[\theta]_{225}$ +15,000° (last reading).

*Anal.* Mass calcd for $C_{18}H_{21}NO_5$: 331.142. Found: 331.140.

**Tazettine (5a).** A solution of 40 mg of 6a-epipretazettine in 10 ml of methanol was made basic with 1 ml of 40% sodium hydroxide and refluxed for 2 hr. The solution was cooled, diluted to 100 ml with water, and extracted three times with chloroform. Evaporation of the solvent under reduced pressure gave 35 mg of tazettine after crystallization from acetone, mp 209–210° (lit.[30] 210–211°); ORD (MeOH): $[\Phi]_{320}$ +3000°, $[\Phi]_{298}$ +1000° tr, $[\Phi]_{280}$ +10,700° sh, $[\Phi]_{251}$ +30,000° pk, $[\Phi]_{238}$ +10,000° tr, $[\Phi]_{225}$ +14,800° (last reading); CD (MeOH): $[\theta]_{290}$ −5500°, $[\theta]_{242}$ +32,000°, $[\theta]_{225}$ +17,000° (last reading).

**Manganese Dioxide Oxidation of 11-Epihaemanthidine.** A solution of 105 mg of 11-epihaemanthidine was stirred at room temperature for 2 hr in 100 ml of chloroform containing 1 g of manganese dioxide. The reaction mixture was filtered, and the filter

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cake was washed repeatedly with chloroform. Evaporation of the combined chloroform filtrates under reduced pressure afforded 88 mg of oil. Two components of this residue were separated by preparative thin layer chromatography. The band at $R_f$ 0.7 provided 23 mg of a compound which was found to be a decomposition product of the material with $R_f$ 0.65. The major band at $R_f$ 0.65 provided 62 mg of 6a-epi-N-demethyl-3-epimacronine (**16**): (amorphous) $[\alpha]^{24}D$ +79° (*c* 0.14, $CHCl_3$); $\lambda_{max}$ (95% EtOH) 228 (25,000), 268 (5800), and 310 mμ (ε 5850); ir ($CHCl_3$): 1711 $cm^{-1}$ (C=O); nmr ($CDCl_3$): δ 6.84 and 7.55 (2s, aromatic protons), 6.04 (s, 2, methylenedioxy), and 3.46 ppm (s, $-OCH_3$).

*Anal.* Mass calcd for $C_{17}H_{19}NO_5$: 315.111. Found: 315.111.

**Haemanthamine.** ORD results (MeOH) were as follows: $[\Phi]_{320}$ +3100°, $[\Phi]_{305}$ +7600° pk, $[\Phi]_{275}$ −6300° sh, $[\Phi]_{252}$ −10,200° tr, $[\Phi]_{230}$ +12,100° (last reading); CD (MeOH): $[\theta]_{290}$ +13,100°, $[\theta]_{245}$ −10,200°, $[\theta]_{225}$ +15,000° (last reading).

**Haemanthamine Methiodide.** ORD results (MeOH) were as follows: $[\Phi]_{320}$ +2000°, $[\Phi]_{305}$ +4300° pk, $[\Phi]_{280}$ −5100° sh, $[\Phi]_{255}$ −8600° tr, $[\Phi]_{230}$ +20,000° (last reading); CD (MeOH): $[\theta]_{295}$ +6300°, $[\theta]_{245}$ −7000°, $[\theta]_{230}$ +8000° (last reading).

**Haemanthidine.** ORD results (MeOH) were as follows: $[\Phi]_{320}$ +3000°, $[\Phi]_{303}$ +5000° pk, $[\Phi]_{280}$ −5000° sh, $[\Phi]_{254}$ −13,600° tr, $[\Phi]_{230}$ +5000° (last reading); CD (MeOH): $[\theta]_{294}$ +11,400°, $[\theta]_{244}$ −7600°, $[\theta]_{225}$ +6000° (last reading).

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# Chlorinated Cyclopentenone Fungitoxic Metabolites from the Fungus, *Sporormia affinis*

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**Abstract:** Three chlorinated metabolites having a structural similarity to terrein have been isolated from fermentations of *Sporormia affinis* Sacc., Bomm and Rouss. Single crystal X-ray analysis was used to show the structure and absolute configuration of the major dichlorinated metabolite to be (1*S*,5*S*)-2-cyclopentene-1-carboxylic acid, 2-*trans*-allyl-3,5-dichloro-1-hydroxy-4-oxo methyl ester (I). Spectral data and ORD studies were then used to assign the structures (1*S*,5*S*)-2-cyclopentene-1-carboxylic acid, 2-*trans*-allyl-3-chloro-1-hydroxy-4-oxo methyl ester (II) and (1*S*,5*R*)-2-cyclopentene-1-carboxylic acid, 2-*trans*-allyl-3-chloro-1-hydroxy-4-oxo methyl ester (III), respectively to the monochlorinated compounds.

Raistrick and others[1,2] isolated and suggested the structure of the metabolite terrein from *Aspergillus terreus*. Several years later Barton and Miller[3] confirmed the structure and determined the absolute configuration of this metabolite. Recently Birch and others[4] have described the biosynthesis of terrein as arising from a polyketide origin with the unusual feature of two linked carboxyl-derived carbons at the 6,7 positions, a distribution which might be explained by contraction of a six-membered precursor (see below). We now wish to report the isolation and complete characterization of three halogenated metabolites from fermentations of *Sporormia affinis* Sacc., Bomm and Rouss each having the terrein skeleton. The structures of the new metabolites are consistent with the proposed biosynthetic pathway for terrein. All of the carbons of the hypothetical precursor appear to be retained in these compounds. In addition these metabolites have interesting antifungal and some antibacterial activity.

For our purposes *Sporormia affinis* was grown in submerged culture under standard conditions of aeration and agitation and the metabolic products were isolated after 120 hr by carbon adsorption followed by at least two further chromatographic steps. Several dihydroisocourmarin products were isolated in addition to the products described in this paper.[5] The most abundant metabolite is an optically active, white, crystalline compound which can readily be crystallized from ether or ethyl acetate as rosettes with a melting range 135.5–136.5°. It is soluble in boiling cyclohexane from which it crystallizes rapidly as long needles on cooling. The crystals from cyclohexane invariably melt at 138–139°. Solutions of this metabolite darken immediately on treatment with basic reagents or nucleophiles such as cyanide ion. However, the material is quite stable to acids. It can be recovered unchanged from solution in concentrated sulfuric acid and after 20 hr of refluxing in dilute acid solution between 30 and 40% of unchanged starting material remains. The molecular formula of the material is $C_{10}H_{10}O_4Cl_2$ (mass spectrum) and because of the presence of the two heavy atoms, it was decided to subject the compound to single crystal X-ray structural analysis.

The crystals were found to be monoclinic, with the unit cell dimensions $a$ = 7.726 Å, $b$ = 13.100 Å, $c$ = 6.277 Å (±0.004 Å), and $\beta$ = 110.75° (±0.10°). The observed density of 1.439 g/cc (23°) is in agreement with a cell content of two molecules $C_{10}H_{10}O_4Cl_2$ ($\rho_c$ = 1.48 g/cc). The only observed extinction rules governed reflections of the type (0*k*0) for which $k = 2n$. This indicated the space group $P2_1$. Three dimensional intensity data were obtained from a crystal of dimensions

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