# Stereochemistry of the Methyne–Methylene Conversion in the Biogeneration of (*S*)-δ-Decanolide and (*S*)-γ-Dodecanolide from (13*RS*)-13-Hydroxyoctadeca-9*Z*,11*E*-dienoic and (10*RS*)-10-Hydroxyoctadec-8*E*-enoic Acid

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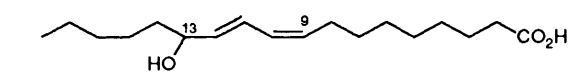
In *Cladosporium suaveolens* the bioconversion of (13*RS*)-13-hydroxy-[9,10,11,12-$^2H_4$]octadeca-9*Z*,11*E*-dienoic acid into axially, 2,3,4-trideuteriated (*S*)-δ-decanolide indicates that protonation of the dienic system occurring at some stage of the degradative sequence takes place on the same face of the molecule. The protonation at position 9 of (10*RS*)-10-hydroxy-[9,10-$^2H_2$]octadeca-8*E*-enoic acid occurring in *Yarrowia lipolytica* during the biogeneration of (*S*)-γ-dodecanolide shows the same steric course.

δ- and γ-Lactones **3** and **4** are flavour components in, *e.g.*, peaches, apricots, strawberries, milk products and fermented foods.[1] They are produced by obscure mechanisms, such as fruit ripening, and are of interest to the food (flavour) industry. Recently there has been legislative discrimination[2] between chemically identical food constituents of synthetic origin and those derived from natural sources. It has therefore become desirable to produce quantities of these lactones, required by the food industry, from the biodegradation of natural products.

The hydroxy acids corresponding to δ-decanolide **3** and γ-dodecanolide **4** are possible degradation products of linoleic and oleic acids, probably *via* oxidation to 13-hydroxyoctadeca-9*Z*,11*E*-dienoic **1** and 10-hydroxyoctadeca-8*E*-enoic **2** acid, respectively. We have therefore screened a number of micro-organisms for their ability to convert acids **1** and **2** into the desired lactones. It was found[3] that *Cladosporium suaveolens* converted the former dienoic acid into δ-decanolide **3** and *Yarrowia lipolytica* degraded the latter hydroxy enoic acid to γ-dodecanolide **4**. These processes are expected to involve β-oxidation, accompanied by the formal conversion at some stage of the sequence of olefinic carbon atoms of the precursors to asymmetric ring methylene groups. To examine these degradations in greater detail we have synthesized (see Experimental section) the appropriately deuterium-labelled precursor hydroxy unsaturated acids **8** and **11**, using published methods. The stereochemistry of the surviving isotopic hydrogen in the corresponding positions of lactones **3** and **4** obtained in feeding experiments in *C. suaveolens* and *Y. lipolytica*, respectively was determined and the results of these studies are reported here.

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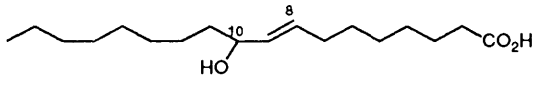


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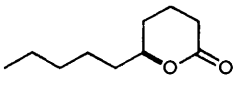

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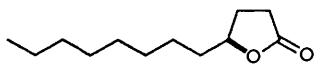
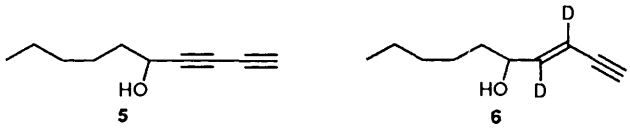
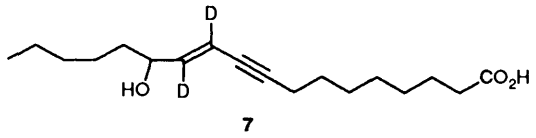


