Structure of the Major Species of Chlorosulfolipid from *Ochromonas danica*. 2,2,11,13,15,16-Hexachloro-*N*-docosane 1,14-Disulfate*

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ABSTRACT: We have recently shown that *Ochromonas danica* elaborates a number of unusual chlorine-substituted docosane and tetracosane disulfates. The previous identification of several members with one and two chlorines per molecule has now been extended to the most abundant species, which contains six covalently bound chlorine atoms. Mild alkali treatment of the hexachlorodocosane-1,14-diol, obtained by acid hydrolysis at the disulfate, eliminated one hydrogen chloride to give a 14,15-epoxide. On further alkali treatment two more chlorines were lost with formation of a 2-ketone. Periodate cleavage gave the twenty-one-carbon noracid, the epoxide ring of which was opened by tosic acid catalyzed acetolysis to give the *vic*-glycol. Oxidative periodate cleavage gave the distal fragment of this molecule as α-chlorooctanoic acid; reduction of the proximal periodate oxidation fragment gave a thirteen carbon dichlorodiol, where the position of the two chlorines on the second and fourth carbon from the distal end was established by a stepwise degradation. From this analysis, supported by mass spectrometry and nuclear magnetic resonance studies, it is concluded that the structure of the original compound is 2,2,11,13,15,16-hexachloro-*n*-docosane 1,14-disulfate.

Chlorosulfolipids are a novel class of lipids recently discovered in *Ochromonas danica* (Elovson and Vagelos, 1969). They are derivatives of docosane 1,14-disulfate, a compound previously described by others (Mayers and Haines, 1967), and of tetracosane 1,15-disulfate, in which one to six chlorine atoms have been substituted for the equivalent number of hydrogens. After acid hydrolysis of the sulfate esters, 13-monochlorodocosane-1,14-diol, 14-monochlorotetracosane-1,15-diol, and 11,15-dichlorodocosane-1,14-diol were identified by chemical degradation and mass spectrometry. The first of these was subsequently characterized as the *threo*-(*R*)-13,(*R*)-14 isomer by Haines *et al.* (1969). As a part of the continuing study of the structure, biosynthesis and function of these unusual compounds the present report describes the structure of the hexachlorodocosane disulfate, which is the most abundant sulfolipid in *O. danica*, when the growth medium contains more than millimolar concentration of chloride ion (Elovson and Vagelos, 1969).

Materials and Methods

O. danica was from the American Type Culture Collection. Dicarboxylic acids, methyl esters, SE-30 silicone gum, HMDSi,[1] and TMSiCl were obtained from Applied Science Laboratories. Silica gel G was from Brinkmann. Dichloroethanol and trichloroethanol were purchased from Aldrich. Perdeuterated BSiA, TMSiCl, and acetic anhydride were obtained from Merck, Sharp and Dohme of Canada. [^{36}Cl]HCl was from New England Nuclear Corp. 2-Hydroxyoctanoic acid was prepared by refluxing 2-bromooctanoic acid (Clark and Taylor, 1964) in 1.5 M potassium hydroxide in acetone–water (1:1) overnight. Both acids were purified on thin-layer chromatography as their methyl esters and their purity and identity were established by gas–liquid chromatography–mass spectrometry. *cis*-9,10-Epoxystearyl alcohol was prepared as previously described (Elovson and Vagelos, 1969).

Most reactions and extractions were performed in 12-ml heavy-walled screw-capped conical centrifuge tubes with Teflon liners. For work-up, reaction mixtures were adjusted to 1:1 water–water-miscible solvents before extracting at least three times with equal volumes of water-immiscible solvent. The extracts were routinely washed with small amounts of water, dried over magnesium sulfate, taken to dryness under a stream of nitrogen, and dried *in vacuo* at room temperature. Volatile short-chain compounds were recovered by allowing the extraction solvent to evaporate from an open tube in a water bath at 42°. Even so, aldehydes, alcohols, and esters with nine carbons or less were lost to a variable and considerable extent.

Periodate Oxidation. Compounds were dissolved in four parts of the indicated alcohol, two parts 1 M sodium acetate (pH 4), and one part 0.5 M sodium periodate to give the final volume indicated in the text. The mixture was incubated at room temperature for 6 hr.

Chromic Acid Oxidation. Compounds were treated for 12 hr at room temperature with at least a 5 molar excess of 1.5% (w/v) CrO_3 in acetic acid containing 2% water.

Acetolysis. Compounds were heated for the indicated time in the indicated volume of 0.6% *p*-toluenesulfonic acid

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[1] Abbreviations used are: TMSi, trimethylsilyl; HMDSi, hexamethyldisilazane; TMSiCl, trimethylsilylchloride; BSiA, bis(trimethylsilyl)-acetamide.

in acetic acid–acetic anhydride (19:1, v/v).

Lithium Aluminum Hydride Reductions. The sample, in a small volume of dry ether, was treated at 0° with an excess of the hydride in ether; after 1 min 1 ml of water and enough 5 N sulfuric acid to make an acid solution were added. The products were extracted with ether.

Substitution with methyl or ethyl mercaptide was performed as previously described using 0.2 ml of the respective mercaptan (Elovson and Vagelos, 1969).

Oxidative ozonolysis was performed essentially according to Klenk and Bongard (1952).

Liquid scintillation counting was performed in a Packard Tri-Carb spectrometer, using Bray's (1960) solution.

TMSi Derivatives. Dry samples (<0.1 mg) were treated with 0.025 ml of pyridine–HMDSi–TMSiCl (2:2:1, v/v) or pyridine-d_{18}-BSiA-d_9-TMSiCl (2:2:1, v/v) at room temperature for 15 min. Acetates were prepared by heating the compound in acetic anhydride–pyridine (1:1, v/v) at 45° for 24 hr.

Thin-Layer Chromatography. Glass plates were coated with slurries of silica gel to nominal 0.25 and 0.5 mm thickness, and air-dried overnight before use. Solvent system compositions are given in milliliters. Compounds were visualized by spraying with 0.1% 2,7-dichlorofluorosceine in methanol and viewing under ultraviolet light. The appropriate zones were either scraped directly into scintillation vials or into fritted glass filter funnels for elution with ether. Trihydroxy and carboxylic compounds were eluted with 10% methanol in ether.

Gas–Liquid Chromatography. Glass columns (6 ft; 1/8 in. i.d.) packed with 1% SE-30 on 60–80 mesh Gas-Chrom Q were used with a Varian 2100-4 instrument equipped with hydrogen flame detectors. Helium pressure was 30 psi, rotameter readings 17. Peak areas were measured with an Infotronic Model CRS-100 digital readout system, or by triangulation.

Gas Chromatography–Mass Spectrometry. A LKB-9000 instrument, equipped with a 6-ft column of 0.2% SE-30 on Gas-Chrom Q, was operated between 100 and 210°. The mass spectrometer was operated at an ionization potential of 70 eV with an ion source temperature of 270°. Several magnetic scans were usually taken over each peak as it eluted from the gas chromatograph. For reasons of space only a selected summary of important ions is tabulated for most compounds. Since the majority of compounds contained chlorine and were analyzed as their TMSi ethers, the ions in the high mass range which permitted estimation of molecular weight usually included the $M^+ - 15$ (CH_3) (Sharkey *et al.*, 1957), $M^+ - 35$ (Cl), and/or $M^+ - 36$ (HCl) ions (McLafferty, 1962). For methyl esters of carboxylic acids the molecular weight was usually indicated by the ion at highest mass $M - 31$ (OCH_3), the acylium ion formed by loss of the methoxy group from the methyl ester (Ryhage and Stenhagen, 1963). Assignments of peaks in the mass spectra to ions containing TMSi groups were verified by the appropriate mass shifts observed in the spectrum of the respective perdeuterio-TMSi ether derivatives (McCloskey *et al.*, 1968). The number of TMSi methyl groups in each fragment determined in that manner was included in the tables. The natural abundance of ^{35}Cl and ^{37}Cl in the ratio of 3:1 gave characteristic splitting patterns to chlorine-containing ions (McLafferty, 1962) and the number of chlorine atoms in a particular fragment which was consistent with the shape of these isotope clusters was also tabulated. In the case of TMSi derivatives such estimates were considerably strengthened by comparison with the spectra of the perdeuterated derivatives, since spurious combinations of unrelated ions with different numbers of TMSi groups often were resolved to show the actual chlorine isotope patterns of their components. Unless otherwise stated the base peak in the spectra of TMSi compounds was the TMSi cation at *m/e* 73. In the case of acetates the base peak was chosen as that second in abundance to the acetyl cation at *m/e* 43.

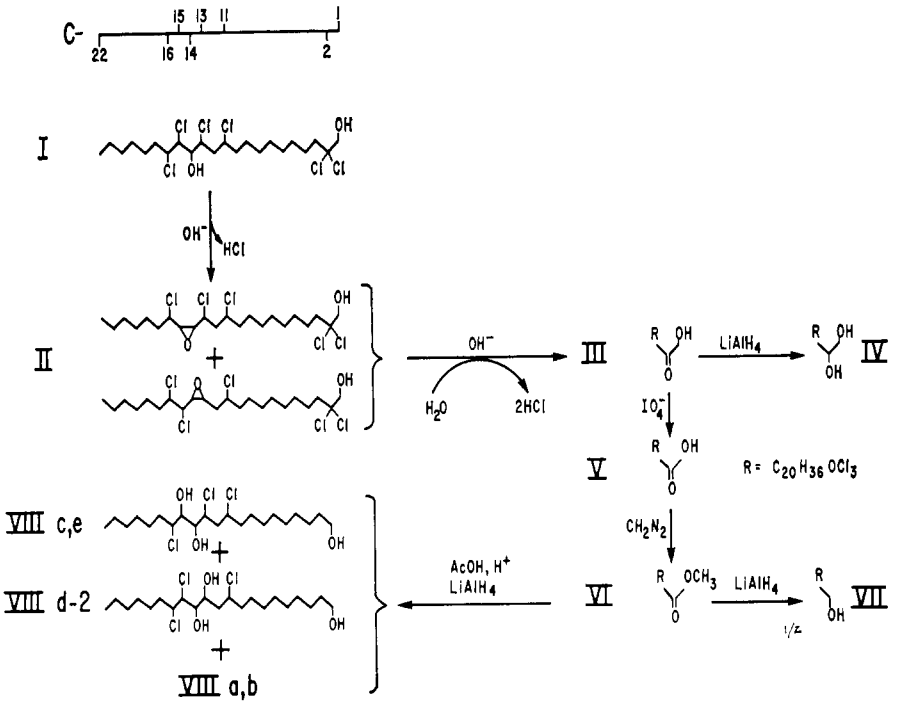
Nuclear Magnetic Resonance Spectra. Samples in 0.5 ml of deuteriochloroform were analyzed on a Varian A-60 and a Varian A-100 spectrometer. Where indicated the sample solution was equilibrated with 0.1 ml of deuterium oxide and centrifuged, and the lower phase was used to obtain spectra free of signals from exchangeable protons. Infrared spectra of sample films between silver chloride disks were recorded on a Perkin-Elmer No. 237 instrument.

Cell Culture. O. danica was grown heterotrophically with aeration in a New Brunswick Fermacell fermentor. The cells were harvested in stationary phase. One-hundred liters of medium contained: nitrilotriacetic acid, 20 g; potassium phosphate, monobasic, 30 g; magnesium carbonate, 80 g; calcium carbonate, 5 g; ammonium sulfate, 50 g; arginine hydrochloride, 10 g; histidine, 20 g; glutamic acid, 1000 g; sodium chloride, 5.85 g; glucose, 1000 g; biotin, 0.1 mg; thiamine hydrochloride, 10 mg; trace metal mixture (Aaronson and Baker, 1959), 50 ml. The final chloride concentration was 1.47 mM; total chloride radioactivity 20 μCi, added as [^{36}Cl]HCl.

