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ATHROTAXIS ALKALOIDS. PART I. ALKALOIDS OF A. *CUPRESSOIDES*

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Abstract - Eleven homo*erythrina*-type alkaloids (1-9, 11, 12) have been isolated from *A. cupressoides* (Taxodiaceae), of which six (6-9, 11, 12) had not previously been reported.

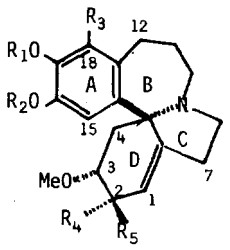
The genus *Athrotaxis* is confined to Tasmania, and comprises the only representatives in the southern hemisphere of the Taxodiaceae, a rather small family that nevertheless contains some of the largest and tallest trees in the world: *Sequoiadendron giganteum* (Big Tree) and *Sequoia sempervirens* (Californian Redwood) respectively. The family is now largely confined to eastern Asia and north America, but was once much more widely distributed as shown by fossil specimens, one of which (*Metasequoia glyptostrobus*) was found in recent times still growing in China. Although some species had been found to give alkaloid tests[1], no alkaloids have previously been isolated from members of the Taxodiaceae[2].

A. cupressoides Don (Pencil Pine) is a tree that grows above 1000 m in central and western Tasmania. The present report describes eleven alkaloids which have been isolated from its bark and foliage.

RESULTS AND DISCUSSION

The extraction of the fresh plant material and isolation of the alkaloids was carried out by standard means and had to be completed as soon as possible to avoid a marked diminution in yields: the alkaloids become undetectable in the plant material after a few days. They proved to have structures of the homo*erythrina*-type, and include five known bases: taxodine (1), which occurs in *Schelhammera* spp. as alkaloid B[3] and in *Cephalotaxus harringtonia* as alkaloid V[4]; 3-epischelhammericine (2), also a constituent of the two latter plants in which it is designated alkaloid E[3] and alkaloid IV[4] respectively, and of three further plants, *C. wilsoniana*[5] and *Dysoxylum lenticellare*[12], and *Phelline comosa* in which it occurs as alkaloid 4[6]; O-methylathrocupressine (3), present in *P. comosa* as alkaloid 5[6]; 2-epi-homoerythratine (4), found in *S. pedunculata* as alkaloid H[3] and in *P. comosa* as alkaloid 3[6]; and finally homoerythratine (5), first isolated from *P. brachyphylla*[7]. The identity of these *Athrotaxis* bases has been confirmed by the agreement of their physical data with reported values, and by a direct comparison with authentic specimens where these were available.

Of the remaining alkaloids, the spectra of 2-hydroxytaxodine (6) showed that it has a phenolic group and two methoxyls which, as in the case of taxodine (1), must be attached to aromatic and aliphatic carbons respectively from their ^1H nmr proton resonances. However, 2-hydroxytaxodine has an extra allylic alcohol group that can readily be removed[6] by conversion to the corresponding chloride followed by LAH reduction. In the process a multiplet signal around 64.3, corresponding in chemical shift to a methine proton in the carbinol group of an allylic alcohol, is eliminated from the ^1H nmr spectrum of the product, which proved to be identical with taxodine (1): the alcohol group in 2-hydroxytaxodine must thus be located at C-7 or C-2. A decision in favour of the latter alternative can be made from mass spectrometry: the ms of 2-hydrotaxodine (6) corresponds very closely with those of homoerythratine[7] (5), 2-epihomoerythratine



1 Taxodine (RI = Me, R_2=R_3=R_4=R_5=H)
2 3-Epischelhammericine (R_1-R_2=CH_2, R_3=R_4=R_5=H)
3 0-Methylathrocupressine (R_1=R_2=Me, R_3=OMe, R_4=R_5=H)
4 E-Epihomoerythratine (R_1-R_2=CH_2, R_3=R_5=H, R_4=OH)
5 Homoerythratine (R_1-R_2=CH_2, R_3=R_4=H, R_5=OH)
6 2-Hydroxytaxodine (R_1=Me, R_2=R_3=R_4=H, R_5=OH)
7 2-Hydroxyisotaxodine (R_1=R_3=R_4=H, R_2=Me, R_5=OH)
8 2-Epihydroxyisotaxodine (R_1=R_3=R_5=H, R_2=Me, R_4=OH)
9 Athrocupressine (R_1=R_2=Me, R_3=OH, R_4=R_5=H)
10 (R_1=R_3=R_4=R_5=H, R_2=Me)
11 2-Acetoxytaxodine R_1=Me, R_2=R_3=R_4=H, R_5=OAc)
12 2-Acetoxyisotaxodine (R_1=R_3=R_4=H, R_2=Me, R_5=OAc)
13 Schelhammerine (R_1-R_2=CH_2, R_3=R_5=H, R_4=OH; Me&at C-3)

