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ATHROTAXIS Alkaloids. PART II. ALKALOIDS OF *A. SELAGINOIDES* AND *A. LAXIFOLIA*

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Abstract - The taxodeaceous plant *A. selaginoides* contains six known homo*erythrina*-type alkaloids and selaginoidine, which has a furan ring replacing the benzene ring of the latter type. Selaginoidine and five of the six above-mentioned alkaloids also occur in *A. laxifolia*.

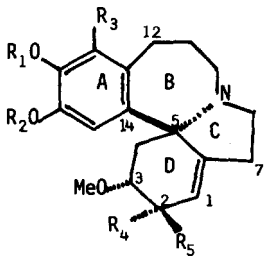
RESULTS AND DISCUSSION

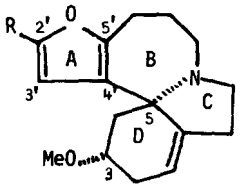
A. selaginoides Don is endemic in Tasmania, where it grows in high rainfall and mountainous country of the centre and west; some specimens attain 40 m in height and are over 1000 years old. The tree is well-known in Tasmania under the name of King Billy Pine for its valuable soft-wood timber[1,2].

As in the case of *A. cupressoides*[3], the fresh plant material must be extracted promptly and the alkaloids separated as rapidly as possible to avoid loss. The presence of eight alkaloids has been established, of which the following homo*erythrina* bases had been found to occur in *A. cupressoides*: taxodine[3-5] (1), 3-epischelhammericine[3-8] (2), homoerythratine[3,9] (3), 2-hydroxytaxodine[3] (4), 2-hydroxyisotaxodine[3] (5), 2-epihydroxyisotaxodine[3] (6), and athrocupressine[3] (7). The first three had also been reported previously from various other plants.

The spectroscopic data of the remaining base, which was named selaginoidine (11), suggested a close relationship to the homo*erythrina* group: its nmr spectra showed signals corresponding in detail to those of carbons and hydrogens associated with rings B, C, and D of the homo*erythrina* bases, and its mass spectrum included peaks derived from fragments of these rings. In particular, a methoxyl proton peak appeared in the ^1H nmr spectrum at $\delta 3.28$, almost identical with the values for taxodine (1) and 3-epishelhammericine (2) which have methoxyl groups attached at C-3 *trans* to the 5-14 bond[4]; on the other hand, the C-3 methoxyl peak in the spectrum of schelhamnericine (8), which has the *cis* arrangement of the methoxyl group, appears at $\delta 2.74$[10].

Apart from these similarities to the usual type of homo*erythrina* alkaloid, selaginoidine (11) also shows some differences: its UV spectrun suggested that it has a furan nucleus, and its ir and nmr spectra gave evidence of a methyl ester group, whose presence was confirmed by intense M-58 and M-59 peaks in its mass spectrun, and by its reduction with LAH to a primary alcohol (12) with one less carbon atom. With the exception of the ester carbonyl and the olefinic group of ring D, selaginoidine has only four sp^2 carbons as shown by its ^{13}C nmr spectrum, in comparison with the six aromatic carbons of the homo*erythrina* alkaloids. The four signals, from three quaternary and one methine carbon, correspond in chemical shift to those of a trisubstituted furan nucleus, which is evidently fused on to ring B in place of the usual aromatic ring A of the homo*erythrina* alkaloids. The methine carbon resonates at 6122.2, and must occupy a β position in the trisubstituted ring[11]. This assignment is supported by the chemical shift of the proton attached to it? the value 66.82 in the ^1H nmr spectrum of 11 is in harmony with that expected for H-3' in a furan bearing a carbomethoxy group at C-2' and fused to a saturated ring at C-4' and C-5'[12]. The signal at $\delta 6.82$ is the only one in the aromatic region, and it undergoes a pronounced nuclear Overhauser effect on irradiation of a broad multiplet around $\delta 3.5$, corresponding to the H-3 proton of ring D. These two methine protons must thus be close





