Biosynthetic Studies on the Xanthone Antibiotics Lysolipins X and I

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Feeding experiments with ^{13}C and ^{18}O-labeled precursors revealed that the molecular framework of the polycyclic xanthone antibiotics, the lysolipins X (1) and I (2), is derived from the polyketide pathway (12 malonate units), the C_1 pool (methionine), molecular oxygen, and the nitrogen pool. Surprisingly, an intact malonate moiety serves as the three-carbon starter unit of the polyketide backbone, and 9 of the 12 oxygen atoms of 1 originate from molecular oxygen, including both of the xanthone oxygen atoms. The orientation of the malonate unit incorporated intact into lysolipin is unique and opposite from those in tetracycline and cycloheximide, i.e., the activated carbon of malonyl CoA is bound to the nitrogen of the lysolipin isoquinoline ring and the CO_2-derived carbon serves as the starter of the polyketide chain. From the biogenetic origin of the oxygen atoms several unusual prearomatic deoxygenation steps early in the biosynthesis have to be postulated.

The polycyclic xanthone antibiotics form a small but distinct family of more than 20 members of microbial natural products. Their biological activities range from antibacterial (mainly Gram-positive bacteria including anaerobes,[1,2] but also Gram-negative bacteria[3]), to anticoccidial,[4] antifungal[1c,5] (e.g. yeasts, dermatophytes), and cytotoxic activities[3c,6] (e.g. HeLa cells).

The lysolipins are antibacterial, antifungal (only 2), as well as cytotoxic compounds.[3] After the albofungins,[1a–c] the lysolipins were the second group of the xanthone family of antibiotics to be discovered. These lipophilic antibiotics act lytically against cell walls, i.e., as inhibitors of cell wall biosynthesis (antagonism against lipid-bound mureine precursors).[3a] Lysolipin X (1) is the natural end product formed by *Streptomyces violaceoniger* (Tü 96) and the immediate precursor of lysolipin I (2), its dehydration product (Figure 1). While the absolute configuration of 2 could be established by X-ray analysis and chemical derivatization, the configuration of C-11 and C-12 in 1 is uncertain, because the instability of the molecule toward weak acids or light does not allow any chemical modification without dehydration to 2 or a 2-derivative, respectively.[3a,d,e]

The polycyclic xanthones are one of the largest subgroups of polyketides being assembled by a type-2 polyketide synthase.[7] The lysolipins attracted our attention because our initial acetate feeding experiments showed a seemingly irregular acetate incorporation pattern into the isoquinoline ring moiety. Formation of the xanthone structures obviously requires some rearrangement of a primary polyketide-derived carbon framework. Oxidation reactions with molecular oxygen often modify primary molecular frameworks extensively during early biosynthetic steps. Examples of such oxidations are epoxidations and successive intramolecular cascade reactions as, e.g., in the biosynthesis of the polyether group of antibiotics[8] or the cyclooxygenation reactions in the biosynthesis of some metabolites of the arachidonic acid cascade, e.g., the formation of the prostaglandins or the thromboxanes.[9] Thus, feeding experiments with $^{18}O_2$ in addition to ones with ^{18}O-labeled biosynthetic building blocks may provide suggestions regarding earlier biosynthetic steps, which can be probed by further biosynthetic investigations. ^{18}O-Incorporation into specific positions can be detected by NMR spectroscopy using the $^{13}C\{^{18}O\}$ shift method introduced by van Etten and Vederas 12 years ago.[10] The application to the xanthones in general and to the lysolipins in particular seemed promising, since these antibiotics carry several oxygen atoms in their structures, and their origin might reveal mechanistic

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(1) (a) Onoprienko, V. V.; Koz'min, Y. P.; Kolosov, M. N. *Bioorg. Khim.* **1978**, *4*, 1418–1422. (b) Gurevich, A. I.; Deshko, T. N.; Kogan, G. A.; Kolosov, M. N.; Kudryashova, V. V.; Onopreinko, V. V. *Tetrahedron Lett.* **1974**, 2801–2804. (c) Gurevich, A. I.; Karapetyan, M. G.; Kolosov, M. N.; Omelchenko, V. N.; Onoprienko, V. V.; Petrenko, G. I.; Popravko, S. A. *Tetrahedron Lett.* **1972**, 1751–1754. (d) Carter, G. T.; Nietsche, J. A.; Williams, D. R.; Borders, D. B. *J. Antibiot.* **1990**, *43*, 504–512.

(2) (a) Ōmura, S.; Nakagawa, A. *J. Am. Chem. Soc.* **1986**, *108*, 6088–6089. (b) Tanaka, H.; Kawakita, K.; Suzuki, H.; Spiri-Nakagawa, P.; Ōmura, S. *J. Antibiot.* **1989**, *42*, 431–439.

(3) (a) Drautz, H.; Keller-Schierlein, W.; Zähner, H. *Arch. Microbiol.* **1975**, *106*, 175–190. (b) Dobler, M.; Keller-Schierlein, W. *Helv. Chim. Acta* **1977**, *60*, 178–185. (c) Pultar, T. Ph. D. Thesis, 1988, University of Tübingen, Germany. (d) Wessels, P. Diplomarbeit, 1988, University of Göttingen, Germany. (e) Gräther, O. Diplomarbeit, 1989, University of Göttingen, Germany.

