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ALKALOIDS OF *CEPHALOTAXUS WILSONIANA*

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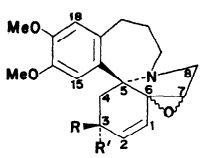
Key Word Index—*Cephalotaxus wilsoniana*; Cephalotaxaceae; homoerythrina alkaloids; wilsonine; cephalotaxine.

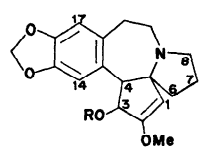
Abstract—Four alkaloids have been isolated from extracts of *Cephalotaxus wilsoniana* Hayata. The major alkaloid (I), for which the name wilsonine is proposed, occurs with its C-3 epimer (II); in addition to these homoerythrina alkaloids, minor amounts of cephalotaxine (III) and acetylcephalotaxine (IV) are also found.

INTRODUCTION

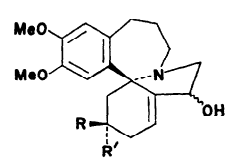
IN CONTINUING our examination of *Cephalotaxus* alkaloids for possible antitumor activity, we now report our findings concerning the alkaloids of *Cephalotaxus wilsoniana* Hayata.[1] Previous work revealed that cephalotaxine (III) is the major alkaloid of *Cephalotaxus drupacea, Cephalotaxus fortunei*[2] and *C. harringtonia.*[3] The structure of cephalotaxine was determined by a combination of NMR[4] and X-ray crystallographic studies.[5] Five homoerythrina alkaloids have also been recorded as minor constituents of *C. harringtonia.*[3] In contrast, the two major alkaloids of *C. wilsoniana* (I and II) belong to the homoerythrina series, and cephalotaxine (III) appears as a minor component accompanied by even smaller amounts of its acetyl derivative (IV). Alkaloid I ('wilsonine') has not previously been reported in the literature although its C-3 epimer ('*epi*-wilsonine', II) occurs in *Phelline comosa* Labill.[6] Also in the seed of *C. harringtonia* var. *drupacea* (Sieb. & Zucc.) Koidzumi, II is a minor constituent.



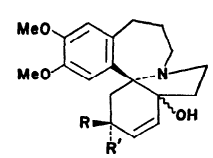
(I) R = OMe; R' = H
(II) R = H; R' = OMe



(III) R = H
(IV) R = Ac



(V) R = OMe; R' = H
(VII) R = H; R' = OMe



(VI) R = OMe; R' = H
(VIII) R = H; R' = OMe

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[1] The collections of *C. wilsoniana* were made in Taiwan (Formosa) during June 1969. *Cephalotaxus harringtonia* seed was harvested in Italy in 1962. This dried plant material was provided by Dr. ROBERT E. PERDUE, JR., U.S. Department of Agriculture, Beltsville, Maryland, as part of a cooperative program with Drug Research and Development, National Cancer Institute (formerly Cancer Chemotherapy National Serivce Center).

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[3] R. G. POWELL, *Phytochem.* **11**, 1467 (1972).

[4] R. G. POWELL, D. WEISLEDER, C. R. SMITH, JR. and I. A. WOLFF, *Tetrahedron Letters* 4081 (1969).

[5] D. J. ABRAHAM, R. D. ROSENSTEIN and E. L. MCGANDY, *Tetrahedron Letters* 4085 (1969).

[6] N. LANGLOIS, B. C. DAS, P. POTIER and L. LACOMBE, *Bull. Soc. Chim. Fr.* 3535 (1970).

RESULTS AND DISCUSSION

Structures of alkaloids I and II were deduced from IR, MS and NMR data. IR shows that neither of these alkaloids contain hydroxyl or carbonyl functions. The MS of I and II are nearly identical, except for minor differences in peak intensities, which indicate that they are closely related structural isomers. Significant features of both spectra include molecular ions at *m/e* 343 ($C_{20}H_{25}NO_4$), *m/e* 328 (base peaks, loss of Me) and *m/e* 312 (loss of OMe).

