cake was washed repeatedly with chloroform. Evaporation of the combined chloroform filtrates under reduced pressure afforded 88 mg of oil. Two components of this residue were separated by preparative thin layer chromatography. The band at R_f 0.7 provided 23 mg of a compound which was found to be a decomposition product of the material with R_f 0.65. The major band at R_f 0.65 provided 62 mg of 6a-epi-N-demethyl-3-epimacronine (**16**): (amorphous) $[\alpha]^{24}D$ +79° (*c* 0.14, $CHCl_3$); λ_{max} (95% EtOH) 228 (25,000), 268 (5800), and 310 mμ (ε 5850); ir ($CHCl_3$): 1711 cm^{-1} (C=O); nmr ($CDCl_3$): δ 6.84 and 7.55 (2s, aromatic protons), 6.04 (s, 2, methylenedioxy), and 3.46 ppm (s, $-OCH_3$).

Anal. Mass calcd for $C_{17}H_{19}NO_5$: 315.111. Found: 315.111.

Haemanthamine. ORD results (MeOH) were as follows: $[\Phi]_{320}$ +3100°, $[\Phi]_{305}$ +7600° pk, $[\Phi]_{275}$ −6300° sh, $[\Phi]_{252}$ −10,200° tr, $[\Phi]_{230}$ +12,100° (last reading); CD (MeOH): $[\theta]_{290}$ +13,100°, $[\theta]_{245}$ −10,200°, $[\theta]_{225}$ +15,000° (last reading).

Haemanthamine Methiodide. ORD results (MeOH) were as follows: $[\Phi]_{320}$ +2000°, $[\Phi]_{305}$ +4300° pk, $[\Phi]_{280}$ −5100° sh, $[\Phi]_{255}$ −8600° tr, $[\Phi]_{230}$ +20,000° (last reading); CD (MeOH): $[\theta]_{295}$ +6300°, $[\theta]_{245}$ −7000°, $[\theta]_{230}$ +8000° (last reading).

Haemanthidine. ORD results (MeOH) were as follows: $[\Phi]_{320}$ +3000°, $[\Phi]_{303}$ +5000° pk, $[\Phi]_{280}$ −5000° sh, $[\Phi]_{254}$ −13,600° tr, $[\Phi]_{230}$ +5000° (last reading); CD (MeOH): $[\theta]_{294}$ +11,400°, $[\theta]_{244}$ −7600°, $[\theta]_{225}$ +6000° (last reading).

Acknowledgment. The authors are grateful to Dr. H. M. Fales at the National Heart Institute for providing high-resolution mass spectrometry data. The HA-100 nuclear magnetic resonance spectrometer used in this research was obtained by a grant from the National Science Foundation to the Department of Chemistry.

Chlorinated Cyclopentenone Fungitoxic Metabolites from the Fungus, *Sporormia affinis*

W. J. McGahren, J. H. van den Hende, and L. A. Mitscher

Contribution from Lederle Laboratories, A Division of American Cyanamid Company, Pearl River, New York 10965. *Received August 5, 1968*

Abstract: Three chlorinated metabolites having a structural similarity to terrein have been isolated from fermentations of *Sporormia affinis* Sacc., Bomm and Rouss. Single crystal X-ray analysis was used to show the structure and absolute configuration of the major dichlorinated metabolite to be (1*S*,5*S*)-2-cyclopentene-1-carboxylic acid, 2-*trans*-allyl-3,5-dichloro-1-hydroxy-4-oxo methyl ester (I). Spectral data and ORD studies were then used to assign the structures (1*S*,5*S*)-2-cyclopentene-1-carboxylic acid, 2-*trans*-allyl-3-chloro-1-hydroxy-4-oxo methyl ester (II) and (1*S*,5*R*)-2-cyclopentene-1-carboxylic acid, 2-*trans*-allyl-3-chloro-1-hydroxy-4-oxo methyl ester (III), respectively to the monochlorinated compounds.

Raistrick and others[1,2] isolated and suggested the structure of the metabolite terrein from *Aspergillus terreus*. Several years later Barton and Miller[3] confirmed the structure and determined the absolute configuration of this metabolite. Recently Birch and others[4] have described the biosynthesis of terrein as arising from a polyketide origin with the unusual feature of two linked carboxyl-derived carbons at the 6,7 positions, a distribution which might be explained by contraction of a six-membered precursor (see below). We now wish to report the isolation and complete characterization of three halogenated metabolites from fermentations of *Sporormia affinis* Sacc., Bomm and Rouss each having the terrein skeleton. The structures of the new metabolites are consistent with the proposed biosynthetic pathway for terrein. All of the carbons of the hypothetical precursor appear to be retained in these compounds. In addition these metabolites have interesting antifungal and some antibacterial activity.

