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ACUTILOLS, POTENT HERBIVORE FEEDING DETERRENTS FROM THE TROPICAL BROWN ALGA, *DICTYOTA ACUTILOBA*

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Key Word Index—*Dictyota acutiloba*; Phaeophyta; marine algae; pachydictyane diterpenes; feeding deterrents; acutilols A and B.

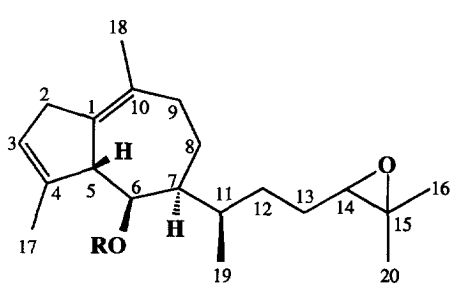
Abstract—The structures of three new diterpenoids, acutilol A, acutilol A acetate and acutilol B have been determined by a combination of spectral methods. The new compounds are based upon the common pachydictyane carbon skeleton but possess unusual $\Delta^{1,10}$ double bonds. The acutilols are potent feeding deterrents against both temperate and tropical herbivorous fishes and sea urchins, suggesting that these molecules provide an effective chemical defence. Copyright © 1996 Elsevier Science Ltd

INTRODUCTION

After over 20 years of intense investigation, it has now become clear that selected families of marine algae produce diverse secondary metabolites that form the foundations for defence against the numerous herbivores found in marine environments [1–3]. Although most investigations have focused on tropical algae, temperate algae can face similar ecological challenges and can also produce chemicals that deter herbivory [4–6]. Some tropical algae, such as brown algae in the family Dictyotaceae (exemplified by the genera *Dictyota*, *Pachydictyon* and *Dictyopteris*) are abundant in tropical regions but can also be common in temperate systems [7]. Thus, the same species will encounter different herbivores in the temperate *versus* tropical portions of its distribution. The question is whether the same class of defensive metabolites can be effective against both temperate and tropical herbivores.

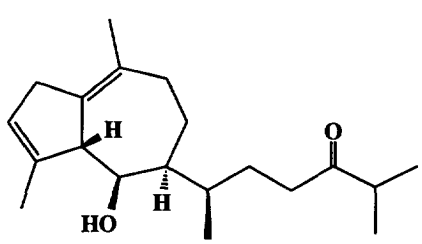
In the present paper, we report the results of a chemical investigation of the herbivore deterrents produced by the tropical alga *Dictyota acutiloba*. The structural determinations of three major metabolites of this alga, acutilol A, acutilol A acetate and acutilol B (**1–3**) are described. The acutilols are potent herbivore deterrents against both temperate and tropical fishes and sea urchins.

In comprehensive biotest [8], the acutilols deterred feeding of the temperate pinfish, *Lagadon rhomboides*, and several species of tropical parrotfish and surgeonfish (*Scarus schlegeli*, *S. sordidus*, *Naso lituratus* and *N. unicornis*) at as low as 20% of their individual concentrations in the algae. Likewise, biotesting using both temperate and tropical sea urchins (*Arbacia punc-*



1, acutilol A R = H

2, acutilol A acetate R = Ac



3, acutilol B

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tulata and *Diadema savignyi*) showed less, but significant activity.

RESULTS AND DISCUSSION

Dictyota acutiloba was collected from the seaward side of a shallow reef platform at Tunnels Beach on the north side of Kauai, Hawaii, in December 1990. The algae were immediately preserved in ethanol and transported to North Carolina where subsequent extraction was performed. The combined extracts were chromatographed by silica gel vacuum flash methods to yield relatively nonpolar mixtures of terpenoids. Subsequent fractionation by silica gel HPLC led to the isolation of three pure oxygenated diterpenoids, acutilol A acetate (**2**, 1.1% of dry weight), acutilol A (**1**) and acutilol B (**3**), each in 0.2% dry weight yield.

The major metabolite, acutilol A acetate (**2**), was obtained as a viscous oil which analysed for $C_{22}H_{34}O_3$ by high-resolution mass spectrometry and ^{13}C NMR methods (Table 1). Two of the six degrees of unsaturation implied by the molecular formula were assigned to carbon–carbon double bonds (δ 140.6 (*s*), 133.9 (*s*), 126.9 (*s*), 124.8 (*d*)), one was assigned as an ester-carbonyl (δ 170.3 (*s*)) (consistent with IR data), and one was assigned as a trisubstituted epoxide (δ 64.3 (*d*), 58.2 (*s*)). Thus, the remaining two degrees of unsaturation were accommodated by a bicyclic carbon skeleton. A three-proton singlet at δ 2.04 in the ^1H NMR spectrum, which correlated to two signals in the ^{13}C NMR spectrum at δ 21.4 (*q*) and 170.3 (*s*), and the IR data, indicated the presence of an acetate ester. The presence of a methine carbon at δ 74.0 (*d*), further indicated that the acetate was secondary. Using comprehensive ^1H and ^{13}C NMR methods, involving COSY, HMQC and HMBC experiments, the acetate and epoxide functionalities were unambiguously placed on a bicyclic hydroazulene diterpene skeleton. NMR analyses allowed the two double bonds to be placed at the C-3, C-4 and C-1, C-10 positions.

