Studies with plant cell cultures of *Catharanthus roseus*. Oxidative coupling of dibenzylbutanolides catalyzed by plant cell culture extracts

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Utilizing appropriate dibenzylbutanolides, for example **7**, obtained via an efficient synthetic route from readily available aldehydes, and cell-free extracts obtained from *Catharanthus roseus* cell cultures, it was possible to achieve enzyme-catalyzed oxidative coupling of **7** to picropodophyllotoxin analogues. Studies are presently underway to convert such compounds to intermediates employed in the synthesis of the commercially important anti-cancer drug, etoposide.

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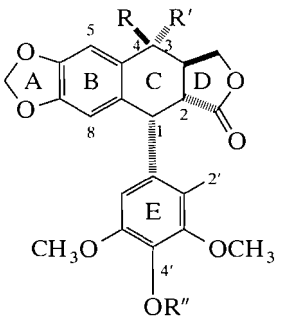
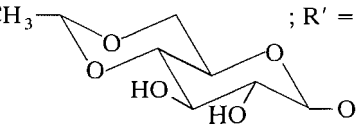
Utilisant des buténolides appropriés, comme le composé **7**, obtenus par une méthode de synthèse efficace à partir d'aldéhydes facilement disponibles, et des extraits sans cellules obtenus à partir des cultures de cellules du *Catharanthus roseus*, on a pu réaliser un couplage oxydant, catalysé par les enzymes, du composé **7** conduisant à des analogues de la picropodophyllotoxine. Des études sont en cours pour transformer de tels composés en intermédiaires utilisés dans la synthèse de l'étoposide, une drogue anticancéreuse commercialement importante.

[Traduit par la rédaction]

The podophyllotoxin family of natural products exemplified by the structures **1**–**5** have received considerable attention over the years. Excellent reviews (1, 2) of the earlier investigations pertaining to their chemistry and pharmacology are available and the most recent interest due to the important anti-cancer activity of the clinical drug, etoposide (**6**), also extensively reviewed (3, 4), has stimulated continued pursuit of synthetic (5–23) and biosynthetic studies (24). The synthesis of **6** from podophyllotoxin (**1**) is also well established (25–27) and presently provides the commercial route to this clinical drug. However, the latter route requires the



Podophyllotoxin **1**: R = H; R′ = OH; R″ = CH_3
Epipodophyllotoxin **2**: R = OH; R′ = H; R″ = CH_3
Deoxypodophyllotoxin **2**: R = OH; R′ = H; R″ = CH_3
Deoxypodophyllotoxin **3**: R = R′ = H; R″ = CH_3
4′-Demethylpodophyllotoxin **4**: R = H; R′ = OH; R″ = H
4′-Demethylepipodophyllotoxin **5**: R = OH; R′ = H; R″ = H
Etoposic **6**: R = CH_3  ; R′ = H; R″ = H

isolation of **1** via extraction of *Podophyllum peltatum* plants, followed by a two-step chemical process to 4′-demethylepipodophyllotoxin (**4**), the latter being the crucial intermediate for synthesis of **6**.

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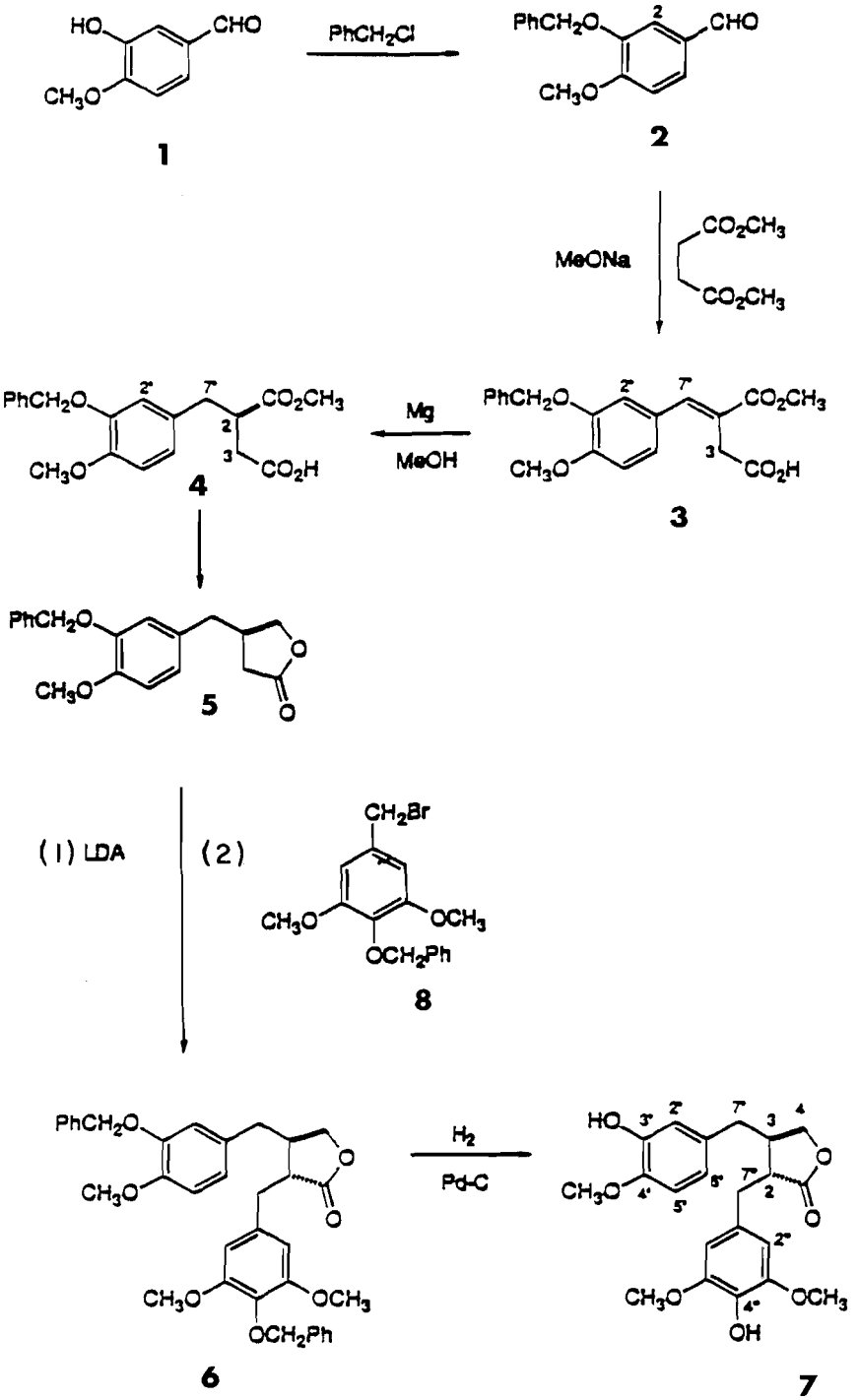
In our program concerning the combined utilization of synthetic chemistry and biotechnological methods, specifically plant cell culture, to develop efficient routes to complex natural products and related biologically active substances (28, 29), we focussed our attention on deriving an efficient route to **4**. In this study, the primary focus was eliminating the dependence on plant extraction and, hopefully, also eliminating some chemical conversions, so as to obtain a more direct synthesis of **4**. This publication presents some results of our studies in this direction.

Our general strategy in utilizing plant cell cultures or enzymes derived from within the cells as "reagents" in organic synthesis, requires initial consideration of "target" synthetic molecules that can subsequently be biotransformed into the desired end products. For this purpose, an understanding of the biosynthetic pathway leading to the podophyllotoxin family is required. It was clear from the detailed studies by Dewick and co-workers (24) that "appropriate" dibenzylbutanolides could act as substrates in enzyme-catalyzed oxidative cyclization reactions to the corresponding podophyllotoxins. In effect, this type of process (see **7** → **9**, Scheme 2) is expected to involve radical intermediates and, within living systems, the latter are usually generated by peroxidase enzymes (30, 31). Indeed such peroxidase enzymes had been produced within our plant cell culture line (coded as AC3) of *Catharanthus roseus* and their role evaluated in the biosynthesis of the clinical anti-cancer drugs vinblastine and vincristine (28). The question as to whether these enzymes could act as "reagents" in catalyzing the cyclization of dibenzylbutanolides to the podophyllotoxin system was of interest and required evaluation.

The first substrate to be studied in incubation experiments with enzymes derived from *C. roseus* plant cell cultures was the butanolide **7** prepared according to Scheme 1.

Isovanillin (**1**) was treated with benzyl chloride and potassium carbonate to give the benzyl ether **2** in quantitative yield and the latter was then subjected to a Stobbe condensation with dimethylsuccinate to afford the conjugated hemisuccinate **3** in 73% yield. Spectral data (see Experimental) indicated that only one geometrical isomer (probably *cis*) was



SCHEME 1. Synthesis of dibenzylbutanolide 7.

present, as expected, by comparison with the results reported for the analogous reaction with piperonal (32). The double bond of the hemiester **3** was reduced with magnesium in methanol (33) to give the unconjugated hemisuccinate **4** in 89% yield. The ^{1}H NMR spectrum of the product showed H(2), H(3), and H(7′) proton signals as either multiplets or doublets of doublets due to the creation of a chiral centre at C-2. The carbonyl frequency in the IR spectrum, in comparison with that of **3**, had increased to 1720 cm^{-1}, demonstrating the loss of conjugation of the ester group with the aromatic ring.

The reductive lactonization of the hemisuccinate **4** to the butanolide **5** was initially performed using the potassium salt of **4** and calcium borohydride prepared *in situ* (34). However, the crude reaction product was very complex and the yields of **5** were low, but by using the stronger reducing agent lithium borohydride (35) directly with the hemisuccinate **4**, satisfactory yields of pure **5** could be realized. The butanolide **5** was characterized by its ^{1}H NMR spectrum, which showed the two new methylene protons (H(4)) as doublets of doublets at δ 3.91 and δ 4.21, respectively. Formation of the lactone ring was demonstrated by the IR spectrum, which showed the carbonyl absorption at a frequency of 1770 cm^{-1}, well above the value observed for the hemisuccinate **4**.

Alkylation of **5** with the bromide **8** (prepared by the literature procedure (36)) gave the dibenzylbutanolide **6** in 80% yield. The ^{1}H NMR spectrum of the product, in comparison with that of **5**, showed the loss of one H(2) signal but the expected *trans* orientation of the two substituents with respect to the lactone ring could not be confirmed owing to the close proximity of the complex H(2) and H(3) signals.

Finally, the benzyl protecting groups of the butanolide **6** were quantitatively cleaved by hydrogenolysis using palladium-on-carbon as a catalyst, to afford the bis(hydroxybenzyl)butanolide **7** in 87% yield. The ^{1}H NMR spectrum of **7** showed the C(3′) and C(4″) phenolic hydroxyl group singlets, which disappeared with D_2O addition, at δ 5.42 and δ 5.58 respectively. The ^{13}C NMR spectrum showed two benzyl methylene carbon signals (C(7′) and C(7″)), which were identified by the attached proton test (APT). The EI mass spectrum of **7** clearly showed the molecular ion at *m/z* 388 and revealed a fragmentation pattern consistent with the structural assignment. In summary, the dibenzylbutanolide **7** was prepared from isovanillin in six steps in 40% overall yield.

With **7** on hand, studies concerning the biotransformation of this compound with an enzyme preparation (cell-free extract, CFE) previously obtained from our *C. roseus* cell line (AC3) (37–40) were initiated. A large number of experiments with **7** and CFE (prepared as indicated in the experimental section) were conducted. The reaction parameters (pH, H_2O_2 as cofactor, units of peroxidase enzyme per mmol substrate, reaction time) were evaluated in small-scale experiments (10–15 mg of **7**) in order to derive optimum conditions for the biotransformation of **7** to **9** (Scheme 2) and only a summary of the most pertinent features is provided here.

One of the important parameters was the pH (pH_h, the pH of the buffer used to prepare the CFE, Fig. 1) and its effect on the enzymatic activity. Figure 1 summarizes the changes in peroxidase activity and specific activity of the CFE with pH of buffer employed (see Experimental for procedures to measure these activities). A value of 6.3 appeared to be a good compromise between high peroxidase activity and specific activity, or the quotient of peroxidase activity and soluble protein concentration. The protein concentration was determined spectrophotometrically by complexing the dissolved protein with a commercial dye solution (41, 42).

The ideal ratio of peroxidase to substrate was determined using an arbitrary amount of 2.0 molar equivalents of hydrogen peroxide and conducting the reaction at pH 6.3. As shown in Fig. 2, **7** was very nearly consumed within 15 min when an amount of CFE corresponding to 400 units peroxidase per millimole of substrate was used. Increasing the peroxidase: substrate ratio or the reaction time (180 min, Fig. 3) failed to completely convert **7** to **9**: instead, the sole effect was to promote the reaction of aryltetralin **9** to **10** (Scheme 2), a species subsequently identified as the dimer of **9** (see later).

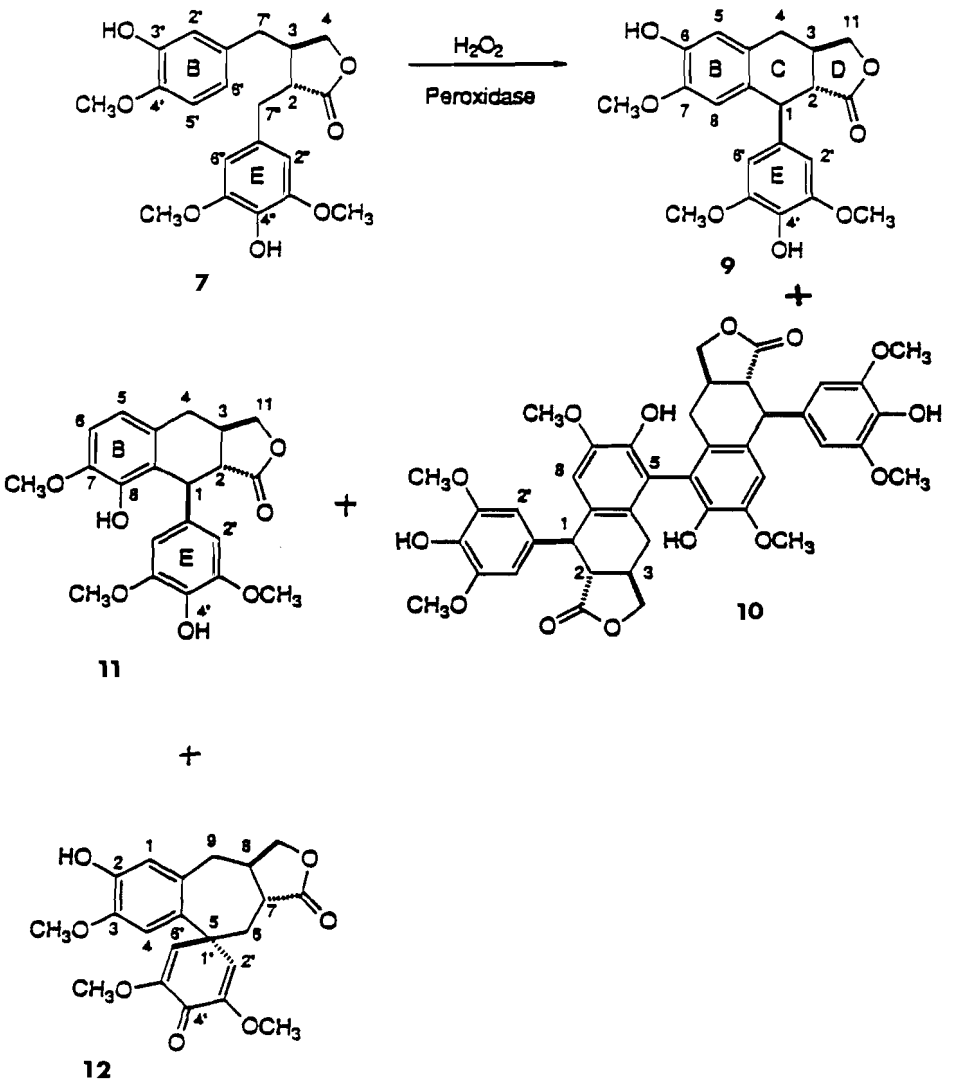
It was concluded that a peroxidase: substrate ratio of approximately 250 units per millimole of butanolide **7** would give optimum yields of the aryltetralin **9**. Higher values promoted dimerization while lower values resulted in incomplete conversion of substrate. The complete structure and stereochemistry of the desired cyclic analogue **9** was established by X-ray diffraction analysis (Fig. 4).

