Structure of cryptosporiopsin: a new antibiotic substance produced by a species of *Cryptosporiopsis*[1]

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Received November 13, 1968

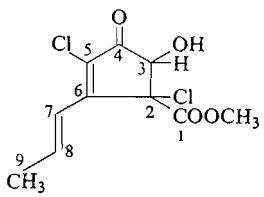
The structure of cryptosporiopsin, a novel chlorine-containing antifungal agent produced by a species of *Cryptosporiopsis*, has been elucidated on the basis of spectral and chemical studies.

Canadian Journal of Chemistry, 47, 2087 (1969)

It was observed by Stillwell (1) that antifungal properties were associated with *Cryptosporiopsis* sp., an imperfect fungus isolated from yellow birch, *Betula alleghaniensis* Britt. Isolation from culture filtrates of the organism of a pure crystalline metabolite has recently been reported by Stillwell *et al.* (2). This compound, which was named cryptosporiopsin, was found to inhibit *in vitro* the growth of a wide variety of microorganisms (2).

In this paper, evidence is presented which allows the assignment of the structure **1** to cryptosporiopsin.

The metabolite was obtained as colorless crystals from *n*-hexane – methylene chloride, m.p. 133–137°, $[\alpha]_D^{25}$ +129°, (*c*, 1.35, chloroform). The molecular formula was established as $C_{10}H_{10}O_4Cl_2$ by elemental analysis and high resolution mass spectrometry. The infrared



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spectrum (KBr disk) of cryptosporiopsin exhibits bands at 1755 and 1730 cm^{-1}, attributed respectively to ester and conjugated five-membered ketone absorption. The relatively high frequency of both peaks is a consequence of chlorine substitution in the α-positions (3). A shoulder at 1737 cm^{-1} may be due to a rotational isomer of the chloroester in which the carbonyl group and the chlorine are staggered. The spectrum, measured at several concentrations in carbon tetrachloride, shows a strong sharp peak at 3500 cm^{-1} attributed to an intramolecularly hydrogen-bonded hydroxyl function. The carbonyl absorptions coalesce in the solution spectrum to a single sharp band at 1746 cm^{-1}.

The high resolution mass spectrum (see Experimental) shows, besides the molecular ion, peaks corresponding to loss from the latter of H_2O, Cl, $COOCH_3$ (base peak), and $COOCH_3$ plus CO.

The nuclear magnetic resonance (n.m.r.) spectrum of cryptosporiopsin (Fig. 1) shows a three-proton singlet at 6.12 τ attributable to the methyl ester hydrogens. A one-proton singlet at 5.31 τ slowly diminishes on addition of deuterium oxide and thus is assigned to the bonded hydroxyl hydrogen. A similar signal at 5.43 τ is shifted downfield to 4.91 τ on acetylation of the antibiotic, and clearly corresponds to the α-proton of a *secondary* alcohol. A three-proton doublet, centered at 7.99 τ (J = 6.2 c.p.s.) and a two-proton multiplet between 2.75 and 3.66 τ constitute an ABX_3-type pattern (4), in accord with the partial structure **2** (J_{AB} = 16.9 c.p.s.; no 1:3 coupling is evident). This assignment was corroborated by spin decoupling.

The metabolite exhibits an ultraviolet chromophore λ_{max}(95% EtOH) at 292 mμ (ε 22 800) indicating a system possessing extended conjugation, such as **3** (*vide infra*). The addition of alkali causes a negligible shift of the chromophore but gives rise to a time-dependent irreversible diminution of the extinction coefficient, demonstrating the lack of stability of the antibiotic in this medium. Since only two vinyl hydrogens appear in the n.m.r. spectrum, R_1 and R_2 must be alkyl substituents or chlorine atoms.

The molecular formula shows that the compound possesses five sites of unsaturation, four

[1]Presented at the Joint Meeting on the Chemistry of Natural Products, Fredericton, New Brunswick, August 1968.