Table 1. ^{13}C NMR data for acutilol A, acutilol A acetate and acutilol B (**1–3**)

C	1	2	3
1	133.8	133.9	133.5
2	36.8	36.0	36.5*
3	123.3	124.8	122.4
4	142.4	140.6	143.7
5	57.9	56.9	55.6
6	74.9	74.0	75.1
7	44.9	42.3	46.4
8	20.7	21.4	21.1
9	35.3	32.8	37.6*
10	126.4	126.9	126.3
11	34.1	34.6	33.5
12	31.0	31.5	26.7
13	26.0	26.4	37.3*
14	64.4	64.3	216.5
15	58.6	58.2	40.8
16	18.6	18.5	18.2
17	16.1	15.5	16.7
18	20.7	20.4	21.0
19	17.5	16.4	17.8
20	24.8	24.7	18.3
21	–	170.3	–
22	–	21.4	–

Spectra recorded at 50 MHz in $CDCl_3$. Assignments for **2** were by two-dimensional heterocorrelation (HMQC and HMBC) experiments, while assignments for **1** and **3** were by analogy.

*Assignments may be interchanged.

The relative stereochemistry at C-5, C-6, C-7 and C-11 was assigned on the basis of proton coupling constant analysis and on the basis of a ^1H two-dimensional NOESY experiment. Results of this experiment, leading to a proposal of the three-dimensional structure for acutilol A acetate (**2**) are shown in Fig. 1. The acetate proton H-6 showed strong correlations with both H-5 and H-7. Although the coupling constants between these protons are undetectable, their proximities are unquestionable. The relative stereochemistry at C-11 was assigned on the basis of the NOE correlation between H-6 and H-11. The relative stereochemistry is identical to the relative stereochemistries observed for other hydroazulene diterpenes isolated from other *Dictyota*, *Dilophus* and *Pachydictyon* species [9]. Unfortunately, no data to define the stereochemistry at C-14 was obtained in this experiment.

The ^1H and ^{13}C NMR spectroscopic data of acutilol A (**1**) were similar to those of **2** but the IR spectrum lacked the ester carbonyl band and, instead, showed strong hydroxyl absorption. The molecular formula for **1**, $C_{20}H_{32}O_2$, derived by HR-mass spectrometry and ^{13}C NMR methods, suggested that this metabolite is the parent alcohol of **2**. Treatment of acutilol A acetate (**2**) with NaOH in methanol smoothly saponified the ester resulting in a high yield of an alcohol, identical to compound **1** in every respect.

The ^1H and ^{13}C NMR spectra of acutilol B (**3**) were very similar to the spectra derived from acutilol A and its acetate (**1**, **2**), but the data clearly showed the epoxide to be absent. The ^{13}C NMR spectrum of **3** showed two new signals at δ 216.5 and δ 40.8 correlating with a ketone and an α-methine-carbon. The presence of ketone was also obvious in the IR spectrum ($\nu_{C=O}$ = 1703 cm^{-1}). Comparison of the spectral data

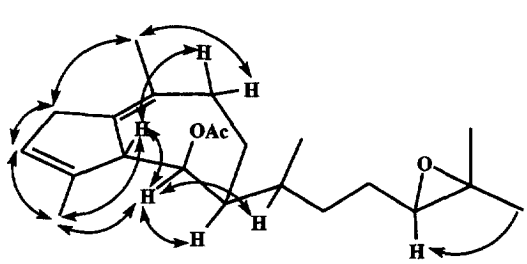

Fig. 1. ^1H NOESY correlations for acutilol A acetate (**2**). The NOESY spectrum was recorded in $CDCl_3$ at 500 MHz with a mixing time t_m = 2 sec according to the relaxation times T_1 of H-3 (2.8 sec), H-5 (2.1 sec) and H-6 (1.2 sec).

for compound **3** with those published for a related ketone possessing unsaturation at the C-10, C-18 positions, confirmed placement of the ketone at C-14 [10].

The acutilols are closely related to numerous pachydictyane diterpenoids, including the first member of this series, pachydictyol A [11]. The acutilols however, possess the endocyclic olefin at C-1, C-10, a feature found only once before in dictyotriene B, the parent hydrocarbon isolated from *Dictyota dichotoma* [10]. This work adds to a growing list of observations showing that diterpenoids of this structural class are potent antifeedants providing a broad defensive adaptation for numerous *Dictyota*, *Dilophus* and *Pachydictyon* species world-wide [3, 6, 9].

EXPERIMENTAL

General. HR-MS were provided by the University of California at Riverside Mass Spectrometry Facility. 1D NMR (^{1}H, ^{13}C and DEPT) were recorded a 200 MHz and 2D NMR (COSY, NOESY, HMQC and HMBC) at 500 MHz.

