DEOXYHARRINGTONINE, A NEW ANTITUMOR ALKALOID FROM *CEPHALOTAXUS*: STRUCTURE AND SYNTHETIC STUDIES*

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Abstract—A new alkaloid (proposed name, deoxyharringtonine) with significant antileukemic activity has been isolated from *Cephalotaxus harringtonia* (Forbes) K. Koch var. *harringtonia* cv. Fastigiata. Its structure has been established as either 2 or 3 by chemical and spectral methods. Total synthesis of the dicarboxylic acid side chain and esterification of its primary carboxyl group with natural cephalotaxine (1) yields 3; therefore, deoxyharringtonine must be 2.

THREE ANTITUMOR ALKALOIDS—harringtonine, homoharringtonine, and isoharringtonine—have previously been isolated from *Cephalotaxus harringtonia* plant materials and characterized.¹ These alkaloids, which show significant inhibitory activity

![Chemical structure of deoxyharringtonine](image)

1, \( R = \text{HO} \)

\[
\begin{align*}
\text{Me} & \quad \text{OH} \\
2, \ R = \text{CH}_3\text{CHCH}_2\text{CH}_2\text{CCH}_2\text{CO}_2\text{Me} & \quad \text{CO}_2
\end{align*}
\]

\[
\begin{align*}
\text{Me} & \quad \text{OH} \\
3, \ R = \text{CH}_3\text{CHCH}_2\text{CH}_2\text{CCH}_2\text{CO}_2 & \quad \text{CO}_2\text{Me}
\end{align*}
\]

\[
\begin{align*}
\text{Me} & \\
4, \ R = \text{CH}_3\text{CHCH}_2\text{CH}_2\text{C} = \text{CHCO}_2 & \quad \text{CO}_2\text{Me}
\end{align*}
\]

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against experimental lymphoid leukemia systems L1210 and P388 in mice,\(^9\) are all esters of cephalotaxine (1). Cephalotaxine\(^{1a,1b,1c}\) accounts for approximately 50\% of the total alkaloids present in *Cephalotaxus* plant extracts, but it is inactive. We now report the structure of a fourth cephalotaxine ester that we propose naming deoxyharringtonine (2) because of its relationship to harringtonine.\(^{1d,1c}\) This new alkaloid has antileukemic activity of the same order of magnitude as that previously reported for the other three cephalotaxine esters.\(^{1d,1c}\)

Since the alkaloid esters of *Cephalotaxus* occur naturally in such small quantities,\(^3\) extensive biological testing might require additional sources of these compounds. Therefore, we decided to synthesize the dicarboxylic acid moieties and esterify them in the proper manner with natural cephalotaxine, which had already been isolated in substantial amounts. This program, if successful, would provide a temporary solution to the availability problem and permit continued testing. Accordingly, we describe the synthesis of the R-group of deoxyharringtonine (2), along with our attempts to esterify it with cephalotaxine.

A brief statement concerning the isolation of alkaloid 2 is given here as a detailed report will be published soon.\(^4\) The IR spectrum of 2 suggested that it was an alkaloid ester with one free hydroxyl group rather than two as in the other three *Cephalotaxus* esters.\(^1c,1d\) High-resolution mass-spectral analysis gave a molecular weight of 515·246 (Calc. 515·251) which is 16 mass units (one oxygen) less than the molecular ion displayed by harringtonine and isoharringtonine and is 30 units (\(\text{CH}_2\text{O}\)) less than that of homoharringtonine. Major fragmentations of 2 give \(m/e\) 314 (\(\text{C}_{18}\text{H}_{20}\text{NO}_4\)), 298 (\(\text{C}_{18}\text{H}_{20}\text{NO}_3\)), 266 (\(\text{C}_{17}\text{H}_{16}\text{NO}_2\)) and 150 derived from the cephalotaxine (1) moiety. Significant ions in the spectrum of 2 at \(m/e\) 173, 159, 141, 99 and 81 are derived from the acyl moiety as revealed by spectra of compounds 9, 10, and 11 (Scheme 1).

