REFERENCES

1. Plouvier, V. (1984) *C. R. Acad. Sci. Paris* **299**, série III, 97.
2. Shimizu, M., Hayashi, T., Shimizu, K. and Morita, N. (1982) *Phytochemistry*, **21**, 245.
3. Chalmers, A. A. and Hall, R. H. (1974) *J. Chem. Soc. Perkin Trans.* 2, 728.
4. Plouvier, V. (1984) *C. R. Acad. Sci. Paris* **299**, série III, 749.

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# ANTIBACTERIAL CONSTITUENTS OF THE RED ALGA *CYSTOCLONIUM PURPUREUM*

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**Key Word Index**—*Cystoclonium purpureum*; Rhodophyllidacea; α-carotene; plastoquinone-9; *trans*-phytol; ubiquinol-9; lutein; fucoxanthin; polysaturated fatty acids; antibacterial acids.

**Abstract**—The red alga *Cystoclonium purpureum* was found to contain α-carotene, plastoquinone-9, *trans*-phytol, ubiquinol-9, lutein and fucoxanthin. The composition of the antibacterial fatty acid fraction was determined by GC/MS.

## INTRODUCTION

An extract of the previously uninvestigated red alga *Cystoclonium purpureum* exhibited strong antibacterial activity. We now report our findings on its chemical constituents.

## RESULTS AND DISCUSSION

*Cystoclonium purpureum*, collected at Lepreau Ledges, New Brunswick, was extracted with methanol in a Soxhlet apparatus and the chloroform soluble portion of the evaporated methanol extract subjected to silica gel CC.

Following elution of free sterols, a fatty acid fraction was obtained which showed significant antibacterial activity in disc diffusion assays. This mixture was fractionated by reversed phase prep. TLC and three fractions (F1, F2 and F3) displayed significant antibacterial activity (Table 1) while the others (F4, F5 and F6) did not. The fatty acid fractions on treatment with ethereal diazomethane provided the corresponding methyl esters which were analysed by GC/MS, the results of which are summarized in Table 2. Examination of the six acid fractions revealed that they were, for the most part, complex mixtures with considerable overlap of components.

In a recent report [1] on the antibacterial acids from the diatom *Navicula delognei* we have noted the presence and antibiotic activity of (6*Z*,9*Z*,12*Z*,15*Z*)-hexadecatetraenoic and an ester of the known antibiotic (5*Z*,8*Z*,11*Z*,14*Z*,17*Z*)-eicosapentaenoic acid. Present indications are that several *cis* polyunsaturated acids are responsible for the antibacterial activity of the *C. purpureum* extract.

## EXPERIMENTAL

*General.* $^1$H NMR spectra were recorded at 200 MHz using TMS as int. standard. An HPLC unit equipped with an absorbance and a refractive index detector, employing a μ-porasil column was used for HPLC purification. Analytical and prep. TLC were performed with precoated silica gel G (Kieselgel 60, F-254) and reverse phase ($KC_{18}F$) plates. GC/MS analysis was carried out on a quadrupole EI-CI system at 70 eV, employing an SP-2330 (30 m) column at 40–160° (10°/min), 160–200° (5°/min) and 14 psi.

Fresh *C. purpureum* Batters (2.3 kg wet wt), collected at Lepreau Ledges, New Brunswick (May 1983), was finely chopped and immersed in MeOH for 2 hr and then extracted in a Soxhlet for 48 hr. The extract was coned under red. pres. and the residue (19 g) dissolved in $H_2O$ (250 ml) and extracted with $CHCl_3$ (3 × 250 ml). Removal of $CHCl_3$ yielded a dark green solid (12.5 g), which was subjected to CC on silica gel G (250 g) eluting with hexane, hexane–EtOAc, $CHCl_3$ and $CHCl_3$–MeOH mixtures in sequence. Fractions were monitored by TLC, combined and purified by prep. TLC and HPLC. In order of elution, the following compounds were obtained and identified by comparison of their spectral and physical characteristics with literature data: α-carotene [2] (0.069 g), plastoquinone-9 [3, 4] (0.044 g, purified by prep. TLC and HPLC with 3% EtOAc in hexane), *trans*-phytol [5] (0.108 g), and ubiquinol-9 [6–8] (0.021 g, purified by HPLC with 1% MeOH in $CHCl_3$). Further elution provided free sterols (0.755 g), fatty acids (0.828 g,

Table 1. Antibacterial assay* results for fatty acid fractions *from Cystoclonium purpureum*

| Organism (source) | Fractions | | | |
|---|---|---|---|---|
| | F1 | F2 | F3 | Control† |
| *Enterobacter cloacea* (ATCC 23355) | — | — | + | +++¶ |
| *Escherichia coli* (ATCC 25922) | — | ++§ | — | +++¶ |
| *Klebsiella pneumoniae* (ATCC 13883) | +‡ | + | ++ | +++** |
| *Proteus vulgaris* (ATCC 13315) | +++ | ++ | — | +++** |
| *Salmonella typhimurium* (ATCC 14028) | + | — | + | +++¶ |
| *Serratia marescens* (ATCC 8100) | — | — | +++‖ | +++†† |
| *Staphylococcus aureus* (ATCC 25923) | +++ | + | ++ | +++¶ |
| *Staphylococcus epidermis* (ATCC 12228) | ++ | ++ | ++ | +++¶ |

*Disc diffusion assay using 1.5 mg test fraction per standard 1/2 in disc.
†Control antibiotic 0.1 mg per standard 1/2 in disc.
‡ + Zone of inhibition noticeable.
§ + + Zone of inhibition > 2 mm.
‖ + + + Zone of inhibition > 4 mm.
¶ Ampicillin.
** Tetracycline.
†† Chloramphenicol.

