Antitumor Alkaloids from *Cephalotaxus harringtonia*: Structure and Activity


Abstract

Cephalotaxine and several of its esters were isolated from *Cephalotaxus harringtonia* K. Koch var. harringtonia. Although cephalotaxine is inactive, harringtonine, isoharringtonine, homoharringtonine, and deoxyharringtonine have shown significant activity against experimental P388 leukemia and against L-1210 leukemia in mice.

Keyphrases

- Cephalotaxus harringtonia alkaloids—structure, antitumor activity
- Harringtonine, isoharringtonine, homoharringtonine, deoxyharringtonine—antitumor activity
- Antitumor alkaloids from *Cephalotaxus harringtonia*—structure, activity
- NMR spectroscopy—identification, *Cephalotaxus* alkaloids

In a search for tumor inhibitors of plant origin, an alcoholic extract of the seed of *Cephalotaxus harringtonia* var. drupacea (Sieb. & Zucc.) Koidzumi showed activity against lymphoid leukemia L-1210 and P388 leukemia in mice. Subsequent fractionation of the seed extract and of an extract obtained from *C. harringtonia* (Forbes) K. Koch var. harringtonia cv. Fastigiata (entire plants) revealed four alkaloids with significant antitumor activity (I). The active *Cephalotaxus* alkaloids are esters of cephalotaxine (I): these include harringtonine (II), isoharringtonine (III), homoharringtonine (IV), and deoxyharringtonine (V).

DISCUSSION

Paudler et al. (2) first isolated cephalotaxine, and their work indicated that two partial structures were possible. Subsequent investigations by other workers, using a combination of NMR (3) and X-ray crystallographic (4) techniques, revealed that cephalotaxine has the structure indicated here (I). We have now characterized the active antitumor alkaloids II-IV and report test data for these and several related alkaloids.

The NMR spectra of alkaloids II-V yielded initial evidence that these compounds are esters of cephalotaxine. This conclusion was based primarily on a comparison of their NMR spectra with the NMR spectra of cephalotaxine and acetylcephalotaxine (VII, Table I). If one disregards signals attributed to the R group, the NMR spectra of the cephalotaxine esters are nearly identical. The number and nature of free hydroxyl groups in alkaloids I-IV were indicated by NMR spectra of dimethyl sulfoxide-d$_6$ solutions before and after deuterium oxide exchange (5). In the mass spectra of these alkaloids, the strongest ion (base peak) is at m/e 298 (C$_{12}$H$_{22}$NO$_3$). This ion corresponds to cephalotaxine minus the appropriate R group.

Transesterification of alkaloids II-IV (sodium methoxide-methanol or sodium ethoxide-ethanol) gives alkaloid I, along with the corresponding dimethyl or diethyl esters (VIII-X or XII-XIV). Structures of Compounds VIII-X were deduced from NMR and mass spectral data.

Significant features of the NMR spectra of dimethyl esters VIII and X (see Experimental section for chemical shift assignments) are

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1. *Cephalotaxus* plant materials were received from Dr. Robert E. Perdue, Jr., U. S. Department of Agriculture (USDA), Beltsville, Md., under a program developed with USDA by Drug Research and Development, National Cancer Institute (formerly the Cancer Chemotherapy National Service Center).


3. The previously used numbering for the cephalotaxine ring system was revised. The revised numbering corresponds closely to that commonly used for the erythrina series of alkaloids.
Table I—NMR Data for Cephalotaxine and Some Cephalotaxine Esters*

<table>
<thead>
<tr>
<th>Proton(s)</th>
<th>I</th>
<th>II</th>
<th>III</th>
<th>IV</th>
<th>V</th>
<th>VII</th>
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<td>s 5.07</td>
<td>s 5.06</td>
<td>s 5.05</td>
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<td>s 5.05</td>
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<td></td>
</tr>
<tr>
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<td>s 6.61</td>
<td>s 6.64</td>
<td>s 6.61</td>
<td>s 6.60</td>
<td>s 6.59</td>
</tr>
<tr>
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<td>s 6.54</td>
<td>s 6.53</td>
<td>s 6.54</td>
<td>s 6.52</td>
<td>s 6.57</td>
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<tr>
<td>Aryl–OCH3</td>
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<td>s 5.85</td>
<td>m 5.82</td>
<td>s 5.85</td>
<td>m 5.84</td>
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<tr>
<td>Vinyl–OCH3</td>
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<td>s 3.68</td>
<td>s 3.67</td>
<td>s 3.67</td>
<td>s 3.66</td>
<td>s 3.71</td>
</tr>
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</table>

