sociation as well as the IN(1)-H···TO(2) hydrogen bond seen in Figure 3 could be utilized by proteins in order to specifically recognize a thymine or a uracil residue. It should be noted that the hydrogen bonding interaction can be made when the uracil or thymine is simultaneously involved in Watson-Crick interaction with adenine. Thus, this study provides a tentative structural basis for the recognition of thymine or uracil by a protein using, in part, specific interactions with a tryptophan residue.

Acknowledgments. I am most grateful to Professor N. Leonard for graciously providing the crystals of Ind[3]-C_3-Thy[1]. I thank Professor K.-I. Tomita for communicating the details of the crystal structure of thymin-1-ylacetic acid-tyramine monohydrate in advance of its publication. This work was supported, in part, by National Institutes of Health Grant GM23966.

Supplementary Material Available: Tables of hydrogen atomic coordinates (Table S1), temperature factors (Table S2), and observed and calculated structure factors (Table S3) (8 pages). Ordering information is given on any current masthead page.

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Biosynthesis of the *Cephalotaxus* Alkaloids. Investigations of the Biosynthesis of Deoxyharringtonine, Isoharringtonine, and Harringtonine[1]

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Abstract: Precursor incorporation experiments have been used to investigate the biosynthesis of the antitumor *Cephalotaxus* alkaloids deoxyharringtonine (**2**), isoharringtonine (**3**), and harringtonine (**4**). It has been established that the acyl portion of deoxyharringtonine is derived from leucine via a pathway that resembles the conversion of valine into leucine in microbial systems. Incorporation experiments with DL-[9-^{14}C]deoxyharringtonic acid (**6**) have also shown that **6** is the precursor of isoharringtonic acid (**12**) and harringtonic acid (**14**) in vivo. Doubly labeled deoxyharringtonine has been synthesized and its ability to serve as an intact precursor of harringtonine has been examined. The results of this experiment indicate, but do not prove, that deoxyharringtonine is directly converted to harringtonine without prior deacylation.

Introduction

Plants of the genus *Cephalotaxus* (Cephalotaxaceae) have been found to contain a number of alkaloids that are esters of the parent alkaloid cephalotaxine (**1**) (Figure 1). These ester alkaloids include deoxyharringtonine (**2**), isoharringtonine (**3**), harringtonine (**4**), and homoharringtonine (**5**).[3] Deoxyharringtonine and its congeners have been shown to exhibit significant antitumor activity against experimental P388 leukemia and L-1210 leukemia in mice.[3] Recent investigations in the People's Republic of China have shown that harringtonine and homoharringtonine are effective in the treatment of human cancers.[4]

The biosynthesis of the ester alkaloids **2–5** poses two interesting problems. The first concerns the origin of the unusual diacids that are attached to cephalotaxine in these alkaloids. The second concerns the stages at which the individual diacids are linked to cephalotaxine. Experiments bearing upon each of these problems are outlined in this paper.

Results and Discussion

The key to the elucidation of the biosynthesis of the acyl portions of the antileukemic *Cephalotaxus* alkaloids was provided by the recognition that the diacid **6** linked to cephalotaxine in deoxyharringtonine, henceforth referred to as deoxyharringtonic acid, bears a close resemblance to a diacid intermediate involved in the biosynthesis of leucine from valine in microorganisms.[5] On the basis of this resemblance, we arrived at the hypothesis for deoxyharringtonic acid biosynthesis shown in Scheme I.

The hypothesis predicts that 3-carboxy-3-hydroxy-5-methylhexanoic acid (**8**) should be an intermediate in the biosynthesis of **6** and that carbon atoms 3–8 of **8** should be derived from L-leucine (**7**). Since the presence of diacid **8** or its derivatives in *Cephalotaxus* plants had never been reported, its presence was sought by isotopic trapping. A synthetic sample of racemic **8** was prepared from 4-methyl-2-pentanone using methods developed for the synthesis of deoxyharringtonic

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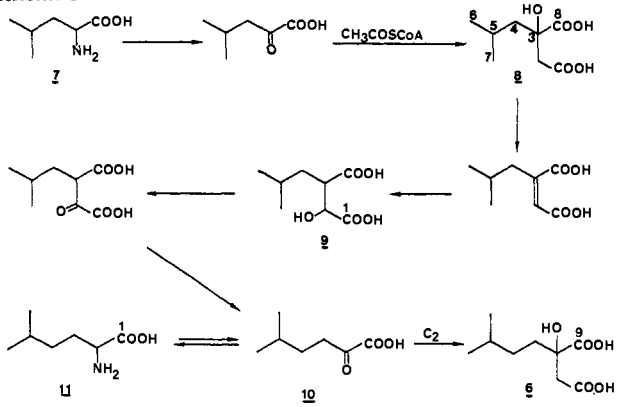
Table I. Feeding Experiments with *Cephalotaxus harringtonia*

expt no.	precursor fed	product isolated	feeding period, days	% incorporation[a]	% distribution of act. in product
1	L-[1-^{14}C]-**7**	**8**[c]	14	0.03	84 at C-8
2	[1-^{14}C]-**9**	**6**[d]	7	0.21	89 at C-9
3	DL-[1-^{14}C]-**11**	**6**[d]	7	3.25	94 at C-9
4	DL-[9-^{14}C]-**6**	**12**[e]	4	0.44	97 at C-9
5	[9-^{14}C]-**13**	**12**[e]	4	0.00	
6	DL-[9-^{14}C]-**6**	**17**	4	1.60	97 at C-9 ^{3}H:^{14}C = 6.50 (**2**)
7	[3′-^{3}H, 1-^{14}C]-**2**, ^{3}H:^{14}C = 6.40	**2, 4**	4	0.60[b]	^{3}H:^{14}C = 15.3 (**4**)

[a] Incorporation figures based on total radioactivity fed. [b] Incorporation figure based on ^{14}C. [c] As bis(*p*-phenylphenacyl) ester. [d] As bis(*p*-bromophenacyl) ester. [e] As dimethyl ester.

Scheme I



acid from 5-methyl-2-hexanone.[6] L-[1-^{14}C]Leucine was administered to rapidly growing *C. harringtonia* plants by the cottonwick method, and the plants were harvested after 14 days. The plant material was worked up by ethanol extraction with the addition of radioinactive **8** as carrier. The crude extract was subjected to alkaline saponification to convert any esters of **8** into the free diacid, and the crude mixture of acids was derivatized with *p*-phenylphenacyl bromide using the crown ether method.[7] The bis(*p*-phenylphenacyl) ester was purified by repeated thin-layer chromatography followed by crystallization to constant radioactivity to give the incorporation level shown in Table I (experiment 1). The incorporation of L-leucine into **8** was shown to be specific by degradation to isolate C-8 of the diacid. Reduction of the bis(*p*-phenylphenacyl) ester with lithium aluminum hydride gave 3-hydroxy-3-hydroxymethyl-5-methyl-1-hexanol, which was purified by chromatography, distilled, and cleaved with periodate to yield radioactive formaldehyde, trapped as its dimedone adduct. The results (Table I, experiment 1) show that most of the radioactivity resides in the expected position of the diacid. Therefore, it appears that the diacid **8** is present in *Cephalotaxus* plants and that it is formed from leucine in the expected fashion.