For our purposes *Sporormia affinis* was grown in submerged culture under standard conditions of aeration and agitation and the metabolic products were isolated after 120 hr by carbon adsorption followed by at least two further chromatographic steps. Several dihydroisocourmarin products were isolated in addition to the products described in this paper.[5] The most abundant metabolite is an optically active, white, crystalline compound which can readily be crystallized from ether or ethyl acetate as rosettes with a melting range 135.5–136.5°. It is soluble in boiling cyclohexane from which it crystallizes rapidly as long needles on cooling. The crystals from cyclohexane invariably melt at 138–139°. Solutions of this metabolite darken immediately on treatment with basic reagents or nucleophiles such as cyanide ion. However, the material is quite stable to acids. It can be recovered unchanged from solution in concentrated sulfuric acid and after 20 hr of refluxing in dilute acid solution between 30 and 40% of unchanged starting material remains. The molecular formula of the material is $C_{10}H_{10}O_4Cl_2$ (mass spectrum) and because of the presence of the two heavy atoms, it was decided to subject the compound to single crystal X-ray structural analysis.

The crystals were found to be monoclinic, with the unit cell dimensions a = 7.726 Å, b = 13.100 Å, c = 6.277 Å (±0.004 Å), and β = 110.75° (±0.10°). The observed density of 1.439 g/cc (23°) is in agreement with a cell content of two molecules $C_{10}H_{10}O_4Cl_2$ (ρ_c = 1.48 g/cc). The only observed extinction rules governed reflections of the type (0*k*0) for which $k = 2n$. This indicated the space group $P2_1$. Three dimensional intensity data were obtained from a crystal of dimensions

(1) H. Raistrick and G. Smith, *Biochem. J.*, 29, 606 (1935).
(2) P. W. Clutterbuck, H. Raistrick, and F. Reuter, *ibid.*, 31, 987 (1937).
(3) D. H. R. Barton and E. Miller, *J. Chem. Soc.*, 1028 (1955).
(4) A. J. Birch, A. Cassera, and A. R. Jones, *Chem. Commun.*, 167 (1965).
(5) W. J. McGahren and L. A. Mitscher, *J. Org. Chem.*, 33, 1577 (1968).

150 × 120 × 80 μ using a General Electric XRD-6, equipped with Eulerian cradle. Cu Kα radiation was used (λ 1.5418 Å, Ni–Co balanced filters) employing the stationary-crystal–stationary-counter peak height method with wide open aperture. The discriminator circuit contained a pulse height analyzer. Of the 864 reciprocal lattice points accessible within a sphere of 1 $Å^{-1}$, 643 were found to exceed the background readings by 50%, and were considered to be observable.

After application of the usual Lorentz and polarization corrections, combined with small absorption corrections, a three-dimensional Patterson vector map was calculated using as input terms the values of $E^2 - 1$. The locations of the two pairs of vectors due to symmetry-induced Cl–Cl interactions were obtained from the Harker section at $v = 1/2$, and these were found to be consistent with a pair of vectors in general position and due to the interactions between the two sets of dissimilar chlorine atoms. Using the thus obtained positions for chlorine to obtain starting phases, the structure was solved in two successive cycles of electron-density map calculations. The identities of the noncarbon atoms were obtained from electron counts in these maps and isotropic refinement lowered R to 0.107. An additional cycle of least-squares refinement yielded a final R of 0.090.[6] At this stage the absolute configuration was determined using the anomalous scattering contributions to the structure factors due to the chlorine atoms. Only six reflections were found to be violating Friedel's law sufficiently to warrant the use of the intensities of pinacoidal Bijvoet pairs (Table I).[7] It was found that the band, as assigned arbitrarily at the outset, was incorrect. In all figures the absolute configuration has been depicted (see Table II for positional parameters and Table III for thermal correction factors).

Table I. Absolute Configuration Comparison of Bijvoet Pairs (Strong Reflections Only)

Reflection	Ratio for $F(h,k,l)/F(-h,-k,-l)$ Calcd	Obsd
0 1 1	0.655	0.428
−2 2 3	0.936	0.876
−2 1 2	0.970	0.941
4 1 1	0.919	0.845
−3 1 5	1.093	1.194
−2 8 2	1.252	1.567

The crystals contain a layer structure with high density occurring parallel to the $20\bar{1}$ planes. Within these planes there exist infinite chains of closely packed molecules in the direction of the b axis; with a short approach between the carbonyl oxygen of one molecule and the ester carbonyl oxygen atom of an adjacent molecule (O–O distance = 3.06 Å), and simultaneous weak hydrogen bonding involving the same carbonyl oxygen and the hydroxyl group of that same neighboring molecule (O–O distance = 3.03 Å). It is of passing interest to note that the carbonyl oxygen atoms have no fewer than ten nearest neighbors located within 4.1 Å.

No other short distances are observed and van der Waals forces are therefore responsible for most of the packing forces, with a nonbonded Cl–Cl distance of 3.51 Å.

The covalent bond lengths indicate the presence of conjugation in the allylo carbonyl chromophore, with some participation of the chlorine atom attached in the 3 position. The covalently bonded interatomic distances are listed in Table IV and the valence angles are given in Table V. The X-ray labeling system and a schematic view of the packing are shown in Figures 1 and 2.

The structure and stereochemistry of the dichlorinated metabolite may be written as (1*S*,5*S*)-2-cyclopentene-1-carboxylic acid, 2-*trans*-allyl-3,5-dichloro-1-hydroxyl-4-oxo methyl ester (I).

