for the limited thermal stability of $(allyl)_2CuLi$ and the products of its decomposition, while demonstrating that with $(allyl)_2Cu(CN)Li_2$ no such problem exists. The extent to which ligand reorganization between α- and γ-termini occurs is a function of temperature and substitution on the allyl unit. The understanding gained from these data now allows for the systematic development of allylic organocopper reagents, in essence, as "new" reagents for organic synthesis.[6]

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On the Mode of Conversion of Racemic, C_{14}–C_{19}, γ-Hydroxy Alkene Fatty Acids into C_7–C_{11}, Optically Active γ- and δ-Lactones in *Cladosporium suaveolens*

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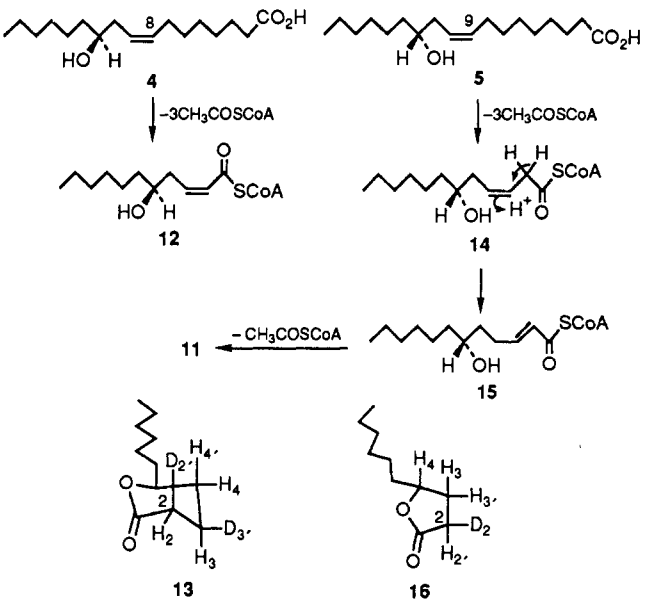
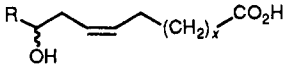
Summary: Growing cultures of *Cladosporium suaveolens* convert the fatty acid derivatives **1–6** either into (*S*)-**7** and -**8** or into (*R*)-**9**, -**10**, and -**11**, depending upon the position of the double bond. Experiments with deuterated substrates show that the hydrogen atom retained α to the carbonyl group in the two series hold the *pro-R* configuration.

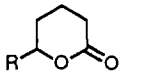
Sir: Optically active γ- and δ-lactones containing up to 12 carbon atoms are widely distributed in nature and play an important role in the flavor industry as aroma components.[1] However, current analytical studies[2,3] indicate that both optical purity and absolute configuration can vary for identical substances isolated from different sources. Moreover, the absolute configuration of the prevalent enantiomer can differ even within a homologous series in the same plant, e.g. the presence of *R* C_8 and C_{10} and *S* C_{12} δ-lactones in cocos meat.[3] These observations give support to the idea[4] that different biosynthetic pathways involving either anabolic or degradative processes can exist for these lipid-derived substances. Degradative processes include β-oxidation of suitably oxygenated intermediates which can be either chiral, such as those involved in the lipoxygenation cascade or those formed in enzymic hydration of methylene-interrupted polyunsaturated fatty acids, or racemic as those formed in photo- or autoxidation.[5] The former set of products is expected to lead to optically active lactones incorporating the chirality of the precursor, as for example in the manufacture of (*R*)-γ-decanolide (**11**) by microbial degradation of natural (*R*)-ricinoleic acid.[6,7] Conversely, racemic precursors are expected to generate educts whose optical purity and absolute configuration may depend upon the susceptibility of the degradative enzyme(s) to the stereochemistry of the hydroxy-bearing carbon atom located in the molecule far away from the place where the β-oxidation starts.

Scheme I

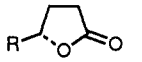




1: R = *n*-C_3H_7; *x* = 5
2: R = *n*-C_3H_7; *x* = 6
3: R = *n*-C_4H_9; *x* = 6
4: R = *n*-C_6H_{13}; *x* = 5
5: R = *n*-C_6H_{13}; *x* = 6
6: R = *n*-C_6H_{13}; *x* = 7



