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A CHEMICAL INVESTIGATION OF TELOSCHISTES FLAVICANS

A THESIS

Presented to

The Faculty of the Graduate Division

by

Vernon Mountcastle Balthis

In Partial Fulfillment

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A CHEMICAL INVESTIGATION OF

TELOSCHISTES FLAVICANS

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GLOSSARY OF ABBREVIATIONS

DMSO	dimethyl sulfoxide
i.d.	internal diameter
n.m.r.	nuclear magnetic resonance
TLC	thin-layer chromatography
TMS	tetramethylsilane

SUMMARY

Chemical investigations of a lichen from the Ascension Islands, Teloschistes flavicans v. minor Crombie, have led to the identification of two readily isolable compounds. The extract obtained from continuous chloroform extraction of the dry lichen represented 3.5% of the dry lichen.

The chloroform extract contained at least three components as analyzed by thin-layer chromatography. These components could not be separated by crystallization; however, after column chromatography using acid-washed alumina, crystallization of the eluted material yielded two crystalline compounds, white needles and gold needles, respectively.

The gold-colored crystalline compound was identified as parietin (4,5-dihydroxy-7-methoxy-2-methylanthraquinone) based on its melting point, elemental analysis, mass spectrum, and the preparation of the acetyl derivative, parietin diacetate. The n.m.r. spectrum of parietin could not be obtained due to its insolubility; however, the n.m.r. spectrum of parietin diacetate was easily analyzed and supported the proposed structure for parietin.

Earlier investigations of lichens of the species Teloschistes flavicans reported the isolation of parietin, teloschistin (4,5-dihydroxy-2-hydroxymethyl-7-methoxyanthraquinone), fallacinal (2-formyl-4,5-dihydroxy-7-methoxyanthraquinone), and a colorless substance, m.p. 240-250°, which was reported to have the formula $C_{17}H_{14}O_5Cl_2$ and was named vicanicin (2,4-dichloro-3-hydroxy-7-methoxy-1,5,8-trimethyldepsidone).

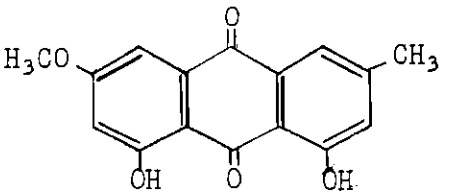
The white crystalline compound obtained in this investigation showed m.p. 239-240° and was first thought to be vicanicin. However, the n.m.r. spectrum of the compound showed four C-methyl groups whereas the reported structure for vicanicin had only three C-methyl groups. Based on the elemental analysis and the mass spectrum, which were satisfactory for the formula $C_{18}H_{16}O_5Cl_2$, and the n.m.r. spectrum, a new structure for vicanicin (2,4-dichloro-3-hydroxy-7-methoxy-1,5,6,8-tetramethyldepsidone) was proposed.

The acetyl derivative, O-acetylvicanicin, was prepared and characterized by elemental analysis and an n.m.r. spectrum. The methyl ether, O-methylvicanicin, and the methyl ester, methyl O-methylvicanicate, were also prepared. These compounds were characterized by elemental analysis, n.m.r. spectra, and mass spectra. The proposed structure of vicanicin was further substantiated by the identification of the expected products from the nitric acid degradation of methyl O-methylvicanicate, methyl 3,5-dichloroeverninate and hydroxytrimethyl-p-benzoquinone.

CHAPTER I

INTRODUCTION

As a result of the first chemical investigation of Teloschistes flavicans Norm, by W. Zopf in 1905, there was reported the isolation of two substances, parietin (I) and a colorless substance, m.p. 240-245° (1). The dried lichen was extracted with benzene, and upon concentration of the filtered solution, a yellow pigment was obtained. After washing the pigment with alcohol, the golden yellow crystals obtained were identical in chemical and physical properties with the known compound parietin: 4,5-dihydroxy-7-methoxy-2-methylanthraquinone (I) (1).

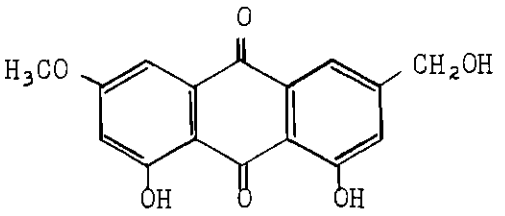


I

When the benzene-alcohol mother liquor of the parietin separation was concentrated and allowed to stand for a few days, a colorless material crystallized in small spherical aggregates (1). After repeated recrystallizations from boiling alcohol, there was obtained colorless crystals, m.p. 240-245° (1). An analysis of this compound was not reported in this work (1).

In 1949 Seshadri and Subramanian reported the isolation of parietin, teloschisten (II), and a colorless substance, m.p. 240-245°, from

an Indian sample of Teloschistes flavicans Norm (2). The first extraction of the lichen using ether as the solvent removed the three components. The colorless substance was separated as an alkali-insoluble fraction. However, in the alkali-soluble portion, they experienced difficulty in purifying the parietin because of the presence of a higher melting, less soluble component (2). This difficulty was eliminated by using petroleum ether and chloroform, successively, for the initial extractions of the lichen. The petroleum ether extract contained the colorless substance and parietin. The chloroform extract contained only the higher melting compound, teloschisten (II), whose structure was established as 4,5-dihydroxy-2-hydroxy-methyl-7-methoxyanthraquinone (2).



II

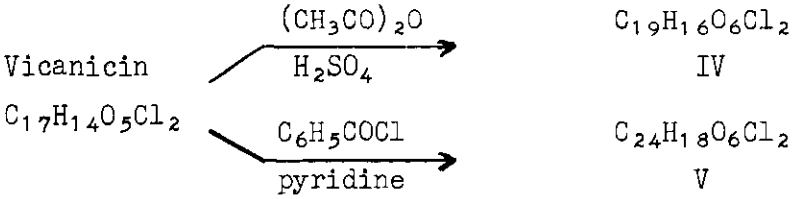
A sample of the same lichen, however, examined in 1955, contained fallacinal (2-formyl-4,5-dihydroxy-7-methoxyanthraquinone) instead of teloschisten (3).

These separate examinations of Teloschistes flavicans Norm established that although the anthraquinone compounds varied in composition, the colorless substance obtained seemed to be the same in all of the lichen samples.

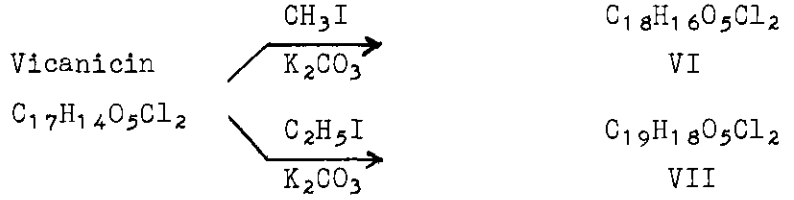
The first work on the identification of this colorless substance, which was named vicanicin (III), was reported in 1962 by Seshadri, et al.

(4). The vicanicin, which was originally isolated from petroleum ether extraction as an alkali-insoluble substance, was isolated in much better yield by column chromatography. Using a column of magnesium carbonate and benzene as the eluting solvent, the vicanicin was obtained pure while the anthraquinone compounds were held on the column (4). Vicanicin was crystallized from boiling benzene as colorless needles, m.p. 248-250°. The analytical data agreed with a molecular formula of $C_{17}H_{14}O_5Cl_2$, and the compound was shown to contain one methoxyl group by the Zeisel method, (found value, 1.06), and three C-methyl groups by the Kuhn-Roth method, (found value, 2.76) (4). The crystalline solid did not give a characteristic color with either alcoholic ferric chloride or concentrated sulfuric acid, nor was it readily soluble in aqueous sodium hydroxide (1,4).

The presence of a free hydroxyl group was established by the formation of a monoacetate (IV) and a monobenzoate (V) by the reaction of vicanicin with acetic anhydride and benzoyl chloride, respectively (4). The



hydroxyl group was shown to be phenolic in nature because vicanicin could be easily methylated (O-methylvicanicin, VI) and ethylated (O-ethylvicanicin,



VII) using the appropriate alkyl iodide and potassium carbonate (4).

