Biosynthesis of the *Cephalotaxus* Alkaloids. Investigations of the Early and Late Stages of Cephalotaxine Biosynthesis[1a]

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Abstract: The biosynthesis of the alkaloid cephalotaxine (**1**) has been investigated by means of precursor incorporation experiments with *Cephalotaxus harringtonia.* It has been established that cephalotaxine is biosynthesized from one molecule each of tyrosine and phenylalanine in a manner consistent with the hypothesis that **1** is a modified 1-phenethyltetrahydroisoquinoline alkaloid. Incorporation experiments with [*p*-^{14}C]- and [*m*-^{14}C]phenylalanine have shown that one of the meta carbon atoms of phenylalanine is lost during the conversion of this amino acid into cephalotaxine. The remaining meta carbon atom is located at C-2 of the alkaloid. Cephalotaxine (**1**), cephalotaxinone (**2**), demethylcephalotaxinone (**3**), and demethylcephalotaxine (**4**) labeled with carbon-14 at C-3 have been synthesized. Incorporation experiments with these labeled alkaloids have established that cephalotaxine and cephalotaxinone are irreversibly demethylated in vivo.

Introduction

Conifers of the genus *Cephalotaxus* (Cephalotaxaceae) contain a group of alkaloids of unique structure. The most abundant member of this group is cephalotaxine (**1**), whose structure and absolute stereochemistry are shown in Figure 1.[2-5] Cephalotaxine is accompanied in nature by small quantitites of related alkaloids[6-9] such as cephalotaxinone (**2**), demethylcephalotaxinone (**3**), and demethylcephalotaxine (**4**) (Figure 1) as well as by several cephalotaxine esters that possess significant antitumor activity.[10] In *C. harringtonia*, the alkaloids derived from the "cephalotaxane" skeleton co-occur with a number of bases possessing the "homerythrina" skeleton.[11] One such compound is 3-epischelhammericine (**5**, Figure 1). Schelhammericine itself is elaborated by *Schelhammera pedunculata* (Liliaceae).[12]

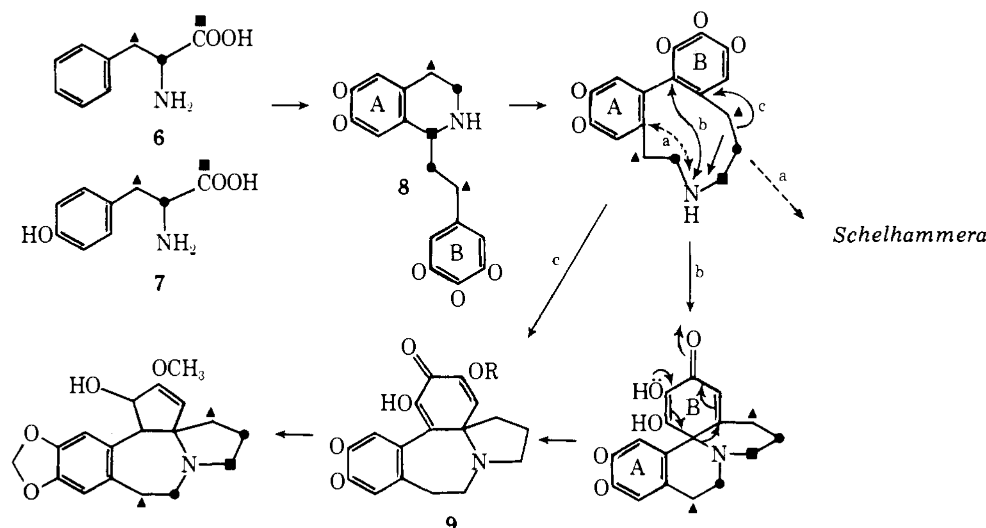
Results and Discussion

Early Stages of Cephalotaxine Biosynthesis. At the outset of our investigations of cephalotaxine biosynthesis, two hypotheses were considered. The favored hypothesis was suggested by the occurrence of "homoerythrina" alkaloids in *Cephalotaxus* plants. This hypothesis predicted that cephalotaxine should be generated from two molecules of phenylalanine (**6**) or tyrosine (**7**) via a 1-phenethyltetrahydroisoquinoline derivative (**8**) and a dienone (**9**) that undergoes ring contraction to yield **1** (Scheme I). Because of the results obtained in investigations of the biosynthesis of colchicine,[13] a modified 1-phenethylisoquinoline alkaloid, it was anticipated that C-6, C-7, and C-8 of cephalotaxine would be derived from C-3, C-2, and C-1, respectively, of the phenylalanine side chain while C-10 and C-11 of the alkaloid would be derived from C-2 and C-3 of the tyrosine side chain.

The alternative hypothesis for cephalotaxine biosynthesis was based upon published investigations of the biosynthesis of the *Erythrina* alkaloids.[14,15] This hypothesis required that cephalotaxine be formed from two molecules of phenylalanine or tyrosine via a 1-benzyltetrahydroisoquinoline. If this hypothesis were correct, then carbon atoms 8 and 10 of **1** will be derived from C-2 of the amino acid side chains while carbon atoms 7 and 11 of cephalotaxine would originate from C-3 of the amino acids.

In order to distinguish between these two hypotheses, it was necessary to develop methods for the degradation of **1** that would provide access to carbon atoms 7, 8, 10, and 11. Our initial attempts at finding a useful degradation were not successful, but they did uncover some interesting cephalotaxine chemistry. Oppenauer oxidation of **1** to cephalotaxinone (**2**, Figure 1) followed by quaternization with methyl iodide and Hofmann elimination gave the seco dienone **10** (Scheme II) [30%, $\bar{\nu}$ 1711, 1600 cm^{-1}; δ 1.60 (2 H, m, allylic H), 2.02 (3 H, s, NMe), 3.82 (3 H, s, OMe), 5.34 (1 H, s, benzylic H), 5.76

Scheme I



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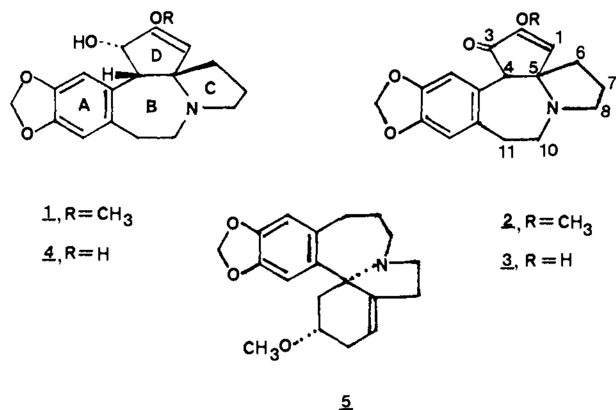


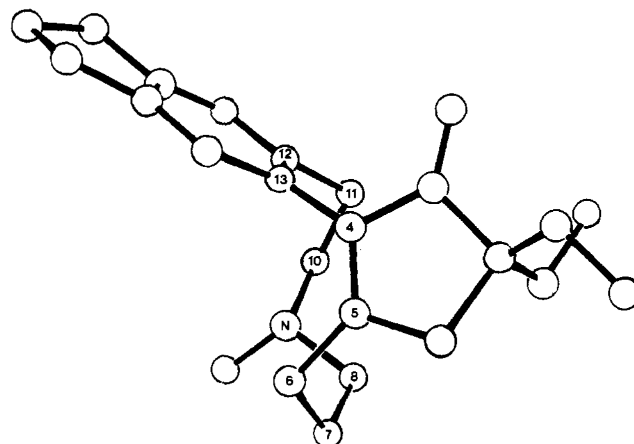
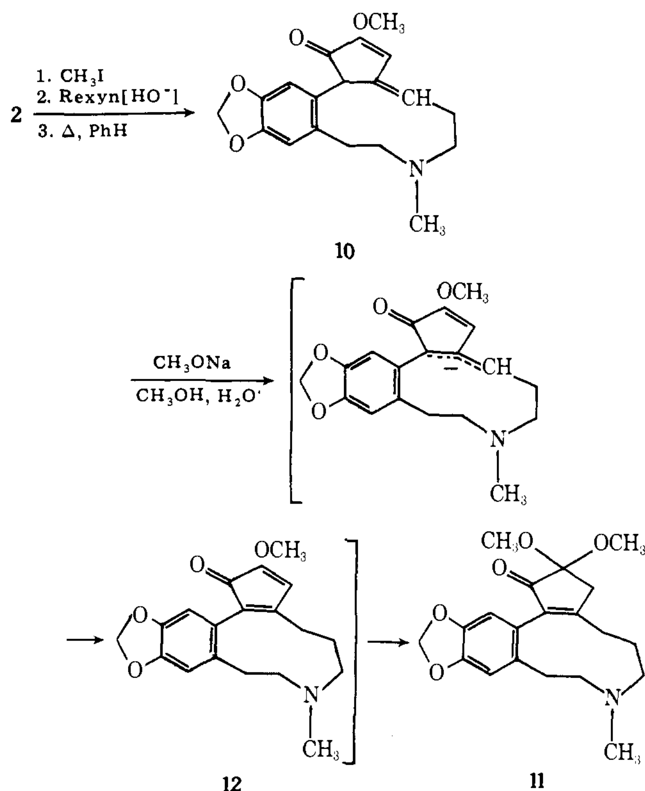


Figure 1. Alkaloids of *Cephalotaxus harringtonia.*

Figure 2. X-ray structure of dimethoxyenone **11**.

Scheme II



(1 H, t, J = 8 Hz, vinyl H, shown by double irradiation to be coupled to allylic H's at δ 1.60), 6.05 (1 H, s, vinyl H). In an attempt to prove the assignment of the absorption at δ 1.60 in the NMR spectrum, **10** was heated with sodium methoxide in a mixture of D_2O and methanol-*O-d*. To our surprise, workup afforded a new, crystalline substance. The reaction was repeated in undeuterated medium to yield (60%) a compound assigned structure **11** (Scheme II) on the basis of spectral data [$\bar{\nu}$ 1717 cm^{-1}; *m/e* 359 (M^+); δ 2.01 (3 H, s, NMe) and 3.39 (6 H, s, OMe)]. Because the formation of **11** required the addition of methoxide to **10** in an unexpected manner, its structure was investigated by X-ray analysis.

Crystal Data:[16] $C_{20}H_{25}NO_5$, monoclinic, space group $P2_1/c$, a = 9.516 Å, b = 16.372 Å, c = 12.590 Å, β = 110.89°, Z = 4. The 1266 independent reflections for which $I/\sigma(I) > 3.0$ were measured on a Syntex $P2_1$ diffractometer (crystal-monochromated Mo Kα radiation). The structure was solved using direct methods and full-matrix least-squares refinement of positional and isotropic thermal parameters for all nonhydrogen atoms converged to a conventional R factor of 0.11. The overall geometry of the molecule is shown in Figure 2. The proposed structure is confirmed including location of the double bond (1.35 Å) between C-4 and C-5.

The ring systems in **11** have two striking features. First, the dihedral angle between the cyclopentenone and benzene ring systems is 78.2°. Inspection of models suggests that this near-orthogonal arrangement is a consequence of the severe steric interactions which develop in the ten-membered ring when the benzene and cyclopentenone rings approach coplanarity. Secondly, the ten-membered ring is in nearly a "chaise lounge" conformation, the dihedral angles between least-squares planes containing atoms C(13)-C(12)-C(11)-C(4), C(11)-C(4)-C(10)-C(5), C(10)-C(5)-N-C(6), and C(6)-N-C(8)-C(7) being 70.7, 27.4, and 82.7°, respectively.

A possible mechanism for the formation of **11** is outlined in Scheme II. Methoxide-catalyzed isomerization of **10** could lead to the cyclopentadienone **12**. 1,6-Addition of methoxide to **12** would then generate **11**.[17a] Although there appears to be no experimental evidence bearing on the mode of addition of nucleophiles to cyclopentadienones, calculations[17b] predict that 1,6-additions should be favored over 1,4-additions. This prediction is indirectly supported by the propensity of cycloheptatrienones toward 1,8-additions of nucleophiles.[17c]

Attempts at degradation of the dimethoxyenone **11** proved fruitless. However, a more successful method for the degradation of cephalotaxine was eventually discovered. Emde reduction[18] of cephalotaxine methiodide yielded two products (Scheme III). The major one (55%) was the cyclopentanone **13** [*m/e* 301 (M^+); $\bar{\nu}$ 1732 cm^{-1}] while the minor one was the methoxyenone **14** [*m/e* 329 (M^+); $\bar{\nu}$ 1709, 1633 cm^{-1}; δ 2.01 (3 H, s, NCH_3), 3.58 (3 H, s, CH_3)]. Treatment of **14** with excess sodium amalgam transformed it into the cyclopentanone **13** suggesting that **14** may be an intermediate in the formation of **13**. The intermediacy of **14** is also consistent with the fact that **13** is optically inactive. Additional proof for the structures of **13** and **14** was obtained by correlation with the dimethoxyenone **11**. Reduction of either **14** or **11** with zinc and sulfuric acid yielded the cyclopentenone **15** ($\bar{\nu}$ 1709 cm^{-1}) (Scheme III).

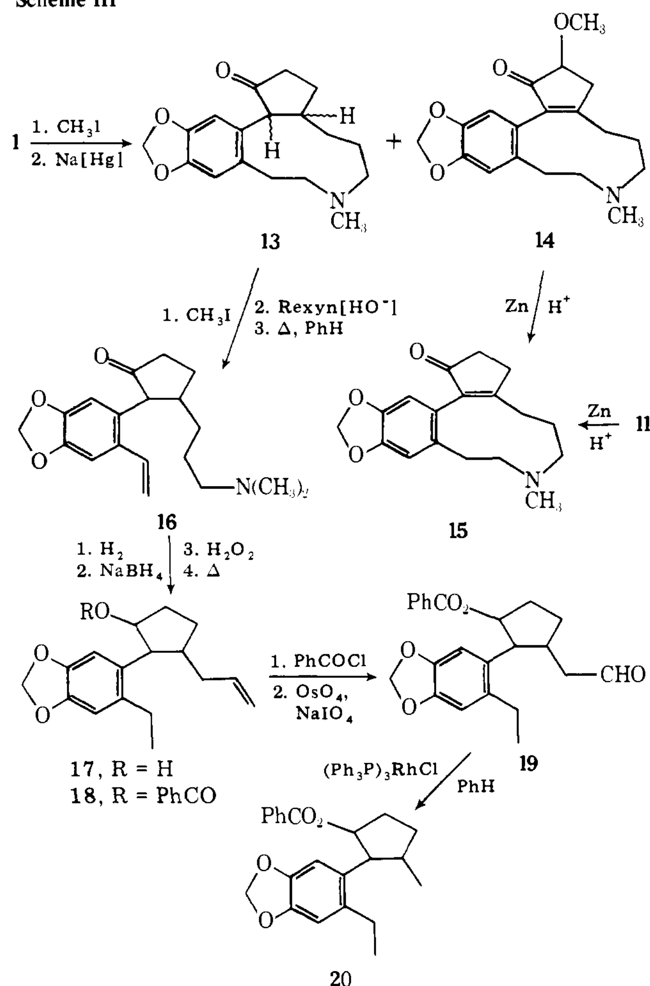
Quaternization of cyclopentanone **13** followed by Hofmann elimination yielded (55%) the styrene **16**, whose identity was revealed by a distinctive ABX system in its NMR spectrum and by characteristic ultraviolet absorption. Availability of styrene **16** allowed the isolation of C-10 of cephalotaxine. Treatment of **16** with osmium tetroxide and sodium periodate[19] yielded formaldehyde, which was readily isolated as its dimedone adduct.

