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Tobacco Chemistry 78.* Biotransformations of Tobacco Cembranoids using Plant Cell Cultures of *Nicotiana Sylvestris*

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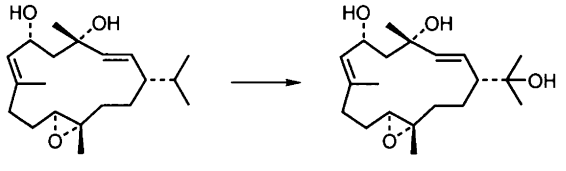
Earlier bioconversion experiments involving (1*S*,2*E*,4*S*,6*R*,7*E*,11*E*)-2,7,11-cembratriene-4,6-diol have been extended to include four related compounds, i.e., the corresponding 11*S*,12*S*-epoxide (**1**) and 11*S*,12*S*-dihydroxy (**2**) derivatives as well as the *seco*-cembranoids (3*E*,7*E*,11*S*,12*E*)-4,8-dimethyl-11-isopropylpentadeca-3,7,12-triene-2,14-dione (**3**) and (4*E*,6*R*,8*S*,9*E*,11*S*)-4,8-dimethyl-11-isopropyl-6,8-dihydroxy-14-oxopentadeca-4,9-dienal (**4**). These have been incubated with plant cell cultures of *Nicotiana sylvestris* or cell homogenates thereof. A novel hydroxylation at the tertiary carbon of the isopropyl group is observed in the case of the epoxide **1**, while five- and six-membered epoxides are derived from the tetraol **2**. The diketone **3**, undergoes epoxidation of the same 7,8-double bond as does the parent diol, while the keto aldehyde **4** undergoes reduction with the formation of a keto triol which is also the obvious intermediate of the second product encountered, a ketohydroxytetrahydrofuran.

The biotransformations of the abundant tobacco constituent (1*S*,2*E*,4*S*,6*R*,7*E*,11*E*)-2,7,11-cembratriene-4,6-diol with plant cell cultures of *Nicotiana sylvestris*, described in the preceding publication,[1] show that the C-10 to C-13 positions are vulnerable to enzymatic attack and that the 11*S*,12*S* epoxide (**1**) was the major product of the many isolated. It was therefore of interest to see whether this epoxide (**1**) and the closely related tetraol, (1*S*,2*E*,4*S*,6*R*,7*E*,11*S*,12*S*)-2,7-cembradiene-4,6,11,12-tetraol (**2**) as well as the *seco*-cembranoids (3*E*,7*E*,11*S*,12*E*)-4,8-dimethyl-11-isopropylpentadeca-3,7,12-triene-2,14-dione (**3**) and (4*E*,6*R*,8*S*,9*E*,11*S*)-4,8-dimethyl-11-isopropyl-6,8-dihydroxy-14-oxopentadeca-4,9-dienal (**4**) could be further biotransformed, the aim being to achieve cleavage of cembranoid ring bonds leading to the formation of lower molecular weight compounds of potential interest as tobacco flavours.

The experiments carried out with the epoxide **1** and 'growing' cells of the previously developed cell line of *N. sylvestris* (T-43-T), using methodology analogous to that described in the accompanying publication,[1] are summarized in Table 1. Rather surprisingly, the sole product obtained could be shown spectroscopically to possess the novel structure **5**, (1*S*,2*E*,4*S*,6*R*,7*E*,11*S*,12*S*)-11,12-epoxy-2,7-cembradiene-4,6,15-triol, cf. Scheme 1. Thus the mass spectrum revealed, when related to earlier mass spectrometric studies of cembranoids,[5] that an additional oxygen was present, obviously as a hydroxy group located on the isopropyl group since the ubiquitous $[M-43]^+$ ion, resulting from elimination of the isopropyl group in typical cembranoid systems, is replaced by a prominent $[M-59]^+$ species. Moreover, the presence of an abundant m/z $58^{+\cdot}$ ion, apparently produced by a McLafferty rearrangement involving the hydroxy-2-propyl group, lends support to this interpretation.

The NMR results confirm the placement of the new hydroxy function at the tertiary isopropyl carbon (C-15) since the C-16 and C-17 methyl signals in the ^{1}H NMR spectrum appear as singlets at 1.18 and 1.11 ppm. In the starting epoxide these methyl proton resonances occur as doublets at 0.80 and 0.85 ppm (J 7 Hz), while all other signals in the two spectra appear at essentially identical positions.

