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Parvibaculum lavamentivorans DS-1^T Degrades Centrally Substituted Congeners of Commercial Linear Alkylbenzenesulfonate to Sulfophenyl Carboxylates and Sulfophenyl Dicarboxylates$^{\nabla}$†

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Commercial linear alkylbenzenesulfonate (LAS) contains 20 congeners of linear alkanes (C_{10} to C_{13}) substituted subterminally with the 4-sulfophenyl moiety in any position from lateral to central. *Parvibaculum lavamentivorans* DS-1^T degrades each of eight laterally substituted congeners [e.g., 2-(4-sulfophenyl)decane (2-C10-LAS); herein, compounds are named systematically by chain length (e.g., C_{10}) and by the position of the substituent on the chain (e.g., position 2)] to a major sulfophenyl carboxylate [SPC; here 3-(4-sulfophenyl)butyrate (3-C4-SPC)] and two minor products, namely, the α,β-unsaturated SPC (SPC-2H, here 3-C4-SPC-2H) and the SPC+2C (here 5-C6-SPC) species (D. Schleheck, T. P. Knepper, K. Fischer, and A. M. Cook, Appl. Environ. Microbiol. 70:4053–4063). The degradation of centrally substituted congeners by strain DS-1 was examined in this work. 5-C10-LAS yielded not only the predicted 4-C8-SPC, 4-C8-SPC-2H, and 6-C10-SPC (about 70% of products) but also sulfophenyl dicarboxylates (SPdC), i.e., C6-, C8-, and C10-SPdC. These were identified by electrospray ionization-mass spectrometry (ESI-MS) after separation by high-pressure liquid chromatography (HPLC). ESI ion-trap MS and ESI-time of flight-MS were used to confirm the identities of key intermediates. Different mixtures of congeners obtained by separation of commercial LAS by HPLC were degraded, and the degradative products were compared. If a congener carried the sulfophenyl substituent on the 5, 6, or 7 position, SPdCs were formed as well as SPC, SPC-2H, and SPC+2C, whereas the substituent on the 2, 3, or 4 position yielded only SPC, SPC-2H, and SPC+2C. Some 50 products were generated from the 20 LAS congeners: 11 major SPCs, each with an SPC-2H and an SPC+2C (i.e., 33 SPC and SPC-2H species), and about 17 SPdC species. A large array of compounds, many in low quantities, is thus generated by *P. lavamentivorans* DS-1 during the degradation of commercial LAS.

The major laundry surfactant in worldwide use is commercial linear alkylbenzenesulfonate (LAS) (6). The preparation in Europe is nominally a mixture of 20 congeners, which are C_{10}-to-C_{13} *n*-alkanes with a subterminal 4-sulfophenyl substituent (e.g., reference 8). Most congeners are chiral (18). Commercial LAS thus represents 38 individual compounds (see also references 2 and 7). We name these compounds systematically by their chain length (e.g., C_{10}) and by the position of the substituent on the chain (e.g., position 2), so 2-C10-LAS represents 2-(4-sulfophenyl)decane (Fig. 1).

LAS is fully biodegradable in oxic environments, as first demonstrated in 1957 (reference 11; cf. references 17 and 18). Degradation involves microbial communities, which can now be examined in defined mixed cultures (5, 14) and whose transient extracellular intermediates include sulfophenyl carboxylates (SPCs) (e.g., references 17 and 18), sulfophenyl dicarboxylates (SPdCs) (e.g., references 1 and 3), and α,β-unsaturated SPCs (SPC-2H) (3, 4, 14). We name SPCs systematically by their chain length (e.g., C_{10}) and by the position of the aromatic substituent relative to the carboxyl group (e.g., position 9), so 9-C10-SPC represents 9-(4-sulfophenyl)decanoate (Fig. 1). Similar nomenclature is used for the SPC-2Hs and SPdCs (Fig. 1).

Parvibaculum lavamentivorans DS-1^T (13, 16) is apparently a representative member of many heterotrophic, LAS-degrading communities (3), in which it catalyzes the first steps of LAS degradation (14). LAS is activated by ω-oxygenation of at least one terminal methyl group, a reaction catalyzed by a putative novel heme monooxygenase (12), and oxidized to the corresponding SPC (3), which is subject to β-oxidation (3, 14), as predicted previously (e.g., references 17 and 18). β-Oxidation ceases at a distance of 3 or 4 carbon atoms from the 4-sulfophenyl substituent (14), and a wide range of SPC (C_4 to C_9) (the major product in each case tested), SPC-2H, and SPdC species is excreted (3, 14). The mixture of those products from commercial LAS is termed SP(d)C.

Strain DS-1 generates several SPC-like species from any one LAS congener (3, 14), a fact which was first recognized when single LAS congeners (e.g., 2-C10-LAS in Fig. 1) were supplied to strain DS-1, and products could be identified by high-pressure liquid chromatography coupled to an electrospray ionization mass spectrometer (HPLC-ESI-MS). Every