[3,6] (4), and schelhammerine[8] (13), all of which have a similar structure to 6 with an allylic alcohol group at C-2 and a methoxyl at C-3. On the other hand, there are significant differences in fragmentation pattern as compared with the ms of analogous bases where the alcohol group is attached to C-7 instead of C-2[6]; moreover, the C-l olefinic protons in the ^{1}H nmr spectra of the latter bases resonate at δ5.92 as compared to 65.55 for 2-hydroxytaxodine (6). At the same time, this chemical shift for the olefinic proton in 6 gives evidence for the configuration of the hydroxyl group attached to the adjacent C-2 position: the corresponding values for the known alkaloids homoerythratine (5) and 2-epihomoerythratine (4), which differ only in the orientation of their C-2 hydroxyls, are 65.52 and 5.78 respectively. On this basis, the configuration shown in 6 *is* proposed for 2-hydroxytaxodine with the alcohol group *trans* to the C-3 methoxyl as in homoerythratine (5), and this stereochemistry is supported by a comparison of the specific rotation of 2-hydroxytaxodine (6, +51.5°) with those of homoerythratine (5, +63.6°) and 2-epihomoerythratine (4, +170°) measured under comparable conditions.

2-Hydroxyisotaxodine (7) is isomeric with 2-hydroxytaxodine (6), and a spectroscopic comparison of the two alkaloids indicated that they have the same functional groups and closely related structures and configurations. Their ms are almost identical, and furthermore, the C-3 methoxyl protons resonate at 63.28 in the ^{1}H nmr spectra of both bases, and also in that of the known alkaloid 2-epihomoerythratine[3] (4). With the epimeric configuration of the methoxyl, a value of around δ2.75 would be expected from analogy with schelhammerine[8] (13), the C-3 diastereomer of 4. Barton oxidation[9] of 2-hydroxyisotaxodine (7) gave a conjugated ketone whose ^{1}H nmr spectrum shows a peak at 66.00 corresponding to an olefinic proton α to the carbonyl group; a B-proton would be expected to resohate distinctly further downfield". The conjugated ketone was closely similar to, although not identical with that formed by a corresponding oxidation of 2-hydroxytaxodina (6). The alcohol group in 7 must thus be located at C-2, and furthermore it must have the same configuration as that in 2-hydroxytaxodine (6) from the optical rotation (+60.7°) of 2-hydroxyisotaxodine (7), and from the chemical shift (65.575) of the olefinic proton in its ^{1}H nmr spectrum. When the allylic hydroxyl was removed[6] from 7, the product (10) proved to be isomeric with taxodine (1) and closely similar to it spectroscopically. The mass spectra of 1 and 10 are virtually indistinguishable; there are, however, small but significant differences in their other spectra, in particular in the chemical shifts of the aromatic singlets in the ^{1}H nmr spectra, and a mixed melting point showed that the two bases are not identical. They evidently differ only in the pattern of substitution in their aromatic rings, and a corresponding difference must also exist between the alkaloids 2-hydroxytaxodine (6) and 2-hydroxyisotaxodine (7). When the H-3 proton (ca. δ3.4) and the H-12 protons (ca. δ2.2) of 7 were separately irradiated, the ^{1}H

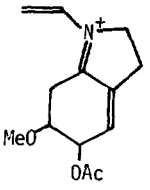
nmr spectrum revealed distinct nuclear Overhauser effects on the two aromatic singlets (δ6.725 and 6.575), which must thus be located at C-15 and C-18 respectively. The phenolic and methoxyl groups of 2-hydroxyisotaxodine are in consequence attached at C-17 and C-16, in the reverse arrangement to that of 2-hydroxytaxodine (6), and the structure and stereochemistry of 2-hydroxyisotaxodine is represented by 7.

2-Epihydroxyisotaxodine (8) could not be completely separated from 2-hydroxytaxodine (6). It proved to be isaneric but not identical with 6 and 2-hydroxyisotaxodine (7), and on Barton oxidation it gave a conjugated ketone corresponding to that from 7, but different to the ketone from 6. 2-Epihydroxyisotaxodine (8) and 2-hydroxyisotaxodine (7) presumably differ only in the configuration of their allylic alcohol groups, and this difference is borne out by the chemical shifts of their C-1 olefinic protons: ~5.75 and δ5.575 respectively (cf. 6, 5 and 4 above).