(4) Lee, T. M.; Carter, G. T.; Borders, D. B. *J. Chem. Soc., Chem. Commun.* **1989**, 1771–1772.

(5) (a) Cooper, R.; Truumees, I.; Gunnarsson, I.; Loebenberg, D.; Horan, A.; Marquez, J.; Patel, M.; Gullo, V.; Puar, M.; Das, P.; Mittelman, S. *J. Antibiot.* **1992**, *45*, 444–453. (b) *Ibid.* **1992**, C-3 (correction).

(6) (a) Kobayashi, K.; Nishino, C.; Ohya, J.; Sato, S.; Mikawa, T.; Shiobara, Y.; Kodama, M. *J. Antibiot.* **1988**, *41*, 741–750. (b) Kobayashi, K.; Nishino, C.; Ohya, J.; Sato, S.; Mikawa, T.; Shiobara, Y.; Kodama, M. *Ibid.* **1988**, *41*, 502–511.

(7) (a) Hopwood, D. A.; Sherman, D. H. *Annu. Rev. Genet.* **1990**, *24*, 37–66. (b) Donadio, S.; Staver, M. J.; McAlpine, J. B.; Swanson, S. J.; Katz, L. *Science* **1991**, *252*, 675–679.

(8) (a) Westley, J. W.; Blount, J. F.; Evans, R. H., Jr.; Stempel, A.; Berger, J. *J. Antibiot.* **1974**, *27*, 597–604. (b) Cane, D. E.; Liang, T.-C.; Hasler, H. *J. Am. Chem. Soc.* **1982**, *104*, 7274–7281. (c) Ajaz, A. A.; Robinson, J.; Turner, D. L. *J. Chem. Soc. Perkin Trans. 1* **1987**, 27–36.

(9) (a) Herbert, R. B. *The Biosynthesis of Secondary Metabolites*, 2nd ed.; Chapman and Hall: London, New York, 1989; p 5, and references therein. (b) Rawn, D. R. *Biochemistry*; Neil Patterson Publ.: Carolina Biological Supply Co., Burlington, NC, 1989; p 447. (c) Samuelsson, B. *J. Am. Chem. Soc.* **1965**, *87*, 3011–3013.

(10) (a) Risley, J. M.; Van Etten, R. L. *J. Am. Chem. Soc.* **1979**, *101*, 252–253; *Ibid.* **1980**, *102*, 4609–4614, 6699–6702. (b) Vederas, J. C. *Ibid.* **1980**, *102*, 374–376. (c) Vederas, J. C.; Nakashima, T. T. *J. Chem. Soc., Chem. Commun.* **1980**, 183–185. (d) Vederas, J. C. *Can. J. Chem.* **1982**, *60*, 1637–1642.

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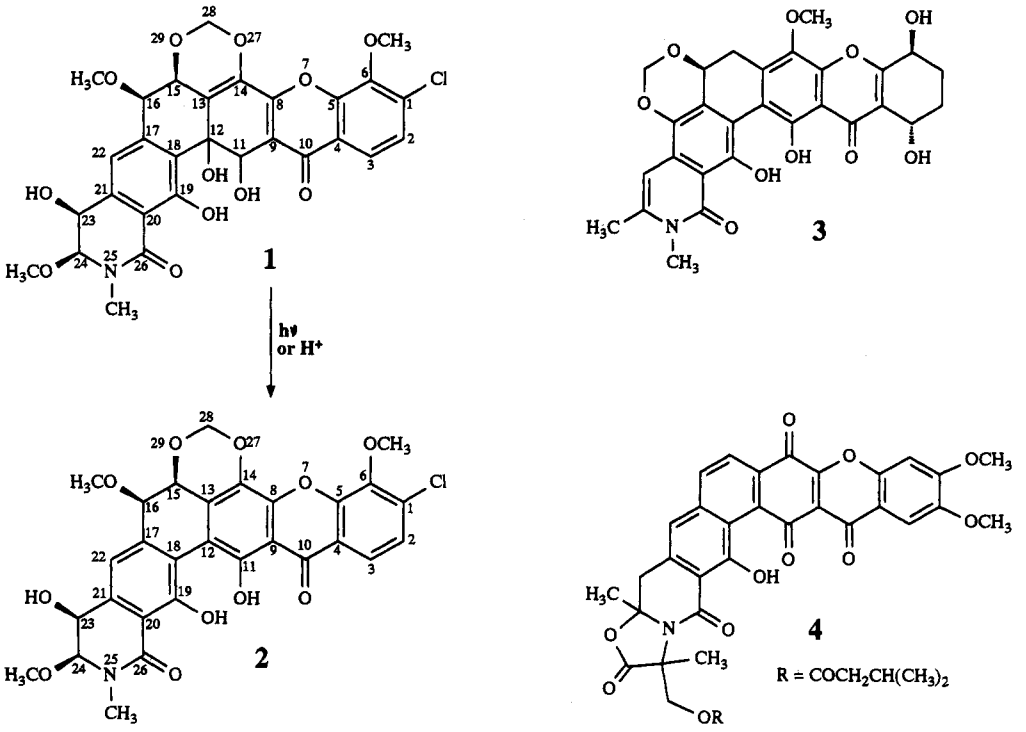
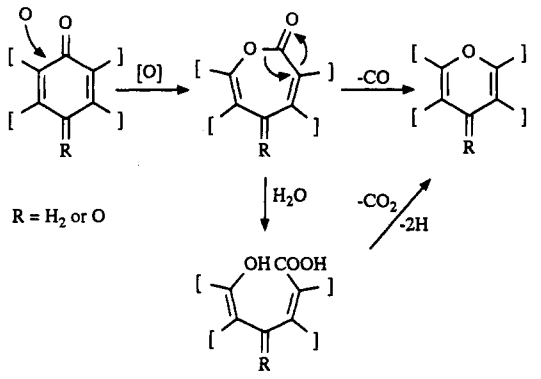