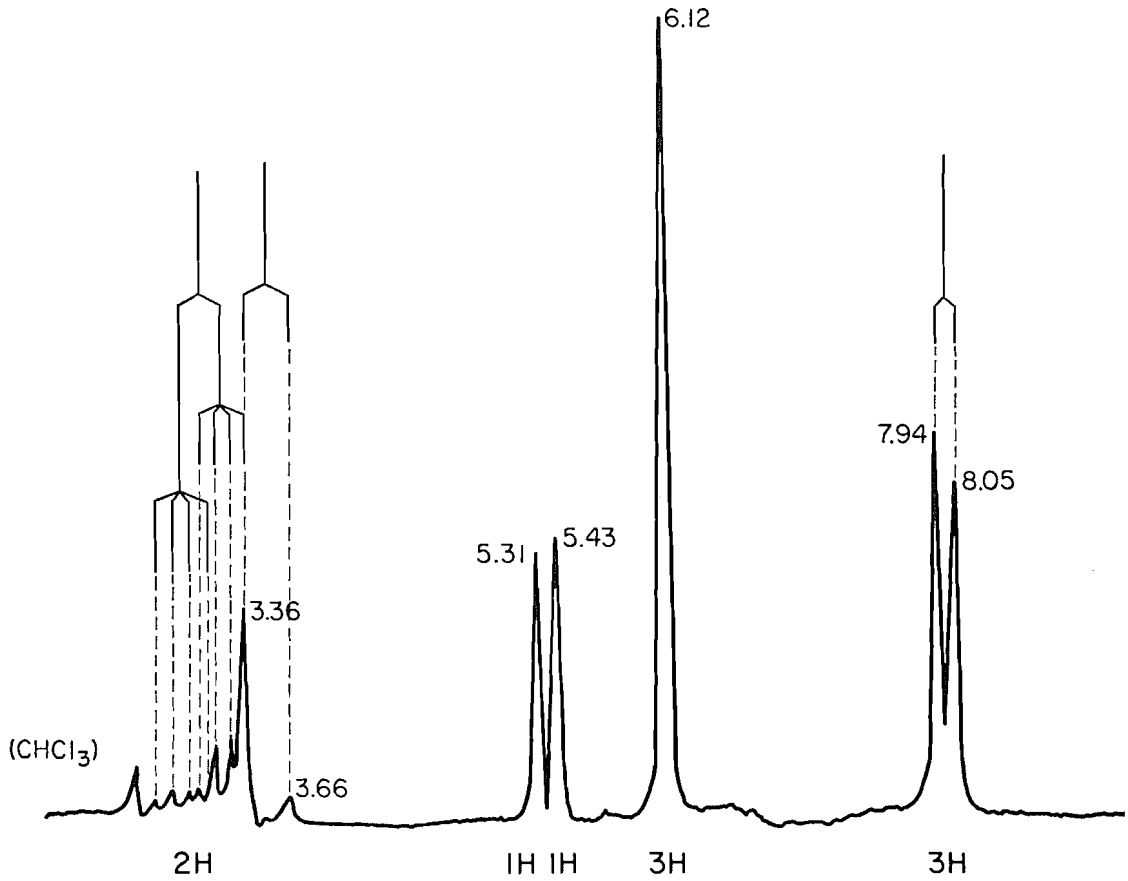
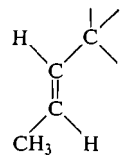


FIG. 1. The 56.4 Mc.p.s. nuclear magnetic resonance spectrum of cryptosporiopsin.

of these being accommodated in the ketone, ester, and double bonds; a ring accounts for the remaining unsaturation. The spectral data and degradative evidence (*vide infra*) eliminate structures possessing a conjugated ester group, and the remaining possiblities consistent with the cited information are represented in the partial formulae **4** and **5**.

Some structural features of the mould metabolite terrein **6** (5) suggested that the latter might be a useful model for spectroscopic comparison with cryptosporiopsin. Terrein shows ultraviolet absorption λ_{max}(EtOH) at 276 mμ (ε 25 000). The presence of a chlorine atom at the α-position would be expected to shift the maximum to 291 mμ (6), which coincides with the observed chromophore of cryptosporiopsin. Furthermore, infrared absorption (KBr disk) at 1635 and 1570 cm^{-1}, associated with the double bond system of cryptosporiopsin is also present in the terrein spectrum, as well as a peak at 965 cm^{-1} for the *trans* double bond (3).

Reduction of cryptosporiopsin with zinc in refluxing ethanol yielded an amorphous product, *m/e* 244, assigned the structure **7**. The relative abundance of the $M^+ + 2$ isotope peak demonstrates that **7** contains a single chlorine atom. The ultraviolet absorption λ_{max}(95% EtOH) at 285 mμ (ε 22 000) shows that the original chromophore is essentially unchanged. The presence of an *ethyl* ester is manifested in the n.m.r. spectrum, and in the mass spectral peak at *m/e* 171 (base peak) corresponding to the loss of the $COOC_2H_5$ fragment. The infrared spectrum (CCl_4) of **7** reveals again the presence of an internally-bonded hydroxyl group, and the carbonyl absorptions, in the absence of the chlorine α to the ester, now appear as a single band at 1730 cm^{-1}.