11 Selaginoidine (R = COOMe)
12 (R = CH_2OH)

1 Taxodine (R_1 = 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 Homoerythratine (R_1-R_2=CH_2, R_3=R_4=H, R_5=OH)
4 2-Hydroxytaxodine (R_1=Me, R_2=R_3=R_4=H, R_5=OH)
5 2-Hydroxyisotaxodine (R_1=R_3=R_4=H, R_2=Me, R_5=OH)
6 2-Epihydroxyisotaxodine (R_1=R_3=R_5=H, R_2=Me, R_4=OH)
7 Athrocupressine (R_1=R_2=Me, R_3=OH, R_4=R_5=H)
8 Schelhammericine (R_1-R_2=CH_2, R_3=R_4=R_5=H; MeO► at C-3)
9 2-Acetoxytaxodine (R_1=Me, R_2=R_3=R_4=H, R_5=OAc)
10 2-Acetoxyisotaxodine (R_1=R_3=R_4=H, R_2=Me, R_5=OAc)

to one another in space; structure 11 which follows from these observations is in full accord with all the spectroscopic data for selaginoidine. Its specific rotation as compared to those of taxodine (1) and 3-epischelhammericine (2) suggests that 11 may also represent the absolute configuration of selaginoidine.

Fran a previous investigation of A. *selaginoides*[2], a mixture of 2-acetoxytaxodine[3] (9) and 2-acetoxyisotaxodine[3] (10) was also isolated, but the plant material used in the former study was collected at a different time of year. It is possible that these compounds are produced by the plant at a certain season only; on the other hand, they could conceivably be artefacts, since acetic acid was used in the course of the extraction and purification of the alkaloids in each case. Further evidence on these questions was sought from a small-scale extraction of fresh twigs and foliage of King Billy Pine collected in March 1983. the same period as the original collection[2], and the separation of the crude alkaloids was carried out with dilute sulphuric acid instead of acetic acid. The presence of 9 and 10 amongst the crude bases was established by the ms technique of multiple metastable peak monitoring[13]: evidently 2-acetoxytaxodine and 2-acetoxyisotaxodine are indeed produced by the plant in autumn at any rate, if not in winter.

A. laxifolia Hook. is a rare species endemic in Tasmania which is intermediate taxonomically between *A. cupressoides* and *A. selaginoides*; it is found associated with these species as isolated trees, and a hybrid origin for it has been postulated, but this has not been substantiated'. From the small sample of plant material available, the following alkaloids were found to occur: taxodine (1), 3-epischelhammericine (2), homoerythratine (3), 2-hydroxytaxodine (4), 2-hydroxyisotaxodine (5), 2-epihydroxyisotaxodine (6), and selaginoidine (11). The alkaloid content is thus the same as that of *A. selaginoides*, except that no athrocupressine (7) was found in *A. laxifolia*.

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 chromatography (hplc) was carried out on a 7.7 mm x 25 cm column with octadecyl silane as stationary phase, and 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/acetonitrile (1:9). Solutions A and 8 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 us). The melting points were recorded on a Yanigimoto Seisakusho micro-melting point apparatus and are uncorrect-

ed. Specific 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, and the carbon-13 magnetic resonance (^{13}C nmr) spectrum was recorded under similar conditions 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.

Voucher specimens of the plant material have been deposited in the collection of dried plant specimens in the Chemistry Beparment, Universsity of Tasmania.

EXTRACTION OF A.' SELAGINOIDES.- Twigs and foliage of King Billy Pine were collected near Zeehan, western Tasmania, in August 1980 and were immersed in 400 litres of methanol as soon as possible after collection. After three days the plant material was removed, drained, and put through a compost shredder. The air-dried material (200 kg) was 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 green syrup, which was dissolved in 10 litres of warm glacial acetic acid and 8 litres of water. The solution was poured in a fine stream into 20 litres of water, which was simultaneously agitated vigorously with a ' mechanical stirrer. The solution was left to stand overnight, then the precipitate that settled out was filtered off, washed with water until free from alkaloids, and discarded. The extract, combined with the washings, was evaporated to dryness in *vacuo* at a temperature below 35°C. The residue was dissolved in 20 litres of water and again evaporated to dryness, and the process of dilution with water and evaporation was repeated once more to get rid of most of the acetic acid. Finally the residue was basified to pH 8-g with aqueous ammonia (d 0.88), whereby a heavy precipitate was produced, which was allowed to settle overnight, then filtered off through Hi-Flo Supercel. The dried precipitate and the filtrate were separately extracted with chloroform until no further alkaloid was removed, then the combined chloroform solutions were extracted with 5% aqueous sulphuric acid (40 x 150 ml) until free fran alkaloids as shown by a negative Mayer's test. The aqueous acid solution was basified with aqueous ammonia (d 0.88) and again thoroughly extracted with chloroform (40 x 150), and the combined chloroform extracts were dried (Na_2SO_4) and evaporated to dryness *in vacuo* to give 14 g of crude alkaloids.

