Phytochemistry, Vol. 21, No. 4, pp. 944–945, 1982
Printed in Great Britain.

0031–9422/82/040944–02$03.00/0

ANTI-BACTERIAL ACTIVITY OF EXTRACTS FROM THE BROWN SEAWEED *STOECHOSPERMUM MARGINATUM*

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(*Received* 22 *June* 1981)

Key Word Index—*Stoechospermum marginatum*; Dictyotaceae; brown seaweed; antibacterial activity; *Staphylococcus aureus*; spatane diterpenoids; 19-acetoxy-5,15,18-trihydroxyspata-13,16-diene.

Abstract—The methanol extract of the brown seaweed *Stoechospermum marginatum* was found to inhibit the growth of *Staphylococcus aureus*. The active constituents were found to be a mixture of diterpenoid monoacetates having the spatane skeleton.

INTRODUCTION

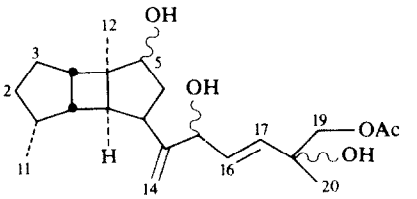
Antibiotic[1], antimicrobial[2], cytotoxic[3] and icthyotoxic[4] agents have been detected and isolated from extracts of marine algae. Spatol, the cytotoxin present in the brown seaweed *Spatoglossum schmittii* has been shown to possess the new tricyclic diterpenoid skeleton spatane[3]. As part of a continuing programme of research on the seaweeds of Sri Lanka, extracts of seaweeds collected from the coastal waters of Sri Lanka were screened for anti-bacterial and anti-fungal activity. In this paper we report the isolation and fractionation of extracts from the brown seaweed *Stoechospermum marginatum* which inhibited the growth of *Staphylococcus aureus*. The active constituents were found to be a mixture of monoacetates belonging to the spatane diterpenoids, the structures of which have been elucidated by Gerwick *et al.* [5]. The two brown seaweeds *Spatoglossum schmittii* [3] and *Stoechospermum marginatum* [5], belonging to the family Dictyotaceae, have been the only sources from which spatane diterpenoids have so far been isolated. Antibacterial activity of spatane diterpenoids has not been reported previously.

RESULTS AND DISCUSSION

The brown seaweed *Stoechospermum marginatum* was collected in Mandativu, Jaffna, in the Northern Province of Sri Lanka. The seaweeds were washed with fresh water and stored in methanol. The finely chopped seaweed was extracted sequentially with methanol, water and then freeze dried. The dry seaweed residue was then extracted with dichloromethane and finally with petrol. The concentrated extracts were tested separately by the standard disc method for activity against *Escherichia coli*, the Oxford strain of *Staphylococcus aureus* and yeast. The methanol extract was found to inhibit the growth of *Staphylococcus aureus*.

The methanol extract was fractionated, as outlined in the Experimental, to obtain an active residue (1.4 g). TLC examination of the active residue showed the presence of at least 12 constituents. The residue was further fractionated by repeated prep. TLC until a product active against *S. aureus* and apparently homogeneous on TLC, was isolated (0.1 g) as a colourless oil ($[\alpha]_D - 10°$).

IR evidence (ν_{max} cm^{-1}: 3500–3200, 1730 and 1230) indicated the presence of hydroxy and acetate functions. The mass spectrum showed peaks at *m/z* 360 and 300 indicating the presence of an acetoxy group. The ^1H NMR spectrum showed signals due to four olefinic protons [δ 5.73 (2H, *d*), 5.35 (1H, *br s*) and 4.93 (1H, *br s*)], three hydroxy groups [2.60 3H, *br m*)] and a single acetoxy group [2.10(3H, *s*)]. Compound **1** was identified by comparison of these spectral parameters with those of the ten new spatane



1

diterpenoids isolated from *Stoechospermum marginatum* [5]. The structure of **1** was found to be similar to that of the mixture of monoacetates whose structure has been established to be 19 - acetoxy - 5,15,18 - trihydroxyspata - 13,16 - diene by Gerwick *et al.* [5] and is possibly stereoisomeric at C-5 and/or C-15.