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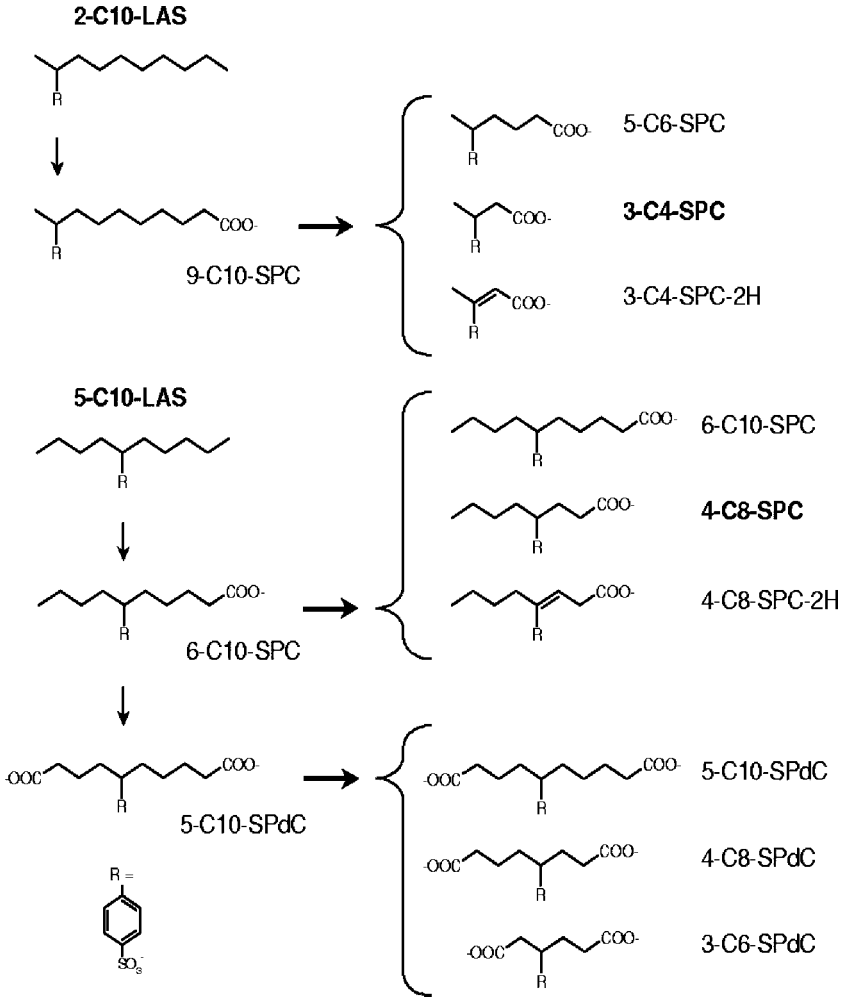

FIG. 1. SPCs and SPC-2H generated during growth of *P. lavamentivorans* DS-1^T with a laterally substituted LAS congener (2-C10-LAS) and SPCs, SPC-2H, and SPdCs generated during growth with a centrally substituted LAS congener (5-C10-LAS). The LAS congeners are transformed to SPCs and SPdCs via ω-oxygenation(s) and ω-oxidation. The intermediates, presumably as coenzyme A thioesters, are subject to β-oxidation and presumably deesterification. SPCs, SPC-2Hs, and SPdCs are excreted into the culture fluid during growth. The set of SPCs (i.e., SPC, SPC-2H, and SPC+2C) generated from 2-C10-LAS was identified in previous work (14), and the set of SPCs and SPdCs from 5-C10-LAS was identified in this work (see text). The SPCs which are excreted in major amounts (major SPC) are indicated in bold letters. The SPC-2H species are drawn as inferred from their UV spectra (see text).

LAS congener was degraded to three compounds. The major product was an SPC (e.g., 3-C4-SPC from 2-C10-LAS) (Fig. 1); the minor products were the corresponding SPC-2H and SPC+2C (3-C4-SPC-2H and 5-C6-SPC, respectively) (Fig. 1). The latter compounds result from α,β-desaturation of 3-C4-SPC (to 3-C4-SPC-2H) and from an earlier round of β-oxidation (SPC+2C).

The data from degradation of single, laterally substituted LAS congeners (14) (as opposed to centrally substituted congeners) allowed the prediction that 11 major SPCs are generated from the 20 LAS congeners: 1 C4-SPC and 2 each of the C5-, C6-, C7-, C8-, and C9-SPCs. Several of these SPCs were thoroughly identified. The generation of SPdCs from commercial LAS as observed by Dong et al. (3) was thus assumed to result from the degradation of centrally substituted LAS congeners present in the mixture.

A major analytical problem in the analysis of intermediates of the degradation of LAS is to determine the position of the sulfophenyl substituent on the alkyl chain (e.g., references 4 and 14). Ion-trap methodology (HPLC-ESI-IT-MS), used to acquire product ion spectra (MS^2), now provides a convenient tool to discriminate positional isomers of LAS (and of its metabolites), because diagnostic fragment ions can be generated by cleavage of the alkyl chain on each side of the sulfophenyl derivative (9). A different problem was the confirmation of the identity of the SPC-2Hs. A precise molecular weight was needed and can now be supplied by time of flight detectors (HPLC-ESI-TOF-MS).

We now confirm the prediction that 11 major SPCs are generated from commercial LAS by observing these previously unknown compounds. The corresponding SPC-2Hs and SPC+2Cs are also detected. SPdCs are shown to be generated from the degradation of centrally substituted LAS congeners. These data allow for the prediction of all SPC, SPC-2H, SPC+2C, and SPdC species generated from commercial LAS by strain DS-1.