Extraction and isolation. Dictyota acutiloba was collected from the seaward side of a shallow reef platform at Tunnels Beach on the north side of Kauai, Hawaii, in December 1990, stored in EtOH, returned to the Institute of Marine Sciences in North Carolina and stored at −30° until processed. A few individuals were kept for identification and the rest of the collection was extracted ×6 with CH_2Cl_2–MeOH (2:1). Individual metabolites were purified using silica gel vacuum flash CC (various ratios of EtOAc in *iso*octane) followed by silica HPLC of the relatively non-polar frs using *iso*octane–EtOAc (4:1) as eluent. The acutilols were isolated as oils. Acutilol A (**1**) was isolated as 0.2% of dry wt, acutilol A acetate (**2**) as 1.1% of dry wt and acutilol B (**3**) as 0.2% of dry wt of the plant.

Acutilol A (**1**). Unstable oil. $[\alpha]_D^{25}$ + 29.8° (*c* 1.5, $CHCl_3$). IR ν_{max}^{film}: 3448, 2959, 2927, 1450, 1378 cm^{-1}. ^{1}H NMR (500 MHz, $CDCl_3$): δ 5.38 (1H, *s*, H-3), 3.87 (1H, *bs*, H-6) 3.22 (1H, *bs*, H-5), 2.90 (2H, *s*, H2), 2.73 (1H, *t*, J = 6 Hz, H_{14}), 2.44 (1H, *m*, H-9β), 2.00 (1H, *m*, H-9α), 1.85 (3H, *s*, H-17), 1.60 (3H, *s*, H-18), 1.80–1.30 (7H, *m*), 1.31 (3H, *s*, H-16), 1.27 (3H, *s*, H-20), 0.96 (3H, *d*, J = 7 Hz, H-19). HREI-MS: $[M]^+$ *m/z* obsd. 304.2410, calc. 304.2402 for $C_{20}H_{32}O_2$.

Acutilol A acetate (**2**). Unstable oil. $[\alpha]_D^{25}$ + 29.6° (*c* 2.0, $CHCl_3$). IR ν_{max}^{film}: 2959, 1737, 1444, 1378, 1239, 1021 cm^{-1}. ^{1}H NMR (500 MHz, $CDCl_3$): δ 5.38 (1H, *s*, H-3), 5.20 (1H, *d*, J = 4 Hz, H-6), 3.25 (1H, *bs*, H-5), 2.85 (2H, centre of AB quartet, H-2), 2.64 (1H, *t*, J = 6 Hz, H-14), 2.44 (1H, *m*, H-9β), 2.04 (3H, *s*, H-22), 1.89 (1H, *m*, H-9α), 1.75 (3H, *s*, H-17), 1.61 (3H, *s*, H-18), 1.58–1.27 (8H, *m*), 1.26 (3H, *s*, H-16), 1.21 (3H, *s*, H-20), 0.80 (3H, *d*, J = 6 Hz, H-19). HRCI-MS: $[M + NH_4]^+$ *m/z* obsd. 364.2852, calc. 364.2852 for $C_{22}H_{34}O_3 \cdot NH_4$.

Acutilol B (**3**). Unstable oil. $[\alpha]_D^{25}$ − 28° (*c* 0.08, $CHCl_3$). IR ν_{max}^{film}: 3479, 2966, 2930, 1703, 1466, 1381 cm^{-1}. ^{1}H NMR (500 MHz, $CDCl_3$): δ 5.36 (1H, *s*, H-3), 4.00 (1H, *d*, J = 7 Hz, H-6), 3.31 (1H, *bs*, H-5), 2.92 (2H, *s*, H-2), 2.70–2.40 (~4H, *m*), 2.10–1.92 (2H, *m*), 1.91 (3H, *s*, H-17), 1.88–1.20 (~6H, *m*), 1.57 (3H, *s*, H-18), 1.09 (6H, *d*, J = 7 Hz, H-16 and H-20), 0.92 (3H, *d*, J = 6 Hz, H-19). HREI-MS: $[M]^+$ *m/z* obsd. 304.2417, calc. 304.2402 for $C_{20}H_{32}O_2$.

Deacetylation of **2**. Acutilol A acetate (**2**, 30 mg) in 10 ml Me_2CO and 10 ml MeOH was stirred with ~0.5 mg NaOH for 18 hr. Another ~0.5 mg NaOH was then added and the mixt. stirred for an additional 5 hr. The soln was dild. with H_2O and extracted ×2 with CH_2Cl_2. The comb. organic phase was washed with satd NaCl soln and dried ($MgSO_4$). After removal of solvent under vacuum, the crude product was purified by silica gel HPLC (*iso*octane–EtOAc, 3:1) to yield pure acutilol A (**1**, 19 mg, 72%) and starting material (**2**, 6 mg, 20%).

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