Dragmacidoside: a new nucleoside from the Red Sea sponge

Dragmacidon coccinea

Dina R. Abou-Husseinab, Jihan M. Badrac and Diaa T.A. Youssefc*

aDepartment of Natural Products and Alternative Medicine, Faculty of Pharmacy, King Abdulaziz University, Jeddah 21589, Saudi Arabia; bDepartment of Pharmacognosy, Faculty of Pharmacy, Cairo University, Cairo 11562, Egypt; cDepartment of Pharmacognosy, Faculty of Pharmacy, Suez Canal University, Ismailia 41522, Egypt

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Chemical investigation of the Red Sea sponge Dragmacidon coccinea led to the isolation of a new nucleoside, dragmacidoside (1), along with eight known compounds: adenosine (2), inosine (3), deoxycytidine (4), methyl-α-D-glucopyranoside (5), clionasterol (6), stigmasterol (7), campesterol (8) and brassicasterol (9). The compounds were isolated from chloroform and ethyl acetate fractions of the methanolic extract of the sponge, and their structures were established based on various spectroscopic data including MS, 1D and 2D NMR (COSY, HSQC and HMBC). Biological testing revealed that the chloroform fraction possesses significant anti-inflammatory activity in the carrageenan-induced hind paw oedema in rats.

Keywords: Red Sea sponges; Dragmacidon coccinea; dragmacidoside; nucleosides; steroids; anti-inflammatory activity

1. Introduction

Marine sponges have gained a significant attention with respect to the diversity of their secondary metabolites. The biological activities of new metabolites from sponges have been reported in hundreds of scientific papers. Sponges have the potential to provide future drugs against important diseases such as cancer (Bai et al. 1993; Blackburn et al. 1999; Hood et al. 2002; Molinski et al. 2009), inflammation (Tan et al. 1997; Pope et al. 1999; Festaa et al. 2012), cardiovascular (Maryanoff et al. 1993; Shuman et al. 1993; Chackalamannil 2001), viral (Müller et al. 1987; Ford et al. 1999; Wellington et al. 2000), plasmodial (Ang et al. 2001) and bacterial (D_Ambrosio et al. 1996). Previous work on another species of the genus Dragmacidon collected from Andaman Sea in Thailand resulted in the isolation of a number of β-carboline alkaloids; some of them are characterised by possessing potent anti-inflammatory and antitumour activities (Pedpradab et al. 2004). In this study, chloroform and ethyl acetate fractions of the methanolic extract of the sponge Dragmacidon coccinea collected from the Red Sea, Hurghada, Egypt were examined. This resulted in the isolation and identification of a new nucleoside (1) along with eight known compounds (2–9) (Figure 1). In addition, the anti-inflammatory activity of the fractions using rat paw oedema test was evaluated.

2. Results and discussion

Compound 1 was isolated as a white precipitate. Combined spectral data of 1 including 1D (1H, 13C) and 2D (1H–1H COSY, HSQC, HMBC) NMR as well as ESI-MS data suggested the
Figure 1. Structures of compounds 1–9.
molecular formula C$_{10}$H$_{13}$N$_{5}$O$_{5}$ requiring seven degrees of unsaturation. $^{13}$C NMR spectrum revealed resonances for ten carbons of which five were detected in the range from 52.9 to 94.8 ppm indicating the presence of a sugar moiety. Comparison of the $^{13}$C NMR data with those reported for sugars (Agrawal 1989) confirmed the identity of the sugar part as $\alpha$-D-ribofuranose. $^{13}$C signal at $\delta$C 52.9 ppm was assigned to C-5 and the relatively low chemical shift (around 10 ppm) suggested an attachment to a nitrogen atom. Five signals in $^{13}$C NMR spectrum indicated the presence of an amide group ($\delta$C 162.8; C-6), one protonated sp$^2$ carbon ($\delta$C 136.0; C-8) and three non-protonated sp$^2$ carbons ($\delta$C 119.1, 142.3 and 152.7; C-5, C-4 and C-2, respectively), suggesting the existence of a guanine nucleotide. Apart from the signals of the sugar moiety, only one proton singlet resonating at $\delta$H 7.79 ppm (H-8) was detected in the $^1$H NMR spectrum. HMBC experiment indicated cross-peaks from H-8 ($\delta$H 7.79) to each of C-4 and C-5 detected at $\delta$C 142.3 and 119.1 ppm, respectively. This provided a further confirmation for the presence of a guanine moiety. The link between the nucleotide and the sugar moiety through C-5 was assigned by HMBC experiment: correlations of H-5 (H-5) to C-4 ($\delta$C 142.3) and C-8 ($\delta$C 136.0) were detected. The linkage was confirmed by the chemical shift value of C-5 (52.9 ppm). Moreover, cross-peaks from H-1 (H-1) to C-3 (C-3) and C-4' ($\delta$C 85.6), from H-2' (H-2') to C-1' ($\delta$C 94.8) and from H-3' (H-3') and H-4' (H-4') to C-5' ($\delta$C 52.9) were displayed by HMBC experiment. A further COSY correlation of a non-interrupted spin–spin coupling from H-1' to H-2'-5' supported and secured the assignment of the signals of the $\alpha$-D-ribofuranose moiety. Compound 1 was reported here for the first time from a natural source and was named, dragmacidoside (Figure 2).