The hypothesis shown in Scheme I also predicts that 2-hydroxy-3-carboxy-5-methylhexanoic acid (**9**) lies on the biosynthetic pathway to **6**. In order to test this prediction, [1-^{14}C]-**9** was synthesized from ethyl 4-methylpentanoate by a modification of literature methods.[5d] The labeled sample of **9** was administered to *C. harringtonia* plants, and the plants were harvested after 7 days. Radioinactive diacid **6** was added to the crude, alcoholic plant extract, which was then subjected to acidic saponification.[8] The crude mixture of acids resulting from the saponification was converted into methyl esters, and the dimethyl ester of **6** was purified by repeated thin-layer chromatography using a system in which the dimethyl ester of the precursor **9** exhibited an R_f value considerably smaller than that for the dimethyl ester of **6**.[9] The purified dimethyl

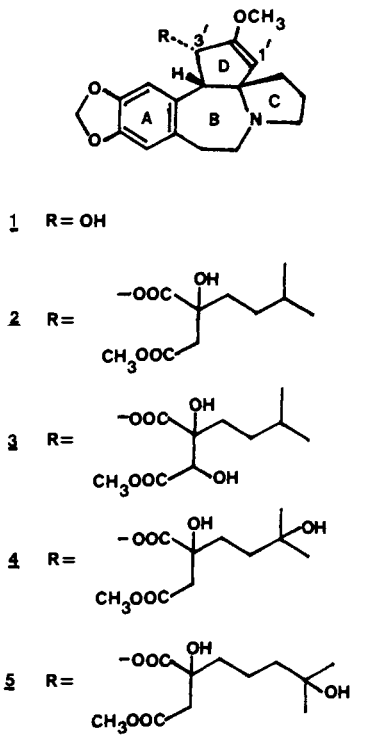
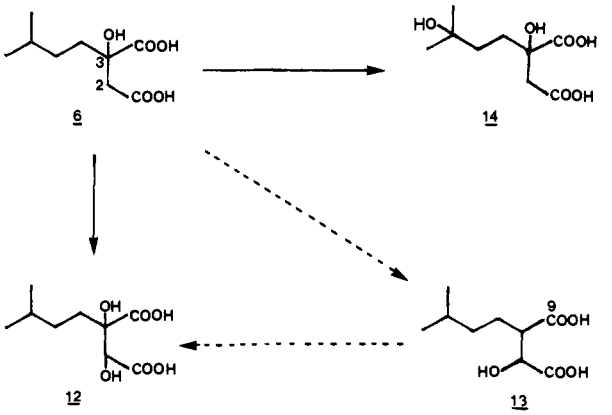


Figure 1.

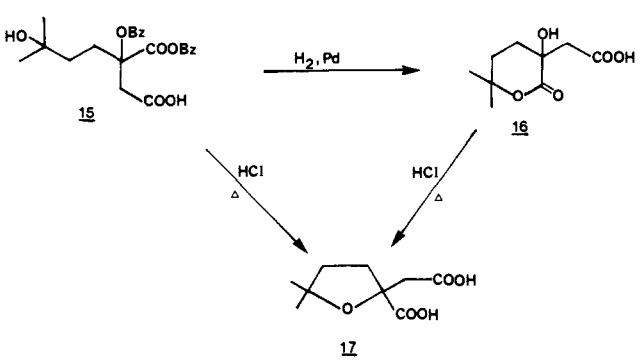
ester of **6** was then hydrolyzed to the free diacid and converted to the bis(*p*-bromophenacyl) ester in the usual way. The bis(*p*-bromophenacyl) ester was purified by repeated thin-layer chromatography using a system in which the bis(*p*-bromophenacyl) ester of the precursor **9** exhibited a lower R_f value than the bis(*p*-bromophenacyl) ester of **6**.[9] The labeled diester was then recrystallized to constant activity to give the incorporation figure shown in Table I (experiment 2). The specific incorporation of the diacid **9** into **6** was demonstrated by reduction of the bis(*p*-bromophenacyl) ester with lithium aluminum hydride and cleavage of the resulting labeled 3-hydroxy-3-hydroxymethyl-6-methyl-1-heptanol with periodate. The results of the degradation (Table I, experiment 2) clearly show that **9** is incorporated into **6** with very little randomization of the label.

On the basis of the hypothesis suggested in Scheme I, 2-oxo-5-methylhexanoic acid (**10**) would be expected to be the immediate precursor of **6**. If this is the case, then homoleucine (**11**) should be specifically incorporated into **6** due to the facile interconversion between α-amino acids and the corresponding α-keto acids. DL-[1-^{14}C]Homoleucine was conveniently prepared from 4-methylpentanal by condensation with potassium [^{14}C]cyanide and ammonium carbonate followed by hydrolysis of the intermediate hydantoin. The resulting amino acid ex-

Scheme II



Scheme III



hibited properties identical with those previously reported.[10] Administration of the labeled homoleucine to *Cephalotaxus* was followed by workup after 7 days in the usual way to give radioactive **6** as its bis(*p*-bromophenacyl) ester. After purification by chromatography, and crystallization to constant activity, the incorporation figure shown in Table I (experiment 3) was obtained. The incorporation level is considerably higher than is usually observed in biosynthetic experiments with higher plants and it demonstrates that homoleucine is a highly efficient precursor of **6**. The specific incorporation of **11** into **6** was verified by reduction of the labeled bis(*p*-bromophenacyl) ester and cleavage of the labeled triol with periodate; the results of the degradation are shown in Table I (experiment 3).

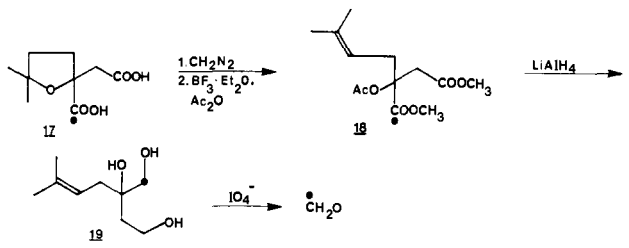
The foregoing precursor incorporation experiments provide convincing evidence that deoxyharringtonic acid is biosynthesized according to the pathway outlined in Scheme I. It appeared that the operation of this pathway could also account for the biosynthesis of the diacids attached to cephalotaxine in alkaloids **3**–**5**. Isoharringtonic acid (**12**) and harringtonic acid (**14**) could be derived from deoxyharringtonic acid by hydroxylation (Scheme II). Homoharringtonic acid could be formed by homologation of deoxyharringtonic acid in the manner of Scheme I, with subsequent hydroxylation. Evidence supporting the formation of isoharringtonic acid and harringtonic acid from deoxyharringtonic acid in vivo was obtained by means of precursor incorporation experiments.

DL-[9-^{14}C]Deoxyharringtonic acid was synthesized from 1-carbethoxy-5-methyl-2-hexanone[6] by treatment with potassium [^{14}C]cyanide followed by acid-catalyzed hydrolysis of the intermediate cyanohydrin. Radioinactive isoharringtonic acid dimethyl ester was prepared using a modification of the method of Weinreb.[11] Administration of DL-[9-^{14}C]deoxyharringtonic acid to *Cephalotaxus* plants for 4 days was followed by workup with the addition of unlabeled dimethyl isoharringtonate as carrier. The crude plant extract was saponified with hydrochloric acid. The mixture of free acids produced by the saponification was then esterified to give a mixture of methyl esters. Isoharringtonic acid dimethyl ester was isolated and purified by column chromatography, thin-layer chromatography, and recrystallization to constant radioactivity to give the incorporation figure listed in Table I (experiment 4). The labeled dimethyl isoharringtonate was degraded by cleavage with sodium periodate to give the unstable methyl ester of 2-oxo-5-methylhexanoic acid, which was immediately reduced to 5-methyl-1,2-hexanediol with lithium aluminum hydride. After purification by bulb-to-bulb distillation, the diol was cleaved with periodate to yield radioactive formaldehyde, which was trapped as its dimedone adduct.[12] The results of the degradation (Table I, experiment 4) demonstrate that isoharringtonic acid is derived in vivo from deoxyharringtonic acid.

