

Isolation and structure of a 357 nm UV-absorbing substance, usujirene, from the red alga *Palmaria palmata* (L.) O. KUNTZE

Isao SEKIKAWA*, Chiharu KUBOTA**, Toshifumi HIRAOKI***
and Isami TSUJINO**¹⁾

* Institute of Immunological Science, Hokkaido University, Sapporo, 060 Japan

** Department of Chemistry, Faculty of Fisheries, Hokkaido University, Hakodate, 041 Japan

*** Department of Polymer Science, Faculty of Science, Hokkaido University, Sapporo, 060 Japan

SEKIKAWA, I., KUBOTA, C., HIRAOKI, T. and TSUJINO, I. 1986. Isolation and structure of a 357 nm UV-absorbing substance, usujirene, from the red alga *Palmaria palmata* (L.) O. KUNTZE. Jap. J. Phycol. 34: 185-188.

Usujirene, a new mycosporine-like amino acid, was isolated from the red alga *Palmaria palmata*. This compound shows a UV-absorption maximum at 357 nm and its structure is determined to be 2.

Key Index Words: *Palmaria palmata*; *Rhodophyta*; *UV-absorbing substance*.

It is well known that compounds having a strong absorption maxima from 310 to 360 nm are present in many marine plants and animals, as reviewed by TSUJINO in 1983. In a previous paper, we described the isolation of shinorine (λ_{\max} 333-334 nm) from the red alga *Chondrus yendoi* and elucidated its structure (TSUJINO *et al.* 1980a). In 1978, TAKANO *et al.* isolated a mycosporine-like amino acid (λ_{\max} 360 nm) from the sea anemone *Palythoa tuberculosa* and named it palythene 1. The stereochemistry of the amino propene moiety of 1 is *trans*-form. In this communication, we will report the isolation of a new mycosporine-like amino acid (λ_{\max} 357 nm) usujirene 2 from the red alga *Palmaria palmata* (L.) O. KUNTZE the stereochemistry of which is *cis*-form.

Materials and Methods

The fronds of *P. palmata* were collected in February at Usujiri, Minami-Kayabe, Hok-

kaido, Japan. They were carefully freed of any foreign matter, and immediately frozen at -18°C . The frozen thalli were immersed in an equal weight of ethanol. After being left for several days with occasional shakes, the mixture was centrifuged. The supernatant was evaporated under reduced pressure at temperatures below 40°C to one-twentieth of its original volume. The resulting slurry was treated with methanol by a method described previously (TSUJINO *et al.* 1980a, 1980b). The aqueous layer was applied to a column of silica gel (Wakogel C-100, Wako Junyaku Co.). The column was first washed with water and then eluted with 50% methanol. The fractions containing a 360 nm UV-absorbing substance were combined and concentrated to dryness under the same condition as the above, dissolved in water and subjected to the second column chromatography similar to the above, using water as the eluent.

Preparative paper chromatography on Whatman No. 3MM paper was employed for purification using solvent A (isopropanol: water=8:2, v/v, multiple ascent) as a

¹⁾ To whom correspondence should be addressed.

solvent system. The areas including UV-absorbing materials were cut and the paper strips were eluted with 80% ethanol. The eluate was concentrated similarly and the residue was dissolved in ethanol. Crude usujirene was precipitated by adding ether to the ethanolic solution.

The same preparative paper chromatography was carried out for further purification, but using solvent B (CHCl_3 : MeOH : water = 35 : 65 : 40, v/v, multiple ascent) as developer. Two UV-absorbing substances were detected on the paper chromatogram. The faster moving substance was extracted with water and lyophilized. The resulting residue was dissolved in ethanol. By adding ether to the ethanolic solution, a refined usujirene preparation was obtained.

The melting point of the purified usujirene was determined on a Shimadzu micromelting apparatus Type MM-2 and was uncorrected. Optical rotation was measured with a Union model PM-101 Automatic Digital Polarimeter. UV-spectra were recorded on a Hitachi 124 Spectrometer. FD-HR-MS spectrometry was performed on a JEOL 01SG-2 spectrometer. Proton NMR spectra were taken in D_2O on JEOL FX-500 and Varian XL-200 spectrometers using DSS as an internal standard. Proton-decoupled and off-resonance decoupling ^{13}C NMR spectra were obtained with a JEOL FX-100 spectrometer using acetone (30.4 ppm) as an internal standard. Paper chromatographic identification was carried out with Toyo No. 50 paper employing the solvent system of A, B (for UV-absorbing materials) and C (pyridine : water = 65 : 35, v/v, for glycine). Further examination of the UV-absorbing materials was performed according to a procedure described previously (TSUJINO *et al.* 1979).