TABLE 1. NMR DATA FOR WILSONINE (I), C-3 *epi*-WILSONINE (II) AND THEIR REDUCTION PRODUCTS*

Protons and assignments	Alkaloid I	II	V	VI	VII	VIII†
H-1	d5·86	q5·77	t5·97	q5·76	t5·97	d5·80
$J_{1,2}$	10·5	10·5	4·0	10·5	3·5	10·5
H-2	m6·06	m6·04	m2·38	q5·97	m2·06	q6·15
$J_{2,3}$	4·0	2·0	—	2·0	—	2·0
$J_{2,4eq}$	1·5	2·0	—	—	—	—
H-3eq	m3·84	—	m3·67	m3·76	—	—
H-3ax	—	m3·30	—	—	m3·35	—
H-4eq	m3·11	m3·14	—	—	q2·72	—
$J_{3eq,4eq}$	1·5	—	4·0	—	—	—
$J_{3ax,4eq}$	—	—	—	—	3·5	—
$J_{4eq,4ax}$	14·0	12·0	14·0	—	11·5	—
H-4ax	q1·67	q1·70	q1·67	—	t1·49	—
$J_{4ax,3eq}$	5·0	—	—	—	—	—
$J_{4ax,3ax}$	—	11·0	—	—	11·5	—
H-15, H-18	s6·82 s6·53	s6·98 s6·61	s7·02 s6·57	s6·96 s6·57	s6·86 s6·66	s7·36 s6·64
C3-OCH_3	s2·96	s3·29	s2·67	s3·30	s3·20	s3·29
Aryl-OCH_3	s3·76 s3·76	s3·79 s3·80	s3·74 s3·81	s3·78 s3·82	s3·76 s3·84	s3·77 s3·86

* Measured in $CDCl_3$ with a Varian HA-100 spectrometer. Chemical shifts (δ) are expressed in ppm from tetramethylsilane.

† Chemical shifts for alkaloid VIII were obtained from the literature[6] for comparison (VIII was not prepared during this study due to lack of sufficient II).

The NMR spectra and chemical shift asssignments for alkaloids I and II are summarized in Table 1. The NMR spectrum of I contains two singlets at δ 6·53 and 6·82 which are assigned to the protons on the aromatic ring. Vinyl protons H-1 and H-2 appear at δ 5·86 and 6·06, respectively. In addition to the vicinal coupling ($J_{1,2}$ = 10·5 Hz), H-2 is coupled to H-3 and to one of the H-4 protons, as indicated by decoupling experiments. Irradiation at the H-2 resonance sharpened the signals centered at δ 3·84 and 3·11. The absorption at δ 3·84 is assigned to H-3, and examination of the spectrum indicates that H-3 is equatorial. This assignment becomes evident in double irradiation experiments. Irradiation at δ 3·84 collapses the H-4ax quartet at δ 1·67 to a doublet ($J_{4eq,4ax}$ = 14·0 Hz). The smaller coupling of 5·0 Hz removed in this experiment is consistent with the equatorial assignment of H-3. The C-3 methoxyl resonance occurs at δ 2·96.

NMR data for alkaloid II are consistent with the C-3 stereochemistry indicated. Coupling between the H-3 proton and the axial H-4 proton is 11·0 Hz, indicative of a *trans* diaxial relationship. In addition, the H-3 resonance of II occurs at higher field (δ 3·30) and the C-3 methoxyl signal at lower field (δ 3·29). These are the only significant shift differences between I and II.