A priori, the conversion of deoxyharringtonic acid (**6**) to isoharringtonic acid (**12**) could occur in one of two ways (Scheme II). The more direct route would proceed via hydroxylation of **6** at C-2. An alternative route could involve the conversion of **6** to the isomeric hydroxy acid **13**, followed by hydroxylation at C-3. The presence of **13** in *Cephalotaxus* plants seems likely, since this compound presumably lies on the pathway from **6** to homoharringtonic acid. Therefore, [9-^{14}C]-**13** was synthesized from isohexyl bromide[13] via formylation[14] of [1-^{14}C]ethyl isoheptanoate and evaluated as a precursor of isoharringtonic acid (**12**). The labeled hydroxy acid **13** was administered to *Cephalotaxus* plants, and dimethyl isoharringtonate was isolated in the usual manner after 4 days. The purified dimethyl ester was radioinactive (Table I, experiment 5). This result supports the derivation of isoharringtonic acid from **6** by hydroxylation at C-2 rather than via the more circuitous route involving hydroxy acid **13**. The *direct* formation of **12** from **6** is also more plausible on stereochemical grounds. (−)-Deoxyharringtonic acid has been assigned the 3*R* configuration,[15] while (+)-isoharringtonic acid has been allotted the 2*S*,3*R* configuration.[16] Consequently, diacid **12** can be derived in one step from **6** by C-2 hydroxylation. In contrast, the conversion of **13** to **12** would be less straightforward. On the basis of the analogy provided by the pathway from valine to leucine, diacid **13** probably possesses either the 2*S*,3*R* or the 2*R*,3*S* configuration. The formation of **12** from the 2*S*,3*R* isomer would require hydroxylation at C-3 with inversion of configuration; most biological hydroxylations proceed with retention of configuration.[17] The formation of **12** from the 2*R*,3*S* isomer of **13** would necessitate inversion of configuration at C-2 in addition to hydroxylation with retention of configuration at C-3.

Having established that deoxyharringtonic acid (**6**) serves as the precursor of isoharringtonic acid (**12**), we then investigated the possibility that **6** is also the precursor of harringtonic acid (**14**) (Scheme II). In order to carry out this phase of the work, radioinactive harringtonic acid was required for dilution purposes. The unlabeled acid was prepared in the form of the δ-lactone **16** by catalytic debenzylation of the known[18] hydroxy acid **15** (Scheme III). The acid stability of **16** was then tested by subjecting the compound to the acid saponification conditions used during the workup of the precursor incorporation experiments. Under these conditions, **16** was cleanly transformed into the ether diacid **17**. Compound **17** could also be obtained more directly by strong acid treatment of hydroxy acid **15**. Diacid **17** proved to be more readily crystallizable than the δ-lactone **16**, and its infrared spectrum showed carbonyl absorptions attributable to carboxylic acid functionality, but no absorptions attributable to an ester function. Treatment of **17** with diazomethane yielded a dimethyl ester whose NMR spectrum exhibited two methyl ester signals at δ 3.60 and 3.71. In contrast, treatment of **16** with diazomethane gave an ester lactone whose NMR spectrum exhibited only one methyl ester signal, at δ 3.62. DL-[9-^{14}C]Deoxyharringtonic acid was administered to *Cephalotaxus* plants for 4 days, and the workup was carried out after addition of radioinactive **16** and **17** as

Scheme IV

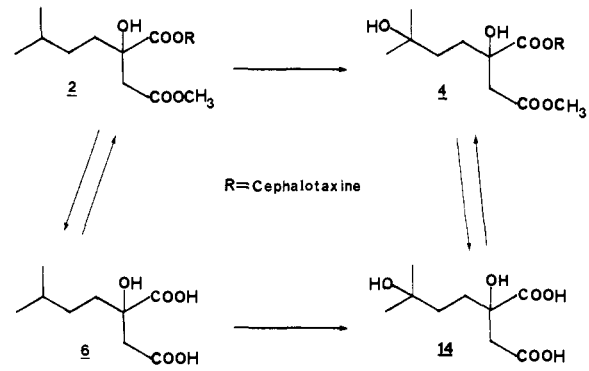


carriers. After acidic saponification, the ether diacid **17** was isolated by column chromatography and then recrystallized to constant radioactivity (Table I, experiment 6). A small amount of the radioactive diacid **17** was also derivatized with *p*-bromophenacyl bromide as a check of its radiochemical purity. The purified bis(*p*-bromophenacyl) ester had an identical specific radioactivity with that of the purified ether diacid **17**. Degradation of the labeled **17** was accomplished as shown in Scheme IV. Conversion of **17** to its dimethyl ester was followed by treatment with boron trifluoride etherate and acetic anhydride[19] to yield the unsaturated acetate **18** as the major product (52%). The NMR spectrum of **18** showed signals attributable to two vinyl methyl groups (δ 1.54, 1.67), an acetoxy group (δ 2.02), and one vinyl hydrogen (δ 4.88–5.13); an AB quartet due to the hydrogens at C-2 was also present (δ 2.75–3.30). Reduction of **18** with lithium aluminum hydride gave the triol **19**, which was cleaved with periodate to give C-9 as dimedoneformaldehyde. The results of the degradation clearly established that harringtonic acid is derived in vivo from deoxyharringtonic acid, probably by direct hydroxylation.

The biosynthesis of the acyl portions of deoxyharringtonine, isoharringtonine, and harringtonine having been clarified, we attempted to determine if deoxyharringtonic acid is hydroxylated at C-6 before or after it is linked to cephalotaxine. In order to accomplish this, deoxyharringtonine was synthesized bearing a tritium label in the cephalotaxine portion of the alkaloid and a carbon-14 label in the ester portion. (−)-[3′-^{3}H]Cephalotaxine was obtained by reduction of cephalotaxinone[20] with potassium [^{3}H]borohydride. The tritiated cephalotaxine was converted to a 2:3 mixture of [3′-^{3}H]-deoxyharringtonine and 3-*epi*-[3′-^{3}H]deoxyharringtonine using the synthesis of Mikolajczak et al.[21] Similarly, a mixture of [1-^{14}C]deoxyharringtonine and 3-*epi*-[1-^{14}C]deoxyharringtonine was obtained by using [1-^{14}C]methyl acetate in conjunction with the same deoxyharringtonine synthesis. The two samples of labeled deoxyharringtonine were mixed to give a tritium to carbon-14 ratio of 6.40. The doubly labeled precursor was purified by repeated preparative TLC without change in the tritium to carbon-14 ratio. The alkaloid was then administered to *Cephalotaxus* plants for 4 days, at the end of which time the plants were harvested and macerated in ethanol. A mixture of radioinactive deoxyharringtonine and 3-*epi*-deoxyharringtonine and a 1:1 mixture[18] of radioinactive harringtonine and 3-*epi*-harringtonine were added as carriers. The deoxyharringtonine mixture and the harringtonine mixture were each reisolated and purified by preparative TLC. The recovered deoxyharringtonine mixture exhibited the same tritium to carbon-14 ratio, within experimental error, as the original precursor (Table I, experiment 7). The recovered harringtonine mixture was found by mass spectral analysis to contain homoharringtonine in addition to 3-*epi*-harringtonine. This was confirmed by LC analysis (Altex Spherisorb ODS, water–methanol–diethylamine[18]). The radioactive mixture containing these three alkaloids was resolved into its components by LC and the purified harringtonine was found to exhibit a tritium to carbon-14 ratio of 15.3 (Table I, experiment 7).

The results of the incorporation experiment with deoxyharringtonine can be interpreted in two ways (Scheme V). One

Scheme V



interpretation is that deoxyharringtonine (**2**) is directly converted to harringtonine (**4**) and that the ^{3}H/^{14}C ratio of the harringtonine is being altered by its deacylation and reacylation during the course of the experiment. This interpretation is consistent with the fact that the ^{3}H/^{14}C ratio in the recovered deoxyharringtonine is unaltered. However, the reasons why reversible deacylation of harringtonine should lead to an *increase* in the ^{3}H/^{14}C ratio are not entirely clear. One possibility is that the radioactivity in the pool of labeled harringtonic acid (**14**) is lowered more rapidly by metabolism than the radioactivity in the pool of labeled cephalotaxine.

An alternative interpretation is that the labeled deoxyharringtonine is deacylated during the course of the experiment to give [^{14}C]deoxyharringtonic acid (**6**) and [^{3}H]cephalotaxine. The tritiated cephalotaxine could then combine with the free, and initially unlabeled, harringtonic acid present in the plant to yield harringtonine with an increased ^{3}H/^{14}C ratio. If this interpretation is correct, then one must account for the fact that the ^{3}H/^{14}C ratio in the recovered deoxyharringtonine is unaltered. Since it is highly probable that deoxyharringtonine is formed by acylation of cephalotaxine with deoxyharringtonic acid, the maintenance of the ^{3}H/^{14}C ratio in deoxyharringtonine that is being deacylated requires that the acylation reaction be inhibited. Inhibition of the acylation reaction could be the consequence of the significant increase in the pool size of deoxyharringtonine resulting from administration of the precursor.

