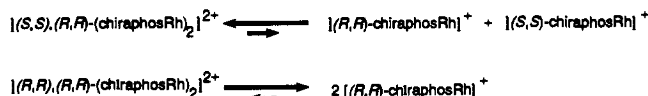
Naturally one anticipates a large number of equilibria in this system, and the "poison" in reality operates by forming several complexes which have reduced rates of catalytic activity. The poison would be expected in practice to deactivate both enantiomers of the active species to some extent. The effectiveness of the poisoning would depend on the quality of the chiral discrimination of the poison.

There is at least one other factor, "chirality amplification", which can contribute significantly to the enantiomeric purity of the product. In our case it appears to be important at lower H_2 pressures. Once the poison preferentially sequesters one enantiomer of the catalyst, there are no longer equal amounts of $[(S,S)$-chiraphos$]Rh^+$ and $[(R,R)$-chiraphosRh$]^+$ in solution. This has an effect on the relative monomer and dimer populations because the stability of the mixed dimer is different than that for the dimer containing identical ligands.



For example, if we consider the idealized case where the poison completely deactivated two-thirds of the $[(S,S)$-chiraphosRh$]^+$ in a racemic mixture, a 1:3 ratio of (S,S) to (R,R) would remain available for catalysis. Very little of the (S,S)–(S,S) dimer would be formed if the formation constant of the homodimer were much less than that of the heterodimer. This would also require that much of the (R,R)–(S,S) dimer would remain associated, whereas the (R,R)–(R,R) would mostly be dissociated. In effect some of the (R,R) monomer sequesters the (S,S) monomer, leaving the remaining pure (R,R) monomer available for catalysis. This idealized case illustrates how a catalyst of low enantiomeric purity could yield a product of higher enantiomeric purity. This type of nonlinear effect was originally investigated by Kagan[11] and recently shown to be important in chiral amino alcohol assisted dialkylzinc addition to carbonyls by Noyori.[12]

This chiral amplification effect contributes in our case under certain conditions. For example, we have found chiral amplification when using nonracemic chiraphos in the formation of dimers in the absence of poisons. The use of dimer prepared from a 1:2 mixture of (S,S)-chiraphos/(R,R)-chiraphos yields a 1:4 mixture of (R)-methylsuccinate to (S)-methylsuccinate.

We anticipate that these chiral poisons can be optimized for use in high-yield asymmetric hydrogenations. We are continuing to try to improve the process; however, at this time we have found that modest increases in the ee of the hydrogenation product were obtained by using THF in place of CH_2Cl_2, increasing the H_2 pressure, and increasing the poison to catalyst mole ratio to greater than $\sim$50%. Increasing this ratio from $\sim$50% to 200% appears to have only a modest effect on the ee. Increasing the temperature from 23 to 32 °C led to a decrease from 49% to 42% ee. These may well not be the optimum poisons nor the optimum conditions: it is plausible that many chiral ligands that have been dismissed in the past because their complexes were poor asymmetric catalysts may actually act as extremely effective poisons. It also follows that the approach described here can be applied to many other types of asymmetric catalysis.[14]

Acknowledgment. We thank the National Science Foundation for support of this research. The synthesis of the (S)-methophos ligand was developed with the support of the National Institutes of Health and in collaboration with Dr. N. Zhang at Yale and Prof. K. Musker and A. Amaro of the University of California at Davis.

Supplementary Material Available: Listing of experimental details of hydrogenations under several conditions (3 pages). Ordering information is given on any current masthead page.

(10) We have not yet elucidated the details of the poisoning.[5] An obvious choice would be the formation of a mixed $[(\text{bisphosphine})_2Rh]^+$ complex with lower activity. $[(\text{bisphosphine})_2Rh]^+$ complexes have seen limited use in chiral catalysis,[11] and to some extent, they may merely provide an alternative source for $[(\text{bisphosphine})Rh]^+$. These bisphosphine complexes are moderately catalytically active, but there are indications that a different path may be involved;[11] nevertheless, they are less efficient catalysts. Regardless, the relative stabilities of $\{[(S)$-methophos$](S,S)$-chiraphos$]Rh\}^+$ and $\{[(S)$-methophos$](R,R)$-chiraphos$]Rh\}^+$ analogues would be different, which implies that (S)-methophos will effectively sequester one hand of the $[$chiraphosRh$]^+$ complex in preference to the other.