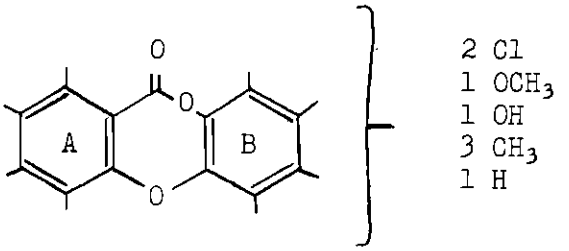
Vicanicin showed the presence of a lactone ring by the reaction of O-methylvicanicin (VI) with 2 N sodium hydroxide in dioxane solution followed by acidification (4). The opening of the lactone ring formed a hy-

$$\underset{\text{VI}}{C_{18}H_{16}O_5Cl_2} \xrightarrow[\text{2.) } H_3O^+]{\text{1.) } 2\ N\ NaOH,\ \text{dioxane}} \underset{\text{VIII}}{C_{18}H_{18}O_6Cl_2}$$

droxy acid, O-methylvicanicic acid (VIII). However, when O-methylvicanicin (VI) was refluxed with absolute methanolic sodium methoxide, methanolysis of the lactone ring produced a methyl ester, methyl O-methylvicanicate (IX) (4).

$$\underset{\text{VI}}{C_{18}H_{16}O_5Cl_2} \xrightarrow{NaOCH_3} \underset{\text{IX}}{C_{19}H_{20}O_6Cl_2}$$

Four of the five oxygen atoms of vicanicin were then accounted for by methoxyl, hydroxyl, and lactone groups. The fifth oxygen atom, being inert, was placed in a diphenyl ether linkage leading to a possible chlorodepsidone structure, as shown by part structure (X) (4). This possible



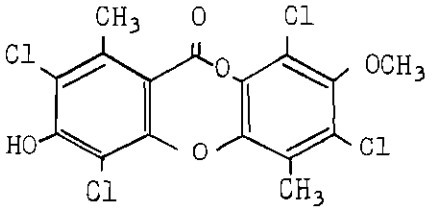
X

structure was supported by spectral studies of vicanicin. The ultraviolet

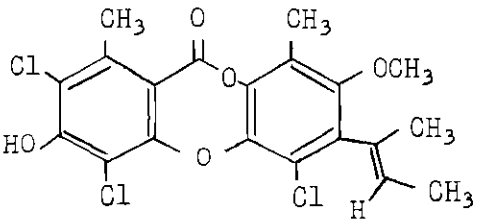
spectrum was similar to those of diploicin (XI), nidulin (XII), and nornidulin (XIII), given in Table 1 (4,5,6). These absorptions have been

Table 1. Ultraviolet Spectra of Some Chlorodepsidones

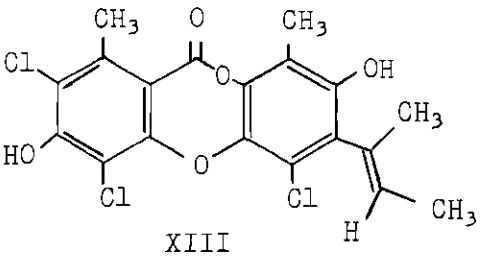
Compound	Absorption: λ_{max} (log ε)
Vicanicin	270 mμ (3.94), 324 (inflexion) (2.48)
Diploicin (XI)	270 mμ (3.79), 325 (inflexion) (3.04)
Nidulin (XII)	267 mμ (3.95), 323 (inflexion) (3.08)
Nornidulin (XIII)	266 mμ (3.91), 323 (inflexion) (2.87)



XI



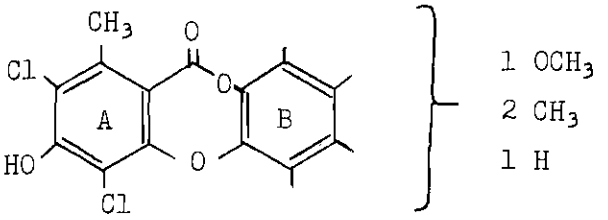
XII



XIII

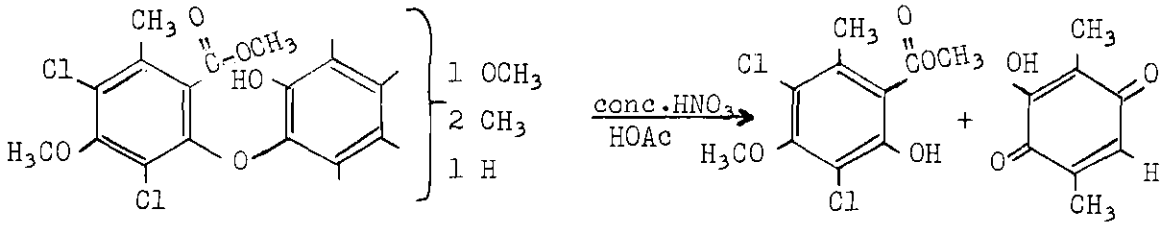
associated with ring A in these compounds (6). The comparable infrared spectra of vicanicin and diploicin led to the partial structure of vicanicin as shown by XIV, indicating a common ring A in all of the compounds

vicanicin, diploicin, nidulin, and nornidulin (4).



XIV

Chlorodepsidones have been degraded by the oxidation of the methyl ester (derived by hydrolysis of the lactone and esterification of the hydroxy acid) with concentrated nitric acid in acetic acid (4,6). When methyl O-methylvicanicate (IX) was degraded, the two products obtained were crystalline methyl 3,5-dichloroeverninate (XV) and an orange-red quinone (XVI), which was never isolated crystalline, but was obtained as a

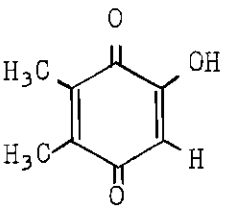


IX XV XVI

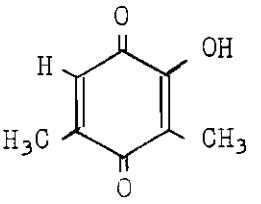
sodium bicarbonate-soluble fraction (4). This quinone (XVI) was identified as 2-hydroxy-3,6-dimethyl-p-benzoquinone based on the comparison of its visible spectrum in buffer solution (purple color, pH 10.4, absorption maximum at 532 mμ) and its infrared spectrum with an authentic sample of the quinone (4,7). The identity of the methyl 3,5-dichloroeverninate (XV) was confirmed by comparison with an authentic sample of the

compound prepared by the chlorination of methyl everninate (4,8).

The other possible structures of the benzoquinone, 2-hydroxy-5,6-dimethyl-p-benzoquinone (XVII) and 2-hydroxy-3,5-dimethyl-p-benzoquinone (XVIII) were eliminated by the following reasoning: 1) the ortho-xyloquinone derivative (XVII) absorbed at 490 mμ in buffer solution (pH 10.4); 2) the meta-xyloquinone derivative (XVIII) absorbed at 523 mμ in buffer solution (pH 10.4) (4,7).

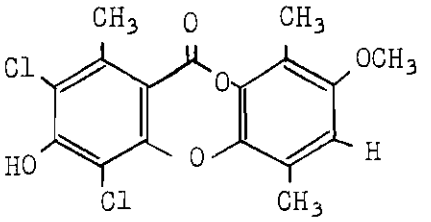


XVII



XVIII

The structure of vicanicin, based on the formation of the two degradation products, was completed as given by III (4). Apparently, the



III

location of the methoxyl group was deduced from analogies with diploicin, nidulin, and nornidulin.

The purpose of this research was to identify the readily isolable compounds from the Ascension Island lichen, Teloschistes flavicans v. minor

Crombie and to compare these compounds with those already isolated and identified from similar lichens of different habitat.