Experimental Section

General. Melting points were determined on a Fisher-Johns apparatus and are uncorrected. Infrared spectra were obtained on a Perkin-Elmer 567 infrared spectrophotometer or on a Beckman 4230 IR spectrophotometer. Peak positions are given in cm^{-1}. The IR spectra of solid samples were measured as potassium bromide dispersions, and the spectra of liquids were determined in chloroform or carbon tetrachloride solutions. NMR spectra were measured on a Varian A-60, Perkin-Elmer R-32, Varian EM-390, or Brüker WH-90 NMR spectrometer. All NMR spectra were recorded in $CDCl_3$, unless otherwise noted, and chemical-shift values are given in parts per million downfield from Me_4Si as an internal standard. Mass spectra were run on an AEI MS-12, Finnigan 3300, or CEC 21-110B mass spectrometer. Elemental analyses were done by Galbraith Laboratory (Knoxville, Tenn.). Samples for liquid scintillation counting were weighed on either a Perkin-Elmer autobalance AD-2, a Cahn G-2 electrobalance, or a Mettler Micro Gram-Atic Balance, and radioactivity was measured using a Beckman LS100-C liquid scintillation counter. Tritium- or ^{14}C-labeled toluene (New England Nuclear Corp.) was used for internal standardization of the radioactive samples. Radioisotopes for syntheses and feeding experiments were purchased from New England Nuclear Corp., Amersham/Searle Corp., ICN, and Schwarz/Mann. A Varian 6000-1 radiochromatogram scanner was employed for measuring the radiochemical purity of radioactive samples. All feeding experiments were conducted within a Lab-Line Biotronette Mark III environmental chamber using Westinghouse Agrolites for illumination. Preparative thin-layer chromatography was accomplished using 750-μm layers of aluminum

oxide HF-254 (type E), aluminum oxide 60 PF-254 (type E), silica gel HF-254 (type 60 PF-254), or silica gel GF-254. Visualization was by short-wave ultraviolet light. Grace silica gel, Grade 923, and Woelm neutral aluminum oxide, activity III, were used for column chromatography. Analytical thin-layer chromatography was run on plastic sheets precoated with aluminum oxide F-254 neutral (type T), 200-μm thick, and on Polygram Sil G/UV_{254} (silica gel), 250 μm on plastic sheets. Visualization was usually by short-wave ultraviolet light, phosphomolybdic acid, or iodoplatinate. Dried *Cephalotaxus harringtonia* var. *fastigiata* was a generous gift of Dr. Robert Perdue of the United States Department of Agriculture and Dr. J. L. Hartwell of the National Cancer Institute. Cephalotaxine was isolated in the manner described by Paudler et al.[27] Samples of deoxyharringtonine and harringtonine were very kindly supplied by Drs. R. G. Powell and K. Mikolajczak.

Synthesis of 3-Carboxy-3-hydroxy-5-methylhexanoic Acid (8). 3-Carboxy-3-hydroxy-5-methylhexanoic acid was synthesized from 4-methyl-2-pentanone using the same method[6] employed in the synthesis of 3-carboxy-3-hydroxy-6-methylheptanoic acid from 5-methyl-2-hexanone. The overall yield of diacid **8**, mp 96–97 °C (CH_2Cl_2–Et_2O–hexane), was 20%: IR (KBr) 3410, 1760; NMR (60 MHz, CD_3CN) δ 0.8–1.2 (m, 7 H, $(CH_3)_2CH$), 1.60 (m, 2 H, C-4 CH_2), 2.4–3.1 (AB q (quartet), 2 H, J = 16 Hz, C-2 CH_2). Anal. ($C_8H_{14}O_5$) C, H.

Bis(*p*-phenylphenacyl)-3-carboxy-3-hydroxy-5-methylhexanoate. The bis(*p*-phenylphenacyl) ester of diacid **8** was prepared by a modification of the method of Durst.[7] The diacid **8** (190 mg, 1 mmol), *p*-phenylphenacyl bromide (810 mg, 3 mmol), anhydrous potassium carbonate (276 mg, 2 mmol), and dibenzo-18-crown-6 (55 mg, 0.15 mmol) were heated with stirring in 50 mL of dry acetonitrile at 60 °C for 8 h. The reaction mixture was allowed to cool, the acetonitrile removed in vacuo, and the residue dissolved in CH_2Cl_2. After removal of the insoluble matter by filtration, the CH_2Cl_2 soluble material was chromatographed on silica gel (5% Et_2O–CH_2Cl_2) to give 434 mg of crystalline derivative (75%), mp 126–127 °C: IR (KBr) 3520, 1750, 1720, 1700, 1600 cm^{-1}; NMR (60 MHz) δ 0.80–1.10 (m, 7 H, $(CH_3)_2CH$–), 1.80 (m, 2 H, C-4 CH_2), 2.75–3.45 (AB q, 2 H, J = 16 Hz, C-2 CH_2), 4.09 (s, 1 H, OH), 5.38 (s, 4 H, CH_2CO), 7.3–8.1 (m, 18 H, ArH). Anal. ($C_{36}H_{34}O_7$) C, H.

Reduction of Bis(*p*-phenylphenacyl)-3-carboxy-3-hydroxy-5-methylhexanoate. The bis(*p*-phenylphenacyl) ester (123 mg, 0.21 mmol) was dissolved in 10 mL by THF, and lithium aluminum hydride (80 mg, 2.11 mmol) was added with stirring. The mixture was stirred for 12 h at room temperature. Ether (15 mL) was then added, followed by a slight excess of 1 N H_2SO_4 (pH 4). The white solid that separated was removed by filtration and washed with ether. The combined ether phases were dried over magnesium sulfate and evaporated to give 76 mg of an oil. The crude mixture was applied to 2 freshly activated preparative thin-layer plates, and the plates were developed 4× with 1:1 benzene–ethyl acetate. The band corresponding to 3-hydroxy-3-hydroxymethyl-5-methyl-1-hexanol was removed and eluted with hot ethyl acetate containing 5% methanol. The recovered triol weighed 27 mg and was still slightly impure by analytical TLC. The triol was therefore applied to one preparative silica plate, and the plate was developed 3× using the same solvent system. Removal of the triol band from the plate and elution gave back ca. 21 mg of triol. This material was then distilled bulb-to-bulb in vacuo (bp 145 °C) to give 20 mg (63%) of pure 3-hydroxy-3-hydroxymethyl-5-methyl-1-hexanol: NMR (90 MHz) δ 1.05 (d, 6 H, J = 6 Hz), *gem*-dimethyl 1.40 (d, 2 H, J = 4 Hz, C-4 CH_2), 1.75 (t, 2 H, J = 6 Hz, C-2 CH_2), 3.46 (s, 3 H, OH), 3.6–3.95 (m, 4 H, C-1, C-8, CH_2). Anal. ($C_8H_{18}O_3$) C, H.

Synthesis of 2-Hydroxy-3-carboxy-5-methylhexanoic Acid (9). Ethyl isohexanoate was converted to the α-formyl derivative by treatment with ethyl formate in the presence of sodium ethoxide.[22] The α-formyl ester obtained was converted to the cyanohydrin, and the cyanohydrin was hydrolyzed utilizing the procedure of Calvo et al.[5d] The crude diacid, a mixture of diastereomers, was recrystallized from ether–methylene chloride–hexane to give white crystals, mp 115–120 °C, in ca. 5% overall yield: IR (KBr) 3500–2900, 1710, 1460, 1380; NMR (90 MHz, CD_3CN) δ 1.90 (6 H, d, J = 5 Hz, *gem*-dimethyl), 1.15–1.90 (m, 3 H, C-4 CH_2 and C-5 H), 1.86 (m, 1 H, C-3 H), 3.92 (br s, 1 H, OH), 4.22 (d, 0.75 H, J = 4 Hz, C-2 H of one diastereomer), 4.40 (d, 0.25 H, J = 4.5 Hz, C-2 H of other diastereomer). Anal. ($C_8H_{14}O_5$) C, H.