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Biosynthesis of Taxoids. Mode of Formation of the Taxol Side Chain†

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Taxol[1] (**1**) and its semisynthetic analog, taxotere,[2] have attracted considerable attention because they show great promise as agents for the treatment of a variety of solid tumors.[3] A major impediment to their development has been the limited supply of these compounds; intensive research efforts are being directed toward finding long-term solutions to this problem. Since for the foreseeable future their commercial production will have to rely on biological systems as the source of at least the diterpene moiety of these compounds, an understanding of the chemistry employed by *Taxus* species to assemble these molecules is extremely pertinent. With this in mind we have embarked on studies on the biosynthesis of taxoids, and we report here on the origin of the phenylisoserine side chain which is essential for the antitumor activity of these compounds.

Leete and Bodem[4] have previously shown that the Winterstein's acid (3-(dimethylamino)-3-phenylpropanoic acid) moiety of taxine is derived from phenylalanine. A similar origin is likely for the phenylisoserine moiety of **1**. Scheme I shows two plausible pathways from phenylalanine to the *N*-benzoylphenylisoserine side chain of **1**, one via β-phenylalanine (path a) and one via cinnamic acid and its epoxide (path b); product stereochemistry would dictate that the latter route proceeds via *cis*-cinnamic acid. Haslam and co-workers[5] found that the conversion of phenylalanine into Winterstein's acid proceeds with retention of the *pro-S* and loss of the *pro-R* hydrogen from C-3 of the side chain, ruling out the involvement of phenylalanine:ammonia-lyase since this enzyme stereospecifically removes the *pro-S* hydrogen from C-3 of phenylalanine. To distinguish between the two pathways shown in Scheme I, we synthesized the possible intermediates **4**–**8** in deuterium-labeled form (**4a**–**8a**; **3a** is commercially available with 99.7 atom % D) (Chart I). Labeled side chain **6a** was prepared

† Dedicated to Professor Meinhart H. Zenk (Munich), a pioneer in plant secondary metabolite biosynthesis, on the occasion of his 60th birthday.

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Table I. Incorporation of Deuterated Precursors into Taxol and Cephalomannine

	taxol	side chain[a]	cephalomannine	side chain[a]
[*benzoyl*-$^2H_5$]side chain (**6a**)	M	P_1	M	P_2
	M + 5 (1.7%)[b]	P_1 + 5	(no M + 5 or P_2 + 5 signals)	
[*ring*-$^2H_5$]-α-phenylalanine (**3a**)	M	P_1	M	P_2
	M + 5 (0.9%)[b]	P_1 + 5	M + 5	P_2 + 5 (weak)
[*ring*-$^2H_5$]-β-phenylalanine (**4a**)	M	P_1	M	P_2
	M + 5 (2.8%)[b]	P_1 + 5	M + 5	P_2 + 5
	M + 10 (2.6%)[b]	P_1 + 10		
[*ring*-$^2H_5$]phenylisoserine (**5a**)	M	P_1	M	P_2
	M + 5 (1.0%)[b]	P_1 + 5	M + 5	P_2 + 5
	M + 10 (0.9%)[b]	P_1 + 10		
[*ring*-$^2H_5$]cinnamic acid (**7a**)	M	P_1	M	P_2
	(no M + 5 or P_1 + 5 signals)		(no M + 5 or P_2 + 5 signals)	
[*ring*-$^2H_5$]cinnamic acid epoxide (**8a**)	M	P_1	M	P_2
	(no M + 5 or P_1 + 5 signals)		(no M + 5 or P_2 + 5 signals)	

[a] P_1, *m/z* 286; P_2, *m/z* 264; no incorporation was observed into the ring fragment *m/z* 569. [b] Enrichments from different precursors are not necessarily comparable since the experiments were carried out at different times, and we have noted substantial seasonal differences in incorporation rates.