ISOLATION, PURIFICATION, AND CHARACTERISATION OF THE ALKALOIDS OF A. SELAGINOIDES.- The crude alkaloid mixture (5 g) was separated by short column chromatography on 150 g of silica gel into six fractions. Elution commenced with chloroform, and was continued with mixtures of chloroform containing gradually increasing amounts of methanol up to 10%. The last two fractions gave no test for alkaloids.

Fraction 1 (0.36 g) contained two components from tlc examination (ethyl acetate/light petroleum : 60/40), which were separated by ptlc with the same solvent system. The less polar component (75 mg) was found to be identical (ms, uv, ir, ^{1}H nmr) with the known alkaloid 3-epischelhammericine[3-8] (2). The more polar constituent (50 mg) proved to be a new alkaloid, selaginoidine, mp 62-63°, $[\alpha]_D^{19}$ 166.7' (C-0.25); λmax: 219 (7680), 269nm (7480); νmax: 2940, 2900, 2850, 1730, 1600, 1550, 1510, 1440, 1300 an-l; ^{1}H nmr: δ6.82 (s, 1H), 5.51 (s, 1H), 3.85 (s, 3H), 3.28 (s, 3H), and an unresolved number of protons between 61.5 and 3.5. When the multiplet at δ3.5 was irradiated, the singlet at 66.82 showed a pronounced nOe; m/z: 317 (M^+, 10); meas.: 317.16253, calc. for $C_{18}H_{23}NO_4$: 317.16280; 301 (10), 286 (18), 259 (100), 258 (90), 244 (10), 217 (15), 175 (16), 165 (5). 146 (5); ^{13}C nmr: 6159.2 (C=O), 156.8 (s, C-2'), 141.3 (s, C-5'), 140.6 (s, C-4'), 127 (s, C-6), 122.2 (d, C-3'), 116.2 (d, C-l), 74.2 (d, C-3), 62.2 (s, C-5), 65.9 (q, *Me*COO), 51.7 (q, MeO), 48.2 and 45.7 (t, C-8 and C-10). 40.0, 31.9, and 28.9 (t. C-2, C-7 and C-12), 26.8 (t, C-14), 18.5 (t, C-11).

Fraction 2 (0.67 g) contained one component only from tlc with several solvent systems.

After purification, this alkaloid proved identical (ms, uv, ir, ^{1}H nmr) with **athrocupressine**[3] (7).

Fraction 3 (0.43 g) also contained only one compound from tlc, which was found to be identical (ms, uv, ir, ^{1}H nmr) with taxodine 3-5 (1).

Fraction 4 (0.47 g) contained at least three components from tlc, and it was separated by triple development ptlc ($CHCl_3$, 5% MeOH) into three subfractions, of which that with the highest *Rf* (37 mg) proved identical (ir, uv, ^{1}H nmr, ms) with **homoerythratine**[3,9] (3). The subfraction of lowest *Rf* (62 mg) was found to be identical (ir, uv, ^{1}H nmr, ms) with **2-hydroxytaxodine**[3] (4). The material from the subfraction of intermediate *Rf* was found by plc to contain three compounds, one of which was separated by hplc. This component proved to be **2-hydroxyisotaxodine**[3] (5) from a comparison of its physical data with those of a sample of 5 isolated in similar fashion from *A. cupressoides*[3].

The mixture of the other two components, which could not be further separated, was found to correspond in its spectroscopic properties with the inseparable mixture of **2-hydroxytaxodine**[3] (4) and **2-epihydroxyisotaxodine**[3] (6) which was obtained by hplc under the same conditions from *A. cupressoides*[3].

LAH REDUCTION OF SELAGINOIDINE.- Selaginoidine (11), 20 mg, 0.063 m mole) was refluxed overnight with a suspension of LAH (30 mg in 20 ml THF) in a current of nitrogen. Excess of LAH was destroyed with water, and the solution was filtered and extracted with chloroform (3 x 30 ml). The combined extracts were dried (Na_2SO_4) and concentrated in *vacuo* to a yellowish oil; purification by ptlc ($CHCl_3$, 7% MeOH, *Rf* 0.3) yielded the alcohol 12 (8 mg, 43%) which could not be crystallised; λmax: 209 (4538), 226 (4538), 285 nm (462); νmax: 3380, 3000, 2900, 2850, 1200 cm^{-1}; ^{1}H nmr: 5.87 (s, 1H), 5.45 (s, 1H), 4.47 (s, 2H), 3.3 (s, 3H), and an unresolved number of protons between δ1.5 and 3.5; m/z: 289 (M^+, 7); meas.: 289.167, calc. for $C_{17}H_{23}NO_3$: 289.160; 250 (10), 232 (18), 231 (100), 230 (50). 214 (30), 200 (18), 178 (16).

