STUDIES WITH PLANT CELL CULTURES OF *CASSIA DIDYMOBOTRYA*. VII. ENZYME CATALYZED BIOTRANSFORMATION OF DIBENZYLBUTANOLIDES TO PODOPHYLLOTOXIN ANALOGUES AND RELATED COMPOUNDS

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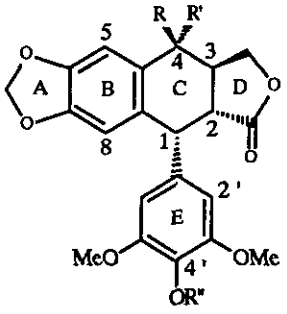
Abstract - Enzyme-catalyzed cyclizations of synthetic dibenzylbutanolides to analogues of the podophyllotoxin family are described. The biotransformations utilize enzymes derived from *Cassia didymobotrya* cell cultures and previously synthesized dibenzylbutanolides which differ in functionality in order to evaluate the course of the cyclization process. The application of this methodology provides additional information relating to the utilization of such "substrates" in the future synthesis of the anti-cancer drug, Etoposide, *via* a combination of synthetic chemistry and biotechnological methods.

The podophyllotoxin family of natural products exemplified by structures (**1-5**) have received considerable attention over the years. Several recent reviews[1,2] provide a summary of the investigations of the chemistry and syntheses of these compounds while other publications[3-5] present an extensive description of the important clinical anti-cancer drug Etoposide (VP-16) (**6**), a podophyllotoxin analogue. The present commercial route to this drug still depends on the isolation of podophyllotoxin (**1**) from *Podophyllum peltatum* plants, followed by a two-step chemical conversion to 4′-demethylepipodophyllotoxin (**4**) and the latter is finally chemically converted to **6**. In an effort to derive a more efficient route to **4**, and which is independent of plant extraction, we have initiated an approach directed toward the utilization of a combination of biotechnological and chemical methods.[6,7] The present publication presents further results relating to the achievement of this objective.



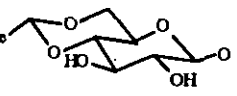
Podophyllotoxin **1**: R=H; R'=OH; R''=Me

Epi podophyllotoxin **2**: R=OH; R'=H; R''=Me

Deoxypodophyllotoxin **3**: R=R'=H; R''=Me

4'-Demethylpodophyllotoxin **4**: R=H; R'=OH, R''=H

4'-Demethyl *epi* podophyllotoxin **5**: R=OH; R'=R''=H

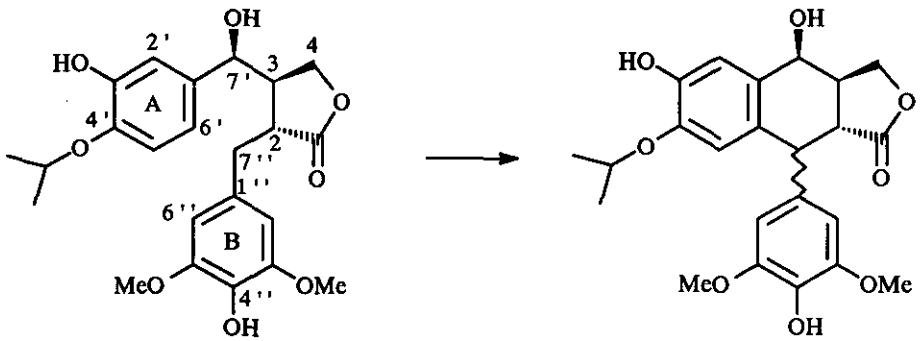
Etoposide **6**: R=  ;R'=R''=H

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. Previous studies[6,7] had revealed that dibenzylbutanolides, structural analogues of the late stage intermediates proposed by Dewick and coworkers[8] in the biosynthetic pathway leading to the podophyllotoxins, were suitable substrates for such purposes. In effect, this type of process (see **7**→ **8**, Scheme 1) is expected to involve radical intermediates and, within living systems, the latter are usually generated by peroxidase enzymes.[9,10] Indeed, such peroxidase enzymes had been produced within our plant cell culture line of *Cassia didymobotrya* and their role evaluated in the biotransformation of 2,2′,4′- and 4,2′,4′-trihydroxychalcones.[11,12]

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 *Cassia didymobotrya* cell cultures was the butanolide (**7**) synthesized according to the published procedure.[7]

Scheme 1.



7 **8**

The enzymic reaction parameters (pH, H_2O_2 as cofactor, units of peroxidase, reaction time) were initially evaluated in small-scale experiments (2-6 mg of **7**) in order to derive optimum conditions for the biotransformation of **7**. The optimum pH was shown to be around 7.5. The protein concentration was determined spectrophotometrically by complexing the dissolved protein with a commercial dye solution.[13,14] The ideal ratio of peroxidase to substrate was 1 mg/ml *vs* 1 mg substrate, corresponding to 118 units of enzymes per one millimole of **7**. A catalytic amount of H_2O_2 was used. After 30 min of incubation at 37 °C, **7** was completely biotransformed. A control experiment was also performed in order to determine if hydrogen peroxide, in the absence of cell free extracts (CFE), was able to transform **7** under the conditions used in the biotransformation. Extraction of the reaction mixture gave a 95% recovery of material consisting exclusively of **7**. From the biotransformation mixture, three products (**8** - **10**) were isolated (Scheme 2).