The uv spectrum of I has a single peak at 289 mμ. Terrein peaks at 275 mμ and both compounds have extinction coefficients of comparable magnitude. The ir spectrum of I has a sharp band at 3420 cm^{-1} in the O–H stretch region and a weak C–H stretch absorption

Table II. Positional Parameters (in Fractions of the Cell Edges) and Their Standard Deviations (in Ångströms)

Atom	x/a	y/b	z/c	$\sigma(x)$	$\sigma(y)$	$\sigma(z)$
Cl(1)	0.83216	0.50000	0.71994	0.005	0.004	0.004
Cl(2)	0.30939	0.54281	0.12639	0.006	0.005	0.005
C(1)	0.71167	0.55335	0.46710	0.017	0.015	0.018
C(2)	0.58859	0.49629	0.27089	0.018	0.016	0.020
C(3)	0.53879	0.56328	0.06643	0.021	0.018	0.019
C(4)	0.57540	0.67397	0.17358	0.013	0.014	0.015
C(5)	0.70735	0.65190	0.41605	0.016	0.013	0.016
O(6)	0.65168	0.73532	0.04610	0.014	0.013	0.012
C(7)	0.39305	0.72129	0.17807	0.022	0.023	0.023
O(8)	0.33075	0.79750	0.06930	0.013	0.013	0.015
O(9)	0.33262	0.67061	0.31658	0.011	0.010	0.012
C(10)	0.81304	0.73145	0.57199	0.016	0.014	0.020
C(11)	0.80607	0.83384	0.54138	0.016	0.015	0.017
C(12)	0.91094	0.90852	0.69967	0.021	0.019	0.026
C(13)	0.15335	0.71068	0.32749	0.017	0.017	0.021
O(14)	0.54014	0.40683	0.27972	0.014	0.011	0.013

Table III. Isotropic and Anisotropic Thermal Correction Factors[a]

Atom	B	B_{11}	B_{22}	B_{33}	B_{12}	B_{13}	B_{23}
Cl(1)	5.33	2595	764	3684	85	677	88
Cl(2)	6.58	2976	1173	4256	118	239	−79
C(1)	4.44	2133	674	4659	−271	1548	−110
C(2)	5.21	1857	928	5603	69	227	−655
C(3)	5.65	2905	755	3376	−27	1008	570
C(4)	4.20	1140	713	3910	64	1477	226
C(5)	4.13	1952	498	2985	311	420	411
O(6)	6.10	2995	1069	4623	−121	1759	−167
C(7)	4.90	2552	1112	3852	102	650	675
O(8)	6.22	2445	1032	5402	128	1101	159
O(9)	5.11	1911	735	4861	−58	957	−241
C(10)	5.08	1752	565	5714	76	1405	103
C(11)	4.45	1744	868	5383	−245	1151	−710
C(12)	6.59	2580	898	6642	374	890	109
C(13)	6.30	2253	1135	9522	−191	2886	−1527
O(14)	5.86	3328	608	3906	389	1099	695

[a] All B_{ii} are multiplied by 10^5. $T = \exp[-(B_{11}h^2 + B_{22}k^2 + B_{33}l^2 + 2B_{12}hk + 2B_{13}hl + 2B_{23}kl)]$.

(6) Tables containing the final structure amplitudes have been deposited as Document No. NAPS-00137 with the ASIS National Auxiliary Publication Service, % CCM Information Sciences Inc., 22 West 34th St., New York, N. Y. 10001. A copy may be secured by citing the document number and by remitting $1.00 for microfiche or $3.00 for photocopies. Advance payment is required. Make checks or money orders payable to: ASIS-NAPS.

(7) J. H. van den Hende and N. R. Nelson, *J. Am. Chem. Soc.*, **89**, 2901 (1967).

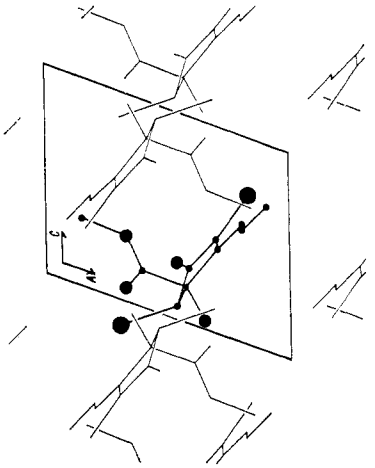


Figure 1. Projection of unit cell parallel to *b*.

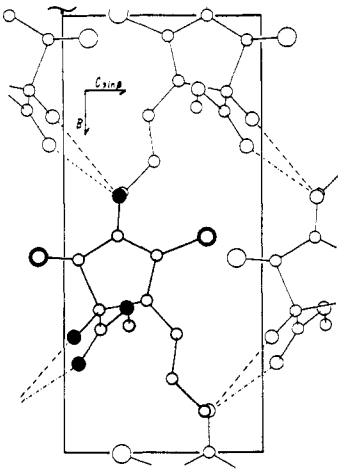
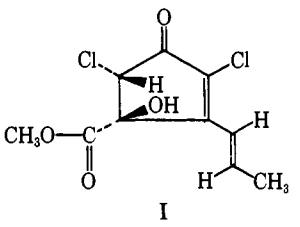


Figure 2. Projection of unit cell parallel to *a*.