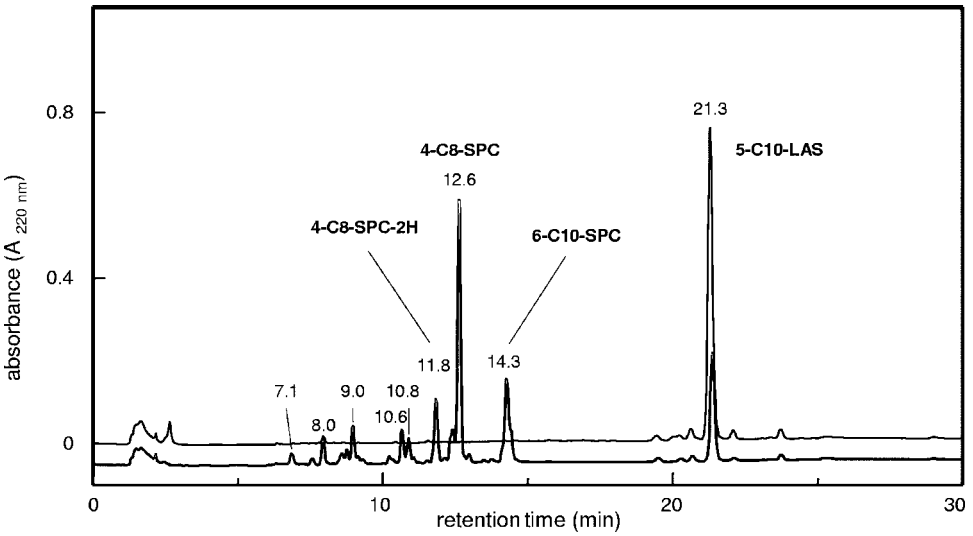

FIG. 2. HPLC-UV chromatograms of 5-C10-LAS in salts medium and of products detected after the growth of strain DS-1 with that LAS congener. The separation was done with gradient system I. The upper curve represents the LAS preparation before growth of strain DS-1^T; the 5-C10-LAS present was identified by HPLC-ESI-MS. The lower curve represents the SPC and SPdC products generated after growth. The retention times (min) of the largest product peaks are shown, and some identifications are indicated (see also Table 1).

MATERIALS AND METHODS

Materials. The commercial LAS in use was Marlon A350 (13). 5-C10-LAS (>93%) was a gift from César Bengoechea (Petresa, San Roque, Cádiz, Spain). The sources of 2-C10-LAS, 2-C11-LAS, 2-C12-LAS, and 3-C12-LAS and the nature of impurities in some samples are described elsewhere (3, 14). Commercial LAS was partially separated by semipreparative HPLC (gradient system I; see below) (14) to give one major congener per fraction. In a first step, the C_{10}, C_{11}, C_{12}, or C_{13} homologues were selected as a subgroup. Each subgroup, in which there was poor separation of isomers, was fractionated further. In most cases, sets of isomers, each with a different dominant congener, were obtained. Each fraction was subjected to treatment in a Rotavap to remove the acetonitrile and then desalted and concentrated by solid-phase extraction (10, 14).

Analytical methods. Gradient system I (0.11 M perchlorate-acetonitrile; reference 13) was used for routine reversed-phase HPLC coupled to detection with a diode array detector (HPLC-UV). Gradient system II was used for HPLC-ESI-MS separations with the detector operating in the negative ion mode; the mobile phase included the volatile ion pair reagent acetic acid-triethanolamine (5 mM). SPC, SPC-2H, and SPdC species were tentatively identified by the *m/z* value of the deprotonated molecular ion, and the identity was confirmed by specific fragmentation patterns (4). The HPLC-ESI-MS chromatograms were from analyses with programs that scanned for deprotonated molecular ions ($[M\text{-H}]^-$) of SPCs (C_4 to C_{13}; *m/z* values of 243, 257, 271, 285, 299, 313, 327, 341, 355, and 369) and SPC-2Hs (C_4 to C_{13}; *m/z* values of 241, 255, 269, 283, 297, 311, 325, 339, 353, and 367) in a first run and of SPdCs (C_4 to C_{13}; *m/z* values of 273, 287, 301, 315, 329, 343, 357, 371, 385, and 399) in a second run; separate chromatograms were used to reduce the complexity of the data sets. No authentic standard for SPdC was available.

HPLC-ESI-IT-MS involved a ThermoFinnigan system. It comprised a Surveyor quaternary pump, a Surveyor autosampler, and an LCQ Advantage ion trap MS equipped with an ESI interface. Separations (gradient system III) of LAS, SPC (≥C6), and SPdC (≥C8) were achieved on a Thermo Hypersil-Keystone BetaBasic-18 column (100 by 2.1 mm; 3-μm particle size) equipped with a guard column (10 by 2.1 mm) of the same packing material. The mobile phases were water acidified with 0.3% formic acid (A) and acetonitrile (B). The gradient program was initiated with 95% A at a flow rate of 200 μl min^{-1}, and after 1 min the portion of A was decreased linearly to 5% over 9.0 min and maintained there for 3.0 min. The initial mobile-phase composition was restored within 0.5 min and maintained for column regeneration for 4.5 min (total run time of 18.0 min); the injection volume was 10 μl (no waste injection). The spray voltage of the ESI interface was −5 kV with a current of 80 μA. The sheath gas flow rate was 30 liter min^{-1}, and the capillary was maintained at 300°C. For MS^2 experiments, the mass width for isolation of precursor ions was 1.5 Da. The relative collision energy was set at 50 to 55% (arbitrary units). The sample of SP(d)C from 5-C10-LAS was desalted by semipreparative HPLC (15) and concentrated to dryness under vacuum (Speedvac). This material (about 1 mg) was dissolved in 1 ml of methanol, from which 100-μl portions were diluted to 1,000 μl with nanopure water prior to injection.

ESI-TOF-MS analyses were done on an LC-ESI-*oa*-TOF MS (Agilent, Santa Clara, CA) equipped with a dual sprayer ESI source for automatic introduction of calibrant and reference solutions. The instrument was run in the negative ion mode at an ion spray voltage applied to the capillary of −3.5 kV, a fragmentor voltage of −120 V, and a skimmer voltage of −60 V. The temperature of the drying gas (flow rate, 12 liter min^{-1}) was held at 350°C. Calibrant masses were simultaneously introduced via the dual sprayer ESI interface.