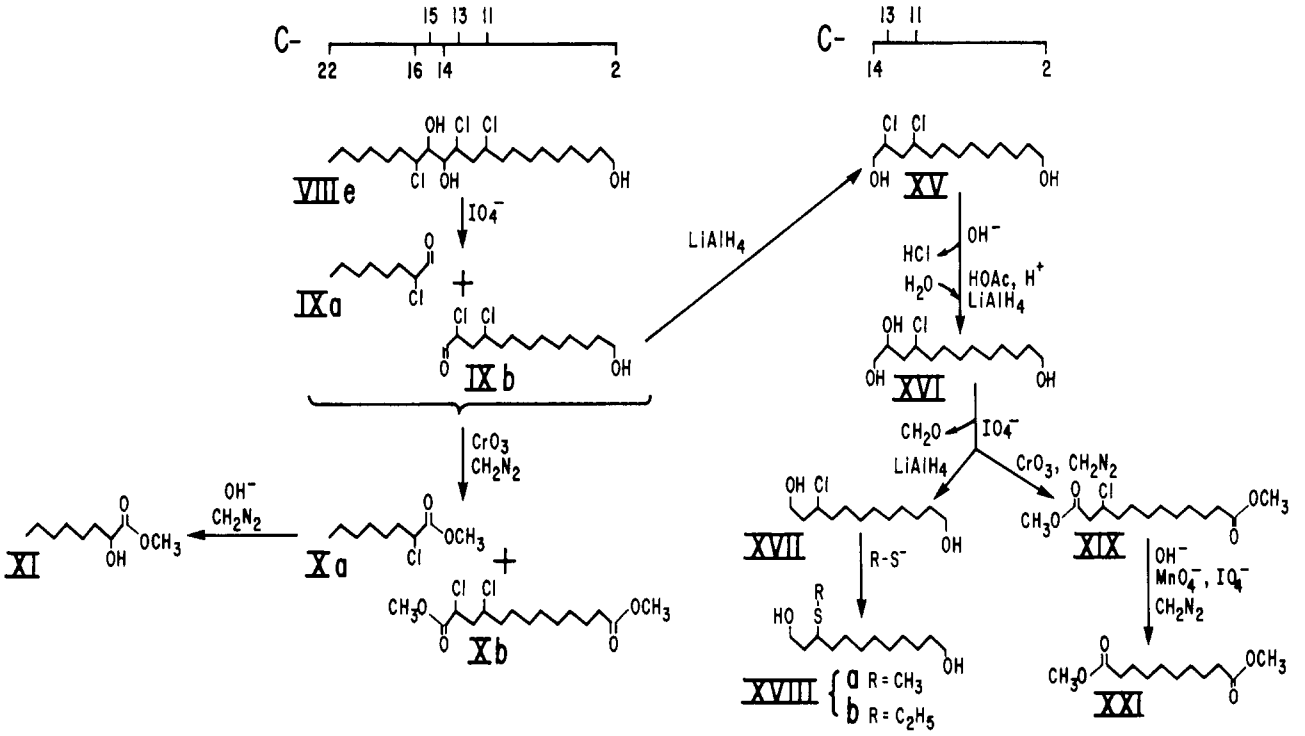
Isolation of Crude Chlorodiols. Batches (50 g; wet weight) of *O. danica* cells were homogenized with 500 ml of chloroform–methanol (1:1, v/v) in a Waring Blendor. After filtering on a suction funnel the residue was reextracted twice with 100 ml of the same solvent, and the combined filtrate was equilibrated overnight against three-tenths volume of distilled water. The dark green-brown chloroform phase was separated and reextracted with two-tenths volume of methanol–water (1:1, v/v). The aqueous phases were serially washed with small amounts of chloroform until free of chloroform-soluble pigment. When necessary, interphase emulsions were separated by centrifugation. The combined aqueous extracts were reduced *in vacuo* at 40° to approximately one-third of original volume on a flash evaporator, and extracted three times with equal volumes of 1-butanol. The butanol extracts were taken to dryness *in vacuo*, and the residue was triturated repeatedly with small amounts of methanol–chloroform (1:1, v/v). The extracts were filtered and taken to dryness. The residue of crude chlorosulfolipids was hydrolyzed at 100° for 60 min in a mixture of 5 ml of water, 10 ml of dioxane, and 1.5 ml of concentrated hydrobromic acid, per g of residue. After cooling, the mixture was transferred to a separatory funnel, diluted with one-half volume water, and extracted three times with one-half volumes of ether. The ether extracts were washed neutral with distilled water, dried over magnesium sulfate, and taken to dryness to yield the crude chlorodiol mixture. Final yield was about 1.1 g of crude chlorodiols/250 g wet weight of cells.

Isolation of Hexachlorodocosanediol. Crude chlorodiols (800 mg) in 60 ml of ether–pentane (1:1, v/v) was distributed into four 30-ml Corex centrifuge tubes and cooled in a meth-

SCHEME I: Stepwise Degradation of 2,2,11,13,15,16-Hexachloro-*n*-docosane-1,14-diol (I).



SCHEME II



anol–Dry Ice bath for 30 min. The precipitate was quickly centrifuged off at full speed in the Sorvall SS-34 head, using rubber adapters precooled on Dry Ice. Material insoluble in ether–pentane (9:1 and 19:1) at Dry Ice temperature was also removed by the same procedure. The precipitates from the latter two steps were refractionated from ether–pentane (19:1, v/v) and the supernatants were pooled with the original supernatant. The concentrate obtained after evaporation of the solvent from this solution contained about 75% hexachlorodocosanediol; the yield of this compound was about 80%.

Aliquots of 50 mg of the concentrate were applied across

20 × 20 cm plates of 0.5 mm of silica gel G and chromatographed in benzene–chloroform–methanol (100:60:5, v/v). Hexachlorodocosanediol was recovered from the upper two-thirds of a band, R_F approximately 0.5, in a yield of about 80% of the amount applied. The material was a colorless oil which gave a white amorphous solid when the last traces of organic solvents were removed *in vacuo*. On gas–liquid chromatography over 90% of the TMSi derivative appeared in a peak with a rentention time of 9 min at 240°. Its mass spectrum was the same as that previously published for the TMSi derivative of hexachlorodocosanediol (Elovson and Vagelos, 1969). The molecular weight of 690 was indicated by the $M^+ - 15$ (CH_3) ion at *m/e* 675 and the $M^+ - 36$ (HCl) ion at *m/e* 654. α cleavage at the secondary TMSi group on C-14 gave the distal fragment of the molecule with two chlorines as the intense ion at *m/e* 283. The proximal fragment at *m/e* 509, which contained two TMSi ethers and four chlorines, was of low abundance, but also gave two peaks at *m/e* 473 and 401 by further loss of HCl and TMSiCl. The material in a small peak which had a retention time of 0.78 relative to that of the hexachloro compound, was the same as that designated compound H in the previous publication (Elovson and Vagelos, 1969), a pentachlorodocosanediol, with two chlorines distal and three chlorines proximal to the secondary TMSi group on C-14. The parent compound chromatographed in the trailing edge of the hexachlorodocosanediol band on preparative thin-layer chromatography and its concentration in the final product varied somewhat depending on how much of that area was discarded. Another small peak with a retention time of 0.68 relative to the major peak, also appeared to be a mixture of two pentachlorodocosanediols, one with one distal and four proximal chlorines, the other with two distal and three proximal chlorines. The concentration of these impurities could not be appreciably reduced by repeating the preparative thin-layer chromatography under a variety of conditions.

In the degradation studies sufficient amounts of hexachlorodocosanediol preparation purified from cells grown on 0.048 mCi/mmole of $^{36}Cl^-$ (Elovson and Vagelos, 1969) were added to give a final specific activity of about 3 μCi/mmole of ^{36}Cl in the starting material.

TABLE I: Important Ions in the Mass Spectra of TMSi II, TMSi III, TMSi IV, VI, and TMSi VII (*cf.* Figure 1 and Scheme I).

	m/e	Cl_n[a]	$(C^2H_3)_n$[b]	Rel Ab (%)[c]	Assignment[d]
TMSi II	582				M^+
	567	5	2	0.07	$M^+ - CH_3$
	546	4	3	0.50	$M^+ - HCl$
	531	4	2	0.40	567 − HCl
	103	0	3	100	*a*
TMSi III	528	3	3	0.6	M^+
	513	3	2	3.4	$M^+ - CH_3$
	477	2	2	1.8	513 − HCl
	103	0	3	100	*a*
TMSi IV	602				M^+
	587	3	5	0.12	$M^+ - CH_3$
	499	3	3	8.5	$M^+ - CH_2$-OTMSi
	205	0	6	7.5	
	147	0	5	42	*b*
VI	456				M^+
	425	3		2.6	$M^+ - CH_3O$
	222	0		30	*d*
	74	0		97	*e*
	55	0		100	C_4H_7
TMSi VII	500				M^+
	485	3	2	1.0	$M^+ - CH_3$
	266	0	3	3.3	*d*

[a] Number of chlorine atoms. [b] Number of TMSi methyl groups. [c] Relative abundance. [d] Tentative.

Results

Schemes I and II outline the sequence of reactions which were used in the stepwise degradation of 2,2,11,13,15,16-*n*-hexachlorodocosane-1,14-diol (I). The specific notation C-1, C-2, etc., to C-22 is used in all intermediates *exclusively* to denote the original position of a carbon atom in the hexachlorodocosanediol starting material.

Formation of Epoxide II. Brief alkali treatment resulted in the loss of one of the six chlorines from I. When a two-phase mixture of I (520 μmoles of ^{36}Cl) in 3 ml of peroxide-free dioxane and 1 ml of 0.5 M KOH was shaken at room temperature under nitrogen for 10 min, 73 μmoles of ^{36}Cl was released as chloride ions. Preparative thin-layer chromatography of the ether-soluble products in benzene–chloroform–methanol (100:65:5, v/v) gave 350 μmoles of ^{36}Cl in II, R_F approximately 0.65, running just ahead of unchanged I (50 μmoles of ^{36}Cl). The mass spectrum (Table I) of TMSi II was consistent with the presence of one TMSi group and five chlorines per a molecular weight of 582, corresponding to loss of TMSiCl from TMSi I. The ions corresponding to the characteristic α cleavage at C-14 in TMSi I were also absent, while α cleavage between C-1 and C-2 gave the base peak at *m/e* 103 (Diekman *et al.*, 1968). These findings indicated that II was formed from I in an intramolecular nucleophilic attack by the secondary C-14 hydroxyl on a chlorine-substituted carbon, with elimination of HCl and formation of a cyclic ether structure. The position and size of the ring was not apparent from the mass spectrum.

Formation of (Epoxy)-2-keto-1-ol III. On slightly more vigorous alkali treatment, I lost a total of three chlorines per molecule. Incubation of I (980 μmoles of ^{36}Cl) in 11 ml of peroxide-free dioxane and 6.8 ml of 0.5 M KOH under nitrogen at 50° for 30 min released 480 μmoles of $^{36}Cl^-$. After neutralization with 6 N HCl the ether-extractable products were recovered and separated by thin-layer chromatography in benzene–chloroform–methanol (100:65:5, v/v). Two narrow bands, R_F 0.7–0.8, 53 μmoles of ^{36}Cl, gave a large number of peaks on gas–liquid chromatography, none of which contained TMSi-reactive groups. Another diffuse band with an R_F of approximately 0.15 contained 32 μmoles of ^{36}Cl in an

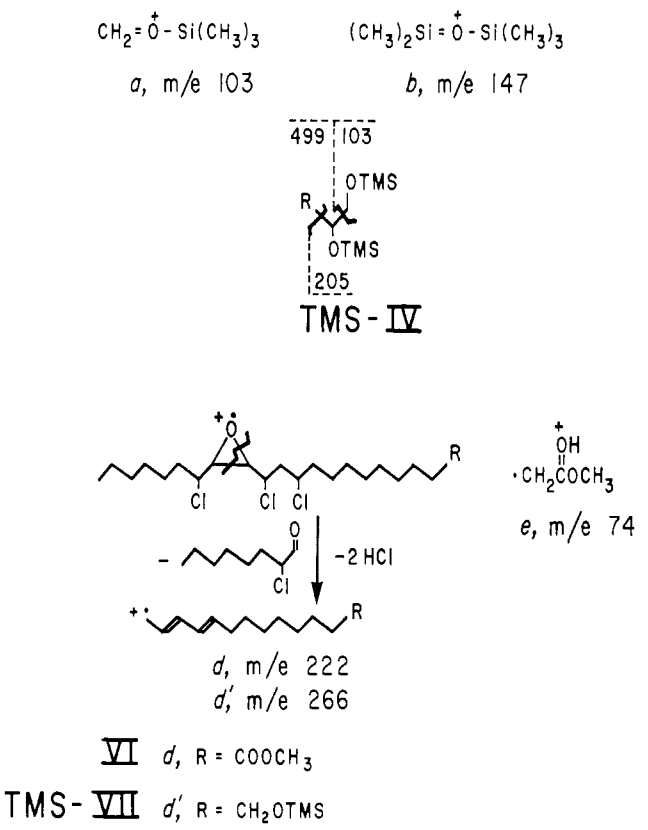


FIGURE 1: Proposed fragmentation of TMSi IV, VI, and TMSi VII. Compare Table I and Scheme I.

acidic compound. After methylation with diazomethane the major product had a mass spectrum indistinguishable from that of VI described below. The major product of the alkaline treatment, III, had an R_F of approximately 0.35 and contained 350 μmoles of ^{36}Cl. The mass spectrum of its TMSi ether (Table I) showed the presence of three chlorines and one TMSi ether group per a molecular weight of 528, corresponding to the substitution of one oxygen atom for two chlorines compared to TMSi II.