Scheme I

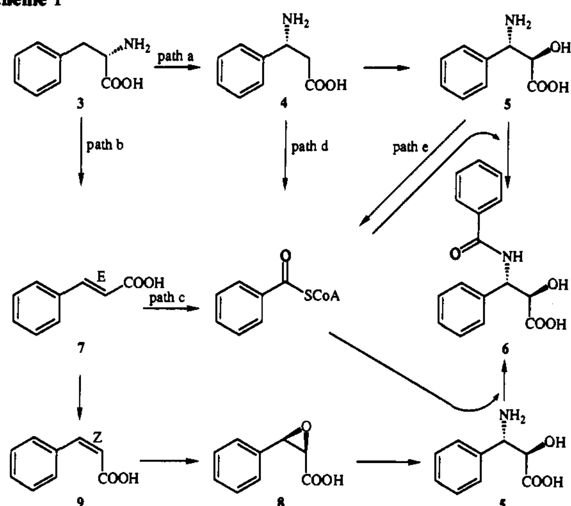


Chart I

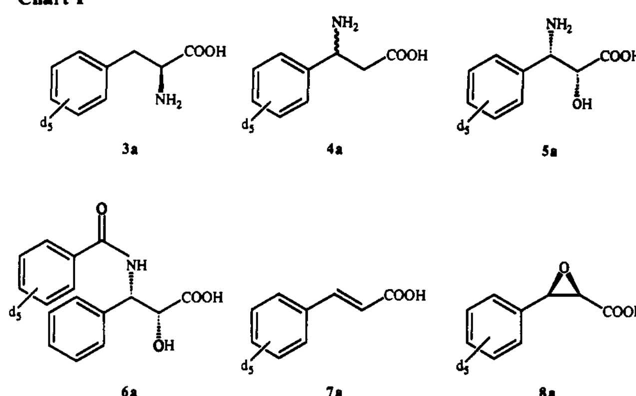
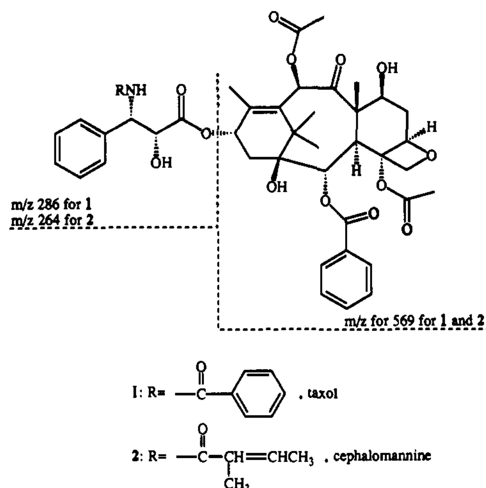


Chart II



by acylating **5** with [*ring*-$^2H_5$]benzoyl chloride obtained from commercial [*ring*-$^2H_5$]benzoic acid (99.4 atom % D). The latter was also reduced to [*ring*-$^2H_5$]benzaldehyde,[6] which was then converted to **7a**[7] and racemic **4a**[8] by literature procedures. Compounds **8a** and **5a** were prepared in 95–100% ee from **7a** by the route of Denis et al.[9] modified to take advantage of recent improvements in the Sharpless asymmetric dihydroxylation method.[10] These precursors were then incubated individually with pieces of bark and cambial tissue of *Taxus brevifolia* in 0.115 M phosphate buffer, pH 7.0 (100 mg of precursor and 30 g of tissue/200 mL), a system similar to that recently described by Strobel et al.[11] After incubation for 96 h, the taxoids were extracted from the cambial tissue using diethyl ether in a Soxhlet extractor and purified by HPLC, separating **1** and **2** (PRP-1 column, 45% CH_3CN/55% H_2O).

