NATURAL PRODUCT RESEARCH, 2016
http://dx.doi.org/10.1080/14786419.2016.1236096

Taylor & Francis
Taylor & Francis Group

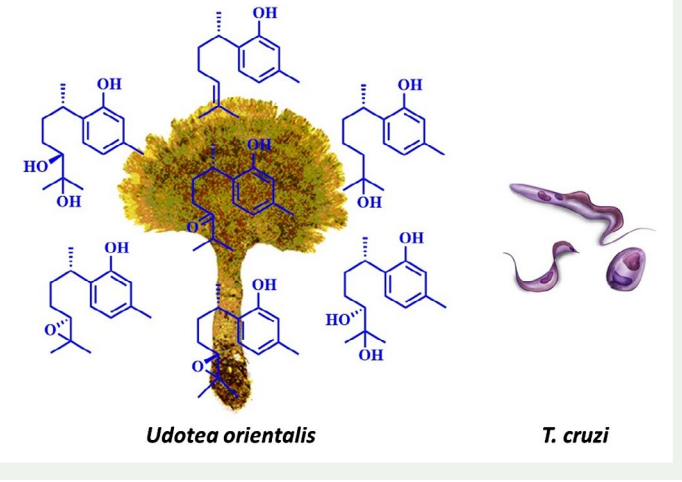
Bioactive new metabolites from the green alga *Udotea orientalis* growing on the Gorgonian coral *Pseudopterogorgia rigida*

Omar M. M. Sabry[a,b], Douglas E. Goeger[a] and William H. Gerwick[a,c]

[a]College of Pharmacy, Oregon State University, Corvallis, OR, USA; [b]Faculty of Pharmacy, Cairo University, Cairo, Egypt; [c]Center for Marine Biotechnology and Biomedicine, University of California, San Diego, CA, USA

ABSTRACT

As part of our continued search for bioactive secondary metabolites from marine sources using a bioassay-guided fractionation technique (Cytotoxic and anti-trypanosome activities), we have examined the organic extract of Papua New Guinean collection of the green alga *Udotea orientalis* growing on the Gorgonian coral *Pseudopterogorgia rigida*. Successive HPLC investigations resulted in isolation of three new compounds, (+) curcuepoxide A, (+) curcuepoxide B and (+)-10α-hydroxycurcudiol. Analysis of different spectroscopic data e.g. UV, IR, LRMS, HRMS, 1D NMR and 2D NMR on the isolated compounds allowed for construction of the planar structures. Stereochemistry assignment at C-7 and C-10 in the new compounds was discussed. Isolated compounds were found to be active in an *in vitro* assay of antitrypanosome activity. The isolated compounds were found to have variable cytotoxic activity in human lung cancer cell lines.



ARTICLE HISTORY
Received 12 May 2016
Accepted 27 August 2016

KEYWORDS
Udotea orientalis; *Pseudopterogorgia rigida*; antitrypanosome; lung cancer

CONTACT Omar M. M. Sabry omar.sabry@cu.edu.eg
Supplemental data for this article can be accessed at http://dx.doi.org/10.1080/14786419.2016.1236096.

1. Introduction

The *Udotea* species grow in the shape of small calcareous fans, and contain calcium carbonate. The calcium carbonate content within *Udotea* gives them a hard body with varying textured plates, and the plates often have lines of calcium carbonate deposits centred around the middle. *Udotea* usually anchors into sandy or muddy silt bottoms or even the walls of coral reefs with a holdfast stemming from or around a single stalk. They are quite common species in tropical near shore marine environments. Their fan plates grow in various shapes, such as in a cup form like that of *Udotea cyathiformis*. These species grow from shallow sea grass flats to deeper depths of 50 feet or more, reaching 3–8 inches in height. Investigations of the different species of the marine green alga *Udotea sp.* have yielded several biologically active diterpene compounds such as udoteal (induced feeding avoidance in the herbivorous fish *Eupomacentrus leucostictus*), udoteal B (antibiotic activity, inhibition of cell division in the fertilised sea urchin egg, and toxicity towards herbivorous damselfish) (Paul et al. 1982; Paul & Fenical 1986; Paul et al. 1990; Iliopoulou et al. 2000). Lectins (antiviral, antibacterial, antifungal, ichthyotoxic, cytotoxic, antitumor, anticoagulant), (Uhlenbruck et al. 1992; Siddhanta & Shanmugam 1999). Petiodial (feeding deterrent) (Fattorusso et al. 1983) and udoteafuran (antimicrobial) (Nakatsu et al. 1981). However, upon searching the literature, we didn't find any research work about investigation of bioactive secondary metabolites from this species, *Udotea orientalis*. Therefore, we investigated this organism for the presence of new bioactive natural products.

