Journal of Natural Products
Vol. 51, No. 1, pp. 158-160, Jan-Feb 1988

PHLOROGLUCINOL DERIVATIVES FROM THREE AUSTRALIAN MARINE ALGAE OF THE GENUS *ZONARIA*

ADRIAN J. BLACKMAN,* GLEN I. ROGERS,

Chemistry Department, University of Tasmania, GPO Box 252C

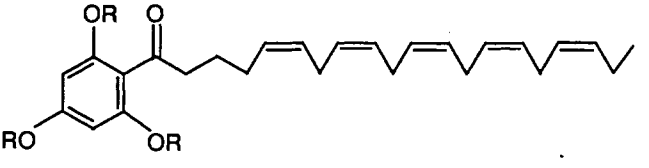
and JOHN K. VOLKMAN

CSIRO Marine Laboratories, Division of Oceanography, GPO Box 1538, Hobart, Tasmania 7001, Australia

Six species of brown seaweeds of the genus *Zonaria* are common in Australian waters (1), and three of these are found in subtidal areas along the Tasmanian coast. These are *Zonaria turneriana* J. Agardh, *Zonaria crenata* J. Agardh, and *Zonaria angustata* Kuetz., none of which has been examined previously for secondary metabolites. We report here the use of conventional chromatographic and spectroscopic techniques in conjunction with a capillary gc-ms technique to study the natural products in these three species. Other species from the same family, Dictyotaceae, have yielded a diverse array of natural products including terpenes (2) and phloroglucinol derivatives (3–7).

Z. turneriana was found to contain the known *C*-acylphloroglucinol derivatives (*all-Z*)-1-(2,4,6-trihydroxyphenyl)-5,8,11,14,17-eicosapentaen-1-one [**1a**] and (*all-Z*)-1-(2,4,6-trihydroxyphenyl)-17-hydroxy-5,8,11,14-eicosatetraen-1-one [**2a**] from comparison of ir, ^{1}H-nmr, ^{13}C-nmr, lrcims, and hrcims data with those reported in the literature (3,7).

Analysis of the solvent extract of *Z. crenata* by tlc showed two uv-absorbing spots at the same R_f as the acylphloroglucinol derivatives from *Z. turneriana*. Compounds **1a** and **2a** were identified in the extract by gc-ms analysis of a portion of the total extract treated with BSTFA to form the TMSi-ether derivatives **1b** and **2b**. TMSi-ether derivatives were chosen because these are rapidly prepared with little possibility for formation of artifacts, and they give Gaussian peak shapes on nonpolar capillary columns. They also have distinctive mass spectra that enable them to be readily identified even when present as trace constituents in complex mixtures.



1a R=H
1b R=Si(CH$_3$)$_3$

2a R=H
2b R=Si(CH$_3$)$_3$

3

Compounds **1a** and **2a** were not detectable in *Z. angustata* using conventional tlc, but a trace quantity of **1a** was identified using the more sensitive gc-ms technique. *Z. crenata* also contained significant amounts of a chromone, (*all-Z*)-5,7-dihydroxy-2-(4,7,10,13,16-nonadecapentaenyl)-4*H*-1-benzopyran-4-one [**3**], which was identified by conventional means (6). This compound was not readily detected using the gc-ms technique, although this procedure should be applicable with some modification to the analysis conditions.

Compounds **1a**, **2a**, and **3** have been reported in other species of *Zonaria* found in the Mediterranean (3-6) and Pacific (7) regions. Studies to date suggest that these compounds are restricted to this genus and may be useful chemotaxonomic markers. Other structurally related compounds have been found in some closely related genera (7). The rapid gc-ms technique described here can be used to identify a range of phloroglucinol derivatives, and it obviates the need for extensive preparation of the sample or for large sample sizes. Using this technique we were able to show that none of the three species contained *C*-acylphloroglucinol derivatives having side chains different from those reported even though these seaweeds contain a variety of possible precursor saturated and polyunsaturated fatty acids.

EXPERIMENTAL

GENERAL EXPERIMENTAL PROCEDURES.—Spectral data were obtained on the following instruments: ir, Hitachi 270-30 infrared spectrometer; ^{1}H nmr, Bruker AM 300; gc, Hewlett-Packard HP5890 equipped with a FID detector operated at 310° and a Shimadzu computing integrator (model CR-3A); gc-ms, Hewlett-Packard HP5890 gas chromatograph connected to a Hewlett-Packard HP5970 mass selective detector. Adsorbents for cc and tlc were Camag Si gel 60.