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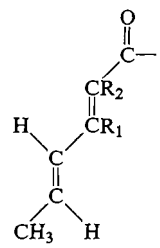

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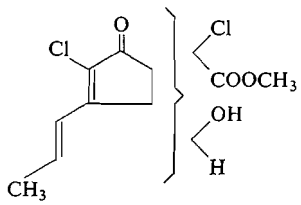

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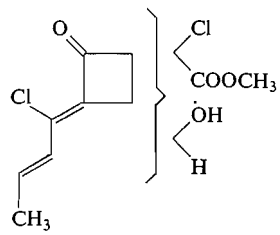

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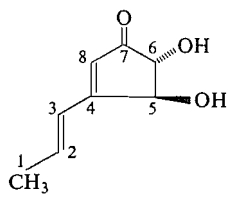

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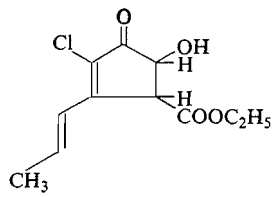
A dihydro product **8**, $C_{10}H_{12}O_4Cl_2$, *m/e* 266, was isolated from hydrogenation of cryptosporiopsin in ethyl acetate with a palladium-on-charcoal catalyst. Infrared absorption (KBr disk) at 1752 and 1738 cm^{-1} accounts for the chloroester and ketone respectively, while the double bond gives rise to a band at 1618 cm^{-1}. The hydroxyl shows again as a sharp peak at 3500 cm^{-1} in the spectrum measured in carbon tetrachloride solution. Ultraviolet absorption λ_{max} (absolute EtOH) at 244 mμ (ε 8300) agrees well with the predicted chromophore (6). The n.m.r. spectrum displays, *inter alia*, a two-proton multiplet at 7.35–7.61 τ, attributed to the allylic hydrogens of **8**. The methyl group appears as a poorly resolved triplet at 9.03 τ and no vinyl hydrogens are in evidence.

When hydrogenation with palladium-on-charcoal was conducted in ethanol, absorption ceased abruptly after uptake of 4 moles of hydrogen, and an oily product, *m/e* 182, corresponding to $C_{10}H_{14}O_3$ was obtained. Hydroxylic absorption is absent in the infrared spectrum



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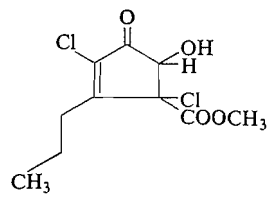

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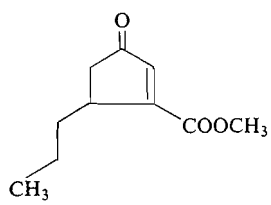
(CCl_4), and in addition to the carbonyl band at 1725 cm^{-1}, the spectrum surprisingly shows double bond absorption at 1610 cm^{-1}. Furthermore, the substance exhibits an ultraviolet maximum at 237 mμ (ε 10 000). This spectroscopic behavior can be readily interpreted in terms of the structure **9**. The primary reduction product is evidently the saturated hydroxy keto ester, which undergoes spontaneous dehydration on work-up.

Further support for structure **9** is found in the n.m.r. spectrum (4), in which the single vinyl hydrogen appears as a narrow doublet at 3.37 τ (J = 1.1 c.p.s.). A broad multiplet at 6.81 τ is attributed to the allylic proton; the low position of this resonance may be due to the anisotropy of the ester carbonyl. The two hydrogens on the saturated carbon α to the ketone constitute the AB part of an ABX system, and the appropriate pattern is discernible centered at 7.54 τ ($|J_{AB}|$ = 18.6 c.p.s.).

The mass spectrum exhibits, in addition to the molecular ion at *m/e* 182, peaks corresponding to the loss of methyl, ethyl, and propyl fragments, as well as $(M—OCH_3)^+$ and $(M—COOCH_3)^+$.

Reduction of **9** with zinc in acetic acid afforded a mixture of epimeric *cis* and *trans* keto esters **10**, not readily separable by thin-layer chromatography (t.l.c.). The mass spectrum of **10** shows



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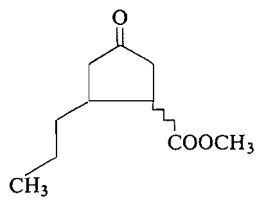

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a molecular ion at *m/e* 184. A strong peak at *m/e* 114, and ions at *m/e* 97 and 87 can be rationalized in terms of the fragmentations shown in Fig. 2.

Separation of the *cis* and *trans* isomers could be effected by preparative vapor phase chromatography (v.p.c.), and the retention times of the