The most polar compound was identical with the cyclization product obtained earlier from the same substrate (**7**) by treatment with cell cultures of *Podophyllum peltatum*.[7]

The ^1H nmr spectrum of the novel compound (**9**, Table 1) was characterized by a low field doublet at 7.59 ppm which was attributed to an olefinic proton, after assigning all of the aromatic proton signals. Irradiation of the above signal affected the complex (*ddt*) signal at 3.66 ppm which was, in turn, related by a decoupling experiment, with the CH_2O proton system of the lactone ring. These findings suggest the formation of a double bond between the C-2 and C-7″ carbons, in which the isolated olefinic proton H-7″ is related to H-3 by a long range coupling (^3J = 2 Hz). The signal of the H-3 proton shows also a second

Table 1. ^{13}C- and ^{1}H-nmr spectral data of novel biotransformation products*

Position	**10**		**9**		**20**	
C=O	176.92		172.28	_	172.36	_
2	53.50	3.45 *dd* (9, 4)	121.71	_	122.82	_
3	49.71	3.23 *m* (SJ=25)	49.80	3.66 *dd t* (8, 4.5, 2x2)	45.48	3.98 *br t* (6.5)
4a		4.39 *dd* (9.5, 5)		4.02 *dd* (10.5, 4.5)		4.40 *br t* (9)
	71.82		62.54		66.85	
4b		4.03 *dd* (9.5, 5)		3.87 *dd* (10.5, 8)		4.21 *dd* (9, 7)
1′	132.1[a]		133.43	_	133.79	_
2′	113.11	6.86 *d* (2.5)	113.03	6.91 *d* (2)	108.28[c]	6.74[d]
3′	144.78		144.69	_	147.84	_
4′	146.97		146.82	_	148.04	_
5′	111.57	6.82 *d* (8.5)	111.69	6.82 *d* (8.5)	106.56[c]	6.74[d]
6′	117.03	6.78 *dd* (8.5, 2.5)	117.03	6.80 *dd* (8.5, 2)	120.04	6.81[e]
7′	84.17[b]	5.32 *d* (4)	81.27	5.54 *d* (2)	73.91	4.97 *d* (6)
1″	131.6[a]		124.96	_	125.28	_
2″, 6″	101.82	6.63 *s*	107.48	6.82 *s*	107.38	7.05 *s*
3″, 5″	147.19		147.20	_	147.13	_
4″	134.17		137.24	_	137.10	_
7″	83.43[b]	5.31 *d* (3.5)	139.92	7.59 *d* (2)	139.51	
OMe	56.39	3.91 *s*	56.45	3.90 *s*	56.41	3.97 *s*
OCH	72.90[b]	4.60 *h* (6)	71.74	4.58 *h* (6)	_	
Me_2	22.09	1.36 *d* (6)	22.11	1.36 *d* (6)	_	
OCH_2O					101.28	5.96, 5.94 *br s*

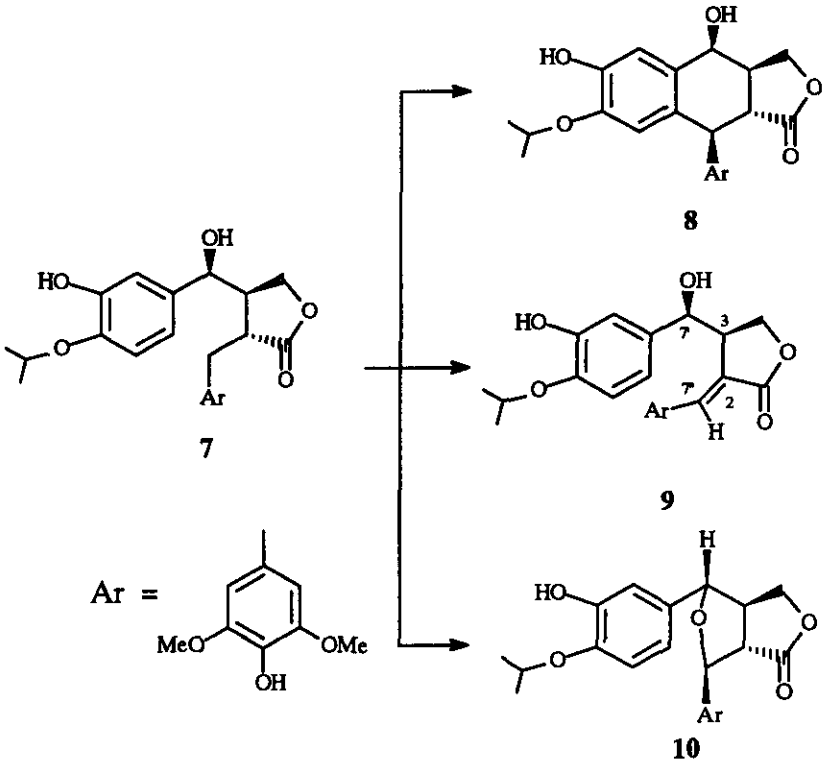
* 75 and 300 MHz, respectively; TMS as internal standard, δ scale. The signals showed the appropriate integrated intensity. Coupling constants, in Hz, are given in parentheses.