Degradation of LAS by *P. lavamentivorans* DS-1^T. Salts medium with a solid support (1 mg glass particles ml^{-1}) for biofilm formation was supplemented with LAS to 0.2 mM and inoculated with *P. lavamentivorans* DS-1^T (DSM 13023^T is NCIMB 13966^T) (16). The degradation of LAS during growth was assayed as the disappearance of foam (16). Samples were taken from the culture medium before and after growth and analyzed by HPLC-UV and HPLC-ESI-MS.

RESULTS AND DISCUSSION

SPC and SPdC generated during degradation of 5-C10-LAS. *P. lavamentivorans* DS-1^T grew reproducibly with 1 mM 2-C10-LAS in salts medium supplemented with glass particles (16). There was no growth with 1 mM 5-C10-LAS, but if the concentration was ≤0.2 mM, rapid growth was obtained. We infer that 5-C10-LAS (with other centrally substituted LAS congeners) is more toxic than the laterally substituted congeners, so the routine total concentration of LAS in growth media in this work was 0.2 mM.

HPLC-UV analysis (gradient system I) of samples taken before and after growth of strain DS-1 with 5-C10-LAS (Fig. 2) showed the formation of three major products of different intensities (peaks at 11.8, 12.6, and 14.3 min), which represented SPCs and an SPC-2H (see below), and of several minor products (peaks at retention times of <11.0 min), which largely represented SPdCs (see below). All products had UV spectra typical of SPCs (not shown), with the exception of the major peak at 11.8 min (see below). Elution profiles with three major

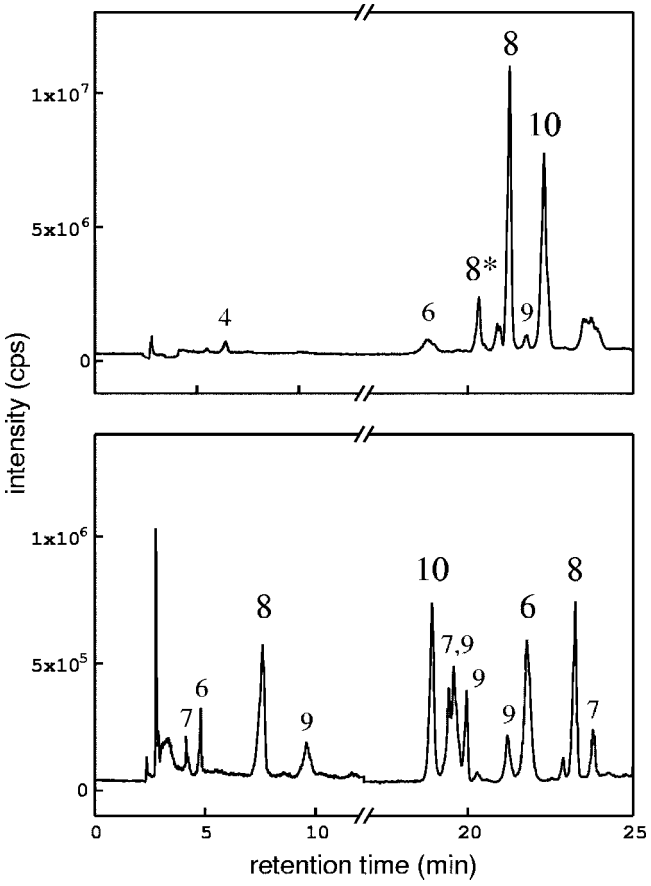

FIG. 3. HPLC-ESI-MS chromatograms of compounds in culture medium after growth of strain DS-1 with 5-C10-LAS. The chromatograms resulted from scanning for deprotonated molecular ions of SPC and SPC-2H (upper chromatogram) and SPdC (lower chromatogram). The numbers above the peaks give the chain lengths of the compound. A mass signal corresponding to an SPC-2H species is marked with an asterisk.

products at different intensities were obtained from the same sample by HPLC-ESI-MS (gradient system II) when scanned for molecular ions corresponding to SPCs and SPC-2Hs (Fig. 3). The mass signals obtained could be assigned to the major peaks (of the same relative intensities and orders of elution) observed by HPLC-UV with gradient system I separation (Fig. 2). The most intense peak at a 12.6-min retention time represented a C8-SPC; the second major peak, at 14.3 min, was a C10-SPC; the peak at 11.8 min showed a mass signal corresponding to C8-SPC-2H in terms of both the UV spectrum and the relative intensity of the peak. The traces of other SPC species detected (Fig. 3) were attributed to the degradation of one impurity (2-C10-LAS) in the preparation to 3-C4-SPC and 5-C6-SPC (8.0 and 10.6 min in Fig. 2, respectively; authentic material was available from earlier work [14]). Other impurities in the LAS were apparently not degraded (Fig. 2); nevertheless, a C9-SPC of unknown provenance (Fig. 3) was detected in low amounts.

We concluded that one portion of the products from the centrally substituted 5-C10-LAS corresponded to those obtained from the laterally substituted LAS congeners, namely, a major SPC, a minor SPC, and an SPC-2H (see the introduction), which together represented about 73% of the total products (retention times of 6.8 to 14.5 min in Fig. 2). The major SPC (12.6 min in Fig. 2) was a C8-SPC (cf. reference 14), the minor SPC (SPC+2C) was a C10-SPC (14.3 min in Fig. 2), and the SPC-2H was a C8-SPC-2H (11.8 min in Fig. 2) (structures are shown in Fig. 1).