Results and Discussion

Usujirene was obtained as an amorphous powder by the repeated precipitation from ethanol by the addition of ether. It exhibited the following physical constants: mp. 129-

132° (dec.), $[\alpha]_D^{25} - 41.3^\circ$ ($c = 1.79$, H_2O) and UV λ_{max} 357 nm (H_2O). Its molecular formula was $\text{C}_{13}\text{H}_{20}\text{N}_2\text{O}_5$ by FD-HR-MS [Found: m/z 285.1448 (MH^+). Calcd: 285.1449] and ^{13}C NMR methods. The ^1H NMR spectra of **2** consisted of two AB patterns due to methylene protons δ 2.81, 2.93 (each 1H, AB_q, $J = 17.4$ Hz), 2.86, 2.89 (each 1H, AB_q, $J = 13.1$ Hz) and three sharp singlets at 3.59 (2H, $-\text{CH}_2-\text{O}-$), 3.70 (3H, $-\text{OCH}_3$) and 4.07 (2H, $>\text{N}-\text{CH}_2-$) ppm. These signals were similar to those of **1**. The signals of olefinic protons were observed at 5.38 (1H, dq, $J = 7.0, 7.0$ Hz) and 6.38 (1H, br. d, $J = 7.0$ Hz) coupled with methyl proton at 1.76 (3H, dd, $J = 1.5, 7.0$ Hz), which obviously indicated the presence of a group, $>\text{N}-\text{CH}=\text{CH}-\text{CH}_3$, in **2**.

The presence of the amino propene moiety was further supported by the catalytic hydrogenation of **2** with palladium black in methanol. The resulting dihydrousujirene [FD-MS: m/z 287 (MH^+)] showed ^1H NMR spectra at δ 0.95 (3H, t, $J = 7.6$ Hz), 1.66 (2H, sextet, $J = 7.6$ Hz) and 3.42 (2H, t, $J = 7.6$ Hz) ppm due to $>\text{N}-\text{CH}_2-\text{CH}_2-\text{CH}_3$. Dihydrousujirene was identified as dihydropalythene by comparing the ^1H NMR and ^{13}C NMR spectra (TAKANO *et al.* 1978). The stereochemistry of the amino propene moiety of **2** was readily determined by ^1H NMR measurement, that is, the coupling constant of two olefinic protons (7.0 Hz) clearly indicated that **2** has a *cis*-form (SAUER und PRAHL 1966). The ^{13}C NMR data are summarized in Table 1.

Furthermore, usujirene yielded glycine by treating with ammonium hydroxide, and palythene (TSUJINO *et al.* 1978) by treating with dilute hydrochloric acid. Based on these results, the structure of usujirene was established to be **2**.

Beside usujirene, palythene **1** was also obtained from the same extract by preparative paper chromatography (solvent B, slower moving substance). Its analytical data (^1H NMR and ^{13}C NMR) were found to be identical to those reported previously for palythene (TAKANO *et al.* 1978). The yields of

Table 1. ^{13}C NMR shifts^a of 1 and 2 in D_2O .

	1		2	
C- 1	161.5 ^c	s ^b	161.9 ^d	s ^b
C- 2	126.4	s	126.2	s
C- 3	154.2 ^c	s	154.4 ^d	s
C- 4	33.8	t	33.3	t
C- 5	71.8	s	71.2	s
C- 6	33.8	t	33.3	t
C- 7	68.4	t	67.7	t
C- 8	60.3	q	59.5	q
C- 9	47.6	t	47.0	t
C-10	175.4	s	174.0	s
C-11	124.5	d	122.1	d
C-12	117.9	d	116.5	d
C-13	15.2	q	14.4	q

a) δ in ppm. Internal standard (1, dioxane; 2, acetone).

b) Multiplicity in the off-resonance decoupled spectrum.

c, d) Each assignment may be exchanged.