The dimer **10**, also obtained in the biotransformation of **7** was characterized by its ^{1}H NMR and mass spectra. The ^{1}H NMR spectrum of **10** strongly resembled that of the monomer **9** but there was a second signal of equal intensity for all protons shown by **9** except H(5), which is not present. This doubling represents the proton signals of the two diastereomers of the dimer molecules since the monomer units are racemic. The dimeric nature of the compound was demonstrated more clearly by the mass spectrum obtained using desorption chemical ionization (DCI) with ammonia, which showed a peak at *m/z* 788 corresponding to $(\mathbf{10} + NH_4)^+$. There was a very weak molecular ion peak in the EI mas spectrum at *m/z* 770, as well as peaks at *m/z* 784, 798, and 812 resulting from the intermolecular transfer of methyl radicals. In addition, the high degree of fragmentation and the low intensity of the monomer peak at *m/z* 386, in relation to the fragments at *m/z* 167 and 154, were in sharp contrast to the pattern observed in the EI spectrum of the monomer **9**. Unfortunately, neither the EI nor the DCI spectra showed any significant fragments in the region spanned by the molecular weights of the monomer and the dimer, suggesting that the bond between the monomer units is very weak.

In larger scale experiments, a third very minor component could be isolated and based on its spectral data was assigned structure **11**. Its EI mass spectrum was virtually identical to that of **9**. The ^{1}H NMR spectrum was also quite similar except for two *ortho*-coupled (J = 8 Hz) proton signals noted at δ 6.77 and 6.85, thereby suggesting that the ring B hydroxyl group is at C(8) of the aryltetralin rather than at C(6) as in **9**. Other significant features of the spectrum were the three separate methoxy proton signals at δ 3.59, 3.80, and 3.89 and the two *meta*-coupled (J = 2.5 Hz) doublets at δ 5.56 and 6.09, which implied nonequivalence of the two sides of ring E due to the loss of its ability to rotate about the C(1)—C(1′) bond. This was not surprising in view of the proximity of the C(8) hydroxyl to ring E. The low yield of this compound (0.53%) is readily explained by these major steric interactions of the C(8) hydroxyl with ring E.

The optimum hydrogen peroxide: substrate ratio was determined using 250 units peroxidase per millimole of **7** in buffer at pH 6.3. In the absence of hydrogen peroxide, no reaction occurred after 15 min (Fig. 5). A similar result was noted when the reaction was allowed to proceed for 180 min. The yield of **9** and **10** then increased with hydrogen peroxide concentration but remained approximately constant at about 80% when more than 2.0 molar equivalents (3.1 nM) were present. However, these results clearly did not represent the exclusive requirements of the oxidative coupling reactions of **7** and **9** in view of the ability of the CFE alone to consume hydrogen peroxide. This property was demonstrated when the CFE was stirred for 15 min with hydrogen peroxide *prior* to precursor addition. The yield of **9** was only 28% (vs. 64% for the control, where precursor **7**, hydrogen peroxide, and CFE were added simultaneously) after 1 h but had increased sharply to 50% 1 h after a further 2.0 equivalents of hydrogen peroxide were added. These competing processes may have involved the catalytic decomposition of hydrogen peroxide (catalyzed by peroxidase, catalase, or metal ions) or the oxidation of cell material.

It was concluded that 2.0 molar equivalents of hydrogen peroxide would give optimum yields of **9**. Lower values resulted in incomplete biotransformation of **7** while higher ratios promoted the dimerization of **9** to **10**. There appeared to be a distinct decrease in enzyme activity when more than 3 equivalents of hydrogen peroxide were used as cofactors.



SCHEME 2. Biotransformation of dibenzylbutanolide **7** with cell free extracts of *Catharanthus roseus* cell cultures.

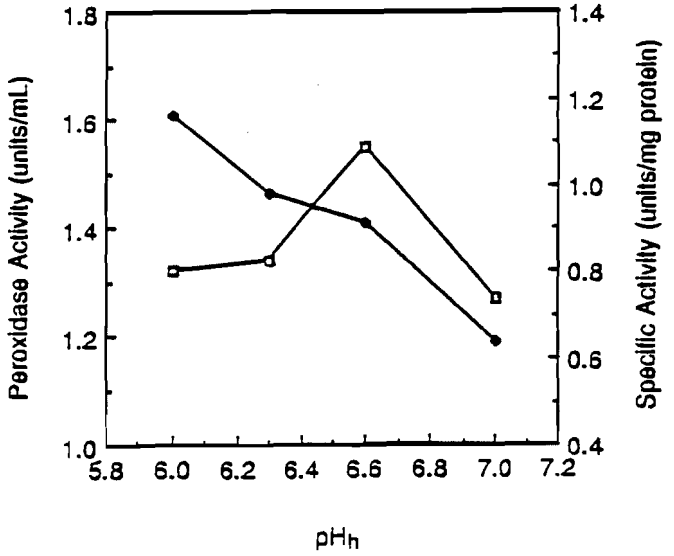


FIG. 1. Effect of pH_h on peroxidase activity and specific activity. □, Peroxidase activity, ◆, specific activity.

The optimum biotransformation pH (pH_r) for the cyclization of **7** to **9** could now be reliably determined, as the ideal peroxidase: substrate and peroxide: substrate ratios had been established. Up to this point, a value of pH 6.3 had been used and found to give good results but it was clearly desirable to identify the optimum value of pH_r more closely. This value (Fig. 6) was found to be about 6.3 based on the yield of **9**, using the optimum conditions established previously. The yield of **9** showed only minor variations over the pH range 6.0–6.6 but decreased markedly at more basic values.

Finally, it was determined that the CFE solution, when stored at 4°C, was quite stable with respect to both the oxidation of pyrogallol (peroxidase assay) and the biotransformation of **7**. The age of the CFE was thus not a significant factor in the reproducibility of individual experiments. In summary, it was found that the biotransformation of **7** to **9** could be accomplished most efficiently using the conditions described in Table 1. The yields of **9** were consistently 70% or higher in the subsequent larger scale experiments (generally 1 g of **7**) and the dimer **10** could be completely suppressed. In these latter studies, a further minor product (usually 1% yield) was isolated and shown to possess the spirodienone structure **12**. The latter was characterized by

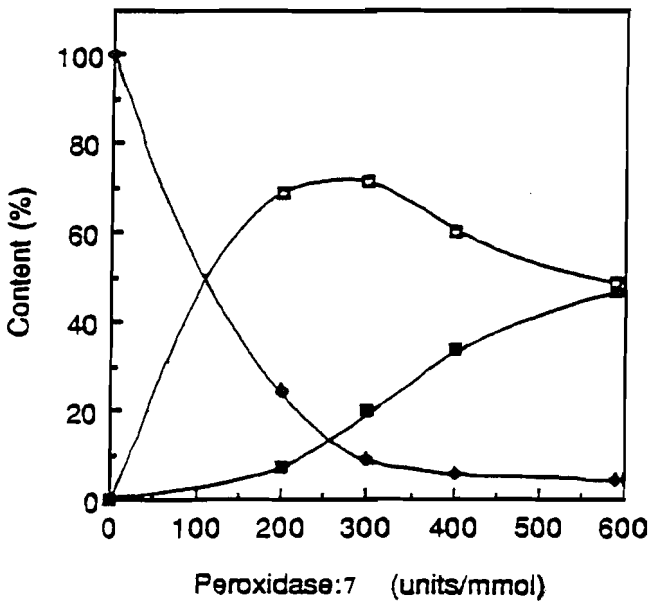


FIG. 2. Effect of peroxidase: substrate ratio on biotransformation of **7**. Reaction time = 15 min; ◆, **7**; □, **9**; ■, **10**.

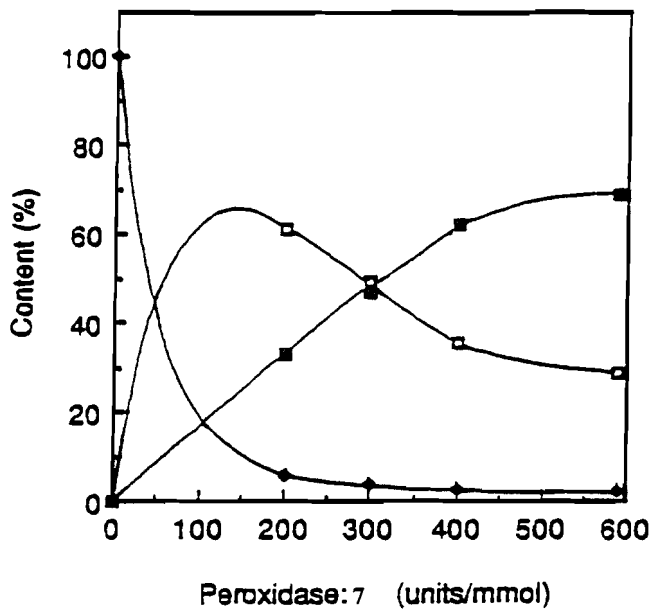


FIG. 3. Effect of peroxidase: substrate ratio on biotransformation of **7**. Reaction time = 180 min; ◆, **7**; □, **9**; ■, **10**.

IR, ^{1}H NMR, and mass spectroscopy. Its IR spectrum showed, in addition to the lactone carbonyl (1774 cm^{-1}), a second carbonyl band at 1673 cm^{-1} that was associated with the C(4′) dienone. The mass spectrum showed the molecular ion as the base peak at *m/z* 386 and virtually no fragmentation. The ^{1}H NMR spectrum showed three distinct methoxy proton signals, two H(6) proton signals (assigned by decoupling H(7)), and two sharp doublets (J = 2.8 Hz) at δ 6.13 and 6.25 that represent H(2′) and H (6′). The chemical shift and coupling constant values of H(2′) and H(6′) were very similar to those reported for a related compound (43).

In summary, it was now clear that the enzymes of *C. roseus* cell cultures, originally developed for the production of indole alkaloids, could tolerate "foreign" substrates of the dibenzylbutanolide family and afford high yields of the desired podophyllotoxin analogues.

It is of interest to note that for the sake of comparison, the biotransformation of **7** to **9** was investigated with commercial horseradish peroxidase. The reaction mixture was complex and the best yields of **9** were 15–19%, thereby revealing that the *C. roseus* derived enzymes are *far superior* for the present study.

In further studies involving enzyme-catalyzed cyclization of dibenzylbutanolides, it was of interest to ascertain whether functionality other than that present in **7** could be tolerated by the *C. roseus* derived enzymes. For this purpose, the butanolide **20** (Scheme 3) was selected as the next substrate to study.

An alternative, superior route to **20** was developed as shown in Scheme 3. The crucial step in the sequence involved a tandem conjugate addition method (44,45) in which the anion of **14** is reacted with butenolide **15** and the resulting intermediate **16**, without isolation, is reacted with **8** to afford **17**.

The dithioacetal **14** was obtained from piperonal (**13**), as shown in Scheme 3, in 98% yield (46). The dithioketal **17** was then prepared in overall 55% yield, as shown, using but-2-en-4-olide (**15**), the latter obtained by a standard procedure (47). The IR spectrum of **17** showed a strong carbonyl band at 1770 cm^{-1}, a frequency substantially higher than that observed for the butenolide (1740 cm^{-1}). The EI mass spectrum showed no significant peaks with *m/z* values greater than 584 ((M$^+$ − SPh) + H) but the fast atom bombardment ionization (FAB) mass spectrum revealed a weak molecular ion at *m/z* 693.

Considerable problems were encountered in the subsequent conversion of the dithioketal **17** to the ketone **18** (R = H or CH_2Ph). Treatment of **17** with perchloric acid (Method 1 in Experimental) (48) gave complex mixtures and poor yields of **18** (R = CH_2Ph) along with the hydroxy ketone **18** (R = H) as a minor product. Varying the reaction time and the amount of perchloric acid failed to improve these results. Treatment of **17** with sulfuryl chloride and wet silica gel (Method 2) (49) gave complex mixtures containing no readily identifiable products. Oxidation with iodine in methanol followed by acid hydrolysis (Method 3) (50), however, gave good yields of the ketone **18** (R = CH_2Ph), which was characterized by ^{1}H NMR, mass, and IR spectroscopy. The ^{1}H NMR spectrum, in comparison with that of the dithioketal **17**, showed a reduction in the number of aromatic protons to five and a considerable downfield shift of the H(3) signal to δ 4.01. The EI mass spectrum exhibited a molecular ion peak at *m/z* 490, and other characteristic fragments consistent with structure **17**.

Hydrogenation (H_2, Pd/C) of **18** (R = CH_2Ph) afforded a mixture of **18** (R = H, 46%) and the alcohol **19** (R = H, 30%), the latter occurring as a mixture of alcohols epimeric at C(7′) (approx. ratio of 3:1). The major isomer was tentatively identified as the alcohol with a β-orientation of the C(7′) hydroxyl for the depicted enantiomer, based on the relative accessibility of the faces of the C(7′) carbonyl group in the most favourable conformation of **18** (R = CH_2Ph) according to Dreiding models, and the results of reductions of closely related dibenzylbutanolides (48). The ^{1}H NMR spectrum of this alcohol mixture showed doublets at δ 1.94

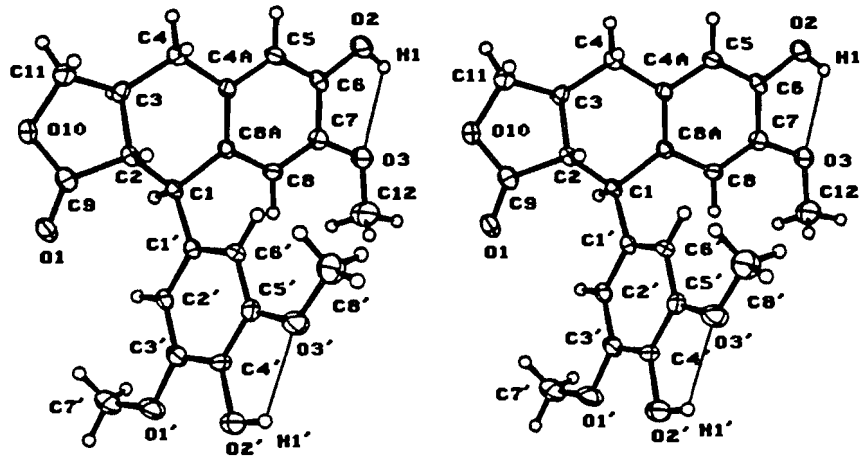


FIG. 4. Stereoview of **9**; 33% probability thermal ellipsoids are shown for the non-hydrogen atoms. Fine lines represent hydrogen bonds.

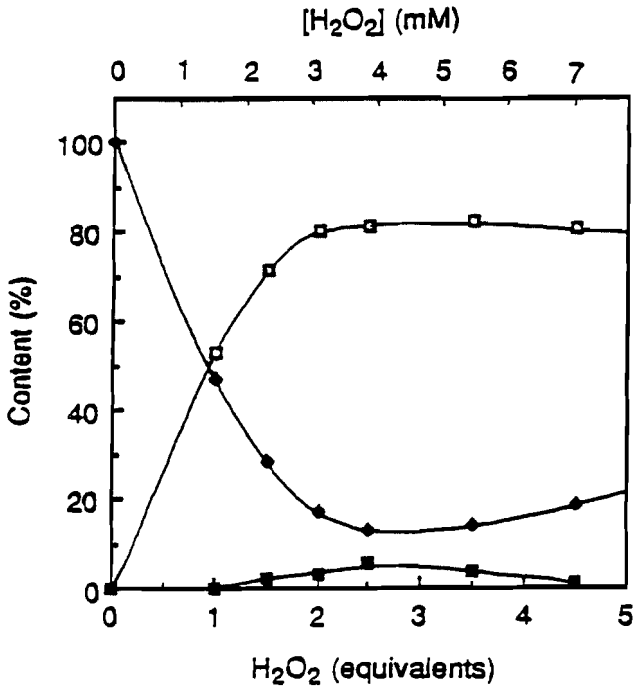


FIG. 5. Effect of hydrogen peroxide concentration on biotransformation of **7**. Reaction time = 15 min; ◆, **7**; □, **9**; ■, **10**.

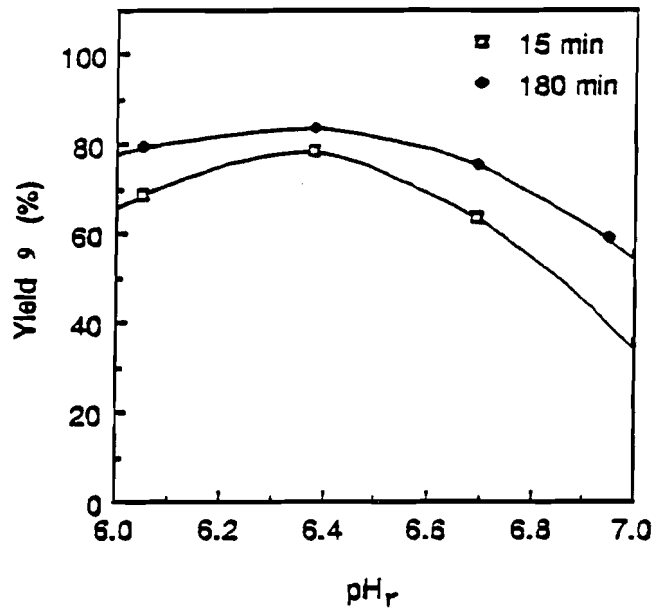


FIG. 6. Effect of pH_r in biotransformation of **7**. □, 15 min; ◆, 180 min.

