## Communications to the Editor

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# KERICEMBRENOLIDES A, B, C, D, AND E, FIVE NEW CYTOTOXIC CEMBRENOLIDES FROM THE OKINAWAN SOFT CORAL *CLAVULARIA KOELLIKERI*

Motomasa Kobayashi,[a] Byeng Wha Son,[a] Yoshimasa Kyogoku,[b] and Isao Kitagawa*,[a]

Faculty of Pharmaceutical Sciences, Osaka University,[a] 1-6, Yamada-oka, Suita, Osaka 565, Japan and Institute for Protein Research, Osaka University,[b] 3-2, Yamada-oka, Suita, Osaka 565, Japan

Five new cytotoxic cembrenolides, kericembrenolides A (7), B (8), C (9), D (10), and E (11), having an α-methylene-γ-lactone moiety were isolated together with neodolabelline (5) and neodolabellenol (6) from the Okinawan soft coral *Clavularia koellikeri*. Their absolute configurations were determined on the basis of chemical and physicochemical evidence.

KEYWORDS —— *Clavularia koellikeri*; soft coral; stolonifer; kericembrenolide A; kericembrenolide B; kericembrenolide C; kericembrenolide D; kericembrenolide E; cembrane diterpene; cytotoxic activity

As part of our continuing studies in search of marine bioactive substances,[1] we have investigated for several years the chemical constituents of the stoloniferan soft coral *Clavularia koellikeri* collected at Kohama-jima, Okinawa Prefecture. We have elucidated so far the absolute stereostructures of trinorsesquiterpenes [clavukerins A (1),[2] B (2),[3] and C (3)[3]], (-)-bicyclogermacrene (4),[4] and methyl-migrated dolabellane diterpene neodolabelline (5).[5] In the course of these investigations, we have found that the composition of these chemical constituents varies depending upon the years of collection although the soft coral was collected at the same waters of Kohama-jima in the same season (July). From the soft coral collected in 1984, we have isolated five new cytotoxic cembrenolides named kericembrenolides A (7), B (8), C (9), D (10), and E (11), together with neodolabelline (5) and neodolabellenol (6).[6] This paper deals with the absolute stereostructure elucidation of these cembrenolides having an α-methylene-γ-lactone moiety.

The acetone extract of the fresh soft coral was partitioned into an AcOEt-$H_2O$ mixture and the AcOEt-soluble portion was subjected to silica gel column chromatography (*n*-hexane-AcOEt) and HPLC (Zorbax ODS, MeOH-$H_2O$) to afford neodolabelline (5), neodolabellenol (6), kericembrenolides A (7), B (8), C (9), D (10), and E (11) (all as colorless oils)[0.7, 1.7, 1.3, 0.8, 1.5, 3.5, and 0.8% respectively from the AcOEt soluble portion]. In the 1984 specimen, the other terpenoid constituents were found only in trace amounts as judged by the TLC examination.

Kericembrenolide D (10), $C_{22}H_{30}O_5$,[7] $[\alpha]_D^{22}$ -71° ($CHCl_3$), was shown by its

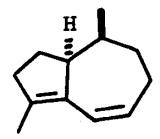
IR spectrum to have hydroxyl [3600, 3435 (br) $cm^{-1}$], acetoxyl [1760 (br), 1250 (br) $cm^{-1}$], and α-methylene-γ-lactone [1723 (br), 1660 $cm^{-1}$] groups. The $^1H$ NMR spectrum (500 MHz, $CDCl_3$) of 10 showed signals assignable to three olefinic protons (δ 5.02, 1H d, J=9.0 Hz, 3-H; δ 5.09, 1H d, J=9.0 Hz, 7-H; δ 4.99, 1H br s, 11-H), three olefinic methyls (δ 1.63, 6H s & 1.86, 3H s, 4,8,12-$CH_3$), one α-methylene-γ-lactone moiety(δ 5.61, 6.33, both 1H d, J=2.0 Hz, 16-$H_2$; δ 5.36, 1H dd, J=9.0, 3.5 Hz, 2-H), one acetoxyl (δ 2.03, 3H s, 6-OAc), one proton geminal to an acetoxyl (δ 5.63, 1H ddd, J=9.5, 9.0, 3.0 Hz, 6-H), and one proton geminal to a hydroxyl (δ 4.07, 1H br dd, J=11.5, 3.0 Hz, 14-H). The $^{13}C$ NMR data for 10 have been assigned as shown in Table I.