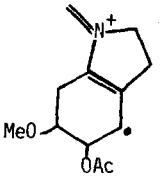
Athrocupressine (9) is a lower homologue of the known alkaloid 0-methylathrocupressine (3), to which it shows a general similarity in spectra. However, it has one less methoxyl, but it has instead a phenolic group from the bathochromic shift in its uv spectrum on addition of alkali, and from its positive Gibbs reaction". The latter observation indicates that the phenolic group has a free para position and is thus attached to C-15 or to C-18. The second alternative is evidently the correct one, since irradiation of the only aromatic proton in the ^{1}H nmr spectrwn of 9 produced an nOe on a methine proton resonating around 63.3, which must-be attached to the aliphatic carbon (C-3) bearing the methoxyl group. The protons of the latter group have the same chemical shift (δ3.28) as those of 0-methylathrocupressine (3), and the stereochemistry at C-3 for athrocupressine (9) must thus be the same as in 3, since the epimeric configuration of the C-3 methoxyl would result in a value around 62.75 (cf. 13[8]). The specific rotation and the remaining spectroscopic data for athrocupressine are in full accord with the structure and stereochemistry shown in 9. The phenolic group in 9 is unreactive to diazomethane, and an attempt to convert 9 to 3 was unsuccessful.

The alkaloids 2-acetoxytaxodine (11) and 2-acetoxyisotaxodine (12) were obtained in very small amounts only, and could not be satisfactorily separated from one another. Their spectra show a close resemblance to those of 2-hydroxytaxodine (6) and 2-hydroxyisotaxodine (7) except that additional absorptions due to acetate ester groups are present: in particular, a peak at 1730 cm^{-1} and a three-proton singlet at 62.11 in their ir and ^{1}H nmr spectra respectively showed that 11 and 12 are esters of alcohols rather than of phenols. Intense ions in their ms which may be formulated as 14 and 15[8] indicated that the acetoxy groups are attached at C-2. On basic hydrolysis, the alkaloids gave products corresponding to 2-hydroxytaxodine (6) and 2-hydroxyisotaxodine (7), and their structures may thus be represented by 11 and 12 respectively.

Since acetic acid was used in the course of the extraction and purification of the alkaloids, 2-acetoxytaxodine (11) and 2-acetoxyisotaxodine (12) could conceivably be artefacts formed by acylation of 6 and 7 respectively. To test this possibility, a rapid small-scale extraction of the fresh plant material was carried out, and the crude alkaloids were isolated using dilute sulphuric instead of acetic acid to separate the basic fraction. The presence of 11 and 12 therein was established by the ms technique of multiple metastable peak monitoring[13].



14 m/z 236



15 m/z 223

EXPERIMENTAL

Thin-layer chromatography (tlc), preparative thin-layer chromatography (ptlc) and column chromatography were performed with Merck silica gel GF_{254} or CAMAG silica gel DSF-5, and the compounds were visualised by spraying with iodoplatinate reagent or by examination under uv light. High-performance liquid chranatography (hplc) was carried out on a 7.7 mm x 25 cm column with octadecyl silane as stationary phase, and with buffered

aqueous acetonitrile for elution at a flow rate of 2 ml/min. The melting points (mp) were recorded on a Yanigimoto Seisakusho micro-melting point apparatus and are uncorrected. Specified rotations were measured in chloroform on a PEPOL 60 spectropolarimeter. Ultraviolet (uv) absorption spectra were recorded on ethanol solutions with a Hitachi-Perkin-Elmer 124 spectrophotometer, and the extinction coefficients are given in parenthesis. Infrared spectra were recorded on chloroform solutions with a Beckman IR-33 spectrometer. Proton magnetic resonance (^{1}H-nmr) spectra were recorded on deuterochloroform solutions with tetramethylsilane as internal standard, at 100 MHz with a Jeol JNM-4H-100 spectrometer unless otherwise specified; the 270 MHz 'H-nmr spectra were recorded with a Bruker HX-270 spectrometer. Chemical shifts are given in ppm, and peaks are described as singlets (s), doublets (d), triplets (t), quartets (q) or multiplets (m). Mass spectra were run on a Vacuum General Micromass 7070 F spectrometer by the direct insertion technique at 200" and 70 eV.