TABLE 1. Optimum conditions for *C. roseus* CFE-catalyzed biotransformation of butanolide **7** to aryltetralin **9**

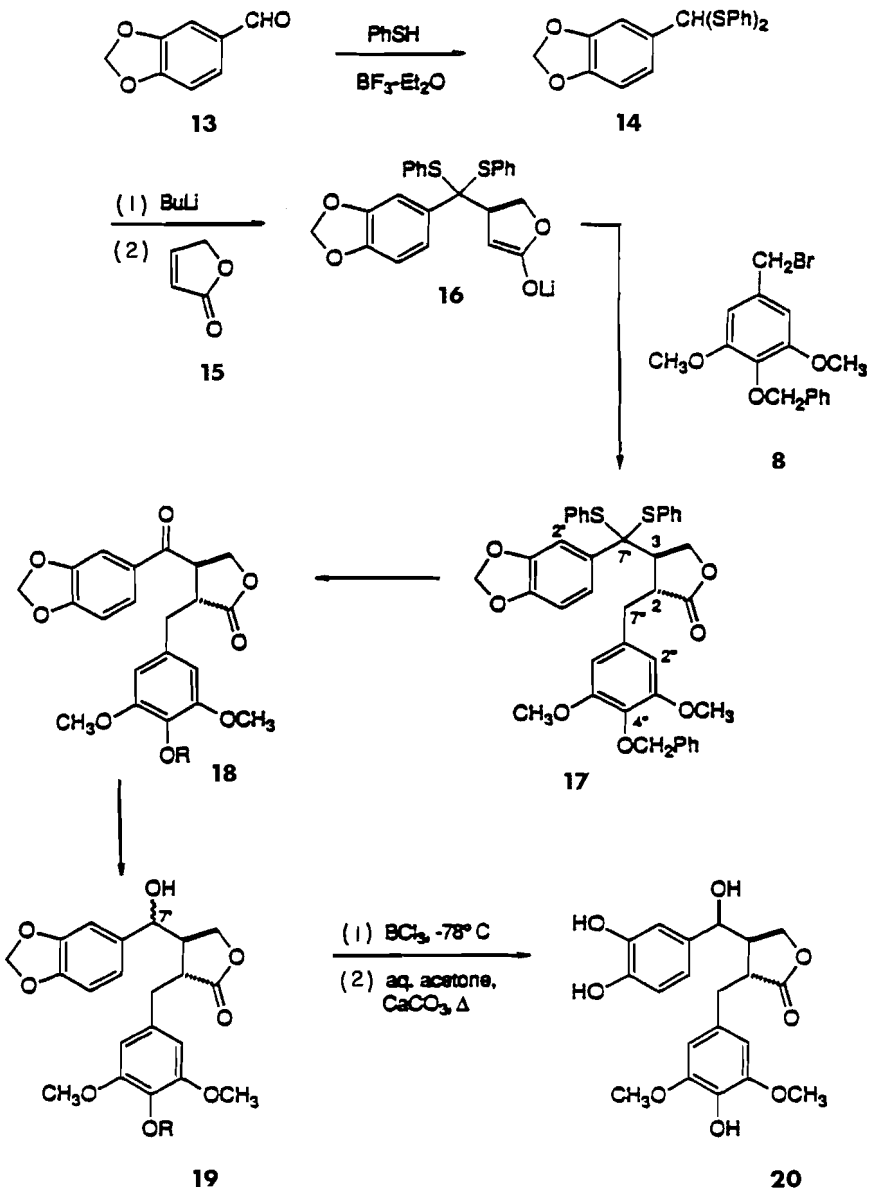
pH_h	6.3
pH_r	6.3
Hydrogen peroxide (equivalents)	2.0
CFE (units peroxidase per mmol 7)	250
Reaction time (min)	180

and 2.00 and a phenolic hydroxyl singlet at δ 5.40, all of which disappeared upon D_2O addition. The doublets correspond to the C(7′) α and β hydroxyl protons, respectively. The H(3)–H(7′) coupling constants of the two epimers (7.6 and 6.7 Hz for minor and major isomers, respectively) were too similar to be useful in assigning the C(7′) stereochemistry of the individual isomers.

A much preferable route to the alcohol **19** was achieved by low-temperature reduction with sodium borohydride to afford a quantitative yield of **19** (R = CH_2Ph) as an epimeric mixture (4.9:1 in favor of the C(7′) β-hydroxyl group). Studies with the sterically hindered reagent, lithium aluminum tri-tertbutoxy hydride at −78°C, improved the epimeric ratio (5.7:1) but the yields were substantially lower (60%).

When the above mixture of alcohol **19** (R = CH_2Ph) was treated under varying conditions with boron trichloride (4–6 equivalents and different time periods, 30–120 min), the desired dibenzylbutanolide **20** was obtained but only in low yield (generally 25%). Difficulties in chromatographic separation of this very polar compound (recovery of 50% on silica and 67% on Fluorosil) and compound instability are major factors influencing the yield of pure product.

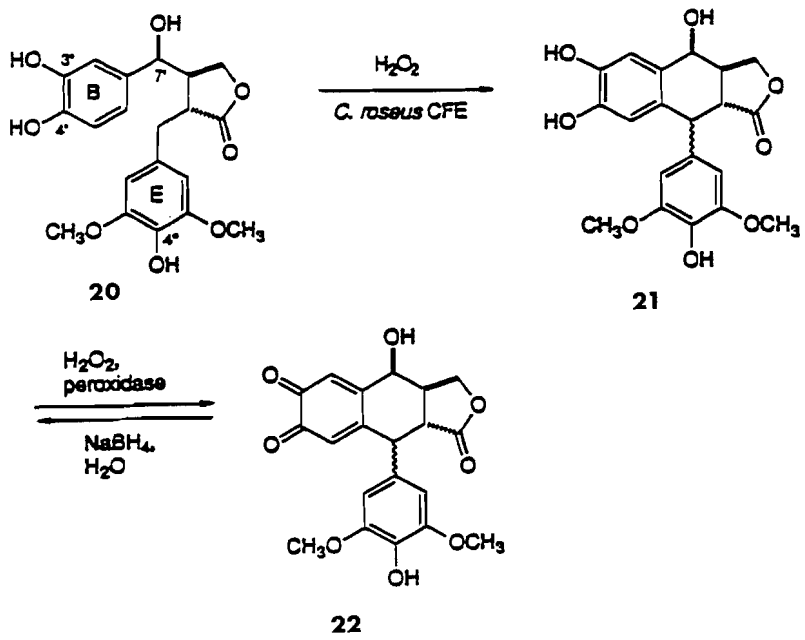
In summary, **20** is available in 12% overall yield for the five-step conversion from piperonal (**13**).



SCHEME 3. Synthesis of dibenzylbutanolide **20**.

We then proceeded to investigate the biotransformation of **20** by the *C. roseus* CFE with the intention of performing the oxidative coupling of **20** to the aryltetralin **21** and (or) the quinone (**22**) (Scheme 4) since both compounds could be converted readily to podophyllotoxin analogues bearing the desired methylene dioxy function in ring B as required for etoposide synthesis. Since catechols are known to react very readily with peroxidase to form *ortho*-quinones (51) and the corresponding diacids (52), the biotransformation of **20**, as described in Table 2, was conducted under oxidizing conditions considerably milder than those employed for **7**. In addition to lower peroxidase:substrate ratio, a higher pH was employed in order to achieve a lower peroxidase reactivity. Nevertheless, the color of the reaction mixture changed from an initial pale yellow to dark purple within 1 min, suggesting extensive oxidation of starting material to quinones. No such color change had been observed in the biotransformation of **7**. A control experiment was also performed in order to determine if hydrogen peroxide, in the absence of CFE, was able to oxidize **20** under the conditions used in the biotransformation. Extraction of the reaction mixture gave 96% recovery of a material consisting exclusively of **20**. No color change was observed. It was concluded that hydrogen peroxide alone was unable to oxidize **20** under the given conditions.

A great deal of difficulty was encountered in the isolation of identifiable products from the various biotransformation experiments with **20**. Recovery of material into organic extracts (ethyl acetate or methanol, for example) was poor and it was clear that extensive decomposition was occurring even in short time incubations (for example, 10 min, Table 2). After much effort, the extraction procedure summarized in Scheme 5 was developed in which sodium borohydride was employed to reduce any resulting unstable quinones and (or) diazomethane was used to convert highly polar and possibly water-soluble carboxylic acids to esters. Under these conditions recovery of identifiable products could be achieved (Table 3).



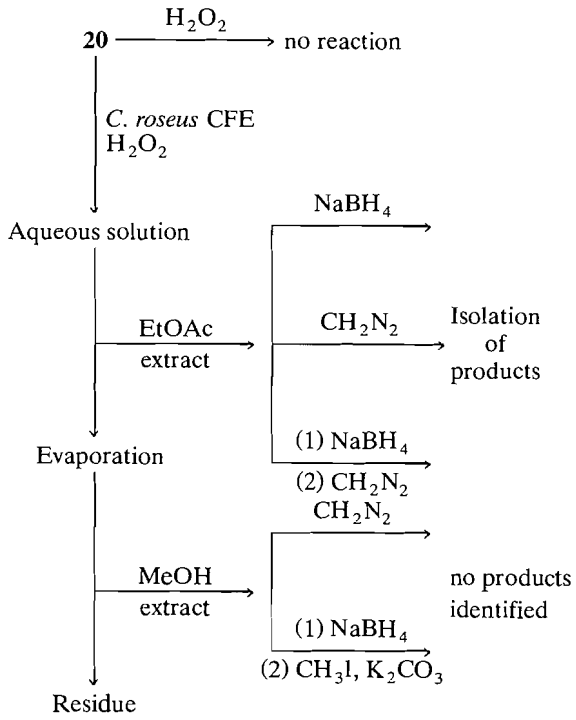
SCHEME 4. Proposed biotransformation of dibenzylbutanolide **20** with cell free extracts of *Catharanthus roseus* cell cultures.

TABLE 2. Catharanthus roseus CFE biotransformation of dibenzylbutanolide **20**

Substrate **20** (mg)	73
pH_h, pH_r	7.0
Hydrogen peroxide (equivalents)	2.0
CFE (units peroxidase per mmol **20**)	120
Reaction time (min)	10
Ethyl acetate extract (mg)	68
Methanol extract (mg)	205

Direct analysis of the ethyl acetate extract by TLC showed the presence of only **20** and very polar unidentified compounds. Addition of sodium borohydride (Table 3, Entry 1) gave a second major component, according to TLC, even prior to the reaction being quenched with dilute hydrochloric acid. The ^{1}H NMR spectrum and other spectral properties were identical with a product obtained in our earlier studies involving the reaction of boron trichloride with **19** (R = H) to which the "retro-lignan" type structure **26** (Scheme 6) had been assigned. The ^{1}H NMR spectrum of **26** showed singlets at δ 3.18 and 3.87 (methoxy protons), singlets at δ 7.12, 7.54, and 7.56 (hydroxyl protons) that disappeared upon D_2O addition, and a total of only four aromatic protons. The trisubstituted ring B aromatic signal pattern was present but the H(2″) signal appeared as only a single proton at δ 6.65. The EI mass spectrum of **26** in contrast to that of **20**, showed a very strong molecular ion peak at m/z 372 and a low degree of fragmentation. The proposed mechanism for the formation of **26** from **20** is shown in Scheme 6.

Only the substrate **20** and partially methylated **20** (**27**) were obtained when the ethyl acetate extract was treated with diazomethane (Entry 2, Table 3). The butanolide **27** was seen as a single spot on TLC analysis and spectroscopic data were consistent with the structural assignment. The ^{1}H NMR spectrum was nearly identical with that of **20** except for two additional methoxy proton signals and an apparent doubling



SCHEME 5. Extraction procedure for reaction mixture obtained in biotransformation of **20** with cell free extracts of *Catharanthus roseus* cell cultures.

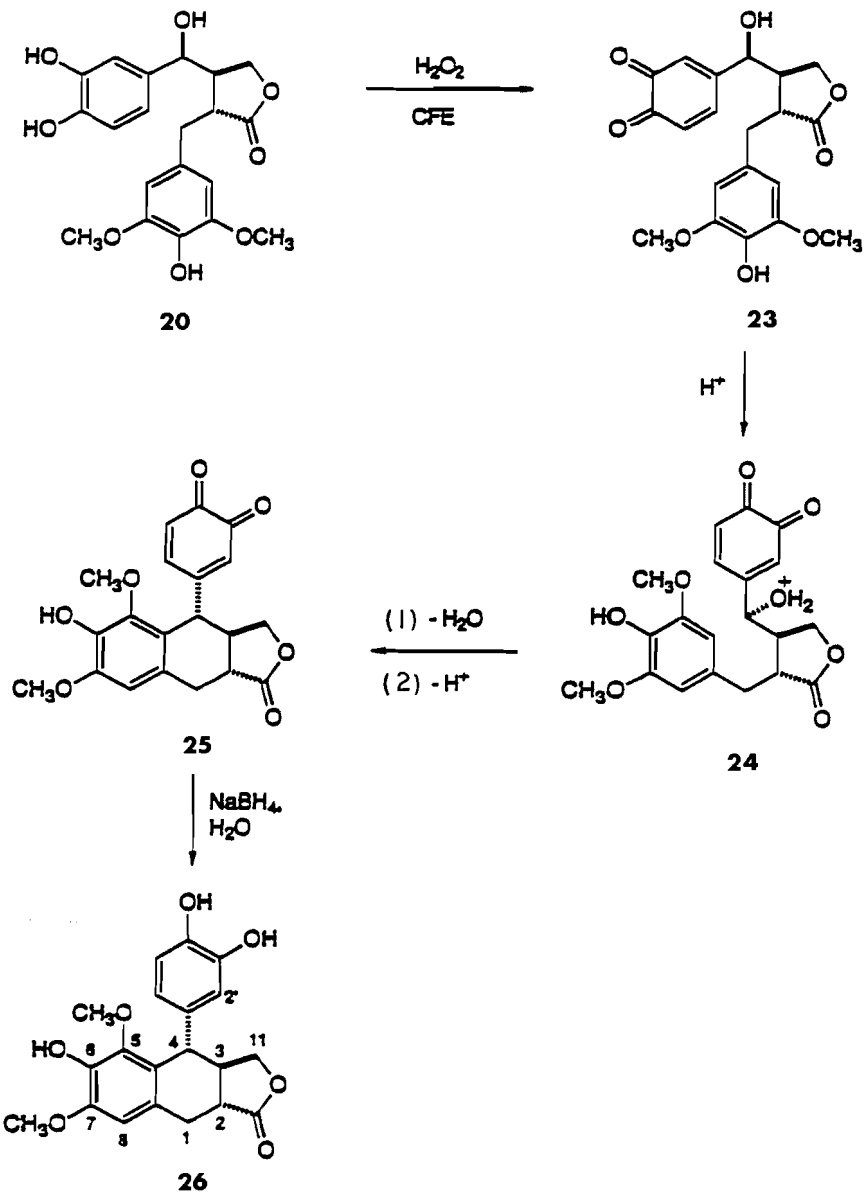
of all signals that suggested two very similar compounds (**27***a*, **27***b*).

Successive treatments of the ethyl acetate extract with large excesses of sodium borohydride and freshly prepared diazomethane (Entry 3, Table 3) afforded two new products. One of these, assigned the structure **28**, was characterized by an ^{1}H NMR spectrum that showed a broad doublet at δ 4.22 (H(4)) and an aromatic signal pattern consistent with the trisubstituted aromatic ring at C(4), and the EI mass spectrum that clearly showed the molecular ion at m/z 414.

TABLE 3. Compounds isolated from ethyl acetate extract of biotransformation of butanolide **20**

Entry	Reagent(s)	Products	Yield (%)	Recovery[a] (%)
1	Sodium borohydride	**20**	12	39
		26	14	
2	Diazomethane	**20**	9	59
		27	15	
3	(1) Sodium Borohydride	**28**	20	82
	(2) Diazomethane	**29**	25	

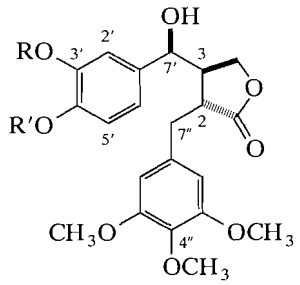
[a] Total recovery of material from chromatographic column or preparative TLC plate.

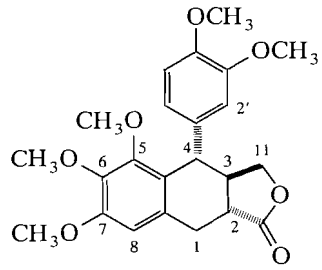


SCHEME 6. Biotransformation of dibenzylbutanolide **20** with cell free extracts of *Catharanthus roseus* cell cultures.