The goal of the degradation then became the isolation of C-8 of cephalotaxine. Hydrogenation of the vinyl group in **16** was followed by reduction of the carbonyl group, amine oxide formation, and pyrolysis. From this reaction sequence, the

Table I. Incorporation of Precursors into Cephalotaxine by *C. harringtonia* var. *fastigiata*

expt no.	precursor	feeding period, weeks	% incorporation	distribution of ^{14}C activity in cephalotaxine
1	[2-^{14}C]-DL-tyrosine	8	0.035 ± 0.0007	37% at C-10, 0% at C-7, C-8
2	[2-^{14}C]-DL-tyrosine	8	0.16 ± 0.003	37% at C-10, 0% at C-7, C-8
3	[ring-^{14}C]-L-tyrosine	8	0.11 ± 0.002	90% in C-12 to C-17, C-18
4	[*p*-^{14}C]-L-tyrosine	8	0.06 ± 0.001	93% in C-12 to C-17, C-18, C-4, C-11
5	[2-^{14}C]-DL-tyrosine	2	0.04 ± 0.0008	49% at C-10
6	[2-^{14}C]-DL-tyrosine	1	0.003 ± 0.000 06	63% at C-10
7	[1-^{14}C]-DL-phenylalanine	2	0.02 ± 0.0004	84% at C-8
8	[1-^{14}C]cinnamic acid	2	0.004 ± 0.0008	9% at C-8
9	[1-^{14}C]cinnamic acid	2	0.006 ± 0.0001	3% at C-8
10	3(*RS*)-[3-^{3}H-2-^{14}C]-DL-phenylalanine, ^{3}H:^{14}C = 4.55	2	0.01 ± 0.0002	^{3}H:^{14}C = 2.12
11	3(*RS*)-[3-^{3}H-*p*-^{14}C]-DL-phenylalanine, ^{3}H:^{14}C = 3.48	2	0.005 ± 0.0001	^{3}H:^{14}C = 1.60, 100% at C-3
12	3(*RS*)-[3-^{3}H-*m*-^{14}C]-DL-phenylalanine, ^{3}H:^{14}C = 2.65	2	0.003 ± 0.000 06	^{3}H:^{14}C = 2.61, 98.5% at C-2

Scheme III



olefin **17** was produced in ca. 28% yield from **16**. If the reduction of the ketone was omitted, pyrolysis of the corresponding amine oxide yielded a multitude of products. Cleavage of **17** with osmate-periodate yielded C-8 of cephalotaxine as dimedone-formaldehyde. However, the other product of the reaction, a hydroxy aldehyde, could not be isolated in acceptable yield. Benzoylation of the hydroxyl function in **17** alleviated this difficulty. Osmate-periodate cleavage of the benzoyl ester **18** gave acceptable yields of both dimedone-formaldehyde and the aldehyde ester **19**. Indirect access to C-7 of cephalotaxine was provided by decarbonylation[20] of **19** with tris(triphenylphosphine)chlororhodium(I) to the ester **20**, which was characterized by a sharp methyl doublet (δ 0.99, J = 6 Hz) in the NMR spectrum.

In 1972 preliminary incorporation experiments with labeled tyrosine and phenylalanine were carried out using *C. harringtonia* var. *fastigiata* growing in a greenhouse. Precursors were administered to cuttings and to whole plants via the cotton wick method. The use of cuttings proved unsatisfactory. Short incorporation periods (<7 days) using intact plants gave insignificant incorporations of phenylalanine and low incorporations of tyrosine. Increasing the length of the incorporation period led to increasing levels of tyrosine incorporation. Table I, experiment 1, shows a typical result: administration of [2-^{14}C]-DL-tyrosine for an 8-week period yielded radioactive cephalotaxine with an incorporation figure of 0.035%. Additional experiments with [2-^{14}C]-DL-tyrosine were then carried out using rapidly growing *C. harringtonia* plants living in an environmental chamber. Under these conditions significantly higher incorporations of tyrosine were observed after an 8-week period (Table I, experiment 2). Degradation of the radioactive cephalotaxine in the manner outlined in Scheme III provided access to C-10, C-8, and C-7 of the alkaloid. The two samples of radioactive cephalotaxine obtained by administration of [2-^{14}C]-DL-tyrosine to *Cephalotaxus* growing either in a greenhouse or in an environmental chamber gave identical results: no significant amount of radioactivity was present at C-8 or C-7 of the alkaloid, and, after correction for the generation of radioinactive formaldehyde from *N*-methyl groups,[21] 37% of the total radioactivity was present at C-10. These observations were surprising since the labeling pattern did not correspond to that expected on the basis of either of our biosynthetic hypotheses.

An explanation for the unusual labeling pattern observed in experiments 1 and 2 was provided by additional investigations. Samples of [*p*-^{14}C]- and [ring-^{14}C]-L-tyrosine were prepared enzymatically using tyrosine phenollyase.[22] The [ring-^{14}C]-L-tyrosine was administered to *C. harringtonia* for 8 weeks and radioactive cephalotaxine obtained. Permanganate oxidation of the labeled alkaloid gave 4,5-methylenedioxyphthalic acid, which was degraded[23] to the corresponding anthranilic acid. The anthranilic acid carried 90% of the total activity of **1** (Table I, experiment 3). A second experiment with the [*p*-^{14}C]-L-tyrosine produced a similar result: 93% of the total radioactivity of the cephalotaxine was present in the 4,5-methylenedioxyphthalic acid obtained by permanganate oxidation (Table I, experiment 4). These experiments established that the ring-labeled tyrosines lead to exclusive labeling of ring A of cephalotaxine under the same conditions which lead to 37% incorporation of the label of [2-^{14}C]tyrosine into C-10 of the alkaloid. The nonexclusive incorporation of label into C-10 could therefore be explained to be the result of catabolism of the tyrosine, possibly by enzyme-catalyzed cleavage to phenol and serine followed by incorporation of the latter substance into cephalotaxine. Evidence favoring such an explanation was obtained by administration of [2-^{14}C]tyrosine to *Cephalotaxus* plants for periods of less than 8 weeks, fol-

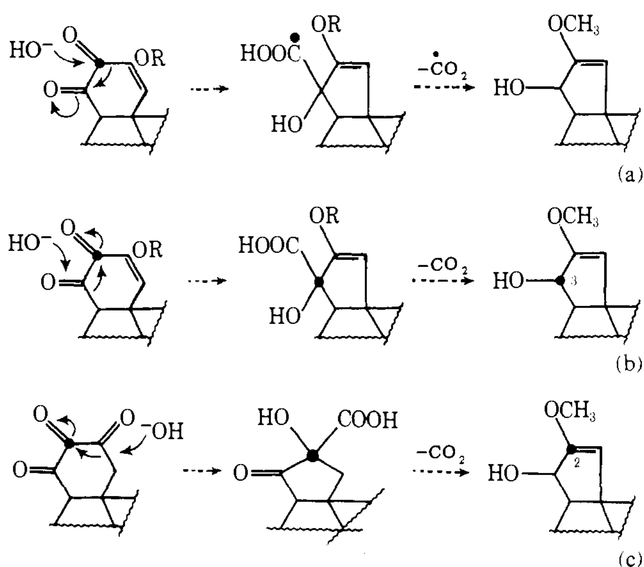
lowed by degradation of the radioactive samples of cephalotaxine to isolate C-10. The results of these experiments (Table I, experiments 5, 6) clearly show that decreasing the length of the feeding period has two effects: it lowers the levels of tyrosine incorporation into **1** and it *raises* the percent of the total radioactivity in the alkaloid that is present at C-10. These observations serve to emphasize an important principle underlying the conduct of biosynthetic investigations, namely, that experimental support for a biosynthetic pathway is derived from the specific incorporation of precursors rather than from the magnitude of the incorporation figures.

The clarification of the role of tyrosine in the biosynthesis of cephalotaxine raised the question of the origin of the remaining carbon atoms of the cephalotaxine skeleton. Although our initial incorporation experiments with phenylalanine had been unpromising, the analogy provided by colchicine required that we reexamine the potential of phenylalanine as a cephalotaxine precursor. An additional impetus was provided by a report from Battersby's laboratory[24] indicating that the biosynthesis of schelhammeridine in *Schelhammera* plants proceeded in accordance with the colchicine analogy. In the event, administration of [1-^{14}C]-DL-phenylalanine to *C. harringtonia* plants growing in an environmental chamber yielded radioactive cephalotaxine after a 2-week period (Table I, experiment 7). Degradation of the alkaloid according to the route outlined in Scheme III revealed that 84% of the total radioactivity was present at C-8. This observation provided substantial evidence that cephalotaxine is a member of the family of phenethylisoquinoline alkaloids which includes the *Schelhammera* and *Colchicum* alkaloids and it suggested that ring D of cephalotaxine is derived from the aromatic ring of phenylalanine by the loss of one carbon atom.

Since it had been established that phenylalanine is incorporated into colchicine via cinnamic acid,[13] the incorporation of [1-^{14}C]cinnamic acid into cephalotaxine was investigated. The results of two feeding experiments are shown in Table I, experiments 8 and 9. Both experiments yielded radioactive alkaloid, but, to our surprise, very little of the radioactivity was present at C-8. The apparent inability of cinnamate to serve as a specific precursor of cephalotaxine raised the question of the nature of the steps associated with the loss of the amino group from the side chain of phenylalanine during its conversion to cephalotaxine. If the amino group were removed by elimination of ammonia, as occurs in the conversion of phenylalanine to cinnamic acid,[25] then a stereospecific loss of one hydrogen atom from C-3 of the amino acid should take place. Administration of 3(*RS*)-[3-^{3}H-2-^{14}C]-DL-phenylalanine to *Cephalotaxus* demonstrated that such a process indeed occurs (Table I, experiment 10): the tritium to carbon-14 ratio in the radioactive alkaloid corresponded to a 54% loss of tritium (expected loss is 50%). This result suggests that an intermediate with α,β unsaturation lies on the pathway between phenylalanine and cephalotaxine. The intermediate may in fact be cinnamic acid, in which case our failure to observe specific incorporation of cinnamate into cephalotaxine could be attributed to an inability of the acid to reach the site of alkaloid biosynthesis.

Alternatively, the loss of tritium observed in experiment 10 could be due to the operation of an exchange process catalyzed by the enzyme tautomerase, rather than to a loss of ammonia via an ammonia lyase reaction. These two possibilities should lead to different stereochemical results with regard to hydrogen removal from C-3: the ammonia lyase reaction should proceed with removal of the 3-pro-*S* hydrogen atom,[25] while the tautomerase reaction should cause loss of the 3-pro-*R* hydrogen atom.[26] In order to examine this point, samples of 3(*R*)-[^{3}H]- and 3(*S*)-[^{3}H]-DL-phenylalanine[25] were administered to *Cephalotaxus* in conjunction with [2-^{14}C]-DL-phenylalanine. These incorporation experiments were carried out under conditions which appeared to be identical with those employed in experiments 7 and 10 (Table I); nevertheless, negligible incorporation of the doubly labeled phenylalanine into cephalotaxine was observed in each case. The failure of phenylalanine to be incorporated into cephalotaxine in these experiments suggests that there are some unknown variables which strongly influence the efficiency of incorporation of this amino acid into cephalotaxine.

Scheme IV



Late Stages of Cephalotaxine Biosynthesis. The incorporation experiments with phenylalanine and tyrosine outlined above support the hypothesis for cephalotaxine biosynthesis shown in Scheme I. This hypothesis requires that cephalotaxine be derived from a dienone (**9**) that undergoes loss of one carbon atom from ring D to yield ring D of cephalotaxine. It has been suggested that the ring contraction may proceed via a benzilic acid rearrangement.[11]

Ring D in dienone **9** is derived from the aromatic ring of phenylalanine. If the loss of one carbon atom from this ring proceeds via a benzilic acid rearrangement, three alternative reaction paths can be envisioned (Scheme IV). The first reaction path, (a), leads to loss of the para carbon atom of the aromatic ring of phenylalanine. The second reaction path, (b), proceeds via loss of one of the meta carbon atoms of phenylalanine to yield cephalotaxine in which the para carbon atom of phenylalanine resides at C-3. The third pathway, (c), which could be operative if R = H in dienone **9**, involves loss of the alternative meta carbon atom of phenylalanine. If reaction pathway (c) obtains, then the para carbon atom of phenylalanine will reside at C-2 of the resulting cephalotaxine. Thus, the three hypothetical pathways shown in Scheme IV should be distinguishable by administration of [*p*-^{14}C]phenylalanine to *Cephalotaxus* plants.

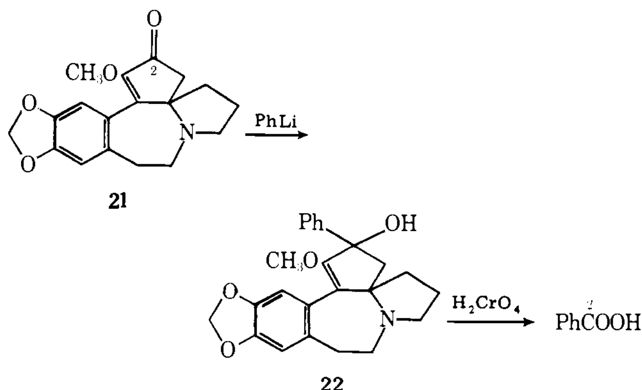
[*p*-^{14}C]-DL-Phenylalanine was synthesized in the following manner. [^{14}C]Carbon dioxide was trapped with methylmagnesium iodide and the resulting [1-^{14}C]acetic acid precipitated as the barium salt. Pyrolysis[27] of the barium acetate gave [2-^{14}C]acetone which was converted into [*p*-^{14}C]aniline by a modification of the procedure of Kratzl and Vierhapper.[28] The labeled aniline was diazotized and converted to [*p*-^{14}C]-DL-α-bromo-β-phenylpropionic acid via the Meerwein arylation reaction.[29] Ammonolysis[30] of the labeled α-bromo acid then gave [*p*-^{14}C]-DL-phenylalanine. The ring-labeled phenylalanine was mixed with [3(*RS*)-^{3}H]phenylalanine so that tritium would serve as a reference label in the event that the para carbon atom was lost.

