The Journal of Organic Chemistry

VOLUME 56, NUMBER 18 AUGUST 30, 1991

© Copyright 1991 by the American Chemical Society

# Communications

## Stereochemistry of the Microbial Generation of δ-Decanolide, γ-Dodecanolide, and γ-Nonanolide from $C_{18}$ 13-Hydroxy, $C_{18}$ 10-Hydroxy, and $C_{19}$ 14-Hydroxy Unsaturated Fatty Acids

Rosanna Cardillo, Giovanni Fronza, Claudio Fuganti,* Piero Grasselli, Andrea Mele, and Domenica Pizzi

*Dipartimento di Chimica del Politecnico, Centro CNR per la Chimica delle Sostanze Organiche Naturali, 20133 Milano, Italy*

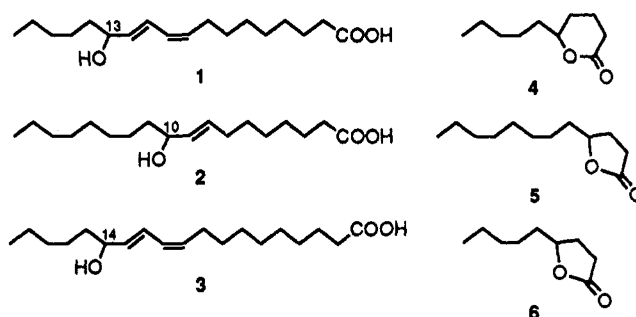
Gianna Allegrone, Massimo Barbeni, and Antonella Pisciotta

*San Giorgio Flavors (Pernod Ricard Group), 10147 Torino, Italy*

*Received April 1, 1991 (Revised Manuscript Received June 19, 1991)*

*Summary:* (*S*)-δ-Decanolide (**4**) was isolated from cultures of *Cladosporium suaveolens* after the microorganism was fed either (*S*)- or (*R*,*S*)-coriolic acid (**1**). Feeding (*R*,*S*)-10-hydroxyoctadec-(8*E*)-enoic acid (**2**) to *Yarrowia lipolytica* produced (*S*)-γ-dodecanolide. When (*S*)-homocoriolic acid (**3**) was fed to *C. suaveolens*, γ-nonalide slightly enriched in the *S* enantiomer was produced. At some stage in the biodegradation of **3**, an inversion of configuration, from *S* to *R*, occurred and was accompanied by the loss of the hydrogen atom originally present on C-14, as GLC/MS analysis of the products of feeding *C. suaveolens* with dideuterated **10** showed.

The need by the flavor industry for large quantities of flavoring compounds that meet the requirements of "naturality" dictated by present rules[1] has stimulated the search for enzymic procedures that enable one to convert intermediates readily available from natural sources into the desired products.[2] A pertinent example of such a procedure is the manufacture of (*R*)-γ-decanolide by the microbial degradation of ricinoleic acid.[3,4] Thus, it seemed reasonable to assume that δ-decanolide (**4**) and γ-dodecanolide (**5**) could be similarly prepared by the β-oxidation of the naturally occurring $C_{18}$ hydroxy fatty acids **1** and **2**—oxidation products of linoleic acid and oleic acid, re-




spectively. Ricinoleic acid occurs in nature only as the *R* enantiomer,[5] thus its biodegradation provides natural (*R*)-γ-decanolide. In contrast, both enantiomers of **1** (coriolic acid) occur in plant glycerides.[6] The *S* enantiomer is accessible by the reduction of the 13-hydroperoxide formed by the lipoxygenation of linoleic acid,[7] whereas (*R*,*S*)-**1** can be generated by reduction of the racemic 13-hydroperoxide formed by autoxidation or photooxidation of linoleic acid.[8] Racemic **2** can be similarly obtained from oleic acid.[9] However, both δ-decanolide and γ-dodecanolide occur in nature as the *R* enantiomers,[10]

