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FORTUNEINE, A HOMOERYTHRINA ALKALOID FROM *CEPHALOTAXUS FORTUNEI**

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Key Word Index—*Cephalotaxus fortunei*, Cephalotaxaceae, homoerythrina alkaloids, epiwilsonine, wilsonine, fortuneine, identification

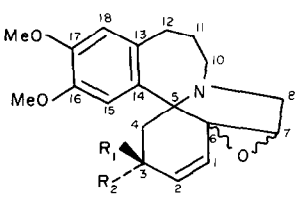
Abstract—Examination of the weakly basic fraction of the twigs and leaves of *Cephalotaxus fortunei*, a plant native to China, has resulted in the isolation and characterization of epiwilsonine, wilsonine and a new alkaloid named fortuneine The structure of fortuneine was deduced by spectral and chemical means In addition, epifortuneine was prepared and spectral data collected

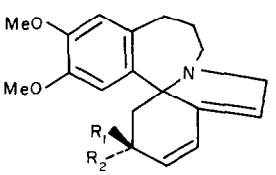
INTRODUCTION

Investigations of the ethanol extract of *Cephalotaxus fortunei* have revealed a number of cephalotaxine-type and homoerythrina-type alkaloids [1–3] Two ester alkaloids, harringtonine and homoharringtonine, derived from cephalotaxine are of particular interest because of their antitumor activity [1–4] Recently, the antitumor alkaloids have been isolated in large scale for clinical use in China [5–7] Investigation of the minor alkaloids of the weak basic fraction obtained at pH 6 constitutes the subject of the present paper

RESULTS AND DISCUSSION

Wilsonine (**1**), epiwilsonine (**2**) and a new alkaloid named fortuneine (**3**) were isolated from the weak basic fraction of *C fortunei* following a gradient pH extraction procedure and repeated prep chromatography on alumina





1 R_1 = OMe, R_2 = H
2 R_1 = H, R_2 = OMe

3 R_1 = OMe, R_2 = H
4 R_1 = H, R_2 = OMe

The alkaloid wilsonine (**1**) has previously been isolated from different species of *Cephalotaxus* namely *C wilsoniana* [8], *C harringtonia* [8] and *C sinensis* [10] Epiwilsonine (**2**) was previously obtained from *C wilsoniana* [8] and from *Phelline comosa* (Aquifoliaceae) [9]

The new alkaloid fortuneine (**3**) was isolated as optically active needle crystals A study of the ^{1}H NMR spectrum of **3** was in agreement with that of homerythrina-type alkaloids [8, 11–13] It shows two signals at δ 6 61 (*s*) and 6 47 (*s*) which are assigned to the two aromatic protons at C-15 and C-18 The C-1, C-2 and C-7 protons absorb at δ 6 60 (*d*, J = 10 Hz) and 5 97 (2H, *m*), respectively Two singlets, appearing at δ 3 86 and 3 76 and integrated for three protons each, were assigned to two methoxy groups at C-16 and C-17 An additional three-proton singlet appearing at δ 3 03 was attributed to the allylic methoxy group present on C-3 The shielded value for the allylic methoxy compares well with that of wilsonine (**1**) indicating that it is axial and that the C-3 hydrogen is quasi-equatorial [8] Two signals at δ 3 16 and 3 61, each appearing as a multiplet, were assigned to the protons at C-4 (equatorial) and C-3, respectively The axial proton at C-4 appeared at δ 1 98 as a double doublet

In addition, the mass fragmentation pattern of fortuneine was characteristic of a homoerythrina alkaloid having a $\Delta^{1,6}$-diene system [12, 13]

Final proof for the structure of fortuneine was obtained by reduction of **1** using zinc and acetic acid [14] The synthetic fortuneine was identical to the natural material (IR, mp, ^{1}H NMR, mass spectrum) Reduction of **2** under the same conditions afforded epifortuneine (**4**) The ^{1}H NMR spectrum of **4** was similar to that of fortuneine, but the allylic methoxy on C-3 appeared somewhat deshielded at δ 3 28 Although we collected ^{13}C NMR data on both fortuneine and epifortuneine, no attempt was made to assign values for specific carbons Fortuneine represents the first alkaloid with a $\Delta^{1,6}$-diene system to be isolated from *Cephalotaxus* species This type of alkaloid, however, has been previously reported in *Schelhammera* species (Liliaceae) [12, 13]