Further evidence was obtained from the HMBC spectrum, which showed cross peaks between C-15 and



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Scheme 1. Biotransformation of the epoxide **1** by T-43-T whole cells.

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Table 1. Biotransformation of (1*S*,2*E*,4*S*,6*R*,7*E*,11*S*,12*S*)-11,12-epoxy-2,7-cembradiene-4,6-diol (**1**) with growing cells of T-43-T cell line.

Exp. No.	SM[a] **1** (mg)	Cell age/d	Medium pH[b]	Incubation time/d	Suspension vol./ml	SM rec.[c] **1** (%)	Product yield, **5** (%)
1	47	8	5.3–5.8	14	500	21	47
2	100	10	5.3–5.8	12	1000	12	54
3	150	10	5.3	16	1500	13	53

[a]SM = starting material. In these experiments, **1** dissolved in ethanol (5 mg ml^{-1} in exp. 1,10 mg ml^{-1} in exp. 2 and 3) was added as one batch. [b]The medium pH was measured during the course of exp. 1 and 2, while in exp. 3 only the initial pH was determined. [c]SM rec. = starting material recovered.

Table 2. Biotransformation of (1*S*,2*E*,4*S*,6*R*,7*E*,11*S*,12*S*)-2,7-cembradiene-4,6,11,12-tetraol (**2**) with a cell homogenate of the T-43-T cell line.

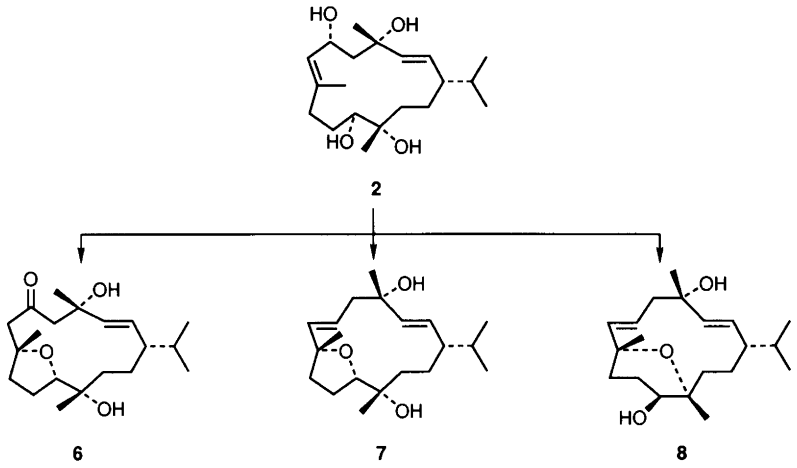
Exp. No.	SM[a] **4** (mg)	Cell age/d	Homogenate pH[b]	Incubation time/h	Homogenate[c] vol./ml	SM rec.[d] **4** (%)	Product yield (%) **6**	**7**	**8**
4	50[e]	20	6.8	120	400	75	—	—	—
5	50	16	6.4	120	400	59	21	—	—
6	50	19	5.8	120	400	52	23	—	—
7	150	18	5.6	96	1200	63	22	—	—
8	100	16	4.4	120	800	58	23	2	2

[a]SM = starting material. In these experiments, **2** dissolved in ethanol (1 mg ml^{-1} unless otherwise stated) was added as one batch at time zero. [b]Initial pH of the homogenate. [c]The homogenate was prepared as described in the Experimental. [d]SM rec = starting material recovered. [e]In this experiment a concentration of 10 mg ml^{-1} of **2** in ethanol was used.

H-16, C-15 and H-17 (two-bond correlations), C-1 and H-16, and C-1 and H-17 (three-bond correlations). The unambiguous assignments of ^{1}H and ^{13}C spectra followed from the COSY and HMQC spectra.

The hydroxylation at C-15 in the epoxide **1** to afford **5** is remarkable when considering the presence of the generally more vulnerable allylic positions. However, since cleavage of skeletal bonds to 'ring-opened' intermediates did not occur, we turned our attention to other analogues.

The 11*S*,12*S*-tetraol (**2**) prepared according to a published procedure[3] was utilized as the substrate in the next series of experiments, which are summarized in Table 2 and involve cell homogenates of the T-43-T cell line prepared as indicated here. The reason for selecting the cell homogenate approach was the earlier observed[1] wider array of products. However, only low yields of biotransformed products and substantial recovery of starting material were encountered. The major component (Scheme 2) obtained was identified as the known[4] cyclic ether **6** by comparison with published spectral data. Very low yields of the known[5,6] cyclic ethers **7** and **8**, were encountered in one experiment (entry 8, Table 2). Since no products generated by ring-cleavage were observed,



Scheme 2. Biotransformation of the tetraol **2** by T-43-T cell homogenate.