Bis(*p*-bromophenacyl)-3-carboxy-3-hydroxy-6-methylheptanoate. The bis(*p*-bromophenacyl) ester of diacid **6** was prepared analogously to the preparation of the bis(*p*-phenylphenacyl) ester of diacid **8**. The yield was 85% of crystalline derivative, mp 103–104 °C: IR (KBr) 2490, 1750, 1720, 1700, 1580 cm^{-1}; NMR (60 MHz) δ 0.95 (d, 6 H, J = 5 Hz, *gem*-dimethyl), 1.10–2.05 (m, 5 H, *i*-Pr methine, C-4, C-5 CH_2), 3.03 (AB q, 2 H, J = 17 Hz, C-2 CH_2), 4.00 (s, 1 H, OH), 5.30 (s, 2 H, CH_2CO), 5.36 (s, 2 H, CH_2CO), 7.5–7.9 (m, 8 H, Ar-H). Anal. ($C_{25}H_{26}BrO_7$) C, H.

Reduction of Bis(*p*-bromophenacyl)-3-carboxy-3-hydroxy-6-methylheptanoate. The bis(*p*-bromophenacyl) ester (200 mg, 0.33 mmol) was dissolved in 20 mL of dry THF, and lithium aluminum hydride (110 mg, 2.9 mmol) was added with stirring. After 17 h, ether and 1 N H_2SO_4 were added cautiously to the stirred mixture to pH 4, and the acidified mixture was then extracted repeatedly with ether. The ether was dried over magnesium sulfate and evaporated in vacuo to give an oil weighing 176 mg. This material was chromatographed on two preparative silica plates using ethyl acetate. The plates were developed 2× and the band corresponding to 3-hydroxy-3-hydroxymethyl-6-methyl-1-heptanol was removed and eluted 5× with hot ethyl acetate containing a trace of methanol. In this way, 75 mg of triol was recovered. Since the triol showed traces of UV-absorbing material by TLC, it was rechromatographed on one preparative silica plate, and the plate was again developed 2×. As a result, 53 mg of triol was obtained. Bulb-to-bulb distillation of the triol gave 51 mg (86%) of pure triol: NMR (90 MHz) δ 0.90 (6 H, d, J = 6 Hz, *gem*-dimethyl), 1.40–1.65 (m, 4 H, C-4, C-5 CH_2), 1.76 (t, 2 H, J = 6 Hz, C-2 CH_2), 3.47 (s, 3 H, OH), 3.70–4.1 (m, 4 H, C-1, C-9 CH_2). Anal. ($C_9H_{20}O_3$) C, H.

Cleavage of 3-Hydroxy-3-hydroxymethyl-5-methyl-1-hexanol with Periodate. The procedure was that of Battersby.[12] Distilled triol (10.4 mg, 0.06 mmol) was dissolved in 1 mL of dry *t*-BuOH (distilled from Na), and 1 mL of water was added. Finely ground sodium periodate (33 mg, 0.15 mmol) was added with stirring over a 1-h period. The mixture was then stirred for ca. 18 h. Additional sodium periodate (4 mg) was then added and stirring continued for another 4 h. The mixture was then poured into 12 mL of saturated aqueous arsenic trioxide solution. The resulting solution was extracted 2× with ether, and the pH of the aqueous phase (pH 3) was adjusted to 8 with solid potassium carbonate. Dimedone (75 mg, 0.54 mmol) was added and the mixture stirred for 15 min. The pH was then readjusted to 6 with 20% aqueous HCl. After we stirred the mixture overnight, it (pH 7) was treated with 20% HCl to lower the pH to 6. The precipitate was filtered off, dried in vacuo, and dissolved in chloroform. The chloroform solution was passed through a 2-in. plug of neutral Woelm aluminum, activity III. Evaporation of the eluent gave 6 mg of crystalline dimedoneformaldehyde (32%), mp 194.5–195 °C (lit.[12] 191.5–192 °C).

Cleavage of 3-Hydroxy-3-hydroxymethyl-6-methyl-1-heptanol with Periodate. The triol was cleaved with periodate using a procedure that was strictly analogous to that employed in the cleavage of the triol derived from **8**. From 51 mg (0.29 mmol) of triol, 59 mg (69%) of dimedoneformaldehyde was obtained.

Synthesis of DL-Homoleucine. Isohexanal (90 mg, 0.90 mmol) was dissolved in 0.60 mL of 50% aqueous ethanol, and 0.04 mL of triethylamine was added. Potassium cyanide (19 mg, 0.29 mmol) and finely ground ammonium carbonate (57 mg) were then added, and the mixture was stirred for 18 h at room temperature. An equal volume of absolute ethanol was then added and the precipitated potassium carbonate removed by filtration. The filtrate was taken to dryness in vacuo and the residue dissolved in 1–2 mL of water. Barium hydroxide (350 mg) was added and the mixture refluxed for 48 h. At the end of this time, the mixture was filtered and the solid washed 2× with hot water. Ammonium carbonate (60 mg) was added to the filtrate, which was then heated to boiling and filtered to remove precipitated barium carbonate. The pH of the filtrate was adjusted to 5 with acetic acid, and the filtrate was then taken to dryness in vacuo. The residue was crystallized from water–ethanol to yield 33 mg (79% based on KCN) of DL-homoleucine. The physical properties of the homoleucine prepared in this fashion were identical with those previously reported.[10] When 1 mCi of [^{14}C]KCN was utilized in this sequence, DL-homoleucine with a specific activity of 0.017 mCi/mg was obtained (radiochemical yield ca. 50%).

Synthesis of [9-^{14}C]Deoxyharringtonic Acid. Ethyl 3-oxo-6-methylheptanoate (63 mg, 0.34 mmol)[6] and [^{14}C]KCN (34 mg, 0.52 mmol, 1 mCi) were stirred together at 0–5 °C, and 45 μL of concentrated HCl was added. The mixture was stirred for 2 h in an ice bath and then

extracted 2× with 1 mL of ether. The ether was removed from the extract in vacuo, and the residue was dissolved in 0.30 mL of concentrated HCl. The acidic mixture was allowed to stand for ca. 12 h and then heated at 100 °C for 2 h. Water (0.6 mL) was added and heating at 110 °C continued for 4 h longer. The solution was cooled and extracted with 2 × 5 mL of ether. The ether extract was dried over magnesium sulfate and then removed. The residue was crystallized 4× from methylene chloride–hexane to yield 15 mg (25%) of pure [9-^{14}C]deoxyharringtonic acid with a specific activity of 0.008 mCi/mg (radiochemical yield = 12%). The labeled compound was at least 99% radiochemically pure based upon radioscanning of its thin-layer chromatogram (silica gel, hexane–ether–acetic acid, 55:45:2).

Dimethyl Isoharringtonate. Isopentylmaleic Anhydride. Deoxyharringtonic acid (**6**)[6] (4 g, 19.6 mmol) was dissolved in acetic anhydride (80 mL, distilled), and the solution refluxed for 3 h with stirring. The excess acetic anhydride was removed in vacuo and the residual oil dissolved in ethyl acetate. The red solution was treated repeatedly with Norit until the solution was light yellow. Removal of the ethyl acetate was followed by vacuum distillation using a Kugelrohr apparatus to give a colorless oil (bp 60–80 °C, ca. 0.5 mm) weighing 2.6 g (79%). The spectral properties of the product were the same as those reported by Weinreb.[11] The isopentylmaleic anhydride was converted to dimethyl isoharringtonate using Weinreb's procedure.[11]

Degradation of Dimethyl Isoharringtonate. 5-Methyl-1,2-hexanediol. Dimethyl isoharringtonate (56 mg, 0.22 mmol) was dissolved in 1.8 mL of distilled water. Finely ground sodium periodate (90 mg, 0.42 mmol) was added over a 45-min period with stirring. After an additional reaction period of 0.5 h, the aqueous solution was extracted 3× with chloroform. The combined chloroform extracts were filtered and the solvent removed in vacuo at room temperature to yield methyl 2-oxo-5-methylhexanoate (25 mg) that showed only a single peak on gas chromatography (6 ft, 10% ucw-98). The α-keto ester was immediately dissolved in anhydrous ether, and lithium aluminum hydride (50 mg, 1.32 mmol) was added. The reaction mixture was then stirred at room temperature overnight. The excess lithium aluminum hydride was destroyed with 40% aqueous potassium hydroxide solution. The mixture was filtered through Celite and the aqueous layer extracted 3× with ether. The combined ether extracts were backwashed with water, dried over magnesium sulfate, and evaporated to give an oil which was vacuum distilled (50–60 °C/ca. 0.5 mm) to yield 14 mg (48%) of 5-methyl-1,2-hexanediol.[26] The compound showed no contaminants by gas chromatography (6-ft ucw-98, 100–200 °C): NMR (90 MHz) δ 0.89 (6 H, d, *gem*-dimethyl), 1.03–1.69 (5 H, m), 2.07 (2 H, br s, OH), 3.25–3.77 (3 H, m, C-1, C-2 Hs).