Brief lithium aluminum hydride reduction of a portion of III converted it into IV without further loss of chlorine. The mass spectrum (Table I) of its TMSi ether was consistent with the presence of three chlorines and two TMSi groups per a molecular weight of 602, corresponding to addition of one hydrogen and a second TMSi group compared with TMS III. The strong peak at $M^+ - 103$, corresponding to loss of C-1 OTMSi, suggested that the new TMSi ether group was located on C-2, *i.e.*, that III had a C-2 keto group.

Formation of (Epoxy)nor Acid V. The 2-keto-1-ol structure was verified by periodate oxidation of III, 300 μmoles of ^{36}Cl, in 8 ml of aqueous *t*-butyl alcohol for 6 hr at room temperature. The product, V, recovered by extraction with pentane–ether (4:1, v/v) was a carboxylic acid. Treatment with diazomethane and preparative thin-layer chromatography in 100% benzene gave the methyl ester, VI, containing 278 μmoles of ^{36}Cl. Its mass spectrum (Table I) showed the typical intense McLafferty-rearrangement peak (McLafferty, 1959) of *m/e* 74; the fragment at highest mass appeared at *m/e* 425 and contained three chlorines, as expected for the acylium ion of

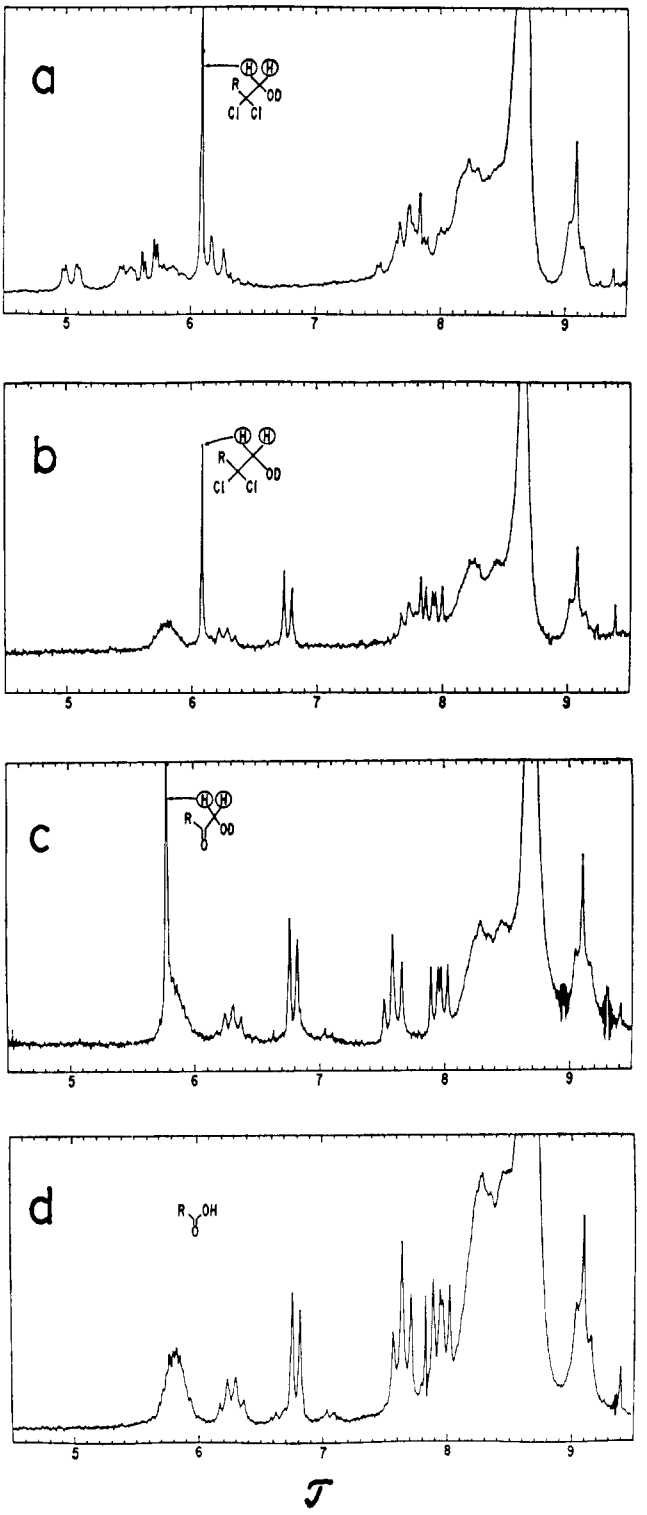


FIGURE 2: 100-Mc nuclear magnetic resonance spectra of (a) I, (b) II, (c) III, and (d) V in deuteriochloroform. The spectra in a, b, and c were recorded after equilibration against deuterium oxide. Compare Scheme I.

the methyl ester of a twenty-one-carbon nor acid derived from III by oxidative cleavage between C-1 and C-2. The intense odd-electron ion *d* at *m/e* 222 could correspond to transannular cleavage of a C-14,C-15-epoxide in VI, as shown

TABLE II: Important Ions in the Mass Spectra of TMSi VIIIa, -b, -d-1, and -d-2. (*cf.* Figure 3 and Scheme I).

	m/e	Cl_n[a]	$(C^2H_3)_n$[b]	Rel Ab (%)[c]	Assignment[d]
TMSi VIIIa	500				M^+
	485	3	2	4.8	$M^+ - CH_3$
	449	2	2	8.1	485 − HCl
	319	1	3	23	$M^+ - (CH_3(CH_2)_5$-$(CHCl)_2)$
TMSi VIIIb	572		6	0.1	M^+
	557	3	5	0.1	$M^+ - CH_3$
	521	2	5	0.2	557 − HCl
	439	2	6	2.0	
	369	0	6	7.1	439 − Cl_2
	235	1	3	85	
TMSi VIIId-2	662				M^+
	647	3	8	0.2	$M^+ - CH_3$
	611	3	8	0.3	647 − HCl
	445	0	9	1.8	481 − HCl
	385	2	6	2.0	
	379	1	6	0.3	
	343	0	6	17	379 − HCl
	283	2	3	1.4	
	213	0	3	100	283 − Cl_2
TMSi VIIId-1	628				M^+
	613	2	8	0.3	$M^+ - CH_3$
	577	1	8	0.2	613 − HCl
	356	0	6	40	$(M^+ - 201) + H$ − 2HCl
	303	0	6	4.3	
	213	0	3	6	303 − TMSiOH
	201	0	3	52	

[a–d] Compare Table I.

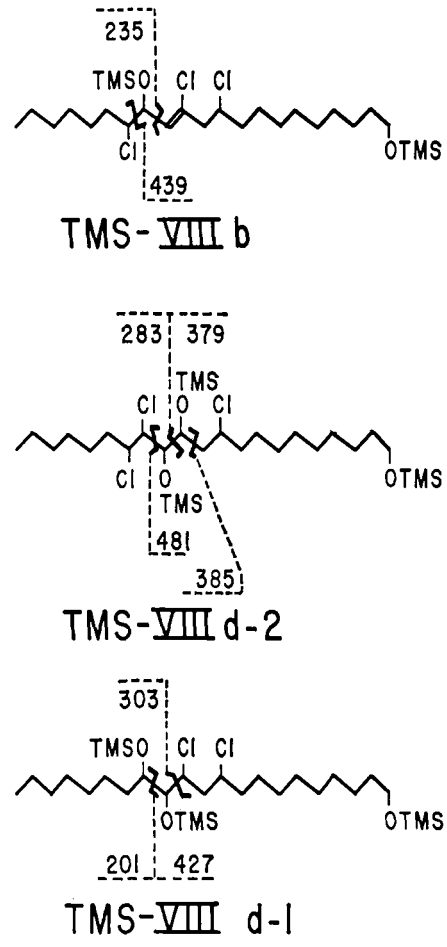


FIGURE 3: Proposed structure and fragmentation of TMSi VIIIb, TMSi VIIId-2, and TMSi VIIId-1. Compare Table II and Scheme I.

in Figure 1. Brief treatment of a portion of VI with lithium aluminum hydride gave the corresponding primary alcohol, VII, without further loss of chlorine; the mass spectrum of its TMSi derivative was consistent with the expected molecular weight of 500, and showed a weaker odd-electron ion d′ equivalent to d.

The *gem*-2,2-dichloro structure in I and II was verified by nuclear magnetic resonance spectrometry. The 60- (not shown) and 100-Mc nuclear magnetic resonance spectra (given in τ values) of I, II, III, and V (Figure 2) all had the three-proton triplet at 9.1 expected for the C-22 methyl hydrogens, and a broad intense band at about 8.7 corresponding to the hydrocarbon methylene hydrogens. The downfield shoulder of this band showed a complex mixture of overlapping peaks, presumably representing signals from methylene and methene protons adjacent to chlorine-substituted carbons. The seven protons on carbons which also carried oxygen or chlorine substituents absorbed at 5–6.5. The sharp singlet at 6.1, which contained two protons by integration, was unaffected by the transition from I to II. The two protons on C-1 of a primary alcohol gave a signal at 6.4; this was shifted downfield to 6.08 in dichloroethanol, and to 5.94 in trichloroethanol. Thus, the chemical shift and the singlet nature of the signal at 6.1 in I and II strongly suggested that it arose from the two isolated protons on C-1 in a *gem*-2,2-dichloro-1-ol structure. This was further supported by the fact that this two-proton singlet was shifted downfield to 5.78 when the two chlorines on C-2 were replaced by a keto group in III, with the accompanying further deshielding of C-1 and C-3; the two protons on the latter probably gave rise to the new triplet at 7.59 in the spectrum of that compound. Finally, the only major change in the spectrum of III when C-1 and its two protons were removed by periodate oxidation to form V was the disappearance of the singlet in question.