The NMR spectrum of deoxyharringtonine is distinctive yet very similar to spectra of the other three alkaloid esters from this plant.\(^1c,1d\) Since the NMR spectrum in DMSO shows one exchangeable proton as a singlet at \(\delta 4·73\), there is only one free hydroxyl group in 2 and it is tertiary.\(^5\)

Base-catalyzed transesterification of 2 further pinpoints its structure: cephalotaxine (1) and a dimethyl ester with the same structure as synthetic compound 9, but presumably optically active,\(^\dagger\) are the products of this reaction. Synthetic compound 9 and dimethyl ester 9 from basic transesterification of 2 give identical IR, NMR, and mass spectral data. Of course, synthetic 9 is racemic. Because of these data, the only elusive structural feature of 2 that remains, other than its absolute configuration, concerns which dicarboxylic acid carboxyl group (primary or tertiary) is esterified with the hydroxyl group of cephalotaxine.

The NMR spectrum of 9 reveals carbomethoxyl signals at \(\delta 3·64\) and 3·77, while half esters 10 and 11 give corresponding signals at \(\delta 3·68\) and 3·76, respectively. Logically, the downfield signal of these two is due to the tertiary carbomethoxyl groups. This evidence is supported by the spectrum of trimethyl citrate, in which

\* Assays were performed under the auspices of Drug Research and Development, National Cancer Institute (formerly Cancer Chemotherapy National Service Center). The procedures were described in *Cancer Chemother. Rep.* 25, 1 (1962).
\dagger Not enough material of sufficient purity for a meaningful ORD analysis was available.
the tertiary carbomethoxyl signal appears at $\delta$ 3.78 and the primary carbomethoxyl signals appear at $\delta$ 3.64. It can also be seen that methylation of 10 shifts the existing primary carbomethoxyl signal from $\delta$ 3.68 to 3.64. In the spectrum of 2, therefore, the $\delta$ 3.64 (downfield of the two signals) is likely due to the vinyl methoxyl while the $\delta$ 3.53 signal results from an upfield shift of the $\delta$ 3.68 primary carbomethoxyl signal of 10: thus in 2, cephalotaxine is shown to be esterified with the tertiary carboxyl group of the acid moiety. This important detail provided the basis for establishing that the other cephalotaxine esters, harringtonine, homoharringtonine and iso­harringtonine,$^{14}$ are also derived by esterification of cephalotaxine with the tertiary carboxyl group of appropriate dicarboxylic acids.

We undertook the synthesis of half esters 10 and 11 (previously unknown compounds) and the esterification of each of them with cephalotaxine isolated from the plant extract. This partial synthesis, in addition to confirming the structure of deoxy­harringtonine, would enhance our supply of 2 for biological testing. The synthesis of dicarboxylic acid 8 (Scheme 1) is straightforward, and yields are good when NaH is used in the initial condensation reaction.$^6$ The absence of a nitrile absorption band in the IR spectrum of 7 was expected because the nitrile group is attached to an oxygen-bearing carbon.$^7$