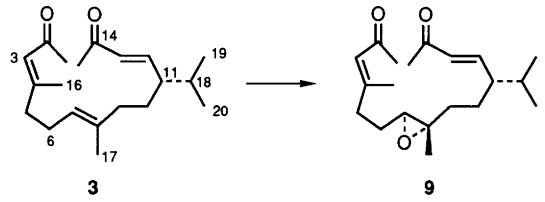
Table 3. Biotransformation of (3*E*,7*E*,11*S*,12*E*)-11-isopropyl-4,8-dimethyl-3,7,12-pentadecatriene-2,14-dione (**3**) with a cell homogenate of the T-43-T cell line.

Exp. No.	SM[a] **3** (mg)	Cell age/d	Homogenate pH[b]	Incubation time/h	Homogenate[c] vol./ml	SM rec.[d] **3** (%)	Product yield **9** (%)
9	50	16	6.4	120	169	21	25
10	100	16	6.5	120	640	84	5
11	50	16	6.6	120	315	57	10

[a]SM = starting material. In these experiments, **3** dissolved in ethanol (10 mg ml^{-1}) was added as one batch at time zero. [b]Initial pH of the homogenate. [c]The homogenate was prepared as described in the Experimental. The cofactors H_2O_2 (4.0 equiv.), FMN (0.5 equiv.) and $MnCl_2$ (0.5 equiv.) were added at time zero. [d]SM rec. = starting material recovered.

further studies with **2** were discontinued. Despite the structural similarity to the epoxide **1**, no compounds possessing a 15-hydroxy group seem to have been produced.

The next cembranoid analogue to be evaluated was the *seco*-diketone **3**, prepared according to a published procedure.[7] The results of three experiments, in which the cell homogenate used was prepared by a modified procedure are detailed in Table 3. In contrast with the above procedure (Table 2), the substrate (**3**) and the cofactors, H_2O_2, FMN and $MnCl_2$ were added to the homogenate simultaneously. Only one product, the novel epoxide **9** (Scheme 3), was isolated in addition to recovered **3**.



Scheme 3. Biotransformation of the *seco*-diketone **3** by T-43-T cell homogenate.

Its mass spectrum, exhibiting a molecular ion at *m/z* 320, suggested that epoxidation had occurred, and the expected enzymatic attack at the 7,8-double bond was readily evident from the ^1H NMR spectrum. Specifically, the C-7 olefinic proton signal in the spectrum of **3** at 5.06 ppm (t, *J* 8 Hz) and the vinyl methyl (C-20) signal at 1.58 ppm (s) were now shifted to 2.60 ppm (t, *J* 6 Hz) and 1.24 ppm (s) respectively.

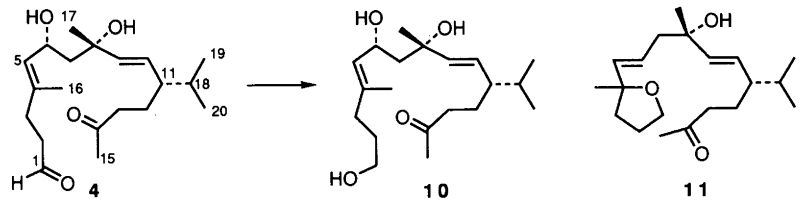
The 7*S*,8*S* configuration of the epoxide **9** was established chemically by oxidation of epoxide **1**, of known absolute configuration, to the corresponding 6-oxo derivative.[4] Upon treatment with potassium carbonate, this compound furnished the retro-aldol cleavage product **9**,[7] identical in every respect with the biotransformation product.

Since only epoxidation and no carbon–carbon bond cleavage in diketone **3** were observed, another *seco*-cembranoid, the known *seco* aldehyde **4**,[3] was evaluated as a possible substrate for the generation of lower molecular weight compounds. The results of two experiments involving incubation of **4** with the aforementioned homogenate are shown in Table 4. The outcome of these two experiments differs considerably in terms of yields of the isolated biotransformation products (**10**, **11**) (Scheme 4) although the only significant difference in the reaction parameters used was that the homogenate: substrate ratio was higher in the latter experiment (entry 13, Table 4).