Acetolysis of Epoxide Ring in VI. As shown below the major components in II–VI had a C-13,C-16-dichloro-C-14,C-15-epoxide structure. At 60 Mc the spectrum of *cis*-9,10-epoxystearyl alcohol had a poorly resolved two-proton peak at 7.1 attributable to the epoxy protons, as shown by its disappearance on reduction with lithium aluminum hydride. It is possible that the chlorines on both adjacent carbons in II–IV shifted that signal downfield to give the two-proton doublet at 6.75. The infrared spectrum of III and V also showed peaks at 8 and 10.9 μ which have been attributed to epoxide ring vibrations (Szymanski, 1967). However, the C-14,C-15-

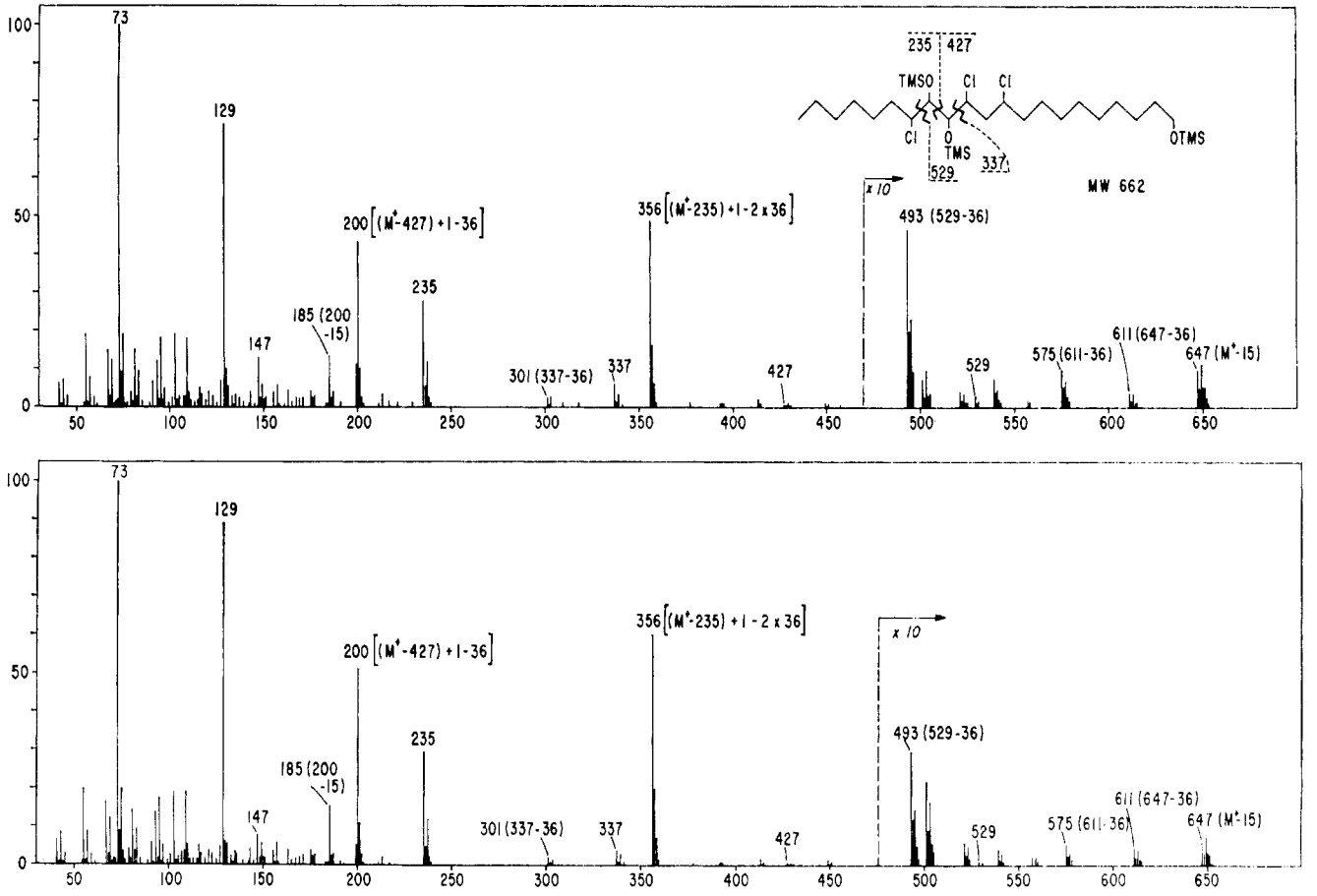


FIGURE 4: Mass spectra of (top) TMSi VIIIe and (bottom) TMSi VIIIc. Abscissa: *m/e*. Ordinate: relative abundance.

epoxide group in these compounds was rather unreactive, presumably due to the presence of the C-13 and C-16 chlorine substituents. Treatment with lithium aluminum hydride at 0° for 1 hr gave only small amounts of products which could be ascribed to reductive opening of an epoxide; the structure of these was not further investigated since they also had lost one or more chlorine. Compounds II and VI also resisted treatment with 2 N sulfuric acid at 80° for 30 min as well as refluxing acetic acid for 12 hr. However, when VI was treated with 2 N sulfuric acid at 100° for 5 hr about equal amounts of two new products were formed. One of these had lost one of the three chlorines originally present in VI but the TMSi ether of the other gave a mass spectrum (see below) consistent with a C-14,C-15-*vic*-diol product of VI. To minimize the formation of dechlorinated by-products the following procedure was adopted. Compound VI, which contained 250 μmoles of ^{36}Cl, was heated at 100° for 18 hr in 1 ml of 0.6% *p*-toluenesulfonic acid in 5% acetic anhydride in glacial acetic acid. The products, which contained 245 μmoles of ^{36}Cl, were recovered by extraction with pentane–ether (4:1, v/v) and treated with an excess of lithium aluminum hydride. The products, containing 235 μmoles of ^{36}Cl, were separated by preparative thin-layer chromatography in chloroform–methanol (200:5, v/v) into five fractions: VIIIa, R_F 0.5, 12 μmoles of ^{36}Cl; VIIIb, R_F 0.44, 35 μmoles of ^{36}Cl; VIIIc, R_F 0.33, 41 μmoles of ^{36}Cl; VIIId, R_F 0.24, 20 μmoles of ^{36}Cl; and VIIIe, R_F 0.15, 130 μmoles of ^{36}Cl.

Properties of Acetolysis Products VIIIa–e. The major component of TMSi VIIIa had a retention time on gas–liquid chromatography of 0.5 relative to that of TMSi VIIIe. Its mass spectrum (Table II) was consistent with the presence of three chlorines and one TMSi ether group per a molecular weight of 500, equivalent to net reduction of the methyl ester to a primary alcohol relative to VI. As discussed below for XXXIV its resistance to acetolysis and the prominent ion at *m/e* 319 would be consistent with a C-11,C-14-substituted tetrahydrofuran structure. TMSi VIIIb had a retention time of 0.64 relative to TMSi VIIIe. Its mass spectrum (Table II) indicated three chlorines and two TMSi ether groups per a molecular weight of 572, a loss of one TMSiOH, compared to TMSi VIIIc, -d-2, and -e. The fragmentation pattern with a strong chlorine-containing ion at *m/e* 235 was consistent with a secondary TMSi ether group at C-15, with one chlorine distal and two chlorines and one double-bond proximal to that position. Its possible α,β-unsaturated alcohol structure (Figure 3) (Gasson *et al.*, 1954) was not further investigated. After TMSi treatment VIIId showed two major components, TMSi VIIId-1 and TMSi VIIId-2, in a ratio of about 1:2, with a retention time of 0.65 and 0.83 relative to TMSi VIIIe. The major components of TMSi VIIIc and TMSi VIIIe accounted for over 90% of the material in these fractions; TMSi VIIIc had a retention time of 0.93 relative to TMSi VIIIe, which eluted after 4.5 min at 230°. The mass spectra of the TMSi derivatives of VIIId-2, VIIIc, and VIIIe were

consistent with the presence of three chlorines and three TMSi ether groups per molecular weight of 662 in all three compounds, equivalent to reduction of the carboxyl function and hydrolytic opening of an epoxide in the acetolysis-reductive deacetylation of VI. The spectrum (Table II) of TMSi VIIId-2 indicated a C-13,C-14-*vic*-glycol with two distal and one proximal chlorines. The primary distal and proximal fragments after cleavage between these two positions appeared as weak ions at *m/e* 283 and 379, respectively; loss of HCl from the latter gave an intense ion at *m/e* 343, while loss of Cl_2 from the former resulted in the base peak at *m/e* 213. The mass spectra of TMSi VIIIc and TMSi VIIIe were almost identical (Figure 4) and consistent with a C-14,C-15-*vic*-glycol structure, with one chlorine distal and two proximal to these positions. Cleavage between C-14 and C-15 gave moderately strong and weak primary ions at *m/e* 235 and 427. Hydrogen transfer to the distal fragment and expulsion of hydrogen chloride gave an intense odd-electron rearrangement ion at *m/e* 200, which also lost a TMSi methyl radical to give the even electron ion at *m/e* 185, as shown by the appropriate metastable ion. Hydrogen transfer to the proximal fragment and expulsion of its two chlorines as two hydrogen chlorides gave an equally intense odd-electron ion at *m/e* 356. Significant ions were also produced by fragmentation on both sides of the *vic*-TMSi ethers (Figure 4). The TMSi derivatives of the C-14,C-15-*vic*-glycol mentioned above, which was obtained in moderate yield by vigorous treatment of VI with aqueous acid, showed the same fragmentation pattern; in this case the C-2 methyl ester structure shifted the two proximal fragment ions from *m/e* 427 and 356 to *m/e* 383 and 312, respectively.

The mass spectrum of TMSi VIIId-1 (Table II) was consistent with the presence of three TMSi ether groups but only two chlorines per a molecular weight of 628. The fragmentation pattern indicated a C-14,C-15-*vic*-glycol with both chlorines proximal to these positions: the proximal fragment appeared as the same intense chlorine-free rearrangement ion at *m/e* 356 as in TMSi VIIIc and -e, while the distal fragment gave an intense α-cleavage ion at *m/e* 201. This compound could be derived from the pentachloro contaminant in the starting material which had only one chlorine beyond C-14, or arise by loss of chlorine in the reductive deacetylation.

Periodate Treatment of vic-Glycols VIIIc and -e. Compound VIIIc, which contained 20 μmoles of ^{36}Cl, and VIIIe, which contained 100 μmoles of ^{36}Cl, were each treated with periodate in 2 ml of aqueous methanol (pH 4) for 6 hr. After addition of 1 ml of water, the mixtures were extracted five times with 2 ml of ether–pentane (1:1) and the pooled extracts were adjusted to 10-ml final volume each. Most of the solvent of 0.05-ml portions was evaporated off at 40°, the concentrates were treated with 0.05 ml of TMSi reagents, and aliquots were analyzed by gas chromatography on a temperature gradient between 80 and 240°. The only volatile product in the VIIIc sample was unchanged starting material, as verified by gas–liquid chromatography–mass spectrometry. In contrast, VIIIe was completely converted into two products which eluted at about 112 and 186°, respectively. Their mass spectra indicated the presence of one chlorine plus one TMSi group, and two chlorines and two TMSi groups per a molecular weight of 266 and 458, respectively. This was consistent with the conclusion that the two aldehydic periodate oxidation products of VIIIe (IXa and -b, Scheme II) were analyzed as the TMSi ethers of their hemiacetals with methanol, the alcohol used in the oxidation. The base peak for both these compounds at *m/e* 133 contained one TMSi ether groups, as expected for the acetal carbon fragment (f, Figure 5). When the periodate oxidation of VIIIe was performed in aqueous ethanol the molecular weight of these two compounds shifted to 280 and 472, respectively, and the base peak appeared at *m/e* 147, in complete agreement with the formation of the respective ethyl hemiacetals.

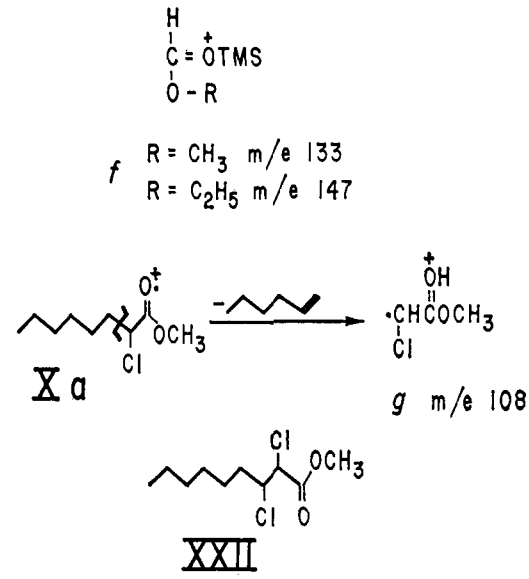


FIGURE 5: Fragmentation of Xa and related compounds. Compare Table III and Scheme III.