[a, b, c] These signals, due to closely similar values, are tentatively assigned and may be interchanged.

[d] B_2, part of AB_2 system.

[e] A, part of AB_2 system.

Scheme 2



small coupling (^2J = 2 Hz) with the vicinal H-7′, thus indicating that the OH function is oriented in a β position (as shown). The values of the H-7″, H-3′ and H2-H4 proton signals are consistent with an E-configuration of the double bond at C-2 - C-7″ (Table 2).[15,16]

The structure **9** is confirmed by the molecular peak in the mass spectrum with a molecular ion peak at *m/z* 430, two units lower than that of the starting **7**.

Table 2. Proton signals (δ) in E- and Z-dibenzylbutanolides [15, 16]

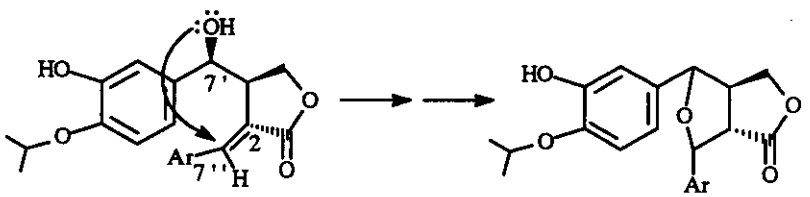
	(E)	(Z)	**9**
H-3	3.79	3.26	3.98
H2-4*	4.25	4.09	4.30
H-7′	7.50	6.65	7.59

*Approximate value of the center of the CH_2O system

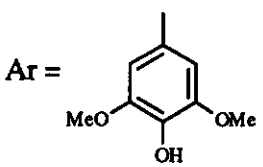
The C-7′ epimer of **7**, isolated as a minor component during the synthesis of **7**, was incubated with the cell free extract of *Cassia didymobotrya* cell cultures, under the same conditions as those used for the biotransformation of **7**. It was rapidly degraded thereby not allowing the isolation of any of the products similar to those obtained from the bioconversion of **7**. Clearly the stereochemistry at C-7′ plays a role in determining the course of biotransformation.

In a separate experiment, **9** is converted, by enzymatic catalysis (30 min incubation), to **10** *via* an intramolecular nucleophilic addition (Michael-type), of the C-7′ hydroxyl group to the activated olefinic double bond (Scheme 3). A control experiment, in which the same reaction was conducted but in the absence of enzyme, allowed the isolation of unreacted **9**.