However, the ^{1}H NMR and mass spectral data for the other product, tentatively assigned structure **29**, did not permit an unambiguous structural assignment to be made. It is proposed that the large excess of sodium borohydride in an aqueous medium may have resulted in the hydrolysis of the lactone to the hydroxy acid **29**. Such a structure would be consistent with the polarity of this compound, as acetone was required to elute **29** from the chromatographic column of silica. Further support is provided by the presence of a broad O–H stretch at 3320 cm^{-1} and a carbonyl band at 1670 cm^{-1}

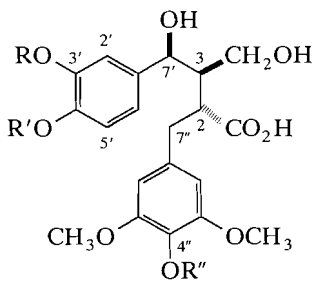
in the IR spectrum, both of which resemble the characteristic stretching frequencies of a carboxylic acid. Both the EI and DCI mass spectra of **29** exhibited a very high degree of fragmentation and peaks of low intensity, as expected for an acyclic compound. However, the acid moiety produced by saponification should have reacted with diazomethane in the subsequent step to afford an ester and this was clearly not the case. Due to its low yield and no direct relevance to





27*a*: R = H; R′ = CH_3
27*b*: R = CH_3; R′ = H

28



R, R′, R″ = H or CH_3

29

the objectives of this study, more detailed investigations to establish a more definitive structure for **29** were not pursued.

The nature of the unrecovered biotransformation products of **20** is not known. The total recovery of material from the chromatographic separation of the ethyl acetate extract, which had been subjected to reduction and methylation (Entry 3, Table 3), was 82% but only 30% of this material showed aromatic proton signals as determined by ^{1}H NMR spectroscopy. Consequently, the methanol extract of the biotransformation mixture was expected to contain a significant portion of the biotransformation products but TLC analysis showed only baseline (R_f 0) material after treatment with sodium borohydride or diazomethane. Treatment with excess sodium borohydride followed by methylation and isolation of material with an R_f value greater than zero by silica TLC analysis gave only minor amounts of an unidentified compound, assumed to be derived from the CFE.

Although the studies with **20** were not entirely conclusive because of the poor recovery of starting material and products, it gave no evidence of the formation of either the desired aryltetralin **21** or its *ortho*-quinone **22** (Scheme 4). It was thus concluded that the oxidation of ring B in **20** to some unidentified species was too rapid to permit the formation of the quinone methide intermediates required for cyclization to **21**. The low recovery values were probably also the result of a highly reactive substrate resulting from the presence of a catechol-like functionality in ring B.

In conclusion, these studies have thus far indicated that *C. roseus* cell culture derived enzymes, originally developed for production of indole alkaloids, are capable of biotransforming selected dibenzylbutanolides, via oxidative coupling, to compounds closely related to the podophyllotoxins. The process can be highly efficient *if* the substrate, as in the case of **7**, possesses a hydroxyl group at C(3′) of the dibenzylbutanolide system. On the other hand, further activation of the ring system, as in **20** where hydroxyl functionality is present at two adjacent centers (C(3′) and C(4′)), leads to competitive facile oxidation and ring closure reactions become of minimal significance. With these data in hand, further studies with various dibenzylbutanolides and cell cultures of *Podophyllum peltatum*, the plant from which the podophyllotoxins are isolated, will be presented in further publications.

Experimental

Melting points were determined using a Reichert melting point apparatus and are uncorrected. The recrystallization solvents are given in parentheses. Infrared spectra were recorded on Perkin Elmer 1710, 710, or 710B spectrometers, using sodium chloride cells, as a chloroform solution (0.1 mm path length), thin film, or Nujol mull. Band frequencies are reported relative to polystyrene (1601 cm^{-1}). Ultraviolet spectra were recorded as methanol solutions on a Cary 15 spectrometer using 1 cm quartz cells. ^{1}H NMR spectra were recorded on Bruker WH-400, Bruker AC 200, or Varian XL-300 spectrometers. Chemical shift values are reported in ppm relative to tetramethylsilane as an internal standard. ^{1}H COSY NMR spectra were recorded on a Bruker AC 200 spectrometer. ^{13}C NMR spectra were recorded on a Varian XL-300 spectrometer at 75.3 MHz or a Bruker AC-200 spectrometer at 50.2 MHz. Signals with a negative intensity in the attached proton test (APT) are indicated as such by "(−)" and imply the attachment of an odd number of protons. Mass spectra were recorded on AEI-MS-9 (low resolution) or Kratos-MS-50 (high resolution) spectrometers, employing electron impact, fast atom bombardment, or desorption chemical ionization methods. Elemental analyses were performed using combustion analysis by Mr. P. Borda, Microanalytical Laboratory, University of British Columbia. Determination of structures by X-ray crystallography was performed by the X-ray Crystallography Laboratory, University of British Columbia.

Cell suspension culture of the AC3 line of *C. roseus* was obtained from Biological Services of the Department of Chemistry, University of British Columbia. Column chromatography (referred to as "flash chromatography") (53) was performed using columns of silica gel (230–400 mesh, Merck art. 9385) or Florisil (60–100 mesh, Fisher F-100) with air or nitrogen gas pressure to obtain a rapid flow rate. Thin-layer chromatography was performed using commercial aluminum-backed silica gel plates (Merck art. 5554). Visualization was accomplished by spraying with 5% ammonium molybdate in 10% aqueous sulfuric acid followed by heating. High-pressure liquid chromatography was performed using a Waters C^{18} "Radial Pak" liquid chromatography cartridge, a Waters 440 absorbance detector set at 280 nm, and a methanol/water eluent. Circular dichroism analysis was performed using a Jasco J-20 automatic recording spectropolarimeter.

3-Benzyloxy-4-methoxybenzaldehyde (2)

A suspension of isovanillin (**1**, 24.9 g, 164 mmol), potassium carbonate (24.8 g, 179 mmol), benzyl chloride (24.8 g, 194 mmol), sodium iodide (1.0 g, 6.7 mmol), and ethanol (75 mL) was refluxed for 4.5 h while stirring with a mechanical stirrer. Water (50 mL) was added and the ethanol removed *in vacuo*. The slurry was poured into a mixture of 1 M sodium hydroxide (125 mL) and

ice (50 g). The solids were filtered off and washed with ice-cold water (3 × 25 mL). Drying *in vacuo* gave the benzyl ether **2** (40.2 g, 100%) as a tan solid. A sample was recrystallized from ethyl acetate/hexanes for analysis.

Physical properties of **2**: mp 60.0–60.5°C (ethyl acetate/hexanes), IR ν_{max} ($CHCl_3$): 1690 cm^{-1}; UV λ_{max} (log ϵ): 273 (4.03), 305 (3.91) nm; ^1H NMR δ: 3.97 (3H, s, $-OCH_3$), 5.20 (2H, s, $-OCH_2Ph$), 7.01 (1H, d, H(5), J = 8 Hz), 7.30–7.49 (7H, m, aromatic), 9.81 (1H, br s, $-CHO$); MS *m/z*: 242 (M^+), 214, 181, 168. High-resolution molecular weight determination calcd. for $C_{15}H_{14}O_3$: 242.0943; found: 242.0942. Anal. calcd. for $C_{15}H_{14}O_3$: C 74.36, H 5.82; found: C 74.25, H 5.82.

E-*2-(3-Benzyloxy-4-methoxybenzylidene)butanedioic acid 1-methyl ester (3)*

A solution of sodium methoxide was prepared by the careful addition of sodium (14.6 g, 633 mmol) to dry methanol (250 mL) under nitrogen. A solution of the benzyl ether **2** (95.7 g, 395 mmol) in dimethylsuccinate (81.6 g, 558 mmol) was added dropwise over 40 min at reflux. After an additional 70 min of stirring at reflux, the bulk of the solvent was removed *in vacuo*. The suspension was cooled to O°C and acidified with 6 M hydrochloric acid. The solids were removed by filtration and the filtrate extracted with dichloromethane (2 × 400 mL). The solids were added to the organic extract, washed with brine (400 mL), dried over magnesium sulfate, filtered, and evaporated *in vacuo* to yield an oily yellow solid, which was recrystallized from ethyl acetate/hexanes to give the hemisuccinate **3** (102.1 g, 73%) as a yellowish powder.

Physical properties of **3**: mp 123–125°C (ethyl acetate/hexanes); IR ν_{max} ($CHCl_3$): 1700 cm^{-1}; UV λ_{max} (log ϵ): 290 (4.03), 307 (4.03) nm; ^1H NMR δ: 3.51 (2H, s, H(2)), 3.86, 3.93 (3H each, s, s, $-CO_2CH_3$, Ar-OCH_3), 5.22 (2H, s, $-OCH_2Ph$), 6.94 (1H, d, H(5′), J = 8 Hz), 7.05 (1H, dd, H(6′), J = 8, 1 Hz), 7.10 (1H, d, H(2′), J = 1 Hz), 7.37–7.45 (5H, m, phenyl), 7.81 (1H, s, H(7′)). MS *m/z*: 356 (M^+), 324, 296. High-resolution molecular weight determination calcd. for $C_{20}H_{20}O_6$: 356.1260; found: 356.1268.

(±)-2-(3-Benzyloxy-4-methoxybenzyl)butanedioic acid 1-methyl ester (4)

Hemisuccinate **3** (96.4 g, 271 mmol) was added to a suspension of magnesium shavings (66.2 g, 2.72 mol) in dry methanol (750 mL) under nitrogen. After a few minutes stirring, the reaction vessel was immersed in an ice bath and stirred at 0°C for 5 h. The suspension was acidified with 6 M hydrochloric acid and the remaining solids were removed by filtration. The filtrate was extracted with dichloromethane (3 × 400 ml), washed with brine (500 mL), dried over magnesium sulfate, filtered, and evaporated *in vacuo* to give the hemisuccinate **4** (86.4 g, 89%) as an amber resin that solidified upon standing at 3°C.

Physical properties of **4**: mp 78–82°C; IR ν_{max} ($CHCl_3$): 1720 cm^{-1}; UV λ_{max} (log ϵ): 279 (3.49) nm; ^1H NMR δ: 2.34 (1H, dd, H(3), J = 18, 4.5 Hz), 2.58–2.72 (2H, m, H(3), H(2)), 2.90–3.10 (2H, m, H(7′)), 3.66 (3H, s, $-CO_2CH_3$), 3.88 (3H, s, Ar-OCH_3), 5.13 (2H, s, $-OCH_2Ph$), 6.70 (2H, m, H(2′), H(6′)), 6.82 (1H, d, H(5′), J = 7.8 Hz), 7.25–7.50 (5H, m, phenyl); MS *m/z*: 358 (M^+), 308, 281. High-resolution molecular weight determination calcd. for $C_{20}H_{22}O_6$: 358.1417; found: 358.1413.

(±)-3-(3-Benzyloxy-4-methoxybenzyl)butanolide (5)

Method 1

The hemisuccinate **4** (2.1 g, 5.7 mmol) was dissolved at 0°C in water (5 mL) with 2 M potassium hydroxide. The solution was neutralized and evaporated *in vacuo* to yield the potassium salt of **4**. A solution of calcium borohydride was prepared by adding a solution of calcium chloride (3.29 g, 28.5 mmol) in methanol (60 mL) to a solution of sodium borohydride (1.62 g, 40.7 mmol) in ethanol (60 mL) at −20°C. Potassium hydroxide (180 mg, 3.2 mmol) was added followed by the potassium salt of **4** dissolved in ethanol (50 mL). The solution was allowed to warm to room temperature and stirred for 4.5 days before being cooled to 0°C, and acidified with 6 M hydrochloric acid. The solution was concentrated *in vacuo*, extracted with dichloromethane (3 × 100 mL), dried over magnesium sulfate, filtered, and evaporated *in vacuo*. Purification by flash chromatography using ethyl acetate/petroleum ether (9:11, v/v) gave the β-benzylbutanolide **5** (1.16 g, 65%) as a yellowish oil that solidified upon standing.

Method 2

Lithium borohydride (0.91 g, 42 mmol) in dry THF (150 mL) was added carefully to a solution of the hemisuccinate **4** (14.7 g, 40.9 mmol) in THF (250 mL) at reflux under nitrogen. The solution was stirred for 2 h at reflux and then cooled to room temperature. Water (2 mL) and 6 M hydrochloric acid (9 mL) were added and the solution was stirred at room temperature for 2.5 h. The bulk of the solvent was removed by evaporation *in vacuo* and the resultant mixture extracted with ether (50 mL). The organic extract was washed with saturated sodium bicarbonate (3 × 25 mL) and water (25 mL) before being dried over magnesium sulfate, filtered, and evaporated *in vacuo* to give the pure β-butanolide **5** (11.4 g, 88%) as a clear oil that solidified upon standing.

Physical properties of **5**: mp 51–54°C; IR ν_{max} ($CHCl_3$): 1770 cm^{-1}; UV λ_{max} (log ϵ): 279 (3.48) nm; ^1H NMR δ: 2.19 (1H, dd, H(2), J = 17.6, 6.7 Hz), 2.50 (1H, dd, H(2), J = 17.6, 7.9 Hz), 2.61–2.80 (3H, m, H(3), H(7′)), 3.89 (3H, s, -OCH_3), 3.91 (1H, dd, H(4), J = 9.1, 6.0 Hz), 4.21 (1H, dd, H(4), J = 9.1, 7.0 Hz), 5.16 (2H, s, -OCH_2Ph), 6.65 (1H, d, H(2′), J = 1 Hz), 6.71 (1H, dd, H(6′), J = 8, 1Hz), 6.85 (1H, d, H(5′), J = 8 Hz); MS *m/z*: 312 (M^+), 222, 137. High-resolution molecular weight determination calcd. for $C_{19}H_{20}O_4$: 312.1362; found: 312.1371.

(±)-trans-*2-(4-Benzyloxy-3,5-dimethoxybenzyl)-3-(3-benzyloxy-4-methoxybenzyl)butanolide (6)*

To a solution of diisopropylamine (2.9 mL, 21 mmol) in dry THF (30 mL) at −78°C was added butyllithium (11 mL, 1.6 M solution, 17 mmol) and stirring continued for 15 min at −78°C and then for 30 min at 0°C. The clear solution was cooled to −78°C, β-butanolide **5** (4.3 g, 14 mmol) in THF (15 mL) was added, and the bright yellow solution stirred for 90 min prior to the addition of the bromide **8** (5.5 g, 16 mmol) in THF (15 mL) and stirring for 8 h at −78°C. The solution was warmed to 0°C, acidified with 1 M hydrochloric acid, and extracted with dichloromethane (2 × 150 mL). The combined organic extracts were washed with water (80 mL), dried over magnesium sulfate, filtered, and evaporated *in vacuo*. Flash chromatography using ethyl acetate/petroleum ether (9:11, v/v) gave the dibenzylbutanolide **6** (6.2 g, 80%) as a yellowish foam.

Physical properties of **6**: mp 35–37°C, IR ν_{max} ($CHCl_3$): 1770 cm^{-1}; UV λ_{max} (log ϵ): 278 (3.55) nm; ^1H NMR δ: 2.32 (1H, dd, H(7″), J = 14.4, 8.2 Hz), 2.45–2.70 (3H, m, H(7″), H(2), H(3)), 3.06 (1H, dd, H(7′), J = 14.0, 8.0 Hz), 3.32 (1H, dd, H(7′), J = 14.0, 5.4 Hz), 3.74 (1H, dd, H(4), J = 9, 8 Hz), 3.80 (6H, s, -OCH_3(3″, 5″)), 3.88 (3H, s, -OCH_3(4′)), 3.99 (1H, dd, H(4), J = 9.2, 7.0 Hz), 5.05, 5.13 (2H each, s, s, -OCH_2Ph), 6.52–6.58 (3H, m, H(2″), H(6′)), 6.69 (1H, s, H(2′)), 6.77 (1H, d, H(5′), J = 8.0 Hz), 7.25–7.49 (10H, m, phenyl); MS *m/z*: 568 (M^+), 402, 312, 167, 137, 91. High-resolution molecular weight determination calcd. for $C_{35}H_{36}O_7$: 568.2461; found: 568.2457.

(±)-trans-*2-(3,5,-Dimethoxy-4-hydroxybenzyl)-3-(3-hydroxy-4-methoxybenzyl)butanolide (7)*

Palladium-on-charcoal (5%, 1.05 g) was suspended in ethanol (40 mL) and stirred under hydrogen at atmospheric pressure for 1.5 h. The dibenzylbutanolide **6** (6.2 g, 11 mmol) was added as an ethyl acetate/ethanol solution (1:3, v/v, 40 mL) and the resultant suspension stirred for 5 h. The catalyst was filtered off and the solvent evaporated *in vacuo* to yield the bis(hydroxybenzyl)butanolide **7** (3.7 g, 87%) as an amorphous white solid after

flash chromatography using ethyl acetate/petroleum ether (11:9, v/v). Small samples were crystallized from diethyl ether/petroleum ether for melting point determination or distilled (240°C, 0.15 Torr; 1 Torr = 133.3 Pa) for microanalysis.