Ozone oxidation of 10 liberated laevulinaldehyde and 2-acetoxylaevulinaldehyde, whereas pyridinium chlorochromate (PCC) oxidation of 10 afforded the cross-conjugated enone (12), colorless oil, $C_{22}H_{28}O_5$, λmax 237 nm (ε 9000), δ 5.90 (1H dd, J=9.0, 2.0 Hz, 2-H), 3.17, 3.50 (1H each ABq, J=15.0 Hz, 13-$H_2$), 2.11 (3H d, J=2.0 Hz, 15-$CH_3$), and the ketone (13), colorless oil, $C_{22}H_{28}O_5$, δ 3.13, 3.22 (1H each ABq, J=13.0 Hz, 13-$H_2$). Deacetylation (1% KOH-MeOH) and subsequent PCC oxidation of 10 provided the keto-enone (14), colorless oil, $C_{21}H_{28}O_5$, λmax 243 nm (ε 8000), 202 (12000), δ 2.89, 3.15 (1H each ABq, J=13.0 Hz, 5-$H_2$), 3.42 (3H s, 16-$OCH_3$). Detailed $^1H$ NMR decoupling experiments with 12, 13, and 14 and comparison of the $^1H$ and $^{13}C$ NMR data for 10 with those reported for 15 (1β-H, 14β-H) obtained from the Australian soft coral *Cespitularia* sp.,[8] have led us to assume a resembled structure of kericembrenolide D (10) to 15. The relative configuration of 10 was determined by NOE experiments in addition to the above findings. NOEs were observed between the following proton pairs among which $J_{1,2}$=3.5 Hz:[9] 1α-H & 3-H (6%), 1α-H & 14α-H (4%), 3-H & 1α-H (5%), 3-H & 6α-H (4%), 6α-H & 3-H (5%), 14α-H & 1α-H (6%), 4-$CH_3$ & 2β-H (8%).

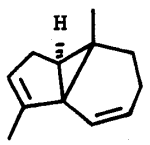
The $^{13}C$ NMR data for kericembrenolide A (7), $C_{22}H_{30}O_4$, $[\alpha]_D^{22}$ -88° ($CHCl_3$), B (8), $C_{22}H_{30}O_4$, $[\alpha]_D^{22}$ +97° ($CHCl_3$), C (9), $C_{24}H_{32}O_6$, $[\alpha]_D^{22}$ -56° ($CHCl_3$), and E (11), $C_{20}H_{28}O_4$, $[\alpha]_D^{21}$ -53° ($CHCl_3$), were assigned as shown in Table I. It became clear that all kericembrenolides (A, B, C, D, and E) have a common 3,7,11,15-tetraen-17,2-olide structure in their cembrenolide skeletons but differ from each other in their oxygen functions. The $^{13}C$ NMR signals of olefinic methyls were always observed at a higher field (Table I), so that their *E* geometries were proved.[10] Respective acetylation of kericembrenolides D (10) and E (11) furnished kericembrenolide C (9), while deacetylation of 9 gave 10 and 11 as minor products.

Reduction of kericembrenolide A (7) with diisobutylaluminum hydride (DIBAL) and subsequent $NaBH_4$ reduction of the product furnished the triol (16), $[\alpha]_D^{29}$ -9° ($CHCl_3$), δ 4.24 (1H dd, J=10.0, 9.0 Hz, 2-H), 4.86 (1H d, J=9.0 Hz, 3-H), 4.03, 4.16 (1H each ABq, J=12.5 Hz, 17-$H_2$). The identical triol (16) (including the specific rotation) was obtained from kericembrenolide D (10) by mesylation in the presence of $Et_3N$ and subsequent reduction with DIBAL and $NaBH_4$.

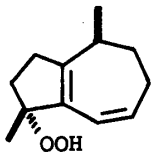
Deacetylation followed by PCC oxidation of kericembrenolide B (8) yielded the enone (17), $C_{21}H_{30}O_4$, λmax 233 nm (ε 8000), δ 6.41 (1H dd-like, J=*ca.* 6.5, 6.0 Hz, 7-H), 3.49, 3.21 (1H each, AB in ABX, $J_{AB}$=14.0, $J_{AX}$=9.0, $J_{BX}$=6.0 Hz, 10-$H_2$), 5.11 (1H, X in ABX, dd, J=9.0, 6.0 Hz, 11-H). On the other hand, deacetylation of 8 and subsequent epoxidation of the product with *t*-BuOOH in the presence of vanadium (IV) oxyacetylacetonate[11] provided the 7,8-epoxy-9-hydroxy derivative (18),



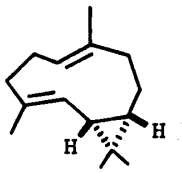
clavukerin A (1)



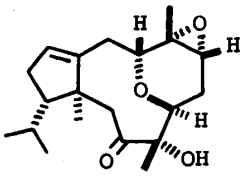
clavukerin B (2)



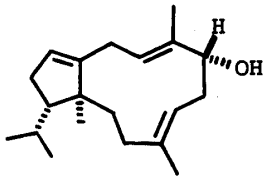
clavukerin C (3)



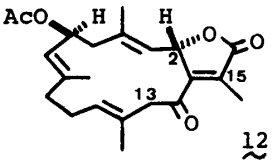
(-)-bicyclogermacrene (4)



neodolabelline (5)



neodolabellenol (6)



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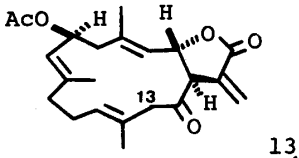