CHAPTER II

EXPERIMENTAL

Apparatus and Techniques

Anhydrous ether was obtained (Fischer reagent E-138) and stored over sodium ribbon. Redistilled benzene was dried by storage over sodium ribbon. Petroleum ether (b.p. 30-60°), methanol, and acetone were always redistilled. Chloroform, except when used for column chromatography, was also redistilled.

Unless otherwise stated, all concentrations and evaporations were performed using a Rinco (Model VE-1000-A) rotating evaporator at water aspirator vacuum and temperatures below 50°. Anhydrous magnesium sulfate (Mallinckrodt AR-6070) was used for drying solutions and extracts in organic solvents. The drying agent was removed by aspirator vacuum filtration through sintered glass funnels and was always washed thoroughly with several fresh portions of the solvent.

All melting points were observed using a Köfler hot stage and are corrected. Microanalyses were performed by Bernhardt Laboratories (Mülheim, West Germany). A Perkin Elmer Model 137 Infracord recording spectrophotometer was used to determine all infrared spectra. Potassium bromide was used for all pellet spectra.

Nuclear magnetic resonance (n.m.r.) spectra were determined using a Varian Model A-60 spectrometer. The magnet temperature was essentially constant at some value between 30 and 40° during the determination of a given spectrum. Chemical shift values are reported in τ units (τ =

10 - δ). Tetramethylsilane (TMS) was used as an internal standard. The spectra were calibrated with certain standards to correct for possible scale width deviations (i. e., TMS (10.00 τ) and chloroform (2.75 τ) for the 500 cps scale). Concentrations given for spectra determined using solutions are per cent by weight.

The mass spectra were obtained through the courtesy of Dr. C. C. Sweeley, Department of Biochemistry, Graduate School of Public Health, University of Pittsburgh. The direct probe inlet system of the LKB Model 9000 mass spectrometer was used. All spectra were obtained at 70 ev.

Alumina chromatography columns were prepared by slowly pouring the indicated amount of the acid-washed alumina (Merck 71695) into a cylindrical column which was already half filled with the indicated solvent. The column had a coarse, fritted glass disc at the bottom and was packed by draining the excess solvent, accompanied by vibration, until the adsorbant was firm. The packed dimensions are given in the text.

Silicic acid chromatography columns were prepared by mixing the indicated amount of silicic acid (100 mesh, Mallinckrodt AR-2847) with chloroform. The slurry was slowly poured into a cylindrical column that had a coarse, fritted glass disc at the bottom. The column was packed by draining the excess chloroform, accompanied by stirring, followed by vibration until the adsorbant was firm. The packed dimensions are given in the text.

Techniques of thin-layer chromatography (TLC) were performed as described previously (9).

Preparation of the Lichen for Chemical Investigation

Extraction Procedures

The lichen, as received, was a damp, rust-colored plant material that smelled faintly of ammonia or a volatile amine. The volcanic ash, sticks, and other debris were removed by hand, and the lichen was then spread out to dry in air. After 24 hrs., the lichen became brittle and was sifted through a sieve (14 mesh) to remove the remaining foreign material. The total weight of dry lichen obtained was 2,751 g.

Petroleum Ether Extractions. An extraction with redistilled petroleum ether (b.p. 30-60°) was started using two soxhlet extractors that contained 206 g. of the lichen equally divided between them. After six hours of extraction, the material from both extractors was combined and evaporated to dryness; this gave 2.54 g. of a pinkish-yellow solid. This material showed seven spots by TLC using the system 3% methanol in chloroform: (R_F values were 0.86, 0.78 (yellow), 0.66, 0.58 (pink), 0.27, 0.19, and 0.12). The spots not designated by a color were detected by iodine. An additional 48 hrs. of extraction gave 1.28 g. of an orange colored solid that showed three spots by TLC using the system 3% methanol in chloroform. R_F values were 0.69 (yellow), 0.56 (pink), and 0.28. The petroleum ether extractions yielded a total of 3.82 g., which is 1.86% of the weight of the dry lichen extracted.

Chloroform Extractions. After the petroleum ether extractions, the lichen was removed from the soxhlets and allowed to dry in air. The lichen was then extracted in the same manner, using chloroform, for 24 hrs. After combining the extracts from the two soxhlets and evaporation of the extract to dryness, 1.74 g. of material was obtained. An additional

48 hrs. of extraction gave 0.33 g. of extract. Chloroform extraction gave 2.07 g. of material, which is 1.01% of the weight of dry lichen extracted.

Ninety-Five Per Cent Ethanol Extractions. After the chloroform extraction, the lichen was again dried in air. The dry lichen was then extracted with 95% ethanol for 24 hrs., which yielded 3.32 g. of material. An additional 48 hrs. of extraction with fresh 95% ethanol gave 0.65 g. of material. The total ethanol extraction gave 3.97 g. of material, which is 1.92% of the weight of lichen extracted.

Isolation of Components by Column Chromatography

A portion of the dry lichen (1,426 g.) was extracted continuously in two large soxhlets with chloroform for two weeks. The extract was evaporated to dryness; the residue was mixed with 95% ethanol, filtered from plant material carried over mechanically, and the filtrate was evaporated to dryness. This dry residue was mixed with 500 ml. of boiling chloroform. The chloroform solution was allowed to cool to room temperature and was then filtered. The filtrate, when evaporated to dryness, contained 38.2 g. (fraction A) of a brownish-orange solid, which showed three spots by TLC using the system 3% methanol in chloroform. The R_F values were 0.75 (yellow), 0.59 (pink), and 0.37 (detected by shortwave ultraviolet light). The above chloroform extraction procedure was repeated three more times; the weights obtained were 5.4 g., 3.3 g., and 2.9 g., respectively. The total weight obtained from the four extractions was 49.8 g., which is 3.5% of the dry lichen extracted.

Five grams of fraction A was dissolved in 75 ml. of boiling chloroform, and 75 g. of acid-washed alumina was added with stirring. After

thorough mixing, the chloroform solvent was allowed to evaporate at room temperature. This dry mixture was then placed on top of a bed of 50 g. of acid-washed alumina (moist with benzene) in a sintered glass funnel, (i.d., 6.7 cm.). The alumina was then eluted with boiling benzene; one-liter fractions were collected. The first two fractions, after being evaporated to dryness, contained 3.5 g. (70% of the material) of a yellow-white solid. Boiling chloroform was then used as the eluting solvent. The next two fractions contained 0.69 g. (14% of the material) of a gold-brown solid. The total weight recovered was 4.19 g. (84% of the material).

The yellow-white solid (3.5 g.) which showed two spots by TLC (using the system 3% methanol in chloroform: R_F values were 0.66 (yellow) and 0.57 (detected by shortwave ultraviolet light)), was chromatographed over another bed of alumina in the same manner as above. The first two one-liter fractions, eluted with boiling benzene, contained 2.72 g. (54% of the original 5 g.) of a yellow-white solid, which showed the same two spots by TLC as the first elution (R_F values were 0.66 and 0.57). The next fraction, B, eluted with boiling chloroform, contained 0.39 g. (8% of the original 5 g.) of a yellow solid. The total weight recovered was 3.11 g. (62% of the original 5 g.).

The above yellow-white solid (2.72 g.) was mixed with 15 g. of alumina and 15 ml. of boiling chloroform, stirred, and the chloroform was allowed to evaporate overnight. This dry mixture was then chromatographed over acid-washed alumina (81 g., column 31 cm. x 1 cm.) using benzene as the eluting solvent (fraction volume, 100 ml.). Fractions 1-4 contained no material. Fractions 5-19 contained 2.07 g. (41% of the original 5 g.) of a white solid. The eluting solvent was changed to

chloroform; fractions 20-24 contained 0.35 g. (7% of the original 5 g.) of a yellow solid. The total weight recovered was 2.42 g. (48% of the original 5 g.).

The alumina remaining in the sintered glass funnels and the column after the chloroform extraction was a light red in color. The alumina in the two sintered glass funnels was combined and extracted continuously in a soxhlet extractor with glacial acetic acid (600 ml.) for three hours. The extract was allowed to cool to room temperature, filtered by gravity, and evaporated to dryness. The dark red material recovered weighed 0.98 g. (20% of the material).