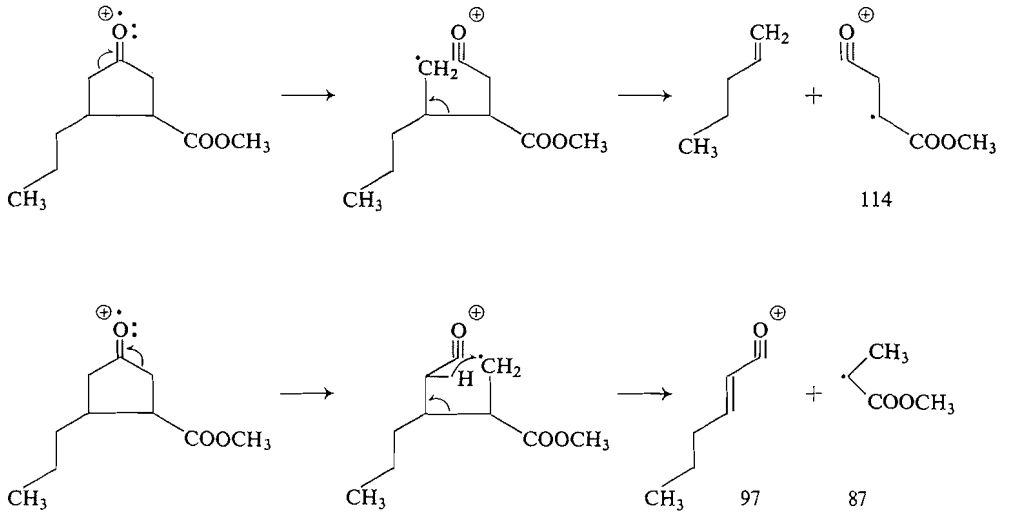


FIG. 2. The fragmentations of **10**

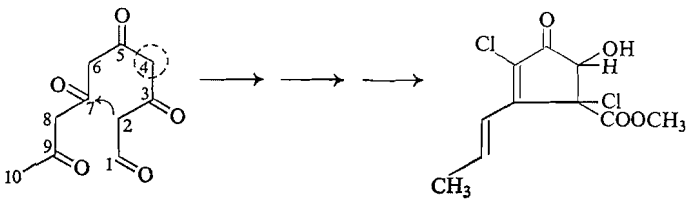
two fractions were identical with the major (*trans*) and minor (*cis*) constituents of a synthetic sample (*vide infra*). Comparison of the n.m.r., infrared, and mass spectra of the natural and synthetic *trans* keto esters confirmed their identities, and these compounds were further characterized as their 2,4-dinitrophenylhydrazones, $C_{16}H_{20}N_4O_6$.

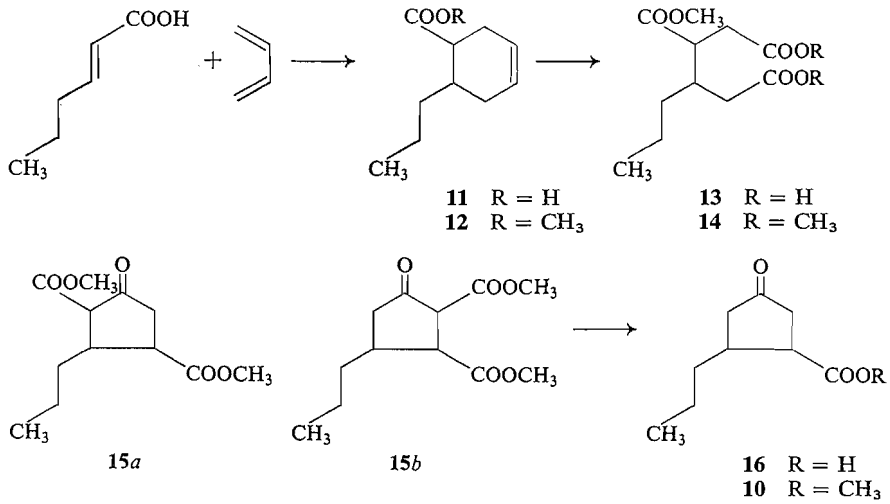
Thus the skeletal structure of cryptosporiopsin is clearly defined, establishing the formulation **1** for the new antibiotic. Inspection of this structure suggests that, like terrein (7), it may arise from a polyketide chain precursor. In any scheme involving a purely polyketide route, the loss of one carbon atom must be accounted for. Birch *et al.* (7) has shown that terrein incorporates polyketide precursors with the unusual feature of two linked "carboxyl" carbons at the 6,7-positions (**6**), and attributes this distribution to a contraction of a six-membered precursor. It is reasonable to speculate that a common pathway is involved in the biosynthesis of cryptosporiopsin and terrein. Cyclization of a five-acetate unit chain with extrusion and the loss of one of the ring carbon atoms is seen to lead readily to the cryptosporiopsin system. Whereas the C-1 carboxyl is retained in cryptosporiopsin, this moiety is lost in the course of the terrein biosynthesis. Studies on further aspects of the chemistry, including stereochemistry, of cryptosporiopsin are in progress.

*Synthesis of 3-carbomethoxy-4-n-propyl Cyclopentanone **10**, cf. (8)*

2-Hexenoic acid, prepared from butyraldehyde and malonic acid by the Knoevenagel–Doebner reaction (9), was heated in a sealed vessel with butadiene to give the Diels–Alder adduct **11**, *m/e* 168. The product, after esterification, was cleaved by ozonolysis affording the ester diacid **13**, $C_{11}H_{18}O_6$. Esterification, followed by Dieckmann cyclization yielded the expected keto esters **15** (*a* and/or *b*). Hydrolysis and decarboxylation, followed by reesterification afforded *trans* and *cis* **10**, *m/e* 184, in the approximate ratio 25:4 (determined by v.p.c.).[2]

[2]This stereochemical assignment is based on the assumption that starting with predominantly *trans* 2-hexenoic acid (see Experimental), the preferred stereochemistry of the centers in question is *trans* throughout the synthetic sequence.