Physical properties of **7**: mp 68–69°C (diethyl ether/petroleum ether); IR ν_{max} ($CHCl_3$): 3540, 1770 cm^{-1}; UV λ_{max} (log ϵ): 279 (3.57) nm; ^1H NMR δ: 2.45–2.65 (4H, m, H(2), H(3), H(7″)), 2.88 (1H, dd, H(7′), J = 14.6, 5.7 Hz), 2.93 (1H, dd, H(7′), J = 14.6, 6.4 Hz), 3.87–3.89 (10 H, m, -OCH_3, H(4)), 4.15 (1H, dd, H(4), J = 7.5, 3.0 Hz), 5.42 (1H, s, -OH(4″)), 5.58 (1H, s, -OH(3′)), 6.49 (1H, dd, H(6′), J = 8.5, 2.1 Hz), 6.62 (1H, d, H(2′), J = 2.1 Hz), 6.73 (1H, d, H(5′), J = 8.5 Hz). All hydroxyl signals disappear upon addition of D_2O. ^{13}C NMR δ: 35.363 (C(7″), 38.147 (C(7′)), 41.891(−), 47.055(−), 56.175(−), 56.531(−) (-OCH_3(3″)), 71.756 (C(4)), 107.549(−) (C(2″)), 112.307(−), 116.348(−), 120.434(−), 129.383, 132.645, 135.354, 146.894, 147.280, 148.544, 179.051 (C(1)); MS *ms/z*: 388 (M^+), 167, 153, 137. High-resolution molecular weight determination calcd. for $C_{21}H_{24}O_7$: 388.1522; found: 388.1520. Anal. calcd. for $C_{21}H_{24}O_7$: C 64.94, H 6.23; calcd. for $C_{21}H_{26}O_8$ (58 + H_2O): C 62.06, H 6.45; found: C 62.31, H 6.25.

*1-Bis(phenylthio)methyl-3,4-methylenedioxybenzene (**14**)*

To a solution of piperonal **13** (1.0 g, 6,7 mmol) in dry chloroform (10 mL) at −40°C under argon was added thiophenol (1.4 mL, 14 mmol) and boron trifluoride etherate (4.6 mL, 45% solution, 17 mmol). The solution was stirred for 15 min at −40°C and then poured into ice-cold water (10 mL). The organic layer was drawn off and the aqueous layer extracted with chloroform (30 mL). The combined organic extracts were washed with 7% potassium hydroxide (2 × 25 mL), water (25 mL), and brine (25 mL) before being dried over potassium carbonate, filtered, and evaporated *in vacuo* to yield the pure dithioacetal **14** (2.3 g, 98%) as a yellowish oil. The physical properties of **14** were determined using a distilled (190°C, 0.15 Torr) sample.

Physical properties of **14**: iR ν_{max} (neat): 2890, 1490 cm^{-1}; UV λ_{max} (log ϵ): 248 (4.14), 282 (3.88) nm; ^1H NMR δ: 5.37 (1H, s, -CH ($SPh)_2$), 5.96 (2H, s, -OCH_2O-), 6.67 (1H, d, H(5), J = 4 Hz), 6.78 (1H, dd, H(6), J = 4, 2 Hz), 6.98 (1H, d, H(2), J = 2 Hz), 7.23–7.32 (6H, m, phenyl), 7.34–7.42 (4H, m, phenyl); MS *m/z*: 352 (M^+), 243. High-resolution molecular weight determination calcd. for $C_{20}H_{16}O_2S_2$: 352.0592; found: 352.0598. Anal. calcd. for $C_{20}H_{16}O_2S_2$: C 68.15, H 4.58; found: C 68.11, H 4.63.

*(±)-trans-2-(4-Benzyloxy-3,5-dimethoxybenzyl)-3-(3,4-methylenedioxy)-α,α-bis(phenylthio)benzyl)butanolide (**17**)*

Butyllithium (3.60 mL, 1.6 M solution, 5.76 mmol) was added to a solution of dithioacetal **14** (2.00 g, 5.69 mmol) in dry THF (10 mL) at −78°C and the purple solution was stirred for 2.5 h. But-2-en-4-olide (**15**, 0.481 g, 5.73 mmol) in THF (8 mL) was added and the solution stirred for 2.5 h at −78°C prior to the addition of the bromide **8** (2.06 g, 6.10 mmol) in THF (10 mL). The solution was allowed to warm slowly to room temperature overnight. Water (25 mL) was added and the mixture extracted with ethyl acetate (160 mL). The organic extract was washed with water (2 × 25 mL) before being dried over magnesium sulfate, filtered, and evaporated *in vacuo*. Purification by flash chromatography using dichloromethane gave the expected dithioketal **16** (replace OLi by carbonyl) (2.16 g. 55%) as an amorphous solid.

Physical properties of **17**: mp 55–57°C (acetone); IR ν_{max} ($CHCl_3$): 1770 cm^{-1}; UV λ_{max} (log ϵ): 279 (3.82), 286 (3.80) nm; ^1H NMR δ: 2.79 (1H, dd, H(7″), J = 14, 5 Hz), 2.85–2.90 (1H, m, H(2)), 3.12 (1H, dd, H(7″), J = 14, 4 Hz), 3.29–3.34 (1H, m, H(3)), 3.49 (1H, dd, H(4), J = 10, 8 Hz), 3.70 (6H, s, -OCH_3), 4.46 (1H, dd, H(4), J = 10, 3 Hz), 5.02 (2H, s, -OCH_2Ph), 6.00, 6.01 (2H total, d, d, -OCH_2O-, J = 1 Hz each), 6.18 (2H, s, H(2″)), 6.73 (1H, d, H(5′), J = 8 Hz), 7.13 (1H, dd, H(6′), J = 8, 2 Hz), 7.21–7.38 (14H, m, phenyl, H(2′)), 7.43–7.47 (2H, m, phenyl); FAB MS *m/z*: 693 (M^+), 584, 473, 383, 218, 185, 167, 154, 135; MS *m/z*: 584, 583, 383, 218, 185, 167, 154, 135. High-resolution molecular weight determination calcd. for $C_{34}H_{31}O_7S$ (M^+−SPh): 583.1790; found: 583.1792. Anal. calcd. for $C_{40}H_{36}O_7S_2$: C 69.34, H 5.24, S 9.25; found: C 69.18, H 5.42, S 9.23.

*(±)-trans-2-(4-Benzyloxy-3,5-dimethoxybenzyl)-3-(3,4-methylenedioxybenzoyl)butanolide (**18**, R = CH_2Ph) and (±)-trans-2-(3,5-dimethoxy-4-hydroxybenzyl)-3-(3,4-methylenedioxybenzoyl)butanolide (**18**, R = H)*

Method 1

Dithioketal **16** (replace OLi by carbonyl) (34.5 mg, 50.0 μmol) was dissolved in ethyl acetate (6 mL). Perchloric acid (70%, 1 drop) was added and the solution stirred for 4 h. Water (1 mL and ethyl acetate (10 mL) were added and the phases separated. The organic extract was washed with brine (2 mL) and saturated sodium bicarbonate (2 × 2 mL), dried over magnesium sulfate, filtered, and evaporated *in vacuo*. Flash chromatography using acetate/dichloromethane (1:19, v/v) gave the ketones **18** (R = CH_2Ph) (10.8 mg, 44%) and **18** (R = H) (2.0 mg, 10%).

Method 2

Sulfuryl chloride (11.8 mg, 87.4 μmol) in dichloromethane (0.4 mL) was added to a mixture of dithioketal **16** (replace OLi by carbonyl) (48.7 mg, 70.3 μmol), silica gel (230–400 mesh, 30 mg), and water (30 mg) in dichloromethane (0.35 mL). After stirring for 2 h, finely powdered anhydrous potassium carbonate was added and stirring continued for 0.5 h. The solids were filtered off and washed with dichloromethane. The filtrate was evaporated *in vacuo* and purified by flash chromatography using acetone/dichloromethane (1:49, v/v) but neither **18** (R = CH_2Ph) nor **18** (R = H) could be identified by ^1H NMR spectroscopy of the major fractions.

Method 3

Iodine (1.46 g, 11.5 mmol) was added to a solution of dithioketal **16** (replace OLi by carbonyl) (1.85 g, 2.68 mmol) in methanol (50 mL) and THF (5 mL). The solution was then stirred at reflux for 1.5 h prior to the addition of saturated sodium thiosulfate (10 mL), brine (10 mL), and water (20 mL). The mixture was extracted with ethyl acetate (100 mL), diluted with water (10 mL) and brine (10 mL), and re-extracted with ethyl acetate (100 mL). The organic extract was washed with water (20 mL), dried over magnesium sulfate, filtered, and evaporated *in vacuo*. THF (5 mL) and 3 M hydrochloric acid (6 mL) were added and the suspension stirred at room temperature for 1 h before being diluted with water (10 mL) and extracted with dichloromethane (2 × 50 mL). The organic extract was washed with water (2 × 20 mL), dried over magnesium sulfate, filtered, and evaporated *in vacuo*. Ether (25 mL) was added and the suspension sonicated for 5 min, stored at 3°C for 3 days, filtered, and washed with ice-cold ether (2 × 5 mL) to give the ketone **18** (R = CH_2Ph) as a white powder (0.920 g). The filtrate was evaporated and purified by flash chromatography using acetone/dichloromethane (0:1 to 1:49 gradient, v/v) to afford **18** (R = CH_2Ph) (134 mg) for a total yield of 80%.

Physical properties of **18** (R = CH_2Ph): mp 43–44°C (diethyl ether); IR ν_{max} ($CHCl_3$): 1770, 1670 cm^{-1}; UV λ_{max} (log ϵ): 275 (3.82), 314 (3.90) nm; ^1H NMR δ: 3.00 (1H, dd, H(7″), J = 14, 6 Hz), 3.09 (1H, dd, H(7″), J = 14, 5 Hz), 3.55–3.61 (1H, m, H(2)), 3.70 (6H, s, -OCH_3), 4.01 (1H, ddd, H(3) J = 8, 8, 8 Hz), 4.10 (1H, dd, H(4), J = 9, 8 Hz), 4.38 (1H, dd, H(4), J = 9, 8 Hz), 4.92 (2H s, -OCH_2Ph), 6.01, 6.04 (1H each, d, d, -OCH_2O-, J = 1 Hz each), 6.28 (2H, s, H(2″)), 6.80 (1H, d, H(5′), J = 8 Hz), 7.24–7.38 (5H, m, H(2′), H(6′), phenyl), 7.47 (2H, dd, phenyl, J = 7, 1 Hz); MS *m/z*: 490 (M^+), 399, 223, 149, 91. High-resolution molecular weight determination calcd. for $C_{28}H_{26}O_8$:

490.1627; found: 490.1627. Anal. calcd. for $C_{28}H_{26}O_8$: C 68.56, H 5.34; found: C 68.78, H 5.56.

Physical properties of **18** (R = H): mp 56–58°C; IR ν_{max} ($CHCl_3$): 3530, 1770, 1670 cm^{-1}; UV λ_{max} (log ϵ): 276 (3.80), 314 (3.86) nm; ^1H NMR δ: 3.01 (1H, d, H(7″), J = 14, 7 Hz), 3.07 (1H, dd, H(7″), J = 14, 6 Hz), 3.53–3.60 (1H, m, H(2)), 3.78 (6H, s, -OCH_3), 4.02 (1H, ddd, H(3), J = 9, 9, 9 Hz), 4.12 (1H, dd, H(4), J = 9, 9 Hz), 4.41 (1H, dd, H(4), J = 9, 9 Hz), 5.37 (1H, s, -OH, disappears with D_2O), 6.07 (2H, m, -OCH_2O-), 6.30 (2H, s, H(2″)), 6.81 (1H, d, H(5′), J = 8 Hz), 7.23 (1H, d, H(2′), J = 2 Hz), 7.27 (1H, dd, H(6′), J = 8, 2 Hz); MS m/z: 400 (M^+), 224, 167, 149. High-resolution molecular weight determination calcd. for $C_{21}H_{20}O_8$: 400.1158; found: 400.1164.

*(±)-trans-2-(3,5-Dimethoxy-4-hydroxybenzyl)-3-(α-hydroxy-3,4-methylenedioxybenzyl)butanolide (**19**, R = H)*

Method 1

Palladium-on-charcoal (10%, 1.676 g) was suspended in ethanol (100 mL) and stirred for 1 h under hydrogen at atmospheric pressure. A solution of the ketone **18** (R = CH_2Ph) (4.50 g, 9.16 mmol) in ethyl acetate (100 mL) was added and the resultant suspension stirred for 7 h. The catalyst was filtered off and the solution evaporated *in vacuo*. The residue was purified by column chromatography using acetone/dichloromethane (1:19 to 1:9 gradient, v/v) to give ketone **18** (R = Ch_2Ph) (0.685 g, 15%), ketone **18** (R = H) (1.686 g, 46%), and alcohol **19** (R = H) (1.113 g, 30%, 3.3:1 mixture of C(7′) epimers according to ^1H NMR spectroscopy).

Method 2

Sodium borohydride (47 mg, 1.2 mmol) was added to a suspension of the ketone **18** (R = H) (464 mg, 1.16 mmol) in dry methanol (50 mL) at 0°C under nitrogen. After stirring for 2.5 h at O°C, 1 M hydrochloric acid (2 mL) was added to the resultant clear solution, which was then concentrated *in vacuo*, diluted with water (5 mL) and brine (5 mL), and extracted with ethyl acetate (2 × 50 mL). The combined organic extracts were washed with water (15 mL), dried over magnesium sulfate, filtered, and evaporated *in vacuo* to give the alcohol **19** (R = H) (443 mg, 95%) as an amorphous white solid. ^1H NMR spectroscopy showed the product to be a 6:1 mixture of C(7′) epimers.

Properties of 3.3:1 mixture of **19** (R = H) (C(7′) β-hydroxyl epimer assumed as major): IR ν_{max} ($CHCl_3$): 3600, 3530, 1770 cm^{-1}; UV λ_{max} (log ϵ): 282 (3.60) nm; ^1H NMR (integrals relative to minor (α) epimer) δ: 1.94 (1H, d, -OH(7′, α), J = 3 Hz), 2.00 (3.3 H, d, -OH(7′, β), J = 3 Hz), 2.51–3.11 (17.2 H, m, H(7″), H(2), H(3)), 3.85 (6H, s, -OCH_3(α)), 3.87 (19.8 H, s, -OCH_3(β)), 3.92–3.96 (6.6 H, m, H(4, β)), 4.17 (1H, dd, H(4, α), J = 9.6, 7.6 Hz), 4.37 (1H, dd, H(7′, α), J = 8.0, 2.5 Hz), 4.40 (1H, dd, H(4, α), J = 9.6, 6.1 Hz), 4.62 (3.3 H, dd, H(7′, β), J = 6.7, 2.9 Hz), 5.40 (4.3 H, s, -OH(4″)), 5.97, 5.98 (3.3 H each, d, d, -OCH_2O-(β), J = 1 Hz), 5.99, 6.00 (1H each, d, d, -OCH_2O-(α), J = 1 Hz), 6.27 (2H, s, H(2″, α)), 6.40 (6.6 H, s, H(2″, β)), 6.61–6.76 (12.9 H, m, H(2′), H(5′), H(6′)). Upon addition of D_2O, all hydroxyl signals disappear and the H(7′) signals collapse to doublets. MS m/z: 402 (M^+), 384, 178, 167, 224, 151, 135, 123. High-resolution molecular weight determination calcd. for $C_{21}H_{20}O_8$: 402.1314; found: 402.1302. Anal. calcd. for $C_{21}H_{22}O_8$: C 62.28, H 5.51; found: C 62.33, H 5.80.

Physical properties of 6:1 mixture of **19** (R = H): mp 43–51°C.

*(±)-trans-2-(3,5-Dimethoxy-4-hydroxybenzyl)-3-(3,4-dihydroxy-α-hydroxybenzyl)butanolide (**20**) and (±)-4-(3,4-dihydroxyphenyl)-5,7-dimethoxy-6-hydroxy-2-hydroxymethyl-1,2,3,4-tetrahydro-2-naphthoic acid γ lactone (**26**)*

Method 1

Alcohol mixture **19** (R = H) (210 mg, 0.52 mmol) dissolved in dichloromethane (10 mL) was added to a solution of boron trichloride (3.1 mmol) in dichloromethane (23.5 mL) at −78°C. After stirring for 2 h at −78°C, saturated potassium bicarbonate (2 mL) was added and the suspension allowed to warm to room temperature. Water (5 mL) and brine (1 mL) were added and the mixture was extracted with ethyl acetate (2 × 50 mL). The organic extract was dried over magnesium sulfate, filtered, and evaporated *in vacuo*. Acetone (4 mL), water (5 mL), and calcium carbonate (330 mg) were added and the suspension stirred for 1.5 h at 65°C. The solids were dissolved by the addition of 6 M hydrochloric acid and the clear solution extracted with ethyl acetate (2 × 50 mL). The organic extract was washed with water (15 mL), dried over magnesium sulfate, filtered, and evaporated *in vacuo*. Flash chromatography using acetone/dichloromethane (2:8 to 3:8, v/v) gave the aryltetralin **26** (78 mg, 40%) and the catechol **20** (49 mg, 24%, single epimer by ^1H NMR spectroscopy) as amorphous white solids. A sample of **26** was recrystallized from methanol/chloroform for analysis.