Alkaloid II is identical in all respects with 'alkaloid 7' previously isolated from *P. comosa*.[6] Hydrogenation of I and II gave as major products V and VII, respectively.[7] The NMR spectra of V and VII contain only one vinyl proton signal, a triplet at δ 5·97. This observation is consistent with the isomerized double bond positions indicated for alkaloids V and VII. Langlois *et al.*[6] obtained VIII by lithium aluminium hydride reduction of II. By analogy we converted I to VI by this reaction. The NMR spectrum of VI still exhibits two vinyl protons like I, and it is again apparent that VI and VIII differ only in their opposite configurations at C-3. Therefore, wilsonine (I) differs from II in being epimeric at C-3. Both compounds are assumed to possess the same absolute stereochemistry at C-5, as all the known homoerythrina alkaloids to date, including II,[6] have the configuration shown for I and II. The stereochemistry of the epoxide oxygen in both I and II remains uncertain.

The occurrence of cephalotaxine (III) as a minor alkaloid in *C. wilsoniana* is in marked contrast to what has been observed in other *Cephalotaxus* species; cephalotaxine normally accounts for 50% or more of the total alkaloids present in *C. harringtonia*, *C. drupacea* and *C. fortunei*. Since both homoerythrina and 'cephalotaxine'-type alkaloids are found in *C. wilsoniana*, these two series of alkaloids may well arise from common precursors.[3]

EXPERIMENTAL

M.ps were determined on a Fisher–Johns block and are uncorrected. IR spectra were made on a Perkin–Elmer Model 137, and UV spectra were recorded on a Beckman DK-2A spectrophotometer. Optical rotations were measured on a Cary Model 60 recording spectropolarimeter at 26° in 0·5 d.m. cells. MS analyses were performed with a Nuclide 12–90G or with a Du Pont (CEC) 21-492-1 spectrometer, and high resolution was employed to establish empirical formulas.

Analyses of all compounds by TLC were accomplished on 0·25 mm Brinkman precoated Silica Gel F254 plates (analytical) or 1 mm Silica Gel G plates (preparative). Analytical plates were developed with the solvent system $CHCl_3$–MeOH (9:1) and spots were visualized with iodine vapor; preparative plates were also developed with $CHCl_3$–MeOH (9:1). However, bands were visualized by spraying with bromothymol blue in EtOH. Respective R_f values for the alkaloids in this solvent system are as follows: I, 0·70; II, 0·85; III, 0·15; IV, 0·48; V, 0·37; and VII, 0·47.

Extraction of alkaloids. Dried twigs and leaves of *C. wilsoniana* Hayata (595 g) were ground and placed in a percolator along with 1 l. of 95% EtOH. After 1 hr the EtOH was drained off and the plant material was extracted 4 × with 800 ml portions of EtOH. The combined extracts were evaporated under reduced pressure (below 40°), and the concentrate (100 ml) was diluted with 0·75 l. of 6% of tartaric acid solution. The acidic solution was extracted repeatedly with $CHCl_3$ (discarding the $CHCl_3$ extracts) and then made basic, to pH 10, by the addition of Na_2CO_3. Repeated extraction of the basic solution with $CHCl_3$ gave 1 g of crude alkaloids (0·17%). Similarly 0·8 g (0·14%) of alkaloidal material was prepared from 570 g of *C. wilsoniana* dried stem (a section of wood 10 cm dia. including bark). When an 800 g sample of *C. harringtonia* seed kernels was ground and defatted with pentane–hexane, 434 g of oil resulted. The remaining meal was then extracted 5 × with 0·5 l. portions of 95% EtOH. Workup of the alcoholic concentrate, as described above, yielded 12·2 g of crude alkaloids (1·52%).

Separation of alkaloids. Alkaloids from the *C. wilsoniana* twig and leaf extracts (483 mg) were separated by preparative TLC on 6 plates. The procedure yielded 220 mg of I, 137 mg of II, 12 mg of III and 38 mg of a mixture of III, IV and an unknown. Similarly, 412 mg of the stem alkaloids gave 76 mg of I, 37 mg of II, 208 mg of III and 23 mg of a mixture of IV and several unknowns. Repeated TLC of the mixtures containing IV yielded 12 mg of relatively pure IV.