Cleavage of 5-Methyl-1,2-hexanediol with Periodate. The procedure used was identical with that employed in the cleavage of 3-hydroxy-3-hydroxymethyl-5-methyl-1-hexanol. From 14 mg of diol, 10 mg (31%) of dimedoneformaldehyde was obtained.

Synthesis of [9-^{14}C]-2-Hydroxy-3-carboxy-6-methylheptanoic Acid (13). [1-^{14}C]Isoheptanoic Acid. Isohexyl bromide[13] (1.0 g, 6 mmol) was dissolved in anhydrous ether (10 mL) and converted to isohexylmagnesium bromide in standard fashion. Titration[23] of the Grignard so prepared indicated a concentration of 0.55 mmol/mL. Two milliliters (1.2 mmol) of the Grignard reagent and 8 mL of ether were added to a dry 25-mL round-bottomed flask equipped with a magnetic stirbar and a carbon dioxide generating apparatus. The carbon dioxide apparatus contained, in three separate tubes, 1 mL of concentrated sulfuric acid, 100 mg (0.50 mmol) of radioinactive barium carbonate, and 50 mg (0.25 mm, 12 mCi) of barium [^{14}C]carbonate. The Grignard solution was cooled in liquid nitrogen, and the entire apparatus was evacuated to 0.05 mm and the sulfuric acid was mixed with the radioactive barium carbonate. When the vigorous bubbling had ceased, the reaction flask was warmed to −20 °C and stirred for 30 min. The flask was then cooled in liquid nitrogen for a second time and evacuated, and the sulfuric acid was mixed with the radioinactive barium carbonate. The reaction flask was then allowed to warm to room temperature with stirring, after which dilute aqueous sodium hydroxide solution was added. The ether phase was removed, and the aqueous phase was extracted with ether. The aqueous phase was then acidified and extracted 3× with ether to yield a colorless oil (75 mg, 75%; 4.0 mCi) that was pure by gas chromatography (6 ft, 10% ucw-98). The properties of the acid were the same as those previously reported:[25] NMR (90 MHz) δ 0.78–0.85 (6 H, d, *gem*-dimethyl), 1.10–1.66 (5 H, m), 2.19–2.35 (2 H, t, J = 7 Hz, C-2 CH_2).

Ethyl 2-[1-^{14}C]Formylisoheptanoate. [1-^{14}C]Isoheptanoic acid (74 mg, 0.66 mmol) was converted to its ethyl ester with diazoethane. The labeled ester was added at −78 °C with stirring to a solution of lithium diisopropylamide (1 mmol) in 1 mL of THF. An additional 75 mg (0.51 mmol) of radioinactive ester was then added. The mixture was stirred under N_2 at −78 °C for 30 min. Distilled ethyl formate (0.17 mL, 2 mmol) in dry HMPA (0.06 mL) was added at the end of this time period. The reaction was allowed to warm to room temperature with stirring, and the solvent was removed in vacuo. Water was added to the residue, and the solution was extracted with ether. The aqueous phase was made acidic and it was then extracted several times with ether. The combined extracts of the acidified aqueous phase were backwashed repeatedly with water to remove HMPA. The ether solution was then dried over $MgSO_4$ and evaporated to give a pale yellow oil that was pure by gas chromatography (6 ft, 10% ucw-98). The yield of α-formyl ester was 77 mg (41%): IR ($CHCl_3$) 2820, 1755, 1650 cm^{-1}; NMR (90 MHz) δ 0.79–0.86 (6 H, d, J = 7 Hz, *gem*-dimethyl), 1.10–1.55 (3 H, two t, J = 6 Hz, OCH_2CH_3), 1.89–2.08 (5 H, m), 3.22 (0.5 H, sextet, C-2 HO), 4.00–4.30 (2 H, q, J = 6 Hz, OCH_2CH_3), 6.80–7.00 (0.5 H, d, J = 12 Hz, =C*H*OH), 9.60 (0.5 H, d, J = 3 Hz, C*H*O). Exact mass calcd for $C_{10}H_{17}O_3$ ($M^+ - 1$): 185.1178. Found: 185.1188.

[9-^{14}C]-2-Hydroxy-3-carboxy-6-methylheptanoic Acid (13). The procedure used was that of Calvo et al.[5d] From 77 mg of α-formyl ester, 36 mg (43%) of [9-^{14}C]-2-hydroxy-3-carboxy-6-methylheptanoic acid (**13**), mp 100–101 °C, was obtained (specific activity, 1.25 × 10^{-2} mCi/mg). The compound, a mixture of diastereomers, was at least 99% pure by radioscanning (silica gel, hexane–ether–acetic acid, 55:45:2): IR (KBr) 1710 cm^{-1}; NMR (90 MHz, CD_3COCD_3) δ 0.87–1.03 (6 H, two d, J = 6.7 Hz, *gem*-dimethyl), 1.18–2.00 (5 H, m), 2.66–3.03 (1 H, M, C-3 H), 4.36–4.66 (1 H, two d, J = 4.7 Hz, C-2 H, ratio of doublets is 4:3). Anal. ($C_9H_{16}O_5$) C, H.

Administration of L-[1-^{14}C]Leucine to *Cephalotaxus harringtonia*. L-[1-^{14}C]Leucine (0.10 mCi, NEN) was administered to two *Cephalotaxus harringtonia* plants by the cottonwick method. After 2 weeks the plants were harvested and ground up in ethanol, and 102 mg of radioinactive 3-carboxy-3-hydroxy-5-methylhexanoic acid was added as carrier. The ethanol was removed in vacuo. Methanol (15 mL) and 1 N sodium hydroxide solution (25 mL) were added to the residue, and the mixture was stirred for 18 h at room temperature. The mixture was then extracted repeatedly with ether, and the aqueous phase was acidified to pH 1 with concentrated HCl. The acidified aqueous phase was extracted repeatedly with ether, the combined ethereal extracts were dried over magnesium sulfate, and the solvent was removed to give 165 mg of a light green oil. This oil was derivatized with *p*-phenylphenacyl bromide using the crown ether method.[7] After repeated TLC of the crude derivatization product (silica gel, 5% EtOAc–benzene), 122 mg of crystalline bis(*p*-phenylphenacyl) derivative was obtained. This derivative was recrystallized repeatedly from methylene chloride–hexane until a constant specific activity was reached (204 dpm/mg, corresponding to 0.03% incorporation).