Compounds 2 and 3 were identified as adenosine and inosine, respectively, by comparing $^1$H NMR data and TLC results with those of reference compounds previously isolated by the authors from Eudistoma laysani (Abou-Hussein et al. 2007).

$^{13}$C NMR spectrum of compound 4 revealed resonances for nine carbons of which five were detected in the range 62.8–88.8 ppm, indicating four oxygenated carbons of a sugar moiety and one deoxygenated carbon that resonated relatively upfield at 41.0 ppm. $^1$H NMR spectrum revealed signals in the range of 2.2–4.38 ppm besides a triplet detected at 6.27 ppm confirming the suggestion of the sugar moiety. HSQC experiment correlated each proton to its corresponding carbon. Comparing $^{13}$C NMR data with those reported for sugars (Agrawal 1989), the sugar moiety could be identified as 2'-deoxyribose. The other four signals detected in the $^{13}$C

Figure 2. HMBC correlations of compound 1.
NMR spectrum indicated the presence of two non-protonated sp² carbons ($\delta_C$ 162.8 and 163.3) and two protonated sp² carbons ($\delta_C$ 101.7 and 143.5 with their corresponding protons detected at $\delta_H$ 5.60, d, $J = 7.8$ Hz and 7.38, d, $J = 7.8$ Hz, respectively). These data confirmed the structure of the nucleobase as cytosine. Accordingly, compound 4 was identified as 2′-deoxycytidine. It was previously detected in marine sediments (Dell’Anno et al. 2002).

1D and 2D NMR data of compound 5 were found to be identical to those reported for methyl-$\alpha$-D-glucopyranoside (Agrawal 1989). Methyl-$\alpha$-D-glucopyranoside was previously isolated from Tulbaghia violacea extract (Lyantagaye 2013).

Comparison of NMR and mass data of compounds 6–9 with previously reported data confirmed their identity as clionasterol, stigmasterol, campesterol and brassicasterol, respectively (Goad & Akihisa 1992). Clionasterol was previously isolated from the Kenyan marine green macroalga Halimeda macroloba (Dzeha et al. 2003), stigmasterol was isolated from the marine microalga Navicula incerta (Kim et al. 2013); whereas campesterol and brassicasterol were reported from coelomic fluid of the marine ripe Nereis succinea (Zeeck et al. 1994).

Biological evaluation indicated the ability of the chloroform fraction to reduce the thickness of the oedema induced by carrageenan in rats. The results of anti-inflammatory activity (Table 1) revealed that a gradual decrease in the thickness of the induced oedema was clearly detected over time. After 24 h of dose administration, the chloroform fraction produced 45.1% of change from the untreated inflamed group of rats thus performing 87% of indomethacin (reference drug) potency. On the other hand, the ethyl acetate fraction displayed only 20.0% of change from the control group, exhibiting 39% of indomethacin activity. Sterols were verified to possess anti-inflammatory activity (García et al. 1999) and the capability of reducing the secretion of pro-inflammatory cytokines and tumour necrosis factor-α (Gupta et al. 1980; Bouic et al. 1996). The significant anti-inflammatory activity produced by the chloroform fraction may then be attributed to its sterol’ contents (compounds 6–9).

3. Experimental

3.1. Biological material

The sponge was collected in July 2009 from the Red Sea, Hurghada, Egypt at a depth of 5–9 m and identified by Prof. Rob van Soest. A voucher specimen, measuring 3.5 cm, has been incorporated in the collections of the Zoological Museum of the University of Amsterdam under registration number ZMAPOR 17592. Another voucher specimen has been deposited in the Red Table 1. Effect of chloroform and ethyl acetate fractions of D. coccinea on carrageenan-induced rat paw oedema measured in mm.