(1) *U.S. Code of Federal Rederal Regulations*, 1985, 21,101.22a.3.
(2) Teranishi, R. In *Flavor Chemistry: Trends and Developments*; Teranishi, R., Buttery, R. G., Shahidi, F., Eds.; ACS Symposium Series 388; American Chemical Society: Washington, DC, 1989; p 1.
(3) U.S. Patent 4,560,656 (to FDO); *Chem. Abstr.* **1983**, *99*, 4080t.
(4) Cardillo, R.; Fronza, G.; Fuganti, C.; Grasselli, P.; Nepoti, V.; Barbeni, M.; Guarda, P. A. *J. Org. Chem.* **1989**, *54*, 4979.
(5) Howling, D.; Morris, L. J.; Gurr, M. I.; James, A. J. *Biochim. Biophys. Acta* **1972**, *260*, 10.
(6) Tallent, W. H.; Hains, J.; Wolff, I. A.; Lundin, R. E. *Tetrahedron Lett.* **1966**, 4329. Kato, T.; Yamagouchi, Y.; Hirano, T.; Yokoyama, T.; Ugehara, T.; Namai, T.; Yamanaka, S.; Harada, N. *Chem. Lett.* **1984**, 409.
(7) Axelrod, B.; Cheesbrough, T.; Laasko, S. *Methods Enzymol.* **1981**, *71*, 441.
(8) Gunstone, F. D. In *The Lipids Handbook*; Gunstone, F. D., Harwood, L. J., Padley, F. B., Eds.; Chapman and Hall: London, 1986; p 453.
(9) Ross, J. *J. Am. Chem. Soc.* **1949**, *71*, 282.

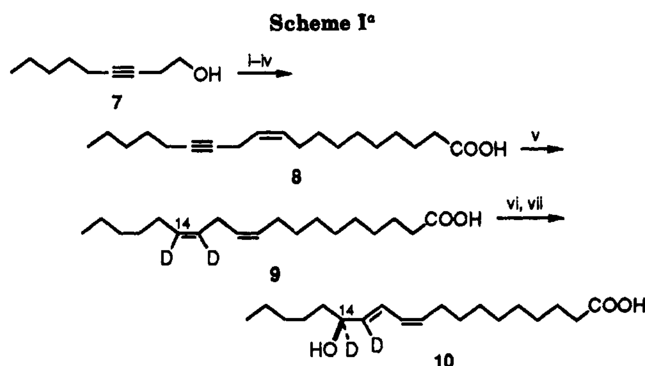
which raises questions about the enantioselectivity of enzymic degradation. We now report on the mode of the microbial generation of **4** and **5** from natural **1** and racemic **2**, respectively, and of **6** from **3**, the unnatural, higher homologue of **1**.

Thus, (*S*)-coriolic acid (**1**)[7] was fed to growing cultures of *C. suaveolens* (CBS 157.58) (100 mg/100 mL, 2% nutrient Merck, 0.02% Tween 80, pH 7, 27–30 °C). After a 48-h incubation, (*S*)-δ-decanolide (**4**) was obtained in ca. 40% yield. The optical purity of the precursor was determined by $^1$H NMR analysis of the derivative formed by the reaction of the methyl ester of (*S*)-**1** with the 2-methoxy-2-(trifluoromethyl)phenylacetic acid [(+)-MTPA].[11] It was shown to be ca. 80%, identical to that of the isolated δ-decanolide, which was determined by GLC analysis of suitable derivatives.[12] Interestingly, feeding racemic coriolic acid[13] to *C. suaveolens* also produced (*S*)-δ-decanolide, of 82% optical purity after 24 h and 79% optical purity after 48 h.

Racemic **2**, obtained as a 1:1 mixture with its isomer racemic (10*E*)-9-hydroxyoctadecenoic acid by the reduction of the mixture of hydroperoxides formed by the photooxidation of oleic acid,[14] when fed to *C. suaveolens* afforded only low yields of the expected γ-dodecanolide (**5**). However, 20–30% conversions of **2** into **5** were observed in *Yarrowia lipolytica* (CBS 2074) after 48 h of incubation. The γ-dodecanolide so obtained was found by GLC analysis with a chiral capillary column[15] to consist predominantly (40% ee) of the *S* enantiomer. Thus, in both microorganisms, the enzymic system(s) that is (are) responsible for the degradation of the $C_{18}$ precursors **1** and **2**, which bear hydroxyl groups at C-13 and C-10, respectively (i.e., at odd- and even-numbered positions), show(s) a clear preference for producing the *S* enantiomers. This behavior appears to be in conflict with the previously reported[4] mode of degradation by *C. suaveolens* of isomeric fatty acids that incorporate the (*Z*)-CH=CHCH$_2$CH(OH)R structural unit (R = *n*-alkyl). By that mode, racemic precursors that bear the hydroxyl group at an even-numbered position gave (*R*)-γ-lactones, whereas (*S*)-δ-lactones were formed from precursors that bear the OH group at an odd-numbered position.