The remainder (33.2 g.) of fraction A was mixed with 300 g. of acid-washed alumina and 300 ml. of boiling chloroform; the mixture was stirred, and the chloroform was allowed to evaporate. This dry mixture was then placed on a column of acid-washed alumina (4,980 g., column 83 cm. x 4.65 cm.) and benzene was used as the first eluting solvent (fraction volume, 1000 ml.). The complete elution sequence of the column is given below.

Solvent	Number of Fractions	Weight Recovered*
100% Benzene	64	0.98 g.
2% Chloroform in benzene	15	0.00 g.
5% Chloroform in benzene	15	0.00 g.
10% Chloroform in benzene	32	0.00 g.
20% Chloroform in benzene	56	9.53 g.
100% Chloroform	27	4.91 g.

The total weight of the material recovered from the column was 15.42 g. (47%). The alumina, which after the chloroform elution was a

---

* The weights of recovered material do not include any fractions that weighed less than 10 mg. These fractions were discarded.

light red in color, was then divided into two parts; both parts were extracted with boiling chloroform on sintered glass funnels (i.d., 15.7 cm.). After twenty-one liters of chloroform extraction, a gold colored preparation was obtained that weighed 3.17 g. Elution of the column using pure chloroform gave 4.91 g. of a gold colored preparation; this was combined with the 3.17 g. The total weight of gold colored preparation was 8.08 g. (24%).

In the elution using 20% chloroform in benzene, fractions 12-56 contained 8.75 g. (27%) of a yellow-white solid. This material was divided in half and each was chromatographed over alumina using benzene as the eluting solvent in two separate columns in the same manner as described previously (p. 13). After crystallization from benzene there was obtained 5.7 g. (17%) of white needles, m.p. 239-240°.

Parietin

Fraction B (0.39 g., p. 13) was crystallized from glacial acetic acid. There was obtained 0.24 g. of yellow-orange crystals, m.p. 207-208° [lit. (10) m.p. 206-207°]. The infrared spectrum (pellet) showed λ_{max} 2.80, 3.30, 5.82, 5.96, 6.12, 6.18, 6.39, 6.78, 7.24, 7.35, 7.59, 8.19, and 8.62 μ, among others. An n.m.r. spectrum could not be obtained because the compound was not sufficiently soluble in any of the common organic solvents. The mass spectrum of parietin had peaks in the molecular ion region at m/e (intensity) 283 (100) and 284 (18). All other observed peaks had relative intensities less than 10.

A small portion of the compound was recrystallized from glacial acetic acid for elemental analysis. The analytical sample showed m.p. 207-208°.

Anal.	$C_{16}H_{12}O_5$	Calc'd:	C, 67.60; H, 4.26; O, 28.14
	(284.26)	Found :	C, 68.24; H, 4.33; O, 27.55

Parietin Diacetate

A solution of parietin (0.24 g., 0.84 mmole), 5 ml. of redistilled glacial acetic acid, and four drops of concentrated sulfuric acid was boiled under reflux for five minutes. The solution was cooled and poured into 300 ml. of distilled water. The solution was filtered by gravity and the water-insoluble material was dried in vacuo at room temperature overnight. The resulting orange crystals showed m.p. 190-210°.

The orange solid was then dissolved in 5 ml. of redistilled acetic anhydride to which was added two drops of concentrated sulfuric acid. The solution was boiled under reflux for five minutes and was then allowed to cool to room temperature. The solution was poured into 300 ml. of distilled water. The water-insoluble material was removed by filtration. After this material was dried in vacuo at room temperature for two hours, a purplish-brown solid (0.28 g.) was obtained.

The solid (0.28 g.) was chromatographed over acid-washed alumina (10 g., column 10.6 cm. x 0.55 cm.) using chloroform as the eluting solvent (fraction volume, 10 ml.). Fraction one contained no material. Fraction two contained 0.22 g. of a bright yellow solid. After recrystallization from redistilled glacial acetic acid, there was obtained 0.070 g. (22%) of yellow crystals, m.p. 187-188°, [lit. (10) m.p. 185-186°].

The n.m.r. spectrum (21%, deuteriochloroform) of the compound showed absorption at 7.56 (6H, singlet), 7.54 (3H, doublet of doublets, J = 0.7, 0.7), 6.03 (3H, singlet), 3.14 (1H, doublet, J = 2.8), 2.82 (1H, double quartet, J = 1.8 and 0.7), 2.35 (1H, doublet, J = 2.8), and 2.01 τ (1H,

double quartet, J = 1.8 and 0.7).

Vicanicin

The white solid (2.07 g., page 13), when crystallized from redistilled benzene, gave 1.17 g. of white needles, m.p. 240-241°, [lit. (4) m.p. 248-250°]. The infrared spectrum (pellet) showed λ_{max} 2.90, 3.35, 5.82, 6.28, 6.91, 7.12, 7.85, 8.25, 8.68, and 9.20 μ, among others. The n.m.r. spectrum (4%, deuteriochloroform) of the compound showed absorptions at 7.71 (3H, multiplet), 7.60 (3H, multiplet), 7.52 (6H, multiplet), 6.25 (3H, singlet), and 3.83 τ (1H, broad singlet). The C-methyl hydrogens were coupled with other hydrogens. The n.m.r. spectrum (21%, DMSO-d_6) of the compound showed absorptions at 7.75 (3H, multiplet), 7.62 (6H, multiplet), 7.52 (3H, multiplet), 6.28 (3H, singlet), and 5.86 τ (1H, broad singlet). The C-methyl hydrogens were coupled with other hydrogens. The mass spectrum of vicanicin had peaks in the molecular ion region at m/e (intensity) 382 (23), 384 (16), and 386 (3). Other intense peaks were present at m/e (intensity) 347 (100), 156 (42), 349 (37), 348 (21), 332 (21), 354 (18), and 77 (18). All other peaks observed had relative intensities less than 15.

A small sample of the compound was recrystallized twice from redistilled benzene for elemental analysis. The analytical sample showed m.p. 239-240°.

Anal.	$C_{18}H_{16}O_5Cl_2$	Calc'd:	C, 56.39; H, 4.21; Cl, 18.52
	(383.31)	Found :	C, 56.55; H, 4.40;
		*Found :	C, 55.80; H, 4.37; Cl, 19.08

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* A sample suspected of being less pure.

O-Acetylvicanicin

Vicanicin (0.198 g., 0.51 mmole) was dissolved in 5.4 ml. of redistilled pyridine and 5.5 ml. of redistilled acetic anhydride was added to the solution. The solution was allowed to stand at room temperature overnight and was then heated on a steam bath under reflux for one hour. The solution was then evaporated *in vacuo* and the dry residue was dissolved in 35 ml. of chloroform. The chloroform solution was washed twice with 5% aqueous sodium carbonate (20 ml.), twice with ice-cold 3 N hydrochloric acid (20 ml.), and twice with redistilled water (20 ml.). The chloroform extract was dried and evaporated, yielding 0.220 g. of a white solid. After crystallization from redistilled ethyl acetate, there was obtained white crystalline O-acetylvicanicin, 0.075 g. (34%), m.p. 208-209°, [lit. (4), m.p. 213-214°].

The n.m.r. spectrum (13%, deuteriochloroform) of O-acetylvicanicin showed absorptions at 7.70 (3H, multiplet), 7.68 (3H, multiplet), 7.62 (3H, singlet), 7.51 (6H, multiplet), and 6.22 τ (3H, singlet). The C-methyl hydrogens were coupled with other hydrogens.

A small sample of the compound was recrystallized twice from redistilled ethyl acetate for elemental analysis. The analytical sample showed m.p. 210-211°.