| Group                  | Dose (mg/kg) | 1 h      | 2 h      | 4 h      | 24 h     | % of change | Potency |
|------------------------|--------------|----------|----------|----------|----------|-------------|---------|
| Control                | –            | 3.3 ± 0.20| 3.38 ± 0.21| 3.6 ± 0.14| 3.5 ± 0.32| –           | –       |
| Chloroform fraction    | 60           | 2.85 ± 0.09*| 2.71 ± 0.11*| 2.30 ± 0.04*| 1.92 ± 0.04*| 45.1        | 0.87    |
| Ethyl acetate fraction | 60           | 3.11 ± 0.25*| 3.10 ± 0.11*| 2.96 ± 0.13*| 2.80 ± 0.09*| 20.0        | 0.39    |
| Indomethacin           | 20           | 1.9 ± 0.09*| 1.81 ± 0.12*| 1.78 ± 0.06*| 1.70 ± 0.06*| 51.4        | 1       |

Note: Results are expressed as mean ± SE, $n = 6$.

*Significantly different from control, $P < 0.01$. 

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3.2. General experimental procedures

NMR spectra were obtained in CDCl₃ and CD₃OD on Bruker Avance DRX 600 Spectrometers (Bruker, Rheinstetten, Germany) at 600 MHz for ¹H NMR and 150 MHz for ¹³C NMR. Mass spectral data were performed on Ion trap LC/MS Agilent. For column chromatography (CC), silica gel (Merck, Darmstadt, Germany, 70–230 mesh ASTM) and Sephadex LH-20 (Pharmacia, New Jersey, United States) were used. Pre-coated silica gel 60 F-254 plates (Merck) were used for TLC. RP-18 (40–63 μm) 200 mg (top), 6 mL standard tubes (extraction tubes), Darmstadt, Germany, were used for the purification of the polar compounds. Spots were visualised by p-anisaldehyde/sulphuric acid.

3.3. Extraction and isolation

The fresh sponge (1.5 kg) was cut into very small pieces and macerated in methanol (3 × 4000 mL) at room temperature. The combined methanolic extracts were dried under vacuum (7 g), dissolved in aqueous methanol and extracted with ethyl acetate (3 × 500 mL). The organic layer was dried under vacuum to afford 1.5 g. The residue left was extracted with chloroform (3 × 2000 mL) and dried to yield 5 g.

3.3.1. CC of the ethyl acetate fraction

Nine hundred milligrams of the ethyl acetate fraction were chromatographed on a silica gel column, eluted, in increasing proportions, with 90–60% CHCl₃/MeOH mixtures. Four subfractions eluted with 85% CHCl₃/MeOH (subfraction 1a), 80% CHCl₃/MeOH (subfraction 1b), 75% CHCl₃/MeOH (subfraction 1c) and 70% CHCl₃/MeOH (subfraction 1d) were obtained. Purification of subfraction 1d (20 mg) on CC of Sephadex LH-20 eluted with 100% MeOH yielded compound 1. Similarly, subfraction 1a (35 mg) yielded compounds 2 and 3, subfraction 1b (50 mg) yielded compound 4 and subfraction 1c (35 mg) yielded compound 5.

The isolated compounds were further purified, separately, on Rp-18 standard tubes with gradient elution using H₂O/MeOH mixtures starting from 100% H₂O then gradually increasing MeOH 10–90 till 100%). Compound 1 (3 mg) was eluted with 90% H₂O/MeOH, while compounds 2–5 (2, 3, 2 mg, respectively) were separately eluted with 20% H₂O/MeOH.

3.3.2. CC of the chloroform fraction

Four grams of the chloroform fraction were chromatographed on a silica gel column packed in hexane and eluted in a stepwise solvent gradient using hexane/CHCl₃ (90–10%), followed by 100% CHCl₃ and CHCl₃/MeOH (99–95%) mixtures. Four subfractions eluted with 20% hexane/CHCl₃ (subfraction 2a, 100 mg), 15% hexane/CHCl₃ (subfraction 2b, 80 mg), 10% hexane/CHCl₃ (subfraction 2c, 120 mg) and 100% CHCl₃ (subfraction 2d, 70 mg) were obtained. Further purification of subfractions a–d separately, on silica gel CC using the same gradient elution system led to the isolation of compounds 6 (20 mg), 7 (14 mg), 8 (30 mg) and 9 (20 mg), respectively.

