# Antineoplastic Agents. 535. 1 Isolation and Structure of Plakorstatins 1 and 2 from the Indo-Pacific Sponge Plakortis nigra

**ARTICLE** *in* JOURNAL OF NATURAL PRODUCTS · OCTOBER 2004

Impact Factor: 3.8 · DOI: 10.1021/np040043j · Source: PubMed



**5 AUTHORS**, INCLUDING:

Toshihiko Nogawa
RIKEN
**38** PUBLICATIONS **466** CITATIONS
SEE PROFILE

John N.A. Hooper
Queensland Museum
**333** PUBLICATIONS **4,351** CITATIONS
SEE PROFILE

# Antineoplastic Agents. 535.[1] Isolation and Structure of Plakorstatins 1 and 2 from the Indo-Pacific Sponge *Plakortis nigra*

George R. Pettit,* Toshihiko Nogawa, John C. Knight, Dennis L. Doubek, and J. N. A. Hooper[†]

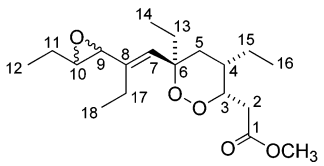
*Cancer Research Institute and Department of Chemistry and Biochemistry, Arizona State University, Tempe, Arizona 85287-2404*

*Received February 19, 2004*

Two new cancer cell growth inhibiting cyclic peroxides, plakorstatins 1 (**1**) and 2 (**2**), were isolated from the Indonesian marine sponge *Plakortis nigra*. The structures of plakorstatins 1 and 2 including relative configuration were elucidated on the basis of mass and 2D NMR spectroscopic interpretations. These are the first plakortides with an epoxy group in the side chain. Plakorstatin 2 was found to differ from plakorstatin 1 only in the configuration of the epoxide group. Both exhibited moderate cancer cell growth inhibition against the murine P388 lymphocytic leukemia cell line with $ED_{50}$ values of 1.1 and 0.91 $\mu$g/mL, respectively, for peroxides **1** and **2**.

While pursuing exploratory sampling of marine invertebrates as potential sources of new anticancer agents in the Celebes Sea off the Indonesian island of Sulawesi in 1995, we collected the sponge *Plakortis nigra*. Extracts of this Indo-Pacific species resulted in confirmed levels of activity against the murine P388 lymphocytic leukemia cell line (PS). Marine sponges of the genus *Plakortis* have become an increasing source of cell growth inhibitors,[2] which include new cytotoxic organic peroxides,[3] lipids,[4] glycolipids,[4b] and a pyridinium alkaloid.[5] Several synthetic approaches to the cyclic peroxides,[6] plakortone D,[7] 6-epiplakortolide E,[8] and chiral isomers of the cancer cell growth inhibitor untenone A[9] have been undertaken. Other recent studies involving *Plakortis* constituents[10] have included antimalarial[11a] and antifungal[11b] leads. The only prior report (2002) of a chemical investigation of *P. nigra* was directed at the $\beta$-carboline and cyclic peroxide constituents of this sponge collected in the Republic of Palau.[2b]

*Plakortis nigra* was extracted with $CH_2Cl_2$−$CH_3OH$ (1:1) to provide a crude extract (11.2 g) that was partitioned between hexane and $CH_3OH$−$H_2O$ (9:1). Interestingly further solvent partition separations (3:2 $CH_3OH$−$H_2O$ with $CH_2Cl_2$ followed by 1-butanol with $H_2O$) guided by PS bioassay did not provide any other active fractions. The PS active ($ED_{50}$ 2.0 $\mu$g/mL) hexane extract was initially subjected to Sephadex LH-20 column chromatography with $CH_2Cl_2$−$CH_3OH$ (3:2) as mobile phase. This was followed by successive column chromatography on Si60-MPLC, Sephadex LH-20, and $C_{18}$ HPLC to afford two new cancer cell growth inhibiting cyclic peroxides designated plakorstatins 1 (**1**, 3.6 mg) and 2 (**2**, 3.3 mg). Plakorstatin 1 was