* Measured in chloroform with a Varian HA-100 spectrometer. Chemical shifts (δ) are expressed in parts per million from tetramethylsilane. In each of these alkaloids, protons H-3 and H-4 are coupled (J = 9.5 Hz), and long-range coupling (J = 0.5 Hz) is observed between protons H-1 and H-3. These protons show strong geminal coupling (J = 16 Hz).

A typical isolation procedure for the Cephalotaxus alkaloids was described elsewhere (8). All attempts to crystallize alkaloids II–IV (from methanol, ether, benzene, petroleum ether, or mixtures of these solvents) failed; but because each gave a single spot on thin-layer chromatograms and a clean NMR spectrum, no contaminants were present in significant quantities.

Cephalotaxine (I)—Cephalotaxine was crystallized by slow evaporation of an ether solution in a loosely capped vial, m.p. 134–136°C; [α]20 = -189° (c 0.51 in chloroform); [α]20 = 209° (c 0.23 in ethanol); λmax: 290 (log ε 3.64), λmax: 260 (log ε 2.75), λmax: 238 nm (log ε 3.56); νmax: 3680, 1650, 1490, 1040, and 934 cm⁻¹. The mass spectra of I yielded prominent ions at m/e 315 (M⁺, 100%), 309 (54), 298 (57), 284 (67), 272 (17), 254 (15), 248 (3), 199 (15), 186 (35), 150 (25), 137 (26), and 115 (16). Anal.—Calc. for C15H20NO4; C, 68.57; H, 6.72; N, 7.22. Found: C, 68.71; H, 6.74; N, 7.20.

The NMR spectrum of cephalotaxine is given in Table I. In dimethyl sulfoxide-d6 solution, cephalotaxine exhibits a one-proton doublet, at δ 4.78, which is coupled to H-3 (δ 4.51). After exchange of an NH group into deuterium oxide, only the H-3 signal is apparent (δ 4.64). A one-proton doublet at δ 4.51 is characteristic of an isopropyl group; a singlet due to an isolated methylene group. slight modification of this moiety significantly affects antitumor activity. The importance of the R group is further emphasized because cephalotaxine (I) and acetylcyleptolaxine (VII) are inactive. On the other hand, pseudo-deoxyharringtonine (VI) gave a T/C ratio of 122 at the highest dose level tested (40 mg./kg. against P388 leukemia) with no apparent toxic effects. Alkaloid VI has not yet been tested at higher levels owing to lack of material. Synthesis of cephalotaxine esters having other R groups may lead to compounds having even more desirable antitumor properties than II–V.

EXPERIMENTAL

Table I—NMR Data for Cephalotaxine and Some Cephalotaxine Esters*

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* Melting points were determined on a Fisher-Johns block and are uncorrected. IR spectra were measured in chloroform solutions on a Perkin-Elmer model 137 instrument, and UV spectra were obtained in absolute ethanol on a Beckman DK-2A spectrophotometer. Optical rotations were determined on a Cary model 60 recording spectrophotometer at 25°C in 0.5-dm. cells. Mass spectral analyses were performed with a Nuclide 12-90G spectrometer. Empirical formulas determined by high resolution are given in parentheses along with relative intensities. NMR spectra were measured with a Varian HA-100 in CDCl₃ solution, unless otherwise specified.
I. R = -OH
   OH OH