The samples of **1** and **2** were then analyzed by electrospray MS–MS in the presence of $CH_3NH_3^+Cl^-$, recording daughter ion spectra for each isotopomer of the methylammonium adduct of the molecular ion (**1**: *m/z* 885, M + $CH_3NH_3^+$; **2**: *m/z* 863, M + $CH_3NH_3^+$). Assignments of fragment ions (**1**: *m/z* 286, side chain; *m/z* 569, ring system; *m/z* 509, ring system – acetate; **2**: *m/z* 264, side chain) are consistent with recently published data[12] (Chart II). Parent ion scans of the unlabeled peak at *m/z* 509 established the percentage of this fragment arising from each isotopomer, revealing the abundance of each isotopomer and hence the isotope enrichment or specific incorporation rate. The results are summarized in Table I.

Neither cinnamic acid nor the epoxide gave any detectable incorporation into **1** and **2**. L-Phenylalanine gave a low but significant enrichment in taxol and cephalomannine. Both β-phenylalanine and phenylisoserine were significantly incorporated into **1** and **2**, with all of the isotope residing in the side chain. This

(6) [*ring*-$^2H_5$]Benzoic acid was reduced to [*ring*-$^2H_5$]benzyl alcohol with $LiAlH_4$ in THF (1 day, reflux, 96%). The alcohol was then oxidized with PCC in CH_2Cl_2 to the corresponding aldehyde (2 h, room temperature, 94%).
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leaves little doubt that the acylated phenylisoserine side chain of these taxoids is synthesized via path a of Scheme I. Unexpectedly, in both experiments **1**, but not **2**, also showed a pronounced M + 10 peak, as well as a P + 10 satellite for the m/z 286 fragment representing the side chain. Hence, the aromatic ring of **4a** and **5a** must have also been incorporated into the benzoyl group of the **1** side chain. Together with the non-incorporation of **7a** this suggests the operation of a new pathway, other than the established[13] path b/c of Scheme I, for the genesis of the benzoate moieties in this plant which proceeds from **3** via **4** (path a/d) or **4** and **5** (path a/e).

In summary, the side chain of **1** and **2** is formed from phenylalanine via β-phenylalanine, presumably generated by an aminomutase reaction,[14] followed by hydroxylation of C-2 and acylation of the nitrogen. The benzoyl moiety is also formed via β-phenylalanine and possibly phenylisoserine rather than via cinnamic acid.

Acknowledgment. We are indebted to Prof. Arthur Kruckeberg, Department of Botany, University of Washington, and Dr. Matthew Loper of this department for guidance and assistance in the collection of plant material and to the American Cancer Society for financial support. P.E.F. was supported by an NIH Predoctoral Training Grant in Biotechnology (GM 08437).

Supplementary Material Available: ES-MS spectra of **1** derived from **3a-6a** and of **2** derived from **3a-5a** and standard ES-MS spectra of **1** and **2** (11 pages). Ordering information is given on any current masthead page.

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The Chemical Nature of Amavadin

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Vanadium is an essential trace element.[1,2] Mushrooms of the genus *Amanita* accumulate vanadium ≤200 ppm[3] wherein vanadium forms the 1:2 complex, Amavadin,[4] with (*S,S*)-2,2'-(hydroxyimino)dipropionic acid, HON{CH(Me)CO_2H}$_2$ (HIDPAH$_3$),[5,6] EPR shows that V^{IV} is present[4,5,7-10] which is reversibly oxidizable to V^V.[11] Initially, a VO^{2+} center was postulated,[5,6] but this is no longer favored. An octacoordinated V^{4+} complex with two HIDPA^{3-} ligands, each bonded via a η^2-N,O group and two unidentate carboxylato groups—as in $[NH_4][NMe_4][V(HIDA)_2]$ (HIDAH$_3$ = 2,2'-(hydroxyimino)diacetic acid (HON{CH_2CO_2H}$_2$))[12-15]—has been accepted, but not proven, for Amavadin. We have prepared and characterized [Δ-V((*S,S*)-HIDPA)$_2$]$^-$, establishing that oxidized Amavadin consists of approximately an equal mixture of the Δ and Λ forms of this anion.