at 2950 cm^{-1}. The α,β-unsatured ring carbonyl absorption is shifted to 1735 cm^{-1} because of halogen substitution on either side and the ester carbonyl



I

appears at 1755 cm^{-1}. Sharp absorption bands at 1635 and 1570 cm^{-1} are readily assigned to the conjugated halogen substituted diene system and the trans nature of the allyl substituent is suggested by the

Table IV. Covalently Bonded Interatomic Distances (Å) (σ 0.01 Å)

C(1)-C(2)	1.466	C(4)-O(6)	1.403
C(2)-C(3)	1.488	C(4)-C(7)	1.549
C(3)-C(4)	1.581	C(7)-O(8)	1.209
C(4)-C(5)	1.530	C(7)-O(9)	1.305
C(5)-C(1)	1.328	O(9)-C(13)	1.505
C(1)-Cl(1)	1.681	C(5)-C(10)	1.464
C(2)-O(14)	1.237	C(10)-C(11)	1.353
C(3)-Cl(2)	1.777	C(11)-C(12)	1.425

Table V. Valence Angles (in degrees) (σ 1.5°)

C(5)-C(1)-C(2)	108.5	C(5)-C(4)-O(6)	114.1
C(5)-C(1)-C(1)	126.2	C(5)-C(4)-C(7)	110.3
C(1)-C(1)-C(2)	124.0	O(6)-C(4)-C(7)	110.4
C(1)-C(2)-C(3)	102.9	C(4)-C(5)-C(1)	111.0
C(1)-C(2)-O(14)	124.2	C(4)-C(5)-C(10)	123.2
O(14)-C(2)-C(3)	127.4	C(1)-C(5)-C(10)	124.8
C(2)-C(3)-C(4)	102.9	C(4)-C(7)-O(8)	120.5
C(2)-C(3)-C(2)	112.9	C(4)-C(7)-O(9)	110.5
C(2)-C(3)-C(4)	114.4	O(8)-C(7)-O(9)	129.2
C(3)-C(4)-C(5)	101.9	C(7)-O(9)-C(13)	113.9
C(3)-C(4)-O(6)	109.3	C(5)-C(10)-C(11)	128.8
C(3)-C(4)-C(7)	110.4	C(10)-C(11)-C(12)	126.7

absorption band at 965 cm^{-1}.[3] The nmr spectrum of I has a split signal at τ 8.09 (J = 6–7 cps) indicative of a methyl group on an olefinic carbon bearing a single proton. A sharp three-proton singlet at τ 6.17 is

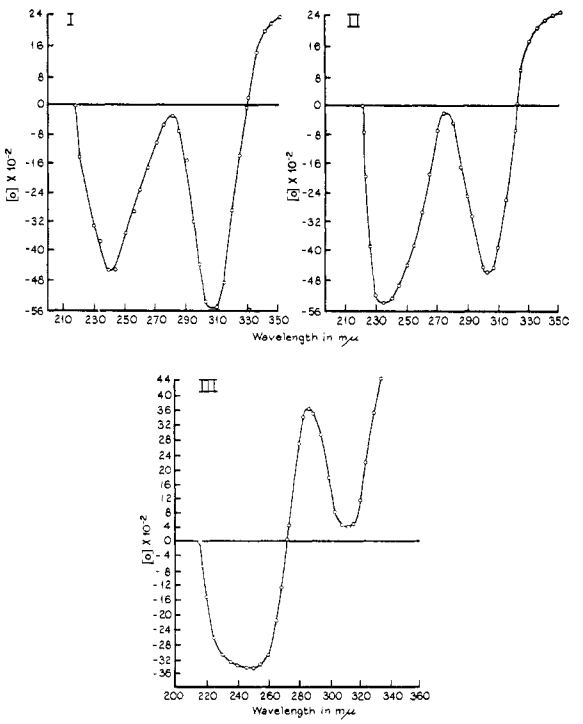
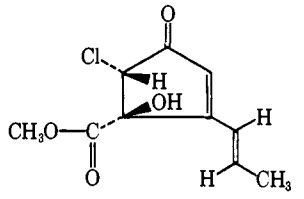


Figure 3. ORD curves of metabolites I, II, and III taken on trifluoroethanol solutions in a 0.1-mm cell using a Cary 60 spectropolarimeter.

attributable to the ester methoxy group. This absorption is shifted downfield by the presence of the α-hydroxy group. The two-proton multiplet centered at τ 3.58 may be assigned to the proton of the side-chain double bond and the magnitude of one of the coupling constants (J = 18 cps) is further evidence of the *trans* nature of the double bond. Ozonolysis of I yielded acetaldehyde, identified as its 2,4-dinitrophenylhydrazone which is confirmation of the allyl side chain. The nmr spectrum also contains a sharp peak with a broadened base at τ 5.83. The broadened base is eliminated on exchange with deuterated methanol and hence is due to an exchangeable hydroxyl proton. The sharp signal remaining is assigned to the single highly deshielded proton on the chlorine-carrying carbon.