Anal. $C_{20}H_{18}O_6Cl_2$ (425.25) Calc'd: C, 56.48; H, 4.26; O, 22.57
Found : C, 56.33; H, 4.23; O, 22.21

O-Methylvicanicin

Vicanicin (1.00 g., 2.6 mmole) was dissolved in a solution of 90 ml. of anhydrous ether and 30 ml. of redistilled methanol. An ice-cold ethereal solution of diazomethane (11) was slowly added by pipette to the

vicanicin solution until the solution retained a faint yellow color. The solution was allowed to stand overnight at room temperature and was then evaporated. After crystallization of the white residue from redistilled benzene, white crystalline O-methylvicanicin (0.750 g., 72%) showed m.p. 193.5-194.5°, [lit. (4), m.p. 193-194°].

The infrared spectrum (pellet) of O-methylvicanicin showed λ_{max} 3.35, 5.75, 5.83, 6.97, 7.12, 7.89, 8.21, 8.69, and 9.21 μ, among others. The n.m.r. spectrum (21%, deuteriochloroform) of the compound showed absorptions at 7.80 (3H, multiplet), 7.57 (3H, multiplet), 7.51 (6H, multiplet), 6.23 (3H, singlet), and 6.17 τ (3H, singlet). The C-methyl hydrogens were coupled with other hydrogens.

The mass spectrum of O-methylvicanicin had peaks in the molecular ion region at m/e (intensity) 396 (26), 398 (18), and 400 (3). Other intense peaks were present at m/e (intensity) 361 (100), 363 (38), and 156 (29). All other peaks observed had relative intensities less than 15.

A small sample of the compound was recrystallized twice from redistilled benzene for elemental analysis. The analytical sample showed m.p. 192-193°.

Anal. $C_{19}H_{18}O_5Cl_2$ (397.24) Calc'd: C, 57.44; H, 4.57
Found : C, 57.66; H, 4.43

Methyl O-Methylvicanicate

A solution of 0.4 g. (1.0 mmole) of O-methylvicanicin in 20 ml. of a sodium methoxide solution (0.5 g. of sodium metal in 50 ml. of redistilled methanol) was heated on a steam bath under reflux for two hours. The residue, after evaporation of the solution, was washed with 20 ml. of ice-cold 1 N hydrochloric acid. The aqueous solution was immediately

extracted three times with 20-ml. portions of ice-cold chloroform. The chloroform extracts were combined, dried, and evaporated. The residue (0.41 g., 95%) was a colorless, glassy solid. After crystallization from redistilled methanol, white crystalline methyl O-methylvicanicate (0.30 g., 70%) showed m.p. 158-160° [lit. (4), m.p. 155-156°].

The infrared spectrum (pellet) of methyl O-methylvicanicate showed λ_{max} 2.88, 3.38, 5.81, 6.92, 7.88, 8.29, and 9.18 μ, among others. The n.m.r. spectrum (21%, deuteriochloroform) of the compound showed absorptions at 7.95 (3H, multiplet), 7.88 (3H, multiplet), 7.80 (3H, multiplet), 7.71 (3H, multiplet), 6.33 (3H, singlet), 6.22 (3H, singlet), 6.20 (3H, singlet), and 3.40 τ (1H, broad singlet). The C-methyl hydrogens were coupled with other hydrogens.

The mass spectrum of methyl O-methylvicanicate had peaks in the molecular ion region at m/e (intensity) 429 (17), 431 (12), and 433 (2). Other intense peaks were present at m/e (intensity) 361 (100), 363 (36), 362 (24), 368 (23), 156 (21), and 370 (15). All other peaks observed had relative intensities less than 15.

A small sample of the compound was recrystallized twice from redistilled methanol for elemental analysis. The analytical sample showed m.p. 154-156°.

Anal.	$C_{20}H_{22}O_6Cl_2$	Calc'd:	C, 55.95; H, 5.17
	(429.29)	Found :	C, 56.70; H, 5.18

Degradation of Methyl O-Methylvicanicate

Methyl O-methylvicanicate (0.2 g., 0.47 mmole) was dissolved in 5 ml. of redistilled glacial acetic acid. Concentrated nitric acid (0.25 ml.) was slowly added to the cooled solution (10°C). The reaction mixture

was allowed to stand at room temperature for 15 min. Ten per cent sodium bicarbonate solution was added dropwise until the solution had pH 6.5. The solution was then extracted with six 50-ml. portions of ether. The extracts were combined, dried, and evaporated. A red oil with an odor of acetic acid was obtained. After drying the oil *in vacuo* overnight, the mixture of an orange-red oil and white crystals weighed 0.16 g.

The mixture (0.16 g.) was dissolved in 5 ml. of redistilled chloroform. The chloroform solution was spread equally on four identical preparative TLC plates (HF_{254}, 0.5 mm. thickness). Redistilled chloroform was used in developing the plates. Five distinct separate bands were observed; four were colored and one was visualized using ultraviolet light. The R_F value and color of each band was as follows: band A (0.66, visualized using ultraviolet light), band B (0.56, dark yellow), band C (streak from 0.3 to 0.4, light yellow), band D (0.18, purple), and band E (streak from 0.0 to 0.15, light brown).

Band A was scraped from the plate; the material was eluted using 10 ml. of redistilled chloroform. The clear oil obtained after evaporation of the solvent was dried *in vacuo* overnight. There was obtained 55 mg. (45%) of white crystalline methyl 3,5-dichloroeverninate, m.p. 76-78°, [lit. (4), m.p. 77-78°]. Recrystallization of the compound showed no change in the m.p.

Band D was scraped from the plate; the material was eluted using 10 ml. of redistilled chloroform and 10 ml. of redistilled acetone. The extracts were combined and evaporated. The residue (14 mg.), which had been dried *in vacuo* overnight, was a yellow-orange material. TLC analysis showed one purple spot at R_F 0.18 using chloroform (Silica Gel HF_{254},

visible detection), [lit. (12), hydroxytrimethyl-p-benzoquinone, R_F 0.22 (Silica Gel G, chloroform)].

The reaction and separation, described above, were repeated using the same scale in order to obtain enough material for a n.m.r. spectrum of band D.

The n.m.r. spectrum (21%, deuteriochloroform) of band D showed absorptions at 8.06 (3H, singlet), 7.96 (6H, singlet), and 2.94 τ (1H, broad singlet).

1,2,4-Trimethyl-3,5,6-triacetoxybenzene

A mixture of 2,3,5-trimethyl-1,4-benzoquinone (3.0 g., 0.02 mole), redistilled acetic anhydride (30 ml.), and concentrated sulfuric acid (3.0 g., 0.03 mole) was allowed to stand at room temperature for two hours. The mixture was then boiled under reflux for two hours. After the reaction mixture had cooled to room temperature, distilled deoxygenated water (11.5 ml.) was added, and the mixture was allowed to stand at room temperature for one hour. The dark purple mixture was then extracted with three 100-ml. portions of ether. The extracts were combined, filtered by gravity, and washed until neutral with ten per cent sodium bicarbonate solution. The purple extract was then washed with distilled water (200 ml.), and the resulting yellow ether extract was dried and evaporated. There was obtained 3.5 g. (59%) of a light yellow solid and orange oil. The addition of anhydrous ether (20 ml.) dissolved the orange oil, leaving a white solid. After allowing the mixture to stand overnight, the white solid was filtered, washed with anhydrous ether, and dried. There was obtained 1.8 g. (30%) of 1,2,4-trimethyl-3,5,6-triacetoxybenzene, m.p. 149-151°, [lit. (13), m.p. 152°].

The n.m.r. spectrum (21%, deuteriochloroform) of the compound showed absorptions at 8.05 (3H, singlet), 7.94 (6H, singlet), 7.72 (6H, singlet), and 7.68 τ (3H, singlet).