3.4. Spectroscopic data

3.4.1. Dragmacidoside C₁₀H₁₃N₅O₅ (1)

White precipitate, m/z: 282 [M − H]^⁺, ¹H NMR (600 MHz, CD₃OD): δH 7.79 (s, H-8), 6.13 (brs, H-1'), 4.02 (brd, J = 6 Hz, H-2'), 4.32 (dd, J = 3.6, 5.4 Hz, H-3'), 4.65 (q, J = 3.6 Hz, H-4'), 1138 D.R. Abou-Hussein et al.
4.73 (dd, $J = 2.4$, 14.4 Hz, H-5'), 3.86 (dd, $J = 3$, 14.4 Hz, H-5'). $^{13}$C NMR (150 MHz, CD$_3$OD): $\delta_c$ 152.7 (C-2), 142.3 (C-4), 119.1 (C-5), 162.8 (C-6), 136.0 (C-8), 94.8 (C-1'), 77.5 (C-2'), 71.8 (C-3'), 85.6 (C-4'), 52.9 (C-5').

3.4.2. Adenosine $C_{10}H_{13}N_5O_4$ (2)
White precipitate, $m/z$: 266 [M – H]$^+$, $^1$H NMR (600 MHz, CD$_3$OD): $\delta_H$ 8.01 (s, H-2), 8.10 (s, H-8), 6.1 (d, $J = 6.6$ Hz, H-1'), 4.16 (q, $J = 4.8$ Hz, H-2'), 4.03 (brd, $J = 5.4$ Hz, H-3'), 3.84 (m, H-4'), 3.51 (m, H-5').

3.4.3. Inosine $C_{10}H_{12}N_4O_5$ (3)
White precipitate, $m/z$: 267 [M – H]$^+$, $^1$H NMR (600 MHz, CD$_3$OD): $\delta_H$ 8.04 (s, H-2), 8.35 (s, H-8), 5.80 (d, $J = 7.2$ Hz, H-1'), 4.13 (br.d, $J = 5$ Hz, H-2'), 4.30 (dd, $J = 5.4$, 1.8 Hz, H-3'), 3.99 (m, H-4'), 3.71 (m, H-5').

3.4.4. Deoxycytidine $C_{9}H_{13}N_3O_4$ (4)
White precipitate, $m/z$: 226 [M – H]$^+$, $^1$H NMR (600 MHz, CD$_3$OD): $\delta_H$ 5.60 (d, $J = 7.8$ Hz, H-5), 7.38 (d, $J = 7.8$ Hz, H-6), 6.27 (t, $J = 1.2$ Hz, H-1'), 2.20 (m, H-2'), 4.38 (t, $J = 3$ Hz, H-3'), 3.90 (d, $J = 4.2$ Hz, H-4'), 3.79 (dd, $J = 3.6$, 12.6 Hz, H-5'), 3.72 (dd, $J = 3.6$, 12.6 Hz, H-5'). $^{13}$C NMR (150 MHz, CD$_3$OD): $\delta_c$ 162.8 (C-2), 163.3 (C-4), 101.7 (C-5), 143.5 (C-6), 86.2 (C-1'), 41.0 (C-2'), 71.1 (C-3'), 88.8 (C-4'), 62.8 (C-5').

3.4.5. Methyl-$\alpha$-D-glucopyranoside $C_7H_{14}O_6$ (5)
Precipitate, $m/z$: 193 [M – H]$^+$, $^1$H NMR (600 MHz, CD$_3$OD): $\delta_H$ 4.69 (d, $J = 3.6$ Hz, H-1), 3.38 (dd, $J = 3.6$, 9.6 Hz, H-2), 3.59 (t, $J = 9$ Hz, H-3), 3.27 (br. s, H-4), 3.51 (m, H-5), 3.80 (dd, $J = 2.4$, 12 Hz, H-6a), 3.65 (dd, $J = 6$, 12 Hz, H-6b), 3.39 (s, OCH$_3$). $^{13}$C NMR (150 MHz, CD$_3$OD): $\delta_c$ 101.2 (C-1), 73.6 (C-2), 75.1 (C-3), 71.7 (C-4), 73.5 (C-5), 62.7 (C-6), 55.5 (OCH$_3$).

3.4.6. Clionasterol $C_{29}H_{50}O$ (6)
White powder, $m/z$: 414 [M]$^+$, $^{13}$C NMR (150 MHz, CDCl$_3$): $\delta_c$ 37.2 (C-1), 31.6 (C-2), 71.8 (C-3), 42.1 (C-4), 140.7 (C-5), 121.6 (C-6), 30.5 (C-7), 31.8 (C-8), 50.0 (C-9), 36.4 (C-10), 21.0 (C-11), 39.7 (C-12), 42.4 (C-13), 56.7 (C-14), 24.2 (C-15), 28.2 (C-16), 55.9 (C-17), 11.8 (C-18), 19.3 (C-19), 36.1 (C-20), 18.9 (C-21), 33.8 (C-22), 26.3 (C-23), 46.0 (C-24), 28.8 (C-25), 19.0 (C-26), 19.5 (C-27), 22.9 (C-28), 12.2 (C-29).