Fragmentation patterns produced by HPLC-ESI-IT-MS were used to determine positions of phenylsulfonate substitution on the SPC and SPC-2H metabolites. 5-C10-LAS (m/z = 297 $[M\text{-H}]^-$) yielded two fragments (m/z = 226 and 240) corresponding to radical anions formed from the loss of either the C_5 alkyl side chain (m/z = 71) or the C_4 alkyl side chain (m/z = 57) and the specific signal for 4-styrenesulfonate (m/z = 183 [reference 4]) (not shown). The major SPC (m/z = 299 $[M\text{-H}]^-$) (Fig. 4A) was identified as 4-C8-SPC by observing the radical anion (m/z = 226) formed by the loss of the C3 carboxylate (m/z = 73) and the 4-styrenesulfonate fragment (m/z = 183); the fragment ion detected at an m/z value of 239 corresponded to the loss of acetic acid (m/z = 60), giving rise to a double bond standing in conjugation to the aromatic ring. The identity of the C10-SPC (m/z = 327 $[M\text{-H}]^-$) was confirmed to be 6-C10-SPC when fragments which corresponded to radical anions formed by (i) the loss of the C_5 carboxylate side chain (m/z = 101) to yield a fragment with an m/z value of 226 and (ii) the loss of the C_4 alkyl side chain (m/z = 57) to yield a fragment with an m/z value of 270 were also detected with the specific 4-styrenesulfonate fragment (m/z = 183) (not shown). The identity of putative 4-C8-SPC-2H (m/z = 297 $[M\text{-H}]^-$) was confirmed by observing the species (m/z = 253) formed by the loss of CO_2 and the radical anion (m/z = 240) formed by loss of the C_4 alkyl side chain: no fragment corresponding to 4-styrenesulfonate was detected from the SPC-2H, as reported previously (reference 4; see also reference 9) (data not shown).

The identification of 6-C10-SPC confirmed ω-oxygenation (and further oxidation) of 5-C10-LAS to the SPC. β-Oxidation was confirmed as the major mechanism of chain shortening by the identification of 4-C8-SPC (the product of the first round) and especially of 4-C8-SPC-2H (the first intermediate of the second round) (discussed in references 3 and 14). The argument is strengthened by the identification of corresponding SPdCs, which were also characterized (see below).

The generation of 3-C8-SPC (peak at 13.0 min in Fig. 2; see also below) from 5-C10-LAS was detectable, but it was a trace product. This indicated that the (first) ω-oxygenation proceeded almost exclusively at the longer C_5 alkyl side chain of 5-C10-LAS, yielding 6-C10-SPC and then 4-C8-SPC as major SPCs after β-oxidation (structures are shown in Fig. 1). We presume that this observation, which is a version of "Swisher's distance principle" (paraphrased "the longest free alkyl chain is attacked first") (18), is generally valid (see below).

The separation of SPdCs (Fig. 3) shows peaks with intensities of about 10% of those obtained for SPCs (Fig. 3): this is in rough agreement with the smaller peaks (7 to 11 min) in Fig. 2. The distribution of the tentatively identified peaks for SPdCs (Fig. 3) would appear anomalous, firstly because there are many more similarly sized signals than are indicated in Fig. 2 and secondly because the signals for C6- to C10-SPdC in the range of 5 to 18 min would appear to recur from 19 to >25