EXPERIMENTAL

Solns were concd to dryness at temps. below 40°. TLC and prep. TLC was carried out with Si gel (Merck Kieselgel 60 $PF_{254+366}$) using mixtures of CH_2Cl_2–EtOAc. Optical rotations were measured at 25° in $CHCl_3$. IR spectra were recorded as films on NaCl discs. ^1H NMR spectra were

recorded at 60 MHz with TMS as int. standard. MS were determined at the Research School of Chemistry, The Australian National University.

Bacteriological testing was carried out using the standard disc method (6 mm discs) at the Faculty of Medicine, University of Peradeniya. MeOH–H_2O solns of the extracts were absorbed on 6 mm filter discs, dried and placed on Müller Hintant Agar (MHA) (Difco) which was subsequently inoculated with cultures of *S. aureus*, *E. coli* and yeast.

Extraction of seaweeds. The finely chopped brown seaweed *Stoechospermum marginatum* (250 g) was placed in a glass column and extracted by the slow percolation of MeOH and H_2O respectively. The extracted seaweeds were freeze dried and then extracted by slow percolation of CH_2Cl_2 and petrol (40–60°) respectively. The MeOH extract (6.7 g), the aq. extract (2.0 g), the CH_2Cl_2 extract (0.2 g) and the petrol extract (0.1 g) respectively were concd to dryness and tested for bactericidal and fungicidal activity. The MeOH extract (6.5 g) was stirred with CH_2Cl_2 at room temp. for 3 hr to yield an insoluble residue (3.0 g, inactive) and a soluble fraction (3.1 g, active). The active fraction was stirred with *n*-hexane at room temp. for 3 hr. An active insoluble residue (1.4 g) and an inactive soluble fraction (1.6 g) were obtained. The insoluble residue on prep. TLC was separated into four fractions (of increasing polarity): a 0.1 g, inactive; b 0.1 g, active; c 0.4 g, active and d 0.3 g, inactive. Repeated prep. TLC of fraction f gave compound **1** (0.1 g, active), which was apparently homogeneous on TLC.

19-*Acetoxy*-5,15,18-*trihydroxyspata*-13,16-*diene* (**1**). This compound was obtained as a colourless oil (0.097 g), $[\alpha]_D$ −10.0° (lit. [5] $[\alpha]_D$ −16°); IR ν_{max} cm^{-1}: 3500–3200, 1960, 1660, 1640, 1375, 1230, 1040, 965, 940 and 900; MS *m/z* (rel. int.): 360.2307 $[M-H_2O]^+$ (2), $C_{22}H_{32}O_4$ requires 360.2300, 300.2085 $[M-H_2O-HOAc]^+$ (23), $C_{20}H_{28}O_2$ requires 300.2089 342 (3), 318 (3), 305 (5), 301 (6), 287 (24), 269 (22), 211 (18), 199 (1.5), 187 (11), 173 (11), 159 (34), 145 (38), 135 (100), 119 (35), 107 (68), 93 (60), 81 (85); ^1H NMR δ 5.73 (2H, *m*, H-16 and H-17), 5.33 (1H, *br s*, H-14), 4.93 (1H, *br s*, H-14), 4.43 (1H, *m*, H-15), 4.16 (1H, *d*, J = 12.0 Hz, H-19), 3.93 (1H, *d*, J = 12.0 Hz, H-19), 3.70 (1H, *m*, H-5), 2.60 (3H, 3 × OH), 2.10 (3H, *s*, $-OCOCH_3$), 1.32 (3H, *s*, H_3-20), 0.98 (3H, *s*, H_3-12), 0.90 (3H, *d*, J = 5.0 Hz, H_3-11).

Acknowledgements—We are grateful to Professor S. N. Arsecularatne, Faculty of Medicine, University of Peradeniya, for permitting the use of facilities for bacteriological testing at the Department of Bacteriology. We thank Professor J. K. MacLeod, Australian National University for high resolution mass spectral data and Dr. W. Fenical, Scripps Institution of Oceanography, California for an authentic sample of **1**. Thanks are also due to Mrs. S. C. Weerasekera for the preparation of the typescript, the National Science Council of Sri Lanka for a research grant and the International Foundation for Science for financial assistance.

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