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Alkaloids of Tylophora II: Structural Studies

KOPPAKA V. RAO

Abstract □ Structural studies on the six alkaloids isolated from *Tylophora crebriflora* (N. O. Asclepiadaceae) are described here. Spectral data indicate that five of these alkaloids (A–E) possess the dibenzo[*f,h*]-pyrrolo[1,2*b*]isoquinoline skeleton known to be present in tylocrebrine. They differ in the number, nature, and distribution of the oxygen-bearing substituents and in the presence or absence of a benzylic-type hydroxyl. An oxygen substitution pattern of 3, 4, 6, and 7 is suggested for Alkaloids A, B, and C and that of 2, 3, 4, 6, and 7 for Alkaloids D and E. Alkaloid F is shown to be a seco analog of tylocrebrine with a 1,2-diphenyl *cis* stilbene skeleton.

Keyphrases □ Alkaloids—*Tylophora crebriflora* □ Structural studies—*T. crebriflora* alkaloids □ NMR spectroscopy—structure □ IR spectrophotometry—structure □ UV spectrophotometry—structure

The isolation of six new alkaloids designated as A, B, C, D, E, and F, together with the known compounds tylocrebrine and tylophorine from *Tylophora crebriflora*, S. T. Blake (N. O. Asclepiadaceae), was described in Part I (1). An examination of the analytical and spectral data indicated that Compounds A–E resemble tylocrebrine (I) in that they possess the dibenzo[*f,h*]-pyrrolo[1,2*b*]isoquinoline skeleton with four or five oxygen-bearing substituents. The studies that provided evidence for the structures of these new members are described in this paper.

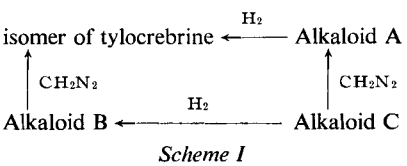
DISCUSSION

Alkaloid A, $C_{24}H_{27}NO_5$, resembles tylocrebrine in its UV and IR spectra and in having four methoxyl groups. The extra oxygen atom is in the form of a hydroxyl group, as shown by the formation of a monoacetate (bands at 1725 and 1225 cm.$^{-1}$ in IR and a sharp 3-proton peak at τ 7.85 in the NMR spectrum). Clemmensen reduction or catalytic hydrogenation converts Alkaloid A to a nonhydroxylic compound $C_{24}H_{27}NO_4$, thus indicating that the hydroxyl is benzylic. In its spectral and chromatographic behavior, the reduction product is almost indistinguishable from tylocrebrine, but the mixed melting (decomposition) point seems to indicate that the two may not be identical.

The presence of the benzylic hydroxyl in Alkaloid A is analogous to the case of tylophorinine (II, R = OH), another known member of this group (2). In this compound, the hydroxyl was placed at 14 instead of 9 (for numbering, see Structure I), because the latter structure would have represented a highly labile carbinolamine system and the stability of the compound was inconsistent with such a structure (2). This was later confirmed by synthesis (3). In an analogous manner, the stability of Alkaloid A strongly suggests the location of the hydroxyl to be 14. This is also supported by the following NMR spectral evidence: the chemical shifts of the benzylic CH(OH) protons in tylophorinine and Alkaloid A are very close: τ 3.96 and 3.87, respectively. The corresponding chemical shift of the same proton in the acetates of both compounds is τ 3.48; in both cases, it is split as a doublet, as would be expected. It is, therefore, concluded that Alkaloid A has the hydroxyl at 14.

Alkaloid B, $C_{23}H_{25}NO_4$, has three methoxyl groups. The fourth oxygen is part of a phenolic group (UV spectral shift in base and band at 3540 cm.$^{-1}$). This is supported further by the formation of a monoacetate (1750 cm.$^{-1}$ in IR and a 3-proton peak at τ 7.59). Methylation with diazomethane leads to a tetramethoxy compound, $C_{24}H_{27}NO_4$, which is almost indistinguishable from tylocrebrine on the basis of spectral and chromatographic data, but the mixed melting (decomposition) point suggests that the two may not be identical.

Alkaloid C, $C_{23}H_{25}NO_5$, shows features similar to both A and B. It has three methoxyls and forms a diacetate (1725 and 1760 cm.$^{-1}$ in IR and 3-proton peaks at τ 7.87 and 7.59 for the alcoholic acetate and phenolic acetate groups, respectively). It can be converted by methylation to Alkaloid A and by Clemmensen reduction to B. Hence, this compound is the desmethyl derivative of Alkaloid A, and the position of the phenolic hydroxyl in B and C is the same. The transformations are shown in Scheme I.