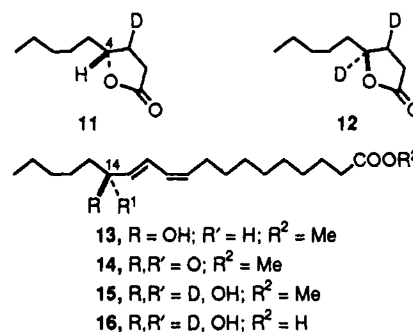
It was then decided to compare the stereochemical outcome of the biodegradation of **1** with that of its higher homologue **3** (homocoriolic acid). After (14*S*)-**3** was fed to *C. suaveolens* (Scheme I), the γ-nonanolide that was isolated after short incubation was found to be the *S* enantiomer. However the ee of the product decreased as incubation was continued. After 48 h, the ee, as determined by GLC analysis on the chiral capillary column, was 20%. However, after the $C_{19}$ precursor had been consumed, the concentration of 4-hydroxynonanoic acid rapidly decreased and the γ-nonanolide that was isolated was predominantly the *R* enantiomer. As time passed, the enantiomeric purity increased, eventually reaching 70% ee. When racemic 4-hydroxynonanoic acid-*4-d* was fed to *C. suaveolens*, rather rapid degradation was observed. The γ-nonalolide that was isolated was enriched in the *R* enantiomer, the ee of which increased with the passage of time. No loss of deuterium was detected. These facts seem to render unlikely the possibility of a bioconversion of the *S* into the *R* enantiomer by way of a redox process. Racemic **3** gave, after a 48-h incubation under the same conditions, (*S*)-γ-nonanolide with an ee of 42%.

**Scheme I**[a]




[a] (i) Ph$_3$P/NBS/CH$_2$Cl$_2$; (ii) Ph$_3$P/toluene/reflux; (iii) Me$_3$COK, then OHC(CH$_2$)$_8$CO$_2$Me; (iv) LiOH; (v) D$_2$/Lindlar; (vi) soybean lipoxygenase/pH 9/0 °C; (vii) HSCH$_2$CO$_2$Na.

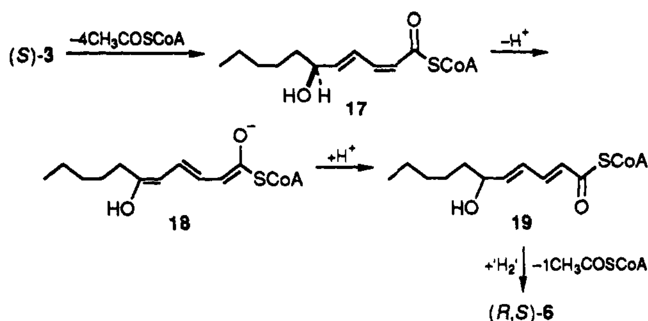
It was then decided to determine the fate of the hydrogen atom originally present at C-14 of (*S*)- and (*R,S*)-**3** during the bioconversion of those compounds into γ-nonanolide (**6**). To this end, (10*Z*,13*Z*)-nonadecadienoic acid-*13,14-d*$_2$ (**9**) was synthesized from non-3-yl-1-ol (**7**) via **8** (Scheme I).[16] This material was rapidly lipoxygenated by treatment with soybean lipoxygenase at pH 9 and 0 °C. Reduction of the hydroperoxide so formed gave the desired (14*S*)-13,14-dideuterated compound **10** (90% ee). The




assignment of *S* configuration to **12** and the estimate of the compound's optical purity are based on the similar behavior, upon $^1$H NMR and HPLC analysis,[17] of the (+)-MTPA derivative of the methyl ester of **10** and that of the corresponding (*S*)-coriolic acid derivative and also on the known *S* enantioselectivity[18] of lipoxygenations by soybean lipoxygenase. The $^2$H NMR spectrum of the γ-nonanolide isolated after a 48-h incubation of **10** with *C. suaveolens* showed signals at 1.30, 0.93, and 3.70 ppm, which corresponded to H-3, H-3′, and H-4, respectively. The H-3:H-3′:H-4 signal ratio was about 6:4:4, which indicated that the labels were located at C-3 and C-4 in a ca. 2:1 ratio. GLC/MS analysis of this material with the chiral capillary column indicated that the *S* enantiomer (58% of the mixture) was 91.9% dideuterated, 5.2% monodeuterated, and 2.9% undeuterated, whereas the *R* enantiomer (42% of the mixture) was 89.5% monodeuterated and 10.5% undeuterated. This information permitted the assignment to the deuterated (*S*)- and