Figure 1. Conversion of lysolipin X (1) into lysolipin I (2) through UV light or acids. The numbering system used refers to the X-ray analysis publication.[3b]

Scheme 1. Mechanistic Hypotheses for the Oxidative Ring Cleavage and Xanthone Formation



features of the biosynthesis. For the formation of the xanthone structural element, a Baeyer–Villiger oxidation of a proposed quinone or anthrone intermediate, followed by lactone opening, and intramolecular OH-attack with decarboxylation can be proposed or, alternatively, a rearrangement of the lactone intermediate with decarbonylation (Scheme 1). A similar mechanism, in analogy to the findings of biosynthetic studies on the ergochromes[11a–c] and on the aflatoxins,[10c,11d,e] was proposed by Carter *et al.*[12] after the first biosynthetic investigations on a polycyclic xanthone antibiotic, simaomicin α (3). The incorporation experiments with labeled acetate showed that the entire molecular backbone derives from a single polyacetate chain which is interrupted at the xanthone ring.[12] But no experiments with ^{18}O-labeled precursors were carried out to prove the hypothesis of biosynthetic xanthone ring formation. In the second study on this class of antibiotics, however, namely on the citreamicins (4, citreamicin α), oxygen-18 experiments showed both xanthone oxygens to be derived from the polyketide building blocks (acetate),[13] a fact which is in clear contrast to the above mentioned xanthone ring formation hypothesis. This result was explained by a rearrangement of the primary carbon skeleton in agreement with the observed acetate incorporation pattern, which was different from the pattern found in 3. This mechanistic diversity in xanthone ring formation made the biosynthesis of the lysolipins particularly interesting.

Material and Methods

Fermentation: *Streptomyces violaceoniger* Tü 96[3a] was cultivated in 10 250-mL triply-baffled Erlenmeyer flasks, each containing 100 mL of a soybean–glycerol medium (2%, 2%; pH adjusted to 8.0 before autoclaving), on a rotary shaker/incubator at 250 rpm for 10 days (at 37 °C for 2 days, and then at 27 °C for another 8 days). The cultures were inoculated directly from slants.

Isolation: The entire cultures were treated with 500 mL of acetone and Celite and centrifuged (2500 rpm). The combined culture filtrate was extracted with 1 L of $CHCl_3$, the extract evaporated, and the residue dissolved in 90% MeOH, defatted by extracting with 100 mL of *n*-pentane, evaporating again, and chromatographing on a Sephadex LH 20 column (2.5 × 100 cm, $CHCl_3$/MeOH = 1:1). Yield: 10–30 mg of lysolipin mixture. Separation of the lysolipins was achieved by HPLC (RP-18 silica gel, CH_3CN/MeOH/H_2O = 4:3:3, relative retention time of lysolipin X: 6 min, and of lysolipin I: 11 min.).

Feeding experiments with labeled precursors: (a) Feeding of sodium [1-^{13}C]acetate, [2-^{13}C]acetate, and [1,2-$^{13}C_2$]acetate, respectively. Each labeled substrate (1g/L culture) was dissolved

(11) (a) Gröger, D.; Erge, D.; Franck, B.; Ohnsorge, U.; Flasch, H.; Hüper, F. *Chem. Ber.* **1968**, *101*, 1954–1969. (b) Franck, B.; Hüper, F.; Gröger, D.; Erge, D. *Ibid.* **1968**, *101*, 1970–1978. (c) Franck, B.; Hüper, F.; Gröger, D.; Erge, D. *Angew. Chem.* **1966**, *78*, 752–753. (d) Sankawa, U.; Shimada, H.; Kobayashi, T.; Ebizuka, Y.; Yamamoto, Y.; Noguchi, H.; Seto, H. *Heterocycles* **1982**, *19*, 1053–1058. (e) See also above,[8a] pp 52–53, and further references cited there.

(12) Carter, G. T.; Goodman, J. J.; Torrey, M. J.; Borders, D. B.; Gould, S. J. *J. Org. Chem.* **1989**, *54*, 4321–4323.