2. Results and discussion

As part of our ongoing search for structurally and pharmacologically interesting substances from marine algae, a detailed exploration of a Papua New Guinean collection of the green alga *U. orientalis* growing on the wall of a coral reef was undertaken. During this study, several metabolites of known identity but unusual to isolate from *Udotea* species were obtained including (+)-10β-hydroxycurcudiol (1), (+) curcuphenol (2) (McEnroe & Fenical 1978; Fusetani et al. 1987; Tasdemir et al. 2003), (+) curcudiol (3) (Manger et al. 1995; Buckner et al. 1996) and (+) curcudiol-10-one (4) (Buckner et al. 1996). In addition, three new compounds, (+) curcuepoxide A (5), (+) curcuepoxide B (6) and (+)-10α-hydroxycurcudiol (7) were discovered in the same organic extract (Figure 1). This research describes the chromatographic isolation and structure elucidation of this new series of *U. orientalis* bioactive compounds. Curcuphenol and related compounds have previously been reported as natural products of the sponge *Didiscus oxeata* (Davis et al. 1983; Fusetani et al.1987; Tasdemir et al. 2003), and the coral *Pseudopterogorgia rigida* (Davis et al. 1983; David et al. 2006)

Analysis of different spectroscopic data e.g. UV, IR, LRMS, HRMS, 1D NMR and 2D NMR of the isolated compounds allowed for construction of their planar structures. HMBC and MS fragmentation were used to confirm their structures.

Compound **5** was isolated as a colourless oil. It showed a HRFABMS $[M + H]^+$ ion at *m/z* 235.1698 for a molecular formula of $C_{15}H_{22}O_2$, and therefore possessed 5° of unsaturation. The IR spectrum displayed bands characteristic for a hydroxyl group (3420 cm^{-1}) as well as the presence of an aromatic system (2976 cm^{-1}). The ^1H NMR spectrum of **5** in C_6D_6 (Table S1) displayed a sharp doublet at δ 7.01 (1H, d J = 7.7 Hz), which was consistent with an aromatic proton ortho to another one that was present as a sharp doublet of doublets at δ 6.76 (1H, dd J = 7.7, 0.8 Hz).

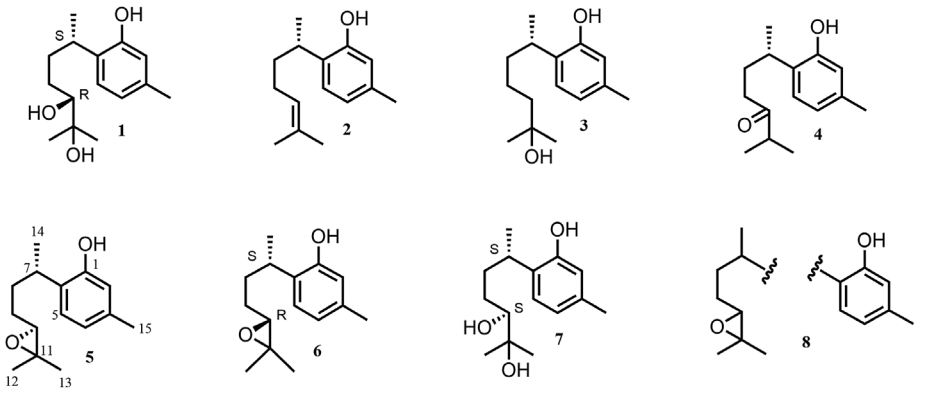


Figure 1. (1–7) Structures of the isolated compounds from *Udotea orientalis*. (8) Partial structures of **5**.

Another sharp doublet at δ 6.81 (1H, d J = 0.8 Hz), which was consistent with an aromatic proton meta to the latter proton, and a sharp singlet at δ 6.9 (1H, s), which was consistent with a phenolic hydroxy proton was detected. We recognised a sharp doublet of doublets at δ 2.42 with J values of 12, 2.8 Hz, which was consistent with a proton attached to a carbon atom attached to an epoxy group. Another two sharp singlets at δ 0.97 and 0.94, assignable to two geminal olefinic dimethyl protons, were also observed. In addition a sharp doublet at δ 1.18, which was consistent with an aliphatic methyl group attached to a methine group, was shown. In addition, the NMR spectrum of **5** showed a multiplet at δ 3.32 (methine proton), four methylene signals (δ 1.60, 1.28, 1.25 and 1.94), and an olefinic methyl at δ 2.13. The ^{13}C NMR spectrum of **5** (Table S1) showed signals for all 15 carbons.