The doubly labeled precursor having been prepared, we experienced some apprehension with regard to the successful

outcome of the incorporation experiment. This apprehension was due to the fact that our two prior attempts to incorporate phenylalanine into cephalotaxine had failed. A successful incorporation experiment was achieved in the present case by utilizing plants that had been removed from an environmental chamber programmed for a short day cycle and placed in a chamber with a long day cycle. After about 2 weeks these plants began to grow vigorously and they were then administered the precursor. It is interesting to note that the plants used in the two experiments where phenylalanine incorporation had failed had been growing on a long day cycle for many months. This may have been the reason why poor phenylalanine incorporations were obtained, but more effort would be required to substantiate this claim.

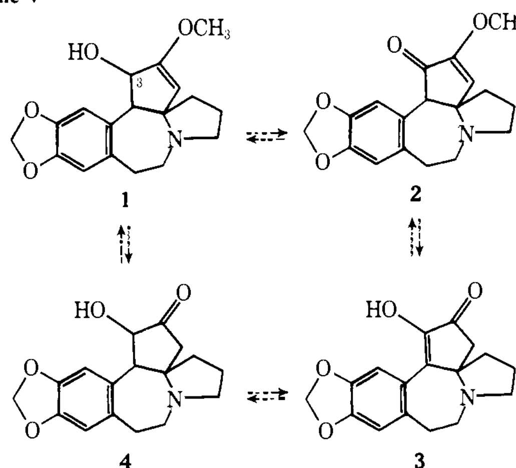
In any event, the results of the incorporation experiment (Table I, experiment 11) with 3(*RS*)-[3-^{3}H-*p*-^{14}C]-DL-phenylalanine established that the ring contraction process proceeds without loss of the para carbon atom of phenylalanine. The expected 50% loss of tritium was observed in this experiment and degradation of the labeled cephalotaxine was accomplished by conversion to the cyclopentanone **13** (Scheme III). Treatment of **13** with phenyllithium followed by oxidation of the adduct with chromic acid yielded radioactive benzoic acid whose specific activity indicated that 100% of the carbon-14 label was present at C-3 of the alkaloid. These observations indicate that, if ring D of cephalotaxine is formed by a benzilic acid rearrangement, the rearrangement follows pathway (b) in Scheme IV.

This conclusion was confirmed by means of a precursor incorporation experiment with [*m*-^{14}C]-DL-phenylalanine. The labeled amino acid was synthesized from [*m*-^{14}C]aniline which was in turn prepared from [1,3-^{14}C]acetone. The labeled acetone was obtained by pyrolysis of [2-^{14}C]barium acetate. The [*m*-^{14}C]-DL-phenylalanine was mixed with 3(*RS*)-[^{3}H]-DL-phenylalanine and the mixture administered to *C. harringtonia* plants. After a 2-week period, the results outlined in Table I (experiment 12) were obtained. The tritium to carbon-14 ratio in the isolated cephalotaxine was found to be the same within experimental error as that of the amino acid precursor. This is precisely the result to be expected if *one* of the two meta carbon atoms is lost. That is to say, loss of one of the meta carbon atoms will lead to a 50% loss of the carbon-14 label, and, since this loss occurs in conjunction with a 50% loss of the tritium label, the original tritium to carbon-14 ratio will be maintained. Furthermore, the results from the incorporation experiment with [*p*-^{14}C]phenylalanine lead one to predict that the remaining 50% of the carbon-14 label should be present at C-7 of cephalotaxine. This was shown to be the case by degradation of the radioactive alkaloid. Conversion[6,31] of the cephalotaxine to isocephalotaxinone (**21**) was followed by

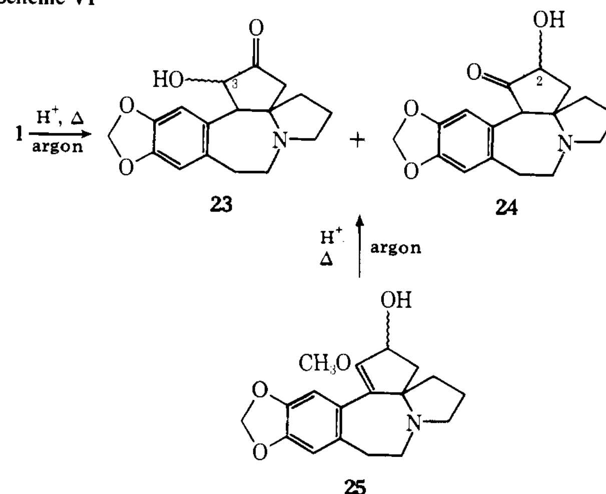


addition of phenyllithium to give the adduct **22**, which was then oxidized with chromic acid to yield benzoic acid. Essentially all of the radioacitivity in the cephalotaxine appeared in the benzoic acid.

Scheme V



Scheme VI



The results of the experiments just described are consistent with the occurrence of a benzilic acid rearrangement during the biosynthesis of cephalotaxine from phenylalanine. If such a rearrangement involves the hypothetical dienone intermediate **9**, the results also suggest that R in **9** may be a methyl group rather than a hydrogen atom since the meta carbon atom bearing the -OR group is retained in the ring contraction step. This possibility is supported by the results of an investigation of the metabolic relationships between cephalotaxine (**1**) and the remaining three alkaloids shown in Figure 1.

The potential metabolic interrelationships between the *Cephalotaxus* alkaloids **1–4** are summarized in Scheme V. These interrelationships have been examined by precursor incorporation experiments. [3-^{14}C]-DL-Cephalotaxine (**1**), [3-^{14}C]-DL-cephalotaxinone (**2**), and [3-^{14}C]-DL-demethylcephalotaxinone (**3**) were synthesized by utilizing [1-^{14}C]-pyruvic acid in conjunction with the Weinreb cephalotaxine synthesis.[31] Each of the radioactive alkaloids was extensively purified by chromatography and by recrystallization to constant specific radioactivity. Difficulty was encountered in our initial attempts to prepare [3-^{14}C]-DL-demethylcephalotaxine. Acid-catalyzed hydrolysis of cephalotaxine according to a published procedure[8] yielded mainly unreacted cephalotaxine accompanied by a small amount of demethylcephalotaxinone. However, when the hydrolysis was carried out under argon at an elevated temperature, the major product of the reaction proved to be an inseparable mixture whose NMR spectrum indicated the presence of four compounds: the two C-3 epimers of demethylcephalotaxine (**23**) and the two C-2 epimers of the isomeric hydroxy ketone **24** (Scheme VI). The NMR spectrum of the mixture indicated that the ratio between the epimeric mixture **23** and the epimeric mixture **24** was ca. 1:4. An iden-

Table II. Administration of Labeled Alkaloids to *C. harringtonia* var. *fastigiata*

expt no.		feeding period, days	alkaloids isolated (% incorporation)	% of label at C-3
1	[3-^{14}C]-DL-cephalotaxine	4	cephalotaxinone (0.44 ± 0.009)	95
			demethylcephalotaxinone (0.045 ± 0.0009)	99
2	[3-^{14}C]-DL-cephalotaxinone	4	cephalotaxine (28 ± 0.6)	99
			demethylcephalotaxinone (4.6 ± 0.09)	102
3	[3-^{14}C]-DL-demethylcephalotaxinone	4	cephalotaxine (0)	
			cephalotaxinone (0)	
4	[3-^{14}C]-DL-cephalotaxine	4	demethylcephalotaxine (0.79 ± 0.02)[a]	100
5	[3-^{14}C]-DL-demethylcephalotaxine[b]	4	cephalotaxine (0)	
6	[3-^{14}C]-DL-demethylcephalotaxinone	5	demethylcephalotaxine (13 ± 0.3)[a]	98

[a] Isolated as a mixture of isomeric hydroxy ketones (see text). [b] Precursor a mixture of isomeric hydroxy ketones (see text).

tical product mixture was obtained by acid-catalyzed hydrolysis of isocephalotaxine (**25**) (Scheme VI), itself prepared from isocephalotaxinone (**21**) by borohydride reduction. Additional proof for the structure assigned to the mixture of **23** and **24** was provided by heating in dilute acid in the presence of air. Under these conditions, demethylcephalotaxinone (**3**) was formed (76%). An epimeric mixture containing [3-^{14}C]-DL-demethylcephalotaxine was synthesized by conversion[31] of [3-^{14}C]demethylcephalotaxinone to [3-^{14}C]isocephalotaxinone, reduction of the labeled isocephalotaxinone with borohydride, and acid-catalyzed hydrolysis of the resulting [3-^{14}C]-DL-isocephalotaxine.

Each of the labeled alkaloids **1–4** was administered to *Cephalotaxus* plants and the related alkaloids were isolated by dilution methods. The results of these experiments are summarized in Table II. The radioactive alkaloids isolated in each precursor incorporation experiment were exhaustively purified by chromatography and recrystallization to constant specific radioactivity. In the case of the isolated demethylcephalotaxine (Table II, experiments 4, 6), extensive chromatographic purification was utilized since the hydroxy ketone mixture is not readily crystallizable. The radioactive cephalotaxine isolated in experiment 2 was degraded by conversion to the cyclopentanone **13**, addition of phenyllithium, and oxidation of the adduct to yield benzoic acid. The radioactive samples of cephalotaxinone and demethylcephalotaxinone (experiments 1, 2) were transformed into cephalotaxine[31] and the latter alkaloid was then degraded in the usual way. The radioactive demethylcephalotaxine (experiments 4, 6) was converted to demethylcephalotaxinone by heating in dilute acid in air. The demethylcephalotaxinone was then degraded in standard fashion. The degradations proved that the observed incorporations were specific in each case. Experiments 1 and 2 in Table II show that cephalotaxine and cephalotaxinone are interconvertible in *Cephalotaxus* and that demethylation of cephalotaxinone to demethylcephalotaxinone also takes place readily. The large difference in the relative magnitude of the incorporation figures for the cephalotaxine–cephalotaxinone interconversion must be interpreted with caution since it may reflect the large pool size for cephalotaxine. Experiment 3 indicates that *Cephalotaxus* plants will not methylate demethylcephalotaxinone to yield cephalotaxinone or cephalotaxine. Similarly, experiments 4 and 5 show that *Cephalotaxus* plants are capable of demethylating cephalotaxine to yield demethylcephalotaxine, whereas they are apparently incapable of carrying out the reverse reaction, the methylation of demethylcephalotaxine to cephalotaxine. Finally, experiment 6 demonstrates that demethylcephalotaxinone is readily reduced by *Cephalotaxus* to demethylcephalotaxine. The reverse reaction, oxidation of demethylcephalotaxine to demethylcephalotaxinone, was not examined owing to the difficulties associated with distinguishing between an in vitro air oxidation and an in vivo biological oxidation.

The following conclusions can be drawn from these experimental results. First, it appears that demethylcephalotaxine is a natural product that occurs in *Cephalotaxus* plants. Previous evidence for the natural occurrence of demethylcephalotaxine appears tenuous in view of the fact that the material isolated from *Cephalotaxus* was identified by comparison with a sample produced by acid-catalyzed hydrolysis in the presence of air.[8] Second, the data suggest that neither demethylcephalotaxine nor demethylcephalotaxinone can be intermediates on the pathway to cephalotaxine. Rather, the enol ether methyl group present in cephalotaxine is probably introduced at an earlier stage in the biosynthesis. Finally, it appears that demethylcephalotaxine and demethylcephalotaxinone represent catabolites of cephalotaxine. The irreversible formation of these compounds by demethylation is analogous to the situation that obtains in the final stages of the biosynthesis of the morphine alkaloids. In the case of the latter alkaloids, it has been shown that codeine and morphine are formed by successive O-demethylations of thebaine.[32-36]

Experimental Section

General. Melting points were determined on a Fisher-Johns apparatus and are uncorrected. Ultraviolet spectra were measured in 95% ethanol using a Perkin-Elmer 25 spectrophotometer. Infrared spectra were obtained on a Perkin-Elmer 567 infrared 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, or a Brüker WH-90 NMR spectrometer. All NMR spectra were recorded in $CDCl_3$, 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 MS12 or MS-9 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-nm 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 nm thick, and on Polygram Sil G/UV$_{254}$ (silica gel), 250 nm on plastic sheets. Visualization was usually by short-wave ultraviolet light or phosphomolybdic acid stain. Dried *Cephalotaxus*

harringtonia var. *fastigiata* was a generous gift of Dr. Robert Perdue of the U.S. Department of Agriculture and Dr. J. L. Hartwell of the National Cancer Institute. Cephalotaxine was isolated in the manner described by Paudler et al.[2]

During the course of these investigations, two new groups of compounds were obtained as products of the chemical degradation of cephalotaxine. The numbering system used for the secocephalotaxane skeleton, as exemplified by *N*-methylseco[*B,C*]cephalotaxan-3-one (**13**, Scheme III), is based upon that used for cephalotaxine. "B" and "C" also refer to the cephalotaxane ring system. Compounds **16–20** are named and numbered as cyclopentanone and cyclopentanol derivatives.

Cephalotaxinone (2). Cephalotaxine (**1**) was oxidized using Oppenauer conditions.[39] Aluminum isopropoxide (309 mg, 1.50 mmol) was placed in a 100-mL round-bottom flask and 20 mL of dry benzene added, followed by 12 mL of dry acetone and 400 mg (1.27 mmol) of cephalotaxine. The solution was degassed by treatment with nitrogen, then heated at reflux and stirred under an atmosphere of nitrogen for 24 h. After cooling to room temperature, volatiles were removed by rotary evaporation and the residue was partitioned between water and ethyl acetate. Following separation of the layers, the aqueous phase was extracted twice more with ethyl acetate and the combined organic layers were dried over sodium sulfate, filtered, and concentrated in vacuo, yielding a yellow gum, 469 mg, smelling strongly of mesityl oxide. Purification by TLC (alumina HF-254, 1:1 benzene-ethyl acetate) gave 257 mg (65%) of a gum which was crystallized from ethyl acetate to yield 230 mg (58%) of colorless crystals, mp 203–208 °C dec (lit.[6] 172–195 °C dec). The NMR and IR data obtained for **2** were identical with those published.[6]

2-Methoxy-*N*-methylseco[*B,C*]cephalotaxa-1,5-diene-3-one (10). Cephalotaxinone (255 mg, 0.81 mmol) was dissolved in 10 mL of methanol and 1 mL of methyl iodide added. The solution was stirred at ambient temperature in the absence of light and after 24 h the volatiles were removed in vacuo. The gummy, yellow residue was dissolved in a minimal amount of 15% aqueous ethanol and applied to a 180 × 25 mm column of Rexyn 201 (OH) in 15% ethanol. Eluate was collected until it was no longer basic and concentrated in vacuo to a volume of a few milliliters. The resulting yellow solution was added portionwise to 50 mL of refluxing benzene (oil bath at 105 °C) in a 100-mL three-necked flask equipped with Dean-Stark trap and a magnetic stir bar. The benzene was stirred vigorously and the aqueous solution added cautiously in order to keep the methohydroxide in suspension and off the walls of the flask. After all the methohydroxide solution had been added, the reaction mixture was stirred and refluxed for an additional 2 h during which time it became lighter in color. The yellow solution was cooled, filtered, and concentrated in vacuo to give 190 mg of brown gum which was purified by preparative TLC (alumina HF-254, 1:1 benzene-ethyl acetate). Elution yielded 148 mg (56%) of noncrystalline dienone: IR (film) 1711, 1600, 1505, 1487 cm^{-1}; NMR (90 MHz) δ 1.61 (2 H, m, allylic H), 2.30 (3 H, s, NCH_3), 3.81 (3 H, s, OCH_3), 5.35 (1 H, s, $ArCHR_2$), 5.72 (1 H, t, J = 8 Hz coupled to H's at 1.61, vinyl H at C-6), 5.80 (1 H, d, H_A of AB quartet, J = 1.5 Hz, OCH_2O), 5.84 (1 H, d, H_B of AB quartet, J = 1.5 Hz, OCH_2O), 6.12 (1 H, s), 6.52 (1 H, s), 6.85 (1 H, s); UV (approximate ϵ) 198 nm (43 000), 233 (sh, 12 200), 298 (11 500), 313 (sh, 9000); MS *m/e* 327 (M^+).