A voucher specimen of the plant material has been deposited in the collection of dried plant specimens in the Chemistry Department, University of Tasmania.

EXTRACTION.- Twigs and leaves of Pencil Pine were collected around the upper reaches of the Ouse River near the Great Lake, Tasmania, in May, 1980, and were immersed as soon as possible after collection in 150 litres of methanol. After three days, the plant material was removed, air dried for one day, then put through a compost shredder. The dry plant material (100 kg) was then percolated with methanol until a test sample gave a negative reaction with Mayer's reagent. The combined extracts were concentrated under reduced pressure at a temperature below 40°C to a thick gummy dark brown syrup, which was dissolved in 10 litres of warm glacial acetic acid. The solution was poured in a fine stream into 50 litres of water, which was subjected simultaneously to vigorous agitation with a vibromixer. The dilute acid extract was left to stand overnight, and the precipitate that settled out was filtered off, washed with water until free from alkaloids, then discarded.

The washings combined with the *acid aqueous* solution were evaporated to dryness under reduced pressure at a temperature below 35°. The residue was dissolved in 10 litres of water and again evaporated to dryness; the process of dilution and evaporation was repeated once more to remove most of the acetic acid. Finally the residue was dissolved in 10 litres of water and the solution was basified *to* pH 8-9 with ammonia (d 0.88). The heavy precipitate that formed was left overnight to settle, then filtered off through Hi-Flo Supercel. The dried precipitate was extracted with chloroform until the residue gave a negative Mayer's test, and the filtrate was likewise extracted with chloroform. The combined chloroform solutions were extracted with 5% (w/v) sulfuric acid (30 x 150 ml) until free from alkaloids. The aqueous acid solution was basified with ammonia (d 0.88) and again thoroughly extracted with chloroform (20 x 150 ml). The combined chloroform extracts were dried (Na_2SO_4) and evaporated under reduced pressure to give 4 g of crude alkaloids.

XSOLATION, PURIFICATION, AND CHARACTERISATION OF THE ALKALOIDS.- The crude alkaloid mixture (2.00 g) was separated by ptlc (7% MeOH/$CHCl_3$) into 7 fractions:

Fraction 1 amounted to 0.12 g, and contained two components from tlc (30% EtOAc/C_6H_6). The mixture was separated by triple development ptlc with the same solvent system. The higher *Rf* component (16 mg) was obtained as a yellowish oil, and proved to be identical with the known homo*erythrina* alkaloid 3-epischelhammericine (2); $[\alpha]_D^{19}$ + 106° (C = 1.6); λmax: 284 (3800), 219 nm (3900); νmax: 3000, 2920, 2840, 1460, 1210, 1120 cm-'; ^{1}H nmr: 6.7 (s, 1H), 6.6 (s, 1H), 5.88 (c, 2H), 5.5 (bs, 1H), 3.21 (s, 3H) and an unresolved number of protons between 1.5 and 3.5 6; m/z: 313 (M^+, 30), 282(35), 255(70), 254(70), 207(20), 178(100), 165(3), 146(32); picrate derivative mp 169-172°, undepressed on admixture with an authentic sample.

The lower *Rf* component (19 mg) was obtained as white needles from hexane, identical with the known homo*erythrina* alkaloid O-methylathrocupressine[3]; mp and mixed mp with an authentic sample: 100-110"; $[\alpha]_D^{19}$ + 91" (C = 1.5); λmax: 228 (1160), 275 nm (260); νmax: 3010, 2920, 2850, 1700, 1540, 1450, 1470, 7220 *cm-'*; ^{1}H nmr: 6.57 (s, 1H), 5.58 (bs, 1H), 3.92 (s, 3H), 3.22

(s, 6H), 3.28 (s, 3H), and an unresolved number of protons between 1.5 and 3.5 6; m/z 359 (M+, 22); meas: 359.2085, calc. for $C_{21}H_{29}NO_4$: 359.2298; 328 (35), 301 (4),286 (15), 254 (10), 178 (100), 165 (30), 146 (25).

