CHAPTER IV

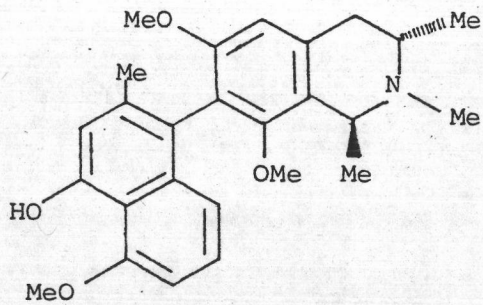
DISSCUSSION

After partition of the ethanol extract, the chloroform fraction was chromatographed on alumina to afford ancistrotectorine (0.016% yield). From its uv spectrum which displayed λ_{max} 234, 286, 306, 323 and 337 nm, ancistrotectorine was a member of the naphthalene-isoquinoline series of alkaloids containing a phenolic hydroxy group (ν_{max} 3375 cm^{-1}), even though no positive ferric chloride test or shift in base (2N NaOH solution) in the uv spectrum was observed. No molecular ion was observed in the electron impact (e.i.) mass spectrum, but a clear $(M^{\cdot} + H^{+})$ was found at m/z 422 in the chemical ionization spectrum using methane as the reagent gas. Important fragments in the e.i. spectrum were observed at m/z 406 and 203.

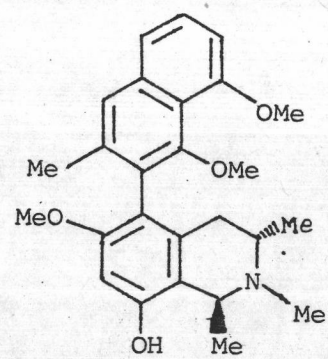
The low-field ^{1}H nmr spectrum of ancistrotectorine firmly established the naphthalene-isoquinoline nature of the compound and indicated the presence of two doublet methyl groups, and aromatic methyl group, an N-methyl group and three aromatic methoxy groups. The aromatic region was complex, although two singlets were apparent.

In order to evaluate the structure more explicitly a detailed examination of the high-field ^{1}H nmr spectrum of ancistrotectorine was undertaken. Two doublet methyl groups were observed at δ 1.268 and δ 1.466 from the 3-CH_3 and the 1-CH_3, respectively and their methine protons were observed at δ 2.544 and δ 3.731.

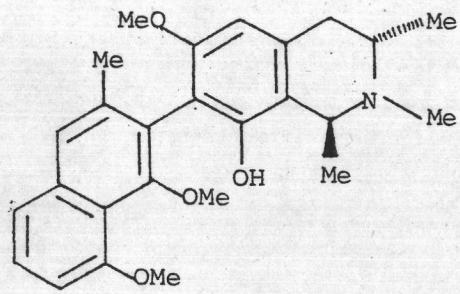
Irradiation at δ 1.27 collapsed the signal at δ 2.544 to a doublet of doublets (J = 3.0, 10.3 Hz) clearly establishing the 3-CH_3 to be equatorial. The aromatic region was now clearly resolved with singlets observed at δ 6.518, and 7.232, doublets at δ 6.689 and 7.343 and a triplet at δ 7.266. The latter three signals were assigned to adjacent protons on the naphthalene nucleus, and on biogenetic grounds a methyl group (δ 2.169) was placed at C-2′ and oxygenation at C-4′ and C-5′. Irradiation of the methoxyl group singlet at δ 3.691 caused an 8.1 % nOe effect in the singlet at δ 6.518, and the reverse irradiation produced an nOe (4%) only in the δ 3.691 singlet. A similar spatial relationship was established between the aromatic methoxy group at δ 3.981 and the doublet at δ 6.689, which could therefore be assigned to a 5′-OCH_3 and its adjacent 6′-H. There were a number of alternatives which could explain the former nOe experiments : i) a 7-1′ linkage, with the OH at C-4′ and the third OCH_3 at C-8, ii) a 7-3′ linkage with the OH/OCH_3 at C-8/C-4′ or reverse, and iii) a 5-3′ linkage with OH at C-8 and OCH_3 at C-4′. A 7-1′ linkage could be specifically excluded by these experiments. Potentially, these structures could be distinguished by the base-induced shift of a proton *ortho* or *para* to the hydroxy group and whether or not the proton shifted is *ortho* to a methoxy group. However, the failure of ancistrotectorine to undergo a bathochromic shift on the addition of 2N sodium hydroxide to the uv spectrum did not augur well for the success of this experiment and consequently the single crystal X-ray crystallographic analysis was carried out.