C. harringtonia seed alkaloids were separated into a series of fractions by countercurrent distribution in 10 tubes with 400 ml of each phase ($CHCl_3$-McIlvaine's buffer, pH 5) per tube.[3] Tubes 1 and 2 were combined (0·61 g), and 0·52 g of the mixture was chromatographed on 50 g of Brockman Grade III neutral alumina in a 2·4 cm i.d. column. With 10 ml fractions the column was eluted successively with 50 ml of benzene–Et_2O (9:1), 50 ml of benzene–Et_2O (3:1), 50 ml of benzene–Et_2O (1:1), 50 ml of benzene–Et_2O (1:3), 50 ml of Et_2O (100%), 50 ml of Et_2O–MeOH (9:1) and 50 ml of Et_2O–MeOH (3:1). Alkaloid I, 129 mg, was concentrated in fractions 20–25 (100% Et_2O).

Wilsonine (I). Wilsonine from *C. wilsoniana* was crystallized by slow evaporation of an ether solution in a loosely capped vial; m.p. 150–151°; $[\alpha]_D$ −51·4° (*c*, 0·55 in $CHCl_3$), $[\alpha]_D$ −36·0° (*c*, 0·55 in C_2H_5OH);

[7] Comparison samples of alkaloid II and its hydrogenation product VII were received as gifts from Dr. PIERRE POTIER, Institute de Chemie des Substances Naturelles, CNRS, 91-Gif-sur-Yvette, France.

λ_{max} 281 (log ϵ 3·41), λ_{min} 258 (log ϵ 2·75), λ_{max} 233 nm (log ϵ 3·90) in EtOH; ν_{max} 1610, 1580, 1460, 1120, 970, 918 and 905 cm^{-1} ($CHCl_3$). MS of I showed prominent ions at *m/e* 343 (M^+, 97%), 328 (100), 312 (46), 300 (25), 284 (21), 283 (30), 282 (14), 256 (24) and 244 (14). Found M^+, *m/e* 343·177; $C_{20}H_{25}NO_4$ requires 343·178 Found: C, 70·18; H, 7·32; N, 4·16. Calc. for $C_{20}H_{25}NO_4$: C, 69·95; H, 7·34; N, 4·08%. The NMR data for alkaloid I are summarized in Table I. Wilsonine from *C. harringtonia* seed, m.p. 150–151°, $[\alpha]_D$ −36·0° (*c*, 0·53 in C_2H_5OH), was identical in all respects with I from *C. wilsoniana.*

C-3epi-*Wilsonine* (II). Alkaloid II gave m.p. 103–104° Et_2O, $[\alpha]_D$ + 60·7 (*c*, 0·55 in $CHCl_3$), $[\alpha]_D$ +75·8° (*c*, 0·52 in C_2H_5OH); λ_{max} 281 (log ϵ 3·44), λ_{min} 258 (log ϵ 2·77), λ_{max} 233 nm (log ϵ 3·92) in EtOH; ν_{max} 1610, 1580, 1460 and 916 cm^{-1} ($CHCl_3$). The MS of II gave prominent ions at *m/e* 343 (M^+, 85%), 328 (100), 312 (41), 300 (28), 284 (24), 283 (33), 282 (15), 256 (27) and 244 (14). Found: M^+, *m/e* 343·179; $C_{20}H_{25}NO_4$ requires 343·178. Found: C, 69·48; H, 7·20; N, 3·92. Calc. for $C_{20}H_{25}NO_4$: C, 69·95; H, 7·43; N, 4·08%. The NMR data for II are summarized in Table 1. An authentic sample of II, obtained from *P. comosa*,[7] was identical in all respects to II from *C. wilsoniana.*

Cephalotaxine (III). Cephalotaxine from *C. wilsoniana*, m.p. 134–136°, $[\alpha]_D$ −184° (*c*, 0·30 in $CHCl_3$) was identical in all respects with III from *C. harringtonia.*[8]