Method 2

Alcohol **19** (R = CH_2Ph) (161 mg, 0.327 mmol) dissolved in dichloromethane (6.5 mL) was added to a stirred solution of boron trichloride (2.0 mmol) in dichloromethane (15.3 mL) at −78°C. After stirring for 2 h, water (3 mL), saturated potassium hydrogen carbonate (1 mL), and brine (1 mL) were added and the mixture allowed to warm to room temperature. The mixture was extracted with ethyl acetate (2 × 40 mL), dried over magnesium sulfate, filtered, and evaporated *in vacuo*. Acetone (3 mL), water (6.5 mL), and calcium carbonate (210 mg) were added and the suspension stirred for 1.5 h at 65°C. The solids were dissolved with 6 M hydrochloric acid and the solution extracted with ethyl acetate (2 × 40 mL). The organic extracts were washed with water (10 mL), dried over magnesium sulfate, filtered, and evaporated *in vacuo* to afford a yellowish foam. Flash chromatography using acetone/dichloromethane (1:4 to 3:2 gradient, v/v) afforded the alcohols **19** (R = H) (42 mg, 32%, 5:1 mixture of epimers by ^1H NMR spectroscopy) and **20** (33 mg, 26%).

Physical properties of **20**: mp 49–56°C; IR ν_{max} (Nujol): 3380, 1740 cm^{-1}; UV λ_{max} (log ϵ): 281 (3.57) nm; ^1H NMR δ: 2.64–2.77, 2.95–3.05 (4H total, m, m, H(7″), H(2), H(3)), 3.80 (6H, s, -OCH_3), 3.93 (1H, dd, H(4), J = 17, 9 Hz), 3.98 (1H, dd, H(4), J = 17, 8 Hz), 4.55 (1H, d, -OH(7′), J = 3.6 Hz), 4.68 (1H, dd, H(7′), J = 6.0, 3.6 Hz), 6.42 (2H, s, H(2″)), 6.68 (1H, dd, H(6′), J = 8, 2 Hz), 6.80 (1H, d, H(5′), J = 8 Hz), 6.88 (1H, d, H (2′), J = 2 Hz), 6.91 (1H, brs, -OH(4″)), 7.80 (2H, brs, -OH(3′), -OH(4′)). Upon addition of D_2O, all hydroxyl signals disappear and the H(7′) signal collapses to a doublet. ^{13}C NMR δ: 35.619 (C(7″)), 43.787(−), 45.709(−), 56.464(−) (-OCH_3), 68.892 (C(4)), 74.217(−) (C(7′)), 107.994(−) (C(2″)), 114.086(−), 115.787(−), 118.432(−), 129.114, 135.743; 145.227, 145.826, 148.339, 179.568 (C(1)); MS m/z: 390 (M^+), 372, 167, 154, 123, 110. High-resolution molecular weight calcd. for $C_{20}H_{22}O_8$: 390.1315; found: 390.1313.

Physical properties of **26**: mp 222–224°C (methanol/chloroform); IR ν_{max} (Nujol): 3400, 3250, 1730 cm^{-1}; UV λ_{max} (log ϵ): 283 (3.70) nm; ^1H NMR δ: 2.30–2.41 (1H, m, H(3)), 2.51 (1H, ddd, H(2), J = 13.6, 11.7, 4.5 Hz), 2.88–2.92 (1H, m, H(1)), 3.03 (1H, dd, H(1), J = 15.3, 4.5 Hz), 3.18 (3H, s, -OCH_3(5)), 3.87 (3H, s, OCH_3(7)), 4.04 (1H, brd, H(4), J = 10.8 Hz), 4.16–4.20 (2H, m, H(11)), 6.50 (1H, dd, H(6′), J = 8, 2 Hz), 6.55 (1H, d, H(2′), J = 2 Hz), 6.65 (1H, s, H(8)), 6.74 (1H, d, H(5′), J = 8 Hz), 7.12 (1H, s, -OH(6)), 7.54, 7.56 (1H each, s, s, -OH(3′), -OH(4′)). All hydroxyl signals disappear upon addition of D_2O. ^{13}C NMR δ: 41.595(−), 46.525(−), 50.499(−), 56.255(−), 58.614(−), 72.246, 101.389, 107.912(−), 114.565(−), 115(779(−), 118.991(−), 127.733, 139.071, 139.320, 143.913, 145.686, 177.2 (C(1)); MS m/z: 372 (M^+), 262. High-resolution molecular weight determination calcd. for $C_{20}H_{20}O_7$: 372.1219;

found: 372.1217. Anal. calcd. for $C_{20}H_{20}O_7$: C 64.51, H 5.41; found: C 63.58, H 5.57.

*(±)-*trans*-2-(4-Benzyloxy-3,5-dimethoxybenzyl)-3-(α-hydroxy-3,4-methylenedioxybenzyl)butanolide (**19**, R = CH_2Ph)*

Method 1

Sodium borohydride (19 mg, 0.51 mmol) was added to a suspension of ketone **18** (R = Ch_2Ph) (173 mg, 0.352 mmol) in dry methanol (25 mL) at 0°C under nitrogen. After stirring for 5 h at 0°C, 1 M hydrochloric acid (3 mL) was added to the resultant clear solution, which was then concentrated *in vacuo*, diluted with brine (2 mL), and extracted with dichloromethane (2 × 40 mL). The combined organic extracts were washed with water (8 mL), dried over magnesium sulfate, filtered, and evaporated *in vacuo* to give alcohol **19** (R = CH_2Ph) (172 mg, 99%) as an amorphous white solid. ^1H NMR spectroscopy indicated that the product was a 4.9:1 mixture of epimers.

Method 2

Lithium aluminum tri-*tert*-butoxy hydride (690 mg, 2.7 mmol) in THF (8 mL) was added to a stirred suspension of the ketone **18** (R = Ch_2Ph) (380 mg, 0.78 mmol) in dry ether (25 mL) and THF (5 mL) under nitrogen at −78°C. The mixture was stirred for 3 h and then acidified with 1 M hydrochloric acid (6 mL), diluted with water (15 mL), and extracted with ethyl acetate (2 × 50 mL). The organic extract was washed with saturated sodium bicarbonate (2 × 15 mL) and water (10 mL), dried over magnesium sulfate, filtered, and evaporated *in vacuo* to afford a foam (329 mg) that, by ^1H NMR spectroscopy, contained the alcohol **19** (R = CH_2Ph) (70%, 5.7:1 mixture of epimers) and the ketone **18** (R = CH_2Ph).

Physical properties of 4.9:1 mixture of **19** (R = CH_2Ph) (C(7′) β-hydroxyl epimer assumed as major): mp 46–49°C, IR ν_{max} ($CHCl_3$): 3450, 1770 cm^{-1}; UV λ_{max} (log ε): 284 (3.55) nm; ^1H NMR δ: 1.87 (1h, d, OH(7′, α), J = 3 Hz), 1.96 (4.9 H, d, -OH(7′, β), J = 2.8 Hz), 2.47–3.10 (23.6 H, m, H(2), H(3), H(7′)), 3.78–3.79 (35.4 H, m, $-OCH_3$), 3.89–3.96 (9.8 H, m, H(4, β)), 4.15–4.17 (1H, m, H(4, α)), 4.33 (1H, dd, H(7′, α), J = 7.1, 3 Hz), 4.37 (1H, dd, H(4, α), J = 9.3, 6.4 Hz), 4.60 (4.9 H, dd, H(7′, β), J = 7.1, 2.8 Hz), 5.00 (11.8 H, s, $-OCH_2Ph$), 5.93–6.01 (11.8 H, m, $-OCH_2O-$), 6.25 (2H, s, H(2″, α)), 6.38 (9.8 H, s, H(2″, β)), 6.57–6.78 (17.7 H, m, H(2′), H(5′), H(6′)), 7.22–7.50 (29.5 H, m, phenyl). Upon D_2O addition, both hydroxyl signals disappear and H(7′) signals collapse to doublets. MS m/z: 492 (M^+), 474, 161, 149, 131, 91. High-resolution molecular weight determination calcd. for $C_{28}H_{28}O_8$: 492.1784, found: 492.1784.

Preparation of C. roseus *cell-free extract (CFE)*

Catharanthus roseus (AC 3 line) cell suspension culture was grown in shake flasks in the dark in a 1B5 medium (54) (500 mL) containing agar (7–8 g) at pH 5.5. The cell line was subcultured every 10 days using a 12% (60 mL) inoculum and harvested at age 11 days. The whole cell suspension was filtered with Miracloth, drained thoroughly, washed with water (75 mL/flask), and drained again. The rest of the procedure was carried out at 0–4°C. Potassium phosphate buffer (0.100 M, pH_h, 0.50 mL/g fresh weight) was added and the suspension homogenized with an Ultra-Turrax blender at 20 000 rpm in four 30-s periods with 30-s intervals. The material was centrifuged, using a Sorvall RC-5B centrifuge and Sorvall GSA rotor, at 10 000 g for 30 min and the supernatant (CFE) analyzed for peroxidase activity (55) and protein concentration (41,42,56) using a Bausch and Lomb Spectronic 20 spectrophotometer.

Determination of hydrogen peroxide consumption by C. roseus *CFE*

An aliquot (3.00 mL) of a solution of **7** (15.3 mg, 39.3 μmol) in ethanol/water (8:17, v/v) was added to water (3.00 mL) and hydrogen peroxide (1.00 mL, 81.6 mM solution, 81.6 μmol). Potassium phosphate buffer (0.100 M, pH 6.3, 10.70 mL) and CFE (pH 6.3, specific activity 0.98 units/mg, 7.30 mL, 9.83 units) were added and the solution (Solution A) stirred for 3 h. An identical solution of water, hydrogen peroxide, buffer, and CFE was stirred for 15 min upon the addition of CFE: an aliquot of **7** was then added as before and the solution (solution B) stirred for an additional 3 h. After each solution had been stirred 1 h following addition of **7**, hydrogen peroxide (1.00 mL, 81.6 μmol) was added to Solution B and water (1.00 mL) to Solution A. HPLC analysis was then performed.

*(±)-1-(3,5-Dimethoxy-4-hydroxyphenyl)-6-hydroxy-3-hydroxymethyl-7-methoxy-1,2,3,4-tetrahydro-2-naphthoic acid γ lactone **9**; (±-5,5′-bis(1-(3,5-dimethoxy-4-hydroxyphenyl)-6-hydroxy-3-hydroxymethyl-7-methoxy-1,2,3,4-tetrahydro-2-naphthoic acid γ lactone (**10**), and (±)-1-(3,5-dimethoxy-4-hydroxyphenyl)-8-hydroxy-3-hydroxymethyl-7-methoxy-1,2,3,4-tetrahydro-2-naphthoic acid γ lactone (**11**)*

Catharanthus roseus CFE (pH 6.3, specific activity 1.5 units/mg, 215 mL, 330 units) was added to a solution containing butanolide **7** (1.860 g, 4.79 mmol) in ethanol (25 mL), water (100 mL), and hydrogen peroxide (20 mL, 0.17 M solution, 3.4 mmol). After stirring for 1 h, further hydrogen peroxide (10 mL, 1.7 mmol) was added and stirring continued for an additional 4.5 h. The reaction mixture was diluted with dichloromethane (500 mL), filtered through Celite, and the organic extract dried over anhydrous magnesium sulfate. The dichloromethane extract was purified by column chromatography using acetone/dichloromethane (1:4–1:0, v/v) to afford aryltetralin **9** (901 mg, 48%), dimer **10** (680 mg, 37%), and aryltetralin **11** (9.8 mg, 0.53%).

Physical properties of **9**: mp 240–241°C (chloroform); IR ν_{max} ($CHCl_3$): 3530, 1778 cm^{-1}; UV λ_{max} (log ε): 282 (3.53) nm; ^1H NMR δ: 2.47 (1H, dd, H(2), J = 14, 11 Hz), 2.59 (1H, m, H(3)), 2.90 (1H, br dd, H(4), J = 15, 11 Hz), 2.97 (1H, dd, H(4), J = 14, 5 Hz), 3.64 (3H, s, $-OCH_3$(7)), 3.85 (6H, s, $-OCH_3$(3″)), 3.98 (1H, dd, H(11), J = 11, 8 Hz), 4.07 (1H, br d, H(1), J = 11 Hz), 4.52 (1H, dd, H(11), J = 11, 7 Hz), 5.43, 5.51 (1H each, s, s, -OH(4′), -OH(6)), 6.34, 6.70 (1H each, s, s, H(5), H(8)), 6.45 2H, s, H(2′)). Both hydroxyl signals disappear upon addition of D_2O. ^{13}C NMR δ: 32.661 (C(4)), 40.68(−), 47.037(−), 48.544(−), 56.106(−), 56.558(−), (−OCH_3(3′)), 71.384 (C(11)), 107.810(−) C(2′)), 113.754(−), 115.696(−), 115.777(−), 176(C(1)). MS m/z: 386 (M^+), 232, 167, 154, 139. High-resolution molecular weight determination calcd. for $C_{21}H_{22}O_7$: 386.1366; found: 386.1366. Anal. calcd. for $C_{21}H_{22}O_7$: C 65.28, H 5.74; found: C 65.12, H 5.72. Circular dichroism analysis: no Cotton effect observed for 0.208 nM methanol solution (250–320 nm). A sample crystallized from chloroform/dichloromethane (1:1, v/v) by slow evaporation was submitted for X-ray analysis. The X-ray data are attached as an Appendix 1.

Physical properties of **10**: mp 156–168°C; IR ν_{max} ($CHCl_3$): 3540, 1780 cm^{-1}; ^1H NMR δ: 2.40–2.52 (3H, m, H(2) (twice), H(4)), 2.55–2.68 (2H, m, H(3)), 2.90 (1H, br dd, H(4), J = 14, 13 Hz), 2.98 (1H, dd, H(4), J = 14, 4 Hz), 3.48–3.55 (1H, m, H(4)), 3.61 (3H, s, OCH_3(7)), 3.67, 3.67 (3H total, s, s, $-OCH_3$(7)), 3.75 (6H, s, $-OCH_3$(3′)), 3.83, 3.83 (6H total, s, s, $-OCH_3$(3′)), 3.95–4.03 (2H, m, H(11)), 4.14 (2H, br d, H(1), J = 12 Hz), 4.48–4.56 (2H, m, H(11)), 5.44, 5.55, 6.02, 6.05 (4H total, s, s, s, s, -OH), 6.18 (1H, s, -OH(6)), 6.36, 6.36 (1H total, s, s, H(8)), 6.45 (2H, br s, H(2′)), 6.51 (2H, br s, H(2′)), 6.73 (1H, s, H(8)). All hydroxyl signals disappear upon addition of D_2O. DCI MS m/z: 788 (83 + $NH_4)^+$, 404. EI MS m/z: 770 (M^+), 400, 386, 167, 154.

Physical properties of **11**: ^1H NMR δ: 2.36 (1H, m, H(2)), 2.59–2.74 (2H, m, H(3), H(4)), 3.08 (1H, dd, H(4), J = 16, 2 Hz), 3.60 (3H, s, $-OCH_3$), 3.74 (1H, d, H(1), J = 11 Hz), 3.80, 3.89 (3H each, s, s, $-OCH_3$), 4.09 (1H, dd, H(11), J = 10, 9 Hz), 4.61 (1H, dd, H(11), J = 10, 7 Hz), 5.38, 5.43 (1 H each, s, s, -OH(6),

-OH(4′)), 5.56, 6.09 (1H each, d, d, H(2′), H(6′), J = 2.5 Hz each), 6.77, 6.85 (1H each, d, d, H(5), H(6), J = 8 Hz each); MS m/z: 386 (M^+), 154, 139. High-resolution molecular weight determination calcd. for $C_{21}H_{22}O_7$: 386.1366; found: 386.1373.

Attempted biotransformation of trans-*2-(3,5-dimethoxy-4-hydroxybenzyl)-3-(3-hydroxy-4-methoxybenzyl)butanolide (7) without hydrogen peroxide*

Catharanthus roseus CFE (ph 6.3, specific activity 2.5 units/mg, 30.4 mL, 47 units) was added to a solution containing butanolide **7** (61.0 mg, 2.32 mmol), ethanol (3.8 mL), potassium phosphate buffer (0.100 M, pH 6.3, 43.0 mL), and water (20.2 mL). The solution was stirred for 3 h and extracted by the above procedure to afford butanolide **7** (59.3 mg, 97% recovery), which was pure by ^{1}H NMR spectroscopy.

Attempted dimerization of 1-(3,5-dimethoxy-4-hydroxyphenyl)-6-hydroxy-3-hydroxymethyl-7-methoxy-1,2,3,4-tetrahydro-2-naphthoic acid γ lactone (9) without CFE

Aryltetralin **9** (15.2 mg, 39.3 μmol) was dissolved in a solution of ethanol (7.3 mL), water (6.7 mL), hydrogen peroxide (2.16 mL, 81.6 mM solution, 1.76 mmol), and potassium phosphate buffer (0.100 M, pH 6.3, 8.9 mL). The solution was stirred for 17 h and analyzed by HPLC; no products were detected.