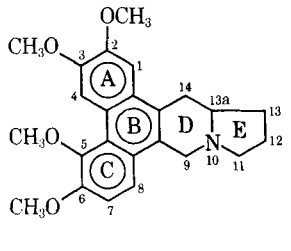


Scheme I

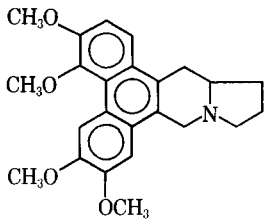
Alkaloids D and E have the compositions $C_{25}H_{29}NO_6$ and $C_{25}H_{29}NO_5$, respectively. They each have five methoxyl groups. Clem-

mensen reduction of Alkaloid D affords E. Based on arguments similar to those already advanced, Compound D is the 14-hydroxy derivative of E. The arrangement of the methoxyl groups will be discussed later in the paper.

At the time they proposed the structure of tylocrebrine, Gellert *et al.* (4) considered two possible alternatives for the arrangement of the methoxyl groups, as shown in Structures I and I*a*. To make a choice, both isomers were synthesized and compared with the natural tylocrebrine. It was observed that the spectral and physical properties of both isomers were very similar to each other, which made the comparison somewhat difficult. Hence, they subjected both synthetic compounds to Hofmann degradation and found that the product from I*a* depressed the melting point of the corresponding derivative of natural tylocrebrine. Thus, Structure I was selected as the correct one for tylocrebrine.



I



I*a*

The NMR spectrum of tylocrebrine (Fig. 1) shows resonance peaks which correspond to four aromatic protons, four methoxyls, and benzylic and methylene protons of expected chemical shifts and splitting patterns. The spectrum, however, cannot distinguish between Structures I and I*a*. The peak at τ 0.7 can be assigned to the proton at 4 (or 5), the peak at τ 2.74 to the proton at 1 (or 8), and the quartet at τ 2.33, 2.49, 2.74, and 2.89 to protons at 7 and 8 (or 1 and 2), depending on whether Structure I or I*a* is being considered.

The spectrum of Alkaloid A (Fig. 2B) shows general similarity to that of tylocrebrine, with an additional peak due to the benzylic hydroxyl and a shifted peak due to the proton at 14. In spite of the otherwise general similarity, one striking feature may be noted; that is the downfield shift (by 44 c.p.s.) of one-half of the AB quartet as compared with its position in the spectrum of tylocrebrine (Fig. 2A). The other half of the quartet also shows a slight downfield shift of 10 c.p.s. In the spectrum of the acetyl derivative of A (Fig. 2C), it can be seen that the larger downfield shift is not present and the positions of the peaks are very close to those of tylocrebrine.

A parallel situation exists in the spectra of Alkaloids B, C, and the diacetyl derivative of C (Figs. 2D, 2E, and 2F). The direction and magnitude of the downfield shift are the same as already noted. Also, in an analogous manner, the spectrum of the diacetate of C (Fig. 2F) is very close to that of Alkaloid B.

It appears that a hydroxyl function in the benzylic position has a strong deshielding effect on one of the two protons which form the AB system; protection of the hydroxyl by acetylation nullifies this effect. Since the hydroxyl group is located at 14 instead of 9 for reasons already stated, and since the 14-hydroxyl group is more likely to affect the proton at 1 than that at 8, it is apparent that the AB system is due to the protons at 1 and 2 rather than those at 7 and 8. This suggests that an oxygen substitution of the type shown in I*a* is present in Alkaloid A.

Very similar arguments have been used recently by Wiegrebe *et al.* (5) in assigning the structure for their Alkaloid A isolated from