Exact mass. Calcd for $C_{19}H_{21}NO_4$: 327.3835. Found: 327.3851.

2,2-Dimethoxy-*N*-methylseco[*B,C*]cephalotax-4-en-3-one (11). A solution of 133 mg (5.8 mmol) of sodium in 4 mL of methanol was added, after dilution with 1 mL of water, to 66 mg (0.20 mmol) of dienone **10**. The resulting solution was stirred at reflux temperature for 2 h, after which time it was cooled and the pH adjusted to 6 with dilute hydrochloric acid. After removal of most of the methanol by rotary evaporation, 1–2 mL of water was added and the mixture extracted thrice with chloroform, which was filtered and concentrated in vacuo to a brown oil (63 mg). Removal of much dark color was effected by passing a chloroform solution of the oil through a plug of activity *III* alumina in chloroform. The resulting yellow gum was crystallized from benzene–hexane to yield 43 mg (59%) of **11** in the form of colorless prisms: mp 175–177.5 °C; IR ($CHCl_3$) 1716, 1637, 1500 cm^{-1}; NMR (90 MHz) δ 2.06 (3 H, s, NCH_3), 3.43 (6 H, s, OCH_3), 5.91 (1 H, d, H_A of AB quartet, J = 1.5 Hz, OCH_2O), 5.96 (1 H, d, H_B of AB quartet, J = 1.5 Hz, OCH_2O), 6.38 (1 H, s, ArH), 6.71 (1 H, s, ArH); UV 198 nm (ϵ 41 500), 236 (15 900), 289 (4300); MS *m/e* 359 (M^+). The proposed structure was confirmed by single-crystal X-ray analysis.

***N*-Methylseco[*B,C*]cephalotaxan-3-one (13) and 2-Methoxy-*N*-methylseco[*B,C*]cephalotax-4-en-3-one (14).** Methyl iodide (1 mL) was added to a solution of cephalotaxine (200 mg, 0.64 mmol) in 8 mL of methanol. The solution was stirred at ambient temperature in the dark, and after 24 h the volatiles were removed in vacuo. The resulting gummy yellow methiodide was dissolved in a mixture of 2 mL of ethanol and 2 mL of water. Another 4.2 mL of water was added followed by 2.56 g of 3% sodium amalgam. The reaction flask, lightly stoppered, was heated on a steam bath, with occasional swirling, for 2 h. During this time a yellow oil separated, which, after cooling of the reaction mixture, was removed by repeated extraction with ether. The combined ether extracts were washed with brine until neutral, dried over sodium sulfate, filtered, and evaporated in vacuo, leaving 186 mg of an orange gum. Preparative TLC (alumina PF-254, 9:1 benzene-ethyl acetate) yielded 105 mg of the ketone **13** (55%) as a colorless gum, which was crystallized from ether to yield colorless plates: mp 140–141 °C; IR ($CHCl_3$) 1732, 1506, 1487 cm^{-1}; NMR (90 MHz) δ 2.09 (3 H, s, NCH_3), 3.82 (1 H, d, J = 10.7 Hz, $ArCHR_2$), 5.84 (d, H_A of AB quartet, J = 1.5 Hz, OCH_2O), 5.88 (d, H_B of AB quartet, J = 1.5 Hz, OCH_2O), 6.28 (1 H, s, ArH), 6.56 (1 H, s, ArH); MS *m/e* 301 (M^+); **13** displays no optical rotation between 325 and 600 nm.

Anal. ($C_{18}H_{23}NO_3$) C, H, N.

Also isolated via preparative TLC was an impure yellow gum (40 mg, 20%), the main component of which was identified as the methoxyenone **14**. Several attempts to further purify **14** (by TLC on alumina and silica gel) met with failure: IR (CCl_4) 1709, 1633, 1505, 1486 cm^{-1}; NMR (90 MHz) δ 2.01 (3 H, s, NCH_3), 3.58 (3 H, s, OCH_3), 4.0 (1 H, m, J = 2.7 Hz, $CHOCH_3$), 5.9 (2 H, m, OCH_2O); MS *m/e* 329 (M^+).

2-(3′,4′-Methylenedioxy-6′-vinyl)phenyl-3-(3″-*N,N*-dimethylaminopropyl)cyclopentanone (16). Methyl iodide (1–2 mL) was added to a stirred solution of 82 mg (0.27 mmol) of **13** in 5 mL of methanol. The flask was wrapped in aluminum foil and its contents stirred at room temperature for 24 h. Volatiles were then removed in vacuo, and the gummy, yellow residue was dissolved in minimal 15% ethanol and applied to a 180 × 15 mm column of Rexyn 201 (OH^-) in 15% ethanol. Eluate was collected until no longer basic and concentrated in vacuo to a volume of a few milliliters. The resulting yellow solution was added portionwise to 50 mL of refluxing benzene (oil bath at 105 °C) in a 100-mL three-necked flask equipped with a Dean-Stark trap and a magnetic stir bar. The benzene was stirred vigorously and the aqueous solution added cautiously in order to keep the methohydroxide in suspension and off the walls of the flask. After all the methohydroxide solution had been added, the reaction mixture was stirred and refluxed for an additional 2 h, during which time it became lighter in color. The yellow solution was cooled, filtered, and concentrated in vacuo to a yellow gum (51 mg) which was purified by TLC (alumina HF-254, 96:4 ethyl acetate-methanol), yielding 47 mg of **16** as a colorless gum (55%): IR (CCl_4) 1745, 1621, 1507, 1486 cm^{-1}; NMR (90 MHz) δ 2.16 [6 H, s, $N(CH_3)_2$], 3.22 (1 H, d, J = 11 Hz, $ArCHR_2$), 5.22 (1 H, d of d, H_A of ABX system, J_{AB} = 1.5, J_{AX} = 10.5 Hz, H trans to Ar), 5.48 (1 H, d of d, H_B of ABX system, J_{AB} = 1.5, J_{BX} = 17 Hz, H cis to Ar), 5.91 (1 H, d, H_A of AB quartet, J = 1.4 Hz, OCH_2O), 5.94 (1 H, d, H_B of AB quartet, J = 1.4 Hz, OCH_2O), 6.43 (1 H, s, ArH), 6.82 (1 H, d of d, H_X of ABX system, J_{AX} = 10.5, J_{BX} = 17 Hz, $ArCH{=}CH_2$), 6.97 (1 H, s, ArH); UV (approximate ϵ) 264 (12 700), 303 nm (6500); MS *m/e* 315 (M^+).

Exact mass. Calcd for $C_{19}H_{25}NO_3$: 315.4161. Found: 315.4125.

Osmium Tetroxide–Sodium Periodate Oxidation of 16. The osmium tetroxide–sodium periodate oxidation of olefin **16** and the derivatization of the product formaldehyde were carried out according to the procedure of Battersby et al.[19] From 30 mg (0.095 mmol) of **16** was obtained 24 mg (86%) of the dimedone derivative of formaldehyde, mp 194.5–195 °C (lit.[19] 191.5–192 °C).

Control Experiment. Determination of the Quantity of Formaldehyde Liberated from *N*-Methyl Groups during Osmium Tetroxide–Sodium Periodate Oxidation of Olefin 16. A 97-mg (0.32 mmol) sample of **13** was dissolved in 3 mL of methanol inside a thick-walled reaction tube with a ground glass joint and a constriction below the joint. Methyl iodide (2 μL) was added and the solution stirred in the dark for several hours. The reaction tube was then connected to a vacuum transfer apparatus and 100 μCi of [^{14}C]methyl iodide (55 mCi/mmol) distilled into the reaction mixture. After several hours the transfer was considered complete and the reaction tube sealed. The contents of the tube were stirred, in the dark, overnight, after

which time the tube was chilled and opened. Unlabeled methyl iodide (1-2 mL) was added and the resulting solution stirred, in the absence of light, at room temperature, for another overnight period, after which time the volatiles were removed in vacuo. The residual ^{14}C-labeled methiodide was converted to the methohydroxide form by ion exchange and subjected to Hofmann conditions as described for the conversion of unlabeled **13** to **16**. The resulting labeled styrene was purified by preparative TLC (45.5 mg, 0.144 mmol, 8.45×10^7 dpm/mmol) and a 15-mg (0.048 mmol) portion oxidized with osmium tetroxide-sodium periodate as described above. The yield of dimedone-formaldehyde was 8 mg (57%), and its specific activity was 8.15×10^6 dpm/mmol, or 9.64% of the specific activity of the precursor styrene. Noting that only one of the *N*-methyl groups of **16** was labeled, it was extrapolated that $(9.64 \times 2)\% = 19.3\%$ of the dimedone derivative of formaldehyde comes from the *N*-methyl groups and 80.7% from the terminal methylene group.

2-(3′,4′-Methylenedioxy-6′-ethyl)phenyl-3-(2″-propenyl)cyclopentanol (17). A. 2-(3′,4′-Methylenedioxy-6′-ethyl)phenyl-3-(3″-*N*,*N*-dimethylaminopropyl)cyclopentanone. Styrene **16** (74 mg, 0.235 mmol) was combined with platinum oxide (7 mg, 0.031 mmol) and 1.6 mL of absolute ethanol. The mixture was stirred at ambient temperature under an atmosphere of hydrogen until rapid uptake of hydrogen had ceased. After filtration to remove the catalyst, volatiles were removed in vacuo and the residue was purified by TLC (alumina HF-254, 96:4 ethyl acetate-methanol). The resulting colorless oil weighed 73 mg (99%): IR (CCl_4) 1741, 1505, 1486 cm^{-1}; NMR (90 MHz) δ 1.14 (3 H, t, J = 8 Hz, CH_2CH_3), 2.25 [6 H, s, $N(CH_3)_2$], 2.50 (2 H, q, J = 8 Hz, CH_2CH_3), 3.13 (1 H, d, J = 11 Hz, $ArCHR_2$), 5.87 (1 H, d, A of AB quartet, OCH_2O), 5.91 (1 H, d, B of AB quartet, OCH_2O), 6.41 (1 H, s, ArH), 6.69 (1 H, s, ArH); MS *m/e* 317 (M^+).

Exact mass. Calcd for $C_{19}H_{27}NO_3$: 317.4319. Found: 317.4345.

B. 2-(3′,4′-Methylenedioxy-6′-ethyl)phenyl-3-(3″-*N*,*N*-dimethylaminopropyl)cyclopentanol. To a stirred solution of 2-(3′,4′-methylenedioxy-6′-ethyl)phenyl-3-(3″-*N*,*N*-dimethylaminopropyl)cyclopentanone (159 mg, 0.50 mmol) in 10 mL of absolute ethanol was added, over a period of 30 min, 95 mg (2.50 mmol) of sodium borohydride. The mixture was stirred at room temperature overnight. After concentration in vacuo to a thick, white slurry, 2-3 mL of freshly prepared 15% NaOH was added, and the aqueous mixture extracted thrice with chloroform. The combined chloroform extracts were washed with brine until neutral and filtered, and the volatiles removed in vacuo, leaving 152 mg of a pale yellow oil. Purification was by TLC (alumina HF-254, 97:3 ethyl acetate-methanol) to give 127 mg (80%) of the alcohol as a colorless oil. The NMR data shows the alcohol to be a mixture of diastereomers: IR ($CHCl_3$) 3580, 1504, 1485 cm^{-1}; NMR (90 MHz) δ 1.17 (3 H, overlapping triplets, J = 7 Hz, CH_2CH_3), 2.48 (s, NCH_3), 2.52 (s, NCH_3), 4.10 (1 H, broad m, $CHOH$), 5.91 (s, OCH_2O), 5.92 (s, OCH_2O), 6.67 (1 H, s, ArH), 6.73 (s, ArH), 6.81 (s, ArH); MS *m/e* 319 (M^+).

C. 2-(3′,4′-Methylenedioxy-6′-ethyl)phenyl-3-(2″-propenyl)cyclopentanol (17). Hydrogen peroxide (30%, 0.54 mL) was added to 2-(3′,4′-methylenedioxy-6′-ethyl)phenyl-3-(3″-*N*,*N*-dimethylaminopropyl)cyclopentanol (135 mg, 0.42 mmol). The mixture was heated on a steam bath to effect solution following which treatment excess H_2O_2 was removed via rotary evaporation. Last traces were removed at steam-bath temperature and aspirator pressure. The flask containing the residual yellow gum was attached to a bulb-to-bulb distillation apparatus and heated under vacuum (170°C, 0.06-0.03 mm). The crude olefin, a yellow oil (99 mg), was collected at −78 °C and purified by TLC (silica gel GF-254, 9:1 benzene-ether) to a colorless gum (42 mg, 36%). The NMR data show it to be a mixture of diastereomers: IR ($CHCl_3$) 3580, 3080, 1640, 1504, 1485, 995, 920 cm^{-1}; NMR (90 MHz) δ 1.17 (3 H, overlapping triplets), J = 7 Hz, CH_2CH_3), 2.60 (2 H, q, J = 7 Hz, CH_2CH_3) 4.15 (1 H, m, $CHOH$), 4.8-5.1 (2 H, m, H_A, H_B of vinyl ABX system), 5.5-5.8 (1 H, m, H_X of vinyl ABX system), 5.90 (2 H, m, OCH_2O), 6.68 (1 H, s, ArH), 6.72 (s, ArH), 6.84 (s, ArH); MS *m/e* 274 (M^+).

Exact mass. Calcd for $C_{17}H_{22}O_3$: 274.3631. Found: 274.3607.