Identification of Distal Portion of VIIIe. FORMATION OF Xa AND XI (SCHEME II). One-third of the ether–pentane extract of periodate-oxidized VIIIe was evaporated at 42° to near dryness and the residue was oxidized with chromic acid. The products were extracted with five 1-ml portions ether–pentane (2:1, v/v) methylated with gaseous diazomethane and separated by thin-layer chromatography in 100% benzene into a monocarboxylic ester, Xa, R_F 0.60, containing 6.9 μmoles of ^{36}Cl, and a dicarboxylic ester, Xb, R_F 0.34, containing 17.3 μmoles of ^{36}Cl. The mass spectrum of the former (Table III, Figure 5) was consistent with the presence of one chlorine per a molecular weight of 192, as expected for the methyl ester of a monochlorooctanoic acid formed from the distal C-15 to C-22 portion of VIIIe. Since the McLafferty-rearrangement ion appeared as a chlorine-containing base peak at *m/e* 108 the chlorine was directly assignable to the C-16 α carbon in this molecule. Treatment of Xa, containing 3 μmoles of ^{36}Cl, in 0.05 ml of acetone with 0.2 ml of 2 M potassium hydroxide at 80° for 8 hr, completely liberated the chlorine as chloride ion. After acidification, the products were recovered by five extractions with 0.5 ml ether, treated with excess diazomethane, and analyzed by gas–liquid chromatography at 78° before and after trimethylsilylation. More than 95% of the volatile material, XI and TMSi XI, had the same retention times as synthetic 2-hydroxyoctanoic acid methyl ester (2.2 min) and 2-*O*-trimethylsilyloctanoic acid methyl ester (4.7 min), respectively. The absence of any peak in the area of elution of standard octanoic acid methyl ester (1 min) showed that no unsaturated product was formed. The mass spectra of

TABLE III: Important Ions in the Mass Spectra of Xa and -b, XIX and XXII (*cf.* Figure 5 and Schemes I and II).

	m/e	Cl_n[a]	Rel Ab (%)[c]	Assignment[d]
Xa	192			M^+
	177	1	0.1	$M^+ - CH_3$
	163	1	9.5	$M^+ - C_2H_5$
	157	0	11	$M^+ - Cl$
	108	1	100	*g*
Xb	340			M^+
	309	2	0.5	$M^+ - CH_3O$
	308	2	0.7	$M^+ - CH_3OH$
	273	1	3.0	309 − HCl
	108	1	11	*g*
	74	0	100	*e*
XIX	292			M^+
	261	1	1.4	$M^+ - CH_3O$
	257	0	6.4	$M^+ - Cl$
	225	0	24	261 − HCl
	219	1	10	$M^+ - CH_2COOCH_3$
	74	0	100	*e*
XXII	240			M^+
	205	1	61	$M^+ - Cl$
	169	0	73	205 − HCl
	108	1	54	*g*
	43	0	100	C_3H_7

[a–d] Compare Table I.

TABLE IV: Important Ions in the Mass Spectra of TMSi XV, TMSi XVI, TMSi XVIa, and TMSi XVII (*cf.* Figure 6 and Scheme II).

	m/e	Cl_n[a]	$(C^2H_3)_n$[b]	Rel Ab (%)[c]	Assignment[d]
TMSi XV	428				M^+
	413	2	5	0.5	$M^+ - CH_3$
	377	1	5	0.9	413 − HCl
	343	0	5	3.0	413 − Cl_2
	269	0	2	7.9	377 − TMSiCl
	103	0	3	66	*a*
TMSi XVI	482				M^+
	467	1	8	0.2	$M^+ - CH_3$
	431	0	8	0.6	467 − HCl
	379	1	6	0.3	
	343	0	6	32	379 − HCl
	205	0	6	29	
	159	0	3	86	*h*
	147	0	5	29	*b*
	103	0	3	23	*a*
TMSi XVIa	448				M^+
	433	0	8	0.6	$M^+ - CH_3$
	345	0	6	14	
	205	0	6	4.2	
	147	0	5	66	*b*
	103	0	3	47	*a*
TMSi XVII	380				M^+
	365	1	5	1.4	$M^+ - CH_3$
	257	0	5	3.8	365 − TMSiCl
	147	0	5	12	*b*
	103	0	3	100	*a*

[a–d] Compare Table I.

TMSi XI and standard 2-*O*-trimethylsilyloctanoic acid methyl ester were indistinguishable and showed the fragmentation pattern characteristic for TMSi ethers of α-hydroxy fatty acid methyl esters (Capella *et al.*, 1968); an intense TMSi-containing ion at *m/e* 187 ($M^+ - 59$) was formed by loss of the ester group, and a rearrangement peak at *m/e* 89 represented a TMSi cation minus one methyl group plus the methyl ester methoxy group. The identity of TMSi XI and the standard showed the absence of branching beyond C-15 in the docosane backbone.

Identification of Proximal Portion of VIIIe. FORMATION OF XV–XVIII (SCHEME II). The mass spectrum (Table III) of the dicarboxylic product Xb was consistent with the presence of two chlorines per a molecular weight of 340, as expected for the C-2 to C-14 proximal fragment of VIIIe. The base peak at *m/e* 74 represented the McLafferty-rearrangement fragment from the C-2 end, while the less intense chlorine-containing ion at *m/e* 108 showed the presence of a chlorine on the C-13 α carbon at the other end of the molecule.

To establish the position of the chlorines in this proximal fragment, the remaining material from the periodate oxidation of VIIIe was reduced with an excess of lithium aluminum hydride, and the products separated by thin-layer chromatography in benzene–chloroform–methanol (100:65:5, v/v). Material with an R_F of 0.30 presumably represented the 2-chlorooctan-1-ol derived from the distal fragment. The mass spectrum (Table IV) of the TMSi ether of the material with an R_F 0.15, XV, containing 41 μmoles of ^{36}Cl, was consistent with the presence of two chlorines and two TMSi groups per a molecular weight of 428, as expected for the reduced proximal C-2 to C-14 fragment. When this material, containing 37 μmoles of ^{36}Cl, was treated with 0.4 ml of 0.5 M KOH in 50% aqueous dioxane for 30 min, 19 μmoles of $^{36}Cl^-$ was released. The ether-soluble products were acetolyzed, deacetylated with lithium aluminum hydride, and chromatographed in chloroform–methanol (150:7.5, v/v). Besides unchanged XV, R_F 0.3, containing 2 μmoles of ^{36}Cl, material in two closely spaced bands at R_F 0.2 contained 3 μmoles of ^{36}Cl. The mass spectra of their TMSi derivatives showed substitution of one hydrogen for one chlorine relative to TMSi XV, presumably by reduction of a ketone formed in the acetolysis of the intermediary epoxide. The major product, XVI, containing 12 μmoles of ^{36}Cl, had R_F 0.08. The mass spectrum of its TMSi derivative (Table IV) was consistent with the presence of one chlorine and three TMSi ether groups per a molecular weight of 482, as expected for replacement of the C-13 chlorine in XV by a hydroxyl. The presence of a terminal *vic*-C-13,C-14-glycol was established by the significant ion at *m/e* 205 (Figure 6) and the intense ion

TABLE V: Important Ions in the Mass Spectra of the TMSi Ethers and Acetates of XVIIIa and -b (*cf.* Figure 7 and Scheme II).

m/e			Rel Ab (%)[c]		
a	b	$(C^2H_3)_n$[b]	a	b	Assignment
		TMSi Ethers			
392	406	6	3.8	2.8	M^+
377	391	5	4.2	2.7	$M^+ - CH_3$
344		6	5.6	5.4	$M^+ - RSH$
302	316	3	5.7	3.3	$M^+ -$ TMSi-OH
287		3	26	17	$M^+ -$ TMSi-OH $-$ R
275	289	3	0.8		*i*
177	191	3	3.0	1.5	*j*
102	116	0	64	30	*k*
m/e			Rel Ab (%)		
a	b	$(C^2H_3)_n$[e]	a	b	Assignment
		Acetates			
332	246	2	0.1	0.5	M^+
272	286	1	12	29	$M^+ - CH_3$-COOH
257		1	20	70	$M^+ - CH_3 -$ COOH $-$ R
245	259	1	2.7	12	*i*
197		0	4.9	22	$M^+ - 2CH_3$-COOH $-$ R
165		0	9.0	20	$M^+ - 2CH_3$-COOH $-$ RS
147	161	1	0.4	0.6	*j*
102	116	0	100	100	*k*

[a–d] Compare Table I. [e] Acetate methyl groups.

at *m/e* 343, formed from the weak ion at *m/e* 379 by loss of HCl, as shown by the appropriate metastable peak. Except for the TMSi cation the most abundant fragment in the spectrum occurred at *m/e* 159 and contained one TMSi group and no chlorine. A possible pathway for its formation is shown in Figure 6. A small peak of chlorine-free material, TMSi XVIa (Figure 6 and Table IV), could arise from a pentachloro contaminant or by dechlorination in the reduction steps.

Compound XVI, containing 10.1 μmoles of ^{36}Cl, was oxidized with periodate in aqueous methanol, and one-half of the products was reduced with lithium aluminum hydride to give XVII, containing 3.9 μmoles of ^{36}Cl. The mass spectrum of its TMSi ether (Table IV) was consistent with the presence of one chlorine and two TMSi groups per the expected molecular weight of 380. Aliquots of XVII, containing 1.5 μmoles of ^{36}Cl, were treated with methyl and ethyl mercaptide, with complete liberation of the chlorine as chloride ion. The products, XVIIIa and -b, were analyzed by gas–liquid chroma-

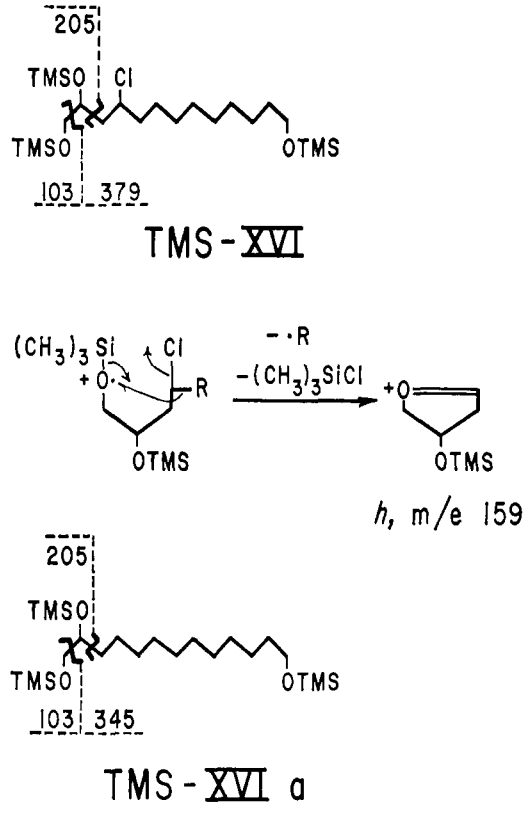
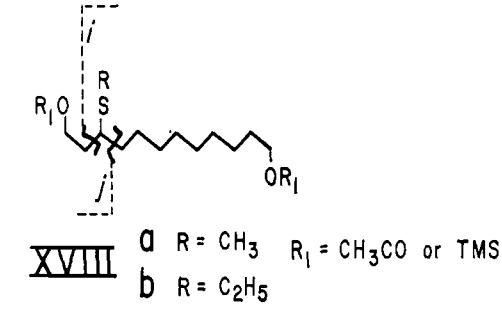


FIGURE 6: Proposed fragmentation of TMSi XVI and TMSi XVIa. Compare Table IV and Scheme II.



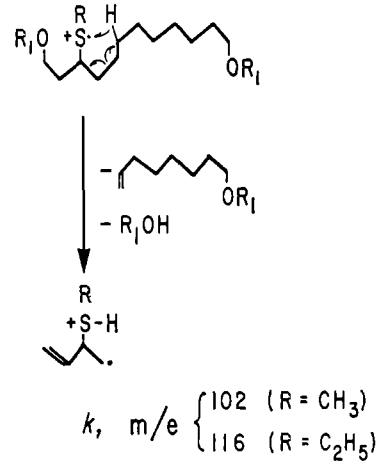


FIGURE 7: Proposed fragmentation of TMSi ethers and acetates of XVIIIa and -b. Compare Table V and Scheme II.