Experimental

Melting points were determined on a hot stage apparatus and are uncorrected. Infrared spectra were determined on a Beckmann IR-10 infrared spectrophotometer. Ultraviolet spectra were obtained on a Beckmann DK-2A spectrophotometer using 95% ethanol as solvent, except where otherwise indicated. Nuclear magnetic resonance spectra were measured in $CDCl_3$ solution with a Varian Associates 56.4 Mc.p.s. spectrometer.

The high resolution mass spectrum was obtained on a CEC 21-110B mass spectrometer, the other mass spectra were determined on a Hitachi Perkin–Elmer RMU-6D mass spectrometer. Microanalyses were performed by Spang Microanalytical Laboratory, Ann Arbor, Michigan, and by Dr. F. Pascher, Mikroanalytiches Laboratorium, Bonn, West Germany.

Cryptosporiopsin (1)

Isolation and purification of cryptosporiopsin have been described elsewhere (2). The metabolite is obtained as colorless crystals from methylene chloride – *n* – hexane, m.p. 133–137°, $[\alpha]_D^{25}$ +129° (*c*, 1.35, chloroform).

Anal. Calcd. for $C_{10}H_{10}O_4Cl_2$: C, 45.30; H, 3.80. Found: C, 45.45; H, 3.90.

Anal. Calcd. for $C_{10}H_{10}O_4Cl_2$ (high resolution mass spectrum): *m/e* 263.9956. Found: *m/e* 263.9943. Isotope peaks as percentages of M^+: $M^+ + 2$, 66%; $M^+ + 4$, 12%.

Acetylation of Cryptosporiopsin

Cryptosporiopsin (100 mg) was dissolved in acetic anhydride (6.0 ml). The solution was cooled to 0° and dry pyridine (0.6 ml) was added by syringe under nitrogen. The initially clear colorless solution was allowed to attain 25°, and was set aside with stirring in a nitrogen atmosphere for 22 h. The resulting brown reaction mixture was then evaporated to dryness and the residue was dissolved in an ether–water mixture. The organic phase was washed with 2% aqueous hydrochloric acid followed by water. The organic solution was subsequently dried over magnesium sulfate and the removal of the solvent under reduced pressure afforded 103 mg of brown gum. After chromatography on silica gel plates, 73 mg of pale-brown amorphous product was isolated. Infrared $\nu_{max}(CCl_4)$: 1741, 1759 cm^{-1} (no OH); n.m.r.: 2.78–3.77 τ (m, 2H), 4.83, 4.91 τ (s, ratio ca 0.4:1 for conformational or stereoisomers, 1H), 6.25 τ (s, 3H), 7.76, 7.83 τ (s, ratio ca 1:0.4 for conformational or stereoisomers, 3H) 7.97 τ (d, 3H); mass spectrum: M^+, 306; $M^+ + 2$, 66%; $M^+ + 4$ 12%.

Zinc–ethanol Reduction of Cryptosporiopsin

To cryptosporiopsin (100 mg, 0.377 mmole) in absolute ethanol (8 ml) was added zinc dust (131 mg, 0.002 g atom). The mixture was set aside with stirring at 25° for 20 h and was then refluxed for 7 h. Filtration removed the solid material, which was washed thoroughly with methylene chloride. The combined filtrates were washed with 2% sulfuric acid followed by water, and after drying over magnesium sulfate, removal of solvent yielded 85 mg of colorless gum. The product, 7, was chromatographed twice on preparative silica gel plates, but failed to crystallize. Infrared $\nu_{max}(CCl_4)$: 3515, 1730, 1642 cm^{-1}; ultraviolet λ_{max}(95% EtOH) 285 mμ (ε 22 000); n.m.r. 3.03–4.05 τ (m, 2H), 5.76 τ (q, J = 6.8 c.p.s., 2H), 6.01 τ (1H), 7.16, 7.22 τ (2H), 8.04 τ (d, 3H), 8.77 τ (t, J = 6.8 c.p.s., 3H); mass spectrum: M^+, 244; $M^+ + 2$, 34%.

Hydrogenation of Cryptosporiopsin, Palladium-on-charcoal – Ethyl Acetate

Cryptosporiopsin (70 mg) in ethyl acetate (30 ml) was stirred under hydrogen at atmospheric pressure and room temperature with a prehydrogenated 10% palladium-on-charcoal catalyst (30 mg) for 35 min. The catalyst was removed by filtration, and was washed thoroughly with ethyl acetate. The combined filtrates were evaporated to dryness furnishing 57 mg of colorless gum, which crystallized on standing. After recrystallization from ether–hexane the product, **8**, melted at 75–91° Spectral details are described in the text. Mass spectrum: M^+, 266; $M^+ + 2$, 66%; $M^+ + 4$, 12%.