Hydroxytrimethyl-p-benzoquinone

A mixture of 1,2,4-trimethyl-3,5,6-triacetoxybenzene (2.0 g., 6.8 mmole), redistilled methanol (15 ml.), and concentrated sulfuric acid (0.5 ml.) was heated under reflux on a steam bath for one hour. Ebullition with dry nitrogen was maintained throughout the reaction. The mixture was cooled, and 30 ml. of distilled, deoxygenated water was added. The methanol was evaporated *in vacuo* at temperatures below 50°. The aqueous solution was then extracted with three 50-ml. portions of ether. The extracts were combined, dried, and evaporated. The resulting cream-white residue was dissolved in 200 ml. of a pH 8 buffer solution (the buffer solution was prepared by dissolving six Coleman certified buffer tablets (Formula No. 30) in 400 ml. of distilled water). The purple solution was then subjected to a slow, steady stream of oxygen at room temperature for 75 min. The dark solution was filtered and placed in an ice-water bath. Concentrated hydrochloric acid was slowly added with stirring until the solution turned light yellow in color; a bright yellow solid precipitated. The solid was immediately filtered with suction and washed with distilled water. After drying the solid *in vacuo* for three hours, there was obtained 0.47 g. (43%) of yellow crystalline hydroxytrimethyl-p-benzoquinone, m.p. 94-95°, [lit. (13), m.p. 95°].

The n.m.r. spectrum (21%, deuteriochloroform) of the compound showed absorptions at 8.23 (3H, singlet), 8.13 (6H, singlet), and 3.46 τ (1H,

singlet).

Attempted Preparations of 2-Hydroxy-3,6-dimethyl-p-benzoquinone

A.) 2,5-Dimethyl-1,3,4-triacetoxybenzene (1.0 g., 3.6 mmole) was mixed with 25 ml. of distilled deoxygenated water. To this suspension 8 ml. of 25% aqueous sodium hydroxide was added, and the mixture was warmed slowly on a steam bath until the solid had dissolved. Then 8 ml. of concentrated hydrochloric acid was added, the solution was filtered, and 8 ml. of 1 N ferric chloride-hydrochloric acid solution was added to the ice-cooled reaction mixture. The yellow-brown solid that precipitated immediately was filtered and dried *in vacuo*. The residue (0.23 g.) was a tan solid, m.p. 180-200°, [lit. (7), m.p. 140-141°]. The solid (0.23 g.) was then chromatographed over silicic acid (10 g., column 9.5 cm. x 0.6 cm.) using chloroform as the eluting solvent (fraction volume, 10 ml.). Fraction three contained 88 mg. of a yellow solid, m.p. 185-195°. The n.m.r. spectrum (21%, deuteriochloroform) of the solid showed absorptions at 8.07 (3H, singlet), 8.00 (3H, singlet), and 2.79 τ (1H, broad singlet).

B.) A mixture of 2,5-dimethyl-1,3,4-triacetoxybenzene (2.0 g., 7.1 mmole), redistilled methanol (15 ml.), and concentrated sulfuric acid (0.5 ml.) was heated under reflux on a steam bath for one hour. Ebullition with dry nitrogen was maintained throughout the reaction. The mixture was cooled, and 30 ml. of distilled deoxygenated water was added. The methanol was evaporated *in vacuo* at temperatures below 50°. The aqueous solution was then extracted with four 50-ml. portions of ether. The extracts were combined, dried, and evaporated. The residue was dissolved

in 200 ml. of a pH 8 buffer solution. (The buffer solution was the same as described on p. 23.) The purple solution was then subjected to a slow, steady stream of oxygen at room temperature for one hour. The dark solution was filtered and placed in an ice bath. Concentrated hydrochloric acid was slowly added with stirring until the solution began to turn yellow in color. The solution then began to darken rapidly and a yellow-brown solid precipitated. The solid was immediately filtered with suction and dried *in vacuo*. There was obtained 0.52 g. of a brown solid, m.p. 180-200°. The solid (0.52 g.) was then chromatographed over silicic acid (20 g., column 9.5 cm. x 1.1 cm.) using 30% acetone in chloroform as the eluting solvent (fraction volume, 25 ml.). Fraction one contained 0.50 g. of a black oil after drying *in vacuo* for four hours.

CHAPTER III

DISCUSSION OF RESULTS

From earlier chemical investigations of the lichen Teloschistes flavicans, three major components have been isolated and identified from each lichen sample studied. Although these samples were from different habitats, they all contained two anthraquinone derivatives and a colorless substance, m.p. 240-250° (1,2,3,4). The predominant anthraquinone derivatives were identified as parietin (I) and teloschisten (II). The colorless substance was identified as being a new chlorodepsidone, 2,4-dichloro-3-hydroxy-7-methoxy-1,5,8-trimethyldepsidone, which was named vicanicin (III) (4).

The purpose of this research was to isolate and identify the major components of a lichen from the Ascension Islands.

The lichen, identified as Teloschistes flavicans v. minor Crombie, was much smaller than the previously investigated samples of Teloschistes flavicans, probably due to the unusual habitat of the Ascension Islands. Small samples of the dry lichen were tested for solubility in various organic solvents so that an efficient solvent system could be utilized in extracting the components of the lichen. It was found that using either low-boiling petroleum ether or chloroform as the solvent in a continuous extraction produced the same components, as identified by R_F values using TLC. Therefore, the majority of crude extract used in the chemical studies was obtained by a continuous soxhlet extraction using chloroform as the solvent. The crude extract, after evaporation of the chloroform, was

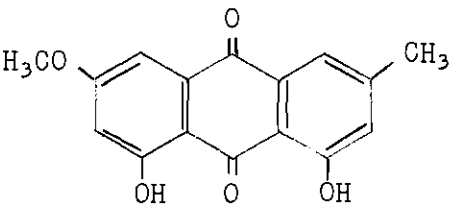
mixed with 95% ethanol which was then filtered, and the filtrate was evaporated to dryness. This residue was extracted with portions of chloroform four successive times. The combined extracts showed three major components by TLC at R_F values of 0.75 (yellow), 0.59 (pink), and 0.37 (detected by ultraviolet light). The components of the crude extract resisted separation by crystallization in all of the many solvent systems tried.

In exploratory attempts to separate the three major components by column chromatography, it was found that the crude extract could be separated into three separate bands, a yellowish-white band, a gold band, and a red band. The eluting solvent system used first was pure benzene, which eluted the yellowish-white band; chloroform eluted the gold-colored material; the red material remained on the column. However, this successful separation was only attained on small samples of the crude extract. When attempted on a large scale, pure benzene would not elute the yellowish-white component, and it was obtained only by using chloroform-benzene mixtures (p. 14). The chloroform-benzene eluant contained some of the gold-colored compound, so these extracts were chromatographed again; pure benzene eluted the yellowish-white component. Pure chloroform was then used to elute the gold-colored material. The alumina, which was pink after the pure chloroform elution, was then extracted continuously with acetic acid. This extract was a red-brown material, which was shown to be a mixture of components by TLC. It was not investigated further.

Extraction of 1,426 g. of the dry lichen with chloroform gave 49.8 g. (3.5%) of crude material. Chromatography of 33.2 g. of this extract gave the following results: 1.) 15.4 g. (47%) of material was recovered; 2.) 8.08 g. (24%) of gold-colored compound was obtained; 3.) 8.75 g. (27%)

of yellowish-white material was obtained. The gold-colored material from the chloroform elutions was recrystallized at least two times from glacial acetic acid and gave pure compound XIX, gold needles, m.p. 207-208°. The yellowish-white material was recrystallized at least twice from benzene and gave pure compound XX, white needles, m.p. 239-240°.

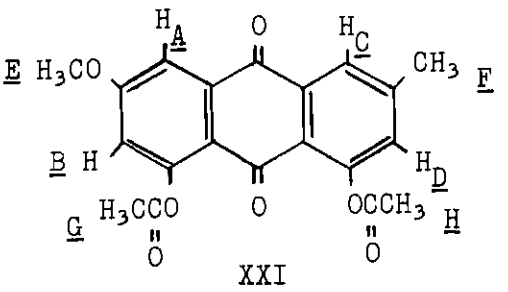
The gold-colored compound XIX was identified as parietin (I) based on its m.p., spectral data, and diacetate derivative. Compound XIX melt-



I

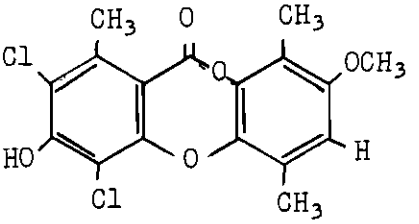
ed at 207-208° and its diacetate derivative melted at 187-188°, which agree with those reported in the literature (10). The mass spectrum of XIX had its most intense peak in the molecular ion region at m/e 283. This supports the formula $C_{16}H_{12}O_5$ for I. There were no other peaks in the spectrum with a relative intensity greater than 18. The n.m.r. spectrum of XIX could not be obtained due to its insolubility in the common organic solvents. However, the n.m.r. spectrum of the diacetate derivative (XXI) of XIX supports the proposed structure of parietin (I) and was easily characterized, as shown below.