Osmium Tetroxide-Sodium Periodate Oxidation of 17. The osmium tetroxide-sodium periodate oxidation of olefin **17** and the derivatization of the product formaldehyde were carried out according to the procedure of Battersby et al.[19] From 10.5 mg (0.038 mmol) of **17** was obtained 10 mg (89%) of the dimedone derivative of formaldehyde, mp 194.5-195 °C (lit.[19] 191.5-192 °C).

2-(3′,4′-Methylenedioxy-6′-ethyl)phenyl-3-(2″-propenyl)cyclopentanol Benzoate (18). Benzoyl chloride (36 μL, 0.31 mmol) was added to a solution of olefin **17** (31 mg, 0.11 mmol) in pyridine (1.1 mL, dried over molecular sieve), and the resulting solution was stirred at ambient temperature for 90 min. Several milliliters of water were added and the resulting mixture was concentrated by rotary evaporation. After the addition of several more milliliters of water, the solution was made strongly basic by the addition of concentrated ammonium hydroxide solution and then extracted three times with chloroform. The chloroform extracts were washed with brine until neutral, filtered, and concentrated in vacuo. The resulting 43 mg of yellow oil was purified via TLC (silica gel GF-254, benzene), affording 39 mg (91%) of ester olefin **18** as a pale yellow gum, which appeared homogeneous by analytical TLC. The NMR data show **18** to be a mixture of diastereomers: IR ($CHCl_3$) 3075, 1710, 1641, 1605, 1585, 1505, 1486, 1272 cm^{-1}; NMR (90 MHz) δ 1.18 (3 H, t, J = 7.5 Hz, CH_2CH_3), 2.67 (2 H, broad q, J = 7.5 Hz, CH_2CH_3), 4.9-5.7 (3 H, multiplets, $CH{=}CH_2$), 5.73 (d, H_A, AB quartet, OCH_2O), 5.85 (d, H_B, AB quartet, OCH_2O), 5.86 (m, OCH_2O), 6.62 (1 H, s, ArH), 6.73 (s, ArH), 6.91 (s, ArH), 7.4 (m, benzoate ArH), 7.9 (m, benzoate ArH); MS *m/e* 378 (M^+).

2-(3′,4′-Methylenedioxy-6′-ethyl)phenyl-3-(2″-oxoethyl)cyclopentanol Benzoate (19). The procedure followed is that of Marion and Sargeant.[37] From 47 mg (0.12 mmol) of olefin **18** was obtained 58 mg of crude aldehyde, which was purified by TLC (silica gel GF-254, 9:1 benzene-ether), giving 31 mg (66%) of **19** as a colorless gum. The NMR data show **19** to be a mixture of diastereomers: IR ($CHCl_3$) 1718, 1506, 1487, 1278 cm^{-1}; NMR (90 MHz) δ 1.18 (3 H, t, J = 7.5 Hz, CH_2CH_3), 2.64 (2 H, q, J = 7.5 Hz, CH_2CH_3), 5.30 (1 H, m, $CHOCOPh$), 5.72 (d, H_A of AB quartet, OCH_2O), 5.83 (d, H_B of AB quartet, OCH_2O), 5.86 (d, H_A of AB quartet, OCH_2O), 5.89 (H_B of AB quartet, OCH_2O), 6.61 (s, ArH), 6.63 (s, ArH), 6.73 (s, ArH), 6.87 (s, ArH), 7.40 (m, benzoate ArH), 7.80 (m, benzoate ArH), 9.61 (t, $J \simeq 1.5$ Hz, CHO), 9.73 (t, $J \simeq 1.5$ Hz, CHO); MS *m/e* 380 (M^+).

Exact mass. Calcd for $C_{23}H_{24}O_5$: 380.4447. Found: 380.4473.

2-(3′,4′-Methylenedioxy-6′-ethyl)phenyl-3-methylcyclopentanol Benzoate (20). The decarbonylation of aldehyde **19** was effected following the procedure of Ohno and Tsuji.[20] The aldehyde (21 mg, 0.055 mmol) was dissolved in benzene (0.9 mL, saturated with nitrogen) and 58 mg (0.063 mmol) of tris(triphenylphosphine)chlororhodium(I) added. The mixture was stirred and heated at reflux under an atmosphere of nitrogen for 2 h. After cooling to room temperature, solvent was removed by rotary evaporation and several milliliters of absolute ethanol were added. The resulting yellow crystals of bis(triphenylphosphine)chlorocarbonylrhodium(I) were removed by filtration and the filtrate was concentrated in vacuo to an orange oil which was applied to a silica gel GF-254 preparative TLC plate. Development in 9:1 benzene-30-60 °C petroleum ether allowed isolation of 18.5 mg (95%) of a colorless gum identified by spectral analysis as **20**. The NMR data show **20** to be a mixture of diastereomers: IR ($CHCl_3$) 1709, 1505, 1487, 1277 cm^{-1}; NMR (90 MHz) δ 0.99 (3 H, d, J = 6.0 Hz, R_2CHCH_3), 1.18 (3 H, t, J = 7.6 Hz, CH_2CH_3), 2.67 (2 H, q, J = 7.6 Hz, CH_2CH_3), 5.3 (1 H, m, $CHOCOPh$), 5.72 (d, H_A of AB quartet, OCH_2O), 5.87 (m, OCH_2O), 6.61 (1 H, s, ArH), 6.71 (s, ArH), 6.88 (s, ArH), 7.40 (m, benzoate ArH), 7.90 (m, benzoate ArH); MS *m/e* 352 (M^+).

Exact mass. Calcd for $C_{22}H_{24}O_4$: 352.1674. Found: 352.1678.

***N*-Methylseco[*B*,*C*]cephalotax-4-en-3-one (15) from 2,2-Dimethoxy-*N*-methylseco[*B*,*C*]cephalotax-4-en-3-one (11).** Dimethoxyenone **11** (35 mg, 0.098 mmol) was dissolved in 0.625 mL of absolute ethanol. The stirred solution was cooled to 0 °C and 0.125 mL of concentrated sulfuric acid added, producing a heavy white precipitate which made stirring quite difficult. On warming to ambient temperature, the precipitate dissolved, leaving an orange solution to which was added, over the course of 1 h, 84 mg (1.29 mmol) of purified zinc dust.[38] The cloudy yellow-gray suspension was stirred overnight. After filtration and copious washing of the collected solids (mostly excess zinc) with absolute ethanol, the combined filtrate was concentrated via rotary evaporation. The resulting yellow oil was dissolved in a few milliliters of water, to which was added concentrated ammonium hydroxide until the solution was strongly basic to pH paper. The mixture was extracted four times with chloroform and the combined chloroform extracts were filtered and concentrated in vacuo, leaving 35 mg of a yellow oil. Preparative TLC (alumina HF-254, 9:1 benzene-ethyl acetate) yielded 16 mg (55%) of a nearly colorless glass, which was crystallized from ether, affording colorless crystals of enone

15: mp (biphasic) 135, 161–162.5 °C; IR ($CHCl_3$) 1688, 1639, 1619, 1504, 1487 cm^{-1}; NMR (90 MHz) δ 2.04 (3 H, s, NCH_3), 5.86 (1 H, d, H_A of AB quartet, OCH_2O), 5.92 (1 H, d, H_B of AB quartet, OCH_2O), 6.30 (1 H, s, ArH), 6.67 (1 H, s, ArH); UV 198 nm (ϵ 44 300), 230 (26 600), 290 (4700); MS *m/e* 299 (M^+).

Anal. ($C_{18}H_{21}NO_3$) C, H, N.

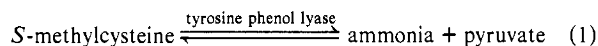
***N*-Methylseco[*B,C*]cephalotax-4-en-3-one (15) from *N*-Methylseco[*B,C*]-2-methoxycephalotax-4-en-3-one (14).** The procedure is the same as that used for zinc reduction of dimethoxyenone **11.** Starting with 65 mg (0.20 mmol) of **14**, 31 mg (53%) of **15** was obtained in the form of a yellow glass, which produced crystals from ether, mp (biphasic) 135–136, 161–162 °C. Samples of **15** produced from **11** and from **14** were mixed, and the resulting mixture melted at 135 and 160.5–163 °C. The NMR spectra of the two samples were identical.

***N*-Methylseco[*B,C*]cephalotaxan-3-one (13) from *N*-Methylseco[*B,C*]-2-methoxycephalotax-4-en-3-one (14).** Methoxyenone **14** (40 mg, 0.12 mmol) was dissolved in 0.75 mL of ethanol. Water (0.7 mL) and 3% sodium amalgam (0.52 g) were added, and the mixture was heated at reflux on a steam bath for 1 h. On cooling to room temperature an oil separated which was extracted by treatment with three aliquots of ether. The combined ether extracts were washed twice with brine, and the extracts were dried over sodium sulfate, filtered, and concentrated in vacuo to afford 29 mg of a yellow gum. Preparative TLC (alumina PF-254, 9:1 benzene–ethyl acetate) yielded 16 mg (44%) of a gum having identical spectral properties with those of ketone **13** prepared by Emde degradation of cephalotaxine methiodide.

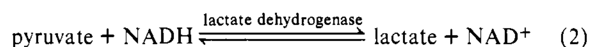
Feeding Experiments. Administration of Labeled Compounds to *Cephalotaxus* and Workup of Plant Material. Whole *Cephalotaxus harringtonia* var. *fastigiata*, 1–2 ft high, were fed via the cotton wick method and the plants allowed to metabolize for a number of weeks. If the plant material were not to be worked up immediately, it was then washed and stored at −20 °C. Plant material was worked up by adding to it a suitable amount (100–1000 mg, depending on the degradation route to be employed) of nonradioactive cephalotaxine as carrier and macerating it in 95% ethanol in a Waring blender. The resulting slurry was poured into a large chromatographic column and extracted with ethanol intermittently over a period of 1 week. The total volume of ethanol employed was perhaps 10 gal. The ethanol extracts were concentrated to a green syrup to which was added several hundred milliliters of 6% tartaric acid solution. The resulting mixture was extracted with chloroform until the chloroform extracts were colorless. After basification with concentrated ammonium hydroxide solution, the aqueous phase was again extracted repeatedly with chloroform. Whenever intractable emulsions were encountered in either extraction process, the entire mixture was filtered through Celite. The chloroform from extraction of the basic aqueous solution was filtered and concentrated by rotary evaporation, affording a viscous, red-brown residue which was applied to alumina PF-254 plates and developed in 9:1 benzene–ethyl acetate. The cephalotaxine isolated thereby was repeatedly crystallized from ether–30–60 °C petroleum ether until constant specific activity was reached.

Isolation of Tyrosine Phenol Lyase. The procedure followed is essentially that of Ellis et al.[22] Three liters of medium (0.5% peptone, 0.5% beef extract, 0.2% NaCl, 0.05 yeast extract, 0.2% L-tyrosine) in six 1-L Erlenmeyer flasks was inoculated from a slant culture of *Escherichia intermedia* AKU 0010 (generously supplied by Professor B. E. Ellis). The flasks were then placed on a rotary shaker at 32 °C for 14 h, after which time the cells were harvested by centrifugation at 6000 rpm for 7 min (Sorvall RC2-B, GSA rotor). All subsequent steps were performed at 0–5 °C. After washing with a dilution buffer comprising 0.01 M potassium phosphate, pH 6.0 and 0.005 M mercaptoethanol, the cells were combined, resuspended in approximately 25 mL of buffer, and disrupted by passage through a French press. The resulting liquid was quickly recooled by immersion in an ice–water bath. Following centrifugation (16 000 rpm, 25 min, SS-34 rotor) the supernatant was brought to 30% of saturation with ammonium sulfate by careful addition of the solid salt. Three hours later the precipitated protein was removed by centrifugation (16 000 rpm, 25 min) and the supernatant brought to 70% of saturation with ammonium sulfate. The suspension was subjected to centrifugation on the following morning and the protein pellet dissolved in minimal buffer solution and dialyzed overnight against 2 L of the same buffer. A small sample of the resulting enzyme solution was reserved for assay and the remainder frozen (−17 °C).

Assay for Tyrosine Phenol Lyase Activity. The procedure used is based on that of Bulos and Handler[40] and was brought to our attention by Dr. J. Henkin, who also made specific suggestions for the adaptation to our enzyme system of the coupled assay system used. The principle of the assay system is as follows:



$$S\text{-methylcysteine} \xrightleftharpoons{\text{tyrosine phenol lyase}} \text{ammonia} + \text{pyruvate} \quad (1)$$



$$\text{pyruvate} + \text{NADH} \xrightleftharpoons{\text{lactate dehydrogenase}} \text{lactate} + \text{NAD}^+ \quad (2)$$

Pyruvate produced by the action of tyrosine phenol lyase is rapidly trapped by lactate dehydrogenase, reducing the keto acid to lactate and oxidizing NADH to NAD^+ in the process. Lactate dehydrogenase is present in excess so that reaction 2 does not become rate limiting. The rate of pyruvate production is measured by the decrease in absorbance at 340 nm, the molar extinction coefficient of NADH at that wavelength being taken as 6.2×10^3.[41] The activity of tyrosine phenol lyase was determined in an incubation mixture containing 0.90 mL of 0.2 M potassium phosphate at pH 7.8, 0.2 μmol of pyridoxal phosphate, 2 μL of lactate dehydrogenase suspension (24.5 mg/mL, 175 units/mg, one unit being the amount of enzyme necessary to catalyze the oxidation of 1 μmol of NADH per min at 25 °C), 5 μL of a solution of 1–2 mg of NADH in 0.1 mL of water, 2.45 mg of *S*-methylcysteine in 100 μL of water, and 5 μL of tyrosine phenol lyase solution. A 1-mL cuvette was placed in the water-jacketed (30 °C) cuvette holder of a Gilford Zeiss spectrophotometer, and the decrease in absorbance at 340 nm plotted against time. Increasing turbidity of the incubation mixture produced a logarithmic flattening of the resulting slope, so that the initial slope was the one used in activity calculations. The slope of a blank control (incubation mixture minus *S*-methylcysteine) was subtracted from that determined for the complete assay solution so as to correct for any extraneous NADH oxidases present in the tyrosine phenol lyase solution. A typical enzyme preparation yielded 108 units of tyrosine phenol lyase (4.68 U/mL), 1 unit of enzyme activity defined as the amount necessary to catalyze the formation of 1 μmol of pyruvate per min.