Scheme 3



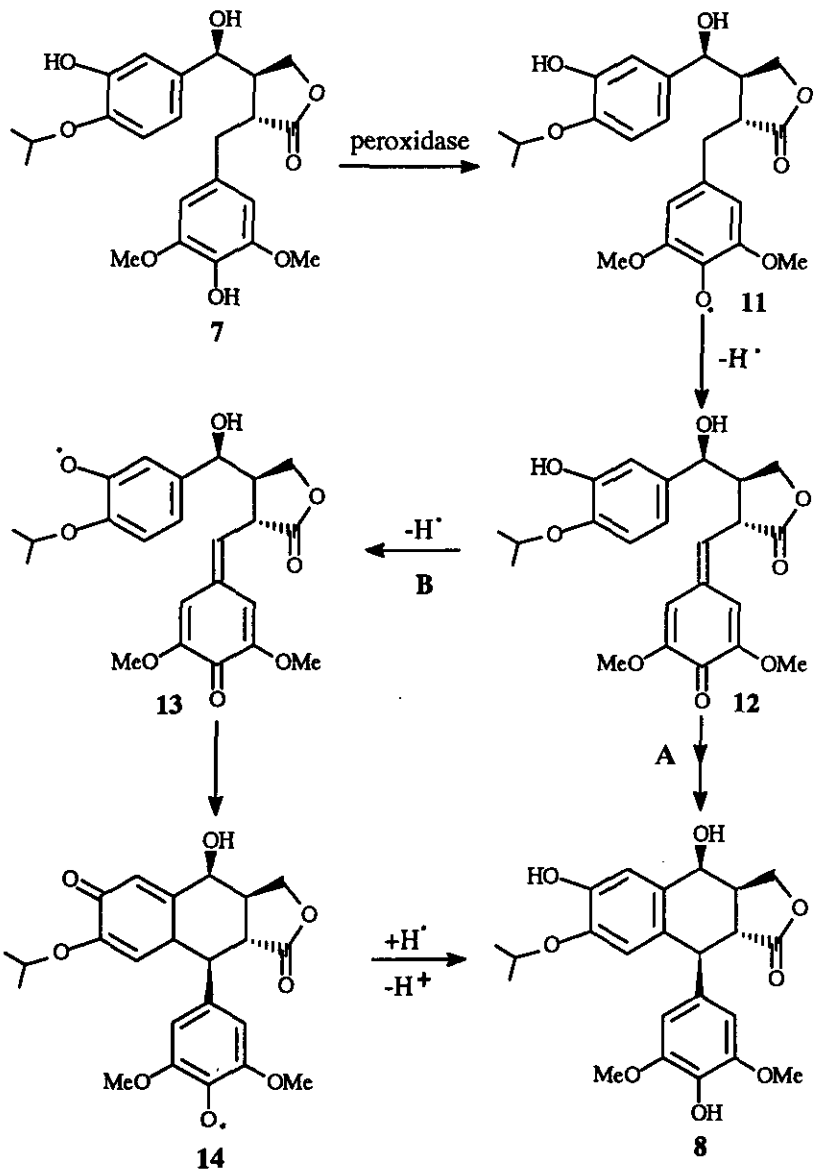
9 **10**



Compound (**10**) exhibited, in ^{1}H- and ^{13}C-nmr spectra (at *ca.* δ 5.3 and 84, respectively) the signals for two oxygenated methine carbon atoms, which were shown by decoupling experiments to be connected through the H-2 and H-3 protons. The molecular peak (at *m/z* 430) in the mass spectrum required the presence of an ethereal bridge between the two methine carbon atoms, which were identified with 7′-CH and 7″-CH. As a result, a tetrasubstituted tetrahydrofuran ring is formed, in which the protons are all *trans* as DIFNOE experiments revealed. Therefore, this compound was assigned the structure (**10**).

With the aim to shed some light on the biosynthetic relationship, if any, between the three products isolated from the biotransformation of **7** (Scheme 2), the compounds (**8**, **9** and **10**) were incubated in separate experiments, with the cell free extract under the same conditions as those used for the bioconversion of **7**.

Scheme 4



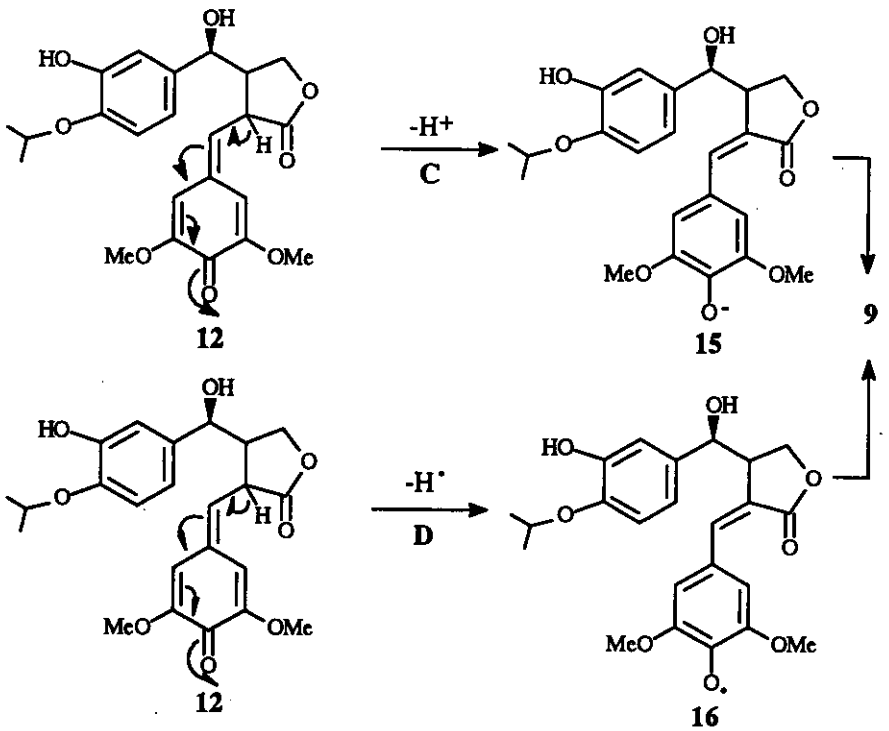
After 24 h of incubation, **8** was not biotransformed at all; **10** was degraded into non-isolable products when a longer reaction time was employed. As noted above, **9** was biotransformed to **10**.

In summary, the above results allow the proposed mechanisms outlined in Schemes 3 and 4. The peroxidase enzymes present in the cell free extract derived from the cell culture of *Cassia didymobotrya*, afford the conversion of **7** to radical **11** and the latter, *via* plausible pathways (paths A and/or B, Scheme 4, and paths C and/or D, Scheme 5) allow the formation of **8** and **9**.

In order to provide further proof for the free radical mechanisms proposed, it was of interest to assess whether ring A functionality within a dibenzylbutanolide system and which effectively "blocks" facile radical formation, will suppress enzyme catalyzed cyclization. For this purpose, the butanolide (**19**) bearing a methylenedioxy function in ring A, was prepared according to a previously published procedure.[17] During this study, the steps, **17** → **18** and **18** → **19** were modified by replacing BCl_3 and $CaCO_3$ with HCl/AcOH and $NaBH_4$ respectively, thereby improving the overall yield (20% versus 12%) (Scheme 6).