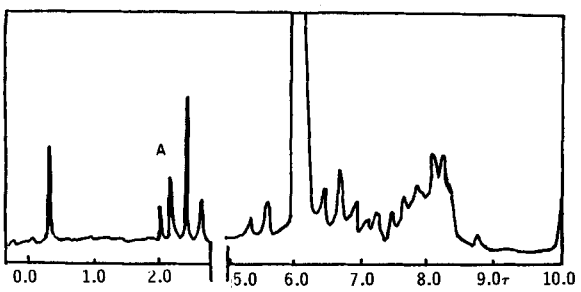


Figure 1—*NMR spectrum of tylocrebrine. Key: A, offset 200 c.p.s.*

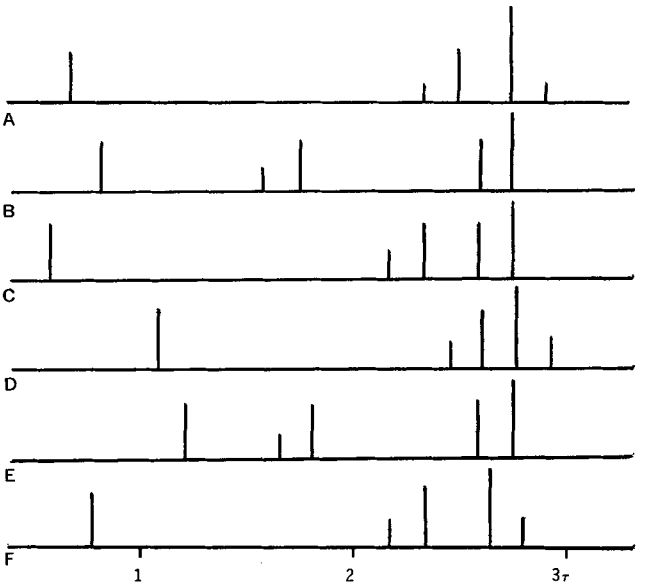
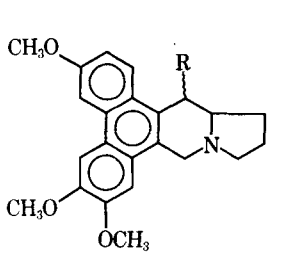


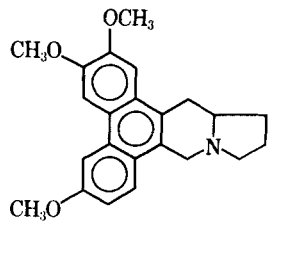
Figure 2—*NMR spectra of tylocrebrine, Alkaloid A,* O*-acetyl A, Alkaloid B, Alkaloid C, and* O*-acetyl C.*

Cyanchum vincetoxicum (L.) Pers. Because of the ambiguity of the NMR spectrum in distinguishing between the two methoxyl patterns: 3, 6, 7 (II, R = H) and 2, 3, 6 (II*a*), they introduced a substituent at 9 (a cyano group). Based on its deshielding influence on the protons at 8 and 7, the choice was made as the 2, 3, 6 isomer (II*a*).

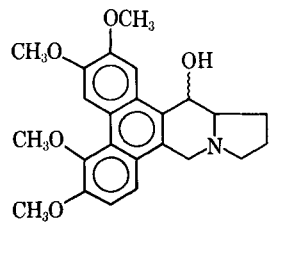
As further evidence for the structure of Alkaloid A (*T. crebriflora*), a comparison of the NMR spectra of deoxytylophorinine (II, R = H), tylophorinine, (II, R = OH), and acetyl tylophorinine (II, R = $OCOCH_3$) was undertaken. The location of the benzylic hydroxyl at 14 and the methoxyl pattern of 3, 6, 7 were established by synthesis by Govindachari *et al.* (3). Portions of the spectra pertinent to the discussion are shown in Figs. 3A, 3B, and 3C. Although the spectra are slightly more complex because of the additional proton in Ring A, one can recognize the half of the AB quartet in question. In deoxytylophorinine (Fig. 3A), these two peaks are located at τ 2.17 and τ 2.02; while in tylophorinine (Fig. 3B), which has the 14-hydroxyl group, these are shifted downfield to τ 1.75 and τ 1.60. In acetyltylophorinine (Fig. 3C), the peaks are shifted upfield to τ 2.24 and τ 2.09. Thus, introduction of the 14-hydroxyl group in deoxytylophorinine brings about a downfield shift of 25 c.p.s. of the peaks due to the proton at 1, and acetylation of the hydroxyl nearly cancels this effect. Based on these strong analogies, it appears that Alkaloid A is represented better by Struc-