(±)-2-Hydroxy-8-hydroxymethyl-6,7,8,9-tetrahydro-3,3′,5′-trimethoxyspiro [5H-benzocycloheptene-5,1′-cyclohexa-2′,5′-dien]-4′-one-7-oic acid γ lactone (12)

Catharanthus roseus CFE (pH 6.3, specific activity 2.5 units/mg, 450 mL, 690 units), was added to a solution containing butanolide **7** (900 mg, 2.32 mmol), ethanol (90 mL), water (150 mL), and hydrogen peroxide (30 mL, 0.17 M solution, 5.1 mmol). The solution was stirred for 3 h and extracted by the standard procedure. The dichloromethane extract was purified by column chromatography using ethyl acetate/chloroform (1:4–9:11, v/v) to afford aryltetralin **9** (633 mg, 71%), butanolide **7** (56 mg, 6%), and spirodienone **12** (9.7 mg, 1.1%).

Physical properties of 12: mp 225–230°C; IR ν_{max} ($CHCl_3$): 3539, 1774, 1673 cm^{-1}; UV λ_{max} (log ε): 272 (3.83) nm; ^{1}H NMR δ: 2.03 (1H, dd, H(6), J = 13.3, 12.8 Hz), 2.32 (1H, dd, H(6), J = 13.7, 2.6), 2.32–2.44 (1H, m, H(8)), 2.68 (1H, ddd, H(7), J = 12.6, 12.6, 2.6 Hz), 2.97 (1H, dd, H(9), J = 15, 2.3 Hz), 3.07 (1H, dd, H(9), J = 14, 10.9 Hz), 3.65 (3H, s, -OCH_3(3)), 3.79, 3.84 (3H each, s, s, -OCH_3(3′), -OCH_3(5′)), 3.98 (1H, dd, -CH_2OCO-, J = 9, 8.4 Hz), 4.51 (1H, dd, -CH_2OCO-, J = 9, 7.6 Hz), 5.55 (1H, br s, -OH(2), disappears upon addition of D_2O), 6.13, 6.25 (1H each, d, d, H(2′), H(6′), J = 2.8 Hz each), 6.78, 6.92 (1H each, s, s, H(1), H(4)). Irradiation of H(7) collapses H(6) signals to doublets (J = 13.6 Hz each) and simplifies H(8) multiplet. MS m/z: 386 (M^+). High-resolution molecular weight determination calcd. for $C_{21}H_{22}O_7$: 386.1366; found: 386.1362.

Control experiment with butanolide 20

Butanolide **20** (4.20 mg, 10.8 μmol) was dissolved in a solution of ethanol (0.28 mL), water (0.87 mL), and potassium phosphate buffer (0.100 M, pH 7.0, 5.16 mL). Hydrogen peroxide (0.28 mL, 81.6 mM solution, 23 μmol) was added and the solution was stirred for 10 min prior to being extracted with ethyl acetate (2 × 50 mL). The organic extract was dried over magnesium sulfate, filtered, and evaporated *in vacuo*. The product (4.01 mg, 95% recovery) was found to be pure butanolide **7** by ^{1}H NMR spectroscopy and TLC.

*Biotransformation of (±)-*trans*-2-(3,5-dimethoxy-4-hydroxybenzyl)-3-(3,4-dihydroxy-α-hydroxybenzyl)butanolide (20)*

Hydrogen peroxide (4.58 mL, 81.6 mM solution, 0.37 mmol) and *C. roseus* CFE (pH 7.0, specific activity 0.51 units/mg, 22.1 mL, 150 units) were added in rapid succession to a solution containing butanolide **20** (73.2 mg, 0.187 mmol), ethanol (4.6 mL), water (14.5 mL), and phosphate buffer (0.100 M, pH 7.00, 63.7 mL). The solution turned dark purple within 1 min. After 10 min of stirring, ethyl acetate (80 mL) and Celite were added and the suspension was stirred vigorously for an additional 5 min and then filtered. Additional ethyl acetate (320 mL) was added and the phases were separated. The organic extract was dried over magnesium sulfate, filtered, and evaporated *in vacuo* to yield a brown solid (60.3 mg). The aqueous residue was subjected to a continuous extraction with ethyl acetate for 2 days. The organic extract was dried over magnesium sulfate, filtered, and evaporated *in vacuo* to afford a brown solid (7.6 mg), which was combined with the previous ethyl acetate extract. The aqueous residue was evaporated *in vacuo* and stirred in refluxing methanol (100 mL) for 16 h. The suspension was filtered while hot and evaporated *in vacuo* to yield brown solids (205.1 mg).

Reduction of ethyl acetate extract (Entry 1, Table 3): (±)-4-(3,4-dihydroxyphenyl)-5,7-dimethoxy-6-hydroxy-2-hydroxymethyl-1,2,3,4-terahydro-2-naphthoic acid γ lactone (26)

A sample of the ethyl acetate extract from the above experiment (Scheme 5, Entry 1, Table 3) (15.47 mg) was dissolved in a mixture of methanol (5 mL) and water (1 mL). Sodium borohydride (1.6 mg, 42 μmol) was added, resulting in an instantaneous color change of the solution from reddish-brown to yellow. After stirring for 5 min, 2 M hydrochloric acid (2 drops) was added, followed by water (2 mL) and brine (1 mL). The mixture was extracted with ethyl acetate (30 mL) and the organic extract was dried over magnesium sulfate, filtered, and evaporated *in vacuo* to afford a brown oil (16.62 mg). Purification by preparative TLC using acetone/dichloromethane (2:3, v/v) gave the aryltetralin **26** (2.23 mg, 14.0%) and **20** (2.06 mg, 12.3%).

Methylation of ethyl acetate extract (Entry 2, Table 3): (±)-2-(3,5-dimethoxy-4-hydroxybenzyl)-3-(3-hydroxy-4-methoxybenzyl-α-hydroxy)butanolide (27a) and (±)-2-(3,5-dimethoxy-4-hydroxybenzyl)-3-(4-hydroxy-3-methoxybenzyl-α-hydroxy)butanolide (27b)

Diazomethane in ethereal solution (approximately 0.4 M) was added to a sample of the ethyl acetate extract from the above experiment (23.19 mg) dissolved in dry methanol (5 mL) until no further effervescence was observed. After stirring for 22 h, aqueous acetic acid (10%, 2 drops) was added and the solution evaporated *in vacuo*. The residue was purified by preparative TLC using acetone/dichloromethane (1:4, v/v) to yield **20** (3.66 mg, 15%) and **27***a* and **27***b* (2.38 mg, 9%, 1:1 mixture of isomers by ^{1}H NMR spectroscopy).

Physical properties of **27***a* and **27***b*: IR ν_{max} ($CHCl_3$): 3600, 3540, 1770 cm^{-1}; ^{1}H NMR δ: 2.64–2.85, 2.85–3.02 (8H total, m, m, H(2), H(3), H(7″)), 4.02–4.15 (4H, m, H(4)), 4.72 (1H, d, H(7′), J = 5.1 Hz), 4.76 (1H, d, H(7′), J = 5.1 Hz), 6.39 (2H, s, H(2″)), 6.40 (2H, s, H(2″)), 6.70–6.92 (6H, m, H(2′), H(5′), H(6′)); EI MS m/z: 418 (M^+), 400, 181, 167, 153. High-resolution molecular weight determination calcd. for $C_{22}H_{26}O_8$: 418.1628; found: 418.1633.

Reduction and methylation of ethyl acetate extract (Entry 3, Table 3): (±)-4-(3,4-dimethoxyphenyl)-3-hydroxymethyl-1,2,3,4-tetrahydro-5,6,7-trimethoxy-2-naphthoic acid γ lactone (28) and unidentified compound (29)

Sodium borohydride (42 mg, 1.1 mmol) was added to a sample of the ethyl acetate extract from the above experiment (26.94 mg) dissolved in a mixture of methanol (4.5 mL) and water (0.5 mL) and the bright yellow solution was stirred at room temperature for 1 h. The solution was acidified with 1 M hydrochloric acid, and water (5 mL) and brine (2 mL) were added. The solution was extracted with ethyl acetate (2 × 60 mL) and the extract was dried over magnesium sulfate, filtered, and evaporated *in vacuo* before

being redissolved in ethanol (5 mL). Freshly prepared ethereal diazomethane (approximately 0.3 g, 7 mmol) was added and the solution stirred for 15 h. Aqueous acetic acid (10%, 0.2 mL) was added and the solution evaporated *in vacuo* to afford a yellow oil (67.5 mg), which was separated by column chromatography using acetone/dichloromethane (1:19 to 1:0 gradient, v/v) to afford 42.92 mg (64%) of eluted products, including **29** (8.56 mg, 25%) and **28** (6.00 mg, 20%). The column packing was removed and stirred in a mixture of acetate (50 mL), water (30 mL), and calcium carbonate (700 mg) at 45°C for 1 h. The mixture was acidified with 2 M hydrochloric acid and the solids filtered and washed with warm acetone (2 × 30 mL). The filtrate was evaporated *in vacuo* and the final traces of silica removed to afford a yellow oil (12.27 mg, no identifiable material by ^{1}H NMR spectroscopy) for a total column recovery of 82%.

Physical properties of **28**: IR ν_{max} ($CHCl_3$): 1770 cm^{-1}; ^{1}H NMR δ: 2.29–2.74 (2H, m, H(2),H(3)), 2.97–3.09 (1H, m), 3.14 (3H, s, -OCH_3(5)), 3.37 (1H, dd, J = 7.0, 6.6 Hz), 3.5–3.6 (1H, m), 3.69–3.91 (12H, m, -OCH_3), 4.04–4.18 (1H, m), 4.22 (1H, br d, H(4), J = 8.0 Hz), 6.60 (1H, m, H(6')), 6.72–6.84 (2H, m, m H(2'), H(8)), 6.90 (1H, d, H(5'), J = 8 Hz); EI MS m/z: 414 (M^+), 400, 181, 151, 137. High-resolution molecular weight determination calcd. for $C_{23}H_{26}O_7$: 414.1679; found: 414.1678.

Physical properties of **29**: IR ν_{max} ($CHCl_3$): 3320, 2970, 2940, 2870, 1670 cm^{-1}; MS m/z: 432 ($M^+ - H_2O$?), 418, 282, 167.

Methylation of methanol extract (Scheme 5)

Diazomethane in ethereal solution (approximately 0.4 M) was added to a sample of the methanol extract obtained from the above experiment and, in accord with Scheme 5 (90.82 mg), dissolved in dry methanol (10 mL) until no further effervescence was observed. The yellow solution was stirred overnight. Analysis by silica TLC showed no new spots in comparison with the initial methanol extract. In both cases no spots with an R_f value greater than zero were observed using acetone/dichloromethane (1:4, v/v).

Reduction and methylation of methanol extract (Scheme 5)

Sodium borohydride (137 mg, 3.6 mmol) was added to a sample of the methanol extract obtained from the above experiment (108.65 mg) dissolved in a mixture of methanol (9 mL) and water (1 mL) and the solution was stirred at room temperature for 1 h. The solution was acidified with 1 M hydrochloric acid, and water (5 mL) and brine (2 mL) were added. The solution was extracted with ethyl acetate (3 × 60 mL) and the extract was dried over magnesium sulfate, filtered, and evaporated *in vacuo* before being redissolved in dry THF (10 mL). Potassium carbonate (180 mg, 1.3 mmol) and methyl iodide (0.5 mL, 8 mmol) were added and the solution stirred for 1 h. Water (2 mL) was added and the mixture acidified with 2 M hydrochloric acid before being extracted with ethyl acetate (2 × 20 mL). The organic extract was dried over magnesium sulfate, filtered, and evaporated *in vacuo* to afford brown solids (43.9 mg). Sonication in dichloromethane followed by filtration and evaporation *in vacuo* gave tan solids (5.35 mg), which exhibited no aromatic proton signals by ^{1}H NMR spectroscopy.

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1. R. S. Ward. Chem. Soc. Rev. **11**, 75 (1982), and references cited therein.
2. I. Jardine. In Anticancer agents based on natural product models. *Edited by* J. M. Cassady and J. D. Douros. Academic Press, New York. 1980, 319.
3. B. F. Issell. Cancer Chemother. Pharmacol. **7**, 73 (1982).
4. B. F. Issell, F. M. Muggia, and S. K. Carter. Etoposide (VP-16) — Current status and new developments. Academic Press, Orlando, Fla. 1984.
5. W. J. Gensler, C. M. Samour, S. Y. Wang, and F. Johnson. J. Am. Chem. Soc. **82**, 1714 (1960).
6. W. J. Gensler and C. D. Gatsonis. J. Org. Chem. **31**, 4004 (1966).
7. J. Renz, M. Kuhn, and A. von Warburg. Liebigs Ann. Chem. **681**, 207 (1965).
8. A. S. Kende, L. S. Liebeskind, J. E. Mills, P. S. Rutledge, and D. P. Curran. J. Am. Chem. Soc. **99**, 7082 (1977).
9. H. P. Plaumann, J. G. Smith, and R. Rodrigo. J. Chem. Soc. Chem. Commun. 354 (1980).
10. R. Rodrigo. J. Org. Chem. **45**, 4538 (1980).
11. A. S. Kende, M. L. King, and D. P. Curran. J. Org. Chem. **46**, 2826 (1981).
12. D. Rajapaksa and R. Rodrigo. J. Am. Chem. Soc. **103**, 6208 (1981).
13. W. S. Murphy and S. Wattanansin J. Chem. Soc. Perkin Trans. 1, 271 (1982).
14. D. I. Macdonald and T. Durst. J. Org. Chem. **51**, 4749 (1986).
15. D. M. Vyas, P. M. Skonezny, T. A. Jenks, and T. W. Doyle. Tetrahedron Lett. **27**, 3099 (1986).
16. J. Van der Eycken, P. De Clerq, and M. Vandewalle. Tetrahedron, **42**, 4297 (1986).
17. H. Yamaguchi, M. Arimoto, S. Nakajima, M. Tanoguchi, and Y. Fukada. Chem. Pharm. Bull. **34**, 2056 (1986).
18. T. Kaneko and H. Wong. Tetrahedron Lett. **28**, 517 (1987).
19. D. W. Jones and A. M. Thompson. J. Chem. Soc. Chem. Commun. 1797 (1987).
20. R. C. Andrews, S. J. Teague, and A. I. Meyers. J. Am. Chem. Soc. **110**, 7854 (1988).
21. D. W. Jones and A. M. Thompson. J. Chem. Soc. Chem. Commun. 1370 (1989).
22. T. Morimoto, M. Chiba, and K. Achiwa. Tetrahedron Lett. **31**, 261 (1990).
23. K. Kanematsu, M. Tsuruoka, Y. Takaoka, and T. Sasaki. Heterocycles, **32**, 859 (1991).
24. A. J. Broomhead, M. M. A. Rahman, P. M. Dewick, D. E. Jackson, and J. A. Lucas. Phytochemistry, **30**, 1489 (1991), and references cited therein.
25. M. Kuhn, A. von Wartburg, and C. Keller-Juslen. Helv. Chim. Acta, **52**, 944 (1969).
26. M. Kuhn and A. von Wartburg. Helv. Chim. Acta, **52**, 948 (1969).
27. C. Keller-Juslen, M. Kuhn, A. von Wartburg, and H. Stahelin. J. Med. Chem. **14**, 936 (1971).
28. J. P. Kutney. Nat. Prod. Rep. **7**, 85 (1990), and references cited therein.
29. J. P. Kutney. Synlett, **1**, 11 (1991), and references cited therein.
30. H. Kohler and H. Jenzer. Free Radical Biol. Med. **6**, 323 (1989).
31. W. D. Hewson and L. P. Hager. *In* The Porphyrins. Vol. 7. *Edited by* D. Dolphin. Academic Press, New York. p. 295.
32. L. S. El-Assal and S. A. M. El-Wahhab. J. Chem. Soc. 849 (1960).
33. R. Brettle and S. M. Shibib. J. Chem. Soc. Perkin Trans. **1**, 2912 (1982).
34. C. Kaneko, A. Sugimoto, Y. Eguchi, S. Yamada, M. Ishikawa, S. Sasaki, and T. Suda. Tetrahedron, **30**, 2701 (1974).
35. H. C. Brown, S. Narasimhan, and Y. M. Choi. J. Org. Chem. **47**, 4702 (1982).
36. E. Brown and A. Dougan. Heterocycles, **26**, 1169 (1987).
37. J. P. Kutney, L. S. L. Choi, T. Honda, N. G. Sato, K. L. Stuart, and B. R. Worth. Helv. Chim. Acta, **65**, 2088 (1982).
38. J. P. Kutney, C. A. Boulet, L. S. L. Choi, W. Gustowski, M.