The next most abundant metabolite of the fungus has the same chromophore as terrein. The substance melts at 91.5–92.5° and has the empirical formula $C_{10}H_{11}O_4Cl$. It is clear from the spectral properties of metabolite II that it is closely related structurally to I. The molecular formula suggests that one of the chlorine atoms has been replaced by a hydrogen atom. Three pieces of evidence confirm this metabolite as (1*S*,5*S*)-2-cyclopentene-1-carboxylic acid, 2-*trans*-allyl-3-chloro-1-hydroxy-4-oxo methyl ester (II).

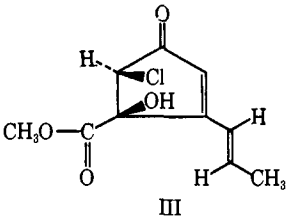


II

The nmr spectrum of II is virtually identical with that of I except that the olefinic region (*ca.* τ 3.6) is now more complicated and integrates for one more proton than in the spectrum of I. Secondly, from Woodward's rules, II would be expected to have a peak in the uv approximately 15 mμ lower than that observed for I. This is almost the case as II has a uv maximum absorption at 277 mμ. Thirdly, since the structural difference between I and II occurs at a site removed from the asymmetric centers one would expect these compounds to have optical rotations of roughly the same order and sign and this is also true.

The third metabolite of the terrein type which we isolated from *Sporormia affinis* was found only in small quantity in some fermentations. This material is isomeric with II and melts at 83.5–84.5° and exhibits a very high optical rotation for such a low molecular weight compound. Because of the similarity of the spectra of this compound with that of II, it is reasoned that this product is structurally identical with II except that one of the asymmetric centers is inverted to give an additive effect to the rotations of the two centers thus accounting for the enhanced optical rotation. A precedent for this line of reasoning has been observed by McGahren and Goodman in their study of tripeptides of the type Z-Aib-L-Phe-DL-AA-OCH_3[8] where AA refers to Ala, Phe, Aib, and PhGly.[9] Tripeptides of the type Z-Aib-L-Phe-D-AA-OCH_3 have lower melting points and higher optical rotations than compounds of the type Z-Aib-L-Phe-L-AA-OCH_3. Optical rotatory dispersion (ORD) measurements were used to verify these ideas concerning the relationships of the asymmetric centers in I and II and the third metabolite (III) as illustrated in Figure 3.

It is well established that the $n \rightarrow \pi^*$ transition of the ketonic carbonyl group gives rise to an absorption band in the 300-mμ region and the Cotton effects associated with this band have been correlated with the stereochemical environment of the carbonyl group in many types of compounds.[10] Compounds I and II have a negative extremum in this region, specifically I at 310 mμ and II at 304 mμ while III has a positive extremum at 289 mμ. These data confirm the suggestion that the asymmetric center adjacent to the carbonyl group is inverted in the third metabolite as compared to the other two. Cotton effects associated with the carboxylic chromophore are observed in the 225-mμ region.[11–13] Each of the three *Sporormia* metabolites has a negative extremum in this region, I at 242 mμ with a lower crossover at 218 mμ, II at 235 mμ with a lower crossover at 221 mμ, and III at 245 mμ with a lower crossover at 216 mμ. Hence all three compounds have the same configuration at the carboxylic asymmetric center, and we may now write the complete structure of the third metabolite as (1*S*,5*R*)-2-cyclopentene-1-carboxylic acid, 2-*trans*-allyl-3-chloro-1-hydroxy-4-oxo methyl ester (III).

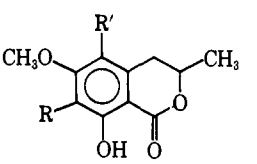


III

The nmr spectra of II and III are almost identical except that in II the hydroxyl proton is observed at τ 5.42 almost overlapping the signal for the single proton of the carbonyl asymmetric center whereas in the spectrum of III, it is located at τ 6.0. This shift of approximately τ 0.6 may be attributed to the deshielding effect of the neighboring chlorine atom in compound III in which the hydroxyl group and the chlorine atom are in what may be described for convenience as pseudo-*cis* positions.

As compounds II and III are epimers, at the center adjacent to a carbonyl group, it is possible that the latter arises by partial epimerization of II. It is to be noted that whereas metabolites I and III are quite stable at room temperature for several months, compound II is relatively unstable even when stored in amber vials in a desiccator. Decomposition occurs in a matter of days and the white crystals become yellow in color. On the other hand the methods of isolation used are quite mild and unlikely to cause any rearrangement. Compound III is found infrequently and in low yield so it is possible that it represents an unnatural epimer of II. However, further work is necessary to eliminate the speculation as to the origin of III.

As already mentioned we have reported elsewhere,[5] the isolation of some chlorinated dihydroisocoumarins



IV, R = R′ = H
V, R = R′ = Cl
VI, R = Cl; R′ = H

from this same culture. It is tempting to draw a biogenetic sequence linking the metabolites of *Sporormia*

(8) W. J. McGahren and M. Goodman, *Tetrahedron*, 23, 2017 (1967).

(9) Abbreviations according to M. Goodman and G. W. Kenner, *Advan. Protein Chem.*, 12, 465 (1957). Aib and PhGly refer to the aminoisobutyryl and phenylglycyl residues, respectively.

(10) J. P. Jennings, W. Klyne, and P. M. Scopes, *J. Chem. Soc.*, 7211 (1965).