*Part 4 in the series "Studies on the Alkaloids of *Cephalotaxus*" For Part 3 see ref [3]

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EXPERIMENTAL

Mps are uncorr UV spectra were recorded in MeOH Optical rotations were carried out in 1 dm cells ^{1}H NMR spectra were recorded in $CDCl_3$ on a 90 MHz instrument using TMS as int standard and are reported in δ (ppm) ^{13}C NMR spectra were obtained at 15 03 MHz using TMS as ref MS were recorded at 70 eV TLC analysis was carried out on precoated (0 25 mm) Si gel 60 F-254 plates and developed with $CHCl_3$–MeOH (9 1) (system A) and $CHCl_3$–EtOAc–NH_4OH (6 4 0 1) (system B) and spots were visualized with I_2 or by spraying with modified Dragendorff's reagent GC analysis was carried out using a N–P detector under the following conditions glass column 2 m × 2 mm i d packed with 2% OV-17, column temp 220° isothermal, detector temp 300°, injection port temp 250°, He at 30 ml/min Caffeine was used as int standard

Plant material Stems and twigs of *C fortunei* Hook f used in this investigation were collected during the spring of 1974 at the foot of Huangshan Mountain, Anhwei Province, People's Republic of China Herbarium specimens are deposited at the Shanghai Institute of Materia Medica, Chinese Academy of Sciences, Shanghai, People's Republic of China

Extraction of alkaloids Dried powdered twigs and leaves (100 kg) were percolated with 95% EtOH and the EtOH extracts partitioned according to a previously published procedure [3] Fractionation of the weakly basic fraction obtained at pH 6 resulted in the isolation of the alkaloids wilsonine, epiwilsonine and fortuneine

Isolation of epiwilsonine (**2**) The alkaloidal residue obtained at pH 6 was dissolved in EtOH and 10% HCl in EtOH was added to bring the pH to 4 The crystalline ppt formed was filtered and recrystallized from EtOH as white needles (10 g), mp 215–216° $[\alpha]_D$ − 74° (MeOH, *c* 0 2) The alkaloid was identical with epiwilsonine (IR, UV, ^{1}H NMR, MS) [8]

Isolation of wilsonine (**1**) The mother liquor of **2** was evaporated and dissolved in H_2O, filtered, then made basic by adding NH_4OH and extracted repeatedly with $CHCl_3$ The $CHCl_3$ layers were combined, washed with H_2O, dried (Na_2SO_4) and evaporated to dryness The alkaloid residue was crystallized from Me_2CO, followed by MeOH to yield 1 g of colorless plates of **1** mp 149–150° (lit [8] 150–151°), $[\alpha]_D^{26}$ − 40 2° (EtOH, *c* 0 46) (lit [8] − 36 0°) The spectral data of **1** were identical (IR, UV, ^{1}H NMR, MS) to those reported in the lit [8]