**4**

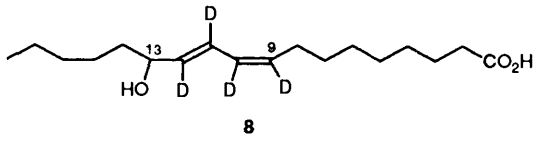
*Synthesis of Regiospecifically Deuteriated Precursors.*—In planning the synthesis of the deuteriated precursors we have taken into account the fact that the enzymic system(s) presiding in *Cladosporium suaveolens* over the bioconversion of acid **1** into δ-decanolide **3** afford, from both (13*S*)- and (13*RS*)-**1**, δ-decanolide **3**, containing *ca.* 90% of the (*S*) enantiomer.[3] This observation simplified the synthetic work by allowing (13*RS*)-13-hydroxy-[9,10,11,12-$^2H_4$]octadeca-9*Z*,11*E*-dienoic acid **8** to be used as the precursor. The hydroxy acid **1**, named 13-HODE or coriolic acid, occurs in the (*R*) enantiomeric form in *Coriaria nepalensis*.[4] Nevertheless it can be easily obtained in the (*S*) form by lipoxygenation of linoleic acid, followed by reduction of the intermediate hydroperoxide.[5] However, this approach to the synthesis of the (*S*) form of compound **8** requires linoleic acid labelled with deuterium atoms at positions 9, 10, 11 and 12 as substrate for the enzymic reaction. This latter compound is not readily available. Of the several syntheses of 13-HODE recently reported,[6] that based on the key intermediacy of deca-1,3-diyn-5-ol **5**[6a] seemed more suited to the preparation of regiospecifically deuteriated compound **8**. 13-HODE was thus prepared from compound **5**, following the reported procedures. Tetradeuteriated compound **8** was obtained from the alcohol **5**, *via* enynol **6** and the acid **7**, by using deuteriated species when reduction of the triple bonds is required.




**5** **6**




**7**




**8**

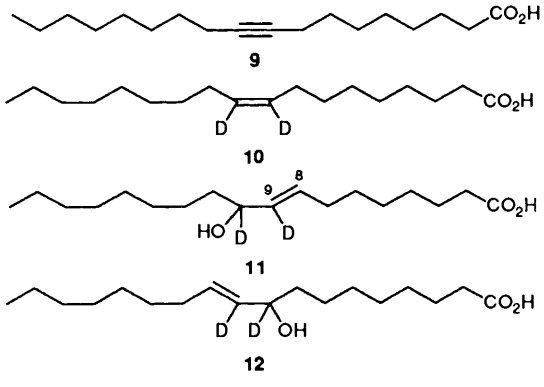
Biodegradation of the 1:1 mixture of racemic 9-hydroxyoctadec-10*E*-enoic and 10-hydroxyoctadec-8*E*-enoic acids in *Yarrowia lipolytica* (obtained in the photooxidation–reduction

**Table 1** $^1$H NMR data for the ring hydrogens of the δ-lactone **3**[a]

| Proton | δ | Coupling | $J_{exp}$/Hz | $J_{calc}$/Hz[b] |
|---|---|---|---|---|
| 2 | 2.06 | J(2,2′) | −17.23 | |
| 2′ | 1.97 | J(3,3′) | −13.50 | |
| 3 | 1.00 | J(4,4′) | −13.80 | |
| 3′ | 1.10 | J(2′,3) | 8.56 | 8.3 |
| 4 | 1.05 | J(2′,3′) | 7.21 | 6.4 |
| 4′ | 0.82 | J(2,3) | 7.24 | 6.0 |
| 5 | 3.60 | J(2,3′) | 5.65 | 5.3 |
| | | J(3,4′) | 10.81 | 10.6 |
| | | J(3′,4′) | 5.77 | 5.1 |
| | | J(3,4) | 5.68 | 5.1 |
| | | J(3′,4) | 4.34 | 3.7 |
| | | J(4′,5) | 10.92 | 10.6 |
| | | J(4,5) | 3.08 | 2.6 |
| | | J(2,4) | 1.08 | |

[a] Chemical shifts with respect to internal $SiMe_4$; solvent $C_6D_6$. The rms error between the experimental and calculated spectrum was 0.095 Hz. [b] Weighted average values of the coupling constants calculated for the equilibria $^3H_4$ (62%) ⇌ $^{2,5}B$ (29%) ⇌ $^4H_3$(9%).