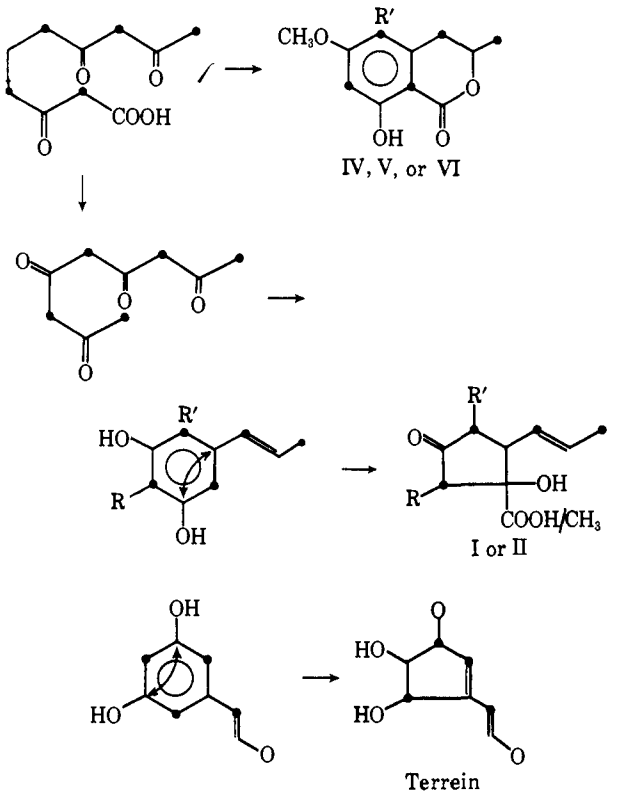
(11) W. Klyne, P. M. Scopes, and A. Williams, *ibid.*, 7237 (1965).

(12) A. Fredga, J. P. Jennings, W. Klyne, P. M. Scopes, B. Sjoberg, and S. Sjoberg, *ibid.*, 3928 (1965).

(13) J. P. Jennings, W. P. Mose, and P. M. Scopes, *ibid.*, 1102 (1967).

affinis and to propose that the carboxyl substituent of metabolites I, II, and III represents the carbon lost in contracting from a six- to a five-membered ring in Birch's proposal for terrein. This question requires confirmation by suitable labeling studies but may be proposed at present as set out in Scheme I. Studies

Scheme I



using labeled acetate would not indicate that ring contraction had occurred in the case of metabolites I and II as they indicate in the case of terrein.

Experimental Section

Nmr spectra were run on a Varian A-60 instrument under normal conditions. Mass spectra were run on an AEI MS9 high-resolution, direct-inlet mass spectrometer. ORD curves were recorded on a Cary 60 spectropolarimeter.

General Isolation Procedure. *Sporormia affinis* Sacc., Bomm and Rouss (Lederle culture N313) was deep fermented using standard conditions of agitation and aeration for 120 hr at 28° on a medium consisting of 2.0 g of molasses, 1.5 g of cornstarch, 1.0 g of cerelose, 0.75 g of soya peptone, 0.5 g of $CaCO_3$, and 0.25 g of distillers grain solubles from corn (Publicker Industries, Inc., Philadelphia, Pa.) per l. of water. The whole mash was filtered, and the filtrate was treated with 10% w/v of active charcoal. The charcoal pad was eluted with 90:10 (acetone–H_2O) at pH 2.0 or neutral pH and the eluate concentrated to the aqueous phase which was extracted with $CHCl_3$. The $CHCl_3$ extracts were concentrated to a gum which was chromatographed over silica gel (Davidson Grade 923) using 2:1 ($CHCl_3$–hexane) as developing solvent. The material eluting in the 12th through 20th holdback volumes on concentration yielded a gum containing a number of uv-absorbing constituents which could be partially separated by tlc on Eastman Chromogram Sheets (Type K 301R) using solvent systems of the type hexane–EtOAc (95:5, 90:10, and 85:15). The gum was partitioned over diatomaceous earth using the partitioning system hexane–EtOAc–MeOH:H_2O (85:15:15:6). Compound I generally was obtained from the third and fourth holdback volumes, compound II was recovered from the fifth through seventh volumes and when present metabolite III was recovered from the eighth holdback volume.

(1*S*,5*S*)-2-Cyclopentene-1-carboxylic Acid, 2-*trans*-Allyl-3,5-dichloro-1-hydroxy-4-oxo Methyl Ester (I). Metabolite I obtained as described in the general isolation procedure has *in vitro* antifungal activity against *Microsporum gypseum* and low-potency antibacterial activity against *Escherichia coli*. It can be recrystallized as characteristic rosettes from ether–hexane or EtOAc–hexane to give yields of up to 130 mg of crystalline material per l. of mash, mp 135.5–136.5°; $[\alpha]^{25}D$ +96.5 ± 2.9° (*c* 1.06, EtOAc). *Anal.* Calcd for $C_{10}H_{10}O_4Cl_2$: C, 45.28; H, 3.62; O, 24.15; Cl, 26.79. Found: C, 45.30; H, 3.77; O, 24.18; Cl, 26.81. Compound I could also be crystallized from hot cyclohexane in a spectacular fashion to get needles, mp 138–139°; $[\alpha]^{25}D$ +61.5 ± 2.9° (*c* 1.023, MeOH); λ_{max}^{MeOH} 289 mμ (ε 22,525); ν_{max}^{KBr} 3400, 2950, 1755, 1735, 1635, 1570, 1430, 1310, 1275, 1212, 1180, 1125, 1112, 1028, 965, 925, 850, 820, 797, 752, and 737 cm^{-1}; nmr in $CDCl_3$; doublet at τ 8.09 (3 H, *J* = 6–7 cps), singlet at 6.20 (3 H), singlet with broadened base at 5.33 (2 H) broadened base obliterated following exchange with CD_3OD, multiplet at 3.58 (2 H); mass spectrum molecular ion at *m/e* 264. The ORD curve of I is shown in Figure 3.