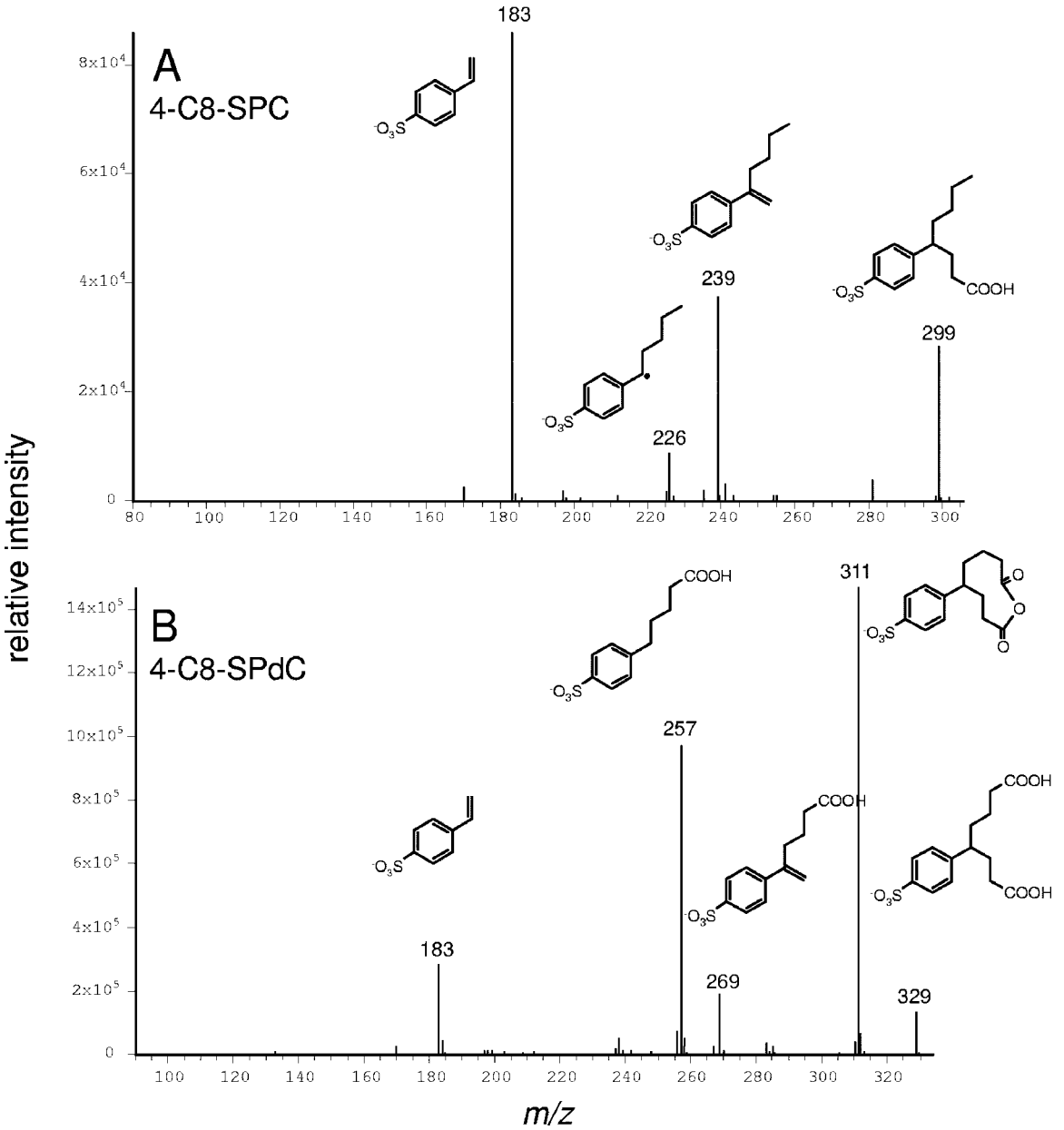

FIG. 4. HPLC-ESI-IT-MS^2 spectra (negative ion mode) of the deprotonated molecular ions of 4-C8-SPC (A) and 4-C8-SPdC (B). The compounds were separated on gradient system III. The structures assigned to the major fragment ions are shown in the spectra. Radical anions with an unpaired electron on the benzylic carbon are likely to be stabilized through delocalization over the aromatic ring.

min. We presume that gradient system II is not robust when separating the tribasic SPdCs in HPLC-ESI-MS, possibly as a function of the ion pair reagent.

The presence and identity of 4-C8-SPdC (m/z = 329 $[M\text{-H}]^-$) (Fig. 4B) were confirmed by HPLC-ESI-IT-MS when fragments corresponding to the loss of H_2O (m/z = 311), presumably due to the formation of a cyclic anhydride and to the loss of the C_3 carboxylate side chain (m/z = 257), were observed. The elimination of acetic acid to give a styrene-like fragment (m/z = 269) corresponded to a fragment from 4-C8-SPC (see above). The specific 4-styrenesulfonate fragment was also observed. SPdCs with chain lengths of $<C_8$ were not detected by HPLC-ESI-IT-MS, presumably due to weak interaction with the stationary phase. 5-C10-SPdC (m/z = 357 $[M\text{-H}]^-$) (not shown) was identified by observing a fragment representing the cyclic anhydride (m/z = 339), while decarboxylation of the deprotonated molecule yielded a fragment ion with an m/z value of 313. The product ion detected at an m/z value of 256 was attributed to the loss of the C_5 carboxylate side chain, leading to a radical anion in analogy to the fragmentation of 6-C10-SPC. The 4-styrenesulfonate fragment was also detected.

The formation of C10-SPdC confirmed that the ω-oxygenation of the second, terminal methyl group of 5-C10-LAS oc-

TABLE 1. SP(d)C species observed after growth of *P. lavamentivorans* DS-1^T with LAS congener(s) and their retention times, mass signals, identities, and origins[a]

Substrate	Products		Interpretation	Identity/origin
	Retention time (min)	Mass signal $[M\text{-H}]^-$ (m/z)		
5-C10-LAS[c]	7.1	301	C6	SPdC from 5-C10-LAS
	9.0	329	C8	SPdC from 5-C10-LAS
	10.8	357	C10	SPdC from 5-C10-LAS
	11.8[b]	297	4-C8-SPC-2H[c]	SPC-2H from 5-C10-LAS
	12.6	299	4-C8-SPC[c]	major SPC from 5-C10-LAS
	14.3	327	6-C10-SPC[c]	SPC+2C from 5-C10-LAS
6-C12-LAS + 5-C12-LAS	7.1	301	C6	SPdC from 6- and 5-C12-LAS
	9.0	329	C8	SPdC from 6- and 5-C12-LAS
	10.8	357	C10	SPdC from 6- and 5-C12-LAS
	12.6[d]	385	C12	SPdC from 6- and 5-C12-LAS
	12.6	299	4-C8-SPC	Major SPC from 5-C12-LAS
	13.0	299	3-C8-SPC	Major SPC from 6-C12-LAS
	14.3–14.4	327	C10-SPCs	SPC+2Cs
	16.2	355	C12-SPC	SPC+4C
7-C13-LAS + 6-C13-LAS + 5-C13-LAS	5.1	287	C5	SPdC from 7-, 6-, and 5-C13-LAS
	7.9	315	C7	SPdC from 7-, 6-, and 5-C13-LAS
	10.0–10.2	343	C9	SPdC from 7-, 6-, and 5-C13-LAS
	11.9[d]	371	C11	SPdC from 7-, 6-, and 5-C13-LAS
	13.2	399	C13	SPdC from 7-, 6-, and 5-C13-LAS
	11.9	285	3-C7-SPC	Major SPC from 5-C13-LAS
	13.5–13.6	313	C9-SPCs	Major SPCs from 7- and 6-C13-LAS
Major SPCs from other LAS congeners	8.0[e]	243	3-C4-SPC	From 2-C12-LAS
	9.2[e]	257	4-C5-SPC	From 2-C13-LAS
	9.3	257	3-C5-SPC	From 3-C13-LAS
	10.4[e]	271	4-C6-SPC	From 3-C12-LAS
	10.7	271	3-C6-SPC	From 4-C12-LAS
	11.5	285	4-C7-SPC	From 4-C13-LAS