of oleic acid) led to the accumulation of (*S*)-γ-dodecanolide **3** of *ca.* 0.40 ee. We therefore prepared [9,10-$^2H_2$]oleic acid **10** for subsequent photooxidation. The material was easily obtained from the reduction of the methyl ester of stearolic acid[7] by deuterium gas in the presence of Lindlar catalyst. $^1$H and $^2$H NMR studies indicated that *ca.* 95% of the deuterium incorporated in the reduction was located in positions 9 and 10 of compound **10**, the remainder being spread along the whole molecule. The methyl ester of acid **10** was photooxidised in the presence of cercosporin, a natural quinonoid photosensitizer.[8] A 1:1 mixture of the isomeric [9,10-$^2H_2$]-labelled 9- and 10-hydroxy acids **11** and **12** was then obtained after reduction with sodium cysteinate and subsequent hydrolysis. The labelling pattern was confirmed through $^1$H, $^2$H and $^{13}$C NMR studies. However, the results of these analyses indicated that the hydroxy-bearing carbon atoms in positions 10 and 9, respectively, of products **11** and **12** contain *ca.* 10% less deuterium than do the adjacent vinylic carbon atoms.




*Feeding Experiments.*—The production of δ-decanolide **3** and γ-dodecanolide **4** in *C. suaveolens* and *Y. lipolytica*, respectively, follows a bell-shaped curve, with the maximum yield reached after 36–48 h. After that time, the metabolism of the two lactones is rapid, as indicated by their disappearance within a few hours. In order to obtain lactones with high ee values in the feeding experiments of labelled acid **8** and of the mixture of acids **11** + **12** (all in racemic form), the cultures were harvested after a short incubation period. By these means trideuteriated (*S*)-δ-decanolide, shown to contain over 90% of the (*S*) enantiomer, and dideuteriated γ-dodecanolide [shown[3] to be in the (*S*) configuration and with a 0.4 ee] were obtained from tetradeuteriated compound **8** and dideuteriated acids **11** + **12**.

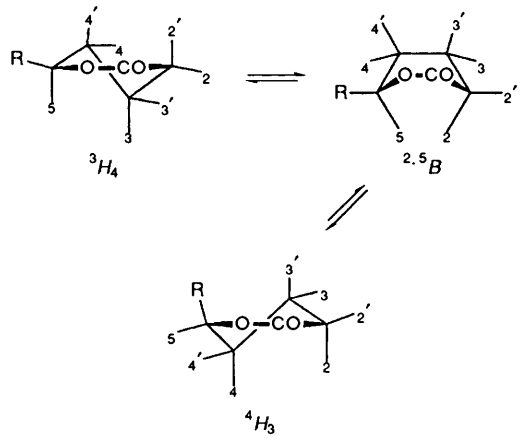
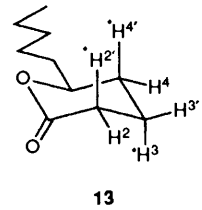



Fig. 1 Conformational equilibrium of δ-decanolide **3** (R = $C_5H_{11}$)

*Assignment of the* $^1$H *NMR Spectra of* δ-*Decanolide and* γ-*Dodecanolide.*—Attempts to probe the steric course of the conversion of the tetradeuteriated precursor **8** into the δ-lactone **13** depend critically on the assignment of the proton spectrum of the full protonated compound **3**. The proton NMR data of compound **3** are reported in Table 1. Since the vicinal coupling constants strongly depend on the conformational features of the lactone ring, the distinction between hydrogens *syn* or *anti* to the alkyl chain at C-5 can be determined if the conformational preference of compound **3** is known. There is general agreement that the C–O–CO–C fragment is planar in δ-lactones;[9] the coplanarity of the lactone ring is attained in both the half-chair and in the boat conformations. Early IR[10] and NMR[11,12] data for some substituted δ-lactones are more consistent with the half-chair conformation. Therefore, assuming as first hypothesis that compound **3** exists in the half-chair conformation, the values of 10.9 and 10.8 Hz for J(4′,5) and J(3,4′), respectively, indicate that protons 5-H, 4-H′ and 3-H are preferentially in an axial orientation, allowing the assignment of 4-H *vs.* 4-H′ and 3-H *vs.* 3-H′. On the other hand the vicinal coupling constants involving protons at carbons C-2 and C-3 clearly cannot be interpreted in terms of a pure half-chair conformation.