Table 2. GC/MS analysis* of methyl esters of free fatty acids from *Cystoclonium purpureum*

| $[M]^+$ | Acid (as Me ester) | Fractions | | | | | |
|---|---|---|---|---|---|---|---|
| | | F1 | F2 | F3 | F4 | F5 | F6 |
| 214 | dodecanoic | | | 1 | | | |
| 242 | tetradecanoic | | | | 15 | 50 | |
| 270 | hexadecanoic | | | | | 27 | |
| 268 | (11Z)-hexadecenoic | | | | 80 | 18 | |
| 268 | hexadecenoic | 20 | | | | | 10 |
| 266 | (7Z,10Z)-hexadecadienoic | | 2 | 10 | | | 1 |
| 266 | (9Z,12Z)-hexadecadienoic | | 15 | 40 | | | 4 |
| 264 | hexadecatrienoic | 45 | 2 | | | | 2 |
| 296 | (10Z)-octadecadienoic | | | | | 5 | |
| 262 | hexadecatetraenoic | 10 | | | | | |
| 294 | octadecadienoic | 7 | | | 2 | <1 | 2 |
| 292 | (6Z,9Z,12Z)-octadecatrienoic | | 1 | | | | |
| 292 | (9Z,12Z,15Z)-octadecatrienoic | | 2 | 20 | | | 1 |
| 290 | octadecatetraenoic | 7 | 4 | | | | 4 |
| 318 | (5Z,8Z,11Z,14Z)-eicosatetraenoic | | | 22 | | | |
| 287 (318 − 31) | eicosatetraenoic | 5 | 50 | | | | |
| 316 | eicosapentaenoic | | | | | | 60 |
| 316 | eicosapentaenoic | | 6 | | | | 5 |
| 316 | eicosapentaenoic | | 3 | | | | 5 |
| 316 | eicosapentaenoic | | 5 | | | | |
| 316 | eicosapentaenoic | | 3 | | | | |

*Assignments are based on comparison with library search data. $^1$H NMR data support assignment of *Z* configuration.

purified by reversed phase silica gel prep. TLC with 10% $CHCl_3$ in MeCN to provide F1, F2 and F5 and with 10% $H_2O$ in MeCN to provide F3, F4 and F6) and lutein [1, 9] (0.047 g purified by prep. TLC with 20% EtOAc in hexane), followed by fucoxanthin [10] (0.328 g, purified by prep. TLC on silica gel with 40% EtOAc in hexane).

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### REFERENCES

1. Findlay, J. A. and Patil, A. D. (1984) *J. Nat. Prod.* **47**, 815.
2. Bjorland, T. and Aguilar-Martinez, M. (1976) *Phytochemistry* **15**, 291.
3. Eck, N. and Trebst, A. (1973) *Z. Naturforsch.* **18B**, 446.
4. Misiti, D., Moore, H. W. and Folkers, K. (1965) *J. Am. Chem. Soc.* **87**, 1402.
5. Sims, J. J. and Pettus, J. A. Jr. (1976) *Phytochemistry* **15**, 1076.
6. Farley, T. M., Daves, G. D. Jr. and Folkers, K. (1968) *J. Org. Chem.* **33**, 905.
7. Muraca, R. F., Whittick, J. S., Daves, G. D. Jr., Friis, P. and Folkers, K. (1967) *J. Am. Chem. Soc.* **89**, 1505.
8. Law, A., Threlfall, D. R. and Whistance, G. R. (1971) *Biochem. J.* **123**, 331.
9. Liaaen-Jensen, S. and Hertzberg, S. (1966) *Acta Chem. Scand.* **20**, 1703.
10. Bommette, R., Mallams, A., Spark, A., Tee, J. and Weedon, B. C. L. (1969) *J. Chem. Soc. (C)* 429.

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# STRUCTURE OF VESUVIANIC ACID FROM *STEREOCAULON* SPECIES

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**Key Word Index**—*Stereocaulon alpinum*; lichen; depsidone; vesuvianic acid.

**Abstract**—Vesuvianic acid, which was previously isolated from *Stereocaulon vesuvianum* var. *pulvinatum* without a full elucidation of structure, has now been isolated from *S. alpinum*. More detailed spectral investigation indicates that vesuvianic acid, which was previously believed to be a new depsidone, is an ethyl ether of stictic acid which itself may be an artefact produced during extraction.

## INTRODUCTION

Depsides and depsidones constitute the largest class of secondary metabolites derived from lichens [1]. Both types of compounds are diphenyl esters, but depsidones also contain a diphenyl ether linkage. Through our present work, which has involved analysis of the chemistry and biological activity of lichens indigenous to Iceland, it has come to our attention that the stability of depsides during extraction with chloroform can be threatened by traces of alcohol present as stabilizer. These findings have been discussed in a previous publication [2].

The isolation of vesuvianic acid from *Stereocaulon vesuvianum* var. *pulvinatum* was previously reported in 1977 [3]. Owing to lack of material, NMR data was not obtained and thus a complete structural elucidation could not be accomplished. However, on the basis of mass spectral, IR and UV data it was considered to be a novel depsidone. During work on *S. alpinum* we have now isolated a compound apparently identical with vesuvianic acid. The structure elucidation of this compound forms the basis of the present communication.

## RESULTS AND DISCUSSION

Extraction of dried *S. alpinum* with petrol followed by chloroform allowed the isolation of a compound having identical mass spectral properties with those of vesuvianic acid [3]. IR and UV data were also comparable and were characteristic of a γ-lactonic depsidone. $^{13}C$ NMR, $^{1}H$ NMR and mass spectral data all implied the presence

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