The number of attached hydrogen atoms was determined from the HSQC spectra in C_6H_6. These data showed that **5** was a sesquiterpene composed of a trisubstituted benzene ring, one epoxy ethylene, one methine, two methylenes and four methyl groups. Since the aromatic ring and the epoxy group accounted for the 5° of unsaturation, compound **5** was inferred to be bicyclic. The complete NMR assignments were established by the combined analysis of ^{1}H–^{1}H COSY, HSQC and HMBC data (Table S1).

The ^{1}H–^{1}H COSY spectrum of **5** contained only two spin systems. The benzylic methine proton (H-7, δ 3.32), which shared a 6.8 Hz coupling with the secondary methyl group (H-14, δ 1.18). Strong couplings were detected between H-7/H-8, H-8/H-9 and also H-9 and H-10. This left C-11 without two bonding partners. The appearance of two methyl groups (H-12 and H-13) as singlets in the ^{1}H NMR spectrum, as well as the HMBC cross peaks observed from both methyls to C-11 (δ 58.7), C-10 (δ 66.2) and also with each other, proved that they were bonded to C-11. This completed Fragment **a** of the structure, as shown in Figure 1. In the aromatic region, H-5 (δ 7.01 d, J = 7.7 Hz) *ortho* coupled to H-4 (δ 6.76 d, J = 7.7 Hz), which in turn showed a weak *meta* coupling with H-2 (δ 6.81, d, J = 0.8 Hz), thus completing the second spin system.

The substitution pattern of the trisubstituted aromatic ring was revealed by a HMBC experiment (J = 8.0 Hz). Correlations were observed from the aromatic methyl group (H-15) to C-2, C-3 and C-4, indicating that H-15 was attached to C-3. Also the residence of the aromatic hydroxy function at C-1 (δ 155.0) was evident from HMBC cross peaks observed between H of the hydroxy group with C-1, C-2 and C-6 as well as H-5/C-1, giving the second

structural fragment, Fragments **a** and **b** (Figure 1) could be bisabolane type sesquiterpene. Partial structures of **5** were connected through the HMBC correlations between H-7/C-6 and H-14/C-6, H-14/C-7 and H-14/C-8. Thus, the gross structure of **5** was determined to be curcuepoxide. The unambiguous assignment of the relative stereochemistry of the two chiral centres (C-7 and C-10) within **5** was not possible by analysis of the 1D NOE experiment. However, the co-occurrence in the same extract of (+) curcuphenol (**2**) and (+) curcudiol (**3**) suggested that **5** possessed *S* configuration at C-7 as well. The *S* configuration at C-7 of (+) curcuphenol and (+) curcudiol had been previously confirmed by chemical synthesis. (Davis et al. 1983)

Compound **6** was isolated as a colourless oil. It showed a HRCIMS $[M]^+$ ion at *m/z* 234.1616 for a molecular formula of $C_{15}H_{22}O_2$, and therefore possessed 5° of unsaturation. The IR spectrum displayed bands characteristic for a hydroxyl group (3392 cm^{-1}) as well as the presence of an aromatic system (2958 cm^{-1}). ^1H NMR experiments were carried out in CD_3OD and C_6D_6 to provide good chemical shift resolution. ^1H NMR signals were well dispersed in CD_3OD. The ^1H NMR spectrum of **6** in CD_3OD (Table S2) displayed a sharp doublet at δ 6.95 (1H, d J = 7.6 Hz), which was consistent with an aromatic proton ortho to another sharp doublet at δ 6.58 (1H, d J = 7.6 Hz), a sharp singlet at δ 6.56 (1H, s). Another sharp triplet was found at δ 2.75 with J values of 13, 6.4 Hz, which was consistent with a proton attached to a carbon atom attached to an epoxy group. Another two sharp singlets at δ 1.22 and 1.14, assignable to two geminal olefinic dimethyl protons were also observed.

Additionally, a sharp doublet at δ 1.18, which was consistent with an aliphatic methyl group attached to a methine group, was observed. In addition, a multiplet at δ 3.16 (methine proton), four methylene signals (δ 1.79, 1.64, 1.45 and 1.42) and an olefinic methyl at δ 2.21 were detected. 2D NMR techniques (^1H–^1H COSY, HSQC, and HMBC) were used to establish the connectivities. An HSQC experiment showed the direct ^1H–^{13}C correlations involving all protonated carbons, while an HMBC experiment allowed the assignment of the nonprotonated carbons.