II. R = CH₂(C(CH₃)₂)₂CH₂CO₂CH₃
    CH₃ CO₂⁻
    OH OH

III. R = CH₂CH(CH₃)₂C₆H₆CO₂CH₃
     CH₃ CO₂⁻
     OH OH

IV. R = CH₂C(CH₃)₂CH₂CH₂CO₂CH₃
    CH₃ CO₂⁻
    OH

V. R = CH₂C(CH₃)₂CH₂CO₂CH₃
    CH₃ CO₂⁻
    OH

VI. R = CH₂(CH₃)₂CH₂CO₂CH₃
    CH₃ CO₂⁻
    OH

VII. R = CH₂CO₂⁻
    R

<table>
<thead>
<tr>
<th>Alkaloid</th>
<th>Dose, mg./kg.</th>
<th>Survivors</th>
<th>Animal Weight Difference (T - C)</th>
<th>Survival Time, Days (T/C)</th>
<th>T/C, %</th>
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<tbody>
<tr>
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<td>-5.2</td>
<td>5.0/9.0</td>
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<td>2.00</td>
<td>6/6</td>
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<td>100</td>
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<td>III</td>
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<td>6/6</td>
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<td>10.9/9.0</td>
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<td>IV</td>
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<td>6/6</td>
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<td>101</td>
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<td>6/6</td>
<td>-0.0</td>
<td>10.9/9.0</td>
<td>101</td>
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</tbody>
</table>

* Data presented are representative of results from several assays with different samples of each alkaloid (Footnote 2). Materials considered active are defined as those treatments (T) whose yields are > 125% of that of the controls (C) (i.e., T/C > 125%).

178 (7), 150 (17), 99 (9), 90 (9), and 81 (9). Found: m/z 531.250; C₆H₆NO₃ requires 531.247. NMR data are given in Table I. In dimethyl sulfoxide-d₆, isoharringtonine gave a one-proton singlet at δ 4.54 and a one-proton doublet at δ 4.94. Both signals are absent after exchange with deuterium oxide.

**Homoharringtonine (IV)**—Evaporation of an ether solution, under vacuum, gave homoharringtonine as an amorphous white solid. [α]₀ = -119° (c 0.45 in chloroform); λₘₚₐₓ = 290 (log ε 3.62), λₘₚₚₐₓ = 261 nm (log ε 2.76); νₚₐₓ = 3850, 1740, 1650, 1480, 1070, and 928 cm⁻¹. The mass spectrum of IV gave ions at m/z 545 (M⁺, 14%), 543 (11), 514 (3), 315 (4), 316 (6), 284 (100), 282 (6), 284 (1), 266 (12), 205 (3), 150 (11), and 116 (5). Found: m/z 545.253; C₆H₆NO₃ requires 545.262. Data from the NMR spectrum of homoharringtonine will be found in Table I. In dimethyl sulfoxide-d₆, IV gave a pair of one-proton singlets at δ 4.75 and 3.97. Both signals are absent after exchange with deuterium oxide.

**Isoharringtonine (II)**—Evaporation of an ether solution, under vacuum, gave isoharringtonine as a white amorphous solid. [α]₀ = -91° (c 0.41 in chloroform); λₘₚₐₓ = 290 (log ε 3.60), λₘₚₚₐₓ = 261 nm (log ε 2.72); νₚₐₓ = 3600, 1740, 1650, 1480, 1080, and 930 cm⁻¹. The mass spectrum of III contains ions at m/z 531 (M⁺, 14%), 516 (1), 500 (4), 318 (8), 314 (7), 298 (100), 284 (7), 282 (6), 266 (13), 150 (11), and 116 (7). Found: M⁺, m/z 531.246; C₆H₆NO₃ requires 531.247.

Data presented are representative of results from several assays with different samples of each alkaloid (Footnote 2). Materials considered active are defined as those treatments (T) whose yields are > 125% of that of the controls (C) (i.e., T/C > 125%).

* Although this analysis is not within normally accepted limits, the empirical formula (C₆H₆NO₃) is established by the high-resolution mass spectrum. In addition, VIII from transesterification of II gave satisfactory elemental analysis, and I was identical to an authentic sample of cephalotaxine.

Table III—Activity of Some Cephalotaxus Alkaloids against P388 Lymphocytic Leukemia

<table>
<thead>
<tr>
<th>Alkaloid</th>
<th>Dose, mg./kg.</th>
<th>Survivors</th>
<th>Animal Weight Difference (T - C)</th>
<th>Survival Time, Days (T/C)</th>
<th>T/C, %</th>
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<td>10.9/9.0</td>
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<td>-0.5</td>
<td>10.9/9.0</td>
<td>109</td>
</tr>
</tbody>
</table>