[a] Either a single centrally substituted LAS congener (5-C10-LAS [cf. Fig. 2]) or mixtures of congeners (C12- or C13-LAS [see Fig. S1, S2, and S3 in the supplemental material]) were used. Shown are their retention times as determined by HPLC-UV (gradient system I), their corresponding signals as determined by HPLC-ESI-MS (gradient system II), and their identities and origins when the datasets were interpreted by following the logic of the degradation principle, ω-oxygenations, and β-oxidation (cf. Fig. 1).

[b] This peak showed a UV spectrum typical of SPC-2H and different from that of SPC.

[c] Identification was confirmed by fragmentation patterns (see the text) obtained from MS after HPLC, ESI, and fragmentation in an ion trap (ESI-IT-MS).

[d] This minor peak coeluted with a major SPC by HPLC-UV (gradient system I).

[e] This major SPC was identified by coelution with authentic material (see the text).

curs, i.e., on the C_4 side chain of 6-C10-SPC (structures are shown in Fig. 1), and that this ω-oxygenation can occur before any β-oxidation occurs. β-Oxidation led to the formation of 4-C8-SPdC and to the trace of putative 3-C6-SPdC (Fig. 3). Given that β-oxidation usually stops at least 3 carbon atoms distant from the sulfophenyl substituent (reference 14; see also above), each carboxylate side chain generated by ω-oxygenation was subject to β-oxidation.

Both C9-SPdC and C7-SPdC were detected (Fig. 3). These products, and possibly the C9-SPC observed above, presumably arose by the often-mentioned but as yet undefined α-oxidation of LAS (or possibly of an SPC), which is seen and discussed elsewhere (14).

The UV spectrum of 4-C8-SPC-2H (maximum at 264 nm) was similar to those of other SPC-2Hs, and it differed from the UV spectrum of SPCs (e.g., maximum at 221 nm for 4-C8-SPC) (14). This shift to longer wavelengths, and the implied wide delocalization of π-electrons compared to that for SPC, led us to draw the structure with a Δ-3 double bond (Fig. 1) rather than with the Δ-2 double bond expected of β-oxidation (compare with the adjacent 3-C4-SPC-2H in Fig. 1). The fragmentation of a β,γ-unsaturated 4-C8-SPC in an HPLC-ESI-IT-MS spectrum can be predicted to yield ions with m/z values of 253 and 240, coincident with those from an α,β-unsaturated C8-SPC. However, cleavage of the conjugated double bond at the benzylic carbon in a β,γ-unsaturated C8-SPC—required to explain the formation of the m/z 240 fragment (loss of carboxylate side chain [see above])—seems unlikely. More of this compound, and of the other SPC-2Hs, must be generated to establish structures and to determine whether an isomerase is present to reposition double bonds (14).

SPCs and SPdCs generated from C12-LAS and from C13-LAS. 5-C10-LAS was unique in being available to us. All other centrally substituted congeners had to be separated from commercial LAS by semipreparative HPLC, which has poor resolution and some tailing (illustrated in reference 12). We could thus separate the sets of homologues (C_{10}, C_{11}, C_{12}, C_{13}), but at best we could obtain fractions containing two congeners, e.g., mainly 6-C12-LAS, mainly 5-C12-LAS, mainly 4-C12-LAS, or mainly 3-C12-LAS, always with several or many other congeners. There followed degradative experiments whose products were examined by HPLC-UV and HPLC-ESI-MS, and the series were compared. The patterns of products and the amounts formed over the series allowed hypotheses to be generated; the validity of these hypotheses could be evaluated when known products appeared in the series (see Electronic