TABLE VI: Important Ions in the Mass Spectra of TMSi VIa and -b and TMSi VIIa and -b (*cf.* Figure 8).

m/e		$(C^2H_3)_n$[b]		Rel Ab (%)[c]		
VIa	VIIa	VIa	VIIa	VIa	VIIa	Assignment[d]
518	562					M^+
503	547	2	5	1.3	6.5	$M^+ - CH_3$
471	515	3	6	2.0	4.0	$M^+ - CH_3S$
347	391	3	6	7.0	3.6	*l*
299	343	3	6	4.2	26	$l - CH_3SH$
231	275	0	3	100	100	*m*
199		0		41		$m - \begin{cases} CH_3OH \\ TMSiOH \end{cases}$
	185		0		52	
VIb	VIIb	VIb	VIIb	VIb	VIIb	
518	562					M^+
503	547	2	5	0.6	1.5	$M^+ - CH_3$
471	515	3	6	0.6	1.1	$M^+ - CH_3S$
404	448	3	6	3.6	4.5	*n*
284	328	0	3	8.5	4.3	*o*
231	275	0	3	10	5.3	*m*
187		3		100		
199		0		5.0		$m - \begin{cases} CH_3OH \\ TMSiOH \end{cases}$
	185		0		3.0	

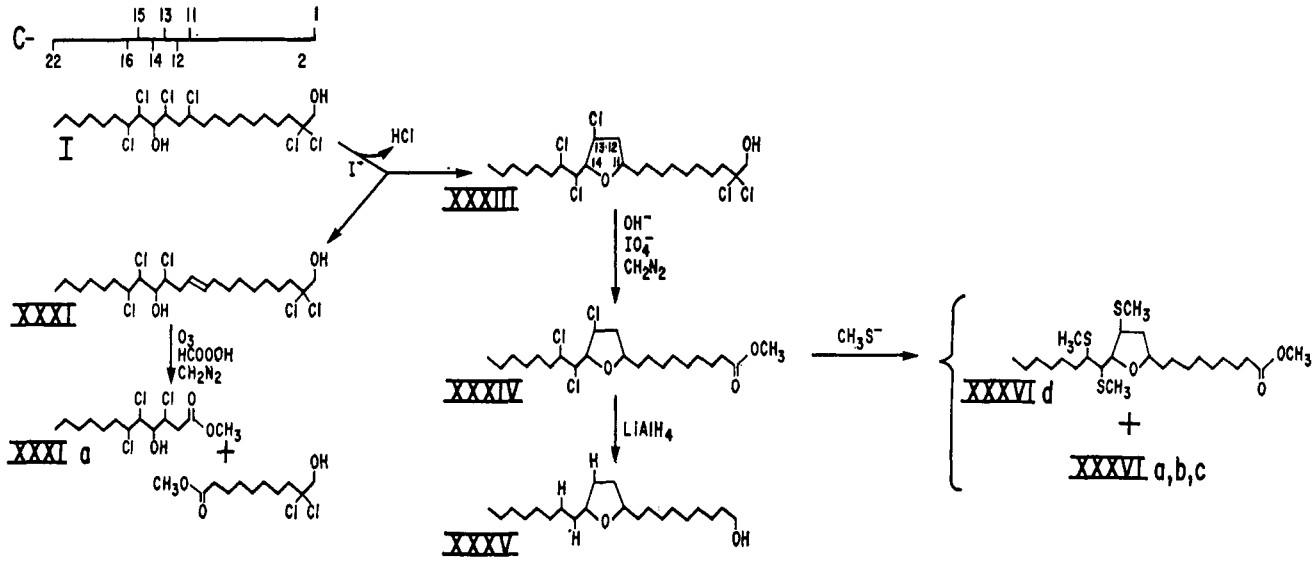
[a–d] Compare Table I.

tography–mass spectrometry as their TMSi ethers and acetates. In both cases more than 80% of the volatile product chromatographed as a single component. Their mass spectra (Table V and Figure 7) showed the molecular weights expected for these products, but the position of the thioether substituents was not shown by any abundant α-cleavage ions. However, for the methylmercaptyl product the most abundant species in both the TMSi and acetate derivative, except for the trivial TMSi and acetyl cations, was an odd-electron rearrangement ion at *m/e* 102. This ion, which did not contain TMSi or acetate groups, was absent in the ethylmercaptyl derivatives, where it appeared instead at *m/e* 116. The formation of these ions, which must incorporate the thioether group and four carbons from the parent molecule, is suggested in Figure 7 as a McLafferty-type β-cleavage involving a C-11 thioether on the third carbon from the distal end of these compounds.

FORMATION OF XIX–XXI (SCHEME II). The remaining periodate oxidation products of XVI were oxidized with chromic acid, methylated with diazomethane, and purified by thin-layer chromatography in 100% benzene to give XIX, containing 3.8 μmoles of ^{36}Cl. The mass spectrum (Table III) was consistent with a molecular weight of 292 as expected for the methyl ester of a twelve-carbon monochlorodicarboxylic acid derived from C-2 to C-13. The complete absence of a chlorine-containing peak at *m/e* 108 showed that the chlorine was not α to one of the carboxyls, while the intense ion at *m/e* 225 and the chlorine-containing ion at *m/e* 219, could be consistent with a β-chloro ester. Treatment of this material in 0.05 ml of acetone with 0.2 ml of 2 M KOH at 80° for 30 min quantitatively liberated $^{36}Cl^-$. The ether-soluble products were oxidized by the von Rudloff (1956) procedure. After acidification and reduction of excess reagent with sodium bisulfite the mixture was extracted five times with ether, the extracts were treated with diazomethane, evaporated to dryness, and the residue was triturated five times with small portions of dry ether. The ether-soluble material showed two peaks on gas–liquid chromatography, XX and XXI, in a ratio of 1:6, with retention times and mass spectra identical with those of standard dimethyl nonanedioate and dimethyl decanedioate. Thus, the product formed in the alkali treatment of XIX was predominantly the α,β-unsaturated dodecanedioate, with smaller amounts of the β,γ isomer, in complete agreement (Johnson *et al.*, 1951) with the assignment of the chlorine in XIX to C-11, *i.e.*, the β-carbon from the distal end. The identity of the products XX and XXI with the straight-chain standards also precluded branching between C-2 and C-11.

PERIODATE TREATMENT OF *vic*-GLYCOLS VIIId. The mixture of VIIId-1 and VIIId-2, containing 10 μmoles of ^{36}Cl, was also oxidized with periodate–chromic acid, and the methyl esters of the products separated on thin-layer chromatography in 100% benzene. The mass spectrum of the material XXII with an R_F of 0.65 (Table III and Figure 5) was consistent with the presence of two chlorines per a molecular weight of 240, as expected for the monocarboxylic ester formed from C-14 to C-22 of VIIId-2. The absence of a significant peak at *m/e* 74, together with the intense chlorine-containing ion at *m/e* 108 showed that one of the chlorines indeed was on the C-15 α position; the material was lost before the position of the

SCHEME III: Characterization of Products Formed from 2,2,11,13,15,16-Hexachloro-*n*-docosane-1,14-diol (I) by Treatment with Sodium Iodide.



second chlorine, presumably on the C-16 β-carbon, could be established. Two dicarboxylic esters with R_F's of 0.37 and 0.25 had mass spectra indistinguishable from those of Xb and XIX, respectively, and presumably represented the C-14 to C-2 and C-13 to C-2 proximal portions of VIIId-1 and -2.

Methyl Mercaptide Treatment of (Epoxy)nor Compounds VI and VII. The presence of the chlorine on C-11 was also demonstrated by direct treatment of VI and VII, containing 6 μmoles of ^{36}Cl each, with methyl mercaptide at 100° for 12 hr, with complete release of $^{36}Cl^-$. In both cases about 80% of the trimethylsilylated product appeared in two major peaks of approximately equal size with relative retention times of about 1.15 (TMSi VIa and TMSi VIIa) and 1.45 (TMSi VIb and TMSi VIIb) compared with those at the starting materials, VI and TMSi VII. The mass spectra of all four products showed molecular weights equivalent to the same net substitution of two methylmercaptyl groups, one hydroxyl as the TMSi ether and one double bond for the three chlorines and one epoxide group originally present in VI and TMSi VII. The new C-11 methyl mercaptide group gave rise to the base peaks at *m/e* 231 and 275 in the spectra of TMSi VIa and TMSi VIIa, respectively (Table VI and Figure 8). These assignments were supported by further loss of methanol and TMSiOH, respectively, from the C-2 ends of these ions to give the intense peaks at *m/e* 199 and 185, as evidenced by the appropriate metastable ions. The most abundant ions beyond the base peaks indicated a C-13 TMSi ether group in these compounds. The ions due to the C-11 methylmercaptyl group also appeared in the spectra of the second pair of products, TMSi VIb and TMSi VIIb, though of lower abundance, while the TMSi-containing base peak for both these molecules at *m/e* 187 only could represent a C-22- to C-16-OTMSi fragment. Significant odd-electron rearrangement ions would be consistent with the presence of a second methylmercaptyl group on C-15: ion *n* (Table VI and Figure 8) presumably was formed by transfer of the C-16 TMSi group to the C-15 methylmercaptyl sulfur with expulsion of C-16 to C-22 as a molecule of heptanal; fragmentation between C-16 and C-15 with hydrogen transfer to the proximal fragment and expulsion of the C-11 substituent as methylmercaptan would give the odd-electron ion *o*, analogously to the formation of the two principal ions in the spectra of VIIIc and -e. No attempt was made to establish the complete structure of VI and VIIa and -b. Nucleophilic attack by mercaptide ions on the C-14,-

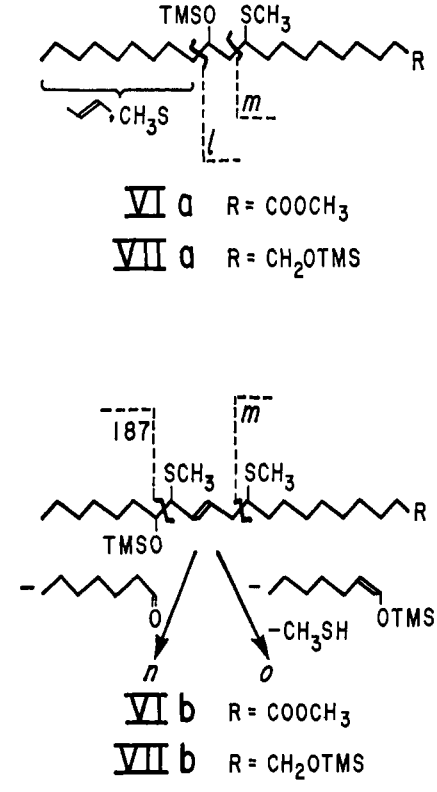


FIGURE 8: Proposed partial structures and fragmentations for the products obtained by methylmercaptan treatment of VI and VII. Compare Table VI.

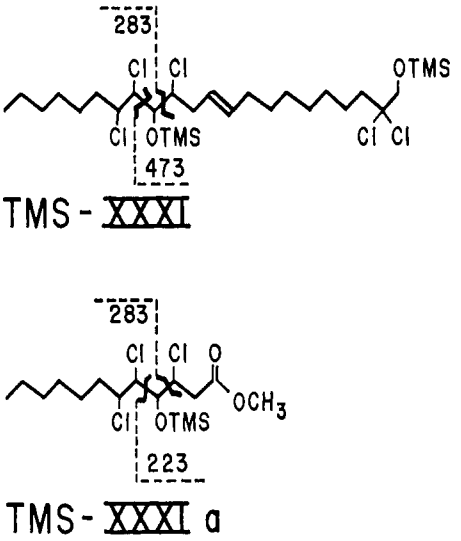


FIGURE 9: Proposed fragmentation of TMSi XXXI and TMSi XXXIa. Compare Table VII and Scheme III.