[1-^{14}C]Aniline Hydrochloride.[42] [1-^{14}C]Benzoic acid (1.0 mCi, 37.5 mg, 0.31 mmol) was placed in a dry 5-mL round-bottom flask with a magnetic stir bar and drying tube and was dissolved in 0.25 mL of chloroform. The flask was immersed in a 40 °C oil bath, and after addition of 0.12 mL of 100% sulfuric acid (made from a mixture of reagent grade sulfuric acid and fuming sulfuric acid, 30% sulfur trioxide) 31 mg (0.48 mmol) of sodium azide was added portionwise over a period of 80 min. Four hours from the start of the reaction, several milliliters of water were added, the contents of the flask cooled (0 °C), and two pellets of solid sodium hydroxide added, yielding a cloudy, strongly basic suspension. Extraction with three aliquots of ether followed by drying over anhydrous sodium sulfate and careful removal of volatiles in vacuo afforded a cloudy yellow oil which was dissolved in chloroform and filtered to remove residual water. Removal of the chloroform in vacuo left a clear yellow oil which was dissolved in 1 mL of methanol and treated with methanolic hydrogen chloride (equivalent to the theoretical yield). Addition of ether produced a precipitate of fine, white crystals (32 mg, 80%) of [1-^{14}C]aniline hydrochloride, which was diazotized directly.

[1-^{14}C]Phenol. To a magnetically stirred solution of 32 mg (0.25 mmol) of [1-^{14}C]aniline hydrochloride in 1.7 mL of 0.5 N hydrochloric acid at 0 °C was added dropwise part of an aqueous solution of sodium nitrite (220 mg in 3 mL). The addition was continued until the reaction mixture produced a strongly positive potassium iodide–starch test which did not abate after several minutes. The acid (5 mL of 12.5% sulfuric acid in water) used to effect the hydrolysis of the diazonium salt was placed in a 15-mL three-necked round-bottom flask to the necks of which had been affixed respectively a 10-mL addition funnel (without side arm), a Vigreux column leading to a short-path distillation apparatus, and a glass stopper. The flask was immersed in a wax bath at 175–180 °C, and, when distillate (which was collected in an Erlenmeyer flask at 0 °C) began to appear, the diazonium salt solution was added several drops at a time through the addition funnel. Care was taken to maintain the temperature of the solution at 0 °C until it entered the refluxing acid solution. Small quantities of water were added to wash through residual diazonium salt, and, when the addition of the diazonium salt solution had been completed, water was added to keep the volume of the hydrolysis mixture constant until 30 mL of distillate had been collected. At this

point the distillation was interrupted, and solid sodium chloride was added to the distillate until saturation had been reached. The aqueous solution was extracted four times with ether and the combined ether extracts were extracted in turn with four portions of 1 N sodium hydroxide. The basic solution was washed thrice with ether to remove neutral impurities, after which concentrated aqueous hydrochloric acid was added until the solution was strongly acidic. The aqueous solution was saturated with sodium chloride and extracted with four portions of ether, which were combined, dried over anhydrous magnesium sulfate, filtered, and concentrated quite carefully in vacuo, leaving a pale yellow oil. The crude phenol was purified by bulb-to-bulb distillation, dissolved in phosphate buffer, and used immediately for the enzymatic synthesis of [*p*-^{14}C]-L-tyrosine. An identical synthesis of phenol yielded 22 mg of product from 33 mg of aniline hydrochloride (94% yield).

[*p*-^{14}C]-L-Tyrosine. The method of Ellis et al.[22] was employed for the incubation at 30 °C of 22 mg (0.23 mmol) of [1-^{14}C]phenol (the presumed yield from the synthesis of [1-^{14}C]phenol from [1-^{14}C]-aniline hydrochloride as described above), 117 mL of 0.5 M potassium phosphate (pH 7.8), 24.3 mg (0.092 mmol) of pyridoxal phosphate monohydrate, 3.16 g (23 mmol) of *S*-methylcysteine, 235 mL of water, and 56.2 units of tyrosine phenol lyase. Four hours after combination of the above components, sufficient concentrated aqueous hydrochloric acid was added to bring the pH to 1, bleaching the formerly yellow solution and producing a sticky, white precipitate of protein, which was removed by centrifugation at 6000 rpm for 10 min. The supernatant was concentrated in vacuo to approximately 20 mL and the liquid (phosphate salts had separated during concentration) applied to a 70 × 1.5 cm column of Dowex 50W × 12 (200-400 mesh) in 2.5 N hydrochloric acid. One hundred and two 17-mL fractions were collected and one of every four samples was analyzed for radioactivity. Fractions 50-86 showed values considerably above background, and comparatively negligible activity was observed in all other fractions. The active fractions were combined and concentrated to dryness in vacuo, and the residue was dissolved in 5 mL of 2 N hydrochloric acid. Addition of solid sodium bicarbonate produced an off-white precipitate at pH 5-6. After sitting at 5 °C for several hours, the precipitated tyrosine was collected by vacuum filtration, washed with water, and dried in vacuo, yielding 28 mg of material which after recrystallization from water was radiochemically and chemically (ninhydrin visualization) homogeneous by paper chromatography (Whatman No. 1, 25:4:10 1-butanol-glacial acetic acid-water). The purified product weighed 22 mg (0.37 μCi), and represented a 39.6% chemical (37.0% radiochemical) yield based on [1-^{14}C]benzoic acid. The same procedure was used for the synthesis of [ring-^{14}C]-L-tyrosine from [U-^{14}C]phenol.

Oxidation of Cephalotaxine to 4,5-Methylenedioxyphthalic Acid and Conversion of the Latter to 4,5-Methylenedioxyanthranilic Acid. Cephalotaxine (223 mg, 0.71 mmol) was subjected to potassium permanganate oxidation according to the method of Leete.[23] Leete's procedure was also followed in conversion of the resulting 4,5-methylenedioxyphthalic acid to 4,5-methylenedioxyanthranilic acid. The following modifications were made: the sublimed *N*-ethylhydrastimide was further purified by preparative TLC (silica gel GF-254, 9:1 benzene-chloroform). Crystallization from absolute ethanol yielded 8 mg of *N*-ethylhydrastimide (5.4% from cephalotaxine), mp 169.5-170.5 °C (lit.[45] 166-167 °C). *N*-Ethylhydrastimide (89 mg, 0.41 mmol) yielded 39 mg of hydrastimide. The 4,5-methylenedioxyanthranilic acid (9 mg) formed from hydrastimide (36 mg) was, without further purification, esterified by treating a methanolic solution with an excess of diazomethane in ethanol-ether. The resulting amino ester was purified by preparative TLC (alumina PF-254, 98:2 benzene-ethyl acetate) and crystallized from cyclohexane, forming tan needles, mp 107-108.5 °C (lit.[43] 108.5 °C).

[1-^{14}C]Cinnamic Acid. Ba^{14}CO$_3$ (17 mg, 0.086 mmol, 4.8 mCi, New England Nuclear) was placed in one of the tubes of a small-scale Grignard CO_2 generator while 35 mg (0.18 mmol) of unlabeled $BaCO_3$ was placed in the second tube. Concentrated sulfuric acid (1 mL) was placed in the third tube. Styrylmagnesium bromide (0.55 mL, 0.25 mmol) in ether was placed in a 10-mL round-bottom flask attached to CO_2 generator. The flask was chilled to −78 °C and briefly evacuated. It was then evacuated to 0.1 mmHg while the flask was cooled in liquid nitrogen. The system was closed and the concentrated sulfuric acid was introduced into the Ba^{14}CO$_3$ to generate $^{14}CO_2$ which was trapped in the reaction flask chilled in liquid nitrogen. When the bubbling of $^{14}CO_2$ had stopped (about 10 min), the reaction flask was gradually warmed to −50 to −60 °C and kept there for 2 h with stirring. The reaction flask was then chilled again in liquid nitrogen, and concentrated sulfuric acid was poured into the tube containing the unlabeled $BaCO_3$. The CO_2 generated was trapped in the reaction flask. After the bubbling of CO_2 had stopped, the reaction flask was warmed to −50 to −60 °C and kept there for 2 h. The temperature was then raised to −20 °C for 1 h and finally to room temperature; the reaction mixture was then stirred for 15 h. Argon was introduced into the apparatus, and 2 mL of 2 N hydrochloric acid was added slowly. The layers were separated and the aqueous layer was extracted three times with chloroform. The combined organic extracts were back-extracted with 2 mL of 1 N sodium hydroxide solution. The aqueous layer, containing the sodium salt of [1-^{14}C]-cinnamic acid, was washed twice with 1-mL portions of chloroform and reacidified with concentrated hydrochloric acid. The cloudy aqueous layer was then extracted three times with chloroform. The extract was filtered and the chloroform removed in vacuo to give a yellow oil. This was redissolved in hot water and treated with Norit. The solution was filtered hot and the resulting colorless liquid was allowed to cool slowly. [1-^{14}C]Cinnamic acid precipitated. It was recrystallized from hot water to yield [1-^{14}C]cinnamic acid (7.5 mg) which had a specific activity of 0.13 mCi/mg. The radiochemical yield was 24% from Ba^{14}CO$_3$. The radiochemical purity was checked by thin layer chromatography (silica, 1:4 methanol-ethyl acetate with trace of acetic acid) and radioscanning and found to be greater than 99.5%.

3(*RS*)-[^{3}H]-DL-Phenylalanine. A. [α-^{3}H]Benzyl Alcohol. Benzaldehyde (106 mg, 1 mmol, freshly distilled) was dissolved in 0.5 mL of absolute ethanol, unlabeled $NaBH_4$ (2 mg) was added, and the mixture was stirred at room temperature under a nitrogen atmosphere for 10 min. The solution was transferred to a vial containing 100 mCi of KB^{3}H$_4$ (17.9 mg, 0.33 mmol, New England Nuclear). The reaction mixture was then stirred at ambient temperature under an argon atmosphere for 24 h. Unlabeled $NaBH_4$ (30 mg, 0.79 mmol) was added at the end of the period, and the mixture stirred for an additional 12 h. The ethanol was removed in vacuo. Sodium hydroxide solution (20%) was added to the residue, and the mixture extracted four times with 1-mL portions of chloroform. The combined extracts were filtered and the chloroform was removed in vacuo. [α-^{3}H]Benzyl alcohol (120 mg) was recovered.

B. 3(*RS*)-[^{3}H]-DL-Phenylalanine. [α-^{3}H]Benzyl alcohol (35 mg, 0.32 mmol, 0.61 mCi/mg) was stirred with 1 mL of thionyl chloride and 2 mL of absolute ether at ambient temperature for 24 h. The excess thionyl chloride and ether were removed in vacuo. Water (1 mL) was added and the mixture stirred for 0.5 h. The mixture was extracted three times with ether, and the ether extracts were dried over anhydrous magnesium sulfate. The ether was removed in vacuo to leave a colorless oil that was [α-^{3}H]benzyl chloride (27.5 mg).

In a 5-mL round-bottom flask, 0.15 mL of sodium ethoxide solution (prepared from 0.89 g of sodium dissolved in 15 mL of absolute ethanol) was added to 47 mg of ethyl acetamidocyanoacetate (0.28 mmol) in 0.3 mL of ethanol. The mixture was added to the [^{3}H]benzyl chloride and heated to reflux for 1 h. It was cooled to room temperature and the ethanol removed in vacuo. Water (1 mL) and ethyl acetate (1 mL) were added, and the organic layer was separated and washed twice with 0.5-mL portions of water; it was dried over anhydrous magnesium sulfate and the ethyl acetate removed in vacuo. The residue was recrystallized from methanol at 0 °C. The ethyl [3-^{3}H]-2-acetamido-2-cyano-3-phenylpropionate (50.5 mg) obtained had a specific activity of 0.22 mCi/mg. The foregoing ester was heated with 0.3 mL of 48% hydrobromic acid to reflux for 1 h. The solution was cooled to room temperature, and 0.5 mL of water added. The aqueous layer was washed twice with 1-mL portions of ether. The water and excess hydrobromic acid were removed in vacuo, and the residual oil was pumped on an oil pump to remove traces of HBr. The oil was taken up in 0.5 mL of water and the pH adjusted to 5.0 with saturated sodium acetate solution. The [3-^{3}H]-DL-phenylalanine precipitated upon cooling in the refrigerator for 24 h. It was recrystallized from aqueous ethanol. The resulting [3-^{3}H]-DL-phenylalanine (7.0 mg) had specific activity of 0.333 mCi/mg. The radiochemical purity of the [3-^{3}H]-DL-phenylalanine was estimated by paper chromatography (BAW, 25:4:10) and radioscanning to be greater than 99.5%.

[*p*-^{14}C]-DL-Phenylalanine. A. [1-^{14}C]-4-Nitrophenol. In a small-scale Grignard CO_2 generator, Ba^{14}CO$_3$ (102.7 mg, 0.52 mmol, 25 mCi, Amersham Searle Co., specific activity 47 mCi/mmol) was

placed in one of the tubes while 100 mg (0.51 mmol) of unlabeled $BaCO_3$ was placed in the second tube. Concentrated sulfuric acid (5 mL) was placed in the third tube. CH_3MgI (4.4 mmol, 2 mL in ether, Alfa Ventron) was added to 10 mL of freshly opened anhydrous ether and the solution stirred and chilled to −78 °C in a 25-mL round-bottom flask attached to the CO_2 generator. The system was briefly evacuated to 0.1 mmHg while the reaction flask was cooled in liquid N_2. The system was then closed and the concentrated sulfuric acid was introduced into $Ba^{14}CO_3$ to generate $^{14}CO_2$ which was trapped in the reaction flask chilled in liquid N_2. When the bubbling of $^{14}CO_2$ had stopped (about 10 min), the reaction flask was gradually warmed to −50 to −60 °C and kept there for 2 h with stirring. The reaction flask was then chilled again in liquid N_2, and the concentrated sulfuric acid was poured into the tube containing the unlabeled $BaCO_3$. The CO_2 generated was trapped in the reaction flask. After bubbling of CO_2 had stopped, the reaction flask was warmed to −50 to −60 °C and kept there for 2 h. The temperature was then raised to −20 °C for 1 h and finally to room temperature; the reaction mixture was then stirred for 15 h. Argon was introduced into the apparatus and 5 mL of water was added, followed by 2 mL of 20% NaOH. The aqueous layer was transferred to a 250-mL three-neck round-bottom flask and distilled (oil bath temperature 125–130 °C) and the radioactivity of the distillate was monitored by liquid scintillation counting; the distillation was stopped when the activity level dropped to 10^3 dpm/μL. About 150 mL of distillate was collected, pH 3.56; it was adjusted to pH 9.60 with saturated $Ba(OH)_2$ solution and the water removed in vacuo. Absolute ethanol (5 mL) was added and the solution evaporated to dryness. The white solid $Ba(OO^{14}CCH_3)_2$ was pumped on with an oil pump for 24 h. It then weighed 112.7 mg (88% yield, specific activity was not measured). The [^{14}C]barium acetate was transferred to a T-shaped quartz pyrolysis tube. The apparatus was evacuated to 0.1 mmHg and then sealed off. The end of the tube which contained [^{14}C]barium acetate was heated at 500 °C for 0.5 h with a cold finger cooled in liquid nitrogen on the other end of the tube. The heated end of the pyrolysis tube was allowed to cool to room temperature and argon was let in to break the vacuum. Water (5 mL) was added to the cold finger to dilute the [2-^{14}C]acetone generated by pyrolysis. It was transferred to a 100-mL round-bottom flask containing 0.47 g of sodium nitromalondialdehyde (3.4 mmol), 10 mL of water, and 0.15 mL of 20% sodium hydroxide solution. The reaction mixture was stirred at 0 °C. The cold finger was rinsed twice with 2-mL aliquots of water and the rinse water was added to the reaction flask. The mixture was stirred at 0–3 °C in the dark for 72 h, then stirred at room temperature for an additional 120 h. At the end of the period, the dark red solution was neutralized with CO_2 gas to pH 6.0 and continuously extracted with ether for 96 h. The bright yellow ether extract was dried over anhydrous sodium sulfate and ether removed in vacuo. A brown oil (194 mg) containing [1-^{14}C]-*p*-nitrophenol was recovered. TLC (silica, 1:1 benzene–ethyl acetate) followed by radioscanning showed no radiolabeled impurities. Preparative TLC (silica PF-254, 1:1 benzene–ethyl acetate. R_f 0.43) gave [1-^{14}C]-4-nitrophenol as off-white crystals (93 mg, specific activity 45 μCi/mg). The radiochemical yield from $Ba^{14}CO_3$ was 17% (there is a 50% loss of carbon-14 in the pyrolysis step).