**1**: 9*S*,10*S* or 9*R*,10*R*
**2**: 9*R*,10*R* or 9*S*,10*S*

isolated as a colorless oil, $[\alpha]_D^{25}$ −177° (*c* 0.1, $CH_3OH$), $R_f$ 0.43 (TLC 9:1 hexane−EtOAc). The molecular formula was determined to be $C_{19}H_{32}O_5$ by HRAPCIMS techniques. The $^{13}C$ NMR spectrum showed 19 signals, which were classified as five methyl, six methylene, five methine, and three quaternary carbons by interpreting the APT and HMQC data. The $^{13}C$ NMR spectrum showed signals at $\delta$ 51.8 and 172.2 for a carbomethoxy group, two oxygenated carbons at $\delta$ 78.6 and 83.9, and two olefin carbons at $\delta$ 125.1 and 140.0 (Table 1). The $^1H$ NMR spectrum revealed a methoxy signal at $\delta$ 3.71 and four methyl triplets at $\delta$ 0.86 (t, $J$ = 7.2 Hz), 0.90 (t, $J$ = 7.6 Hz), 1.04 (t, $J$ = 7.6 Hz), and 1.05 (t, $J$ = 7.6 Hz), which suggested that plakorstatin 1 had four terminal ethyl groups (Table 1). The $^1H$ and $^{13}C$ NMR spectra overall were reminiscent of the particular pattern shown by plakortides and suggested that plakorstatin 1 contained a 3,4,6,6-tetrasubstituted six-membered cyclic peroxide ring system.

The $^1H$−$^1H$ COSY spectrum revealed the connectivities of C-2 to C-5, C-9 to C-12, C-13 to C-14, C-4 to C-16, and C-17 to C-18. The epoxy group at C-9 and C-10 was deduced from the $^1H$ and $^{13}C$ NMR chemical shifts of C-9 and C-10, which were $\delta_H$ 3.06 (d, $J$ = 1.6 Hz) and $\delta_C$ 60.0, and $\delta_H$ 2.67 (td, $J$ = 5.2, 2.0 Hz) and $\delta_C$ 62.8, respectively. That premise was supported by the molecular formula. In the HMBC spectrum, the correlations from the methoxy signal at $\delta$ 3.71 to C-1 and H-2 to C-1 gave the connectivity of the carbomethoxy group to C-2. The correlations from H-13 to C-5 and H-14 to C-6 showed that a terminal ethyl group was attached at C-6 on the peroxide ring and confirmed the connectivities of C-5 to C-14. The correlations of H-17 to C-9 and H-18 to C-8 suggested that the side chain from C-9 to C-12 and the ethyl group of C-17 to C-18 were attached at C-8, which was connected to C-7 through a double bond. That conclusion was further established by the correlations of H-7 to both C-9 and C-17. The H-7 signal was also correlated with C-5, and this suggested C-7 was connected to C-6, which was supported by the observed correlation of H-13 to C-7.

The relative configuration of the peroxide ring was established from NOESY spatial correlations as shown in Figure 1. The correlations between both H-2s and H-5 at $\delta$ 1.31 showed that they were on the same face and showed the peroxide ring to be in the chair conformation, allowing H-5 at $\delta$ 1.31 and the carbomethoxy side chain at C-3 to be assigned as axial. The H-2 at $\delta$ 2.39 also correlated with

* To whom correspondence should be addressed. Tel: 480-965-3351. E-mail: bpettit@asu.edu.
[†] Queensland Museum, P.O. Box 3300, S. Brisbane, Queensland 4101, Australia.