The white crystalline compound XX, when first obtained, was thought to be the chlorodepsidone, vicanicin (III), even though the m.p. of XX was ten degrees below that reported in the literature for III (4). This



XXI

H	τ	J, cps
A	3.14	Ab = 2.8
B	2.35	
C	2.01	CD = 1.8
D	2.82	CF = 0.7
E	6.03	DF = 0.7
F	7.54	
G,H	7.56	



III

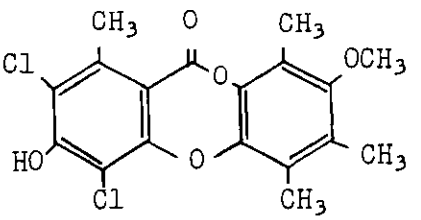
possibility was eliminated, however, when the n.m.r. spectrum of XX clearly showed four C-methyl group absorptions and no aromatic hydrogen absorption. Although it was reported that Kuhn-Roth analysis showed three C-methyl groups in III, the found value of 2.76 would only be in 31% error for four C-methyl groups (4). There has been no previous n.m.r. spectrum reported for vicanicin.

The elemental analysis of compound XX was satisfactory for the formula $C_{18}H_{16}O_5Cl_2$, which is one carbon and two hydrogens more than is represented by III. The mass spectrum of compound XX also supported the formula $C_{18}H_{16}O_5Cl_2$, M.W. = 383.31. The molecular ion region had m/e

(intensity) 382 (23), 384 (16), and 386 (3). The ratio of these intensities is in good agreement with the expected ratio for a compound with two chlorine atoms (14). The most intense peaks in the spectrum were at m/e 347 (100), 156 (42), and 349 (37). The peaks at mass 347 and mass 349 are attributed to the loss of a chlorine atom, which produces the fragmentation species $[C_{18}H_{16}O_5Cl]^+$. The peak at mass 156 (42) corresponds to a species $[C_7H_5O_2Cl]^+$. This peak was accompanied by a peak at mass 158 (13) which confirms that the species must have a chlorine atom rather than being an ion produced from the loss of both chlorine atoms.

The n.m.r. spectrum of compound XX (saturated deuteriochloroform solution) showed absorptions at 7.71 ($-CH_3$, multiplet), 7.60 ($-CH_3$, multiplet), 7.52 ($2-CH_3$, multiplet), 6.25 ($-OCH_3$, singlet), and 3.83 τ (-OH, broad singlet). The n.m.r. spectrum of compound XX (21%, DMSO-d_6) showed a substantial shift of the hydroxyl proton absorption. The absorptions were 7.75 ($-CH_3$, multiplet), 7.62 ($2-CH_3$, multiplet), 7.52 ($-CH_3$, multiplet), 6.28 ($-OCH_3$, singlet), and 5.86 τ (-OH, broad singlet). There was no aromatic proton absorption in either spectrum.

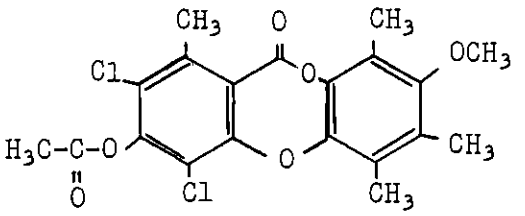
A new structure, XX, for vicanicin was proposed based on the evidence obtained from the elemental analysis, mass spectrum, and n.m.r.



XX

spectrum. However, it seemed desirable to substantiate this structure by the preparation of derivatives and the identification of degradation products.

The acetyl derivative of vicanicin (XX), O-acetylvicanicin (XXII), was a white crystalline compound, m.p. 210-211°. The elemental analysis was satisfactory with the formula $C_{20}H_{18}O_6Cl_2$. The n.m.r. spectrum of O-acetylvicanicin showed absorptions at 7.70 (CH_3, multiplet), 7.76 ($-CH_3$,

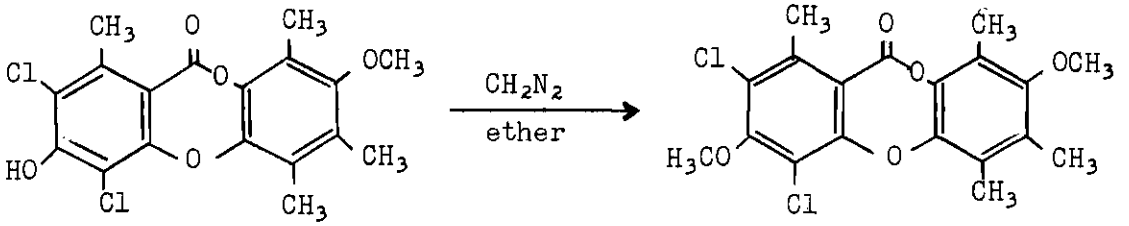


XXII

multiplet), 7.62 ($-O-\overset{O}{\overset{\|}{C}}-CH_3$, singlet), 7.51 ($2-CH_3$, multiplet), and 6.22 τ ($-O-CH_3$, singlet). There was no aromatic proton absorption in the spectrum.

The reaction sequence for the preparation and degradation of methyl O-methylvicanicate (XXIV) is outlined below.

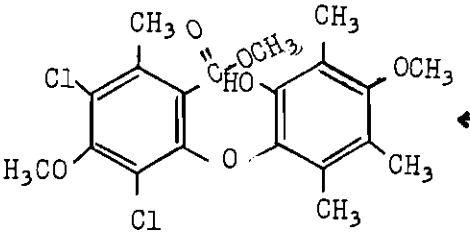
The methyl ether of vicanicin was easily prepared by reacting an ethereal diazomethane solution with an ethereal solution of vicanicin (XX). O-Methylvicanicin (XXIII) was obtained in at least 70% yield, m.p. 192-193°. Elemental analysis supported the formula $C_{19}H_{18}O_5Cl_2$. The mass spectrum of O-methylvicanicin showed peaks in the molecular ion region at m/e (intensity) 396 (26), 398 (18), and 400 (3). The ratio of these intensities is in good agreement with the expected ratio from a



XX

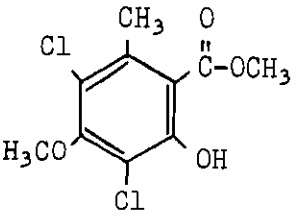
XXIII

NaOMe
MeOH



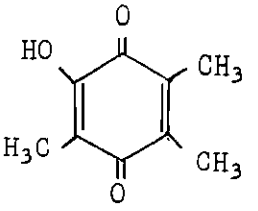
XXIV

HNO_3
HOAc



XXV

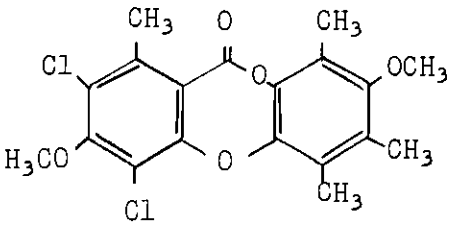
+



XXVI

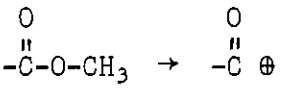
compound with two chlorine atoms (14). The most intense peaks in the spectrum were 361 (100) and 363 (38), which are attributed to the species $[C_{19}H_{18}O_5Cl]^+$. There were peaks at 156 (29) and 158 (9), $[C_7H_5O_2Cl]^+$, which were also present in the spectrum of vicanicin.