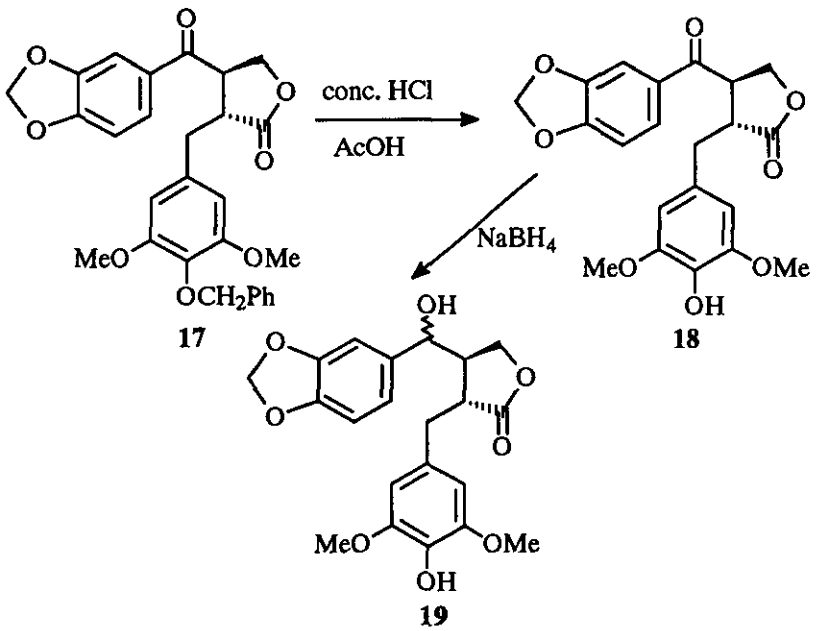
Precursor(**19**)did not give any cyclization product but was converted by the enzyme mixture into **20** in a 38% yield. Nmr spectra (^1H and ^{13}C) of **20** revealed a close similarity with those of **9** (Table 1). The chemical shifts of protons H-3, H2-H4 and H-7" were in agreement with an E configuration.

Scheme 5



The next objective was to study the enzyme specificity for dibenzylbutanolides bearing different substitution patterns in rings A and B, respectively (Scheme 7).

Scheme 6



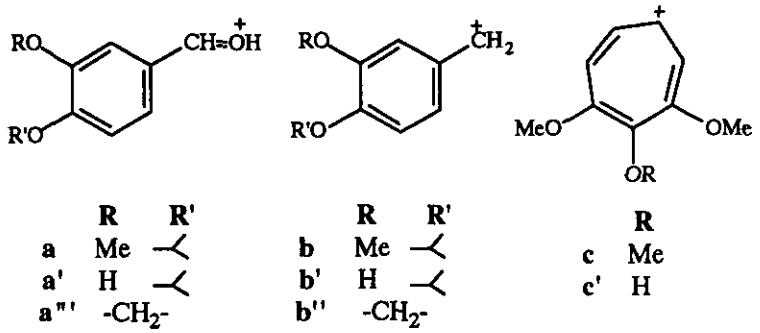
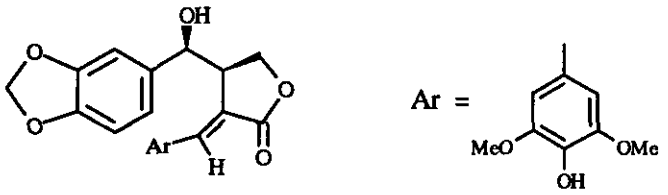


Figure 1. Mass fragmentation of dibenzylbutanolides.

Treatment of a methanol solution of **7** with a saturated solution of CH_2N_2, in Et_2O gave two methyl derivatives, which were separated by preparative tlc. In ^{13}C nmr spectra of the two compounds obtained, a signal at *ca.* 60 ppm, attributable to a methoxyl group at the C-4″ position, was present. Similarly, in their mass spectra the base peak at *m/z* 181 was attributed to the ring B tropylium ion **c** (Figure 1). It was therefore clear that both compounds carried the C-4″ methoxyl functions. However the ring A fragments (Figure 1) differed by 14 mu units in one of the compounds thereby indicating that one of the products isolated from the CH_2N_2 reaction still carried a free hydroxyl group in ring A. On this basis, the products were assigned structures (**21**) and (**22**).



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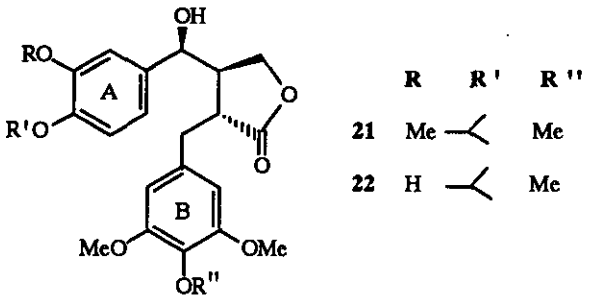
Scheme 7



Incubation of **21** and **22** with the cell free extract of *Cassia didymobotrya* followed by analysis of the fermentation mixture, revealed that unreacted starting materials were completely recovered.