Compound **7** was isolated as a colourless oil. It showed a LRCIMS $[M + NH_4]^+$ ion at *m/z* 270 for a molecular formula of $C_{15}H_{24}O_3$, and therefore possessed 4° of unsaturation. The IR spectrum displayed bands characteristic for a hydroxyl group (3340 cm^{-1}) as well as the presence of an aromatic system (2960 cm^{-1}). The ^1H NMR spectrum in CD_3OD of **7** (Table S3) displayed a sharp doublet at δ 6.95 (1H, d J = 7.5 Hz), which was consistent with an aromatic proton coupled to another proton in the ortho position. Another sharp doublet at δ 6.85 (1H, d J = 7.5 Hz), consistent with an aromatic proton coupled to the previous proton, was recognised. A sharp singlet at δ 6.57 (1H, s), a sharp doublet of doublet at δ 3.36 with J values of 10.5, 1.5 Hz, which was consistent with a proton attached to a carbon atom attached to hydroxy group was detected. Two sharp singlets at δ 1.45 and 1.47, assignable to two geminal olefinic dimethyl protons attached to quaternary carbon were also displayed. Another sharp doublet at δ 1.19 with J value of 7.2 HZ, which was consistent with methyl aliphatic protons attached to a methine group were observed. In addition, a multiplet at δ 3.09 (methine proton), four signals for protons attached to methylene groups (δ 1.82, 1.63, 1.60 and 1.36) and an olefinic methyl at δ 2.21 attached to an aromatic nucleus were obvious. 2D NMR techniques (^1H–^1H COSY, HSQC, and HMBC) were used to establish the connectivities and finally to the planar structure of **7**.

(*S*)-(+)-Curcuphenol (**2**) $[\alpha]_D^{25}$ = +29.7° provided the starting point for the assignment of the unknown stereochemistry at C-10 of metabolites **5**–**7**. The presence of two freely rotating

methylene groups between the C-7 stereocentre and the distant additional stereogenic carbon at C-10 of **5**–**7** minimises any direct chiroptical perturbation between stereocentres and allows for application of van't Hoff's principle (asymmetric centres separated by several single bonds make simple additive contributions to the molar rotation angle) of optical superposition (El Sayed et al. 2002). The molar rotations ($[M]_D$) of curcuphenol and metabolites **5**–**7** were determined by experimental procedure. (*S*)-(–)-3-Ethyl-22-dimethyl-oxirane, (*R*)-(+)-3-ethyl-22-dimethyl-oxirane (Davis et al. 1983) and (*S*)-(–)-2-methylpentane-23-diol were used as references in the analysis of the configuration at C-10 for **5**–**7**, respectively, because the substitution pattern around the stereocentre in these compounds is closely related to the compounds isolated from *U. orientalis*.

Since the *S*-configuration at C-7 in curcuphenol and in **5**–**7** is constant, the expected molar rotations for all possible diastereomers of **5**–**7** were readily derived (Table S4). Stereochemical assignments were possible due to the considerable differences in the observed and calculated molar rotations between the different stereoisomers. The (7*S*,10*S*) and (7*S*,10*R*) assignments for **5** and **6,** respectively, provided the best agreement between the observed data and the values obtained by additional calculations for the two stereocentres (Table S4). However, the observed molar rotation of compound **7** (7*S*,10*S*) deviated to some extent from the calculated value, but it may be considered within the range. Absolute stereochemistry was not determined due to insufficient quantities of the previous compounds. The previously reported metabolites **1**–**4** (McEnroe & Fenical 1978; Tasdemir et al. 2003) were also isolated from this algal species. Full investigation of the spectroscopic data of these compounds were carried out a in the course of this work.

Compounds **1**–**4** were found to possess the same physical and spectroscopic data as reported in the literature (McEnroe & Fenical 1978; Tasdemir et al. 2003). Stereochemistry assignment of compound **1** was found to be the same as that reported in the literature (El Sayed et al. 2002) by comparing the ^{1}H, ^{13}C NMR data and specific angle of rotation, and finally compared with calculated values for the molar rotation (Table S5). Isolation of these three new metabolites from the organic extract of a Papua New Guinean collection of the green alga *U. orientalis* growing on coral wall in addition to the four known compounds from this single collection, demonstrates the capability of marine ecology to produce a remarkable variety of structures in the same organism.