7: R = *n*-C_3H_7
8: R = *n*-C_6H_{13}



9: R = *n*-C_3H_7
10: R = *n*-C_4H_9
11: R = *n*-C_6H_{13}

In this context, we now describe the results of a study on the mode of degradation of the racemic C_{14}–C_{19} fatty acids **1–6**, all of which contain the structural unit (*Z*)-CH=CHCH$_2$CH(OH)R, to C_7–C_{11} lactones by growing cultures of *Cladosporium suaveolens*. The choice of the precursors **1–6** originates from the consideration that mid-chain hydroxy acids without conjugated unsaturation occur in nature[8] and that knowledge of the mode of their

(1) Belitz, H. D.; Grosch, W. *Food Chemistry*; Springer Verlag: Berlin, 1987; p 270.
(2) Schuring, V. In *Bioflavour '87*; Schreier, P., Ed.; de Gruyter: Berlin, 1988; p 35.
(3) Mosandl, A.; Günther, C.; Gessner, M.; Deger, W.; Singer, G.; Heusinger, G. In *Bioflavour '87*; Schreier, P., Ed.; de Gruyter: Berlin, 1988; p 55.
(4) Tressl, R. In *Biogeneration of Aromas*; Parliment, T. H., Croteau, R., Eds.; ACS Symposium Series 317; American Chemical Society: Washington, DC, 1986; p 114.
(5) Gunstone, F. D. In *The Lipid Handbook*; Gunstone, F. D., Harwood, L. J., Padley, F. B., Eds.; Chapman and Hall: London, 1986; p 453.
(6) U.S. Patent 4,560,656 (to Fritzsche, Dodge, and Olcott); *Chem. Abstr.* **1983**, *99*, 4080*t*.
(7) Ital. Appl. 67742 (to San Giorgio Flavors).

0022-3263/89/1954-4979$01.50/0

Table I. Enantiomeric Excess Values of Lactones 7–11 Obtained from Racemic 1–6 in Growing Cultures of *C. suaveolens*[a]

entry	precursor	lactone	R	ee values 24 h	48 h	120 h	198 h
1	1	(*S*)-7	n-C_3H_7	0.58	0.50	0.38	
2	2	(*R*)-9	n-C_3H_7		0.30	0.26	
3	3	(*R*)-10	n-C_4H_9			0.22	
4	4	(*S*)-8	n-C_6H_{13}		0.88		
5	5	(*R*)-11	n-C_6H_{13}		0.54		
6	6	(*S*)-8	n-C_6H_{13}				0.8

[a] The ee values were determined by GLC analysis of the derivatives obtained with (*S*)-tetrahydro-5-oxo-2-furancarboxylic acid (Doolittle, R. E.; Heath, R. R. *J. Org. Chem.* **1984**, *49*, 5041) according to: Gessner, M.; Deger, W.; Mosandl, A. *Z. Lebensm. Unters. Forsch.* **1988**, *186*, 417, and comparison with authentic samples, obtained according to: Francke, A. *Biochem. J.* **1965**, *95*, 633. Tuynenburg, Muys. G.; Van der Ven, B.; De Jonge, A. P. *Appl. Microbiol.* **1963**, *11*, 389. Utaka, M.; Watabu, H.; Takeda, A. *J. Org. Chem.* **1987**, *52*, 4363.

conversion by microbial degradation into γ- and δ-lactones could be useful in the current context of the production of aroma components by microbial methods.[9]

Compounds **1–6** were prepared by an extension of the procedure reported for **5**[10] and fed (1 g/100 mL, 2% nutrient Merck, 0.02% Tween 80, pH 7, 27–30 °C) to *Cladosporium suaveolens* (CBS 157.58). As expected,[11] in agreement with C_2 degradation by β-oxidation (Scheme I), the C_{14}, C_{17}, and C_{19} hydroxy acids **1**, **4**, and **6** afforded C_8 and C_{11} δ-lactones, whereas the C_{15}, C_{16}, and C_{18} acids **2**, **3**, and **5** gave rise to C_7, C_8, and C_{10} γ-lactones. However, the absolute configuration of the prevalent enantiomer in the two series differs, the *S* δ-lactones **7** and **8** and the *R* γ-analogues **9**, **10**, and **11** being obtained. Moreover, the optical purity (Table I) is higher within the first set and decreases in each series on shortening the *n*-alkyl side chain. Furthermore, the ee values in entries 1 and 2 suggest operation, within the biodegradation, of kinetic resolution. The yields (not optimized) ranges from 5 to 20%.