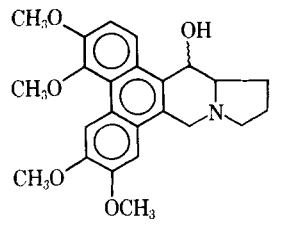
II



II*a*



III



IIIa

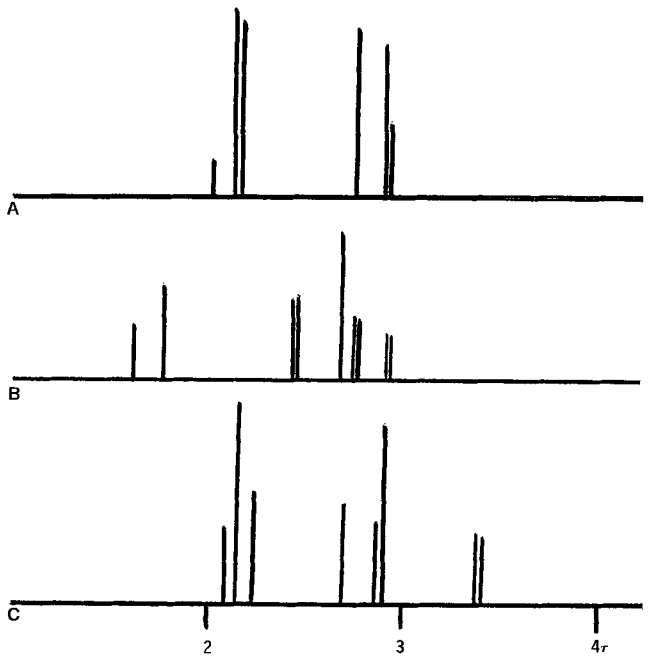
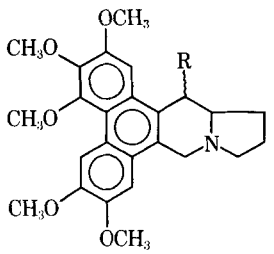


Figure 3—*NMR spectra of deoxytylophorinine, tylophorinine, and O-acetyltylophorinine.*

ture III*a* instead of III. It is thus possible that the reduction product of Alkaloid A will be the isomer of Structure I*a*.

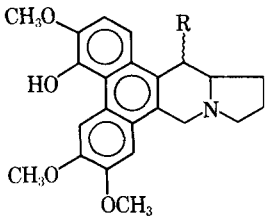
Alkaloids D and E contain five methoxyls each, and it has already been shown that D is the 14-hydroxy derivative of E. Their UV spectra bear much closer resemblance to the spectra of tylocrebrine and Alkaloid A than that of tylophorine, the 2, 3, 6, 7-tetramethoxy derivative. In the NMR spectra of both D and E (Figs. 4B and 4A), the familiar AB pattern characteristic of tylocrebrine and its derivatives is lacking. This suggests that the vicinal proton system (1, 2 or 7, 8) is absent and that the additional methoxyl is located at one of these. The 2-position was chosen for the following reasons. In the spectrum of E (Fig. 4A), the peak at τ 0.79 can be assigned to the proton at 5, and the signal at τ 2.87 (2 protons) can be assigned to those at 1 and 8. In the spectrum of D (Fig. 4B), the peak at τ 1.0 is equal to two protons (5 and 1), and the signal at τ 2.17 is equal to one proton (8). Thus the signal due to one of the protons is shifted downfield (105 c.p.s.) as a result of the introduction of the hydroxyl at 14. The most probable explanation appears to be that the additional methoxyl is located at 2 and the downfield shift (105 c.p.s.) observed is that due to the proton at 1 under the deshielding influence of the 14-hydroxyl. The NMR spectrum of the acetate of D (Fig. 4C) supports this view; the peak at τ 0.92 is again equal to one proton (5) and the other two peaks due to the protons 1 and 8 can be clearly seen upfield at τ 2.80 and 2.89, almost at the same positions as they are in E. The structures of E and D are represented in IV and V.



IV; R = H
V; R = OH

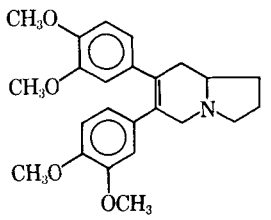
The magnitude of the downfield shift of the H-1 peak under the influence of the 14-hydroxyl appears to be roughly a function of the extent of substitution in Ring A. When there is only one methoxyl and three protons (at 1, 2, and 4), the shift is 25 c.p.s.; with two methoxyls and two protons (at 1 and 2), the shift is 44 c.p.s.; and with three methoxyls and one proton (at 1), the shift is 105 c.p.s. This suggests that the 14-hydroxyl group might be involved in some type of a hydrogen bonding with the π-electron system of Ring A. In addition to the observations of Wiegrebe *et al.* (5) already mentioned, similar effects were noted in the catechin series where they were attributed to the presence of a pseudoequatorial hydroxyl in close proximity to the aromatic system (6).