(13) Carter, G. T.; Borders, D. B.; Goodman, J. J.; Ashcroft, J.; Greenstein, M.; Maiese, W. M.; Pearce, C. J. *J. Chem. Soc., Perkin Trans. 1* **1991**, 2215–2219.

in 100 mL of sterile water and neutralized with 0.1 M HCl. One milliliter of this solution was added every 6 hours (in 10 portions, from 80 h until 140 h after inoculation) to each of the 10 Erlenmeyer flasks containing 100 mL each of the growing culture of *S. violaceoniger* Tü 96. (b) Feeding of [1,3-$^{13}C_2$]malonic acid, [2-^{13}C]malonic acid, and [U-$^{13}C_3$]malonic acid, respectively (each 1 g/L), as in a with 5 N NaOH as neutralization reagent. (c) Feeding of [1,3-$^{13}C_2$, $^{18}O_2$]malonic acid (1.2 g/L) as in b. To avoid an ^{18}O-exchange with the solvent, each 120 mg of the labeled material was dissolved instantly before the feeding in 10 mL of sterile water and neutralized immediately with 5 N NaOH. (d) Feeding of [$^{13}CH_3$]methionine (100 mg/L): Since experiments with unlabeled methionine had shown that this amino acid supresses the secondary metabolism of *S. violaceoniger* Tü 96, the labeled methionine (100 mg) was dissolved in 100 mL of water and added to a 1-L culture as in a. (d) Fermentation of a 1-L culture in an $^{18}O_2$-containing atmosphere: The fermentation was carried out in a closed system using a previously described apparatus.[14,15] The fermentation was started with normal air, and consumed oxygen was replaced by $^{16}O_2$ during the first 140 h. Then the fermentation atmosphere in the apparatus was replaced by a mixture of 80% N_2, and 20% oxygen (50% $^{18}O_2$, total volume: 7 L). An additional 2.5 L of 50% $^{18}O_2$ was added during the following 65 h of fermentation. For the last 25 h of fermentation, again $^{16}O_2$ was used.

Labeled compounds: Sodium [1-^{13}C]-, [2-^{13}C]-, and [1,2-$^{13}C_2$]acetate, [S-$^{13}CH_3$]methionine as well as [1,3-$^{13}C_2$]- and [2-^{13}C]-malonic acid (each 99% ^{13}C), and the $^{18}O_2$ (50%) were obtained from Cambridge Isotope Laboratories (CIL, Cambridge, MA). The [1,3-$^{13}C_2$,$^{18}O_2$]malonic acid was prepared *via* malonic acid dichloride[16] from [1,3-$^{13}C_2$]malonic acid, with dichloromethyl methyl ether and $ZnCl_2$ as the catalyst, and by hydrolysis of the dichloride with a stochiometric amount of $H_2{}^{18}O$ (97% ^{18}O, obtained from Isotec Inc., Miamisburg, OH). [U-$^{13}C_3$]Malonic acid was synthesized from K^{13}CN (99% ^{13}C, from CIL) and [1,2-$^{13}C_2$]acetic acid (99% ^{13}C, supplied by the Los Alamos Stable Isotope Resource) *via* bromoacetic acid.[17]

NMR experiments: ^1H and ^{13}C NMR spectra were recorded in $CDCl_3$ at 200, 300, or 500 MHz and at 50.3, 75.4, or 125.7 MHz, respectively. The ^1H and ^{13}C NMR signals of the lysolipins were assigned unequivocally based on chemical shift theory and on a variety of 1D and 2D heteronuclear and homonuclear correlations, such as H/D exchange, 2D-H,H-COSY and 2D-NOESY experiments for ^1H assignments, and phase-sensitive DEPT and C,H-COSY or HMQC (*h*eteronuclear *m*ultiple-*q*uantum connectivity) for the carbon multiplicities and the proton bearing carbons, respectively. The quarternary carbons were assigned by 2D long-range coupling experiments (COLOC = *co*rrelation spectroscopy *via lo*ng range *c*ouplings and HMBC = *h*eteronuclear *m*ultiple-bond connectivity) showing $^2J_{C,H}$ and $^3J_{C,H}$ long-range couplings as illustrated in Figures 2 and 3.

Results

Acetate feedings: The specific incorporation rates[18] obtained from the acetate feedings were rather low (1–2%). The experiment with [1-^{13}C]acetate showed 12 carbon atoms of **2** to be enriched, namely C-2, C-4, C-6, C-9, C-12, C-14, C-15, C-17, C-19, C-21, C-24, and C-26. The other 12 carbons (C-1, C-3, C-5, C-8, C-10, C-11, C-13, C-16, C-18, C-20, C-22, and C-23) of the molecular backbone of **2** were enriched by feeding [2-^{13}C]acetate. These findings are in agreement with the expected single chain polyketide assembly (as found also for simaomicin α, **3**).[12] The only difference compared with the biosynthesis

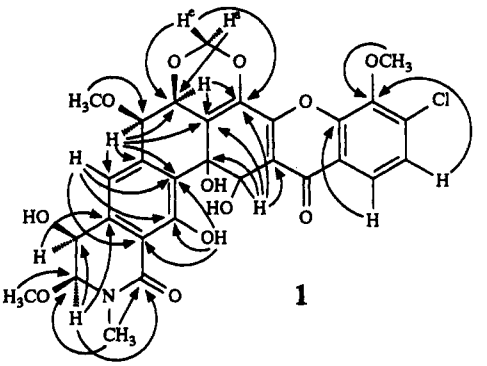


Figure 2. $^2J_{C,H}$ and $^3J_{C,H}$ Long-range couplings in lysolipin X (**1**) observed in the COLOC experiment.