In summary, we may conclude that the CFE of *Cassia didymobotrya* cell cultures contains several enzyme systems: i) one of these is able to catalyze the C-6′—C-7″ bond formation (**7**→**8**). As suggested in Scheme 4, this requires free hydroxyl groups in each aromatic rings (rings A and B) and a peroxidase enzyme is involved; ii) another enzyme system which is able to convert compounds such as **7** and **19** to **9** and **20** respectively by removal of H_2. This latter conversion could be achieved by a peroxidase or dehydrogenase enzyme. This dehydrogenative process requires only a free hydroxyl group in ring B; iii) a further enzyme, responsible for the biotransformation of **9** to **10**, is also present. A control experiment was also performed in order to determine if hydrogen peroxide, in the absence of CFE, was able to convert **9** to **10** under the conditions used in the biotransformation. Extraction of the reaction mixture gave complete recovery of unreacted **9**. Although a radical mechanism for the conversion of **9** to **10** is possible, we favour the Michael-type cyclization proposed in Scheme 3.

EXPERIMENTAL

General biotransformation procedure

Butanolides (2 mg) were incubated at 37 °C with CFE from *Cassia didymobotrya* (10 ml, 5.6 U/ml), Tris-HCl buffer (5 ml, pH 7.5) and H_2O_2 12.3 mM (120 ml). After 30 min, the reaction mixture was extracted with EtOAc and purified as indicated below.

Biotransformation of *trans*-2-(4-hydroxy-3,5-dimethoxybenzyl)-3-(α,3-dihydroxy-4-*iso*propoxybenzyl)butanolide (7):

The pooled EtOAc extracts of several small-scale biotransformations (40 mg of substrate in total) gave by preparative tlc with $CHCl_3$/EtOAc/MeOH, 90:7:3, the compounds (in order of increasing R_F), **8** (14 mg, 35 %), **9** (8 mg, 20 %) and **10** (3 mg, *ca* 8 %). Compound **8** (co-tlc, ^{1}H nmr and ms spectra) was identical with an authentic sample of the cyclization product obtained by the biotransformation of **7** with cell cultures of *Podophyllum peltatum.*

***trans*-(E)-2,2′-(4-Hydroxy-3,5-dimethoxybenzal)-3-(α,3-dihydroxy-4-*iso*propoxybenzyl)butanolide (9):**

^{1}H- and ^{13}C Nmr spectra in **Table 1**; EIms *m/z* (rel. int.): 430 $[M]^+$ (100), 412 $[M - H_2O]^+$ (17), 399 (17), 388 $[M - C_3H_6]^+$ (22), 370 (9), 368 (12), 357 (32), 339 (5), 250 (16), 249 (15), 222 (58), 181 $[a']^+$ (21), 167 (21), 154 (32), 143 (11), 139 $[a' - C_3H_6]^+$ (23), 137 (53), 123 $[b']^+$ (13).

6-(3-Hydroxy-4-*iso*propoxyphenyl)-8-(4-hydroxy-3,5-dimethoxyphenyl)-3,7-dioxabicyclo[3.3.0]-octan-2-one (10):

^{1}H- and ^{13}C-Nmr spectra in **Table 1**; EIms *m/z* (rel. int.): 430 $[M]^+$ (100), 388 $[M - C_3H_6]^+$ (22), 368 (7), 275 (17), 222 (58), 181 (68), 167 $[b']^+$ (22), 149 (46), 137 (53),131 (22), 123 $[b' - C_3H_6]^+$ (35).

Methyl derivatives: To a solution of 7 (50 mg) in MeOH (5 ml), a saturated solution of CH_2N_2 was added portionwise until disappearance of the starting material (tlc). Purification by preparative tlc with CH_2Cl_2-hexane-EtOAc,7:2:1, gave dimethyl derivative **21** *(40 mg) and monomethyl derivative 22 (22 mg).*

***trans*-2-(3,4,5-Trimethoxybenzyl)-3-(α-hydroxy-3-methoxy-4-*iso*propoxybenzyl)butanolide (21):**

^{1}H Nmr: δ 6.84 (1H, *d*, J= 8 Hz, H-5′), 6.78 (1H, *d*, J= 2 Hz, H-2′), 6.74 (1H, *dd*, J= 8 and 2 Hz, H-6′), 6.41 (2H, *s*, H-2″, H-6″), 4.63 (1H, *d*, J=7 Hz, H-7′), 4.52 (1H, *d*, J= 6 Hz, OCH), 3.91, 3.88 (1H each, *dd*, J= 13 and 8 Hz, H_2-4), 3.82 (12H, *br s*, 4xOMe), 3.14 (1H, *br dd*, J= 13 and 5 Hz, H-7″A), 3.01 (1H,

dt, J= 8 and 5x2 Hz, H-2), 2.90 (1H, *br dd*, J= 13 and 6 Hz, H-7''B), 2.65 (1H, *d*, J= 8 Hz, H-3), 1.37, 1.36 (1H each, *d*, J= 6 Hz, 2xMe); ^{13}C nmr: δ 179.12 (*s*, CO), 153.00 (*s*x2, C-3'', C-5''), 150.49 (*s*, C-3'), 147.30 (*s*, C-4'), 136.52 (*s*, C-1''), 134.31 (*s*, C-4''), 133.32 (*s*, C-1'), 118.18 (*d*, C-6'), 115.22 (*d*, C-2'), 109.55 (*d*, C-5'), 106.68 (*d*x2, C-2'', C-6''), 75.32 (*d*, OCH), 71.35 (*d*, C-7'), 68.20 (*t*, C-4), 60.80 (*q*, 4''-OMe), 56.00, 55.89 (*q*x2, q, 3xOMe), 44.82 (*d*, C-3), 43.84 (*d*, C-2), 35.24 (*t*, C-7''), 21.97 (*q*x2, 2xMe); EIms *m/z* (int.rel.): 460 $[M]^+$ (74), 418 $[M-C_3H_6]^+$ (14), 238 (13), 195 **[a]**$^+$ (28), 181 **[c]**$^+$ (100), 179 **[b]**$^+$ (19), 153 $[\mathbf{a}-C_3H_6]^+$ (69), 151 (28), 137 $[\mathbf{b}-C_3H_6]^+$ (20).