(1*S*,5*S*)-2-Cyclopentene-1-carboxylic acid, 2-*trans*-allyl-3-chloro-1-hydroxy-4-oxo methyl ester (II) was isolated as indicated under the general procedure and recrystallized from ether–hexane to get white crystals, mp 91.5–92.5°; $[\alpha]^{25}D$ +105.0 ± 3.0° (*c* 1.00, EtOAc) (*Anal.* Calcd for $C_{10}H_{11}O_4Cl$: C, 52.06; H, 4.77; O, 27.76; Cl, 15.40. Found: C, 52.95; H, 5.29; O, 27.83; Cl, 15.40); λ_{max}^{MeOH} 277 mμ (ε 23.040); ν_{max}^{KBr} 3475, 2900, 1742, 1710, 1635, 1570, 1433, 1328, 1295, 1265, 1208, 1180, 1165, 1125, 1070, 1005, 970, 955, 925, 878, 838, 822, 798, 765, 695, and 678 cm^{-1}; nmr in $CDCl_3$: doublet at τ 8.0 (3 H, *J* = 6–7 cps), singlet at 6.17 (3 H), singlet at 5.42 (1 H) exchangeable with CD_3OD, singlet at 5.33 (1 H), multiplet at 3.55 (3 H); mass spectrum molecular ion at *m/e* 230. The ORD curve of II is shown in Figure 3.

The material turns yellow after a few weeks even if stored in amber vials in a desiccator. It is impossible to remove this coloration by recrystallization once it appears. No attempt has been made as yet to investigate this color change. Yields of this material were up to 65 mg of crystalline material per l. of mash. It has slightly lower antifungal activity than I.

(1*S*,5*R*)-2-Cyclopentene-1-carboxylic acid, 2-*trans*-allyl-3-chloro-1-hydroxy-4-oxo methyl ester (III) was obtained as outlined in the general isolation procedure as an oil which after much effort yielded yellowish crystals from ether. Several recrystallizations from ether–hexane and EtOAc–hexane gave white crystals, mp 83.5–84.5°; $[\alpha]^{25}D$ +322 ± 2.9° (*c* 1.00, EtOAc) (*Anal.* Calcd for $C_{10}H_{11}O_4Cl$: C, 52.06; H, 4.77; O, 27.76; Cl, 15.40. Found: C, 52.66; H, 5.26; O, 27.69; Cl, 15.57); λ_{max}^{MeOH} 277 mμ (ε 22,010); ν_{max}^{KBr} 3350, 2930, 1750, 1712, 1635, 1575, 1435, 1275, 1242, 1170, 1130, 1042, 1008, 963, 906, 870, 848, 795, 772, and 697 cm^{-1}; nmr in $CDCl_3$: doublet at τ 8.09 (3 H, *J* = 6–7 cps), singlet at 3.13 (3 H), singlet at 6.00 (1 H) exchange with CD_3OD, multiplet at 3.58 (3 H). Mass spectrum molecular ion at *m/e* 230. The ORD curve of III is shown in Figure 3.

The maximum yield of III was 10 mg of crystalline product per l. of mash. It had approximately the same antifungal activity as II.

Reaction of I with Cyanide Ion. The earliest spectral data obtained on I were ir and uv curves and these could be interpreted in terms of an α-pyrone structure. Since α-pyrones react smoothly with cyanide ion[14] to give good yields of cyanohexenoic acid, this test was tried. About 132 mg was dissolved in 5 ml of MeOH, and 30 mg of solid NaCN was added. The suspension darkened rapidly. After 15 min the reaction mixture was concentrated, diluted with H_2O, and extracted with $CHCl_3$. The $CHCl_3$ extract in concentration yielded a dark brown gum which could not be resolved by chromatography.