**13**

H = D

MMX molecular mechanics calculations* showed that the minimum-energy conformation of compound **3** corresponds to the half-chair $^3H_4$ (Fig. 1) with the substituent at C-5 equatorially orientated. Two other minima, corresponding to the $^{2,5}B$ boat and $^4H_3$ half-chair conformations (Fig. 1), display higher energy with respect to the $^3H_4$ structure by *ca.* 0.5 and 1.0 kcal mol$^{-1}$,† respectively.

The populations $P^I$, $P^{II}$ and $P^{III}$ of each conformer can be calculated from the experimental vicinal coupling constants through the time-average equation $J_{av} = P^I J^I + P^{II} J^{II} + P^{III} J^{III}$ where $J^I$, $J^{II}$ and $J^{III}$ are the reference coupling constants

* PCMODEL, 1990, Serena Software, BOX 3076, IN47402-3076. Calculations were performed at Chiesi Farmaceutici, Parma, Italy.
† 1 cal = 4.184 J.

**Table 2** $^1$H NMR data for the ring hydrogens of the γ-lactone **4**[a]

| Proton | δ | Coupling | $J_{exp}$/Hz | $J_{calc}$/Hz[b] |
|---|---|---|---|---|
| 2 | 1.78 | J(2,2′) | −17.5 | |
| 2′ | 1.91 | J(3,3′) | −12.5 | |
| 3 | 1.29 | J(2,3) | 9.4 | 8.7 |
| 3′ | 0.93 | J(2,3′) | 9.2 | 8.2 |
| 4 | 3.71 | J(2′,3) | 4.0 | 3.5 |
| | | J(2′,3′) | 9.5 | 8.9 |
| | | J(3,4) | 6.3 | 6.2 |
| | | J(3′,4) | 8.0 | 8.2 |

[a] Chemical shifts with respect to internal $SiMe_4$; solvent $C_6D_6$. [b] Weighted average values of the coupling constants calculated for the conformational equilibrium $^3E$ (30%) ⇌ $E_3$ (70%).

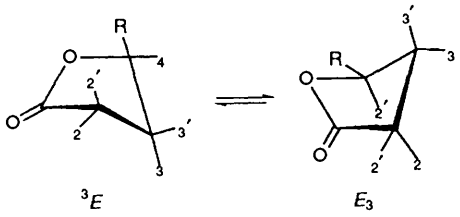
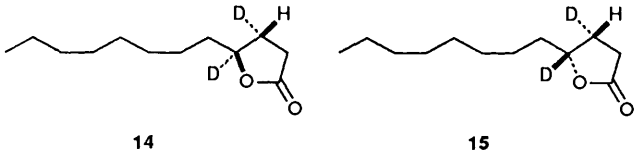



**Fig. 2** Conformational equilibrium of γ-dodecanolide **4** (R = $C_8H_{17}$)

of the pure conformations. We have employed the coupling constants computed from the dihedral angles extracted from the modelled conformations using the generalised Karplus equation developed by Altona and co-workers,[13] as reference values. According to this procedure the structures $^3H_4$, $^{2,5}B$ and $^4H_3$ were found to account for *ca.* 62, 29 and 9% of the total population, respectively. The values of the coupling constants computed according to these populations are also reported in Table 1 for comparison.

Further observations substantiating the predominance of the half-chair conformation for compound **3** are: (i) it is known[14] that the value of the geminal coupling constant J(2,2′) is *ca.* 18 and 15 Hz for the half-chair and boat conformations, respectively. Compound **3** displays a value of 17.2 Hz, in agreement with a prevalence of the half-chair conformer. (ii) The long-range coupling constant J(2,4) of 1.1 Hz suggests a near planar W-arrangement of the H(2)–C(2)–C(3)–C(4)–H(4) fragment which is present in the $^3H_4$ half-chair but not in the boat conformation and allows the assignment of 2-H *vs.* 2-H′. This assignment was confirmed by irradiation of 5-H, which produced an NOE of *ca.* 1% on 2-H. The weighted-average distance $r_{av}$ (2,5) is *ca.* 3.5 Å for the aforementioned conformational equilibrium, while $r_{av}$ (2′,5) is *ca.* 4.1 Å. This is consistent with the small but selective NOE displayed by 2-H with respect to 2-H′ upon irradiation of 5-H.