10.1021/np040043j CCC: $27.50 
Published on Web 08/12/2004

**Table 1.** $^1H$ and $^{13}C$ NMR Chemical Shift Assignments for Plakorstatins 1 and 2 in $CDCl_3$ at 400 MHz

| | **1** | | **2** | |
|---|---|---|---|---|
| position | $\delta^1H$ (*J* in Hz) | $\delta^{13}C$ | $\delta^1H$ (*J* in Hz) | $\delta^{13}C$ |
| 1 | | 172.2 | | 172.2 |
| 2 | 2.39 dd (16.0, 3.6) | 31.2 | 2.39 dd (16.0, 3.6) | 31.2 |
| | 3.02 dd (16.0, 9.2) | | 3.02 dd (16.0, 9.6) | |
| 3 | 4.47 ddd (9.2, 5.2, 3.6) | 78.6 | 4.49 | 78.7 |
| 4 | 2.02 | 35.3 | 2.10 | 35.3 |
| 5 | 1.31 ax | 35.7 | 1.30 ax | 35.8 |
| | 1.70 eq | | 1.70 eq | |
| 6 | | 83.9 | | 83.9 |
| 7 | 5.38 s | 125.1 | 5.40 s | 125.2 |
| 8 | | 140.0 | | 140.3 |
| 9 | 3.06 d (1.6) | 60.0 | 3.08 d (2.0) | 60.1 |
| 10 | 2.67 td (5.2, 2.0) | 62.8 | 2.66 td (5.6, 2.0) | 62.6 |
| 11 | 1.62 2H | 25.3 | 1.62 2H | 25.3 |
| 12 | 1.04 t (7.6) | 9.8 | 1.03 t (7.2) | 9.8 |
| 13 | 1.60 2H | 33.0 | 1.56 2H | 33.0 |
| 14 | 0.86 t (7.2) | 7.6 | 0.84 t (7.2) | 7.6 |
| 15 | 1.18 2H | 25.0 | 1.18 2H | 25.0 |
| 16 | 0.90 t (7.6) | 10.9 | 0.91 t (7.2) | 10.9 |
| 17 | 2.20 | 21.6 | 2.15 | 21.3 |
| | 2.43 | | 2.54 | |
| 18 | 1.05 t (7.6) | 12.9 | 1.03 t (7.2) | 13.0 |
| $OCH_3$ | 3.71 s | 51.8 | 3.71 s | 51.8 |

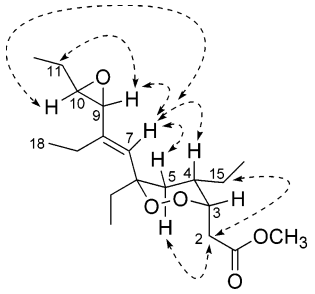



**Figure 1.**

H-15 and pointed to the ethyl group at C-4 being on the same face of the molecule. Furthermore, H-7 was found to correlate with H-4, again supporting a chair conformation for the peroxide ring. In addition, this indicated that H-4 was on the same face as the side chain containing H-7 and was axial. The latter assignment for H-4 was also supported by its coupling constant ($J = 5.2$ Hz) with H-3. Since H-7 also correlated with H-5 at $\delta$ 1.70, it was assigned a $\beta$-orientation. The *E*-configuration of the double bond between C-7 and C-8 was established by NOESY correlations between H-7 and both H-9 and H-10. The epoxide at C-9 and C-10 was confirmed as a *trans*-epoxide by the small coupling constants ($J = 1.6$ and 2.0 Hz for H-9 and H-10) in the $^1H$ NMR spectrum (Table 1). Two stereoisomers at C-9 and C-10, namely, 9*S*, 10*S* or 9*R*, 10*R*, are possible for compound **1**, and this was also supported by the NOESY correlations between H-9 and H-11. However, the NOESY data did not distinguish between the two configuration sets. The correct stereochemistry of the epoxy ring at C-9 and C-10 and the absolute configuration will require an X-ray crystal structure determination of a suitable derivative for unequivocal assignments. However, the few milligrams isolated were not useful for this purpose. Thus, the structure of plakorstatin 1, except for the relative configuration of the epoxide, was assigned as shown in Figure 1.