C-15-epoxide in VI and VII followed by formation and opening of secondary epoxides involving the chlorines on C-13 or C-16 could presumably account for the hydroxyl group found on these positions in the products. The mechanism for the formal loss of Cl_2 or dimethyl disulfide (rather than two HCl or two methylmercaptans) which is required for formation of only one double bond in these products, is unknown. This does not invalidate the compeling mass spectrometric evidence for the presence of a methylmercaptyl group on C-11 in these products, and the results are presented as independent support for the position of the C-11 chlorine atom in the original hexachlorodocosanediol. Furthermore, the mass spectra of TMSi VI and VIIa and -b did not indicate chlorine substitution on any positions other than those already established by the stepwise degradation, *e.g.*, C-11, C-13, C-15, and C-16.

Iodide Treatment of Hexachlorodiol I (Scheme III). Dehydrochlorination to Unsaturated Pentachlorodiol XXXI and Substituted Tetrahydrofuran XXXIII. The rate of reaction of the chlorine atoms in I with iodide ions was slow, but when I, containing 450 μmoles of ^{36}Cl, was treated with 350 mg of dry sodium iodide in 2.5 ml of dry acetone at 105° for 29 hr, 61 μmoles of $^{36}Cl^-$, were released. Thin-layer chromatography in benzene–chloroform–methanol (100:60:5, v/v) gave 263 μmoles of ^{36}Cl in the major product, XXXIII, R_F 0.65 (Scheme III). A minor fraction, R_F 0.5, contained 83 μmoles of ^{36}Cl; gas–liquid chromatography–mass spectrometry of its TMSi derivative showed that it contained about 60% of a new compound, TMSi XXXI, with a retention time of 0.63 relative to that of unchanged TMSi I, which constituted about 20% of the fraction. The mass spectrum of TMSi XXXI (Table VII) showed the presence of five chlorines and two TMSi groups per a molecular weight of 654, equivalent to loss of HCl from TMSi I, with the double bond proximal to C-14. An indication of its position was obtained by oxidative ozonolysis (Klenk and Bongard, 1952). After methylation and trimethylsilylation analysis by gas–liquid chromatography–mass spectrometry showed complete disappearance of XXXI with formation of several much more volatile products. The most abundant of these, TMSi XXXIa, eluted at about 165° and had a mass spectrum (Table VII and Figure 9) consistent with the structure expected for the methyl ester of a carboxylic acid formed from the distal portion of XXXI by cleavage of a C-10,C-11 double bond. A smaller component which eluted at about 150° showed the presence of one TMSi ether and two chlorines per a molecular weight

TABLE VII: Important Ions in the Mass Spectra of TMSi XXXI and TMSi XXXIa (*cf.* Figure 9 and Scheme III).

	m/e	Cl_n[a]	$(C^2H_3)_n$[b]	Rel Ab (%)[c]	Assignment[d]
TMSi	654	5	6	0.5	M^+
XXXI	639	5	5	0.2	$M^+ - CH_3$
	618	4	6	0.9	$M^+ - HCl$
	473	3	6	1.3	
	437	2	6	16	473 − HCl
	283	2	3	17	
TMSi	404				M^+
XXXIa	389	3	2	2.2	$M^+ - CH_3$
	373	3	3	3.4	$M^+ - CH_3O$
	283	2	3	27	
	223	1	3	100	

[a–d] Compare Table I.

TABLE VIII: Important Ions in the Spectra of TMSi XXXIII, XXXIV, and TMSi XXXV (*cf.* Figure 10 and Scheme III).

	m/e	Cl_n[a]	$(C^2H_3)_n$[b]	Rel Ab (%)[c]	Assignment[d]
TMSi	582				M^+
XXXIII	567	5	2	0.8	$M^+ - CH_3$
	546	4	3	3.0	$M^+ - HCl$
	401		3	2.2	
	293	2	0	20	401 − TMSiCl
	103	0	3	100	
XXXIV	456				M^+
	425	3		1.3	$M^+ - CH_3O$
	421	2		1.0	$M^+ - Cl$
	349	0		16	421 − 2HCl
	285	3		3.0	
	275	1		39	
	249	2		4.5	285 − HCl
	243	1		34	275 − CH_3OH
	207	0		24	243 − HCl
	55	0		100	C_4H_7
TMSi	398	0	3	3.7	M^+
XXXV	383	0	2	3.1	$M^+ - CH_3$
	285	0	3	68	
	197			0.9	
	183	0	0	78	

[a–d] Compare Table I. [e]

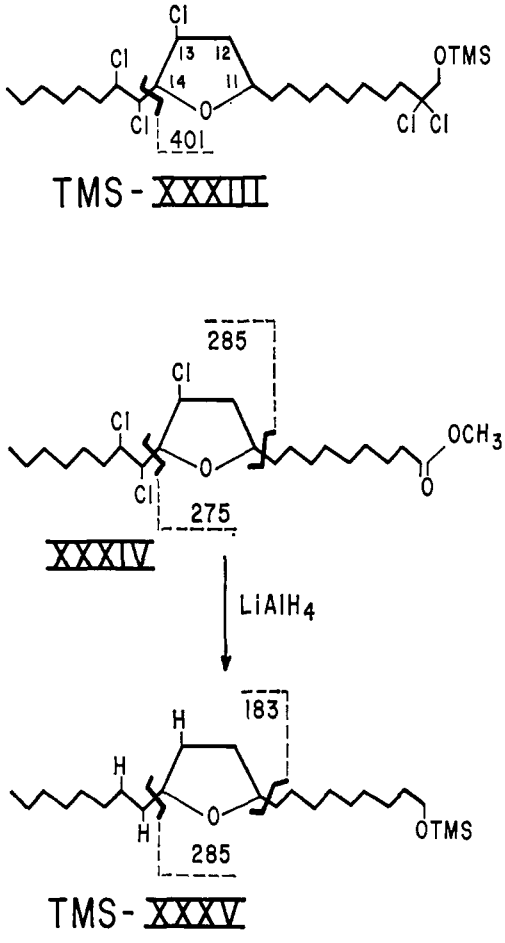


FIGURE 10: Proposed fragmentation of TMSi XXXIII, XXXIV, and TMSi XXXV. Compare Table VIII and Scheme III.

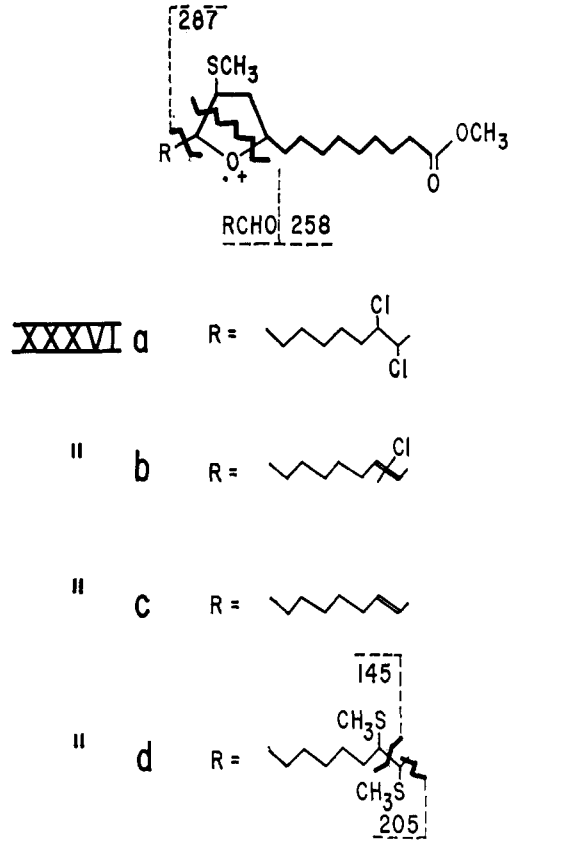


FIGURE 11: Proposed fragmentation of XXXVIa, -b, -c, and -d. Compare Table IX and Scheme III.

of 342 as expected for the methyl ester of the carboxylic acid formed from the proximal portion of XXXI.

Formation of (Substituted Tetrahydrofuran)nor Acid Methyl Ester XXXIV. The TMSi derivative of the major product, XXXIII, had a mass spectrum (Table VIII and Figure 10) consistent with five chlorines and one TMSi group per a molecular weight of 582, the same overall composition as TMSi II. However, an intense peak at *m/e* 293, which contained two chlorines and no TMSi group, indicated a cyclic ether structure extending from C-14 to C-11 or C-13. Alkali treatment of XXXIII, containing 190 μmoles of ^{36}Cl, as described above for II, gave 80 μmoles of ^{36}Cl in a 2-keto-1-ol; periodate oxidation and methylation gave 68 μmoles of ^{36}Cl in XXXIV, the methyl ester of the noracid. Its mass spectrum (Table VIII and Figure 10) was consistent with the expected three chlorines per a molecular weight of 456. Cleavage between C-15 and C-14 gave the proximal fragment as an intense ion at *m/e* 275, which lost methanol and hydrogen chloride to give intense ions at *m/e* 243 and 207, as shown by the appropriate metastable ions. The weak ions at *m/e* 285 and 249 were consistent with a substituted tetrahydrofuran structure, with the oxygen bridge between C-14 and C-11. Such a structure, rather than a C-14,C-13-epoxide, was supported by the refractility of XXXIV to acetolysis under conditions which opened the epoxide ring in VI. To obtain direct evidence for the five-membered ring structure, a portion of XXXIV was completely dechlorinated by refluxing with lithium aluminum hydride in tetrahydrofuran for 2 hr. A large number of products were formed which had undergone ring opening with formation of a secondary hydroxyl and/or double bonds as judged by the mass spectra of their TMSi ethers. However, the mass spectrum of the single largest component, XXXV (Table VIII and Figure 10), which represented about one-fourth of the material, was consistent with that of the expected product: the position of the C-11,C-14-substituted tetrahydrofuran ring was conclusively shown by the two intense ions at *m/e* 285 and 183, the α-cleavage ions with charge retention on the ring oxygen. The absence of a significant fragment at *m/e* 197 also excluded the presence of an isomeric C-14,C-10-substituted tetrahydropyran structure.

Methyl Mercaptide Treatment of XXXIV. Treatment of XXXIV with methyl mercaptide at 100° for 12 hr gave about equal amounts of three major products, XXXVIa, -b, -c, and small amounts of a fourth product, XXXVId, with retention times on gas–liquid chromatography of 1.53, 0.95, 0.65, and 2.34, respectively, relative to that of XXXIV. The mass spectra and proposed structures for these compounds are summarized in Table IX and Figure 11. All four compounds showed a prominent ion at *m/e* 287, corresponding to the proximal fragment after cleavage between C-14 and C-15, with a methyl mercaptide group substituted for the ring chlorine on C-13; loss of methanol and methylmercaptan from this fragment

TABLE IX: Important Ions in the Mass Spectra of XXXVIa, -b, -c, and -d (*cf.* Figure 11 and Scheme III).

m/e				Rel Ab (%)[c]				
a	b	c	d	a	b	c	d	Assignment[d]
468 (Cl_2)	432 (Cl_1)	398	492	1.4	7.0	7.0	4.2	M^+
		350	444			7.5	44	$M^+ - CH_3SH$
397				20				$M^+ -$ (Cl + HCl)
	397				26			$M^+ -$ Cl
			397				56	$M^+ -$ ($CH_3S + CH_3SH$)
	287			100	7	12	32	
	258			14	15	36	3	
	255			47	5	5	27	287 − CH_3OH
	243			11	21	22	3	258 − CH_3
	210			40	78	100	9	258 − CH_3SH
	207			12			7	255 − CH_3SH
	178			31	71	78	6	210 − CH_3OH
			158				63	(M^+ − 287) + H − CH_3SH
			145				100	

[a–d] Compare Table I.

gave secondary ions at *m/e* 255 and 207, accompanied by the appropriate metastable ions. All four compounds also showed a series of odd-electron ions derived from the proximal part of the molecule. The primary fragment at *m/e* 258 would arise by transannular cleavage between C-13 and C-14 and between C-11 and the ring oxygen, with expulsion of the distal fragment as the appropriately substituted nonanal. Further expulsion of the methylmercaptylmethyl radical gave the even-electron ion at *m/e* 243, while sequential expulsion of methylmercaptan and methanol gave rise to intense odd-electron ions at *m/e* 210 and 178; all these secondary reactions gave metastable ions at the appropriate positions. In XXXVIa, -b, and -c the tentative structure beyond C-14 was only inferred from the molecular weight and number of chlorines per molecule, taking the C-15,C-16 *vic*-dichloride structure of XXXIV for granted; formation of only one double bond in this portion of the molecule in XXXVIc required the same elimination of Cl_2 or dimethyl disulfide, rather than simple loss of two HCl or two methylmercaptans, as mentioned above for VI and VII, a and -b. However, in XXXVId the position of the new substituents on C-15 and C-16 was directly verified by the fragmentation pattern. The base peak at *m/e* 145 corresponded to the distal fragment of the molecule after cleavage between C-15 and C-16, while the intense odd-electron rearrangement ion at *m/e* 158 would be formed by cleavage between C-14 and C-15 with hydrogen transfer to the distal fragment and expulsion of the substituent on C-16 as a molecule of methylmercaptan.