Acetylcephalotaxine (IV). Repeated TLC gave IV[8] with an unknown impurity (5–10%) that was not completely removed by repeated TLC and the sample failed to crystallize from ether. Comparison of the sample (IR, NMR and MS) with a sample of synthetic IV[8] was sufficient to identify the major component of the mixture positively as acetylcephalotaxine. The NMR spectrum gave characteristic one-proton singlets at δ 5·05, 6·57 and 6·59; one-proton doublets at δ 3·77 and 5·80 (J = 9·5 Hz); a two-proton singlet at δ 5·85; and a pair of three-proton singlets at δ 1·58 and 3·71. Minor signals, due to the impurity, were noted at δ3·20, 3·83, 6·60 and 6·75. The MS of IV from *C. wilsoniana* was essentially identical to that of synthetic IV except for the presence of an additional peak at *m/e* 178 (20%). The *m/e* 178 peak, attributed to the impurity, is significant in that a number of homoerythrina alkaloids give *m/e* 178 as the base peak.[3]

Hydrogenation of wilsonine (I). A 64-mg sample of I in 5 ml of EtOH was hydrogenated at atmos. pres. (2 hr) with palladium-on-carbon (10%) catalyst. The product, 59 mg, was recovered by filtering off the catalyst and evaporating the solution to dryness. Preparative TLC gave V as the major alkaloid (26 mg) along with three minor unidentified products. Alkaloid V gave ν_{max} 3600, 1610, 1575, 1460, 1330, 1150, 1120, 1070 and 915 cm^{-1}. MS had prominent ions at *m/e* 345 (M^+, 18%), 314 (14), 288 (22), 287 (100), 286 (34), 272 (20), 270 (36), 256 (10), 194 (28) and 181 (5). The NMR spectrum of V is summarized in Table 1.

Hydrogenation of C-3epi-*Wilsonine* (II). A 53-mg sample of II was hydrogenated in the same manner as I. After the resulting mixture (48 mg) was subjected to preparative TLC, the major product (VII) amounted to 23 mg. 3 minor unidentified products were also isolated. Alkaloid VII gave ν_{max} 3600, 1610, 1575, 1460, 1330, 1150, 1120, 1035, 985, 957 and 915 cm^{-1}. The MS had prominent ions at *m/e* 345 (M^+, 18%), 314 (13), 288 (21), 287 (100), 286 (34), 272 (19), 270 (34), 256 (9), 194 (39) and 181 (7). The NMR spectrum of VII is summarized in Table 1. The MS of an authentic sample of VII[7] gave *m/e* 345 (M^+, 13%), 314 (13), 288 (20), 287 (100), 286 (32), 272 (18), 270 (35), 256 (9), 194 (39) and 181 (10).

Lithium aluminium hydride reduction of (I). Cautiously, an excess of $LiAlH_4$ was added to soln of 32 mg of I in 2 ml of dry tetrahydrofuran at 0°. The ice bath was removed and the mixture was stirred for 30 min while it warmed to room temp. and it was then refluxed for 4 hr. Excess $LiAlH_4$ was destroyed by cautious addition of water at 0°. After the resulting mixture was filtered, the solids were rinsed repeatedly with benzene. Evaporation of the combined benzene extracts gave 28 mg of product. Preparative TLC of this mixture produced VI (11 mg) as the major compound. Alkaloid VI gave λ_{max} 3600, 1610, 1575 and 1460 cm^{-1}. MS of VI had prominent ions at *m/e* 345 (M^+, 83%), 330 (100), 314 (30), 233 (13), 232 (30) and 220 (21). The NMR spectrum of VI is summarized in Table 1.

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[8] R. G. POWELL, D. WEISLEDER, and C. R. SMITH, JR., *J. Pharm. Sci.* in press.