Plakorstatin 2 was also isolated as a colorless oil, $[\alpha]_D^{25}$ −220° (*c* 0.1 $CH_3OH$), $R_f$ 0.41 (TLC 9:1 hexane−EtOAc), and its molecular formula was found to correspond to $C_{19}H_{32}O_5$ by HRAPCIMS and to be isomeric with plakorstatin 1. The $^1H$ and $^{13}C$ NMR spectra exhibited by plakorstatin 2 were almost identical with those of peroxide **1** (Table 1). The relative configuration was inspected by using the NOESY spectrum, which showed the same correlations as already described above for peroxide **1**. The epoxide ring was also confirmed as *trans* by the coupling constant between H-9 and H-10 ($J = 2.0$ Hz) in the $^1H$ NMR spectrum and suggested that the relative configuration of the epoxide ring was again 9*S*, 10*S*, or 9*R*, 10*R*. The NOESY spectrum showed the same correlations around the epoxide ring as with those of plakorstatin 1. However, the epoxide chirality was found to be definitely opposite in isomers **1** and **2**. That observation was further supported by different $[\alpha]_D$ and $R_f$ values (on TLC). Therefore, plakorstatins 1 and 2 differ by having the opposite configuration at the epoxide ring.

**Table 2.** Cancer Cell Growth Inhibitory Activity ($ED_{50}$ values for P388 and $GI_{50}$ values for other cancer cell lines) for Plakorstatins 1 and 2

| | cancer cell lines[a] | | | | | | |
|---|---|---|---|---|---|---|---|
| compd | P388 | BXPC3 | MCF7 | SF268 | NCIH460 | KM20L2 | DU145 |
| **1** | 1.1 | >10 | >10 | 1.8 | >10 | >10 | >10 |
| **2** | 0.91 | 6.4 | 3.8 | 1.6 | >10 | 6.7 | 1.7 |

[a] Cancer cell type: P388 (lymphocytic leukemia); BXPC3 (pancreas adenocalcinoma); MCF7 (breast adenocarcinoma); SF268 (CNS glioblastoma); NCIH460 (lung large cell); KM20L2 (colon adenocarcinoma); DU145 (prostate carcinoma).

Plakorstatins 1 and 2 represent the first isolation of plakortides with an epoxy moiety in the side chain which have been evaluated for cancer cell growth inhibition against murine P388 lymphocytic leukemia and a minipanel of human tumor cell lines. Both plakorstatins 1 and 2 exhibited moderate inhibition especially against the P388 and CNS glioblastoma SF268 cell lines, as shown in Table 2. Even though they have the same planar structure and very close overall configuration, they showed different inhibition values against breast adenocarcinoma MCF7 and prostate carcinoma DU145. Only plakorstatin 2 exhibited significant inhibition against both of these human cancer cell lines. Clearly the chirality of the epoxide is very important and a good clue for future SAR research.

## Experimental Section

**General Experimental Procedures.** Solvents used for extraction and isolation were redistilled. Silica gel Uniplates for TLC were developed in hexane−EtOAc (9:1) and were visualized by spraying with 3% ceric acid in 3 N sulfuric acid reagent and heated at approximately 150 °C. Sephadex LH-20 was obtained from Pharmacia Fine Chemicals AB. MPLC was carried out using a 310 mm × 25 mm i.d. Lobar LiChroprep Si60 (40−63 $\mu$m) column. HPLC was performed with a 250 mm × 9.4 mm i.d. Agilent Zorbax column packed with a 5 $\mu$m C-18. The optical rotation values were measured using a Perkin-Elmer 241. High-resolution mass spectra were obtained with a JEOL LC-Mate equipped with an APCI inlet. NMR experiments were conducted with INOVA-400 or -500 spectrometers with tetramethylsilane as an internal reference.

***Plakortis nigra* Levi (1959).** The marine sponge *P. nigra* was collected using scuba equipment in the summer of 1995 in the Celebes Sea (at 7−40 m) northwest of Tanjung Batuanguf, Sulawesi, Indonesia. About 1 kg of the sponge was preserved in methanol−water and shipped by air to our Institute. *P. nigra* is grouped in the subclass Homoscleromorpha and family Plakinidae. The taxonomic identification was completed by one of us (J.N.A.H.), and a voucher specimen is maintained in the Queensland Museum.