Fraction 2 (0.199 g) also contained two components from tlc (5% MeOH/$CHCl_3$); the mixture was separated by double development ptlc with the same solvent system. The component of lower *Rf* (51.7 mg) proved to be a new *homoerythrina* alkaloid, athrocupressine (9), mp 152-153° (Me_2CO), $[\alpha]_D^{19}$ + 102.23 (C = 0.43), xmax: 227 (4204), 280 nm (940); after addition of a drop of 5% aqueous sodium hydroxide: 237 and 298 nm; νmax: 3500, 3400, 3000, 2920, 2840, 1600, 1490, 1310, 1120, 750 cm^{-1}; ^1H nmr (270 MHz): 6.39 (s, 1H), 5.65 (bs, 1H), 3.94 (s, 3H), 3.82 (s, 3H), 3.28 (s, 3H), and an unresolved number of protons between 1.5 and 3.5 6. When the singlet proton at δ 6.39 was irradiated, the proton signal around δ 3.3 showed a nuclear Overhauser effect; m/z: 345 (M^+, 25); meas.: 345.1944, calc. for $C_{20}H_{27}NO_4$: 345.0378; 314 (30), 287 (45), 286 (25), 272 (20), 178 (100), 165 (35), 146 (35); found: C 69.19; H 8.33; N 4.05; $C_{20}H_{27}NO_4$ requires: C 69.56; H 7.82; N 4.05%. Athrocupressine gave a positive test with Gibbs' reagent.

The component of higher *Rf* (20.7 mg) contained two compounds present in very small amounts, insufficient for satisfactory separation. From spectroscopic and chemical data, the mixture appeared to consist of the two isomeric acetyl derivatives 11 and 12; xmax: 270 and 207 nm (no change on addition of dil. NaOH); νmax: 3400, 3000, 2920, 2840, 1730, 1680, 1510, 1240 cm^{-1}; ^1H nmr: 6.80 (m, 2H), 5.55 (m, 2H), 3.92 (s, 1H), 3.84 (s, 3H), 3.28 (s, 3H), 211 (s, 3H) and an unresolved number of protons between 1.5 and 3.5 6; m/z: 373 (M^+, 40); meas.: 373.1912, calc. for $C_{21}H_{27}NO_5$: 373.1910; 342 (35), 341 (20), 330 (20), 314 (75), 313 (70), 297 (40), 282 (42), 272 (72), 261 (75), 260 (70), 260 (70), 236 (100), 223 (60), 176 (25), 162 (70), 132 (72).

Fraction *3* (0.149 g) contained two components from tlc (8% MeOH/$CHCl_3$), which were separated by ptlc with the same solvent system. The component of higher *Rf* was non-alkaloidal, and that of lower *Rf* (0.5, 6.6 mg) proved identical with taxodine (1); mp 150-152" (Me_2CO); $[\alpha]_D^{19}$ + 111.0 (C = 0.9); λmax: 209 (4720), 230 (1890), 291 nm (861); after addition of a dron of 5% aqueous sodium hydroxide: 210, 250, 298 nm; νmax: 3380, 3000, 2920, 2840, 2500, 2450, 1210 cm^{-1}; ^1H nmr: 6.78 (s, 1H), 6.63 (s, 1H), 5.52 (bs, 1H), 3.87 (s, 3H), 3.22 (s, 3H) and an unresolved number of protons between 1.5 and 3.5 δ; m/z: 315 (M^+, 35), meas.: 315.1833, calc. for $C_{19}H_{25}NO_3$: 315.1834; 284 (40), 257 (80), 256 (79), 240 (15), 178 (100), 165 (39), 146 (35), 137 (25).

Fraction 4 (0.165 g) appeared to be a mixture from its ^1H nmr spectrum, but a variety of solvent systems failed to separate it by ptlc. The separation was finally achieved by hplc with acetonitrile/water (18:82) buffered to pH 3.0 (0.1M $NH_4H_2PO_4$ and H_3PO_4) for elution. The mixture of alkaloids (15 mg) was dissolved in 1 ml of the solvent system and injected in 200 μl aliquots; each run required 32.4 min. The separated fractions were bulked and basified with aqueous ammonia (d 0.88), then extracted with dichloromethane, evaporation of which yielded the two separated components. The minor component (4.9 mg) was identified as 2-epihomoerythratine (4), mp 184-185° (Me_2CO); $[\alpha]_D^{19}$ + 170" (C = 1.4); λmax: 240 (5500), 290 nm (5000); νmax: 3420, 3000, 2920, 2850, 1500, 1460, 1456, 1260 cm^{-1}; ^1H nmr: 6.60 (s,1H), 6.58 (s, 1H), 5.92 (s, 2H), 5.78 (bs, 1HO, 4.38 (bs, 1H), 3.32 (s, 3H), and an unresolved number of protons between 1.5 and 3.5 6; m/z: 329 (M^+, 50); meas.: 329.16407, calc. for $C_{19}H_{23}NO_4$: 329.16778; 298 (15), 271 (65), 255 (50), 194 (100), 181 (30), 162 (32).