B. **[*p*-^{14}C]-DL-Phenylalanine.** [1-^{14}C]-4-Nitrophenol (93 mg, 0.67 mmol) was dissolved in 3 mL of absolute ethanol and 5% palladium on carbon catalyst (20 mg) was added. Catalytic hydrogenation at atmospheric pressure was carried out until the calculated quantity of hydrogen had been absorbed. The catalyst was filtered off under an argon atmosphere and the ethanol was removed in vacuo. Acetone (3 mL) was added to the residue followed by 120 mg of 5-chloro-1-phenyltetrazole (0.66 mmol) and 320 mg of anhydrous K_2CO_3. The reaction mixture was flushed with argon and heated to reflux for 24 h. The inorganic salts were filtered off through a sintered glass funnel and washed thoroughly with acetone. The acetone was removed in vacuo to leave 210 mg of crude [1-^{14}C]-1-(1′-phenyltetrazoyl) 4-aminophenyl ether. Recrystallization of the crude ether from benzene gave 174 mg of pure material. This was suspended in 3 mL of benzene with 40 mg of 5% palladium on carbon catalyst and the mixture hydrogenated at 35 °C at atmospheric pressure. After 9 h, the expected quantity of hydrogen had been absorbed. The catalyst was then removed by filtration and washed with benzene. The combined benzene extracts were evaporated in vacuo below room temperature and the crude [*p*-^{14}C]aniline was separated from 1-phenyltetrazolone by preparative TLC (alumina PF-254, benzene, R_f 0.55) under an argon atmosphere. The [*p*-^{14}C]analine was quickly eluted from the alumina by extraction with warm ethyl acetate. Dry HCl gas was bubbled through the ethyl acetate solution, and the resulting mixture was taken to dryness in vacuo to leave [*p*-^{14}C]aniline hydrochloride (33 mg). This was dissolved in 0.5 mL of acetone, the solution cooled to 0 °C, and hydrobromic acid added (0.1 mL, 48%). Aqueous sodium nitrite solution (5 M, 0.11 mL) was added with stirring and cooling. After 10 min at 3–5 °C, acrylic acid (0.12 mL) and cuprous bromide (2 mg) were added. The light yellow reaction mixture turned dark red immediately and bubbles of nitrogen gas were visible. The reaction mixture was slowly warmed to room temperature and held there for 1 h. Water (1 mL) was added and the brown oil that separated was extracted with 4 × 1 mL of ethyl acetate. The combined ethyl acetate extracts were dried over anhydrous sodium sulfate and the ethyl acetate was removed in vacuo. The brown residue was pumped on with an oil pump for 24 h to remove excess acrylic acid. Crude [*p*-^{14}C]-α-bromo-β-phenylpropionic acid (102 mg) was recovered. The compound was examined by analytical TLC (silica, 1:1 methanol–ethyl acetate plus trace of acetic acid) and radioscanning. The radiochemical purity was at least 95%. The crude [*p*-^{14}C]-α-bromo-β-phenylpropionic acid was stirred with concentrated ammonium hydroxide at ca. 3 °C for 48 h, then at room temperature for 24 h. At the end of the period, the reaction mixture was extracted thrice with chloroform. The aqueous phase was then acidified with 1 N hydrochloric acid and extracted again with chloroform. Upon removal of chloroform, [*p*-^{14}C]cinnamic acid (6 mg) was recovered as a minor product. The aqueous phase contained [*p*-^{14}C]-DL-phenylalanine, which was purified by preparative paper chromatography (1-butanol–acetic acid–water, 20:5:12, R_f 0.60). Radioscanning was used to locate the [*p*-^{14}C]phenylalanine on the paper. The band containing [*p*-^{14}C]phenylalanine was cut into small pieces and extracted three times with 10-mL portions of water. The extract was filtered through a sintered glass funnel and the water removed in vacuo. A light yellow oil (17 mg) was obtained. It was crystallized from 90% aqueous ethanol in a Craig tube. The final crystalline product (11 mg), [*p*-^{14}C]-DL-phenylalanine, had a specific activity of 25.1 μCi/mg. The radiochemical yield from $Ba^{14}CO_3$ was 2.7%. The radiochemical purity was estimated by radioscanning to be greater than 99% ([*p*-^{14}C]phenylalanine chromatographed on a paper strip using 1-butanol–acetic acid–water, 25:4:10).

[*m*-^{14}C]-DL-Phenylalanine. [*m*-^{14}C]Phenylalanine was synthesized on the same scale as that used in the preparation of [*p*-^{14}C]phenylalanine. The same reaction sequence was also used for making [*m*-^{14}C]phenylalanine, starting with [2-^{14}C]barium acetate (5 mCi, 65–70 mCi/mmol, Amersham Searle Co.).

***N*-Methylseco[*B*,*C*]-3-phenylcephalotaxan-3-ol.** A solution of phenyllithium (0.7 mL, 2.2 M solution in hexane) was added to a stirred solution of 50 mg (0.166 mmol) of ketone **13** in 2 mL of anhydrous ether at ambient temperature under an argon atmosphere. The mixture was stirred for 7 h and the color of the solution changed from brown to light yellow and finally to orange. Water (2 mL) was added and the mixture stirred for 10 min. The layers were partitioned and the aqueous layer was extracted repeatedly with ether. The ether extract was washed with brine until neutral and then dried over anhydrous sodium sulfate. Volatiles were removed in vacuo; the residue weighed 123 mg and had a distinct odor of biphenyl. Preparative thin layer chromatography (alumina PF-254, 5% ethyl acetate in benzene, R_f 0.29) yielded 43 mg of alcohol as a colorless gum (68% yield). It was crystallized from ether–hexane to give colorless crystals: mp 148–149 °C; IR ($CHCl_3$) 3570, 1500, 1476 cm^{-1}; NMR (90 MHz) δ 1.99 (3 H, s, NCH_3), 4.24 (1 H, d, J = 8.0 Hz, $ArCHR_2$), 5.59 (2 H, s, OCH_2O), 6.13 (1 H, s, ArH), 6.69 (1 H, s, ArH), 6.99 (5 H, m, PhH); MS *m/e* 379 (M^+).

Anal. ($C_{24}H_{29}NO_3$) C, H, N.

Chromic Acid Oxidation of *N*-Methylseco[*B*,*C*]-3-phenylcephalotaxin-3-ol. A 50-mL three-neck round-bottom flask was equipped with a magnetic stirrer, a nitrogen inlet, and a short-path distillation condenser. A solution of 2 g of purified CrO_3 in 7 mL of 10% sulfuric acid was stirred into the flask and heated at 115–130 °C in an oil bath. A solution of 50 mg of *N*-methylseco[*B*,*C*]-3-phenylcephalotaxan-3-ol (0.13 mm) in 2 mL of 10% sulfuric acid was added. The reaction mixture was distilled for 3 h with a moderate nitrogen flow. The distillate was collected in a receiving flask that contained 5 mL of 10% sodium hydroxide solution cooled in an ice-salt bath. Water was added to the reaction flask periodically to keep the total volume the same. At the end of a 3-h period, about 35 mL of distillate had been collected. It was concentrated to a volume of 5 mL, acidified with 6 N hydro-

chloric acid to pH 1, and extracted repeatedly with ether. The ether extract was dried over anhydrous magnesium sulfate and the ether was removed in vacuo. Benzoic acid (7.0 mg) was obtained (43.5% yield). It was recrystallized twice from hot water, mp 122 °C.

2-Phenylisocephalotaxine (22). Isocephalotaxinone (**21**,[31] 26 mg, 0.08 mmol) was dissolved in 1 mL of freshly opened anhydrous ether, the solution was stirred at 0 °C, and 0.5 mL of 2.2 M phenyllithium solution (Alfa Ventron) was syringed in under an argon atmosphere. The yellow solution was slowly warmed to room temperature and then stirred for 7 h. Water (1 mL) was added to the orange solution, the ether layer separated, and the aqueous layer extracted thrice with ether. The organic phase was back-washed with brine until neutral and dried over anhydrous sodium sulfate and the ether was removed in vacuo. Preparative TLC of the residue (alumina, 5% methanol in ethyl acetate, R_f 0.28) gave 20 mg (62%) of **22:** IR ($CHCl_3$) 3555, 1650, 1500, 1490 cm^{-1}; NMR (90 MHz) δ 2.22 (2 H, AB quartet, $J \simeq$ 13 Hz, C-1 CH_2), 3.45 (3 H, s, OCH_3), 4.00 (1 H, broad s, OH), 5.92 (2 H, s, OCH_2O), 6.59 (1 H, s, ArH), 6.87 (1 H, s, ArH), 7.25–7.62 (5 H, m, PhH); MS *m/e* 391 (M^+).

Anal. ($C_{23}H_{25}NO_4$) C, H, N.

Chromic Acid Oxidation of 2-Phenylisocephalotaxine. The oxidation was carried out in a manner completely analogous to the oxidation of *N*-methylseco[*B*,*C*]-3-phenylcephalotaxan-3-ol. From 21 mg (0.055 mmol) of 2-phenylisocephalotaxine, 2.5 mg (34%) of benzoic acid was obtained.

Demethylcephalotaxine (23) from Cephalotaxine (1). Cephalotaxine (100 mg, 0.32 mmol) isolated from *Cephalotaxus harringtonia* was heated with 1 N sulfuric acid (5 mL) under an argon atmosphere at 85 °C for 17 h. The mixture was allowed to cool to room temperature and then neutralized with solid sodium bicarbonate. The solution was extracted with 4 × 1 mL of $CHCl_3$, the combined $CHCl_3$ extracts were filtered, and the solvent was removed in vacuo. The residual oil (71 mg) was purified by preparative TLC (silica PF-254, 1:4 methanol–ethyl acetate) to give demethylcephalotaxine (45 mg, 47%, R_f 0.11) and demethylcephalotaxinone (12 mg, 14%, R_f 0.23). Spectral data indicated that the demethylcephalotaxine was a mixture of four isomeric compounds; IR ($CHCl_3$) 3470, 1710, 1503, 1455 cm^{-1}; NMR (90 MHz) δ 3.53 (d, 0.1 H, J = 4 Hz, C-4 H of one epimer of **23**), 3.59 (d, 0.1 H, J = 4 Hz, C-4 H of other epimer of **23**), 4.25–4.43 (m, 0.8 H, C-3 H of both epimers of **23** accounting for 0.2 H plus C-2 H of one epimer of **24** accounting for 0.6 H), 4.53–4.68 (t, 0.2 H, $J \simeq$ 4 Hz, C-2 H of other epimer of **24**), 5.85 (s, 0.25 H, OCH_2O of **23**), 5.89 (s, 0.75 H, OCH_2O of **24**), 6.36–6.71 (singlets totaling 2 H, Ar-H); MS *me/* 301 (M^+).

Anal. ($C_{17}H_{19}NO_4$) C, H, N.

Isocephalotaxine (25). Isocephalotaxinone (**21**, 17 mg, 0.06 mmol) was dissolved in 1 mL of absolute ethanol contained in a 10-mL round-bottom flask. Sodium borohydride (14 mg, 0.37 mmol) was added in one portion under an argon atmosphere at ambient temperature. The mixture was stirred for 24 h. The solvent was removed in vacuo, 1 mL of 15% sodium hydroxide solution added, and the mixture extracted three times with chloroform. The combined chloroform extracts were filtered and the chloroform was removed in vacuo. The resulting colorless oil was purified by thin layer chromatography (alumina, 10% ethyl acetate in benzene, R_f 0.15). Isocephalotaxine was recovered as a colorless oil (16 mg, 95% yield). It failed to crystallize from ether or ethyl acetate: IR 3545, 3300, 1650 cm^{-1}; NMR (90 MHz) δ 3.74 (3 H, s, OCH_3), 3.91 (1 H, broad s, OH), 4.63–4.81 (1 H, m, C*H*OH), 5.96 (2 H, s, OCH_2O), 6.66 (1 H, s, ArH), 6.75 (1 H, s, ArH).

Exact mass. Calcd for $C_{18}H_{21}NO_2$: 283.1572. Found: 283.1567.

Demethylcephalotaxine (23) from Isocephalotaxine (25). Isocephalotaxine (**25**, 94 mg, 0.3 mmol) was heated with 5 mL of 1 N H_2SO_4 at 90 °C under an argon atmosphere for 17 h. At the end of the reaction period, the mixture was allowed to cool to room temperature and then neutralized with solid sodium bicarbonate. The mixture was extracted repeatedly with chloroform, the chloroform filtered, and the solvent removed in vacuo. Preparative TLC of the residue (silica, 20% methanol in ethyl acetate) gave 43 mg of a yellow oil (R_f 0.11) (47% yield). It was identical in every aspect with the product derived from cephalotaxine by hydrolysis under the same conditions.

Demethylcephalotaxinone (3) from Demethylcephalotaxine (23). Demethylcephalotaxine (85 mg) was dissolved in 1 N H_2SO_4 (5 mL) and the solution heated at 85 °C in the presence of air for 14 h. At the end of this time, the solution was cooled, neutralized with solid sodium bicarbonate, and extracted repeatedly with $CHCl_3$. Evaporation of the $CHCl_3$ gave the crude product, which was purified by preparative TLC (silica, 20% MeOH in EtOAc) to give **33**. Recrystallization of the chromatographed material from methanol yielded 65 mg (76%) of pure demethylcephalotaxinone which was identical in all respects with material prepared by acid-catalyzed hydrolysis[6] of cephalotaxinone.