The same procedure has been followed for the elucidation of the steric course of the conversion of the deuteriated precursor

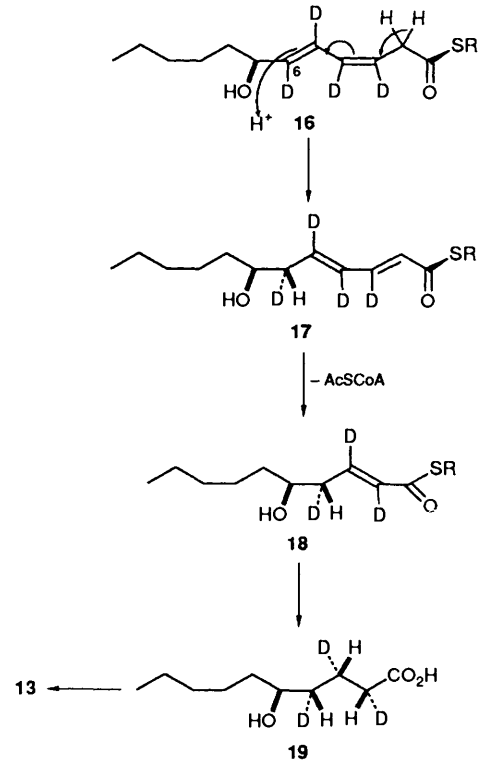



**14** **15**

**11** + **12** into the γ-lactone **14** + **15**. The NMR data for the ring hydrogens of the fully protonated γ-lactone **4** are reported in Table 2. Owing to the planarity of the lactone group only the two envelope conformations $^3E$ and $E_3$ should be present in solution under fast-exchange conditions (Fig. 2).

On the basis of molecular mechanics calculations* the $E_3$ conformation (with the pseudoequatorial alkyl chain) would predominate over the $^3E$ conformation (with a pseudoaxial substituent) with a gain in stability of *ca.* 0.2 kcal mol$^{-1}$. The population of the $^3E$ conformer deduced as described above for the δ-lactone is *ca.* 70% of the total population, in good agreement with that found for the 4-vinylbutyrolactone (68%).[15] The agreement between the experimental coupling constants and those calculated by averaging of the two pure modelled conformations are within 1 Hz. The assignment of proton 2-H *vs.* 2-H′ has been confirmed by irradiation of 4-H at δ 3.71, which produced a selective NOE enhancement by 1.5% of the signal of 2-H at δ 1.78.

*Stereochemistry of the Deuteriated Lactones and Mechanistic Considerations.*—In the light of the aforementioned studies the appearance in the $^2$H NMR spectrum of (*S*)-δ-decanolide, biogenerated in *C. suaveolens* from tetradeuterio **8**, of three signals at δ 0.79, 1.00 and 1.95, respectively, allowed the trideuteriated product to be assigned to the structure depicted by formula **13**, with the three deuterium atoms axially located. The mechanistic consequences of the above results might be as follows. The conversion of acid **1** into lactone **3** by β-oxidation is expected to involve the intermediates shown in Scheme 1. The



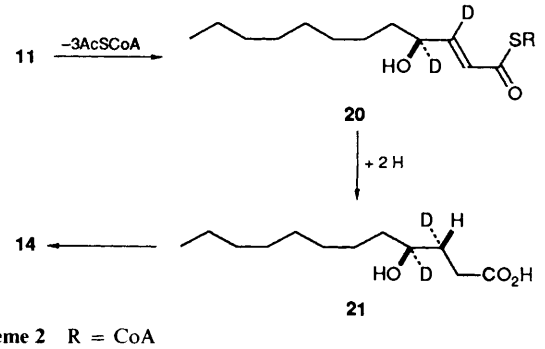

**Scheme 1** R = CoA

stereochemistry of the hydrogen atoms added stepwise through different chemical operations along the sequence **1** ⟶ **3** is that indicated in the (penultimate) intermediate **19**. Therefore, a formal saturation of the (9*Z*,11*E*) dienic system of compound **1** takes place on the same face of the molecule, on the side of the allylic hydroxy group. Also, the indicated steric course of the protonation at position 6 of the C-12 intermediate **16**, with an (*S*)-allylic alcohol moiety, required in order to conjugate the double bonds and to assess the α-*trans* double bond stereochemistry for the further β-oxidation of diene **17** to ene **18**, is identical with that known to occur[16] in the biodegradation of ricinoleic acid to (*R*)-γ-decanolide, through an intermediate with an homoallylic alcohol of (*R*) absolute configuration. Furthermore, saturation of the *E* double bond of compound **18**