Half esters 10 and 11 are prepared by reactions that attack the least hindered
carboxyl group. Mass spectral, NMR and IR data support the structures shown for compounds 9, 10 and 11. An interesting fragmentation pattern is observed in the mass spectra of 10 and 11. Ions involved are those of m/e 173 (M - 45) and m/e 159 (M - 59), corresponding to loss of \(-\text{CO}_2\text{H}\) and \(-\text{CO}_2\text{Me}\), respectively. Relative intensities of ion 173 to 159 (3:1 in 10 and 1:8 in 11) clearly demonstrate that preferred cleavage occurs at the tertiary position. The spectrum of 2 displays m/e 173 and 159 ions in essentially the same ratio (3:1) as that of 10; these data support NMR results and the conclusion that deoxyharringtonine consists of cephalotaxine esterified with the tertiary carboxyl of 10 as depicted in structure 2. An m/e 159 ion in the spectrum of 10 could arise by splitting off the carboxymethoxyl, but this reasoning does not account for a 159 ion in the spectrum of 2. We feel that this ion results from ester scrambling under electron impact in both spectra. A certain amount of cleavage removing the cephalotaxine fragment of 2 must have occurred before scrambling of the methyl ester takes place. Simple cleavage could produce the m/e 173 ion from 11, but it might also arise from ester scrambling followed by cleavage as well. The base peak m/e 99 in the spectra of 8, 9, 10 and 11 is probably \((\text{CH}_3)_2\text{CHCH}_2\text{CH}_2\text{C}≡\text{O}\); the base peak m/e 298 in the spectra of 2, 3 and 4 is the stabilized allylic cation from the cephalotaxine-minus-hydroxyl (315-17) moiety.

Synthesis of alkaloid ester 3 from the acid chloride of 11 and cephalotaxine proceeded readily as expected. Even though two diastereomers are formed because 11 is racemic, spectral data reveal that neither of them is deoxyharringtonine (2). These two diastereomers give rise to the duplicate signals of slightly differing chemical shifts and multiplicities observed for the vinyl, methine and aromatic protons in the NMR spectrum of 3. These data thereby provide important evidence that structure 2 is correct for deoxyharringtonine because it is the only alternative.

A major byproduct of the reaction producing 3 is the corresponding dehydrated ester 4. We have no evidence to distinguish whether dehydration occurs during acyl chloride preparation or during the actual esterification reaction; either route seems possible. Absence of a chiral center in the acyl moiety of 4 precludes formation of a diastereomeric mixture\(^\star\) and this point is confirmed by the NMR spectrum. Although alkaloid esters 3 and 4 have been subjected to preliminary antitumor screening, available results are inadequate for a meaningful interpretation.

Attempts to synthesize deoxyharringtonine by esterifying cephalotaxine with the hindered tertiary carboxyl group of 10 via the acid chloride have given complex mixtures which contain none of the desired product. We are actively pursuing other approaches to the problem and hope to report a solution soon.

**EXPERIMENTAL**

A Beckman DK-2A\(^\dagger\) recording spectrophotometer was used to record UV spectra, and IR analyses were done on 1\% solutions in CHCl\(_3\) with a Perkin-Elmer Model 137 instrument. NMR spectra were obtained on CDCl\(_3\) solutions (unless otherwise specified) with a Varian HA-100 spectrometer, and chemical

\(^\star\) Only the positional isomer indicated in 4 was detected here. But when 9 is subjected to purposeful dehydration with SOCl\(_2\) and pyridine, both possible positional isomers (double bond conjugated with both carboxyl groups as in 4, and double bond conjugated with only the tertiary carboxyl group) are isolated in a 55:45 ratio.

\(^\dagger\) The mention of firm names or trade products does not imply that they are endorsed or recommended by the Department of Agriculture over other firms or similar products not mentioned.
shifts are given relative to internal TMS. Mass spectral analyses were performed at 70 eV with a Nuclide 12-90 G or with a CEC 21-492-1 spectrometer, and ORD spectra were recorded with a Carey Model 60 spectropolarimeter. M.ps were determined with a Fisher-Johns block and are uncorrected.

All compounds were analyzed by TLC with appropriate solvent systems on either 0.25 mm Silica Gel G plates or on Brinkmann precoated 0.25 mm Silica Gel F-254 plates. Spots were visualized either by staining them with iodine vapour or by spraying the plates with an ethanolic solution of bromothymol blue. All preparative separations were made on 1 mm Silica Gel G layers with bromothymol blue visualization. Ether and CHCl₃ extracts were routinely dried over MgSO₄.