The site of demethylation in Alkaloids B and C will now be considered. They both have the 3, 4, 6, 7-substitution pattern, and one o the four groups is a phenolic hydroxyl. A comparison of the NMR spectra of A and C shows that the only significant difference between the two is the upfield shift (by 25 c.p.s.) of the signal due to the proton at 5. On acetylation, this shift becomes absent. The effect appears to be due to the increased electron density as a result of the close proximity to the phenolic hydroxyl. An examination of models indicates that the effect will be more pronounced if the hydroxyl is present at 4 than at 6. As a further support, the sharp band at 3540 cm.$^{-1}$ in IR suggests the presence of a hindered phenolic hydroxyl (7). Alkaloids B and C also give a positive Gibb's test (2,6-dibromo-*N*-chloroquinonimine), and this requires the phenolic group to be present at 4 since it is the only one with a free *para*-position. Also, the ready oxidizability to quinonoid products indicates the presence of a hindered phenolic group. Thus, B and C are represented by VI and VII.



VI; R = H
VII; R = OH

Alkaloid F differs from all the hitherto known members of the tylophora alkaloids in its physical properties. Chief among these is the UV spectrum, which shows a relatively broad maximum at 288 mμ (log ϵ, 4.03) and a shoulder at 240 mμ (log ϵ, 4.2), in contrast with the sharp maximum in the region 257–263 mμ (log ϵ, 4.8) shown by all the other members. However, the molecular formula, $C_{24}H_{29}NO_4$, differs from that of tylocrebrine by only two hydrogen atoms and they both contain four methoxyl groups. The mass spectra of both show intense peaks at M-69 mass units, which can be explained as being the result of a retro Diels-Alder type of fission with the expulsion of a neutral fragment, C_4H_7N. This result gives strong support to the presence of the indolizidine system in F. The NMR spectrum shows only two signals in the aromatic region at τ 3.39 and τ 3.43 in a ratio of 2:1 and shows general similarity to the spectrum of tylocrebrine otherwise. The absence of signals below τ 1.0 indicates alteration of the phenanthrene part of the skeleton. On oxidation with permanganate, veratric acid is isolated as the most significant product. Based on these results, Structure VIII is proposed. The physical properties and the proposed structure show that it is identical with septicine isolated earlier from *Ficus septica* (8).



VIII

Tylocrebrine and Alkaloids A, B, and C give dark-red solutions when treated with a number of oxidizing agents such as ceric sulfate, nitric acid, bromine water, or chromic acid. In contrast, tylophorine and tylophorinine do not respond to this reaction. In the cases of the two members (B and C) that possess the phenolic hydroxyl group, this oxidation can be achieved not only by the mentioned reagents but also by nitrous acid, periodate, and lead tetra-

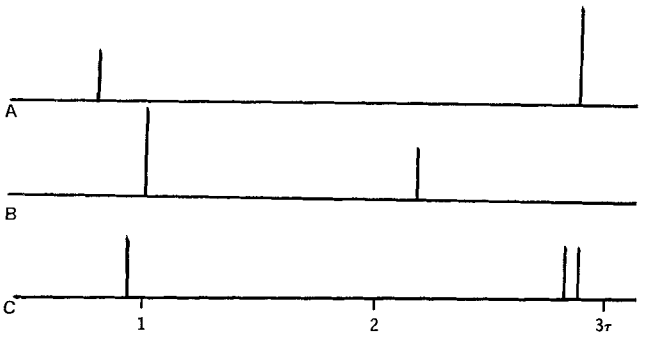
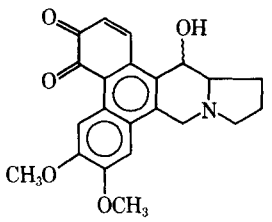


Figure 4—*NMR spectra of Alkaloid E, Alkaloid D, and* O-*acetyl D.*

acetate. The reaction product from Alkaloid A has the composition $C_{22}H_{21}NO_5$ with only two methoxyl groups. The compound appears to be an orthoquinone formed by the loss of two methoxyl groups, and the spectral evidence is in agreement with its formulation as IX. The requirement for the hindered methoxyl (or hydroxyl) group is indicated by the absence of the oxidation with tylophorine or tylophorinine.