McHugh, J. Nakano, T. Nikaido, H. Tsukamoto, G. M. Hewitt, and R. Suen. Heterocycles, **27**, 613 (1988).
39. J. P. Kutney, C. A. Boulet, L. S. L. Choi, W. Gustowski, M. McHugh, J. Nakano, T. Nikaido, H. Tsukamoto, G. M. Hewitt, and R. Suen. Heterocycles, **27**, 621 (1988).
40. J. P. Kutney, B. Botta, C. A. Boulet, C. A. Buschi, L. S. L. Choi, J. Golinski, M. Gumulka, G. M. Hewitt, G. Lee, M. McHugh, J. Nakano, T. Nikaido, J. Onodera, I. Perez, P. Salisbury, M. Singh, R. Suen, and H. Tsukamoto. Heterocycles, **27**, 629 (1988).
41. Bio-Rad Chemical Division Bio-Rad Protein Assay, Bio-Rad, Richmond, Calif.
42. B. C. Saunders, A. G. Holmes-Siedle, and B. P. Stark. *In* Peroxidase. Butterworth, London. 1964. p. 135.
43. B. F. Bowden, R. W. Read, and W. C. Taylor. Aust. J. Chem. **33**, 1823 (1980).
44. F. E. Ziegler and J. A. Schwartz. Tetrahedron Lett. **52**, 4643 (1975).
45. A. Pelter, R. S. Ward, P. Satyanarayana, and P. Collins. J. Chem. Soc. Perkin Trans. **1**, 643 (1983).
46. M. Tanoguchi, T. Kashima, H. Saika, T. Inoue, M. Arimoto, and H. Yamaguchi. Chem. Pharm. Bull. Jpn. **37**, 68 (1989).
47. C. C. Price and J. M. Judge. Org. Synth. Collect. Vol. 5, 255 (1973).
48. A. Pelter, R. S. Ward, M. C. Pritchard, and I. T. Kay. J. Chem. Soc. Perkin Trans. **1**, 1603 (1988).
49. M. Hojo and R. Masuda. Synthesis, **10**, 678 (1976).
50. B. M. Trost, T. N. Salzmann, and K. Hiroi. J. Am. Chem. Soc. **98**, 4887 (1976).
51. C. E. M. Pugh and H. S. Raper. Biochem. J. **21**, 1370 (1927).
52. H. W. Schmidt, S. D. Haemmerti, H. E. Shoemaker, and M. S. Leisola. Biochemistry, **28**, 1776 (1989).
53. W. C. Still, M. Kahn, and A. Mitra. J. Org. Chem. **43**, 2923 (1978).
54. O. L. Gamborg and D. E. Eveleigh. Can. J. Biochem. **46**, 417 (1968).
55. B. Chance and A. C. Maehly. *In* Methods in enzymology. Vol. 2. *Edited by* S. P. Colowick and N. O. Kaplan. Academic Press, New York. 1955, p. 773.
56. M. M. Bradford. Anal. Biochem. **72**, 248 (1976).
57. International tables for X-ray crystallography. Vol. IV. Kynoch Press, Birmingham, U.K. (present distributor: Kluwer Academic Publishers, Dordrecht, The Netherlands). 1974. pp. 99–102 and 149.

TABLE A1. Crystallographic data[a]

Compound	**9** · $CHCl_3$
Formula	$C_{21}H_{22}O_7 \cdot CHCl_3$ ·
fw	505.78
Crystal system	Monoclinic
Space group	$P2_1/a$
a, Å	27.957(1)
b, Å	7.427(2)
c, Å	11.204(1)
β°	99.129(5)
V, Å^3	2296.8(7)
Z	4
ρ_{calc}, g/cm^3	1.462
$F(000)$	1048
μ(Mo-K_α), cm^{-1}	4.39
Crystal size, mm	0.10 × 0.15 × 0.23
Transmission factors	0.92–1.00
Scan type	ω
Scan range, deg in ω	1.25 + 0.35 tan θ
Scan speed, deg/min	16 (9 rescans)
Data collected	$+h, +k, \pm l$
$2\theta_{max}$, deg	55
Crystal decay	Negligible
Total reflections	5544
Total unique reflections	5277
R_{merge}	0.43
No. of reflcns with $I \geq 3\sigma(I)$	1564
No. of variables	297
R	0.045
R_w	0.040
gof	1.89
Max Δ/σ (final cycle)	0.02
Residual density, e/Å^3	−0.28 to +0.27

[a]Temperature 294 K, Rigaku AFC6S diffractometer, Mo-K_α radiation (λ = 0.71069 Å), graphite monochromator, takeoff angle 6.0°, aperture 6.0 × 6.0 mm at a distance of 285 mm from the crystal, stationary background counts at each end of the scan (scan/background time ratio 2:1, up to 9 rescans), $\sigma^2(F^2) = [S^2(C + 4B) + (0.01F^2)^2]/Lp^2$ (S = scan rate, C = scan count, B = normalized background count), function minimized $\Sigma w(|F_o|-|F_c|)^2$ where $w = 4F_o^2/\sigma^2(F_o^2)$, $R = \Sigma||F_o|-|F_c||/\Sigma|F_o|$, $R_w = (\hbar w(|F_o|-|F_c|)^2/\Sigma w|F_o|^2)^{1/2}$, and gof = $[\Sigma w(|F_o|-|F_o|)^2/(m-n)]^{1/2}$. Values given for R, R_w, and gof are based on those reflections with $I \geq 3\sigma(I)$.

Appendix: X-ray crystallographic analysis of compound 9

Experimental

Crystallographic data appear in Table A1. The final unit-cell parameters were obtained by least squares on the setting angles for 25 reflections with 2θ = 79.5–91.1°. The intensities of three standard reflections, measured every 150 reflections throughout the data collection, remained essentially constant. The data were processed[2] and corrected for Lorentz and polarization effects, and absorption (empirical, based on azimuthal scans for four reflections).

The structure was solved by direct methods, the coordinates of the non-hydrogen atoms being determined from an E-map. There is one molecule of chloroform in the asymmetric unit along with the molecule of **9**. The non-hydrogen atoms were refined with anisotropic thermal parameters, the OH hydrogen atoms were refined with isotropic thermal parameters, and the remaining hydrogen atoms were fixed in calculated positions (C—H = 0.98 Å, $B_H = 1.2\ B_{bonded\ atom}$). Neutral atom scattering factors and anomalous dispersion corrections for the non-hydrogen atoms were taken from the *International tables for X-ray crystallography* (57). Final atomic coordinates and equivalent isotropic thermal parameters, bond lengths, bond angles, and hydrogen-bond data appear in Tables A2-A5, respectively. Hydrogen atom parameters, anisotropic thermal parameters, bond lengths and angles involving hydrogen, torsion angles, packing dia-

[2]TEXSAN/TEXRAY structure analysis package, which includes versions of the following: MITHRIL, integrated direct methods, by C. J. Gilmore; DIRDIF, direct methods for difference structures, by P. T. Beurskens; ORFLS, full-matrix least-squares, and ORFFE, function and errors, by W. R. Busing, K. O. Martin, and H. A. Levy; ORTEP II, illustrations, by C. K. Johnson.

TABLE A2. Final atomic coordinates (fractional) and B_{eq} (Å^2)[a]

Atom	x	y	z	B_{eq}
Cl(1)	0.22269(7)	0.3256(3)	0.7077(2)	7.2(1)
Cl(2)	0.23922(7)	0.6537(4)	0.5851(2)	9.2(1)
Cl(3)	0.31943(7)	0.4453(4)	0.6991(2)	11.4(2)
O(1)	0.5330(1)	0.1945(5)	1.0847(3)	4.0(2)
O(2)	0.4895(1)	0.2557(6)	0.2992(3)	3.7(2)
O(3)	0.5787(1)	0.1425(5)	0.3924(3)	3.8(2)
O(10)	0.4552(1)	0.2602(5)	1.0248(3)	3.4(2)
O(1′)	0.7000(1)	0.1819(6)	1.0868(3)	4.0(2)
O(2′)	0.7241(1)	0.4966(7)	1.0153(4)	4.7(2)
O(3′)	0.6664(1)	0.6896(6)	0.8494(3)	4.2(2)
C(1)	0.5420(2)	0.2039(7)	0.8114(4)	2.1(2)
C(2)	0.5034(2)	0.2901(7)	0.8754(4)	2.2(2)
C(3)	0.4518(2)	0.2510(7)	0.8153(4)	2.4(2)
C(4)	0.4422(2)	0.3411(8)	0.6925(4)	2.8(3)
C(4A)	0.4812(2)	0.2856(7)	0.6204(4)	2.2(2)
C(5)	0.4704(2)	0.2950(8)	0.4942(5)	2.7(3)
C(6)	0.5031(2)	0.2461(7)	0.4218(4)	2.6(3)
C(7)	0.5489(2)	0.1873(7)	0.4739(5)	2.5(2)
C(8)	0.5605(2)	0.1798(7)	0.5981(4)	2.4(2)
C(8A)	0.5272(2)	0.2274(7)	0.6737(4)	2.0(2)
C(9)	0.5014(2)	0.2402(7)	1.0046(5)	3.0(3)
C(11)	0.4245(2)	0.3137(8)	0.9130(5)	3.2(3)
C(12)	0.6266(2)	0.097(1)	0.4396(5)	6.5(4)
C(13)	0.2607(2)	0.511(1)	0.7039(5)	5.9(4)
C(1′)	0.5919(2)	0.2796(7)	0.8595(4)	2.1(2)
C(2′)	0.6230(2)	0.1856(7)	0.9464(4)	2.5(2)
C(3′)	0.6667(2)	0.2599(8)	0.9982(5)	2.9(3)
C(4′)	0.6801(2)	0.4270(8)	0.9613(5)	2.8(3)
C(5′)	0.6493(2)	0.5225(8)	0.8745(4)	2.7(3)
C(6′)	0.6053(2)	0.4481(8)	0.8237(4)	2.5(3)
C(7′)	0.6864(2)	0.017(1)	1.1344(5)	5.4(4)
C(8′)	0.6368(2)	0.7919(8)	0.7616(6)	4.7(3)

[a] $B_{eq} = (8/3)\pi^2\Sigma\Sigma U_{ij} a_i^* a_j^* (a_i \cdot a_j)$.

TABLE A3. Bond lengths (Å) with estimated standard deviations

Atom	Atom	Distance	Atom	Atom	Distance
Cl(1)	C(13)	1.742(7)	C(2)	C(3)	1.521(6)
Cl(2)	C(13)	1.734(7)	C(2)	C(9)	1.504(7)
Cl(3)	C(13)	1.722(6)	C(3)	C(4)	1.515(6)
O(1)	C(9)	1.205(6)	C(3)	C(11)	1.503(7)
O(2)	C(6)	1.368(5)	C(4)	C(4A)	1.513(6)
O(3)	C(7)	1.371(5)	C(4A)	C(5)	1.399(6)
O(3)	C(12)	1.401(6)	C(4A)	C(8A)	1.398(6)
O(10)	C(9)	1.354(6)	C(5)	C(6)	1.364(6)
O(10)	C(11)	1.456(5)	C(6)	C(7)	1.391(6)
O(1′)	C(3′)	1.377(6)	C(7)	C(8)	1.379(6)
O(1′)	C(7′)	1.412(7)	C(8)	C(8A)	1.400(6)
O(2′)	C(4′)	1.383(6)	C(1′)	C(2′)	1.386(6)
O(3′)	C(5′)	1.374(6)	C(1′)	C(6′)	1.383(7)
O(3′)	C(8′)	1.404(6)	C(2′)	C(3′)	1.381(6)
C(1)	C(2)	1.528(6)	C(3′)	C(4′)	1.379(7)
C(1)	C(8A)	1.543(6)	C(4′)	C(5′)	1.387(7)
C(1)	C(1′)	1.522(6)	C(5′)	C(6′)	1.386(6)

gram, and measured and calculated structure factor amplitudes are included as supplementary material.[3]

[3]Supplementary material mentioned in the text may be purchased from: The Depository of Unpublished Data, Document Delivery, CISTI, National Research Council Canada, Ottawa, Canada K1A 0R6.

Tables of hydrogen atom parameters, bond lengths and angles involving hydrogen, and the packing diagram have also been deposited with The Cambridge Crystallographic Data Centre and can be obtained on request from The Director, Cambridge Crystallographic Data Centre, University Chemical Laboratory, Lensfield Road, Cambridge, CB2 1EW, U.K.

TABLE A4. Bond angles (deg) with estimated standard deviations

Atom	Atom	Atom	Angle	Atom	Atom	Atom	Angle
C(7)	O(3)	C(12)	116.9(4)	C(1)	C(8A)	C(4A)	123.1(4)
C(9)	O(10)	C(11)	109.5(4)	C(1)	C(8A)	C(8)	118.5(4)
C(3′)	O(1′)	C(7′)	116.5(4)	C(4A)	C(8A)	C(8)	118.3(4)
C(5′)	O(3′)	C(8′)	116.6(4)	O(1)	C(9)	O(10)	120.9(5)
C(2)	C(1)	C(8A)	108.9(4)	O(1)	C(9)	C(2)	130.7(5)
C(2)	C(1)	C(1′)	110.8(4)	O(10)	C(9)	C(2)	108.4(5)
C(8A)	C(1)	C(1′)	113.5(4)	O(10)	C(11)	C(3)	104.2(4)
C(1)	C(2)	C(3)	113.8(4)	C(1)	C(13)	Cl(2)	11.3(3)
C(1)	C(2)	C(9)	119.0(4)	C(1)	C(13)	Cl(3)	111.5(4)
C(3)	C(2)	C(9)	101.6(4)	C(2)	C(13)	Cl(3)	111.4(4)
C(2)	C(3)	C(4)	109.6(4)	C(1)	C(1′)	C(2′)	120.0(5)
C(2)	C(3)	C(11)	100.0(4)	C(1)	C(1′)	C(6′)	120.4(5)
C(4)	C(3)	C(11)	119.2(4)	C(2′)	C(1′)	C(6′)	119.5(4)
C(3)	C(4)	C(4A)	109.1(4)	C(1′)	C(2′)	C(3′)	120.5(5)
C(4)	C(4A)	C(5)	117.9(4)	O(1′)	C(3′)	C(2′)	125.9(6)
C(4)	C(4A)	C(8A)	123.2(4)	O(1′)	C(3′)	C(4′)	114.2(5)
C(5)	C(4A)	C(8A)	118.9(5)	C(2′)	C(3′)	C(4′)	119.9(5)
C(4A)	C(5)	C(6)	122.0(5)	O(2′)	C(4′)	C(3′)	118.1(5)
O(2)	C(6)	C(5)	118.6(5)	O(2′)	C(4′)	C(5′)	121.7(6)
O(2)	C(6)	C(7)	121.8(5)	C(3′)	C(4′)	C(5′)	120.2(5)
C(5)	C(6)	C(7)	119.6(5)	O(3′)	C(5′)	C(4′)	114.1(5)
O(3)	C(7)	C(6)	114.4(4)	O(3′)	C(5′)	C(6′)	126.2(5)
O(3)	C(7)	C(8)	126.3(4)	C(4′)	C(5′)	C(6′)	119.7(5)
C(6)	C(7)	C(8)	119.3(5)	C(1′)	C(6′)	C(5′)	120.3(5)
C(7)	C(8)	C(8A)	121.9(4)				

TABLE A5. Hydrogen bond geometry

Interaction	O—H (Å)	H···O (Å)	O···O (Å)	O—H···Cl/O (°)
O(2)—H(1)···O(1)($x,y,z-1$)	0.84(5)	2.19(5)	2.896(5)	142(5)
O(2)—H(1)···O(3)	0.84(5)	2.23(5)	2.680(5)	114(5)
O(2′)—H(1′)···O(3′)	0.77(5)	2.23(5)	2.677(6)	118(5)
O(2′)—H(1′)···O(1′)($3/2-x$, $1/2+y$, $2-z$)	0.77(5)	2.34(5)	2.911(6)	132(5)
C(13)—H(14)···O(2′)($1-x$, $1-y$, $2-z$)	0.98	2.34	3.108(7)	134
C(13)—H(14)···O(1′)($1-x$, $1-y$, $2-z$)	0.98	2.47	3.332(8)	146

Discussion of crystal structure

The crystal structure of **9** · $CHCl_3$ is dominated by weak hydrogen bonding. The two hydroxyl hydrogen atoms (H(1) and H(1′)) and the chloroform hydrogen atom (H(14)) are each involved in bifurcated hydrogen bonding arrangements (see Table A5). H(1) is intramolecularly H-bonded to O(3) and intermolecularly H-bonded along the *c* axis to O(1). H(1′) is similarly hydrogen-bonded, intramolecularly to O(3′) and intermolecularly to O(1′) of a molecule related by the twofold screw axis along *b*. The chloroform solvate molecule is linked by a pair of C—H···O hydrogen bonds to O(1′) and O(2′) of the same molecule. The molecule **9** is shown in Fig. 1. The junction between the five-membered lactone ring and the central six-membered ring is *trans*. The lactone ring has a C(3)-envelope conformation and the central cyclohexene ring has C(2) and C(3) displaced in opposite directions from the plane defined by C(1), C(8a), C(4a), and C(4). The bulky 4-OH-3,5-$(OMe)_2$-C_6H_2 substituent at C(1) occupies a pseudo-equatorial position. The two aromatic rings are planar to within experimental error. Bond lengths and angles (Tables A3 and A4) are as expected.