**Deoxyharringtonine (2).** The original material extracted from the plant with 95% EtOH was dissolved in 5% tartaric acidaq.: extraction with CHCl₃ removed the neutral and acidic components. Basification of the resultant aqueous phase, followed by a second CHCl₃ extraction, afforded a crude alkaloid mixture that was subjected to a 10-tube countercurrent distribution between CHCl₃ and pH 5 McIlvaine buffer. Deoxyharringtonine (2), along with some other alkaloids, remained in tubes 1 and 2. Finally, 2 was purified by column chromatography and subsequent prep TLC of material from selected column fractions: generally, a yield of <0.01%, of the plant dry weight or about 2%, of the crude alkaloid mixture was realized. Although apparently pure as evidenced by a single TLC spot and clean NMR spectrum, deoxyharringtonine existed as an amorphous solid, which could not be induced to crystallize: IR 3600 (→OH), 1740 (ester C=O), 1650 (trisubstituted double bond) and 925-950 cm⁻¹ (methyleneedioxy); ⁷⁶S⁹⁰CH₃ 90 µmol (log e = 3.66) and 261 µmol (log e = 2.86); [α]D₂₀ = −151° (c 0.03 EtOH) and [α]D₂₀ = −119° (c 0.06 CHCl₃); NMR δ 0.82 (d, J = 6 Hz, isopropyl), 2.06 (q, 2, J = 16 Hz, methylene between carboxyl and asymmetric carbon in R-group of 2), 3.53 and 3.64 (2 s, each 3, vinyl methoxyl and carbomethoxy), 5.01 (s, 1, vinyl), 5.82 (m, 2, methylenedioxy), 5.96 (d, 1, J = 10 Hz, methine on oxygen-bearing carbon), and 6.50 and 6.59 (2, s, each 1, aromatic); in DMSO at 1 H singlet at δ-4.73 disappeared when the sample was treated with D₂O (tertiary-OH): mass spectrum m/e (rel intensity) M⁺ 515(5), 484(8), 314(12), 298(100), 266(27), 173(10), 159(3), 150(35), 141(3), 99(20) and 81(12).

**Methyl 3-carbomethoxy-3-hydroxy-6-methylheptanoate (9) and cephalotaxine (1) from transeserification of deoxyharringtonine (2).** Alkaloid 2 was placed in a vial and dried overnight at 78° in an Abderhalden tube with P₂O₅. The sample (0.092 g) was treated with 2.5 ml of 0.5 M NaOMe in MeOH, and the vial was capped and placed in a dry atmosphere at room temp for 5 hr. After AcOHaq (30 ml of 5%) was added, the solution was extracted with 30 ml of CHCl₃. After being washed with 5% AcOH and then Na₂CO₃ solutions, the CHCl₃ extract yielded 0.036 g of crude 9, which was purified on an alumina column: IR 3595 (→OH), 1740 (ester C=O), 1100, 990 and 972 cm⁻¹; NMR δ 0.85 (d, 6, J = 6 Hz, isopropyl), 1.0-1.8 (m, 5, methylenes and methine of isovaleryl branch), 2.79 (q, 2, J = 16 Hz, methylene adjacent to carboxyl) and 3.64 and 3.77 (2 s, each 3, carbomethoxy groups): mass spectrum m/e (rel intensity) 173(75), 141(10), 99(100) and 81(35). Extraction of the basified AcOHaq. phase (from isolation of 9 above) with CHCl₃ yielded cephalotaxine (1): IR, UV, NMR and [α]D₂₀ entirely consistent with those of pure cephalotaxine.¹⁶²