It is noteworthy when considering our earlier findings with tobacco cell homogenate, that the enzymatic reactions involving reduction and possibly cyclization are predominant. However, such reactions are consistent with earlier biosynthetic predictions. Literature precedent for a cyclization similar to the formation of the novel cyclic ether **11** under acid-catalyzed conditions in the laboratory, is available within the cembranoid series.[3]

The structure of **10** was readily established when the aldehyde **4**, on sodium borohydride reduction, afforded a product identical with that obtained in the above biotransformation experiments.

The structural assignment of the cyclic ether **11** is based on spectral data. The peak at the highest mass in the chemical ionization spectrum occurred at *m/z* 323 $[M+1]^+$ thereby indicating a structure possessing 18 mass units less than that of **10**. However, a prominent ion at *m/z* 197, encountered in related *seco*-cembranoids and characteristic of a unit comprising carbons 8–15, 17–20[2] indicated that this portion of the structure of **11**



Scheme 4. Biotransformation of the *seco* aldehyde **4** by T-43-T cell homogenate.

Table 4. Biotransformation of (4*E*,6*R*,8*S*,9*E*,11*S*)-6,8-dihydroxy-11-isopropyl-4,8-dimethyl-14-oxo-4,9-pentadecadienal (**4**) with cell homogenate of T-43-T cell line.

Exp. No.	SM[a] **4** (mg)	Cell age/d	Homogenate pH[b]	Incubation time/h	Homogenate[c] vol./ml	SM rec.[d] **4** (%)	Product yield (%) **10**	**11**
12	50	15	6.4	120	90	73	10	—
13	100	16	6.4	120	422	2	29	32

[a]SM = starting material. In these experiments, **3** dissolved in ethanol (10 mg ml^{-1}) was added as one batch at time zero. [b]Initial pH of the homogenate. [c]The homogenate was prepared as described inthe Experimental. The cofactors H_2O_2 (2.4 equiv. in exp. 12 and 4.0 equiv. in exp. 13), FMN (0.5 equiv.) and $MnCl_2$ (0.5 equiv.) were added at time zero. [d]SM rec. = starting material recovered.

was unaltered. Comparison of the ^{1}H NMR spectra of **4** and **11** showed that the C-16 methyl proton singlet is shifted from 1.71 ppm in the former to 1.29 ppm in the latter. However, the C-5 olefinic proton signal (5.25 ppm, *J* 8 Hz) and the C-6 proton signal (4.78 ppm) in **4** are replaced by two new sets of signals for C-5 (5.48 ppm, *J* 16 Hz) and C-6 (5.31 ppm, m) protons in the spectrum of **11**. In the ^{13}C NMR spectrum, the C-4 signal occurs at 135.7 ppm in the case of **4** but at 82 ppm in the case of **11**. Also the C-5, C-6 and C-16 resonances (128.7, 66.1 and 19.1 ppm, respectively) in the spectrum of **4** are shifted in that of **11** (138.7, 129.2 and 20.7 ppm, respectively).

In conclusion the regioselective hydroxylation at C-15 of the cembranoid **1** constitutes an interesting and novel enzymatic bioconversion. In contrast, the structurally closely related tetraol **2** is enzymatically modified at the C-8–C-12 portion of the cembranoid skeleton to afford several cyclic ethers. The *seco*-diketone **3** is subject to regioselective epoxidation, as is the parent 2,7,11-cembratriene-4,6-diol, while the other *seco*-cembranoid examined (**4**) undergoes reduction of the aldehyde function and subsequent cyclization involving the resulting primary alcohol function. It is remarkable to note the completely different modes of enzymatic attack when the different cembranoids studied (**1**–**4**) are exposed to the enzymes of *N. sylvestris* cell cultures. However, this is in agreement with earlier biogenetic proposals, forwarded to account for the structural diversity among the numerous cembranoids encountered in tobacco.[8]

Experimental

The instruments and standard techniques are as in Ref. 9. The T-43-T cell line of *Nicotiana sylvestris* was propagated and maintained as described in the accompanying publication.[1] All culture manipulations and biotransformation experiments were performed under aseptic conditions.

General procedure for experiments 1 to 3 (single addition of the epoxide **1** *to the growing T-43-T cell suspension culture).* The suspension culture of T-43-T was grown in MS medium for varying time periods, as shown in Table 1. An alcoholic solution of **1** was added in a single batch to the cells at the ages indicated and incubated for the time periods shown. The resulting biotransformation mixture was harvested by extraction with ethyl acetate and the products were separated by column chromatography on silica gel using ethyl acetate as the eluent.