[3-^{14}C]-DL-Demethylcephalotaxinone. The procedure was derived from Weinreb and Auerbach.[31]

A. [1-^{14}C]-2-Oxo-1-(5,8,9,10-tetrahydro-6*H*-1,3-dioxolo[4,5-*h*]pyrrolo[2,1-*b*][3]benzazepin-11-yl)-1-propanone. [1-^{14}C]Sodium pyruvate (6.4 mg, 0.06 mmol, 0.5 mCi, NEN) was dissolved in 0.4 mL of CH_3CN, 15 μL of ethyl chloroformate was added, and the mixture was stirred at 0 °C for 2 h. In a separate round-bottom flask, 92 mg (1.05 mmol) of pyruvic acid was dissolved in 0.5 mL of CH_3CN and 0.27 g of anhydrous sodium bicarbonate added. The mixture was stirred at room temperature for 0.5 h and then chilled at 0 °C. Ethyl chloroformate (100 μL, 0.83 mmol) was added and the solution stirred for 2 h. In the meantime, a solution of 100 mg (0.44 mmol) of 5,8,9,10-tetrahydro-6*H*-1,3-dioxolo[4,5-*h*]pyrrolo[2,1-*b*][3]-benzazepine in 0.5 mL of CH_3CN was added to the first flask containing the [^{14}C]pyruvate adduct. The mixture turned brown immediately. The brown solution was stirred at 30 °C for 1 h. The mixture in the second flask was added to the first flask and the resulting mixture stirred for another 2 h at 30 °C. The solution was filtered through a sintered glass funnel to remove the inorganic salts, the salts were washed thoroughly with $CHCl_3$, and the combined organic solutions were concentrated to a total volume of 2 mL. The concentrated organic phase was washed twice with 0.5 mL of water, twice with a 0.5-mL portion of saturated sodium bicarbonate solution, then twice with water again. The organic layer was dried over anhydrous magnesium sulfate and the $CHCl_3$ removed in vacuo to yield 105 mg of a dark brown oil. Preparative TLC (silica PF-254; 1:1 benzene–ethyl acetate, R_f 0.4) of the oil yielded 81 mg of a bright orange solid. It was recrystallized from ethyl acetate–hexane in a Craig tube. Orange crystals (77 mg) were recovered after drying in vacuo in the Abderhalen at 55 °C for 4 h (64% yield), mp 151.5–153 °C, specific activity 5.93 μCi/mg (91% radiochemical yield), lit. mp 155 °C.

B. [3-^{14}C]-DL-Demethylcephalotaxinone. [1-^{14}C]-2-Oxo-1-(5,8,9,10-tetrahydro-6*H*-1,3-dioxolo[4,5-*h*]pyrrolo[2,1-*b*][3]benzazepin-11-yl)-1-propanone (70 mg, 415 μCi) was dissolved in 0.75 mL of dry methanol in a 10-mL round-bottom flask. The flask was equipped with a drying tube and flushed with argon, and the mixture was stirred at 0–3 °C. Four milliliters of freshly prepared 1 N $Mg(OCH_3)_2$ in methanol was added and the reaction flask was kept at 0–3 °C for 2 h. Concentrated H_2SO_4 (0.4 mL) was added followed by 0.2 mL of water and the mixture was stirred at room temperature for 1 h. The pH was adjusted to 8.0 by addition of solid sodium bicarbonate, 2 mL of water was added, and the suspended solid was filtered off and washed with chloroform. The aqueous layer was then extracted thrice with chloroform. The combined $CHCl_3$ extracts were filtered and the chloroform was removed in vacuo. Preparative TLC (silica, 1:4 methanol–ethyl acetate, R_f 0.37) of the residue yielded 55 mg of a light yellow oil which crystallized from methanol at −20 °C to give 38.5 mg of demethylcephalotaxinone (55% yield, specific activity 5.92 μCi/mg, radiochemical yield 55%). Radiochemical purity was found by radioscanning to be greater than 99.5%.

[3-^{14}C]-DL-Cephalotaxinone. The preparation was adapted from Weinreb.[31] In a 10-mL round-bottom flask equipped with a Dean-Stark trap and reflux condenser, a mixture of 27.5 mg of [3-^{14}C]-demethylcephalotaxinone (162 μCi), 2.0 mL of freshly distilled *p*-dioxane, 2.0 mL of distilled 2,2-dimethoxypropane, and 76 mg of *p*-toluenesulfonic acid monohydrate was heated at 95–100 °C for 1 h. When the temperature was raised to 125 °C for 0.5 h, some liquid collected in the Dean-Stark trap. The temperature was brought down to 95–100 °C again and was kept there for 18 h. At the end of an 18-h period, the temperature was raised to 135 °C to distill off more liquid; the remaining dark red residue was then taken up in $CHCl_3$ and extracted thrice with 1-mL portions of 1 N HCl. The combined aqueous extracts were neutralized with solid sodium bicarbonate and extracted thrice with chloroform, and the extract was dried over anhydrous sodium carbonate. The dried extract was filtered and the chloroform was removed in vacuo. Preparative TLC (alumina PF-254, 7% ethyl acetate in benzene, R_f 0.30) recovered 21.5 mg of colorless oil. It was crystallized from ethyl acetate–ether in a Craig tube to yield 16.1 mg of off-white crystals (specific activity 5.90 μCi/mg, 56.5% yield, 59%

radiochemical yield). The radiochemical purity was estimated by radioscanning to be greater than 99.5%.

[3-^{14}C]-DL-Cephalotaxine. The procedure was adapted from Weinreb and Auerbach.[31] [3-^{14}C]-DL-Cephalotaxinone (9.0 mg, 53 μCi) was reduced with 10 mg of $NaBH_4$ in 1 mL of absolute ethanol at ambient temperature for 24 h. The ethanol was removed in vacuo, and the residue taken up with 1 mL of 15% sodium hydroxide solution. The aqueous suspension was extracted four times with 1-mL portions of chloroform. The combined chloroform extracts were filtered and the chloroform was removed in vacuo. A colorless oil (9.1 mg) was recovered. Preparative TLC (alumina PF-254, 7% ethyl acetate in benzene, R_f 0.13) yielded 5 mg of product which was still an oil. Attempts to crystallize it from ether, ethyl acetate-ether, or hexane were fruitless. Finally, 10 mg of (−)-cephalotaxine (isolated from *Cephalotaxus harringtonia*) was added and the combined cephalotaxine crystallized from ether-hexane to give 14.5 mg of product, specific activity 1.86 μCi/mg, radiochemical yield 51%. The radiochemical purity was shown by radioscanning to be greater than 99.5%.

[3-^{14}C]-DL-Demethylcephalotaxine. [3-^{14}C]Demethylcephalotaxinone (5 mg, specific activity 5.9 μCi/mg) and 10 mg of unlabeled demethylcephalotaxinone were dissolved in 1 mL of methanol. Diazomethane (in ether-ethanol) was added dropwise until the solution was a permanent bright yellow; it was then stirred for an additional 1 h. The excess diazomethane was removed by bubbling nitrogen through the solution. The solvent was removed in vacuo to leave 17 mg of [3-^{14}C]isocephalotaxinone as a yellow oil. This was reduced with 14 mg of $NaBH_4$ in 1 mL of absolute ethanol under an argon atmosphere at ambient temperature for 24 h. The solvent was removed in vacuo, 1 mL of 15% sodium hydroxide solution added, and the mixture extracted thrice with chloroform. The combined chloroform extracts were filtered and the chloroform was removed in vacuo. The resulting [3-^{14}C]isocephalotaxine was heated with 4 mL of 1 N sulfuric acid under an argon atmosphere at 85 °C for 17 h. The reaction mixture was neutralized with solid sodium bicarbonate after cooling to room temperature. Extraction with 4 × 1 mL of chloroform gave a distinctive pinkish aqueous layer. The combined chloroform extracts were filtered and the solvent was removed in vacuo to give 9 mg of a brown oil. Preparative TLC (silica PF-254, 1:4 methanol-ethyl acetate, R_f 0.11) yielded 5.5 mg of a mixture of [3-^{14}C]demethylcephalotaxine (**23**) and the labeled isomeric hydroxy ketones **24**. The specific activity of the mixture was 1.0 μCi/mg (19% radiochemical yield) and the radiochemical purity estimated by radioscanning was >99%.

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References and Notes

(1) (a) Portions of this work have been the subject of preliminary communication: Parry, R. J.; Schwab, J. M. *J. Am. Chem. Soc.* **1975**, *97*, 2555. Schwab, J. M.; Parry, R. J.; Foxman, B. M. *J. Chem. Soc., Chem. Commun.* **1975**, 906. Schwab, J. M.; Chang, M. N. T.; Parry, R. J. *J. Am. Chem. Soc.* **1977**, *99*, 2368. (b) Recipient of a Public Health Service Research Career Development Award (GM-00143/GM-00472) from the National Institute of General Medical Sciences, 1975–1980. (c) Ph.D. Thesis, Brandeis University, 1979. (d) Ph.D. Thesis, Brandeis University, 1976. (e) In part: Department of Chemistry, Brandeis University, Waltham, Mass. 02154.

(2) Paudler, W. W.; Kerley, G. I.; McKay, J. B. *J. Org. Chem.* **1963**, *28*, 2194.

(3) Powell, R. G.; Weisleder, D.; Smith, C. R., Jr.; Wolff, I. A. *Tetrahedron Lett.* **1969**, 4081.

(4) Abraham, D. J.; Rosenstein, R. D.; McGandy, E. L. *Tetrahedron Lett.* **1969**, 4085.

(5) Arora, S. K.; Bates, R. B.; Grady, R. A.; Powell, R. G. *J. Org. Chem.* **1974**, *39*, 1269.

(6) Powell, R. G.; Mikolajczak, K. *Phytochemistry* **1973**, *12*, 2987.

(7) Powell, R. G.; Weisleder, D.; Smith, C. R., Jr.; Rohwedder, W. K. *Tetrahedron Lett.* **1970**, 815.

(8) Paudler, W. W.; McKay, J. *J. Org. Chem.* **1973**, *38*, 2110.

(9) Mikolajczak, K. L.; Powell, R. G.; Smith, C. R., Jr. *Tetrahedron* **1972**, *28*, 1995.

(10) Powell, R. G.; Weisleder, D.; Smith, C. R., Jr. *J. Pharm. Sci.* **1972**, *61*, 1227.

(11) Powell, R. G. *Phytochemistry* **1972**, *11*, 1467.

(12) Johns, S. R.; Lamberton, J. A.; Sioumis, A. A. *Aust. J. Chem.* **1969**, *22*, 2219.

(13) Battersby, A. R.; Herbert, R. B.; McDonald, E.; Ramage, R.; Clements, J. H. *J. Chem. Soc., Perkin Trans. 1* **1972**, 1741.

(14) Barton, D. H. R.; James, R.; Kirby, G. W.; Turner, D. W.; Widdowson, D. A. *J. Chem. Soc. C* **1968**, 1529.

(15) Barton, D. H. R.; Boar, R. B.; Widdowson, D. A. *J. Chem. Soc. C* **1970**, 1213.

(16) A detailed presentation of the crystallographic analysis is in preparation: Foxman, B. M.; private communication.

(17) (a) When the conversion of **10** to **11** was carried out with MeO^- in $MeOD-D_2O$ and the reaction interrupted before it was complete, the NMR spectrum of the recovered methine showed that no exchange of the vinyl proton at C-6 had taken place. Therefore, if the cyclopentadienone **12** is an intermediate in the conversion, it undergoes 1,6-addition of MeO^- at C-2 much more readily than methoxide-catalyzed deprotonation at C-6. (b) Fratev, F., *Natura (Plovdiv, Bulg.)* **1968**, *2*, 35. (c) Pietra, F. *Chem. Rev.* **1973**, *73*, 293.

(18) Humber, L. G.; Kondo, H.; Kotera, K.; Takagi, S.; Takeda, K.; Taylor, W. I.; Thomas, B. R.; Tsuda, Y.; Tsuka, K.; Uyeo, S.; Yajima, H.; Yanaihara, N. *J. Chem. Soc.* **1954**, 4622.

(19) Battersby, A. R.; Binks, R.; Lawrie, W.; Parry, G. V.; Webster, B. R. *J. Chem. Soc.* **1965**, 7459.

(20) Ohno. K.; Tsuji, J. *J. Am. Chem. Soc.* **1968**, *90*, 99.

(21) Battersby, A. R.; Parry, R. J. *Chem. Commun.* **1971**, 901.

(22) Ellis, B. E.; Major, G.; Zenk, M. H. *Anal. Biochem.* **1973**, *53*, 470.

(23) Leete, E. *J. Am. Chem. Soc.* **1963**, *85*, 473.

(24) Battersby, A. R.; McDonald, E.; Milner, J. A.; Johns, S. R.; Lamberton, J. A.; Sioumis, A. A. *Tetrahedron Lett.* **1975**, 3419.

(25) Wightman, R. H.; Staunton, J.; Battersby, A. R.; Hanson, K. R. *J. Chem. Soc., Perkin Trans. 1* **1972**, 2355.

(26) Retey, J.; Bartl, K.; Ripp, E.; Hull, W. E. *Eur. J. Biochem.* **1977**, *72*, 251.

(27) Logan, A. V.; Murray, J. *J. Am. Chem. Soc.* **1952**, *74*, 2436.

(28) Kratzl, K.; Vierhapper, F. W. *Monatsh. Chem.* **1971**, *102*, 224.

(29) Cleland, G. H. *J. Org. Chem.* **1961**, *26*, 3362.

(30) Cleland, G. H. *J. Org. Chem.* **1969**, *34*, 744.

(31) Weinreb, S. M.; Auerbach, J. *J. Am. Chem. Soc.* **1975**, *97*, 2503.

(32) Parker, H. I.; Bloschke, G.; Rapoport, H. *J. Am. Chem. Soc.* **1972**, *94*, 1276.

(33) Rapoport, H.; Stermitz, F. R.; Baker, D. R. *J. Am. Chem. Soc.* **1960**, *82*, 2765.

(34) Stermitz, F. R.; Rapoport, H. *J. Am. Chem. Soc.* **1961**, *83*, 4045.

(35) Battersby, A. R.; Harper, B. J. T. *Tetrahedron Lett.* **1960**, *No. 27*, 21.

(36) Battersby, A. R.; Martin, J. A.; Brochmann-Hanssen, E. *J. Chem. Soc. C* **1967**, 1785.

(37) Marion, L.; Sargeant, K. *Can. J. Chem.* **1957**, *35*, 301.

(38) Tsuda, K.; Ohki, E.; Nozoe, S. *J. Org. Chem.* **1963**, 28, 783.

(39) Djerassi, C. *Org. React.* **1951**, *6*, 207.

(40) Bulos, B.; Handler, P. *J. Biol. Chem.* **1965**, *240*, 3283.

(41) Horecker, B. L.; Kornberg, A. *J. Biol. Chem.* **1948**, *175*, 385.

(42) Fields, M.; Rothchild, S.; Leaffer, M. A. *J. Am. Chem. Soc.* **1952**, *74*, 2435.

(43) Dallacker, F. *Monatsh. Chem.* **1959**, *90*, 846.