Administration of [1-^{14}C]-2-Hydroxy-3-carboxy-5-methylhexanoic Acid to *C. harringtonia*. [1-^{14}C]-2-Hydroxy-3-carboxy-5-methylhexanoic acid (2.6 mg, 0.025 mCi) was dissolved in water, and the pH was adjusted to 6 with 1 equiv of sodium bicarbonate. The resulting solution was administered to two *C. harringtonia* plants. After 7 days, the plants were harvested and macerated in ethanol, and deoxyharringtonic acid (150 mg) was added as carrier. The ethanolic extract was taken to dryness in vacuo and the residue suspended in 8 mL of concentrated HCl. The acidic mixture was heated at 100 °C for 3 h, 16 mL of water was added, and heating at 115 °C was continued for 4 h. The black mixture was allowed to cool, and it was then extracted repeatedly with ether. The combined ether extracts were dried with magnesium sulfate and evaporated to give a residue weighing 300 mg. The examination of this residue (silica, hexane–ether–acetic acid, 55:45:2) showed the presence of deoxyharringtonic acid. The crude product was dissolved in 25 mL of methanol, 0.7 mL of sulfuric acid was added, and the mixture was refluxed for 7 h. The decanted supernatant was filtered, the methanol was removed in vacuo, and aqueous bicarbonate was added to the residue; extraction with ether recovered 284 mg of a colored oil. This material was chromatographed on three preparative silica plates (5% EtOAc–benzene), and the dimethyl deoxyharringtonate was removed. Since the dimethyl ester was still impure by TLC, it was distilled bulb-to-bulb (bp 120 °C, 0.1 mm) to give a colorless oil (40 mg). The distilled dimethyl ester (40 mg) was diluted with 59 mg of radioinactive dimethyl deoxyhar-

ringtonate, and the diluted ester was rechromatographed on 2 silica preparative plates that were developed 3× (5% EtOAc–benzene). The recovered dimethyl deoxyharringtonate was chromatographed for a second time on 2 silica plates, and the plates were developed 3×. Each side of these preparative plates carried a small quantity of dimethyl 2-hydroxy-3-carboxy-5-methylhexanoate as a marker. The zone corresponding to the radioactive dimethyl deoxyharringtonate ran well ahead of the marker spots. Recovery of the dimethyl deoxyharringtonate gave 81 mg. This was diluted with 80 mg of radioinactive dimethyl deoxyharringtonate, and the diluted ester was dissolved in 1.7 mL of concentrated HCl. The solution was heated at 100 °C for 2 h, after which time 3.4 mL of water was added. The mixture was then heated at 110 °C for 4 h. The aqueous solution was allowed to cool, and it was then extracted repeatedly with ether. The combined ether extracts were dried over magnesium sulfate and evaporated to yield 152 mg of deoxyharringtonic acid. The free diacid was derivatized with *p*-bromophenacyl bromide using the crown ether method to yield 148 mg of crystalline bis(*p*-bromophenacyl) ester. This was recrystallized until a constant specific radioactivity was reached (final value was 156 dpm/mg).

Administration of DL-[1-^{14}C]Homoleucine to *C. harringtonia*. DL-[1-^{14}C]Homoleucine (2.6 mg, 0.04 mCi) was dissolved in 0.5 mL of distilled water and administered to two *C. harringtonia* plants by the cottonwick method. After 7 days the plants were harvested and macerated in ethanol, and 104 mg of deoxyharringtonic acid was added as carrier. Alkaline saponification of the crude plant extract was followed by ether extraction, acidification, and additional ether extraction. The ethereal extract of the acidified aqueous phase yielded 233 mg of a dark green oil which was derivatized with *p*-bromophenacyl bromide using the crown ether method. After extensive preparative TLC (silica, 5% EtOAc–benzene), 61 mg of the bis(*p*-bromophenacyl) ester was recovered. After two recrystallizations from methylene chloride–hexane, 26 mg of crystalline bis(*p*-bromophenacyl) ester was obtained. This was diluted with 105 mg of radioinactive ester and the total recrystallized to constant specific radioactivity (final specific activity was 5905 dpm/mg).

Harringtonic Acid δ-Lactone (16). 3-Benzyloxy-3-benzyloxycarbonyl-6-hydroxy-6-methylheptanoic acid (**15**)[18] (1.11 g, 2.8 mmol) was dissolved in 15 mL of 2-propanol containing 60 mg of 10% palladium on carbon catalyst and 3 drops of concentrated HCl. The mixture was hydrogenated at atmospheric pressure for 24 h. After removal of the catalyst by filtration, the filtrate was taken to dryness, and the residue was recrystallized from acetone–benzene to give 298 mg of crystalline lactone (53%), mp 132–135.5 °C: IR (KBr) 3300–2500, 1770, 1735, 1720 cm^{-1}; NMR (60 MHz) δ 1.47 (6 H, s, *gem*-dimethyl), 1.70–2.55 (4 H, m, C-4, C-5 CH_2), 2.90 (2 H, AB q, J = 17 Hz, C-2 CH_2).

Preparation of 2-Carboxy-2-carboxymethyl-5,5-dimethyltetrahydrofuran (17). Hydroxy acid **15** (270 mg, 0.67 mmol) was dissolved in concentrated HCl (13 mL) and heated at 85 °C with stirring for ca. 12 h. The acid was removed in vacuo, and the residual brown oil was purified by column chromatography (Silicar CC-4, $CHCl_3$) to yield 103 mg of light brown crystals. Recrystallization of this material from $CHCl_3$–hexane yielded 82 mg (61%) of colorless crystals, mp 134 °C: IR ($CHCl_3$) 3680, 3300–2500, 1770, 1720, 1600, 1385 cm^{-1}; NMR (90 MHz) δ 1.25 (3 H, s, CH_3), 1.40 (3 H, s, CH_3), 1.70–2.55 (4 H, m), 2.55–3.25 (2 H, AB q, J = 14 Hz, CH_2COOH). Anal. ($C_9H_{14}O_5$) C, H.

Treatment of diacid **17** with excess diazomethane gave the corresponding dimethyl ester (bp 95–105 °C, ca. 0.1 mm): NMR (90 MHz) δ 1.18 (3 H, s, CH_3), 1.27 (3 H, s, CH_3), 1.49–2.60 (4 H, m), 2.60–3.01 (2 H, AB q, J = 15 Hz, $CH_2CO_2CH_3$), 3.60 (3 H, s, CO_2CH_3), 3.71 (3 H, s, CO_2CH_3).

Bis(*p*-bromophenacyl) Ester of Diacid 17. Using the method of Durst,[7] diacid **17** (12 mg) was converted into 22 mg (62%) of the crystalline bis(*p*-bromophenacyl) ester, mp 147.5–148 °C, after recrystallization from $CHCl_3$–hexane: IR ($CHCl_3$) 1745, 1700 cm^{-1}; NMR (90 MHz) δ 1.30 (3 H, s, CH_3), 1.40 (3 H, s, CH_3), 1.7–2.7 (4 H, m), 2.8–3.3 (2 H, AB q, J = 9 Hz, CCH_2), 5.2 (2 H, s, CH_2CO), 5.3 (2 H, s, CH_2CO), 7.5–7.8 (8 H, Ar-H).

Methyl 3-Acetoxy-3-carbomethoxy-6-methyl-5-heptenoate (18). The procedure used was due to Kitahara et al.[19]

The dimethyl ester of diacid **17** (105 mg, 0.46 mmol) was dissolved in dry benzene (2.1 mL) and freshly distilled acetic anhydride (0.10 mL). Boron–trifluoride etherate (distilled, 4 drops) was added slowly with stirring. The reaction mixture was stirred at room temperature for 2.75 h. The reaction was then quenched with aqueous sodium bicarbonate, ether was added, and the two phases were separated. The aqueous phase was extracted 3× with ether. The combined ether extracts were dried with anhydrous magnesium sulfate and evaporated to give a yellowish oil that was purified by preparative TLC (silica, 9:1 $CHCl_3$–EtOAc) to yield 50 mg of the unsaturated acetate **18**, 9.4 mg of saturated diacetate, and 38 mg of starting material. Treatment of the recovered starting material with acetic anhydride and BF_3 etherate gave an additional 16 mg of unsaturated acetate **18**. The two lots of **18** were combined and distilled bulb-to-bulb (bp 115 °C, ca. 0.1 mm) to yield 65 mg (52%) of pure unsaturated acetate: IR ($CHCl_3$) 1742, 1605, 1370 cm^{-1}; NMR (90 MHz) δ 1.54 (3 H, s, CH_3), 1.67 (3 H, s, CH_3), 2.02 (3 H, s, CH_3CO), 2.59–2.75 (2 H, m), 2.75–3.30 (2 H, AB q, J = 14.6 Hz, C-2 H), 3.62 (3 H, s, CO_2CH_3), 3.65 (3 H, s, CO_2CH_3), 4.88–5.13 (1 H, br t, J = 7.3 Hz, $=CHCH_2$). Exact mass calcd for $C_{11}H_{16}O_4$ (M^+ − HOAc): 212.1048. Found: 212.1060.