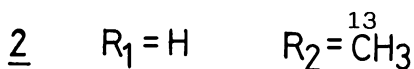
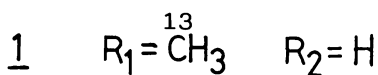
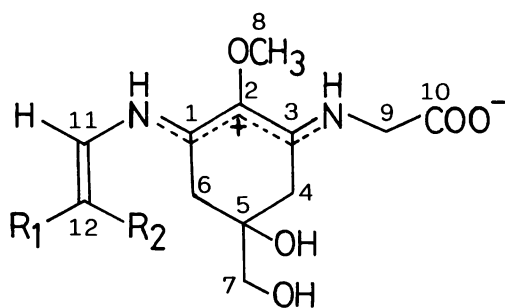


Fig. 1. Structures of palythene (1) and usujirene (2).

1 and 2 compounds were 0.0007% and 0.0029%, respectively. These formulas are biogenetically plausible if it is derived from

porphyra-334 (TAKANO *et al.* 1979) through decarboxylation followed by dehydration.

The physiological roles, the distribution and the composition of mycosporine-like amino acids in marine organism are under investigation.

Acknowledgements

We wish to thank Prof. T. MASAKI for valuable comments on the manuscript and to Dr. K. WATANABE for FD-HR-MS analysis.

References

- SAUER, J. und PRAHL, H. 1966. Isomerisierungen von N,N-Dialkylallylaminen mit Basen zu *cis*-Enaminen. *Tetrahedron Lett.* 2863-2866.
- TAKANO, S., UEMURA, D. and HIRATA, Y. 1978. Isolation and structure of two new amino acids, palythanol and palythene, from the zoanthid *Palythoa tuberculosa*. *Tetrahedron Lett.* 4909-4912.
- TAKANO, S., NAKANISHI, A., UEMURA, D. and HIRATA, Y. 1979. Isolation and structure of a 334 nm UV-absorbing substance, porphyra-334 from the red alga *Porphyra tenera* KJELLMAN. *Chemistry Lett.* 419-420.
- TSUJINO, I., YABE, K., SEKIKAWA, I. and HAMANAKA, N. 1978. Isolation and structure of a mycosporine from the red alga *Chondrus yendoi*. *Tetrahedron Lett.* 1401-1402.
- TSUJINO, I., YABE, K. and SAKURAI, M. 1979. Presence of the near 358 nm UV-absorbing substances in red algae. *Bull. Fac. Fish. Hokkaido Univ.* 30: 100-108. (In Japanese)
- TSUJINO, I., YABE, K. and SEKIKAWA, I. 1980a. Isolation and structure of a new amino acid, shinorine, from the red alga *Chondrus yendoi* YAMADA et MIKAMI. *Bot. Mar.* 23: 65-68.
- TSUJINO, I. and YABE, K. 1980b. Purification and crystallization of a 320 nm absorbing substance from the red alga, *Chondrus yendoi*. *Bull. Japanese Soc. Sci. Fish.* 46: 1113-1115. (In Japanese)
- TSUJINO, I. 1983. UV-absorbing compounds. p. 78-89. *In Japanese Soc. Sci. Fish. [ed.] Biochemistry and Utilization of Marine Algae.* Koseisha-Koseikaku, Tokyo. (In Japanese)

関川 勲*・久保田千春**・平沖敏文***・辻野 勇**：紅藻ダルス (*Palmaria palmata*
(L.) O. KUNTZE) の 357 nm 紫外線吸収物質ウスジレンの単離と構造

白尻産ダルスの近紫外線吸収物質について研究した。シリカゲルクロマトグラフィーにより 360 nm 吸収区分を集め、更に調製用紙クロマトグラフィーにより精製し、2種の化合物 (1, 2) を分離した。核磁気共鳴スペクトル、質量スペクトルにより研究し、1はパリセン (palythene)、2は新化合物でパリセンのシス型であることを決定し、2をウスジレン (usujirene) と命名した。(*060 札幌市北区北15西7 北海道大学免疫科学研究所 **041 函館市港町 3-1-1 北海道大学水産学部水産化学科 *** 060 札幌市北区北10西8 北海道大学理学部高分子学科)

News

International Society for Diatom Research

This new Society welcomes all diatomists as members. The first part of the journal "Diatom Research" (152 pages) is published and Part 2 will appear shortly. Volume 2 (Parts 1 and 2) will appear in 1987. The subscription due now for 1986 is £20 (\$30). The subscription for 1987 is due on January 1st 1987.

Please send following subscription form to Miss Patricia A. Sims, Botany Department, British Museum (Natural History), Cromwell Road, London SW7 5BD, U. K.

Please include me as a member of the International Society for Diatom Research. I enclose my initial subscription of £20 (or dollar equivalent).

Signed

Address

.....
.....