The major component of fraction 4 (7.8 mg) proved to be homoerythratine (5), mp 178-179° (Me_2CO); $[\alpha]_D^{19}$+ 63.61 (C = 3.63; λmax: 219 (2757), 288 nm (1316); νmax: 3380, 2920, 2840, 1490, 1470, 1240, 1040 cm^{-1}; ^1H nmr: 6.82 (s, 1H), 6.65 (s, 1H), 5.93 (s, 2H), 5.52 (bs, 1H), 4.35 (m, 1H), 3.3 (s, 3H) and an unresolved number of protons between 1.5 and 3.5 δ; m/z: 329 (M^+, 50); meas.: 329.16407, calc. for $C_{19}H_{23}NO_4$: 329.16778, 298 (18), 271 (25), 270 (15), 255 (25), 254 (20), 242 (10), 194 (100), 181 (24), 162 (25); found: C 69.38, H 7.46, N 4.21; $C_{19}H_{23}NO_4$ requires: C 69.30, H 6.99, N 4.25.

Fractions *5* and *6* appeared from tlc to contain the same two components in different proportions. The combined fractions (0.157 g) were separated by ptlc (5% MeOH/$CHCl_3$, double development), and the subfraction of lower Rf (10.7 mg) yielded 2-hydroxytaxodine (6), mp 192-193° (Me_2CO);

$[\alpha]_D^{19}$ + 51.47 (C = 1.03); λmax: 212 (4062), 230 (1812), 283 nm (750); after addition of a drop of 5% aqueous sodium hydroxide: 218, 250, and 298 nm; νmax: 3380, 3000, 2920, 2840, 1560, 1448, 1270, 1200, 1150 cm^{-1}; ^1H nmr: 6.86 (s, 1H), 6.65 (s, 1H), 5.55 (s, 1H), 4.3 (bin, 1H), 3.85 (s, 3H), 3.28 (s, 3H) and an unresolved number of protons between 1.5 and 3.5 δ; m/z: 331 (M^+, 60); meas.: 331.1785, calc. for $C_{19}H_{25}NO_4$: 331.1783; 300 (18), 273 (35), 272 (30), 257 (38), 256 (30), 194 (100), 178 (35), 162 (30).

The component of higher Rf (80 mg) from fractions 5 and 6 was separated by hplc with a solvent system made up of two solutions, A and B: solution A contained acetonitrile/water (1:9) and a buffer solution (0.02M of $NH_4H_2PO_4/H_3PO_4$, pH 2.5, and triethylamine 0.007M), and solution B consisted of THF/acetonitirle (1:9). Solutions A and B were mixed in the ratio 93.5:6.5. The mixture of alkaloids was dissolved in 5 ml of mixed solvent and injected on to the column (maximum volume per injection 225 μl). The mixture was separated into three subfractions, which were bulked and basified with aqueous ammonia (d 0.88), then extracted with dichloromethane. The first subfraction (53.6 mg) yielded 2-hydroxyisotaxodine (7) which could not be crystallised; $[\alpha]_D^{19}$ + 60.69 (C = 1.0); λmax: 240 (1900), 283 nm (850); after addition of a drop of 5% aqueous sodium hydroxide: 218, 250, and 298 nm; νmax: 3400, 3120, 2950, 2870, 1558, 1520, 1450, 1280, 1226, 1050 cm^{-1}; ^1H nmr (270 MHz): 6.725 (s, 1H), 6.575, 5.575 (bs, 1H), 4.3 (bm, 1H), 3.85 (s, 3H), 3.28 (s, 3H) and an unresolved number of protons between 1.5 and 3.5 6; when the multiplet at 63.4 was irradiated, the singlet at 66.725 showed an nOe, and when the multiplet at 62.2 was irradiated, the singlet at 66.575 showed an nOe; m/z: 331 (M^+, 60); meas.: 331.1785, calc. for $C_{19}H_{25}NO_4$: 331.1783; 300 (18), 273 (75), 272 (3), 257 (38), 256 (30), 194 (100), 178 (35), 162 (30).

The second subfraction (9.3 mg) consisted of a mixture of 6 and 8 which could not be further separated. The mass spectrum of the mixture showed differences in intensity only as compared with those of 6 and 7. ^1H nmr of 8 (after elimination of the proton signals from 6): 6.675 (s, 1H), 6.55 (s, 1H), 5.75 (bs, 1H), 4.3 (bm, 1H), 3.75 (s, 3H), 3.25 (s, 3H) and an unresolved number of protons between 1.5 and 3.5 δ.