The above observations are interesting from a further point of view. A research area in applied enzymology[12] attempts to define empirical structural rules which would enable one to predict the steric course of enzymic transformations of nonconventional substrates. What emerges in the present case is that the inversion of configuration, associated with the lactone ring size, is a consequence of the shift of the γ-hydroxy alkene moiety along the fatty acid chain and that the length of the *n*-alkyl side chain influences the optical purity of the educts. Advanced intermediates in the β-oxidation of (*S*)-**4** and (*R*)-**5** are thought to be the products **12** and **14** (Scheme I). The conversion of **12** into lactone **8** requires double-bond saturation prior to or after ring closure. By means of feeding experiments with [8,9-$^2H_2$]-(*R*,*S*)-**4** we have shown that double-bond saturation formally takes place with syn hydrogen addition onto the *si* face of the double bond as indicated by NMR studies which allow assignment of structural formula **13** to the dideuteriated lactone.[13] The value of 10.5 Hz for J(4′,5) shows that H-5 is pseudoaxial. Irradiation of H-5 produces a small but clean NOE enhancement of 1.3% of H-2 at 2.08 ppm. This effect permits discrimination between H-2 and H-2′ and establishes the relative stereochemistry at C-2 with respect to C-5 for **13**. The ^2H spectrum of **13** displays two signals at 1.98 and 1.13 ppm. The peak at 1.13 ppm is a broad doublet (J-(^{2}H-3′,H-3) = 2.0 Hz). The total width of the signal is 5 Hz corresponding to a proton signal ca. 33 Hz wide (6.51 is the factor to convert J(H,^{2}H) to J(H,H), which is appropriate for an equatorially oriented hydrogen (H-3′).

Similarly, protonation of the double bond of the *R* intermediate **14** occurs on the *si* face during the conversion into **15**, the penultimate product on the pathway from **5** to **11**. Indeed, feeding experiments with [9,10-$^2H_2$]-(*R*)-**5** yielded a monodeuterated lactone with the stereochemistry depicted in **16**.[14] The relative stereochemistry of C-2 vs C-5 rests on the observation in the NMR spectrum of **16** of a 1.1% enhancement in the H-2 signal by irradiation of H-4. Furthermore, double-bond isomerization occurring during the conversion of **14** to **15** is apparently not accompanied by a intramolecular 1,3 hydrogen shift, because biotransformation of [8-$^2H_2$]-**5** afforded (*R*)-**11** devoid of deuterium. Thus, in the enantiomeric lactones **13** and **16**, the hydrogen atoms originally attached to the double bond of the precursors survived not only in the position α to the carbonyl group but appear in the same *pro-R* configuration. The last feature, together with the preparative significance, adds interest to the present biodegradation, whose mechanism will be further investigated.

Acknowledgment. We thank Rosanna Bernardi for the helpful analytical assistance.

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(12) Whitesides, G. M.; Wang, C. H. *Angew. Chem., Int. Ed. Engl.* **1985**, *24*, 617.

(13) ^1H NMR (500 MHz, C_6D_6): δ 2.08 (m, 1 H, H-2, J(2,2′) = 17.0 Hz, J(2,3) = 7.0 Hz, J(2,3′) = 5.5 Hz), 1.98 (m, 1 H, H-2′, J(2′,3) = 8.5 Hz, J(2′,3′) = 7.0 Hz), 1.14 (H-3′), 1.05 (H-3), 0.87 (H-4′), 1.09 (H-4), 3.63 (m, 1 H, H-5, J(4,5) = 2.5 Hz, J(4′,5) = 10.5 Hz) (the chemical shifts of H-3, H-3′, H-4, and H-4′ have been determined by COSY experiments). ^2H NMR (46 MHz, C_6D_6): δ 1.98 (dd, ^{2}H-2′, J(^{2}H-2′,H-2) = 2.6 Hz, J(^{2}H-2′,H-3) = 1.2 Hz), 1.13 (m, H-3′, J(^{2}H-3′,H-3) = 2.0 Hz, $\Delta\nu$(1/2) = 4.0 Hz).
(14) ^1H NMR (500 MHz, C_6D_6): δ 3.74 (m, 1 H, H-4, J(3,4) = 6.5 Hz, J(3′,4) = 8.0 Hz), 1.31 (m, 1 H, H-3, J(3,3′) = 12.5 Hz, J(2,3) = 9.5 Hz, J(2′,3) = 4.0 Hz), 0.96 (m, 1 H, H-3′, J(2,3′) = 9.5 Hz, J(2′,3′) = 9.5 Hz), 1.91 (m, 1 H, H-2′, J(2,2′) = 17.5 Hz), 1.80 (m, 1 H, H-2). ^2H NMR (46 MHz, C_6D_6): δ 1.78 (m, ^{2}H-2).