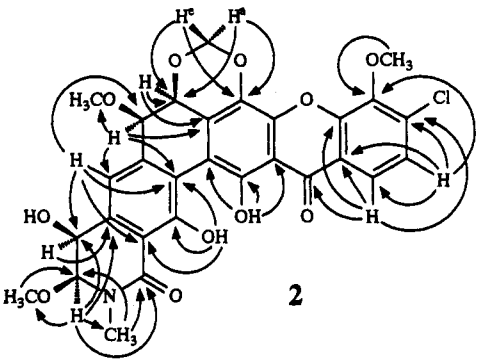


Figure 3. $^2J_{C,H}$ and $^3J_{C,H}$ Long-range couplings in lysolipin I (**2**) observed in the COLOC and the HMBC experiments.

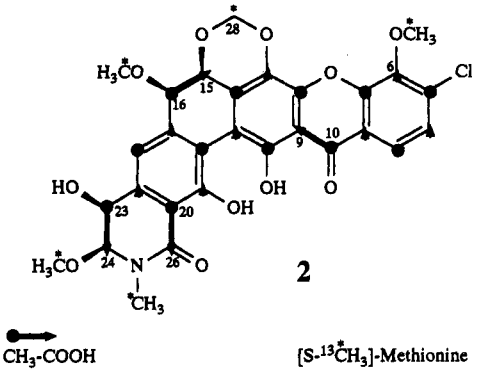


Figure 4. Results from incorporation experiments into lysolipin I (**2**) with labeled ([1-^{13}C]-, [2-^{13}C]-, and [1,2-$^{13}C_2$]-) acetate, labeled ([1,3-$^{13}C_2$] and [2-^{13}C]-) malonic acid, and methionine.

of **3** seemed to be that C-2 of the starter acetate unit was lost. The feeding with [1,2-$^{13}C_2$]acetate, however, showed a coupling between C-23 and C-24. Due to the low specific incorporation, only four intact acetate derived units could be identified unambiguously, namely C-9/C-10, C-15/C-16, C-20/C-26, and C-23/C-24 (Figure 4). Since the acetate unit C-23/C-24 is oriented in the opposite direction to the other three, in disagreement with the expected biosynthesis, a different mode of formation of the isoquinoline moiety involving an amino acid was considered. Thus, a biosynthetic relationship to the polycyclic benznaphthacenequinone antibiotics (e.g., the benanomicins and pradimicins)[19] seemed possible, since these are assembled from twelve acetate and one amino acid unit.

(14) Udvarnoki, G.; Henkel, T.; Machinek, M.; Rohr, J. *J. Org. Chem.* **1992**, *57*, 1274–1276.
(15) Vederas, J. C. In *Mycotoxins and Phytotoxins*; Steyn, P. S., Vleggaar, R., Eds.; Elsevier Science: Amsterdam, 1986; pp 97–108.
(16) Rieche, A.; Gross, H. *Chem. Ber.* **1959**, *92*, 83–91.
(17) Murray, A. W., III; Williams, D. L. *Organic Synthesis with Isotopes*; Interscience: New York, 1958; pp 780–781.
(18) Scott, A. I.; Townsend, C. A.; Okada, K.; Kajiwara, M.; Cushley, R. J.; Whitman, P. J. *J. Am. Chem. Soc.* **1974**, *96*, 8069–8080.
(19) (a) Kakushima, M.; Sawada, Y.; Nishio, M.; Tsuno, T.; Oki, T. *J. Org. Chem.* **1989**, *54*, 2536–2539. (b) Gomi, S.; Sezaki, M.; Hamada, M.; Kondo, S.; Takeuchi, T. *J. Antibiot.* **1989**, *42*, 1145–1150.

Table 1. ^1H NMR Data of the Lysolipins X (1) and I (2); δ in ppm Relative to Internal TMS in $CDCl_3$ (J in hertz)

	1[a]	2[b]
2-H	7.40 d (8.8)	7.40 d (8.7)
3-H	7.91 d (8.8)	7.96 d (8.7)
11-H	5.60 s	–
11-OH	n.o.[c]	13.00 s[d]
12-OH	n.o.[c]	–
15-H	4.95 d (2.5)	5.03 d (2.9)
16-H	4.56 d (2.5)	4.47 d (2.9)
19-OH	13.57 s[d]	13.15 s[d]
22-H	7.10 d (0.6)	7.10 s
23-H	5.03 dd (4.0, 0.6)	5.04 dd (11.9, 4.2)
23-OH	2.05 s[d,e]	2.57 d (11.9)[d]
24-H	4.74 d (4.0)	4.71 d (4.2)
28-H_2	5.32 d (5.6)	5.41 d (5.6)
28-H_e	5.59 d (5.6)	5.65 d (5.6)
6-OCH_3	4.10 s	4.17 s
16-OCH_3	3.38 s	3.35 s
24-OCH_3	3.56 s	3.55 s
NCH_3	3.32 s	3.35 s