Typical procedure for biotransformation of (1S,2E,4S,6R,7E,11S,12S)-11,12-epoxy-2,7-cembradiene-4,6-diol (**1**) *with T-43-T whole cells (entry 3, Table 1).* The epoxide **1** (150 mg) was dissolved in ethanol (15 ml) and divided into three equal portions. Each portion was added to an Erlenmeyer flask containing the growing cell suspension culture of T-43-T (500 ml, 10 days old). The resulting suspensions were incubated at 26°C in the dark on a rotary shaker (135 rpm). After being shaken for 16 days, the cell suspensions were filtered through Miracloth and the filtrate was extracted with ethyl acetate (3 × 500 ml). The combined organic extracts were washed with water (500 ml), brine (500 ml) and dried over magnesium sulfate. Concentration *in vacuo* afforded the crude broth extract (145 mg). Ethyl acetate (500 ml) was added to the cell material and the resulting suspension was homogenized with an IKA Ultra-Turrax Disperser T-25 fitted with an S25N-25F rotor/stator (Jankie and Kunkel GmbH and Co. KG) at 24000 rpm for 5 min. The homogenate was filtered through Miracloth and the filtrate was washed with water (300 ml) and brine (300 ml) and dried over magnesium sulfate. Concentration *in vacuo* gave the crude cell extract (124 mg). The broth and cell extracts (269 mg) were combined and submitted to chromatography on silica gel (75 g), using ethyl acetate as the eluent, to afford first the recovered epoxide **1** (20 mg, 13%) followed by (1*S*,2*E*,4*S*,6*R*,7*E*,11*S*,12*S*)-11,12-epoxy-2,7-cembradiene-4,6,15-triol (**5**) (83 mg, 53%): m.p. 74–75°C; $[\alpha]_D^{25}$ +93.6°(*c* 0.22, $CHCl_3$); IR ν_{max} ($CHCl_3$): 3600, 3400 cm^{-1}; ^{1}H NMR ($CDCl_3$): δ 1.11 (s, 3 H, H-17), 1.18 (s, 3 H, H-16), 1.24 (s, 3 H, H-20), 1.28 (s, 3 H, H-18), 1.80 (s, 3 H, H-19), 2.86 (dd, *J* 1 and 5 Hz, 1 H, H-11), 4.48 (dt, *J* 1 and 5 Hz, 1 H, H-6), 5.40 (d, *J* 10 Hz, 1 H, H-7), 5.48 (m, overlapped, 2 H, H-2/H-3); ^{13}C NMR ($CDCl_3$): δ 15.8 (C-20), 16.5 (C-19), 25.0 (C-10), 25.6 (C-14), 26.9 (C-16), 27.2 (C-17), 29.9 (C-18), 36.0 (C-13), 36.6 (C-9), 52.0 (C-1), 53.0 (C-5), 59.5 (C-11), 61.1 (C-12), 66.1 (C-6), 72.2 (C-15), 72.3 (C-4), 126.4 (C-2), 132.6 (C-3), 135.7 (C-8), 141.2 (C-7); MS [*m/z* (%)]: 320 (0.2), 302 (0.2), 287 (0.5), 279 (3.7),

262 (2.5), 149 (15.5), 121 (11.8), 87 (50.3), 58 (30.2) and 43 (100). HRMS calcd. for $C_{20}H_{32}O_3$: 320.2352 $[M-18]^+$: found: 320.2341. Anal. calcd. for $C_{20}H_{34}O_4$: C 70.97, H 10.12; found: C 70.77, H 9.96.

General procedure for the preparation of cell homogenate for experiments 4–8 (Table 2). All procedures were performed at 0–4°C and followed the preparation according to Fig. 1 and Table 2. The cell suspension taken directly from the Erlenmeyer flask was homogenized with an IKA Ultra-Turrax Disperser T-25 fitted with an S25N-25F rotor/stator (Jankie and Kunkel GmbH and Co. KG) at 24000 rpm for 3 × 20 s. In order to avoid overheating the suspension, a 1 min break was allowed between each operation. The homogenate thus obtained was used directly for the experiments outlined in Table 2. Peroxidase activity and protein concentration were determined by the procedures outlined in the accompanying publication.[1]