* See footnote on p. 2978.

proceeded with formal *syn* hydrogen addition. The proton added at position 2 of compound **18**, eventually located at position 2 of compound **13**, retains the same stereochemistry at that H-atom added at position 2 of the *Z*-configuration intermediate analogue of compound **18**. This latter compound is believed to participate in the degradative pathway leading, in *C. suaveolens*, from the C-19 unnatural homologue of ricinoleic acid to (*S*)-δ-undecanolide.[16]

The $^2H$ NMR spectrum of dodecanolide (7:3 mixture of *S* and *R* enantiomers), obtained from *Y. lipolytica*-derived 9,10 dideuterio (*R,S*)-**11**, showed three signals, at δ 3.72, 1.30 and 0.93, indicating that the deuterium atoms have replaced hydrogens at positions 4, 3 and 3′. However, the intensity proportions of the three signals were ~42:42:16, respectively, thus suggesting a difference in the extent of deuteriation between positions 4 and 3 of biogenerated γ-dodecanolide, seemingly exceeding that already observed at positions 10 and 9 of the precursor **11**. GLC/MS analysis of the biogenerated lactone on a chiral capillary column indicated that the deuterium content of the two enantiomers was nearly the same. Although the different extents of the deuteriation are so far unexplained, NMR studies indicate that the (*S*) and (*R*) forms of [$^2H_2$]-γ-dodecanolide biogenerated from acid (*RS*)-**11** possess the structural formulae **14** and **15**, respectively. The reported deuterium stereochemistry therefore suggests the following mechanistic considerations. The (*S*) C-12 intermediate **20** formed from compound **11** by removal of three acetyl CoA units is thought to be the key intermediate in the biogeneration of γ-dodecanolide **4** from compound **11**. Saturation of the double bond of intermediate **20** occurs at position 3 by formal addition on the *re* face (Scheme 2), to yield compound **21**, which eventually cyclised to compound **14**. The same steric course has been observed in the corresponding position of compound **18** in its conversion into isomer **19**. Future feeding experiments with [8-$^2H_1$]-**11** should clarify the mode of saturation at the α-position of compound **20** in its conversion into compound **21**




**Scheme 2** R = CoA

The results of our present and previous[16] experiments with regiospecifically deuteriated precursors indicate that in the biogeneration of γ- and δ-lactones of (*S*) or (*R*) absolute configuration from structurally similar hydroxy fatty acids, some identical operations onto the double bond occur with the same absolute stereochemistry although different intermediates are involved. This represents a common biosynthetic link between lactones of opposite absolute configuration. However, further studies designed to define the influence of the position in the fatty acid chain of the hydroxy-bearing carbon atom and of the adjacent double bonds on the absolute configuration of the lactones obtained as microbial degradation products are necessary, especially in the light of the current discussion on chirality and naturality of aroma components.[17]

## Experimental

$^1H$ NMR spectra were acquired on a Bruker CXP 300 or a Bruker AC250 spectrometer, except for δ-decanolide **3** and γ-dodecanolide **4** whose data were recorded on a Bruker AMX 600 spectrometer. The $^2H$ NMR spectra were run on a Bruker CXP 300 spectrometer (46.1 MHz) in the gated $^1H$ broad-bond decoupling mode. The spectrum of δ-decanolide **3** was simulated using the iterative program PANIC on a Bruker Aspect 2000 computer. All the volatile materials mentioned in this paper were submitted to GLC–linear retention index analysis. This was performed on a Hewlett-Packard 5890 gas chromatograph equipped with two fused silica capillary columns (DB-1 and DB-1701, J & W, 30 m × 0.25 mm id), mounted in the same injector port, and two flame ionisation detectors. Injector (split ratio 50:1) and detector (FID) point heaters were 280 and 300 °C, respectively. Helium carrier gas was used (1 $cm^3$ $min^{-1}$) and the temperature program was 50 °C for 3 min, followed by an increase at 5 °C $min^{-1}$ to 285 °C for the remainder of the run. The double column signals were recorded simultaneously and elaborated on a Hewlett-Packard 5895A GC-workstation, connected to the gas chromatograph. Linear retention indices of peaks, referred to n-alkanes, were calculated and compared with those of authentic standards chromatographed under identical conditions on DB-1 and DB-1701 columns.