* See Footnote a, Table II.
Acetyllephalotaxine (VII)—A solution of 1.0 g. of cephalotaxine in 2 ml. of acetic anhydride–pyridine (1:1) was allowed to stand at room temperature for 18 hr. The resulting solution was evaporated to dryness, and the remaining oil was chromatographed on a column of Brockmann grade III neutral alumina. This procedure gave 0.9 g. of acetyllephaloxine, m.p. 144–145°; [α]D = -99° (c 0.52 in chloroform), [α]D = -133° (c 0.05 in ethanol); λmax: 290 (log ε 6.62), 251 nm. (log ε 2.69); νmax: 1734 cm. -1. The mass spectrum of VII gave prominent ions at m/e 357 (M+ 58%), 342 (10), 326 (19), 314 (22), 298 (100), 282 (14), 266 (31), 254 (8), 214 (10), 150 (22), 137 (11), and 115 (9). Found: M,mie 357.164; C6H12NO2 requires 357.158. Data from the NMR spectrum are recorded in Table I.

Transesterification Reactions—A typical transesterification reaction involved ≈100 mg. of thoroughly dried alkali II–V as appropriate and 2.5 ml. of 0.5 M base (sodium methoxide–methanol or sodium ethoxide–ethanol). The reagents were placed in a capped vial and allowed to stand in a dry atmosphere at room temperature for 5 hr. Aqueous 5% acetic acid (30 ml.) was then added, and the solution was extracted repeatedly with 30-ml portions of chloroform. The chloroform extracts were washed with 5% acetic acid and 5% sodium carbonate solutions and dried over sodium sulfate; upon evaporation, they yielded the appropriate aldehyde ester.

Methyl 3-Carbomethoxy-3,6-dihydroxy-7-methyloctanoate (VIII) and Ethyl 3-Carbomethoxy-3,6-dihydroxy-7-methyloctanoate (XII)—Alkaloid II (102 mg.) was transesterified (sodium methoxide-methanol) and yielded two products: I, 70 mg., m.p. 135-136°, [α]D = -183° (c 0.23 in chloroform); and VIII, 28 mg., colorless liquid, [α]D = -189° (c 0.51 in chloroform). The mass spectrum of VIII showed no molecular ion but gave ions at m/e 357 (M+), 342, 326, 314, 298, 282, 266, 254, 214, 150, 137, and 115 (9). Found: M,mie 357.164; C6H12NO2 requires 357.158.

The NMR spectrum of VIII gave prominent signals at 3.73 and 3.79 (25, 3H each, carbomethoxyl groups). Two hydroxyl protons were observed (δ 2.00 and 3.80) and were readily exchanged with deuterium oxide. The mass spectrum of X gave ions at m/e 245 (14%); 129 (C6H10O5), 185 (C6H10O4), 169 (C6H10O4, 30), 167 (73), 162 (20), 145 (C6H10O4, 100), 129 (C6H10O5, 55), 116 (C6H10O4, 34), 113 (C6H10O4, 44), and 111 (C6H10O2, 27). No molecular ion was observed; however, an M* + 1 ion was detected with an excessive sample pressure, m/e 263 (2%).

Transesterification of IV (100 mg.) with sodium ethoxide gave I (54 mg.) and the diethyl ester XIV (22 mg.). The mass spectrum of X and XIV are quite similar, except that XIV exhibits two overlapping quartets at δ 4.22 and two overlapping triplets at δ 1.28 (two ethyl ester groups) rather than the methyl ester signals present in the spectrum of X.

REFERENCES


ACKNOWLEDGMENTS AND ADDRESSES

Received October 18, 1971, from the Northern Regional Research Laboratory, Agricultural Research Service, U. S. Department of Agriculture, Peoria, IL 61604
Accepted for publication April 19, 1972.
Presented at the 162nd National Meeting of the American Chemical Society, Washington, D. C., September 1971.

The authors are indebted to Mrs. M. Wakeman and Mr. R. Freiding for technical assistance; to Mrs. C. E. McGrew for elemental analyses; to Dr. D. J. Abraham and Dr. W. K. Rohwedder for mass spectra; to Dr. W. W. Paudler for an authentic sample of cephalotaxine; and to Dr. W. H. Tallent, Dr. I. A. Wolff, Dr. R. B. Bates, Dr. J. L. Hartwell, and Mr. K. L. Mikolajczak for helpful discussions.

References to specific equipment or commercial firms are made for clarity and do not necessarily constitute endorsement by the U. S. Department of Agriculture over other products or firms not mentioned.

▲ To whom inquiries should be directed.