3-Hydroxy-3-hydroxymethyl-6-methyl-5-hepten-1-ol (19). The unsaturated acetate **18** (65 mg, 0.24 mmol) was dissolved in dry THF (7 mL), the mixture was stirred, and lithium aluminum hydride (70 mg, 1.8 mmol) added. After 0.5 h, the reaction was quenched by adding ether and 1 N H_2SO_4 to pH 4. The ether layer was removed and the aqueous phase extracted 3× with ether. The combined ether extracts were dried over $MgSO_4$ and evaporated to yield a colorless oil (48 mg). Preparative TLC of the oil (silica, EtOAc, developed 2×) gave 72 mg (77%) of triol **19**: IR ($CHCl_3$) 3640, 3500, 1085, 1010 cm^{-1}; NMR (90 MHz) δ 1.56 (3 H, s, CH_3), 1.68 (3 H, s, CH_3), 2.07–2.31 (2 H, br d, $J \simeq$ 6.7 Hz, CH_2CH_2OH), 3.44 (2 H, s, CH_2OH), 3.75 (2 H, t, J = 5.3 Hz, CH_2CH_2OH), 5.02–5.24 (1 H, br t, $J \simeq$ 7.3 Hz, vinyl H). Exact mass calcd for $C_8H_{15}O_3$ (M^+ − CH_3): 159.1021. Found: 159.1032.

Periodate Cleavage of 3-Hydroxy-3-hydroxymethyl-6-methyl-5-hepten-1-ol (19). The procedure used was the same as that employed in cleavage of 3-hydroxymethyl-5-methyl-1-hexanol. From 32 mg of 3-hydroxy-3-hydroxymethyl-6-methyl-5-hepten-1-ol, 25 mg (48%) of dimedoneformaldehyde was obtained.

Synthesis of [1-^{14}C]Deoxyharringtonine. Synthesis of [1-^{14}C]Methyl Acetate. The procedure used was adapted from Ropp.[24] Sodium [1-^{14}C]acetate (5 mg, 0.06 mmol, 4 mCi) and radioinactive sodium acetate (95 mg, 1.12 mmol) were washed into a 5-mL pear-shaped flask with distilled water, and the water was removed in vacuo. The diluted sodium acetate was dried overnight at 110 °C (0.1 mm). The reaction flask was equipped with a small plug of dry glass wool and with a reflux condenser that was connected at the top with Tygon tubing to two traps in sequence. Trimethyl phosphate (0.25 mL, 2.15 mmol) was introduced into the reaction flask, and the reaction mixture was heated at 165–180 °C for 1 h. The reaction was allowed to cool, and the first trap was then cooled to −18 °C in a dry ice–acetone bath while the second trap was cooled in liquid nitrogen. The system was evacuated (0.1 mm), and the labeled methyl acetate distilled from the reaction flask at 60 °C. [1-^{14}C]Methyl acetate collected in the second trap, while unreacted trimethyl phosphate remained in the first trap. The yield of labeled methyl acetate was 60 mg (69%); gas chromatographic analysis showed that the purity of the methyl acetate was at least 99%.

[1-^{14}C]Deoxyharringtonine. [1-^{14}C]Methyl acetate (60 mg, 0.81 mmol) was washed from the collection trap with dry THF (1.8 mL) into a round-bottomed flask containing the ester[21] of 2-oxo-5-methylhexanoic acid and cephalotaxine (185 mg, 0.42 mmol). The resulting solution was dried for 12 h over 4A molecular sieves.

Lithium isopropylcyclohexylamide was generated under N_2 in a 25-mL 3-necked round-bottomed flask using isopropylcyclohexylamine (0.15 mL, 0.81 mmol), THF (1 mL), and *n*-butyllithium (0.34 mL, 2.2 M, 0.75 mmol). The solution containing the labeled methyl acetate and the cephalotaxine ester was then syringed into the lithium isopropylcyclohexylamide solution with stirring at −78 °C over a 20-min period. The reaction mixture was stirred for 1.5 h at −78 °C and for 0.5 h at 0 °C. It was quenched with pH 7 buffer (20 mL, MCB) and extracted 3× with methylene chloride. The combined organic layers were backwashed with brine and dried over Na_2SO_4, and the solvent was removed in vacuo to yield 176 mg of a dark yellowish oil. The oil was purified by preparative TLC (silica, 8:2:1 benzene–methanol–NH_4OH) to yield 59 mg (27%) of a diastereomeric mixture of [1-^{14}C]deoxyharringtonic and 3-*epi*-[1-^{14}C]deoxyharringtonine (0.11 mCi): MS *m/e* 515 (M^+).

Synthesis of [3′-^{3}H]Deoxyharringtonine. [3′-^{3}H]Cephalotaxine.

Cephalotaxinone[20] (85 mg, 0.26 mmol) was dissolved in methanol (6 mL), and ca. 5 mg of KB^3H_4 (0.09 mmol, ca. 25 mCi) was added with stirring. The solution was stirred overnight, and sodium borohydride (250 mg, 6.6 mmol) was then added. The resulting solution was stirred an additional 0.5 h, and the solvent was then removed in vacuo. Aqueous NaOH (15%) and $CHCl_3$ were added to the residue, and the aqueous solution was extracted 5× with $CHCl_3$. The combined organic layers were washed with brine, dried with Na_2SO_4, and evaporated to give 60 mg (71%) of crystalline cephalotaxine. This was diluted with radioinactive cephalotaxine (35 mg), and the lot was recrystallized from ether to yield 80 mg of pure cephalotaxine (3.9 mCi).

[3′-^{3}H]Deoxyharringtonine. [3′-^{3}H]Cephalotaxine (80 mg, 0.24 mmol, 3.9 mCi) was converted into a mixture of [3′-^{3}H]deoxyharringtonine and 3-*epi*-[3′-^{3}H]deoxyharringtonine using the procedure of Mikolajczak et al.[21] The yield was 25 mg (26%) of product with a total radioactivity of 0.052 mCi: MS *m/e* 515 (M^+).

Administration of [1-^{14}C,3′-^{3}H]Deoxyharringtonine to *Cephalotaxus*. A mixture of [1-^{14}C,3′-^{3}H]deoxyharringtonine and its C-3 epimer (16 mg, $^3H/^{14}C$ = 6.40, 0.002 mCi of ^{14}C) was dissolved in distilled water containing an equivalent of acetic acid. The water was removed in vacuo, and the residue redissolved in a small volume (ca. 1 mL) of distilled water. The resulting solution was administered to four *Cephalotaxus* plants via the cottonwick method. After 4 days the plants were harvested and macerated with 95% ethanol, and 50 mg each of radioinactive mixtures of harringtonine and 3-*epi*-harringtonine and of deoxyharringtonine and 3-*epi*-deoxyharringtonine were added. The alkaloids were isolated using the procedure of Powell et al.[3] to yield 340 mg of crude bases. A mixture of deoxyharringtonine and 3-*epi*-deoxyharringtonine was isolated from the crude alkaloid fraction by preparative TLC (silica, 8:2:0.1, benzene–MeOH–NH_4OH). The isolated deoxyharringtonine–epideoxyharringtonine mixture was subjected to a second preparative TLC purification to yield a total of 25 mg of the pure alkaloid ($^3H/^{14}C$ = 6.50): MS *m/e* 515 (M^+). A mixture of harringtonine and 3-*epi*-harringtonine weighing 182 mg was also isolated by preparative TLC of the crude alkaloid mixture on silica gel. Analytical TLC of this mixture on alumina (4:1 EtOAc–benzene) showed the presence of cephalotaxine. The cephalotaxine was removed by preparative TLC on alumina (plates developed 2×) to give 48 mg of a mixture of harringtonine and 3-*epi*-harringtonine that was homogeneous by analytical TLC. A mass spectrum of this mixture indicated the presence of homoharringtonine. This was confirmed by LC analysis (Altex 4.6 mm × 250 mm Spherisorb 005 10-μm column, 55:45:0.05, H_2O–CH_3OH–Et_2NH).[18] Pure harringtonine was isolated from this mixture by repeated injections and collection of the eluant.

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References and Notes

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