IX

EXPERIMENTAL

General Method of Acetylation—A solution of the alkaloid (0.2–0.5 g.) in acetic anhydride (5–10 ml.) and pyridine (0.5–1 ml.) was heated at 100° for 2 hr. The cooled solution was diluted and made slightly basic. The precipitated solid was filtered and crystallized from methanol.

The acetate of Compound A is a colorless crystalline solid, m.p. 197–198°.

Anal.—Calcd. for $C_{26}H_{29}NO_6$: C, 69.16; H, 6.47; N, 3.10. Found: C, 69.13; H, 6.52; N, 3.03.

The acetate of Compound B is a colorless crystalline solid, m.p. 226–228°.

Anal.—Calcd. for $C_{25}H_{27}NO_5$: C, 71.25; H, 6.46; N, 3.32. Found: C, 70.75; H, 6.51; N, 3.36.

The diacetate of Compound C is a colorless crystalline solid, m.p. 216–218°.

Anal.—Calcd. for $C_{27}H_{29}NO_7$: C, 67.63; H, 6.10; N, 2.92. Found: C, 67.78; H, 6.17; N, 2.83.

The acetate of Alkaloid D is crystallized from ether–isopropyl ether. It is a colorless crystalline solid, m.p. 188–190°.

Anal.—Calcd. for $C_{27}H_{31}NO_7$: C, 67,34; H, 6.49; N, 2.91. Found: C, 67.27; H, 6.51; N, 2.79.

General Procedure for Clemmensen Reduction—Zinc dust (50 g.) was washed twice with acetone. It was then suspended in water (250 ml.) and treated with concentrated hydrochloric acid (25 ml.) and aqueous mercuric chloride (5 g. in 100 ml.). After standing for 10 min., the clear solution was decanted off and the zinc amalgam washed with water twice and kept under water.

A mixture of the alkaloid (0.5 g.), 2 *N* hydrochloric acid (50 ml.), and zinc amalgam (approximately 10 g.) was boiled under reflux for 24–36 hr. Aliquots were tested by paper chromatography for completion of the reaction. At the end, the clear solution was decanted off and enough sodium acetate added to pH 4–5. The solution was extracted twice with chloroform and the solvent extract concentrated to dryness. The solid was crystallized from a mixture of chloroform and methanol.

When reduced by this method, Alkaloid A gave a colorless crystalline solid, m.p. 219–220°. Mixed melting point with tylocrebrine was 210–215°.

Alkaloid C gave a colorless crystalline solid identical with Alkaloid B, and Alkaloid D gave a crystalline solid identical with Alkaloid E.

Hydrogenation of Alkaloid A—A solution of Alkaloid A (0.5 g.) glacial acetic acid (10 ml.) was hydrogenated in the presence of Adanis catalyst (0.2 g.) at 50 p.s.i. for 48 hr. The reaction mixture was filtered, diluted with water, heated with a slight excess of ammonia, and extracted with chloroform. Paper chromatography of the extract showed the presence of unchanged starting material, which was removed by countercurrent distribution in the chloroform–ethyl acetate–3% aqueous acetic acid (7:3:10) system. The hydrogenation product was recovered and crystallized twice from chloroform–methanol. It was identical with the product obtained from Clemmensen reduction.

Oxidation of Alkaloid A—A solution of Alkaloid A (1 g.) in 1 *N* HCl (10 ml.) was cooled in an ice bath and treated with 5% ceric sulfate (15 ml.). The dark-red soluton was extracted with chloroform in the presence of sodium acetate. The dark-red extract was concentrated to dryness, and the solid crystallized from methylene chloride. The quinone hydrochloride is a maroon-red crystalline solid, m.p. >300°.

Anal.—Calcd. for $C_{22}H_{21}NO_5 \cdot HCl$: C, 63.53; H, 5.33, N, 3.36, and $OCH_3(2)$, 14.91. Found: C, 63.72; H, 5.52; N, 3.26, and OCH_3, 15.32.

SUMMARY

Of the six alkaloids, A, B, C, D, E, and F, isolated from *T. crebriflora*, S. T. Blake, five (A–E) retain the dibenzo[*f,h*]-pyrrolo[1,2*b*]isoquinoline skeleton present in tylocrebrine. The distribution of the oxygen-bearing substituents in these compounds is discussed based on the NMR spectral evidence. An oxygen substitution pattern of 3, 4, 6, and 7 is suggested for Alkaloids A, B, and C and that of 2, 3, 4, 6, and 7 for Alkaloids D and E. Alkaloid F is an interesting member, and a structure based on tetramethoxy stilbene is proposed for this.

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