***trans*-2-(3,4,5-Trimethoxybenzyl)-3-(α,3-hydroxy-4-*iso*propoxybenzyl)butanolide (22):**
^{1}H Nmr: δ 6.81 (1H, *d*, J= 8 Hz, H-5'), 6.79 (1H, *d*, J= 2 Hz, H-2'), 6.72 (1H, *dd*, J= 8 and 2 Hz, H-6'), 6.42 (2H, *s*, H-2'', H-6''), 4.61 (1H, *d*, J= 7 Hz, H-7'), 4.58 (1H, *d*, J= 6 Hz, OCH), 3.91, 3.88 (1H each, *dd*, J= 13 and 8 Hz, H2-4), 3.82 (9H, *br s*, 3xOMe), 3.17 (1H, *br dd*, J= 13 and 5 Hz, H-7''A), 3.01 (1H, *dt*, J= 8.5 and 5x2 Hz, H-3), 2.88 (1H, *br dd*, J= 13 and 6 Hz, H-7''B), 2.65 (1H, *d*, J= 7.5 Hz, H-2), 1.43 (6H, *d*, J= 6 Hz, 2xMe); ^{13}C nmr:δ 179.21 (*s*, CO), 153.09 (*s*x2, C-3'', C-5''), 149.47 (*s*, C-3'), 147.35 (*s*, C-4'), 136.04 (*s*, C-1''), 134.38 (*s*, C-4''), 133.18 (*s*, C-1'), 118.06 (*d*, C-6'), 115.25 (*d*, C-2'), 110.12 (*d*, C-5'), 106.09 (*d*x2,C-2'', C-6''), 75.05 (*d*, OCH), 71.64 (*d*, C-7'), 68.11 (*t*, C-4), 60.81 (*q*, 4''-OMe), 56.01 (*q*x2, 2xOMe), 44.52 (*d*, C-3), 43.68 (*d*, C-2), 34.98 (*t*, C-7''), 22.01 (*q*x2, 2xOMe); EIms *m/z* (int. rel.): 446 $[M]^+$ (53), 404 $[M-C_3H_6]^+$ (7), 238 (16), 181 **[c]** $^+$ and **[a']**$^+$ (100), 165 **[b']**$^+$ (8), 139 $[\mathbf{a}'-C_3H_6]^+$ (19), 137 (7), 123 $[A\text{-}Trop\text{-}C_3H_6]^+$ (7).

***trans*-2-(3,5-Dimethoxy-4-hydroxybenzyl)-3-(3,4-methylenedioxybenzoyl)butanolide (18):**
Concentrated HCl (1.5 ml) was added to a solution of the ketone (**21**) (243 mg, 0.5 mmol) in AcOH (35 ml). The solution was then stirred at reflux for 30 min. After cooling, a saturated solution of $NaHCO_3$ was added with caution and the mixture was then extracted with $CHCl_3$ (3x100 ml). The organic extracts were dried over Na_2SO_4, filtered and evaporated *in vacuo*. The residue was treated with carbon tetrachloride to eliminate the HCl in excess. The purification by column chromatography ($CHCl_3$/EtOAc, 6%) gave 190 mg (96%) of a white solid **18.** mp 137-138°C. ^{1}H Nmr: δ 7.26 (1H, *dd*, J= 8 and 2Hz, H-6'), 7.23 (1H, *d*, J= 2Hz, H-2'), 6.80 (1H, *d*, J= 8 Hz, H-5'), 6.29 (2H, *s*, H-2'', H-6''), 6.08, 6.07 (1H each, *d*, J= 1.3 Hz, OCH_2O), 4.41, 4.11 (1H each, *t*, J= 8.5 Hz, H_2-4), 4.01 (1H, *q*, J= 9 Hz, H-3), 3.75 (6H, *s*, 2xOMe), 3.55 (1H, *dt*, J= 9 and 6x2 Hz, H-2), 3.04 (1H, *dd*, J= 14.5 and 6 Hz, H-7''A), 2.98 (1H, *dd*, J=14.5 and 6.5 Hz, H-7''B); ^{13}C nmr: δ 194.45 (*s*, 7'-CO), 177.16 (*s*, 1-CO), 152.73 (*s*, C-4'), 148.58 (*s*, C-3'), 146.99 (*s*x2, C-3'', C-5''), 133.47 (*s*, C-4''), 130.34 (*s*, C-1'), 128.00 (*s*, C-1''), 124.70

(*d*, C-6'), 107.93, 107.82 (d each, C-2', C-5'), 105 79 (dx2, C-2'', C-6''), 102.20 (*t*, OCH_2O), 68.28 (*t*, C-4), 56.10 (*q*x2, 2xOMe), 46.67 (*d*, C-3), 44.74 (*d*, C-2), 34.68 (t, C-7''); EIms *m/z* (int. rel.): 400 $[M]^+$ (100), 399 $[M-H]^+$ (43), 224 (40), 167 $[c']^+$ (56), 149 (45), 121 (14).