Discussion

Linear molecules such as the hexachlorodocosanediol and its various derivatives are obviously well suited for structural analysis by mass spectrometry, and the spectrum of the original compound directly showed the presence of the secondary hydroxyl on C-14 and the distribution of four chlorines proximal and two distal to that carbon (Elovson and Vagelos, 1969). However, since the electronegative chlorines do not promote α cleavage at the carbon on which they are located (McLafferty, 1962), their exact positions could only be determined after suitable substitution. The work presented here was not intended as a systematic investigation of the mass spectrometric characteristics of the many compounds analyzed. Rather, the structural evidence obtained by mass spectrometry was used to guide a stepwise degradation of the starting material which permitted assignment of six chlorines to six positions on the carbon chain. Still, although the medium resolution spectrometer in principle did not permit absolute ion assignments, the internal consistency of the data and the general agreement with studies by others (Capella *et al.*, 1968; Diekman *et al.*, 1968; Budzikiewicz *et al.*, 1967; Esselman and Clagett, 1969; Samuelsson and Samuelsson, 1969) seemed sufficient to include spectra from other derivatives which were less completely characterized chemically.

In the stepwise degradation of I the docosanediol backbone was cleaved adjacent to all chlorine-substituted carbons except C-16. The presence of the most distal chlorine on that position was conclusively established when it was made the α-carbon in Xa. First, the McLafferty rearrangement ion from that compound, which only incorporates the carboxyl and α-carbons, contained the chlorine atom; the equivalent fragment was also found in the spectrum of the synthetic α-bromo analog of Xa.[2] Second, as expected for an α-halo acid (Johnson *et al.*, 1951), alkali treatment of Xa yielded the α-hydroxy acid rather than an unsaturated product. The presence of a chlorine atom on C-16 was also clearly indicated by the substitution of hydroxyl and methylmercaptyl groups on that position in VIb and VIIb, and XXXVId, respectively.

[2] Unpublished experiments.

In the stepwise degradation of I, the position of the C-11-chlorine was established by its reactivity to alkali in the C-2- to C-13-dicarboxylic fragment XIX, clearly that of a β-substituted halogen (Johnson *et al.*, 1951); this was also supported by the mass spectral analysis of XVIIIa and -b, which indicated a C-11-chlorine in XVII, the diol equivalent of that fragment. Mass spectrometry also conclusively showed a C-11 methylmercaptyl group in the major products formed by direct methyl mercaptide substitution on the C-2- to C-22-noracid and alcohol VI and VII. Further evidence for this chlorine position was obtained from the iodide substitution experiment. The cyclic ether formed in that reaction was completely resistent both to the acid-catalyzed acetolysis and vigorous substitution with mercaptide which opened the epoxide ring in VI, the product of alkali treatment. Since a C-14,C-12-substituted trimethylene oxide structure would be sensitive to acid (Pritchard and Long, 1958), the remaining alternatives would be a C-14,C-11-tetrahydrofuran or C-14,C-10-tetrahydropyran structure. These should be equally susceptible to ring opening in the treatment with hot lithium aluminum hydride; however, although moderate yield of the substituted tetrahydrofuran XXXV was obtained in that procedure, there was no formation of the equivalent tetrahydropyran product.

The position of the C-13-chlorine was demonstrated by formation of the C-13,C-14-*vic*-glycol XVI from the terminal chlorohydrin XV. It was also supported by the C-13,C-14-*vic*-glycol structure in VIIId-2, which showed that small amounts of C-13,C-14-epoxide were formed in the alkali treatment of I.

The yield of III in the alkali treatment of I was about 70%. The conversion probably involved a C-1,C-2-epoxy,C-2-chloro intermediate[2] (Kirrmann *et al.*, 1964). If atmospheric oxygen was not excluded, considerable amounts of a new acidic product were formed. The TMS derivative of its methyl ester had a molecular weight of 558, and since brief treatment with lithium aluminum hydride reduced it to a compound indistinguishable from IV, its structure could only be that of a 22-carbon α-hydroxy acid, presumably formed by auto-oxidation of III.[2] Besides III about 5% of the noracid V was directly formed in the extended alkali treatment of I by an unknown mechanism which did not require the presence of atmospheric oxygen.

The acetolysis of VI was designed to open any epoxide ring in that compound, and 80% of the products were recovered in VIIIc, -e, and -d-2, which showed the expected new *vic*-glycol grouping. The C-13,C-14-*vic*-glycol in VIIId-2 was clearly indicated by the fragmentation pattern of its TMSi derivative, and was conclusively established by oxidative cleavage. Although the distal and proximal fragments from that reaction were not completely degraded, their mass spectra agreed with the C-11,15,16-trichloro structure for VIIId-2. The structure of VIIIe, the major product from VI, was unambiguously shown by the stepwise degradation. A plausible structure for VIIIc is that of a diasteromer of VIIIe, less polar than the latter on thin-layer chromatography, and more volatile on gas–liquid chromatography. Diasteromeric products should be formed in the acetolysis of VI, since nucleophilic attack on the epoxide ring can occur on either C-14 or C-15, and the adjacent carbons are asymmetric. Depending on the unknown relationship between the configuration of the chlorines on C-13 and C-16 and the epoxide ring, one or the other product could also be favored, to account for the fact that VIIIc and -e were formed in a ratio of about 1:3. There can be no doubt that VIIIc had a C-13,C-16-dichloro-C-14,C-15-*vic*-glycol structure. The mass spectrum of its TMSi derivative was almost completely identical with that of VIIIe, with striking odd-electron ions. On mild alkali treatment VIIIc lost two-thirds of its chlorine with formation of two intramolecular cyclic ethers involving the two glycol hydroxyls, while acid hydrolysis of this compound gave a major component, the TMSi derivative of which had a mass spectrum consistent with that of a C-13,14,15,16-tetrahydroxy product of VIIIc.[2] The unexpected resistance of VIIIc to periodate oxidation cannot be explained at present. Perhaps that isomer of the crowded C-14,C-15-dihydroxy-C-13,C-16-dichloro structure was sterically hindered in forming the *cis*-diester intermediate in periodate oxidation (Bunton, 1965) as well as being less free to interact with the stationary phases on absorption and gas–liquid chromatography. These questions will presumably be resolved when the configuration around the asymmetric carbon atoms becomes known.

Since no evidence was found for more than six loci of chlorine substitution in the hexachlorodocosanediol, it appears to be predominantly a single positional isomer. The nature of the reactions leading to the final degradation products XI and XXI, and the identity of these with their respective straight-chain synthetic standards also precluded branching of the hydrocarbon backbone. Thus, the compound analyzed here had the 2,2,11,13,15,16-hexachloro-*n*-docosane-1,14-diol structure present in the cell as the disulfate (Elovson and Vagelos, 1969). The unchlorinated diol has previously been shown to have the unbranched *n*-docosane structure (Mayers *et al.*, 1969).

The six aliphatic chlorines in I could be distinguished by their different chemical reactivity. Under mild alkali conditions epoxide formation occurred more readily with displacement of the chlorohydrin chlorine on C-15 than of that on C-13; epoxide formation involving the *gem*-chlorines on C-2 required more vigorous conditions and went on to formation of the 2-keto group under the conditions used. Of the two nonchlorohydrin chlorines on C-16 and C-11 the latter was also the only one on an isolated position; the fact that it alone reacted with iodide ion at a significant rate indicated that the C-16 and the chlorohydrin chlorines were more hindered.

Inspection of models of chlorosulfolipids suggests that these strongly amphiphilic molecules would tend to a globular rather than extended conformation at interphases, with the two sulfates close together facing the aqueous solution, and the proximal and distal hydrocarbon loop and tail fitting together to form the hydrophobic back surface of the molecule. In this configuration all six chlorines in the hexachloro compound, including the isolated position of C-11, would be at the polar surface of the molecule. The significance of this for the biosynthesis and function of these unusual halogen-containing molecules remains to be clarified.

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References

Aaronson, S., and Baker, H. (1959), *J. Protozool. 6*, 282.

Bray, G. A. (1960), *Anal. Biochem. 1*, 279.

Budzikiewicz, H., Djerassi, C., and Williams, D. (1967), Mass Spectrometry of Organic Compounds, San Francisco, Calif., Holden-Day, p 471.

Bunton, C. A. (1965), *in* Oxidation in Organic Chemistry, Part A, Wiberg, K. E., Ed., New York, N. Y., Academic, p 367.

Capella, P., Galli, C., and Fumagalli, R. (1968), *Lipids 3*, 431.

Clark, H. T., and Taylor, E. R. (1964), *in* Organic Synthesis, Coll. Vol. 1, 2nd ed, New York, N. Y., Wiley, p 115.

Diekman, J., Thomson, J. B., and Djerassi, C. (1968), *J. Org. Chem. 33*, 2271.

Elovson, J., and Vagelos, P. R. (1969), *Proc. Nat. Acad. Sci. U. S. 62*, 957.

Esselman, W. J., and Clagett, C. O. (1969), *J. Lipid Res. 10*, 234.

Gasson, E. J., Graham, A. R., Millidge, A. F., Robson, K. M., Webster, W., and Young, P. (1954), *J. Chem. Soc.*, 2170.

Haines, T. H., Pousada, M., Stern, B., and Mayers, G. L. (1969), *Biochem. J. 113*, 565.

Johnson, A. W., Dalgliesh, C. E., and Walker, J. (1951), *in* Chemistry of Carbon Compounds, Vol. 1, Rodd, E. H., Ed., New York, N. Y., Elsevier, p 618.

Kirrmann, A., Duhamel, P., and Mouri Bimorghi, M. R. (1964), *Bull. Soc. Chim. Fr.*, 3264.

Klenk, E., and Bongard, W. (1952), *Hoppe-Seyler's Z. Physiol. Chem. 290*, 181.

Mayers, G. L., and Haines, T. H. (1967), *Biochemistry 6*, 1665.

Mayers, G. L., Pousada, M., and Haines, T. H. (1969), *Biochemistry 8*, 2981.

McCloskey, J. A., Stillwell, R. N., and Lawson, A. M. (1968), *Anal. Chem. 40*, 233.

McLafferty, F. W. (1959), *Anal. Chem. 31*, 82.

McLafferty, F. W. (1962), *Anal. Chem. 34*, 2.

Pritchard, J. G., and Long, F. A. (1958), *J. Amer. Chem. Soc. 80*, 4162.

Ryhage, R., and Stenhagen, E. (1963), *in* Mass Spectrometry of Organic Ions, McLafferty, F. W., Ed., New York, N. Y., Academic, p 399.

Samuelsson, B., and Samuelsson, K. (1969), *J. Lipid Res. 10*, 41.

Sharkey, A. G., Friedel, R. A., and Langer, S. H. (1957), *Anal. Chem. 29*, 770.

Szymanski, H. A. (1967), Interpreted Infrared Spectra, Vol. 3, New York, N. Y., Plenum Press, p 155.

von Rudloff, E. (1956), *Can. J. Chem. 34*, 1413.