**Collection, Extraction, and Isolation Methods.** All the separations were guided by bioassay results using the murine P388 lymphocytic leukemia and a minipanel of human cancer cell lines. *Plakortis nigra* was extracted with $CH_2Cl_2$−$CH_3$-

OH (1:1). The solvent was evaporated under reduced pressure to obtain the residue (11.23 g), which was dissolved in 1 L of $CH_3OH-H_2O$ (9:1) and extracted with hexane (3 × 1 L). Removal of solvent from the hexane extract gave 4.57 g (PS $ED_{50}$ 2.0 $\mu$g/mL) of a dark brown oily residue, which was chromatographed on Sephadex LH-20 columns successively using $CH_2Cl_2-CH_3OH$ (3:2) and hexane−*i*-PrOH−$CH_3OH$ (8:1:1) solvent systems. The active (PS $ED_{50}$ 1.6 $\mu$g/mL) fraction was subjected to an MPLC on Si gel with hexane−EtOAc (97.5:2.5) as eluent to provide four fractions. The PS active ($ED_{50}$ 1.2 $\mu$g/mL) fraction was again chromatographed on a Sephadex LH-20 column with hexane−EtOAc−$CH_3OH$ (8:1:1) as eluent, and the most active (PS $ED_{50}$ 0.36 $\mu$g/mL) fraction was separated on a $C_{18}$ HPLC column monitored by UV at 235 nm with $CH_3CN-H_2O$ (6:4) as mobile phase. Finally, the next active (PS $ED_{50}$ 1.9 $\mu$g/mL) fraction (at 26.7 min retention time, 4.0 mL/min) on HPLC was subjected to MPLC on Si gel with hexane−EtOAc (95:5) and provided pure plakorstatins 1 (3.6 mg) and 2 (3.3 mg).

**Plakorstatin 1:** colorless oil; $[\alpha]_D^{25}$ −177° (*c* 0.1, $CH_3OH$); TLC $R_f$ 0.43 with hexane−EtOAc (9:1); $^1H$ and $^{13}C$ NMR spectra, Table 1; HRMS (APCI$^+$ *m*/*z* 341.2251 $[M + H]^+$ (calcd for $C_{19}H_{33}O_5$, 341.2328).

**Plakorstatin 2:** colorless oil; $[\alpha]_D^{25}$ −220° (*c* 0.1, $CH_3OH$); TLC $R_f$ 0.41 using hexane−EtOAc (9:1); $^1H$ and $^{13}C$ NMR spectra, Table 1; HRMS (APCI$^+$) *m*/*z* 341.2326 $[M + H]^+$ (calcd for $C_{19}H_{33}O_5$, 341.2328).

**Acknowledgment.** The very important financial support (greatly appreciated) was provided by Outstanding Investigator Grant CA44344-06-12 and Grant RO1 CA90441-01-03 with the Division of Cancer Treatment and Diagnosis, NCI, DHHS; Dr. Alec D. Keith; the Fannie E. Rippel Foundation; the Arizona Disease Control Research Commission; the Robert B. Dalton Endowment Fund; Dr. Alec Keith; Diane and Bruce Halle; Jack W. and Carol D. Whiteman; Gary L. and Diane R. Tooker; Dr. John C. Budzinski; and Polly Trautman. We also thank the Government of Indonesia; Mr. Makmur, Dr. H. M. K. Moosa, and Ms. D. Soenarijadi, the Indonesian Institute of Sciences (LIPI), Oceanology Division; Profs. F. Hogan and J. M. Schmidt; Drs. J.-C. Chapuis, E. Hamel, V. J. R. V. Mukku, and R. Nieman; and F. Craciunescu, L. Tackett, D. Nielsen-Tackett, C. Weber, and L. Williams for other helpful assistance.

## References and Notes

(1) For part 534, refer to: Pettit, G. R.; Zhang, Q.; Pinilla, V.; Hoffmann, H.; Doubek, D. L.; Chapuis, J.-C.; Pettit, R. K.; Schmidt. *J. Nat. Prod*., in preparation.