EXTRACTION OF A. LAXIFOLIA.- Twigs and foliage were collected in November 1983 near Pine Lake, central Tasmania, from two trees of *A. laxifolia* growing in association with a stand of *A. cupressoides*. The material was immersed as soon as possible after collection in litres of methanol, and after a week it was removed from the solvent, drained, and milled. The dried plant material (1 kg) was percolated with methanol until a test sample gave a negative reaction with Mayer's reagent. The combined extracts were concentrated *invacuo* at a temperature below 40° to a thick gummy dark green syrup, which was dissolved in 500 ml of glacial acetic acid and 1 litre of water. The solution was left to stand overnight, and the precipitate that settled out was filtered off, washed with water until free from alkaloids, then discarded. The aqueous solution and washings were evaporated to dryness under reduced pressure at a temperature below 35". The residue was dissolved in 1 litre of water, and the solution was again evaporated to dryness; finally the residue was redissolved in 1 litre of water, and the solution was basified to pH 8-9 with aqueous ammonia (d 0.88). The heavy precipitate that formed was left to settle overnight, then filtered off through Hi-Flo Supercel. The dried percipitate and the solution were separately extracted with chloroform until they gave a negative Mayer's test, and the combined chloroform solutions were extracted with aqueous sulphuric acid (5%, w/v, 20 x 100 ml) until free from alkaloids. The aqueous acid solution was basified with ammonia and again thoroughly extracted with chloroform (30 x 50 ml). The combined chloroform extracts were dried ($MgSO_4$) and evaporated under reduced pressure to give 254 mg of crude alkaloids.

ISOLATION, PURIFICATION, AND IDENTIFICATION OF THE ALKALOIDS OF A. LAXIFOLIA.- The crude mixture of alkaloids (254 mg) was separated by ptlc ($CHCl_3$, 7% MeOH) into six fractions.

Fraction 1 (11.5 mg) contained two bases from tlc (C_6H_6, 30% EtOAc). The mixture was separated by double development ptlc with the same solvent system. The higher *Rf* component was obtained as a yellowish oil, and proved identical with **3-epischelhammericine**[3-8] (2) (ir, uv, ^{1}H nmr, ms). The component of lower *Rf* (4.0 mg) was obtained as a yellowish oil, which was found to be identical (ir, uv, ^{1}H nmr, ms) with selaginoidine (11).

Fraction 2 (19.0 mg) contained two components from tlc ($CHCl_3$, 5% MeOH), which were separated by double development ptlc with the same solvent system. The higher *Rf* component (7.0 mg) proved to be non-alkaloidal. The component of lower *Rf* (8.0 mg) crystallised as colourless

uv, ^{1}H nmr, ms, mixed mp) with taxodine[3-5] (1).

Fraction 3 (27.5 mg) contained two components from tlc ($CHCl_3$, 5% MeOH), and the same solvent system was used to separate them by double development ptlc. The lower *Rf* component (13.0 mg) was obtained as white needles, mp 178-179° (Me_2CO), identical (ir, uv, ^{1}H nmr, ms, mixed mp) with hanoerythratine[3,9] (3). The component of higher *Rf* (9.0 mg) proved to be non-alkaloidal.

Fraction 4 (14.0 mg) contained one component only as shown by tlc ($CHCl_3$, 2%, 5%, 7% MeOH), which proved identical (ir, uv, ^{1}H nmr, ms) with homoerythratine[3,9] (3).

Fraction 5 (13.0 mg) was devoid of alkaloids.

Fraction 6 (25.0 mg) contained at least two bases from tlc ($CHCl_3$, 5% MeOH), and was separated into two fractions by ptlc using the same solvent system. The component of lower *Rf* (7.0 mg) was obtained as a colourless oil and was found to be identical (ir, uv, ^{1}H nmr, ms) with 2-hydroxytaxodine[3] (4). The higher *Rf* material (10 mg) could not be further separated by ptlc, but its ^{1}H nmr spectrum showed it to consist predominantly of a mixture of 2-hydroxyisotaxodine[3] (5) and 2-epihydroxyisotaxodine[3] (6) by comparison with the spectra of the corresponding fractions from *A. selaginoides* and *A. cupressoides*[3].

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