Isolation of fortuneine (**3**) The mother liquor of **1** was chromatographed on a grade IV neutral Al_2O_3 column using $CHCl_3$–MeOH (99 1) as eluting solvent Fractions containing fortuneine were combined, the solvent evaporated and the residue crystallized from Et_2O Recrystallization from Me_2CO afforded 50 mg of needle crystals of **3**, mp 110°, R_f 0 26 and 0 15 (systems A and B, respectively, *RR*, 5 7, $[\alpha]_D^{26}$ − 121° (MeOH, *c* 0 2) UV λ_{max}^{MeOH} nm (log ε) 282 (sh) (3 37), 233 (4 05), IR ν_{max}^{KBr} cm^{-1} 1675, 1600, 1578, 1510, 1460, 1260, 1212, 1118, 1030, 970, 925, 860 and 775 EIMS (probe) 70 eV, *m/z* (rel int) 327 $[M]^+$ (24), 312 $[M - Me]^+$ (18), 297 (11), 296 (47), 294 (8), 269 (5), 243 (11) and 132 (100), Found M^+ *m/z* 327 1802 (HRMS) for $C_{20}H_{25}HNO_3$, calc 327 1805 ^{1}H NMR ($CDCl_3$) δ 6 61 (1H, *s*) and 6 47 (1H, *s*) (H-15 and H-18), 6 60 (1H, *d*, *J* = 10 Hz, H-1), 5 97 (2H, *m*, H-2 and H-7), 3 86 (3H, *s*) and 3 76 (3H, *s*) (for OMe on C-16 and C-17) 3 61 (1H, *m*, H-3), 3 16 (1H, *m*, H-4 equatorial), 1 98 (1H, *dd*, *J* = 11 and 5 Hz, H-4 axial) ^{13}C NMR ($CDCl_3$) δ 147 5 (*s*), 146 60 (*s*), 142 62 (*s*), 131 20 (*d*), 129 10 (*s*), 128 00 (*d*), 125 07 (*d*), 124 20 (*d*), 114 94 (*d*), 113 90 (*d*), 73 70 (*d*), 69 52 (*s*), 58 80 (*t*), 56 20 (*q*), 55 90 (*q*, double intensity), 49 20 (*t*), 37 42 (*t*), 32 22 (*t*) and 22 80 (*t*)

Reduction of **1** *with zinc and acetic acid (conversion of* **1** *to* **3** To 120 mg **1** in 9 ml HOAc was added 1 g Zn dust and the reaction mixture stirred for 4 hr at 60° The reaction product was filtered and the residue rinsed repeatedly with HOAc The combined acidic soln was diluted with 10 ml H_2O, made basic with Na_2CO_3 and extracted with $CHCl_3$ Evaporation of the $CHCl_3$ extract gave 102 mg of a mixture consisting of 87% **3** and 13% **1** The mixture was further purified by prep TLC on Si gel G using system A The required band was located by spraying the edge of the chromatogram with Dragendorff's reagent The zone was removed, eluted with $CHCl_3$–MeOH (1 1) and evaporated to dryness (yield 80 mg) The purified material was crystallized from Et_2O–MeOH (50 1) to yield pale yellow needles (40 mg) The synthetic fortuneine was identical in all respects with the natural material (mp, mmp, IR, UV, ^{1}H NMR, MS, R_f and RR_t)

Reduction of **2** *with zinc and acetic acid (preparation of epifortuneine* (**4**) Reduction of **2** (120 mg) under the same conditions described above afforded epifortuneine as a pale yellow amorphous solid (80 mg) $[\alpha]_D^{26}$ + 134 4° (MeOH, *c* 0 06), *RR*, 6 6, R_f 0 41 and 0 24 (in systems A and B, respectively), IR ν_{max}^{KBr} cm^{-1} 1675, 1598, 1570, 1505, 1458, 1250, 1110 and 1030 EI-MS (probe) 70 eV, *m/z* (rel int) 327 (27), 312 (17), 296 (39), 144 (17), 132 (37), 117 (10), 97 (12), 91 (17), 85 (65) and 83 (100) ^{1}H NMR ($CDCl_3$) δ 6 67 and 6 53 (1H each, *s*, for H-15 and H-18), 6 50 (1H, *d*, *J* = 10 Hz, H-1), 5 95–5 74 (2H, *m*, H-2 and H-7), 3 87 and 3 77 (3H each, *s*, OMe on C-16 and C-17) and 3 30 (3H, *s*, C-3 OMe) ^{13}C NMR ($CDCl_3$) δ 147 70 (*s*), 146 51 (*s*), 143 33 (*s*), 131 90 (*s*), 131 44 (*d*), 127 93 (*s*), 123 71 (*d*), 122 60 (*d*), 115 45 (*d*), 113 77 (*d*), 76 21 (*d*), 71 21 (*s*), 59 71 (*t*), 55 94 (*q*), 55 70 (*q*), 49 44 (*t*), 39 90 (*t*), 32 74 (*t*) and 23 40 (*t*)

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