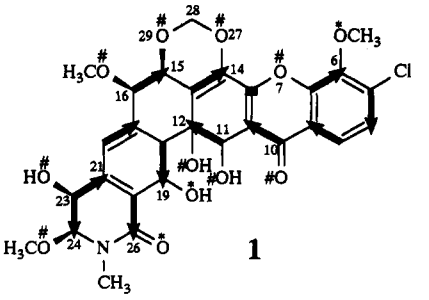
[a] At 200 MHz. [b] At 300 MHz. [c] Not observed. [d] Exchangeable with D_2O. [e] Broad.

Table 2. ^{13}C NMR Data of the Lysolipins X (1) and I (2) at 125.7 MHz in $CDCl_3$; δ in ppm Relative to Internal TMS ($^1J_{C-C}$ in hertz from samples biosynthesized from [1,2-$^{13}C_2$]acetate or [U-$^{13}C_3$]malonate)

	1[a]	2[a]
C-1	132.5 s	134.4 s (63.5)
C-2	126.2 d	125.6 d (63.7)
C-3	120.6 d	120.8 d (n.o.[b])
C-4	124.4 s	120.7 s (n.o.[b])
C-5	149.3 s	149.9 s (78.4)
C-6	145.4 s	145.2 s (78.4)
C-8	151.6 s	143.7 s –
C-9	119.5 s	108.9 s (55.8)
C-10	175.8 s	181.6 s (56.0)
C-11	65.3 d	151.7 s (76.1)
C-12	72.9 s	110.9 s (75.7)
C-13	115.4 s	127.6 s (71.1)
C-14	143.5 s	133.3 s (71.1)
C-15	72.7 d	75.4 d (42.4)
C-16	79.5 d	78.9 d (42.4)
C-17	139.2 s	139.1 s (59.9)
C-18	123.6 s	118.8 s (71.5)
C-19	159.4 s	159.0 s (71.7)
C-20	110.0 s	110.4 s (63.3)
C-21	140.0 s	139.6 s (44.2)
C-22	117.0 d	116.1 d (60.0)
C-23	67.5 d	67.8 d (44.2, 44.5)[c]
C-24	92.3 d	92.0 d (44.4)
C-26	168.1 s	168.2 s (63.5)
C-28	90.4 t	91.1 t –
6-OCH_3	61.8 q	61.8 q –
16-OCH_3	58.1 q	57.7 q –
24-OCH_3	58.4 q	58.1 q –
NCH_3	36.2 q	36.6 q –

[a] Multiplicities from the phase-sensitive DEPT experiment. [b] Not observed (signals collapse into a singlet). [c] Signal appears as quintuplet.

Malonic acid feedings: The specific incorporations[18] were clearly higher (5–10%), and the feeding experiments with [1,3-$^{13}C_2$]- and [2-^{13}C]malonic acid confirmed the results obtained with [1-^{13}C]- and [2-^{13}C]acetate, respectively. The feeding with [U-$^{13}C_3$]malonic acid confirmed that all the "acetate units" are incorporated intact and revealed the surprising result that an intact malonate unit acts as the starter moiety of the lysolipins (Table 2, Figure 5). Thus the lysolipins belong to those rare polyketides which are assembled from malonate building blocks only. Malonate starter units were previously found for tetracycline[20] and cycloheximide.[21]



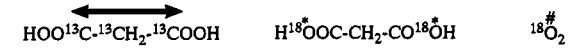


Figure 5. Results from incorporation experiments into lysolipin X (1) with labeled ([U-$^{13}C_3$]- and [1,3-$^{13}C_2{}^{18}O_2$]-) malonic acid and from the fermentation in $^{18}O_2$-containing atmosphere.

Table 3. Upfield Shifts ($\Delta\delta$ $^{13}C-^{18}O$ in ppm) Observed in Lysolipin X (1) after Feeding [1,3-$^{13}C_2{}^{18}O_2$]Malonic Acid (A) and from the Fermentation with $^{18}O_2$ (B) at 125.7 MHz in $CDCl_3$ (^{18}O Enrichments in %[a])

	A	B
C-5	–	0.03 (13)
C-6	0.01	–
C-8	–	0.03 (14)
C-10	–	0.03 (11)
C-11	–	0.01 (17)
C-12	–	0.01 (14)
C-14	–	0.02 (16)
C-15	–	0.02 (16)
C-16	–	n.o.[b]
C-19	0.01	–
C-23	–	0.02 (16)
C-24	–	0.02 (17)
C-26	0.03	–
C-28	–	0.02 (23)
6-OCH_3	0.02	–
16-OCH_3	–	0.02 (15)
24-OCH_3	–	0.03 (18)

[a] ^{18}O Enrichment = $(I_{^{13}C-^{18}O}/I_{^{13}C-^{16}O} + I_{^{13}C-^{18}O}) \times 100\%$. [b] Not observed.