(10) Engel, K. H. In *Bioflavour'87*; Schreier, P., Ed.; De Gruyter: Berlin, 1988; p 75.
(11) Van Os, C. P. A.; Veute, M.; Vliegenthart, J. F. G. *Biochim. Biophys. Acta* **1979**, *574*, 103.
(12) Gessner, M.; Deger, W.; Mosandl, A. *Z. Lebensm. Unters. Forsch.* **1988**, *186*, 417.
(13) Rama Rao, A. V.; Reddy, E. R.; Sharma, G. V. M.; Yadagiri, P.; Yadav, J. S. *J. Org. Chem.* **1986**, *51*, 4158.
(14) Arnone, A.; Assante, G.; Caronna, T.; Di Modugno, V.; Nasini, G. *Phytochemistry* **1988**, *27*, 1669.
(15) A Megadex 1 (permethylated β-cyclodextrin-coated fused silica capillary column) obtained from Mega srl, 20035 Legnano, Italy.
(16) Bradshaw, R. W.; Day, A. C.; Jones, E. R. H.; Page, C. B.; Thaller, V.; Vere Hodge, R. A. *J. Chem. Soc. C* **1971**, 1156.
(17) Andre, J. C.; Funk, M. O. *Anal. Biochem.* **1986**, *158*, 316.
(18) Zhang, P.; Kyler, K. S. *J. Am. Chem. Soc.* **1989**, *111*, 9241.

Scheme II




(R)-γ-nonanolides biosynthesized from dideutero (14S)-10 the structures 11 and 12, respectively. Thus, during the degradation of $C_{19}$ (14S)-10 to (4R)- and (4S)-4-hydroxydecanoic acid, the loss of the hydrogen atom originally located on the hydroxyl-substituted carbon atom occurs, at some point, only from that species that undergoes inversion of configuration. In support of this view are the results of feeding experiments with (14R,S)-16-*14-d*, prepared from 13 by way of 14 and 15.[17] The γ-nonanolide that was isolated after a 34-h incubation was a 72:28 mixture of the S enantiomer (95.2% monodeuterated, 4.8% undeutrated) and the R enantiomer (38.9% monodeuterated, 61.1% undeuterated). NMR analysis indicated that the retained deuterium atom is located on C-4 of 6. It thus seems that both enantiomers of homocoriolic acid (3) are converted into γ-nonanolide (6), but at different rates and by different mechanisms. The S enantiomer of 3 is metabolized at a faster rate, and the deuterium atom at C-14 is lost from that fraction of the material that is converted into (R)-6. The R enantiomer of 3 is degraded at a slower rate directly to (R)-γ-nonanolide and retains throughout the hydrogen atom originally present on the hydroxyl-substituted carbon atom.

Possible intermediates in the degradation of 3 to 6 are shown in Scheme II. It is possible that the $C_{11}$ species 17, which possesses Z,E stereochemistry, could undergo isomerization, by way of 18, to 19, which incorporates the α-E-configured double bond that apparently is required for further β-oxidation.[19] It may be that a satisfactory explanation for the loss of deuterium is to be found in knowledge of mechanisms of the conversion of (S)-17 into (R)-19 and in the conformational changes, which accompany that conversion.

**Acknowledgment.** We thank Rosanna Bernardi for analytical assistance. This work was supported financially by Progetto Strategico CNR Sviluppo Tecnologico P & M Imprese.

**Registry No.** 1, 10219-69-9; 2, 115511-53-0; 3, 135106-69-3; 4, 59285-67-5; 5, 69830-92-8; 6, 104-61-0; 9, 135106-70-6; 10, 135106-71-7; 11, 135106-72-8; 12, 135106-73-9; (±)-16-14*d*, 135106-74-0.

(19) Galliard, T. In *Recent Advances in the Chemistry and Biochemistry of Plant Lipids*; Galliard, T., Mercer, E. I., Eds.; Academic Press: Lnodon, 1975; p 318.

# Production of 2-Octenyl Radicals from the Fe(III)•Bleomycin-Mediated Fragmentation of 10-Hydroperoxy-8,12-octadecadienoic Acid

Anand Natrajan and Sidney M. Hecht*

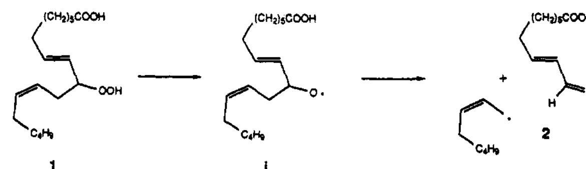
*Departments of Chemistry and Biology, University of Virginia, Charlottesville, Virginia 22901*

*Received May 16, 1991*

*Summary:* The Fe(III)·BLM-mediated fragmentation of 10-hydroperoxy-8,12-octadecadienoic acid was demonstrated unambiguously to occur via homolytic O–O bond scission.