***trans*-2-(4-Hydroxy-3,5-dimethoxybenzyl)-3-(α-hydroxy-3,4-methylenedioxybenzyl)butanolide (19).** $NaBH_4$ (0.19 g, 50 mmol) was added to a suspension of the ketone (**18**) (46 mg, 01 mmol) in MeOH (6 ml) at 0°C under nitrogen. The solution was then stirred at 0 °C for 2.5 h before the addition of 0.1 M HCl (1 ml). After evaporation of MeOH and dilution with water (2 ml) and brine (2 ml), the mixture was extracted with EtOAc (3x10 ml). The organic extracts were washed with water, dried over Na_2SO_4, filtered and evaporated *in vacuo*. The purification of the reaction mixture by column chromatography (CH_2Cl_2/EtOAc/MeOH, 90:7:3) gave 46 mg (quantitative) of alcohol (**19**) as a mixture of epimers (7:3). **4-β:** ^1H Nmr: δ 6.74 (1H, *d*, J= 8 Hz, H-5'), 6.69 (1H, *br d*, J= 8Hz, H-6'), 6.66 (1H, *br s*, H-2'), 6.37 (2H, *s*, H-2'', H-6''), 5.97, 5.9 (1H each, *d*, J= 1.2 Hz, OCH_2O), 4.62 (1H, *d*, J= 6.5 Hz, H-7'), 3.95, 3.93 (1H each, *dd*, J= 11 and 7 Hz, H_2-4), 3.84 (6H, *s*, 2xOMe), 3.02, 2.88 (1H each, *dd*, J= 12 and 5 Hz, H_2-7''), 2.93 (1H, *dt*, J=7 and 5x2 Hz, H-2), 2.60 (1H, *br d*, J= 7 Hz, H-3); ^{13}C nmr: δ 170.04 (*s*, C=O), 148.03 (*s*, C-4'), 147.47 (*s*, C-3'), 146.80 (*s*x2, C-3'', C-5''), 135.40 (*s*, C-4''), 133.29 (*s*, C-1'), 128.41 (*s*, C-1''), 119.18 (*d*, C-6'), 108.12 (*d*, C-5'), 106.24 (*d*x2, C-2'', C-6''), 106.06 (*d*, C-2'), 101.29 (*t*, OCH_2O), 75.15 (*d*, C-7'), 68.39 (*t*, C-4), 56.20 (2xOMe), 44.80 (*d*, C-3), 43.72 (*d*, C-2), 35.37 (*t*, C-7''). EIms *m/z* (rel. int.): 402 $[M]^+$ (84), 401 $[M-H]^+$ (63) 384 $[M-H_2O]^+$ (9), 229 (19), 178 (22), 167 [B-Trop]$^+$ (100), 151 [A-CHOH]$^+$(54), 135 [A-Trop]$^+$(28), 122 (14). **4-α:** ^1H Nmr: δ 6.72 (1H, *d*, J= 8 Hz, H-6'), 6.62 (1H, *dd*, J= 8 and 1.5 Hz, H-2'), 6.25 (2H, *s*, H-2'', H-6''), 5.94, 5.93 (1H each, *d*, J= 1.2 Hz, OCH_2O), 4.97 (1H, *d*, J= 6 Hz, H-7'), 4.39, 4.21 (1H each, *dd*, J= 9.5 and 6 Hz, H_2-4), 3.96 (6H, *s*, 2xOMe), 3.04, 2.92 (1H each, *m*, H_2-7''), 2.65 (1H, *dt*, J= 7x2 and 4.5 Hz, H-2), 2.54 (1H, *br d*, J= 6.5 Hz, H-3); ^{13}C nmr: δ 176.35 (*s*, CO), 147.90 (*s*, C-4'), 147.09 (*s*, C-3'), 146.89 (*s*x2, C-3'', C-5''), 135.40 (*s*, C-1'), 133.39 (*s*, C-4''), 128.41 (*s*, C-1''), 119.58 (*d*, C-6'), 107.87 (*d*, C-5'), 105.68 (*d*x2, C-2'', C-6''), 106.31 (*d*, C-2'), 101.37 (*t*, OCH_2O), 74.28 (*d*, C-7'), 68.90 (*t*, C-4), 56.37 (*q*x2, 2xOMe), 45.88 (*d*, C-3), 43.82 (*d*, C-2), 35.29 (*t*, C-7''). EIms *m/z* (rel, int.): 402 $[M]^+$ (40), 401 $[M-H]^+$ (27) 384 $[M-H_2O]^+$ (42), 224 (25), 178 37), 167 $[c']^+$ (100), 151 $[a'']^+$(50), 135 $[b'']^+$(25), 122 (12).

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