Typical procedure for biotransformation of (1S,2E,4S,6R,7E,11S,12S)-2,7-cembradiene-4,6,11,12-tetraol (**2**) *with T-43-T cell homogenate (entry 8, Table 2).* The tetraol **2** (100 mg) was dissolved in ethanol (200 ml) and divided into two equal portions. Each portion was placed in a 1 l Erlenmeyer flask containing T-43-T cell homogenate (400 ml, containing 976 units of peroxidase and 357 mg of protein) prepared from cell suspension culture (16 days old) by direct homogenization as indicated above. The mixture was incubated at room temperature with stirring (magnetic stir bar) for 120 h. Then ethyl acetate (125 ml) was added and the mixture was allowed to stir for another 5 min. The resulting mixture was filtered through Celite and the filtrate was extracted with ethyl acetate (3 × 500 ml). The Celite was sonicated with ethyl acetate (300 ml) for 30 min and filtered. The combined organic extracts were washed with water (400 ml), brine (400 ml) and dried over magnesium sulfate. Concentration of the extracts *in vacuo* afforded the crude product mixture (197 mg). Chromatography over silica gel (50 g) using ethyl acetate as the eluent gave (1*S*,2*E*,4*S*,8*R*,11*S*,12*S*)-4,12-dihydroxy-8,11-epoxy-2-cembren-6-one (**6**) (23.3 mg, 23%) followed by (1*S*,2*E*,4*S*,6*E*,8*R*,11*S*,12*S*)-8,11-epoxy-2,6-cembradriene-4,12-diol (**7**) (1.6 mg, 2%), (1*S*,2*E*,4*S*,6*E*,8*R*,11*S*,12*S*)-8,12-epoxy-2,6-cembradiene-4,11-diol (**8**) (1.5 mg, 2%) and recovered **2** (58.4 mg, 58%). Comparison of spectral data (MS and NMR) of **6–8** with those described in the literature[2,4–6] established their identity.

General procedure for the preparation of cell homogenate for experiments 9–13 (Tables 3 and 4).[1] All procedures were performed at 0–4°C. T-43-T cell suspension culture was harvested by filtration through a Büchner funnel equipped with Miracloth and the filtrate was collected for pH and refractive index measurements. The cells were washed with distilled water and allowed to dry by suction. The fresh weight was determined. Phosphate buffer (0.1 M, pH 6.6, 140 ml/100 g fresh weight) was added to the cells and the resulting suspension was then homogenized with an IKA Ultra-Turrax Disperser T-25 fitted with an S25N-25F rotor/stator (Jankie and Kunkel GmbH and Co. KG) at 24000 rpm for 30 s. The same procedure was repeated three times. In order to avoid overheating the suspension, a 1 min break was allowed between each operation. The resulting homogenate was used directly in the biotransformation experiments. As in the above study, peroxidase activity and protein concentration were measured for each cell homogenate used.

Typical procedure for biotransformation of (3E,7E,11S,12E)-11-isopropyl-4,8-dimethyl-3,7,12-pentadecatriene-2,14-dione (**3**) *with T-43-T cell homogenate (entry 9, Table 3).* The *seco*-diketone **3** (50 mg) was dissolved in ethanol (5 ml) and added to a 1 l Erlenmeyer flask containing T-43-T cell homogenate (169 ml, containing 752 units of peroxidase and 189 mg of protein) prepared from cell suspension culture (16 days old) as described above. To this mixture were added phosphate buffer (175 ml, pH 6.6), distilled water (75 ml), H_2O_2 (4.0 equiv.), FMN (0.5 equiv.) and $MnCl_2$ (0.5 equiv.) and the mixture was incubated at room temperature with stirring for 120 h. The reaction mixture was processed as above to afford the crude product mixture (129 mg). Chromatography on silica gel (30 g) using ethyl acetate–hexane (1 : 3) as the eluent afforded recovered **3** (10.5 mg, 21%) and (3*E*,7*S*,8*S*,11*S*,12*E*)-4,8-dimethyl-7,8-epoxy-11-isopropyl-3,12-pentadecadiene-2,14-dione (**9**) (12.6 mg, 25%) as an oil: $[\alpha]_D^{25}+18.9°$ (*c* 0.28, $CHCl_3$); IR ν_{max} ($CHCl_3$): 1685, 1675 cm^{-1}; ^1H NMR ($CDCl_3$): δ 0.81 (d, *J* 6.5 Hz, 3 H)/0.84 (d, *J* 6.5 Hz, 3 H) (H-19/H-20), 1.24 (s, 3 H, H-17), 2.12 (s, 3 H, H-16), 2.18 (s, 3 H, H-15), 2.22 (s, 3 H, H-1), 2.60 (t, *J* 6 Hz, 1 H, H-7), 5.59 (s, 1 H, H-3), 6.02 (d, *J* 10 Hz, 1 H, H-13), 6.50 (dd, *J* 15 and 10 Hz, 1 H, H-12); MS [*m/z*, (%)]: 320 (0.8), 305 (0.2), 302 (0.3), 295 (0.4), 277 (1.5), 262 (2.4), 194 (2.6), 137 (14.3), 123 (20.3), 109 (19.2), 95 (21.8), 81 (28.1), 69 (16.0), 55 (22.8) and 43 (100). HRMS calcd. for $C_{20}H_{32}O_3$ $[M]^{+\cdot}$: 320.2351; found: 320.2345.