3. Conclusions

Compounds **1** and **4** were found to have strong cytotoxic activities (EC_{50} = 2, 4 µg/mL), respectively, in human lung cancer (NCI-H460) cell lines. In addition, they both displayed sodium channel blocking activity at this dose but not at the lower dose of 1 µg/mL. This activity was 72 and 82%, respectively, compared to 87% for saxitoxin at 0.05 µg/mL. Curcudiol showed a strong anti-trypanosomal activity in comparison to other isolated compounds with IC_{50} values of 10 µM/mL.

Acknowledgement

We gratefully acknowledge Jeff. Moore (Chemistry, OSU) for mass spectral data, the Government of Papua New Guinea for permission to make the collection.

Disclosure statement

No potential conflict of interest was reported by the authors.

Funding

This work was supported by the National Institute of Health [grant number GM 63554], [grant number CA 52955].

ORCiD

Omar M. M. Sabry http://orcid.org/0000-0002-5796-2708

References

Buckner FS, Verlinde CLMJ, La Flamme AC, Van Voorhis WC, Buckner FS, Verlinde CLMJ, La Flamme AC, Van Voorhis WC. 1996. Efficient technique for screening drugs for activity against *Trypanosoma cruzi* using parasites expressing galactosidase. Antimicrob Agents Chemother. 40:2592–2597.

David M, Amade P, Funel-Le Bon C, Fernández R, Reyes F. 2006. Acerolide, a new cytotoxic cembranolide from the soft coral *Pseudopterogorgia acerosa*. Nat Prod Res. 20:548–552.

Davis FA, Harakal ME, Awad SB. 1983. Chemistry of oxaziridines. 4. Asymmetric epoxidation of unfunctionalized alkenes using chiral 2-sulfonyloxaziridines: evidence for a planar transition state geometry. J Am Chem Soc. 105:3123–3126.

El Sayed KA, Yousaf M, Hamann MT, Avery MA, Kelly M, Wipf P. 2002. J. Nat. Prod. 65:1547–1553.

Fattorusso E, Magno S, Mayol L, Novellino E. 1983. Petiodial, a new monocyclic diterpenoid from the Mediterranean green alga *Udotea petiolata*. Experientia. 39(11):1275–1276.

Fusetani N, Sugano M, Matsunaga S, Hashimoto K. 1987. Isolation of a hexaprenylhydroquinone sulfate from the marine sponge *Dysidea sp*. as an H, K-ATPase inhibitor. Experientia. 43:1234–1235.

Iliopoulou D, Vagias C, Harvala C, Roussis V. 2000. Udoteal B, a new linear diterpenoid from the green alga *Udotea petiolata*. Nat Prod Lett. 14:373–378.

Manger RL, Leja LS, Lee SY, Hungerford JM, Hokama Y, Dickey RW, Granade HR, Lewis R, Yasumoto T, Wekell MM. 1995. Detection of sodium channel toxins: directed cytotoxicity assays of purified ciguatoxins, brevetoxins, saxitoxins, and seafood extracts. J AOAC Int. 78:521–527.

McEnroe FJ, Fenical W. 1978. Structures and synthesis of some new antibacterial sesquiterpenoids from the gorgonian coral *Pseudopterogorgia rigida*. Tetrahedron. 34:1661–1664.

Nakatsu Tetsuo, Ravi BN, Faulkner D John. 1981. Antimicrobial constituents of *Udotea flabellum*. J Org Chem. 46:2435–2438.

Paul VJ, Fenical W. 1986. Chemical defense in tropical green algae, order *Caulerpales*. Mar Ecol. 34:157–169.

Paul VJ, Nelson SG, Sanger HR. 1990. Feeding preferences of adult and juvenile rabbitfish *Siganus argenteus* in relation to chemical defenses of tropical seaweeds. Mar Ecol. 60:23–34.

Paul VJ, Sun HH, Fenical W. 1982. Udoteal, a linear diterpenoid feeding deterrent from the tropical green alga *Udotea flabellum*. Phytochemistry. 21:468–469.

Siddhanta AK, Shanmugam M. 1999. Metabolites of tropical marine algae of the family Codiaceae (*Chlorophyta*): chemistry and bioactivity. J Ind Chem Soc. 76:323–334.

Tasdemir D, Bugni TS, Mangalindan GC, Concepcion GP, Harper MK, Ireland CM. 2003. Bisabolane type sesquiterpenes from a marine *Didiscus* sponge. Turk J Chem. 27:273–279.

Uhlenbruck G, Hanisch FG, Kljajic Z, Poznanovic S, Schroeder HC, Mueller WEG. 1992. The lectin from the algae *Udotea petiolata*: isolation, characterization and sugar binding properties. Behring Inst Mitt. 91:67–77.