[$^{13}CH_3$]Methionine feeding: The experiment (specific incorporation 5–8%) showed the expected result that the three OCH_3 groups, the NCH_3, and the methylenedioxy group (C-28) originate from methionine (Figure 4).

^{18}O-Experiments: Both $^{18}O_2$ gas and malonic acid were evaluated as sources of the oxygens in the lysolipins. To get information about the origin of the 12-OH group, the more unstable lysolipin X (1) had to be isolated after the ^{18}O feeding experiments. The usual synthesis of an ^{18}O-labeled acid[22] could not be used to prepare ^{18}O-labeled malonic acid, since heating in acidic $H_2{}^{18}O$ would lead to decarboxylation to acetic acid. The problem was circumvented by synthesizing malonic acid dichloride[16] and hydrolyzing it with 2 equiv of $H_2{}^{18}O$. The disadvantage is that one can reach only an ^{18}O enrichment of at most 50% of that of the $H_2{}^{18}O$ used.

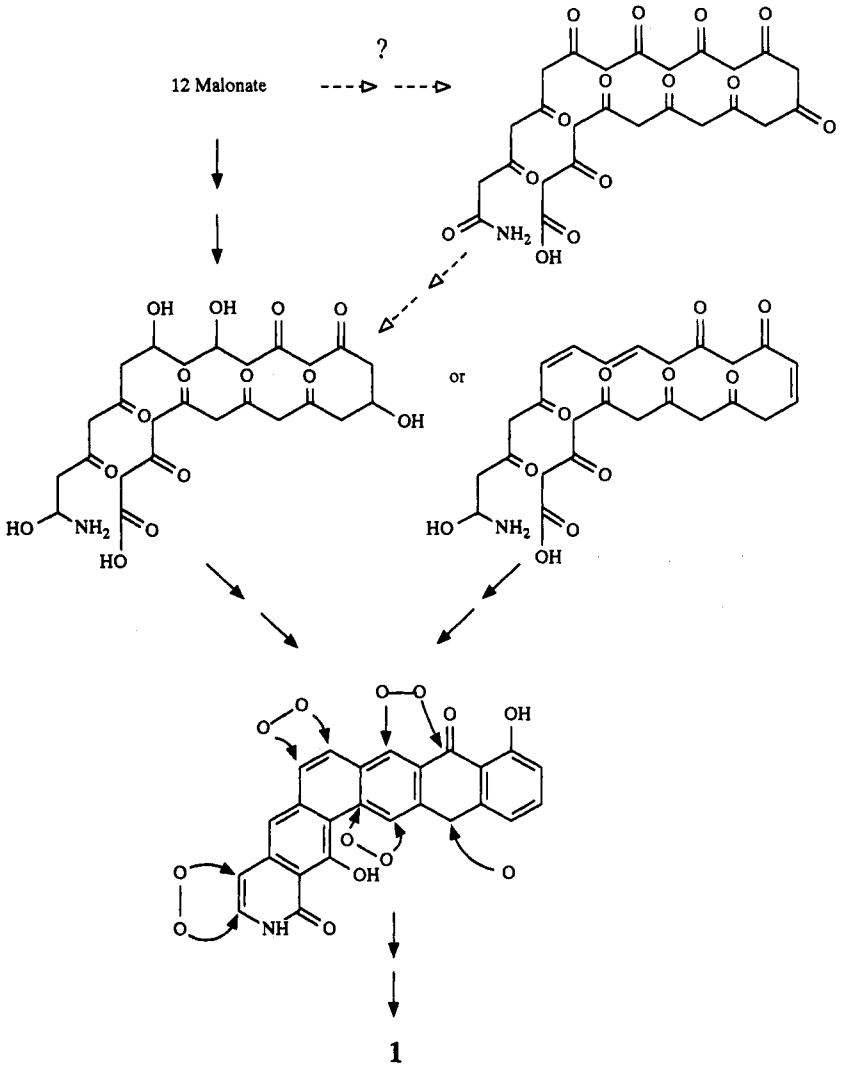
The upfield shifts observed in the proton noise decoupled ^{13}C NMR spectra of 1 from the two feeding exper-

(20) (a) Mitscher, L. A. *The Chemistry of the Tetracycline Antibiotics*; Medical Research Series; Marcel Dekker Inc.: New York, 1978; Chapter 3, Vol. 9, pp 65–90. (b) Thomas, R.; Williams, D. J. *J. Chem. Soc., Chem. Commun.* **1983**, 677–679; *Ibid.* **1985**, 802–803; and references therein.

(21) (a) Jeffs, P. W.; McWilliams, D. *J. Am. Chem. Soc.* **1981**, *103*, 6185–6192, and references therein. (b) Shimada, H.; Noguchi, H.; Iitaka, Y.; Sankawa, U. *Heterocycles* **1981**, *15*, 1141–1146.