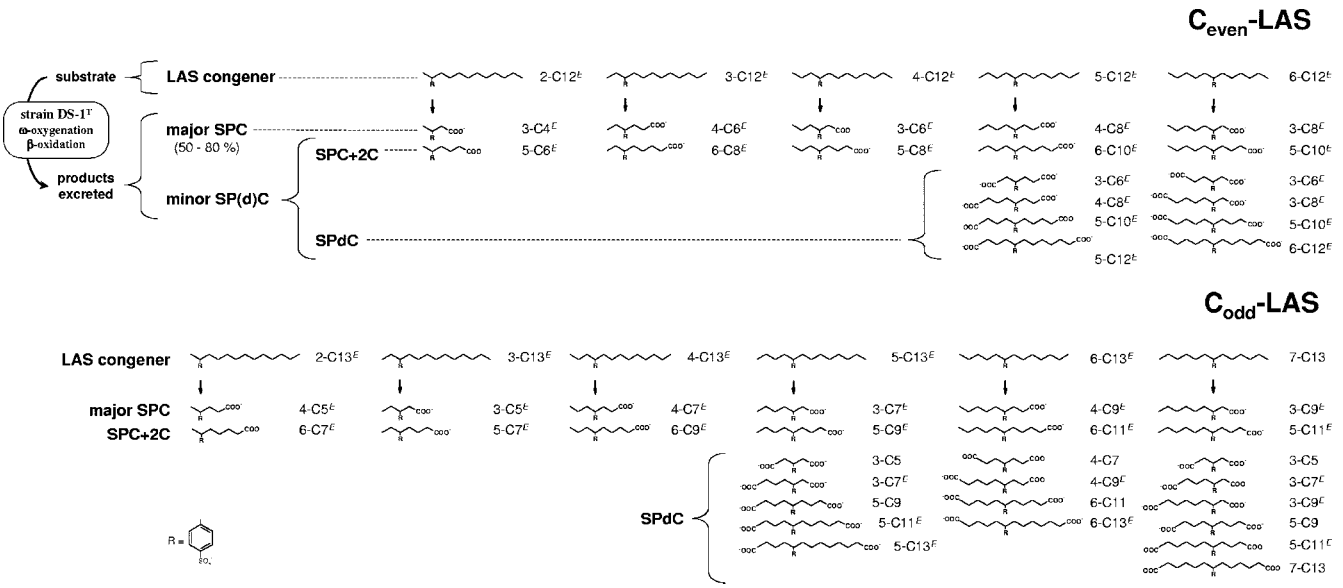

FIG. 5. Diagrammatic representation of the longest LAS congeners present in European commercial LAS, with the SPCs, and, if appropriate, SPdCs. The upper panel shows C12-LAS species (whose products include and expand on the products from C10-LAS species), and the lower panel shows C13-LAS species (whose products include and expand on those from the C11-LAS species). For simplification, the corresponding SPC-2H species, which emerge with each major SPC (Fig. 1), are not included in this diagram. The SPCs generated from pure 2-C12-LAS, 3-C12-LAS, and 2-C11-LAS (from 2-C13-LAS in this diagram) were identified in previous work (13, 14). SPCs and SPdCs generated from pure 5-C10-LAS (from 5-C12-LAS in this diagram) were identified in this work. The products generated from all other C12- and C13-LAS congeners were identified by using fractions of commercial LAS which were strongly enriched in individual C12- and C13-LAS congeners (see Electronic Supplementary Data and Fig. S1, S2, and S3 in the supplemental material). *E*, racemic mixture of enantiomers.

Supplementary Data and Fig. S1, S2, and S3 in the supplemental material). Thus, 4-C8-SPC and 6-C10-SPC were formed from 5-C12-LAS, as they were from 5-C10-LAS (see above), as predicted from earlier work (14). The other standards included 4-C6-SPC formed from 3-C12-LAS, 4-C5-SPC formed from 2-C11-LAS, and 3-C4-SPC formed from 2-C10-LAS (14). The results of these experiments are summarized in Table 1 and Fig. 5.

Identification by ESI-TOF-MS of an SPC-2H species. The structural characterization of the metabolite 3-C4-SPC-2H was achieved by LC-ESI-TOF analysis (not shown). The molecular ion observed was conclusive, with the deprotonated ion at *m/z* 241.0186 having a deviation of only 4.07 ppm from the exact mass (241.0176) calculated for $C_{10}H_9O_5S$ ($[M\text{-}H]^+$). In addition to the high mass accuracy, the observed isotope ratio of ^{12}C to ^{13}C and the fragmentation pattern also fit perfectly to the proposed structure (not shown).

Conclusions. A new understanding of the degradative pathway of LAS has emerged through the availability of a pure culture (13, 14, 16) and the application of three advanced MS methods. The HPLC-ESI-IT-MS data identify the positions of the 4-sulfophenyl substituent in SPCs, SPC-2Hs, and SPdCs in the work with 5-C10-LAS. The method allows structures to be attributed when very little material is available. Under these conditions, nuclear magnetic resonance spectra are both difficult to obtain and, with the multiplicity of neighboring methylene groups, difficult to interpret. The HPLC-ESI-TOF-MS data allowed us to derive the molecular composition of a compound unequivocally and thus confirm the conclusions drawn in earlier papers (3, 4, 14) and followed up in the present work. With this background, along with earlier data and identifications (14), the longer trains of logic in the identifications made in the supplemental material were made possible.

Figure 5 illustrates the array of SP(d)Cs, which we show (references 3 and 14 and this work) to be excreted by *P. lavamentivorans* DS-1^T during growth with commercial LAS. The attack is initiated by ω-oxygenation (a putative heme monooxygenase [12]) following the "distance principle" (18) and oxidation to the corresponding SPC. This SPC can be subject to β-oxidation or to a second ω-oxygenation (if the sulfophenyl substituent is at the 5, 6, or 7 position) or it can be excreted unchanged. Long-chain SPCs (C_{10} to C_{13}) can appear as transient intermediates (16). β-Oxidation generally proceeds to within 4, or a minimum of 3, carbon atoms from the sulfophenyl substituent. If β-oxidation is active on only one of the two portions of the side chain, two types of excretion product, an SPC and an SPC-2H, are possible; these can obviously be excreted in any cycle (3), but the largest amount excreted is usually the SPC which cannot undergo a further round of β-oxidation (major SPC), along with the corresponding SPC-2H and SPC+2C. When β-oxidation is active on both portions of the side chain, major and minor SPCs and SPdCs are excreted: in the case of SPdCs, the SPdC species which cannot undergo further β-oxidation is the least common excretion product.

The number of compounds excreted from commercial LAS is considerable (Fig. 5). The 20 LAS congeners yield 11 major SPCs, 11 SPC-2Hs, and 11 SPC+2Cs, most (>22) of them chiral. The formation of 17 SPdCs, most (12 of 17) chiral, can be inferred (Fig. 5). Relatively few of these compounds seem to have been detected in the environment, whereas their disappearance in samples from many environments shows their

degradation (3). SPCs are degraded by bacteria which, where known, have narrow substrate ranges (14). It remains to be seen how many organisms are needed to complement an organism like *P. lavamentivorans* in the completion of the mineralization of LAS.

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