**Methyl 3-carbomethoxy-3-hydroxy-6-methylheptanoate (9).** Synthetic. NaH (120 g), as a 55% dispersion in mineral oil, was placed in a 1-liter three-necked flask and was triturated five times with hexane. All manipulations were done in a dry N₂ atmosphere and all glassware was dried dry just before use. The NaH was suspended in anhyd tetrabutylammonium (65 ml) and the suspension was stirred with a sealed constant-torque stirrer while 60 ml of 0.5 mol of diethyl carbonate was added. Then 28.5 g (0.25 mol) of 5-methyl-2-hexanone (5), from Chemical Samples Co., Columbus, Ohio) in ether (65 ml) was added slowly over 6 hr: the mixture was refluxed (with constant stirring) on a steam bath during this addition. The mixture stood overnight at room temp. Glacial AcOH (40 ml) was added slowly (1.5 hr) to the stirred, cold ice bath mixture: the resulting NaOAc was dissolved by addition of water. The acidic aqueous phase was extracted thoroughly with ether, and the extract was washed to neutrality with dilute NaHCₐ₃ aq and then water. Crude 6, 47.5 g, was obtained by removal of the ether in vacuo: IR 1710 and 1735 cm⁻¹ (C=O); TLC with ether–hexane (9:10) revealed one spot, Rf 0.53.

The β-ketoester 6 (35.0 g) was stirred thoroughly at 0° with about 30 g of KCN. Conc HCl (40 ml) was added slowly (1.5 hr) from a dropping funnel and stirring at 0° was continued an additional 2.5 hr. The yield of crude cyanohydrin 7 obtained by ether extraction of this mixture was 38.2 g: IR 3630 cm⁻¹ (→OH). Hydrolysis of this cyanohydrin 7 was achieved by treating it overnight at room temp with conc. HCl (200 ml) and heating it on a steam bath for 2 hr. Water (400 ml) was added and the mixture refluxed for 4 hr. Ether extraction of this acidic solution gave 41.3 g of crude 3-carboxy-3-hydroxy-6-methylheptanoic acid (8). Recrystallization from hexane–CHCl₃ (1:2.5) at −20° afforded 24.6 g of a white waxy solid: m.p.
78-80°: IR 3580 (—OH), 2500-3300 (free carboxyl groups) and 1720 cm⁻¹ (C=O); mass spectrum m/e (rel intensity) no M⁺, 159(83), 141(50), 99(100) and 81(32). Dimethyl ester 9 was prepared by treating 8 with CH₂N₂ or by refluxing 8 with 3% H₂SO₄ in MeOH followed by the usual ether extraction. This diester was a viscous liquid: IR 3595 (—OH), 1740 (ester C=O), 1100, 990 and 972 cm⁻¹; NMR δ 0.85 (d, 6, J = 6 Hz, isopropyl), 1.0-1.8 (m, 5, methylenes and methine of isovaleryl branch), 2.79 (q, 2, J = 16 Hz, methylene adjacent to carboxyl), 3.64 and 3.77 (2 s, each 3, carboxymethoxy groups); mass spectrum m/e (rel intensity) M⁺ 232(1), 173(60), 141(10), 99(100) and 81(35).

Methyl 3-carboxy-3-hydroxy-6-methylheptanoate (10). A typical reaction involved treating 2.250 g of diacid 8 (dissolved in 4 ml of anhyd dioxane) with 0.84 ml of anhyd MeOH and two drops of cone H₂SO₄ for 7 to 14 days at room temp. The product was recovered by ether extraction. TLC analysis with ether-hexane-AcOH (55:45:2) revealed only one spot, Rf 0.87 (d, 6, J = 6 Hz, isopropyl), contained the components of 8 and H₂SO₄ in MeOH followed by the usual ether extraction. This diester was a viscous liquid: IR 3589 (—OH), 1740 (ester C=O), 1100, 990 and 972 cm⁻¹; NMR δ 0.85 (d, 6, J = 6 Hz, isopropyl), 1.0-1.8 (m, 5, methylenes and methine of isovaleryl branch), 2.79 (q, 2, J = 16 Hz, methylene adjacent to carboxyl), 3.64 and 3.77 (2 s, each 3, carboxymethoxy groups); mass spectrum m/e (rel intensity) M⁺ 232(1), 173(60), 141(10), 99(100) and 81(35).

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