The third subfraction (5.7 mg) was obtained in too small a quantity for adequate study. Fraction 7 proved to be devoid of alkaloids.

HYDROLYSIS OF 2-ACETOXYTAXODINE (11) and 2-ACETOXYISOTAXODINE (12).- The mixture of alkaloids 11 and 12 (10 mg) from fraction 2 was dissolved in aqueous methanol (1:1, 10 ml) and dilute sodium hydroxide (1%, 2 ml) was added. The solution was refluxed for 4 hours, then cooled to room temperature and neutralised with dilute sulfuric acid (5%). The neutral solution was exhaustively extracted with chloroform, and the extract on evaporation left a residue (5 mg) whose components gave the same Rf values and ms as those of authentic specimens of 2-hydroxytaxodine (6) and 2-hydroxyisotaxodine (7) respectively.

OXIDATION OF 2-EPIHOMOERYTHRATINE (4).- A sample of 4 (13 mg, 0.04 m mole) was oxidised with μ-oxo bis(triphenyl bismuth dichloride)' (40 mg, 0.04 m mole) and potassium carbonate (50 mg) in chloroform (5 ml) at room temperature overnight. After separation by ptlc (5% MeOH/$CHCl_3$), the ketone derivative of 4 was obtained (7 mg), mg 172-173" (Me_2CO); λmax: 230 (9343) 288 nm (1094); vmax: 2910, 2820, 1680, 1470, 1220, 1110, 1020 cm^{-1}; ^1H nmr: 6.77 (s, 1H), 6.67 (s, 1H), 6.1 (s, 1H), 6.0 (s, 2H), 3.54 (s, 3H), and an unresolved number of protons between 1.5 and 3.5 δ; m/z 327 (M^+, 65); meas.: 327.14669, calc. for $C_{19}H_{21}NO_4$: 327.14668; 284 (50), 269 (75), 268 (17), 241 (60), 240 (100), 211 (20), 192 (85), 179 (10), 160 (38), 149 (25).

DEHYDROXYLATION OF 2-EPIHOMOERYTHRATINE (4).- A sample of 4 (13.5 mg, 0.04m mole) was dissolved in 5 ml of dry benzene and treated with freshly distilled thionyl chloride (2 ml). The reaction mixture was stirred at room temperature for 2 hr, then evaporated to remove excess of thionyl chloride and solvent. The residue from the reaction mixture was redissolved in benzene/THF (3 ml: 100 ml) and treated with LAH (100 mg). The mixture was refluxed overnight, then excess of LAH was destroyed by adding water The precipitate was removed by filtration, and washed until free from alkaloid. The filtrate was extracted with chloroform (3 x 50 ml), and the combined extracts were dried (Na_2SO_4) and evaporated under reduced pressure. The residue was separated by ptlc (7% MeOH/$CHCl_3$) to give'a product (10 mg) identical (ir, uv, ^1H nmr, ms) with 2. The mp of the picrate

derivative was not depressed on admixture with the corresponding derivative of 2.

OXIDATTON OF HOMOERYTHRATINE (5).- A sample of 5 (13 mg, 0.04 m mole) was oxidised with μ-oxo-bis(triphenyl bismuth chloride)' (40 mg, 0.04 m mole) and potassium carbonate (50 mg) in chloroform (5 ml) at room temperature overnight. After separation by ptlc (5% MeOH/$CHCl_3$), a ketone identical (ir, uv, ^1H nmr, ms) with that formed by a corresponding oxidation of 4 was obtained (8 mg).

DEHYDROXYLATION OF HOMOERYTHRATINE (5).- A sample of 5 (50 mg, 0.14 m mole) was converted to the corresponding chloride with thionyl chloride (2 ml) in dry benzene (5 ml) as described above. The crude product was reduced with LAH (200 mg) in 3 ml of dry benzene and 10 ml of THF. The product (25 mg) was identical (ir, uv, ^1H nmr, ms) with 2.

DEHYDROXYLATION OF 2-HYDROXYTAXODINE (6).- A sample of 6 (28 mg, 0.084 m mole) was converted to the chloride derivative with thionyl chloride (1.5 ml) in dry benzene (3 ml) as described above. The crude product was reduced with LAH (100 mg) in 3 ml of dry benzene and 10 ml of THF. The product (4.1 mg) was identical (ir, uv, ^1H nmr, ms) with taxodine (1).