(13RS)-[9,10,11,12-$^2H_4$]*Octadeca*-9*Z*,11*E*-*dienoic Acid* **8**.—Deca-1,3-diyn-5-ol **5** was converted[6a] with $LiAlD_4$ into [3,4-$^2H_2$]dec-3*E*-en-1-yn-5-ol **6** in 50% yield, $\delta_H(CDCl_3)$ 0.9 (3 H, dist. t, Me), 1.05–1.60 (8 H, m, 4 × $CH_2$), 2.84 (1 H, d, HC≡C), 4.12 (1 H, t, CHOH) and 2.20 (1 H, br s, OH). An *analytical sample* was obtained by bulb-to-bulb distillation (oven: 140–150 °C; 0.05 mmHg) (Found: C, 77.9; H, 11.7. $C_{10}H_{14}{}^2H_2O$ requires C, 77.86; H, 11.76%).

Coupling of this material with 8-bromooctanoic acid to give compound **7** was carried out as reported.[6a] Product **7**, obtained in 65% yield, was soon reduced with deuterium gas in the presence of Lindlar catalyst to give, upon evaporation of the filtered solution, oily 13-hydroxy-[9,10,11,12-$^2H_4$]octadeca-9*Z*,11*E*-dienoic acid **8** quantitatively, $\delta_H(CDCl_3)$ 0.89 (dist. t, Me), 1.20–1.70 (18 H, m, 9 × $CH_2$), 2.18 (2 H, t, $CH_2C$=C), 2.28 (2 H, t, $CH_2CO_2$) and 4.10 (1 H, t, C*H*OH); $\delta_D(CHCl_3)$ 5.42 (9-D), 5.65 (12-D), 5.98 (10-D) and 6.49 (11-D). A sample of the methyl ester (diazomethane) was submitted to bulb-to-bulb distillation (oven: 150–170 °C; 0.01 mmHg) and was shown, by the above GLC/linear retention index analysis, to be identical with an authentic sample of methyl coriolate and to be >95% pure.

10-*Hydroxy*-[9,10-$^2H_2$]*octadec*-8*E*-*enoic Acid* **11** + [9,10-$^2H_2$] 9-*Hydroxy*-[9,10-$^2H_2$]*octadec*-10*E*-*enoic Acid* **12**.—Stearolic acid **9**[7] was converted into the oily methyl ester upon treatment with ethereal diazomethane, $\delta_H(CDCl_3)$ 0.89 (3 H, dist. t, Me), 1.20–1.70 (22 H, m, 11 × $CH_2$), 2.14 (4 H, m, 2 × $CH_2C$≡C), 2.30 (2 H, t, $CH_2CO_2$) and 3.66 (3 H, s, OMe).

A solution of the latter material (14 g, 50 mmol) in hexane–ethyl acetate (1:1; 80 $cm^3$) was stirred at room temperature in the presence of deuterium gas with Lindlar catalyst (2 g). When deuterium (1 mol equiv.) had been adsorbed, evaporation of filtered solution quantitatively afforded methyl [9,10-$^2H_2$]octadec-9*Z*-enoate **10** (methyl ester) as an oil, $\delta_H(CDCl_3)$ 0.89 (3 H, dist. t, Me), 1.20–1.70 (22 H, m, 11 × $CH_2$), 2.01 (4 H, dist. t, 2 × $CH_2C$=C), 2.30 (2 H, t, $CH_2CO_2$), 3.66 (3 H, s, OMe); $\delta_D(CHCl_3)$ 5.37 (CD=CD).