3.4.7. Stigmasterol $C_{29}H_{48}O$ (7)
White powder, $m/z$: 412 [M]$^+$, $^{13}$C NMR (150 MHz, CDCl$_3$): $\delta_c$ 140.9 (C-5), 138.5 (C-20), 129.4 (C-21), 121.9 (C-6), 72.0 (C-3), 56.9 (C-14), 56.1 (C-17), 50.3 (C-9), 46.0 (C-22), 42.5 (C-4), 42.5 (C-13), 40.0 (C-18), 39.9 (C-12), 37.4 (C-1), 36.3 (C-10), 32.2 (C-7), 32.1 (C-8), 31.8 (C-2), 29.3 (C-25), 29.1 (C-16), 26.2 (C-23), 25.6 (C-15), 23.2 (C-19), 21.3 (C-11), 20.0 (C-27), 19.6 (C-26), 19.0 (C-28), 12.2 (C-24), 12.0 (C-29).

3.4.8. Campesterol $C_{28}H_{48}O$ (8)
White powder, $m/z$: 400 [M]$^+$, $^{13}$C NMR (150 MHz, CDCl$_3$): $\delta_c$ 37.2 (C-1), 31.7 (C-2), 71.7 (C-3), 42.2 (C-4), 140.7 (C-5), 121.6 (C-6), 31.8 (C-7), 31.6 (C-8), 50.2 (C-9), 36.5 (C-10), 21.0 (C-11), 39.7 (C-12), 46.0 (C-13), 56.8 (C-14), 24.3 (C-15), 28.2 (C-16), 56.7 (C-17), 11.8 (C-18),
19.5 (C-19), 36.1 (C-20), 18.8 (C-21), 33.6 (C-22), 31.6 (C-23), 39.0 (C-24), 33.8 (C-25), 19.3 (C-26), 18.9 (C-27), 15.4 (C-28).

3.4.9. *Brassicasterol* C$_{28}$H$_{46}$O (9)

White powder, *m/z*: 398 [M]$^+$, $^{13}$C NMR (150 MHz, CDCl$_3$): $\delta$C 36.9 (C-1), 31.2 (C-2), 70.8 (C-3), 41.9 (C-4), 140.8 (C-5), 120.9 (C-6), 31.5 (C-7), 32.8 (C-8), 49.7 (C-9), 36.1 (C-10), 20.6 (C-11), 39.7 (C-12), 40.1 (C-13), 56.4 (C-14), 25.9 (C-15), 28.5 (C-16), 56.3 (C-17), 11.9 (C-18), 19.2 (C-19), 39.9 (C-20), 22.6 (C-21), 135.7 (C-22), 131.3 (C-23), 42.7 (C-24), 33.4 (C-25), 19.0 (C-26), 18.6 (C-27), 17.7 (C-28).

3.5. Evaluation of the anti-inflammatory activity

The hind paw oedema method was used for the detection of the anti-inflammatory activity of the chloroform and ethyl acetate fractions. Hind paw oedema (skin oedema) was induced by 0.1 mL of 1% carrageenan (Sigma-Aldrich, Schnelldork, Germany) injected into the subplantar region of the left hind paw (Panthong et al. 1995). Adult male albino rats (24) of Sprague Dawley strain (130–150 g b.wt.) were purchased from the breeding unit of the Egyptian Organization for Biological Product and Vaccines (Cairo). The inflamed animals were divided randomly into four groups (six for each): the first group received 1 mL saline and served as control. The second group was treated with the reference drug indomethacin (EIPICO, Cairo, Egypt) at a dose of 20 mg/kg b.wt. *p.o*. The tested fractions were given, separately, to the third and fourth groups of inflamed animals at a dose of 60 mg/kg b.wt. *p.o*. The change in paw thickness in all tested animals was measured at 1, 2, 4 and 24 h after carrageenan injection. The data obtained from the study were statistically analysed using the Student’s *t* test (Snedecor & Cochran 1971). All values are expressed as mean ± SE of the number of experiments (*n*).

4. Conclusion

To the best of our knowledge, dragmacidoside (1) is a new nucleoside isolated from the Red Sea sponge *D. coccinea*. This is the first report on the isolation of compounds 2–9 from the sponge. The chloroform fraction demonstrated significant anti-inflammatory activity while the ethyl acetate fraction revealed weak activity. The results need a further investigation concerning the safety of the chloroform fraction and its validity to be used for the treatment of inflammation.

Supplementary material

Supplementary material relating to this article is available online.

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