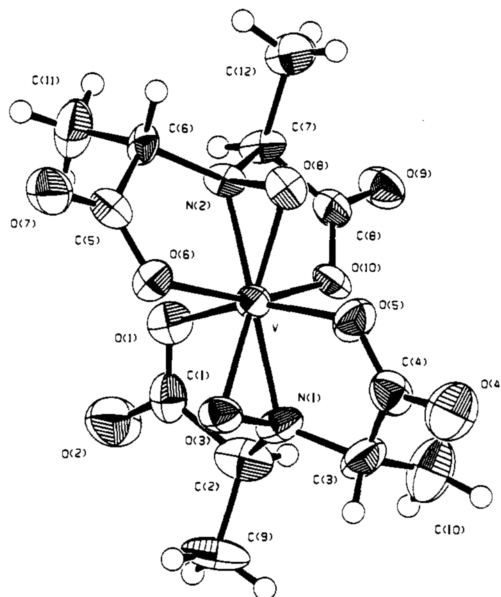

Figure 1. Structure of the anion of $[PPh_4]$[Δ-V((*S,S*)-HIDPA)$_2$]·H_2O.

Table I. Comparison of the Vanadium-Ligand Distances[a] for $[V(HIDA)_2]^{n-}$ (n = 1, 2) and [Δ-V((*S,S*)-HIDPA)$_2$]$^-$

bond	length (Å)		
	$[V(HIDA)_2]^{2-}$	$[V(HIDA)_2]^-$	[Δ-V((*S,S*)-HIDPA)$_2$]$^-$
V-O(1)	2.071 (3)	1.991 (7)	1.993 (9)
V-O(5)	2.065 (3)	1.955 (7)	1.96 (1)
V-O(6)	2.063 (3)	1.922 (8)	1.977 (9)
V-O(10)	2.070 (3)	1.936 (7)	1.941 (9)
V-O(3)	1.973 (3)	1.963 (8)	1.926 (9)
V-O(8)	1.976 (3)	1.977 (8)	1.973 (9)
V-N(1)	2.003 (4)	2.016 (8)	2.02 (1)
V-N(2)	2.002 (3)	2.028 (9)	2.00 (1)

[a] The labeling scheme adopted is defined in Figure 1 for [Δ-V((*S,S*)-HIDPA)$_2$]$^-$, and the comparisons are made with respect to the equivalent bonds in $[V(HIDA)_2]^-$ and $[V(HIDA)_2]^{2-}$.[14] O(1), O(3), and O(5) belong to the same ligand molecule, and O(3) and O(8) are the hydroxamato O atoms.

HIDAH$_3$ and HIDPAH$_3$ were synthesized (see ref 16a), the latter from L(or D)-alanine via D(or L)-bromopropionic acid.[16b] The acid forms of the V^{IV} complexes were prepared from [VO(pentane-2,4-dionate)$_2$] in H_2O, with chromatographic separation on a Dowex column and evaporation to dryness. The V^{IV} complexes were oxidized by $[NH_4]_2[Ce(NO_3)_6]$, and the V^V anion was precipitated with $[PPh_4]^+$. Red, prismatic crystals[17,18] of

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(17) $[PPh_4][V(HIDA)_2]·CH_2Cl_2$ crystallizes in the monoclinic space group $P2_1/c$, a = 791.5 (1), b = 2810.0 (5), c = 1532.0 (3) pm, β = 91.13 (1)°, V = 3406.7 × 10^6 pm^3, Z = 4; $F(000)$ = 1576; ρ_{calcd} = 1.50 g cm^{-3}; μ = 6.07 cm^{-1}; Mo Kα radiation; θ range = 2-20°; 3199 reflections measured, 1789 observed ($I > 3\sigma I$); number of parameters 443; R = 0.061, R_w = 0.052. Full details of the crystallographic characterization have been deposited at The Cambridge Crystallographic Data Centre, University Chemical Laboratory, Lensfield Road, Cambridge CB2 1EW, UK.

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