Anal. Calcd. for $C_{10}H_{12}O_4Cl_2$: C, 44.96; H, 4.53; Cl, 26.55. Found: C, 45.00; H, 4.69; Cl, 26.49.

TABLE I

Cryptosporiopsin: accurate mass measurements of major peaks in the higher mass region of the spectrum

Peak*	Relative abundance	Observed mass	Composition	Calcd. mass (10)	Error (obs.–calcd.)	Fragment
264	36	263.9943	$C_{10}H_{10}O_4Cl_2$	263.9956	−0.0013	M^+
246	6	245.9865	$C_{10}H_8O_3Cl_2$	245.9850	+0.0015	$(M—H_2O)^+$
229	8	229.0268	$C_{10}H_{10}O_4Cl$	229.0268	0.0000	$(M—Cl)^+$
205	100	204.9828	$C_8H_7O_2Cl_2$	204.9823	+0.0005	$(M—COOCH_3)^+$
185	22	185.0375	$C_9H_{10}O_2Cl$†	185.0369	+0.0006	$(M—Cl \text{ and } CO_2)^+$
177	38	176.9871	$C_7H_7OCl_2$‡	176.9874	−0.0003	$(M—COOCH_3 \text{ and } CO)^+$

*Isotope peaks with appropriate relative intensities are present in each case.
†One of several plausible mechanistic rationales for a fragment with this composition involves migration of the ester methyl group to the carbon vacated by chlorine, through a four-center mechanism, with concomitant extrusion of CO_2. An alternative fragmentation pathway however appears also to be available for the $(M—Cl)^+$ ion. The spectrum shows a metastable peak at *m/e* 126.5 which corresponds to the decomposition 229 → 170, i.e. loss from $(M—Cl)^+$ of the $COOCH_3$ (mass, 59) fragment.
‡Metastable peak at *m/e* 153.5 (205 → 177).

Hydrogenation of Cryptosporiopsin, Palladium-on-charcoal – 95% Ethanol

Cryptosporiopsin (200 mg, 0.755 mmoles) in 95% ethanol (40 ml) was stirred under hydrogen at atmospheric pressure and 25° with a 10% prehydrogenated palladium-on-charcoal catalyst (90 mg). Absorption ceased abruptly after uptake of 4 moles of hydrogen. The catalyst was removed by filtration, and was washed thoroughly with ethanol. The combined filtrates upon the removal of solvent afforded 144 mg of pale-brown oil. Chromatography of 56 mg of this product on a silica gel preparative layer plate yielded 32 mg of colorless oily unsaturated keto ester **9**.

Spectral details are described in the text.

Due to asymmetric induction the hydrogenation product **9** was optically active, $[\alpha]_D^{30}$ −26.5° (*c*, 1.29, chloroform).

Zinc – Acetic Acid Reduction of 9

To a refluxing solution of the keto ester **9** (200 mg) in glacial acetic acid (30 ml) was added zinc dust (2.0 g) in three portions during 20 min. The resulting suspension was heated under reflux with stirring for 5 h. The solid material was removed by filtration, and was washed thoroughly with ether. The combined filtrates were evaporated to dryness, and the residue was redissolved in ether–water. The ether solution was washed with water and dried over magnesium sulfate. Removal of solvent *in vacuo* furnished 197 mg of pale-yellow gum. Chromatography on a preparative silica gel plate yielded 170 mg of colorless oil, consisting of a mixture of epimeric keto esters **10**. Although it was subsequently shown that t.l.c. did effect a partial separation of the isomers, their presence was not readily discernible on inspection of t.l.c. plates, but was however clearly manifested in the n.m.r. spectrum, in particular by the presence of two methoxyl peaks (6.24, 6.27 τ) together integrating for three protons. Separation of the epimers was effected by means of a Varian Aerograph Autoprep Model A-700; 20 ft by 3/8 in. column (30% SE 30 on Chromosorb W) at 205°, with helium as the carrier gas at 133 ml/min. Under these conditions the retention times of the two stereoisomers were (**10***a*) 21 min 15 s and (**10***b*) 22 min 55 s, identical with those of the major and minor epimeric constituents of an authentic synthetic product (considered to be *trans* and *cis* respectively—see text).

The infrared spectrum of **10***a* shows a broad carbonyl band at 1745 cm^{-1}. In the n.m.r. spectrum the methyl ester protons give rise to a sharp singlet at 6.28 τ, five deshielded hydrogens are included in a multiplet whose principal peaks are at 7.44 and 7.55 τ and the methyl protons of the propyl side chain appear as a poorly resolved triplet at 9.07 τ. The mass spectrum is similar to that of the epimer mixture **10**, and exhibits ions at *m/e* 184 (molecular ion, 5%), 114 (66%), 97 (7%), and 87 (32%) as described in the text. The base peak at *m/e* 55 corresponds to the ion $C_3H_3O^+$. In addition, peaks at *m/e* 155 (4%), 153 (5%), 141 (17%), and 125 (18%) correspond to the loss from the molecular ion of C_2H_5, OCH_3, C_3H_7, and $COOCH_3$ fragments respectively.