Effect of Acidic and Basic Reagents on I. a. Concentrated H_2SO_4. About 200 mg of I were dissolved in 0.5 ml of concentrated H_2SO_4. The solution had a faint greenish color and was allowed to remain at room temperature for about 60 hr. It was then diluted with H_2O and the resulting suspension was extracted with $CHCl_3$. After evaporation of the $CHCl_3$, the remaining solid was recrystallized from ether–hexane to yield 150 mg of white crystals, mp 135–136°. The ir spectrum of this material was identical with that of I.

b. Refluxing Dilute HCl. Before the structure of I was known to contain a methyl ester, the material was refluxed for 4 hr in 2.5 *N* HCl and recovered unchanged. In addition, 100 mg of I was dissolved in 5 ml of dioxane and 1 ml of 4 *N* HCl added, and the solution was refluxed for 20 hr. Approximately 36 mg of solid were recovered from the work-up and recrystallized from ether–

(14) G. Vogel, *J. Org. Chem.*, 30, 203 (1965).

hexane to yield 28 mg of white crystals, mp 135–136° whose ir was identical with that of the starting material. Some tar was also recovered from the work-up which could not be resolved chromatographically.

c. Basic Reagent. Addition of a few drops of dilute NaOH or 5% $NaHCO_3$ to a solution of I caused rapid darkening of the solution. Work-up attempts yielded only tars. Addition of a few drops of pyridine to a solution of I also caused darkening. Addition of hydrazine hydrate gave a precipitate of a tacky off-white product which gummed and darkened in a matter of minutes.

Ozonolysis of I. Approximately 250 mg of I was dissolved in 10 ml of CH_2Cl_2 and cooled to around −5°. The solution was then subjected to a stream of ozonized oxygen from a Welsbach generator for 20 min. The solvent was removed under reduced pressure and the yellow-green ozonide gum was decomposed by H_2O and Zn dust. The suspension was steam distilled into Brady's reagent and the resultant precipitate was chromatographed over alumina and eluted with benzene to get approximately 70 mg of a 2,4-DNP derivative, mp 163.5–165°. The literature melting point for the 2,4-DNP of acetaldehyde is 165–166°.[15] The ir spectrum of the ozonolysis derivative from I was identical with that of freshly prepared acetaldehyde 2,4-dinitrophenylhydrazone.

2,4-Dinitrophenylhydrazone of I. To a solution of 0.5 g of I in 30 ml of MeOH and 6 ml of concentrated HCl approximately 0.6 g of 2,4-dinitrophenylhydrazone was added. The reaction mixture was refluxed for 4 hr and the resultant solution was concentrated to 10 ml and refrigerated overnight. The precipitated solid was recovered to yield 0.9 g of red solid which was stirred with 100 ml of ether for 15–20 min. The insoluble material was discarded and the ether solution was concentrated to an oil which was chromatographed over silica gel. A major component of 200 mg was eluted with benzene–EtOAc (95:5) and recrystallized successively from MeOH–H_2O and ether to get 140 mg of reddish crystals, mp 188–189° (*Anal.* Calcd for $C_{16}H_{14}N_4O_7Cl_2$: C, 43.14; H, 3.14; N, 12.58; Cl, 15.95. Found: C, 43.53; H, 3.50; N, 12.16; Cl, 16.13); λ_{max}^{MeOH} 385 mμ (36,500), 303 (10,460), and 269 mμ (ε 13,800); ν_{max}^{KBr} 3410, 3290, 1750, 1620, 1592, 1530, 1430, 1362, 1340, 1315, 1270, 1215, 1140, 1118, 1100, 970, 924, 876, 833, and 742 cm^{-1}; nmr in $CDCl_3$: doublet at τ 8.12 (3 H, J = 6–7 cps), singlet at 6.23 (3 H), singlet at 4.88 (1 H), broad band at 4.58 (1 H), exchangeable with CD_3OD, doublet at 3.60 (3 H, J = 2–3 cps), doublet at 1.93 (1 H, J = 7–8 cps), split doublet at 1.63 (1 H, J = 7–8, 2–3 cps), doublet at 0.97 (1 H, J = 2–3 cps), and singlet at −1.77.

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Interpretation of the Titration Curve of Oxyhemoglobin. Detailed Consideration of Coulomb Interactions at Low Ionic Strength[1]

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Abstract: An extension of the Tanford–Kirkwood theory of protein titration curves has been used to fit the titration curve of horse oxyhemoglobin at low ionic strengths in the pH range 4.5–9.0. Site coordinates were taken from a model built according to Perutz' 1965 description. The effect of Coulomb interactions on the titration of the individual sites was obtained from the calculations. The sensitivity of the fit to variations of intrinsic pK's, protein dielectric constant, and site depth was investigated. The possibility that some sites might be masked was also considered, and a plausible interpretation of the Bohr effect was obtained. An attempt to correlate the results with short-range interactions was only partially successful, suggesting that longer range interactions also contribute to the variation of site environment.

The titration curve of a protein is the sum of the titration curves of all of its proton-binding groups. The total number of protons bound at each pH may be calculated from the experimental titration curve, isoionic pH, and composition of the protein. In addition, the number of protons bound to a particular type of group may be obtained from a spectrophotometric titration if the group has an observable spectral change with ionization, or from reaction rates if a catalytic effect changes with ionization. The difficulty with the latter two methods is that they are not generally applicable from the practical point of view. Experimental determination of the titration curves of the individual sites seems even more remote at this time.

The theoretical approach to the problem was initiated by Linderstrom-Lang[2] who calculated the Coulomb interactions of the charges bound to the protein by assuming all charges to be spread uniformly over the surface of a spherical protein. Scatchard[3] found that this model gave a satisfactory representation of the interactions of eight binding sites at the vertices of a cube. In a recent analysis, Tanford and Nozaki[4] have

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