The n.m.r. spectrum of O-methylvicanicin (XXIII) showed absorptions at 7.80 ($-CH_3$, multiplet), 7.57 ($-CH_3$, multiplet), 7.51 ($2-CH_3$, multiplet), 6.23 ($-OCH_3$, singlet), and 6.17 τ ($-OCH_3$, singlet).



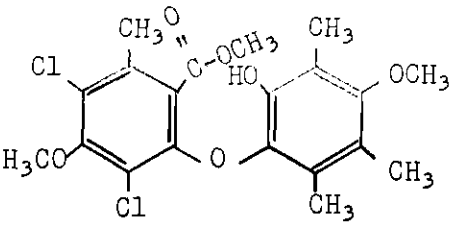
XXIII

The reaction of O-methylvicanicin (XXIII) and sodium methoxide solution produced a glassy solid after work-up. Crystallization of this solid from methanol gave methyl O-methylvicanicate (XXIV) m.p. 154-156°, in at least 70% yield. The elemental analysis and mass spectrum of XXIV gave satisfactory results for the formula $C_{20}H_{22}O_6Cl_2$. The molecular ion region in the mass spectrum showed peaks at m/e (intensity) 429 (17), 431 (12), and 433 (2). The ratio of these intensities compares favorably with the expected chlorine atom ratio. The most intense peaks in the spectrum were 361 (100) and 363 (36). These fragmentation ions, $[C_{19}H_{19}O_5Cl]^+$, are produced through the loss of a chlorine atom and a



methoxy unit. The peaks attributed to $[C_7H_5O_2Cl]^+$ were also present in this spectrum at 156 (21) and 158 (9).

The n.m.r. spectrum of methyl O-methylvicanicate (XXIV) showed absorptions at 7.95 ($-CH_3$, multiplet), 7.88 ($-CH_3$, multiplet), 7.80 ($-CH_3$,



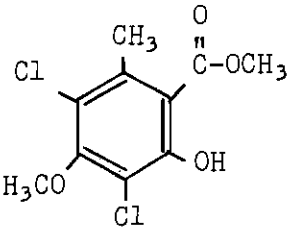
XXIV

multiplet), 7.71 ($-CH_3$, multiplet), 6.33 ($-OCH_3$, singlet), 6.22 ($-OCH_3$, singlet), 6.20 ($-OCH_3$, singlet), and 3.40 τ (-OH, broad singlet).

The nitric acid degradation of methyl O-methylvicanicate (XXIV) gave, after work-up, a mixture of a red oil and a white crystalline solid. A few crystals of the solid were separated manually. The solid showed a m.p. 76-78° which agrees with the m.p. of methyl 3,5-dichloroeverninate reported in the literature (4). However, the reported separation (4) of the quinone component in the degradation of vicanicin (III) as a sodium bicarbonate-soluble fraction was not successful when attempted on this mixture from vicanicin (XX). Chromatography over silicic acid did not separate the components; however, there was 100% recovery of material from the column. Analytical TLC showed that separation of the components was possible, thereby establishing preparative TLC as a means to obtain the degradation products. Using the R_F values of authentic samples, the ester component (R_F 0.66) and the quinone component (R_F 0.18) were located and recovered. The detailed separation and isolation of each

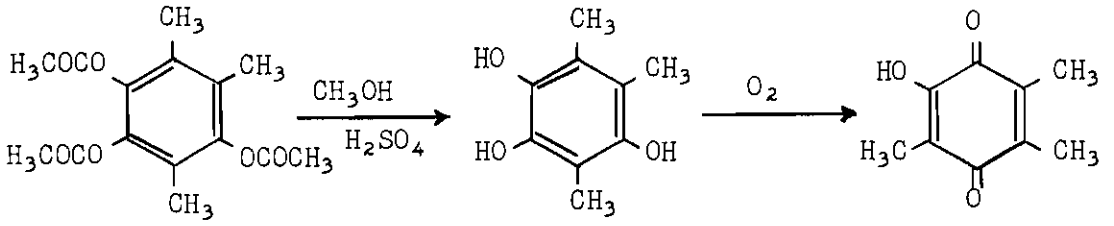
component is discussed in the experimental section of this thesis.

Methyl 3,5-dichloroeverninate (XXV), m.p. 76-78°, was obtained as a white crystalline solid. The isolation and identification of this compound proved that the A ring of vicanicin was the same in both structures, III and XX.



XXV

Following the procedure of Anslow and Raistrick (15), an authentic sample of hydroxytrimethyl-p-benzoquinone (XXVII) was prepared by hydrolysis of 1,2,4-trimethyl-3,5,6-triacetoxybenzene and then oxidation of the resulting phenol. Hydroxytrimethyl-p-benzoquinone (XXVII) was a yellow

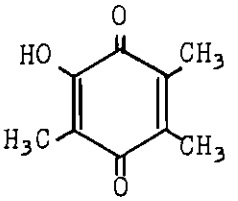


XXVII

crystalline solid, m.p. 94-95°, which agreed with that reported in the literature (13). However, another report stated that hydroxytrimethyl-p-benzoquinone was a red crystalline solid, m.p. 172° (16). It is

suggested that the compound, m.p. 172°, was probably the o-benzoquinone isomer. The n.m.r. spectrum of XXVII showed the expected absorptions at 8.23 ($-CH_3$, singlet), 8.13 ($2-CH_3$, singlet), and 3.46 τ (-OH, singlet).

Although a crystalline compound could not be obtained from the R_F band containing the quinone component, two degradations of methyl-O-methylvicanicate (XXIV) provided enough material, XXVI, for an n.m.r. spectrum. The n.m.r. spectrum of XXVI was identical with the spectrum of an authentic sample of hydroxytrimethyl-p-benzoquinone. The absorptions of XXVI were at 8.06 ($-CH_3$, singlet), 7.96 ($2-CH_3$, singlet), and



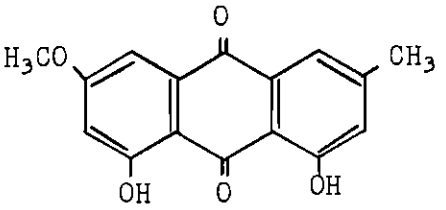
XXVI

2.94 τ (-OH, broad singlet). The R_F value (0.18) of hydroxytrimethyl-p-benzoquinone (XXVI) compares favorably with the R_F value of the synthetic quinone and also with the R_F value reported in the literature (12).

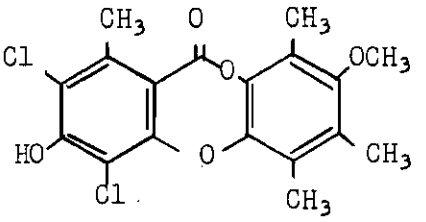
Attempts were made to synthesize 2-hydroxy-3,6-dimethyl-p-benzoquinone (XVI), which was the quinone reported from the degradation of vicanicin (III) (4). The hope of comparing the n.m.r. spectrum of XVI with the n.m.r. spectrum of XXVI was not realized, however, because the quinone could not be obtained from 2,5-dimethyl-1,3,4-triacetoxybenzene using either the procedure of Fieser and Ardo (7) or the procedure of Anslow and Raistrick (15). The n.m.r. spectrum of the residue obtained in the

reaction using Fieser's method showed two C-methyl absorptions at 8.07 (singlet) and 8.00 τ (singlet) but only one low field hydrogen at 2.79 τ (broad singlet).

Two compounds, isolated from the Ascension Islands lichen Teloschistes flavicans v. minor Crombie, were found to be parietin (I) and vicanicin (XX). The identification of these compounds was based on anal-



I



XX

ytical data, derivatives, and spectral evidence. The new structure proposed for vicanicin (XX) was also supported by obtaining the expected products, methyl 3,5-dichloroeverninate (XXV) and hydroxytrimethyl-p-benzoquinone (XXVI), from the nitric acid degradation of methyl O-methylvicanicate (XXIV).

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