(2) (a) Zampella, A.; Giannini, C.; Debitus, C.; D'Auria, M. V. *Tetrahedron* **2001**, *57*, 257−263. (b) Cafieri, F.; Fattorusso, E.; Taglialatela-Scafati, O.; Ianaro, A. *Tetrahedron* **1999**, *55*, 7045−7056. (c) Cafieri, F.; Fattorusso, E.; Taglialatela-Scafati, O.; Di Rosa, M.; Ianaro, A. *Tetrahedron* **1999**, *55*, 13831−13840.

(3) (a) Rudi, A.; Afanii, R.; Gravalos, L. G.; Aknin, M.; Gaydou, E.; Vacelet, J.; Kashman, Y. *J. Nat. Prod*. **2003**, *66*, 682−685. (b) Sandler, J. S.; Colin, P. L.; Hooper, J. N. A.; Faulkner, D. J. *J. N. Nat. Prod*. **2002**, *65*, 1258−1261. (c) Fattorusso, E.; Taglialatela, S. O.; DiRosa, M.; Ianaro, A. *Tetrahedron* **2000**, *56*, 7959−7967. (d) Fontana, A.; Ishibashi, M.; Kobayashi, J. *Tetrahedron* **1998**, *54*, 2041−2048. (e) Ichiba, T.; Scheuer, P. J.; Kelly-Borges, M. *Tetrahedron* **1995**, 45, 12195−12202. (f) Varoglu, M.; Peters, B. M.; Crews, P. *J. Nat. Prod*. **1995**, *58*, 27−36. (g) Rudi, A.; Kashman, Y. *J. Nat. Prod*. **1993**, *56*, 1827−1830. (h) Kobayashi, J.; Takeuchi, S.; Ishibashi, M.; Shigemori, H.; Sasaki, T. *Tetrahedron Lett*. **1992**, *33*, 2579−2580. (i) Davidson, B. S. *J. Org. Chem*. **1991**, *56*, 6722−6724. (j) Davidson, B. S. *Tetrahedron Lett*. **1991**, *32*, 7167−7170. (k) Sakemi, S.; Higa, T.; Anthoni, U.; Christophersen, C. *Tetrahedron* **1987**, *43*, 263−268.

(4) (a) Costantino, V.; Fattorusso, E.; Mangoni, A.; Di-Rosa, M.; Ianaro, A. *Tetrahedron* **2000**, *56*, 1393−1395. (b) Costantino, V.; Fattorusso, E.; Mangoni, A.; Di-Rosa, M.; Ianaro, A. *Bioorg. Med. Chem. Lett*. **1999**, *9*, 271−276. (c) Carballeira, N. M.; Shalabi, F. *Lipids* **1990**, *25*, 835−840.

(5) Campagnuolo, C.; Fattorusso, C.; Fattorusso, E.; Ianaro, A.; Pisano, B.; Taglialatela-Scafati, O. *Org. Lett*. **2003**, *5*, 673−676.

(6) Yao, G.; Steliou, K. *Org. Lett*. **2002**, *4*, 485−488.

(7) Hayes, P. Y.; Kitching, W. *J. Am. Chem. Soc*. **2002**, *124*, 9718−9719.

(8) Jung, M.; Ham, J.; Song, J. *Org. Lett*. **2002**, *4*, 2763−2765.

(9) Miyaoka, H.; Watanuki, T.; Saka, Y.; Yamada, Y. *Tetrahedron* **1995**, *51*, 8749−8756.

(10) (a) del Sol Jiménez, M.; Garzón, S. P.; Rodriguez, A. D. *J. Nat. Prod*. **2003**, *66*, 655−661. (b) Hu, J.-F.; Goa, H.-F.; Kelly, M. *Tetrahedron* **2001**, *57*, 9379−9383. (c) Harrison, B.; Crews, P. *J. Nat. Prod*. **1998**, *61*, 1033−1037.

(11) (a) Gochfeld, D. J.; Hamann, M. T. *J. Nat. Prod*. **2001**, *64*, 1477−1479. (b) Chen, Y.; McCarthy, P. J.; Harmody, D. K.; Schimoler-O'Rourke, R.; Chilson, K.; Selitrennikoff, C.; Pomponi, S. A.; Wright, A. E. *J. Nat. Prod*. **2002**, *65*, 1509−1512.

NP040043J