The infrared spectrum of the 2,4-dinitrophenylhydrazone of "natural" (dextrorotatory) **10***a*, was identical in all details with that of the derivative of racemic synthetic **10***a*.

An analytical sample, after four crystallizations from methanol, melted at 107–115°. Mass spectrum: *m/e* 364.

Anal. Calcd. for $C_{16}H_{20}N_4O_6$: C, 52.74; H, 5.53; N, 15.38. Found: C, 53.23; H, 5.61; N, 15.03.

2-Hexenoic Acid

2-Hexenoic acid was prepared according to the procedure of Niemann and Redemann (9). The crude reaction product crystallized on standing and was used in the subsequent Diels–Alder reaction without further purification. The n.m.r. spectrum indicates clearly that it consists principally of the *trans* isomer. A pair of partially superimposed triplets in the region 2.72–3.23 τ and a doublet at 4.25 τ (peaks slightly broadened due to 1,3-splitting) constitute the AB part of an ABX_2 spectrum. The coupling constant J_{AB} is 15.8 c.p.s., the correct magnitude for *trans* olefinic protons.

Preparation of 11 and 12

2-Hexenoic acid (14.3 g, 0.125 moles) was heated with butadiene (ca 7 g, 0.13 moles) and hydroquinone (100 mg) in a Parr bomb at 170–190° for 5 h.

Distillation of the brown reaction product afforded 11.7 g of colorless oily **11**, b.p. 140–150° /9 mm. In the

n.m.r. spectrum the two vinyl protons appear as a somewhat broadened singlet at 4.39 τ; the mass spectrum shows a molecular ion peak at *m/e* 168.

Conversion to the methyl ester **12** was effected by refluxing **11** (7.1 g) with anhydrous methanol (40 ml) in the presence of concentrated sulfuric acid (3 ml), monitoring the progress of the reaction by t.l.c. After work-up in the usual manner, the product was distilled, and the pure ester **12** was collected at 99–102° /11 mm. Infrared $\nu_{max}(CCl_4)$ 1737, 1658 cm^{-1}; n.m.r.: 4.39 τ (broad s, 2H), 6.33 τ (s, 3H); mass spectrum: molecular ion *m/e* 182.

*Preparation of **13** and **14***

An ozone–oxygen mixture was passed by means of a sintered glass bubbler into a solution of **12** (3.75 g, 20.6 mmoles) in ethyl acetate (100 ml) cooled in a dry ice – acetone bath. (Welsbach Model T-23 ozonator, operating at 90 V, 8 lb pressure, flow rate 0.02 ft^3/min). The end point was indicated by the development of a blue color in the solution, whereupon the system was flushed with nitrogen to remove excess ozone. The solution was then stirred vigorously under hydrogen at atmospheric pressure with a prehydrogenated 10% palladium-on-charcoal catalyst (304 mg), until the calculated volume of hydrogen had been taken up. The catalyst was removed by filtration, and the solvent was stripped off *in vacuo* below 30°.

The resulting dialdehyde ($\nu_{max}(CCl_4)$ 2720 cm^{-1}, broad carbonyl 1738–1715 cm^{-1}) was dissolved in acetone (200 ml), and the solution was cooled in an ice-bath. A solution of potassium permanganate (4.88 g, 30.9 mmoles) in distilled water (200 ml) was added slowly with stirring during 40 min, and the reaction mixture was set aside with stirring at 0–10° for a further 30 min. After addition of 20% sulfuric acid (2 ml), sulfur dioxide gas was passed in until a clear solution resulted; this was extracted exhaustively with ether. After drying over magnesium sulfate, removal of solvent under reduced pressure furnished 3.767 g of colorless oil, which partially crystallized on standing. Recrystallization from ether – *n*-hexane afforded colorless crystals of **13**, m.p. 128–132°.

Anal. Calcd. for $C_{11}H_{18}O_6$: C, 53.65; H, 7.37. Found: C, 53.83; H, 7.38.

Esterification was effected by sulfuric acid-catalyzed reaction with anhydrous methanol in the usual manner.

The pure triester **14** was eluted from a silica gel column with benzene–ether (95:5).

A somewhat broadened singlet at 6.32 τ in the n.m.r. spectrum accounts for the nine methoxyl protons; $\nu_{max}(CCl_4)$ 1740 cm^{-1}.

The same product was obtained by direct oxidation of the potassium salt of **11** with aqueous permanganate, followed by esterification.