Typical procedure for biotransformation of (4E,6R,8S,9E,11S)-4,8-dimethyl-6,8-dihydroxy-11-isopropyl-14-oxo-4,9-pentadecadienal (**4**) *with T-43-T cell homogenate (entry 13, Table 4).* The aldehyde **4** (100 mg) was dissolved in ethanol (10 ml) and added to a 1 l Erlenmeyer flask containing T-43-T cell homogenate (422 ml, containing 1498 units of peroxidase and 662 mg of protein) prepared from a cell suspension culture (16 days old) as described above. To this mixture, phosphate buffer (350 ml, pH 6.6), distilled water (150 ml), H_2O_2 (4.0 equiv.), FMN (0.5 equiv.) and $MnCl_2$ (0.5 equiv.), were added and the mixture was incubated at room temperature with stirring for 120 h. The reaction mixture was processed as above to afford the crude product mixture (285 mg). Chromatography on silica gel (70 g) using ethyl acetate–hexane (1 : 1) as the eluent afforded, in order of elution, (4ξ,5*E*,8*S*,9*E*,11*S*)-4,8-dimethyl-1,4-epoxy-

8-hydroxy-11-isopropyl-5,9-pentadecadien-14-one (**11**) (31.9 mg, 32%), recovered substrate **4** (2.3 mg, 2%) and (4*E*,6*R*,8*S*,9*E*,11*S*)-4,8-dimethyl-1,6,8-trihydroxy-11-isopropyl-4,9-pentadecadien-14-one (**10**) (29.3 mg, 29%). The physical properties of these products are as follows: Trihydroxy ketone **10** is an oil: $[\alpha]_D^{25} + 33.6°$ (*c* 0.96, $CHCl_3$); IR ν_{max} ($CHCl_3$): 3600, 1710 cm^{-1}; ^1H NMR ($CDCl_3$): δ 0.82 (d, *J* 8.0 Hz, 3 H)/0.86 (d, *J* 8.0 Hz, 3 H) (H-19/H-20), 1.40 (s, 3 H, H-17), 1.70 (d, *J* 1 Hz, 3 H, H-16), 2.12 (s, 3 H, H-15), 3.65 (t, *J* 6 Hz, 2 H, H-1), 4.79 (dt, *J* 4 and 10 Hz, 1 H, H-6), 5.25 (d, *J* 12 Hz, 1 H, H-5), 5.35 (dd, *J* 12 and 16 Hz, 1 H, H-10), 5.50 (d, *J* 16 Hz, 1 H, H-9); ^{13}C NMR ($CDCl_3$): δ 16.5 (C-16), 19.1 (C-20), 20.6 (C-19), 26.2 (C-17), 27.0 (C-7), 29.7 (C-3), 30.3 (C-15), 32.0 (C-19), 35.7 (C-2), 42.1 (C-13), 47.6 (C-12), 48.6 (C-11), 62.4 (C-1), 66.2 (C-6), 73.1 (C-8), 127.9 (C-10), 128.6 (C-5), 137.5 (C-4), 139.9 (C-9), 210.0 (C-14); MS [*m/z*, (%)]: 304 (39.6), 289 (9.8), 261 (12.1), 246 (6.4), 231 (7.6), 197 (30.1), 136 (49.8), 121 (92.0), 111 (89.1), 93 (100), 85 (51.6), 71 (23.6), 43 (5.1). HRMS calcd. for $C_{20}H_{32}O_2$ $[M-36]^{+\cdot}$: 304.2402; found: 304.2403. The spectral data of **10** were identical with those of the chemical product obtained by reduction of **9** with sodium borohydride (see below). The epoxy ketone **11** was an oil; $[\alpha]_D^{25} - 3.0°$ (*c* 0.96, $CHCl_3$); IR ν_{max} ($CHCl_3$): 1710 cm^{-1}; ^1H NMR ($CDCl_3$): δ 0.82 (d, *J* 8.0 Hz, 3 H)/0.86 (d, *J* 8.0 Hz, 3 H) (H-19/H-20), 1.25 (s, 3 H, H-17), 1.29 (s, 3 H, H-16), 2.12 (s, 3 H, H-15), 5.31 (ddd, *J* 2,7 and 16 Hz, H-6), 5.48 (d, *J* 16 Hz, H-5), 5.60 (m, overlapped, 2 H, H-9/H-10); ^{13}C NMR ($CDCl_3$): δ 19.1 (C-19), 20.7 (C-20), 25.7 (C-16), 26.2 (C-12), 30.0 (C-15), 32.0/32.1 (C-17/18), 37.7 (C-13), 42.1 (C-7), 45.8 (C-3), 48.6 (C-2), 48.7 (C-11), 67.4 (C-1), 72.2 (C-8), 82.0 (C-4), 122.4 (C-10), 129.2 (C-6), 138.7 (C-5), 140.3 (C-9), 209.5 (C-14); MS [*m/z*, (%)]: 304 (21.0), 289 (7.0), 286 (2.8), 261 (9.9), 246 (4.6), 203 (15.2), 177 (22.4), 197 (10.9), 111 (10.0), 93 (46.1), 85 (50.8) and 71 (20.3). HRMS calcd. for $C_{20}H_{32}O_2$ $[M-18]^+$: 304.2402; found: 304.2403. Chemical ionization-MS, revealed the following significant peaks at [*m/z* (%)]: 323 ($[M+1]^+$, 7.8), 305 (100), 197 (19.0), 111 (16.2) and 85 (39.4).