(i)

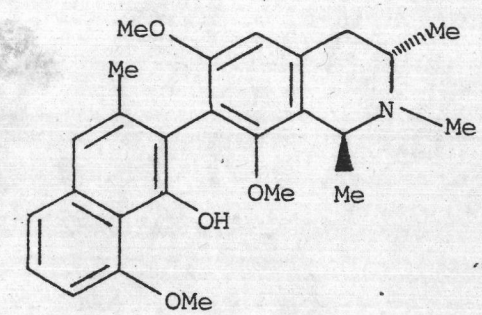


(iii)



(ii)

or



(ii)

Ancistrotectorine crystallised in the monoclinic $P2_1$ space group with unit cell dimensions of $\underline{a}$ = 11.858 (14), $\underline{b}$ = 7.043 (7) and $\underline{c}$ = 14.693 (28) Å. The crystal structure was elucidated using the program MULTAN[45] and refined using the program SHELX[46]. The hydrogen bond lengths were restricted to 1.0 Å during refinement and the largest peak on a final electron density difference map was 0.12 eÅ^{-3}. The final R = 8.0% over 1087 independent reflexions using a unit weighting scheme.

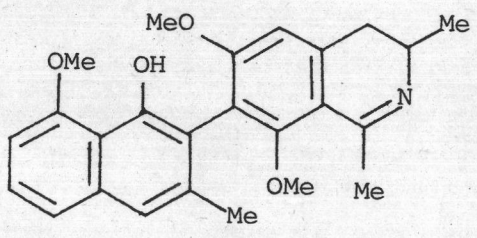
Final positional parameters, bond lengths, valency angles and torsion angles are listed in Tables 2-5 (pp. 85-89) respectively. The structure deduced for ancistrotectorine indicates that it is indeed 7-3′ linked naphthalene-isoquinoline alkaloid having the molecular

array shown in 2 (p.50) in which the C-1 and C-3 methyl groups are *cis* and the N-containing ring of the isoquinoline moiety adopts a sofa conformation with C-3 the out-of-plane atom. Steric hindrance about the diaryl linkage results in a dihedral angle of 56.4° between the two aromatic rings.

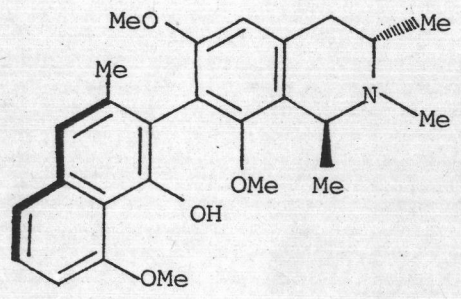
It now became possible to assign the ^{1}H spectrum completely. From the nOe experiments the singlets at δ 3.691 and δ 3.985 could be assigned to the 6- and 5′-OCH_3, respectively, leaving the singlet at δ 3.232 to be a somewhat shielded methoxy group at C-8. Similarly, the aromatic singlet at δ 6.518 could be assigned to H-5 and that δ 7.232 to H-1′. Examination of the coupling constants permitted the doublets of doublets at δ 2.655 and δ 2.787 to be assigned to H-4β and H-4α respectively. The complete assignments are shown on structure 3. Ancistrotectorine (2) is therefore a member of the rare 7-3′ linked naphthalene-isoquinoline alkaloids of which ancistrocladidine (1)[33], is the only other example.

The mass spectrum of ancistrotectorine also merits some additional comment. A facile loss of 15 amu is apparent to give the stable species at m/z 406. The most interesting ion though is m/z 203 since this does not correspond to either half of the molecule as might be expected, although it clearly is derived from the isoquinoline unit. In our estimation this ion may have the structure 4 in which one of the aliphatic methyl groups has been lost together with one of the methyls from an aromatic methoxy group. Ancistrocladidine (1) shows a loss of 15 amu from the molecular ion at m/z 405, but no stable species are observed below this mass.

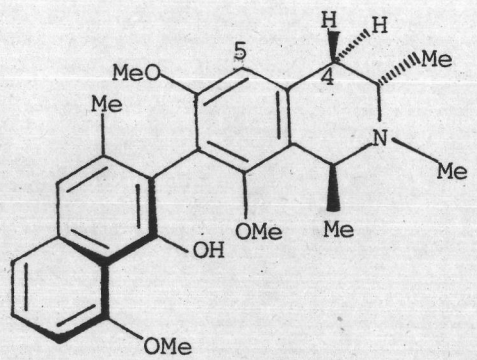
The absolute configuration of naphthalene-isoquinoline alkaloids has been studied previously[24,32]. The cd spectrum of ancistrotectorine in methanol showed a negative first Cotton effect ($[\theta]_{234}$ -34, 179 and a positive second Cotton effect ($[\theta]_{218}$ + 70, 167) which clearly demonstrates the absolute configuration of ancistrotectorine to be that shown in 2.



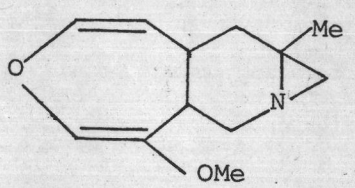
1



2



3



4 m/z 203