DEHYDROXYLATION OF 2-HYDROXYISOTAXODINE (7).- A sample of 7 (28 mg, 0.084 m mole) was converted to the corresponding chloride with thionyl chloride (1.0 ml) in dry benzene (3 ml) as described above. The crude product was reduced with LAH (100 mg) in 3 ml of dry benzene and 10 ml of THF. The product (6 mg) had mp 142-145°; λmax: 219 (11550), 230 (10920), 283 nm (5460); after addition of a drop of 5% aqueous sodium hydroxide: 220, 253 and 295 nm; νmax: 3400, 2920, 2840, 1855, 1500, 1450, 1270, 1210, 1100, 1080 cm-l; ^1H nmr: 6.70 (s, 2H), 5.55 (bs, 1H), 3.8 (s, 3H), 3.25 (s, 3H) and an unresolved number of protons between 1.5 and 3.5 6; m/z: 315 (M^+, 35); meas.: 315.1833, calc. for $C_{19}H_{25}NO_3$: 315.1834; 284 (4), 257 (80), 256 (79), 240 (15), 178 (loo), 165 (39), 146 (35), 137 (25).

OXIDATION OF 2-HYDROXYTAXODINE (6).- A sample of 6 (28 mg, 0.084 m mole) was oxidised with μ-oxo-*bis*(triphenyl bismuth dichloride)' (0.12 g, 0.12 m mole) and potassium carbonate (50 mg) in chloroform (5 ml) at room temperature overnight. After separation by ptlc (5% MeOH/$CHCl_3$), the ketone derivative of 6 (15 mg) was obtained as a colourless oil; λmax: 230 (12,502), 283 nm (4,167); after addition of a drop of 1% aqueous sodium hydroxide: 237 and 298 nm; νmax: 3500, 3400, 3010, 2920, 2850, 1670, 1580, 1500, 1450, 1350, 1310, 1270, 1210, 1160, 1120, 1050 cm^{-1}; ^1H nmr: 6.77 (s, 1H), 6.55 (s, 1H), 6.05 (s, 1H), 3.725 (s, 3H), 3.425 (s, 3H), and an unresolved number of protons between 1.5 and 3.5 6; m/z: 329 (M^+, 30); meas.: 329.1668, calc. for $C_{19}H_{23}NO_4$: 329.1627; 286 (35), 271 (45), 243 (38), 242 (40), 228 (35), 212 (25), 192 (50), 160 (30), 85 (60), 83 (100).

OXIDATION OF 2-HYDROXYISOTAXODINE (7).- A sample of 7 (28 mg, 0.084 m mole) was oxidised with μ-oxo-*bis*(triphenyl bismuth dichloride)' 129 mg, 0.12 m mole) and potassium carbonate (50 mg) in chloroform (5 ml) at room temperature overnight. After separation by ptlc (5% MeOH/$CHCl_3$), the ketone derivative of 7 was obtained (17 mg) as a colourless oil; λmax: 230 (12,500), 283 (4,160); after addition of a drop of 1% sodium hydroxide: 237 and 298 nm; νmax: 3500, 3400, 3010, 2920, 2850, 1670, 1570, 1500, 1450, 1310, 1270, 1210, 1160, 1120, 1050 cm^{-1}; ^1H nmr: 6.675 (s, 2H), 6.0 (s, 1H), 3.85 (s, 3H), 3.425 (s, 3H), and an unresolved number of protons between 1.5 and 3.5 6; m/z: 329 (M^+, 70); meas.: 329.1658, calc. for $C_{19}H_{23}NO_4$: 329.1627; 286 (50), 271 (68), 270 (30), 243 (25), 242 (43), 240 (75), 226 (20), 192 (100), 160 (28), 85 (50), 83 (80).

OXIDATION OF 2-HYDROXYTAXODINE (6) and 2-EPI-HYDROXYISOTAXODINE (8).- The mixture of 6 and 8 (3 mg) from fractions 5-6 was oxidised with μ-oxo-*bis*(triphenyl bismuth dichloride)' (10 mg) and 10 mg of potassium hydrogen carbonate in 1 ml of chloroform for 2 hours. Two oxidation products were obtained, which were separated by multiple development ptlc (5% MeOH/$CHCl_3$). The product with the higher *Rf* value corresponded to the ketone obtained by oxidation of 7 when they were chromatographed under the same conditions, and the product of lower *Rf* corresponded to that formed from 6.

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