A solution of the latter material (6 g, 20 mmol) in acetonitrile (60 $cm^3$) was irradiated in the presence of cercosporin (0.5 g) while oxygen was bubbled through in a Rayonet RPR-100 apparatus, equipped with 16 fluorescent lamps (Sylvania F8T5/CW and F8T5/D9 and a merry-go-round), exactly as indicated.[8] The concentrated solution was stirred at room

temperature with a solution of L-cysteine (3.6 g, 30 mmol) in 1 mol $dm^{-3}$ NaOH until the hydroperoxide had been completely reduced (TLC). The recovered organic residue was chromatographed on silica gel with hexane–ethyl acetate and the purified esters were boiled with 1 mol $dm^{-3}$ LiOH (100 $cm^3$) for 4 h to yield, after acidification and solvent extraction, a mixture of acids **11** and **12** (3 g, ~ 50% overall) as an oil, which solidified on storage, $\delta_H(CDCl_3)$ 0.90 (3 H, dist. t, Me), 1.20–1.80 (22 H, 11 × $CH_2$), 2.03 (2 H, q, $CH_2CH{=}C$), 2.37 (2 H, t, $CH_2CO_2$) and 5.62 (1 H, m, C=CH); $\delta_D(CHCl_3)$ 4.03 (CDOH) and 5.47 (C=CD); $\delta_C(CDCl_3)$ 13.8 (Me), 22.4–36.7 (17 resonances due to the 13 methylene groups), 72.3 (br t, CDOH), 131.3 and 131.6 (=CH), 132.0 and 132.3 (2 br t, =CD) and 178.2 and 178.3 ($CO_2H$).

*Feeding Experiments.*—Cultures of *C. suaveolens* and *Y. lipolytica* were performed in 300 $cm^3$ conical flasks, containing 2% Nutrient Merck (100 $cm^3$) and 0.02% Tween 80; pH 7; 27–30 °C, and the precursor (100 mg), maintained on a rotatory shaker. The inoculum was made from 24-h-old cultures, grown on solid medium. Harvesting was performed at 24 or 48 h intervals. The acidified cultures (pH 1 with 6 mol $dm^{-3}$ HCl) were filtered on a Celite pad, and the latter was washed twice with dichloromethane, used subsequently to extract the aq. phase. The dried ($Na_2SO_4$) organic phase was evaporated (20 cm Vigreux) and the residue was chromatographed on a short silica gel column with hexane–ethyl acetate; the desired fraction was subsequently submitted to bulb-to-bulb distillation (130–150 °C oven temp.; 0.1 mmHg). Typically, for *C. suaveolens*, feeding of compound **8** (500 mg) gave compound **13** (~ 50 mg), shown to be > 95% chemically pure by GLC analysis. Similarly, from *Y. lipolytica* fed with a mixture **11** + **12** (1 g), γ-dodecanolide **4** (60 mg) was isolated (94% pure by GLC) after 48 h incubation.

*Absolute Configuration and ee-Values of Biogenerated Lactones* **3** *and* **4**.—The above information for biogenerated δ-decanolide **3** were obtained by GLC analysis of the diastereoisomeric esters obtained from reaction of the isopropyl ester of 5-hydroxydecanoic acid, prepared from the lactone by methanolic KOH treatment, followed by treatment with 2-bromopropane in dimethylformamide, with the chloride of (*S*)-tetrahydro-5-oxofuran-2-carboxylic acid in the presence of dry pyridine, by exactly following the reported procedure.[18] δ-Decanolide obtained at 24 h from tetradeuteriated acid **8** was found, by comparison with authentic samples of (*R*)- and (*S*)-materials, to contain 90% of the (*S*)-enantiomer. γ-Dodecanolide obtained in *Y. lipolytica* was submitted to GLC analysis on a chiral capillary column (Megadex 1, permethylated β-cyclodextrin-coated fused silica capillary column, 25 m × 0.25 mm id). Analysis conditions: 110 °C for 3 min, then 1 °C $min^{-1}$ up to 180 °C. Comparison of this material with authentic samples indicated the *S* absolute configuration and *ca.* 0.42 ee.

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