PLANT MATERIAL.—*Z. turneriana* and *Z. crenata* were collected at 5-m depth at Tinderbox, Tasmania (43°3.6′S, 147°20′E), in September 1985. *Z. angustata* was obtained at 3-m depth from Sisters Beach, Tasmania (40°6.5′S, 145°39.4′E) in October 1986. Each sample analyzed was free of obvious epiphytes. Voucher specimens (MUCV 1926, 1928, and 2035, respectively) are lodged at the Botany Department Herbarium, Monash University, Melbourne, Australia.

EXTRACTION AND ISOLATION.—A freeze-dried sample of *Z. turneriana* (630 g) was milled and extracted successively by percolation at room temperature with EtOAc (4 liters) and then MeOH (3 liters). The concentrated crude extract was then dissolved in Et_2O-CH_2Cl_2 (1:10) and passed through a Si gel plug to yield a dark brown oil (37.4 g). A portion of this oil (3.5 g) was purified by preparative Si gel tlc (CH_2Cl_2-EtOAc, 10:1) to give the acylphloroglucinols **1a** as a yellow oil (359 mg, 0.60% dry wt) and **2a** as a pale yellow oil (185 mg, 0.30% dry wt).

Z. angustata (170 g), when treated in a similar way, yielded a brown oil (3.2 g), a portion of which (1.66 g) gave the chromone **3** as a pale yellow oil (50 mg, 0.06% dry wt).

GC AND GC-MS ANALYSIS.—A methylsilicone-fused silica capillary column (20 m×0.31 mm i.d.) was used for gc analysis. Operating conditions were as follows: cool on-column injection (SGE OCI-3), 1 min isothermal period, oven program rate 40° to 120° at 30°/min and then 4°/min to 310°. The carrier gas was H_2 with a linear velocity of 40 cm/sec. Gc-ms analysis was carried out using a methylsilicone-fused silica capillary column (10 m×0.31 mm i.d.) with similar operating conditions to those described above. The carrier gas was He at a flow rate of approximately 2 ml/min.

A sample of wet alga (ca. 1 g) was extracted successively with iPrOH (10 ml) and $CHCl_3$-MeOH (2:1, 4×10 ml). The combined extracts were partitioned between H_2O (pH 3) and CH_2Cl_2. The organic phase was evaporated under reduced pressure, and the residue was silylated using BSTFA.

Gc-ms analysis of *Z. crenata* gave the derivatives **1b** and **2b** in respective yields (dry wt) of 0.79% and 0.47%. **1b**: Rt 40.51 min; ms of TMSi-ether derivative $[M]^+$ 626 (7%), 611 (29), 369 (100), 343 (21), 73 (66). **2b**: Rt 44.18 min; ms of TMSi-ether derivative $[M]^+$ 716 (3%), 701 (14), 397 (7), 369 (92), 343 (12), 73 (100).

Gc-ms analysis of *Z. angustata* in a similar manner showed the presence of the compound **1a**.

Compounds were identified by comparison with published spectroscopic data (3,5,7). Full details of the isolation and identification are available upon request to the senior author.

ACKNOWLEDGMENTS

We thank Dr. Julie Phillips, Botany Department, Monash University, for identification of

plant material and the Central Science Laboratory, University of Tasmania for spectroscopic analysis. A CSIRO-University of Tasmania postgraduate award is gratefully acknowledged.

LITERATURE CITED

1. H.B.S. Womersley, *Aust. J. Bot.*, **15**, 189 (1967).
2. W. Fenical, in: "Marine Natural Products: Chemical and Biological Perspectives." Ed. by P.J. Scheuer, Academic, New York, 1978, vol. II, pp. 173–245.
3. V. Amico, R. Currenti, G. Oriente, M. Piattelli, and C. Tringali, *Phytochemistry*, **20**, 1451 (1981).
4. V. Amico, G. Nicolosi, G. Oriente, M. Piattelli, and C. Tringali, *Phytochemistry*, **21**, 739 (1982).
5. C. Tringali and M. Piattelli, *Gazz. Chim. Ital.*, **112**, 465 (1982).
6. C. Tringali and M. Piattelli, *Tetrahedron Lett.*, **23**, 1509 (1982).
7. W. Gerwick and W. Fenical, *Phytochemistry*, **21**, 633 (1982).

Received 23 June 1987