Preparation of (1S,2E,4S,7E,11S,12S)-*11,12-epoxy-4-hydroxy-2,7-cembradien-6-one* (**12**). To a stirred cool (ice-bath) solution of pyridinium chlorochromate (35.6 mg, 0.165 mmol) in dichloromethane (15 ml) was added a solution of epoxide **1** (50 mg, 0.155 mmol) in dichloromethane (1 ml). The mixture was stirred at 0°C for 2.5 h. Water (50 ml) was added to the mixture and stirring was continued for a further 5 min. Extraction with ethyl acetate (3 × 50 ml) followed by solvent removal and chromatography on silica gel (25 g) using ether–hexane (2 : 1) gave the known[4] (1*S*,2*E*,4*S*,7*E*,11*S*,12*S*)-11,12-epoxy-4-hydroxy-2,7-cembradien-6-one (**12**) (34.2 mg, 68%) and recovered epoxide **1** (8.9 mg, 18%).

Preparation of (3E,7S,8S,11S,12E)-*4,8-dimethyl-7,8-epoxy-11-isopropyl-3,12-pentadecadiene-2,14-dione* (**9**). The keto epoxide **12** (30 mg, 0.094 mmol) was dissolved in methanol (10 ml) and water (1 ml). Powdered potassium carbonate (75 mg, 0.54 mmol) was added and the mixture was stirred at room temperature for 2 h. Water (50 ml) was added and the mixture was extracted with ethyl acetate (3 × 50 ml). The extract was washed with water until neutral, then with brine, and was finally dried over magnesium sulfate. Concentration *in vacuo* and chromatography using ethyl acetate–hexane (1 : 1) as the eluent gave (3*E*,7*S*,8*S*,11*S*,12*E*)-4,8-dimethyl-7,8-epoxy-11-isopropyl-3,12-pentadecadiene-2,14-dione (25.2 mg, 84%) which was identical with the biotransformation product **9**.

Preparation of (4E,6R,8S,9E,11S)-*4,8-dimethyl-1,6,8-trihydroxy-11-isopropyl-4,9-pentadecadien-14-one* (**10**). Sodium borohydride (5.0 mg, 0.13 mmol) was added to a solution of **4** (50 mg, 0.148 mmol) in methanol (5 ml) at 0°C. After being stirred for 5 min, the reaction mixture was diluted with ethyl acetate (50 ml) and the resulting solution was washed with water and brine and dried over anhydrous sodium sulfate. Concentration *in vacuo* and chromatography on silica gel (25 g) using ethyl acetate–hexane (1 : 2) gave first the starting aldehyde **4** (11 mg, 22.0%) followed by (4*E*,6*R*,8*S*,9*E*,11*S*)-4,8-dimethyl-1,6,8-trihydroxy-11-isopropyl-4,9-pentadecadien-14-one (35 mg, 69.5%) which was identical with the biotransformation product **10**.

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