The bleomycins (BLMs) are a family of glycopeptide-derived antibiotics with clinically useful antitumor activity.[1] In the presence of metal ions such as $Fe^{2+}$, bleomycin forms a binary complex [Fe(II)·BLM] that can reductively activate molecular oxygen.[2] The resulting unstable and reactive species termed "activated bleomycin" is believed to be an oxygenated metallobleomycin.[3] Activated bleomycin degrades DNA[2,3] and RNA[4] and also oxidizes and oxygenates low molecular weight substrates such as styrene and naphthalene.[5] Burger et al. have shown that the same activated bleomycin is accessible from either Fe(II)·BLM + $O_2$ or Fe(III)·BLM + $H_2O_2$;[3f] the latter reaction is analogous to the "peroxide shunt" pathway in cytochrome P-450 activation by various oxygen transfer agents.[6]

**Scheme I. Decomposition of 10-Hydroperoxy-8,12-octadecadienoic Acid (1) to 10-Oxo-8-decenoic Acid (2) via Homolytic O–O Bond Scission**




(1) (a) Umezawa, H. In *Bleomycin: Current Status and New Developments*; Carter, S. K., Crooke, S. T., Umezawa, H., Eds.; Academic: New York, 1978. (b) Umezawa, H.; Maeda, K.; Takeuchi, T.; Okami, Y. *J. Antibiot.* **1966**, *19*, 200. (c) Umezawa, H. *Pure Appl. Chem.* **1971**, *28*, 665. (d) Umezawa, H. *Biomedicine* **1973**, *18*, 459. (e) Hecht, S. M. In *Bleomycin: Chemical, Biochemical and Biological Aspects*; Hecht, S. M., Ed.; Springer-Verlag: New York, 1979.

(2) (a) Dabrowiak, J. C. *Adv. Inorg. Chem.* **1982**, *4*, 70. (b) Hecht, S. M. *Acc. Chem. Res.* **1986**, *19*, 83. (c) Stubbe, J.; Kozarich, J. W. *Chem. Rev.* **1987**, *87*, 1107.

(3) (a) Sausville, E. A.; Peisach, J.; Horwitz, S. B. *Biochem. Biophys. Res. Commun.* **1976**, *17*, 814. (b) Sausville, E. A.; Stein, R. W.; Peisach, J.; Horwitz, S. B. *Biochemistry* **1978**, *17*, 2746. (c) Burger, R. M.; Horwitz, S. B.; Peisach, J.; Wittenberg, J. B. *J. Biol. Chem.* **1979**, *254*, 12299. (d) Sugiura, Y.; Kikuchi, T. *J. Antibiot.* **1979**, *31*, 1310. (e) Kuramochi, H.; Takahashi, K.; Takita, T.; Umezawa, H. *J. Antibiot.* **1981**, *34*, 576. (f) Burger, R. M.; Peisach, J.; Horwitz, S. B. *J. Biol. Chem.* **1981**, *256*, 11636. (g) Burger, R. M.; Kent, T. A.; Horwitz, S. B.; Munck, E.; Peisach, J. *J. Biol. Chem.* **1983**, *258*, 1559.

(4) (a) Magliozzo, R. S.; Peisach, J.; Ciriolo, M. R. *Mol. Pharmacol.* **1989**, *35*, 428. (b) Carter, B. J.; de Vroom, E.; Long, E. C.; van der Marel, G. A.; van Boom, J. H.; Hecht, S. M. *Proc. Natl. Acad. Sci. U.S.A.* **1990**, *87*, 9373.

(5) (a) Murugesan, N.; Ehrenfeld, G. M.; Hecht, S. M. *J. Biol. Chem.* **1982**, *257*, 8600. (b) Ehrenfeld, G. M.; Murugesan, N.; Hecht, S. M. *Inorg. Chem.* **1984**, *22*, 1496. (c) Murugesan, N.; Hecht, S. M. *J. Am. Chem. Soc.* **1985**, *107*, 493. (d) Heimbrook, D. C.; Mulholland, R. L., Jr.; Hecht, S. M. *J. Am. Chem. Soc.* **1986**, *108*, 7839. (e) Heimbrook, D. C.; Carr, S. A.; Mentzer, M. A.; Long, E. C.; Hecht, S. M. *Inorg. Chem.* **1987**, *26*, 3836.

 © 1991 American Chemical Society