(22) Boyer, P. D.; Koeppe, O. J.; Luchsinger, W. W. *J. Am. Chem. Soc.* **1956**, *78*, 356–357.

Scheme 2. Possible Prearomatic Reduction Steps during the Assembly of the Polyketide Chain and One of the Alternative Oxygen-Introduction Hypotheses into a Putative Lysolipin Precursor via Mono- (O) and Dioxygenases (O–O)



iments were in the expected range and indicate that only three oxygens in lysolipin X (1) are derived from the polyketide building blocks (Table 3), namely 6-O, 19-O, and 26-O. All other oxygen atoms (O-7, 10-O, 11-O, 12-O, O-27, O-29, 16-O, 23-O, and 24-O) in 1 originate from atmospheric oxygen, none from water, which is a rather surprising result (Figure 5). Even though an upfield shift on C-16 could not be observed, the origin of 16-O from molecular oxygen is clearly indicated by the upfield shift on 16-OCH_3.

The results of the feeding experiments with the ^{13}C- and ^{18}O-labeled precursors are summarized in Figures 4 and 5.

Discussion

The biosynthetic studies on the lysolipins revealed a similar biosynthesis of the carbon backbone as found for simaomicin α (3),[12] i.e., an assembly from a single polyketide chain in contrast to the findings on citreamicin α (4).[13] Besides the corrected assignments of the proton and carbon-13 NMR signals for 1 and 2[23] (Tables 1 and 2), two remarkable results should be emphasized.

First, the function of an intact malonate as a starter unit is quite unusual. In this respect 1 and 2 differ from all other known polycyclic xanthone antibiotics in that the lysolipins are biosynthesized from malonate building blocks only. By comparing the incorporations of the [1,2-$^{13}C_2$]acetate (labeling C-23/C-24 only, Figure 4) with the [U-$^{13}C_3$]malonate unit (labeling C-21/C-23/C-24, Figure 5) into the nitrogen heterocyclic moiety of the lysolipins, it is evident that the activated carbon of the malonyl CoA which is incorporated intact is attached to the ring nitrogen, whereas the CO_2-derived carbon is the one which initiates the polyketide chain. This is consistent with formation of malonamide as an intermediate; first, the nitrogen is introduced at the activated carbon and then the free carboxyl group of malonamide must undergo activation again. The examination of this hypothesis by a feeding experiment with [1,3-$^{13}C_2$,^{15}N]malonamide is currently under way. An intact malonate was also shown to be the polyketide starter unit in tetracycline[20] as well as cycloheximide[21] biosynthesis, but its orientation in tetracycline as well as in cycloheximide is reversed, i.e., the activated carbon of malonyl CoA serves as the initiator of the polyketide chain, and the CO_2-derived carbon is linked to the nitrogen of the carboxamide and the glutarimide moiety, respectively. For tetracycline, a malonamide

(23) Some of the earlier assignments[3b] were incorrect.

intermediate was also discussed as one alternative, but an incorporation experiment with [1,3-$^{14}C_2$]malonamic acid failed.

Second, the oxygen incorporations are unexpected and surprising. The finding that both xanthone oxygens originate from molecular oxygen is in agreement with the proposed mechanism shown in Scheme 1. On the basis of the results, one can speculate about the enzymes introducing the various oxygens and their mechanisms. For example, it is possible that the oxygen pairs O-29/16-O (or O-27/O-29) and 23-O/24-O are introduced into 1 *via* diooxygenases (perhaps also 11-O/O-27 or 11-O/12-O), whereas O-7, 10-O, 12-OH, and O-27 (or 16-O) are probably introduced *via* monooxygenases. For C-10, a nonenzymatic oxidation at the anthrone stage may also be considered. From the results one can also infer several prearomatic deoxygenation steps, preferably already initiated as reductions during the assembly of the polyketide chain, as depicted in Scheme 2. This is quite unusual for the polycyclic aromatic type-2 polyketides. Most of the studies give evidence for only one prearomatic deoxygenation step as was shown in recent studies on the angucyclinones deoxyrabelomycin[24] and aquayamycin,[25] but a second prearomatic deoxygenation step was also proven for the biosynthesis of PD 116740.[24] Thus we will study the origin of the oxygen atoms of other polycyclic aromatic polyketide antibiotics in order to seek more evidence for prearomatic deoxygenation steps. Our further investigations into the biosynthesis of the lysolipins will be aimed at identifying aromatic (quinone) intermediates (e.g., see Scheme 2) accumulated by blocked mutants obtained by mutagen treatment of *Streptomyces violaceoniger* (Tü 96). The discovery of such mutants should be facilitated by the fact that the expected aromatic products should be more intensely colored than lysolipin; hence one can select for darker colonies.

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(24) Gould, S. J.; Cheng, X.-C.; Halley, K. A. *J. Am. Chem. Soc.* **1992**, *114*, 10066–10068.

(25) Rohr, J.; Schönewolf, M.; Udvarnoki, G.; Eckardt, K.; Schumann, G.; Wagner, C.; Beale, J. M.; Sorey, S. D. *J. Org. Chem.* **1993**, *58*, 2547–2551.