*Dieckmann Condensation of **14***

To sodium hydride (306 mg, 12.75 mmoles, washed free of mineral oil with light petroleum) was added anhydrous tetrahydrofuran (30 ml) containing 5 drops of absolute methanol. The suspension was cooled in an ice-bath, and a solution of triester **14** (1.75 g, 6.37 mmoles) in dry tetrahydrofuran (70 ml) was added during 20 min with stirring. Moderate evolution of hydrogen occurred. When the addition was complete, and effervescence ceased, a nitrogen atmosphere was introduced, and the mixture was stirred at 25° for 15 h; it was then heated under reflux for 7 h. After cooling, methanol was added cautiously to decompose any unreacted hydride, and the solution was poured on to ice. The pH was adjusted to 2–3 by addition of 5% hydrochloric acid, and the mixture was extracted thoroughly with ether. The extracts were washed with sodium bicarbonate solution, dried over magnesium sulfate, and evaporated, yielding 1.214 g of crude oily product **15** (*a* and/or *b*). The cyclized material, which was not purified further exhibits ultraviolet absorption at λ_{max}(95% EtOH) 250 mμ (ε 2000). The addition of 1 drop 5% sodium hydroxide solution shifts and enhances the chromophore to 279 mμ (ε 15 000).

*Preparation of **10***

The crude Dieckmann product **15** (*a* and/or *b*) (3.4 g) was hydrolyzed and decarboxylated by refluxing with 4 *N* hydrochloric acid (25.2 ml) for 18 h. When cool, the mixture was extracted with ether, and the combined extracts were shaken with saturated sodium bicarbonate solution. Acidification of the aqueous phase, and extraction afforded 1.97 g of acidic product **16**.

Esterification was effected by refluxing **16** (1.97 g) with methanol (75 ml) and concentrated sulfuric acid (4 ml) for 3 h. After removal of the methanol under reduced pressure, work-up in the usual manner yielded 1.5 g of pale-yellow oil, which appeared homogeneous on t.l.c.

Vapor phase chromatographic analysis of synthetic **10** thus obtained revealed the presence of two components in the approximate ratio 25:4, corresponding to the *trans* and *cis* epimers **10***a* and **10***b* respectively (*vide supra*).

The infrared, n.m.r., and mass spectra of synthetic **10***a* were completely identical with the purified "natural" dextrorotatory *trans* epimer **10***a*.

Similarly the infrared spectrum of the 2,4-dinitrophenylhydrazone of racemic synthetic **10***a* was identical with that of the corresponding derivative of the "natural" keto ester.

An analytical sample, after four crystallizations from methanol melted at 103–109°.

Anal. Calcd. for $C_{16}H_{20}N_4O_6$: C, 52.74; H, 5.53; N, 15.38. Found: C, 52.83; H, 5.55; N, 15.31.

Acknowledgments

The authors thank Dr. P. Levins, Arthur D. Little Inc., Cambridge, Massachusetts for the high resolution mass spectrum, and Mr. J. E. Anderson, Harvard University for the spin decoupling study. Gratitude is expressed to Mrs. H. Moore, Mr. H. J. Liu, and Mr. K. T. Kan, all of the University of New Brunswick, for their contributions to this work in the form of nuclear magnetic resonance, mass spectra, and optical rotation measurements respectively.

1. M. A. STILLWELL. Can. J. Bot. **44**, 259 (1966).
2. M. A. STILLWELL, F. A. WOOD, and G. M. STRUNZ. Can. J. Microbiol. **15**, 501 (1969).
3. L. J. BELLAMY. The infrared spectra of complex molecules. 2nd ed. John Wiley and Sons, Inc., New York. 1958; K. NAKANISHI. Infrared absorption

spectroscopy, practical. Holden-Day, Inc., San Francisco. 1962.
4. L. M. JACKMAN. Applications of nuclear magnetic resonance spectroscopy in organic chemistry. The Pergamon Press, Ltd., New York. 1959; N. S. BHACCA and D. H. WILLIAMS. Applications of n.m.r. spectroscopy in organic chemistry. Holden-Day, Inc., San Francisco. 1964.
5. D. H. R. BARTON and E. MILLER. J. Chem. Soc. 1028 (1955); J. F. GROVE. J. Chem. Soc. 4693 (1954).
6. A. I. SCOTT. Interpretation of the ultraviolet spectra of natural products. The Pergamon Press, Ltd., New York. 1964.
7. A. J. BIRCH, A. CASSERA, and A. R. JONES. Chem. Commun. 167 (1965).
8. K. TOKI. Bull. Chem. Soc. Japan, **32**, 233 (1959).
9. C. NIEMANN and C. T. REDEMANN. J. Am. Chem. Soc. **68**, 1933 (1946).
10. J. H. BEYNON and A. E. WILLIAMS. Mass and abundance tables for use in mass spectrometry. American Elsevier Publishing Co. Inc., New York. 1963.