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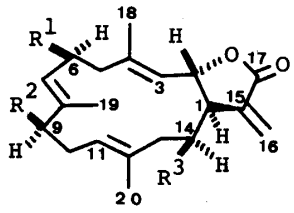
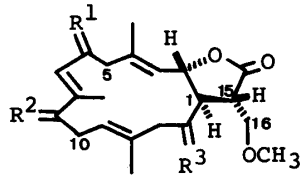
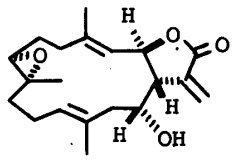


| | | $R^1$ | $R^2$ | $R^3$ |
|---|---|---|---|---|
| kericembrenolide A | (7) | OAc | H | H |
| " B | (8) | H | OAc | H |
| " C | (9) | OAc | H | OAc |
| " D | (10) | OAc | H | OH |
| " E | (11) | OH | H | OH |



14: $R^1=R^3=O$, $R^2=H_2$
17: $R^1=R^3=H_2$, $R^2=O$



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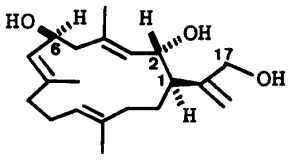
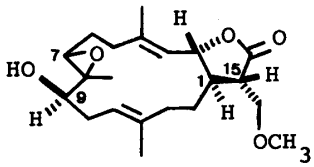
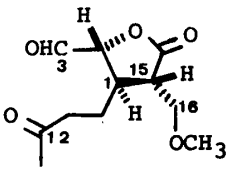


16



18



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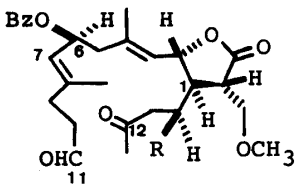

20: R=OBz
21: R=H

Table I. $^{13}C$ NMR Data[a)]

| Carbon | 7 | 8 | 9 | 10 | 11 |
|---|---|---|---|---|---|
| 1 | 42.7 | 43.1 | 45.7 | 47.5 | 47.6 |
| 2 | 79.2[b)] | 79.6[b)] | 73.5[b)] | 73.5[b)] | 73.8[b)] |
| 3 | 124.5[b)] | 124.5[b)] | 124.8[b)] | 124.8[b)] | 123.8[b)] |
| 4 | 139.1[c)] | 139.4[c)] | 136.8 | 138.7 | 138.3 |
| 5 | 42.7 | 38.5 | 42.7 | 42.6 | 45.6[d)] |
| 6 | 69.6 | 23.8 | 69.4 | 69.7 | 66.7 |
| 7 | 124.3[b)] | 121.4[b)] | 124.6[b)] | 124.8[b)] | 129.3[b)] |
| 8 | 140.6[c)] | 134.6[c)] | 140.3[c)] | 140.5[c)] | 140.8[c)] |
| 9 | 38.6 | 78.4 | 38.3 | 38.4 | 38.6 |
| 10 | 23.7 | 29.8 | 23.4 | 23.4 | 23.5 |
| 11 | 125.7[b)] | 130.3[b)] | 128.4[b)] | 127.6[b)] | 128.0[b)] |
| 12 | 132.2 | 131.5 | 129.7 | 130.7 | 130.5 |

| Carbon | 7 | 8 | 9 | 10 | 11 |
|---|---|---|---|---|---|
| 13 | 35.8[d)] | 36.4[d)] | 42.1 | 45.4 | 45.4[d)] |
| 14 | 32.1[d)] | 32.7[d)] | 74.1[c)] | 72.8 | 72.8[c)] |
| 15 | 140.8[c)] | 140.3[c)] | 139.7[c)] | 139.2[c)] | 139.0[c)] |
| 16 | 121.2[e)] | 122:1 | 122.9[e)] | 122.4 | 122.3 |
| 17 | 169.6[f)] | 170.2[f)] | 169.9[f)] | 170.5[f)] | 170.6[f)] |
| 18 | 15.6[f)] | 15.4[f)] | 15.6[f)] | 15.6[f)] | 15.4[f)] |
| 19 | 19.3[f)] | 10.9[f)] | 19.2[f)] | 19.3[f)] | 19.7[f)] |
| 20 | 15.8[e)] | 15.4[f)] | 15.7[f)] | 15.9[f)] | 16.0[f)] |
| $CH_3CO$- | 170.1[e)] | 170.2 | 169.8[e)] | 170.1 | |
| | 21.3 | 21.3 | 169.4[e)] | 21.2 | |
| | | | 20.8 | | |
| | | | 21.1 | | |

a) Measured at 22.5 MHz in $CDCl_3$. b~f) Assignments may be interchangeable within the same column.

$C_{21}H_{32}O_5$, δ 3.71 (1H d-like, 9-H), 1.05 (3H s, 8-$CH_3$). Detailed $^1H$ NMR decoupling experiments with 17 and 18 revealed the presence of the 9-OAc function in 8. Furthermore, the keto-aldehyde (19), $C_{11}H_{16}O_5$, $[\alpha]_D^{18}$ -5.0° ($CHCl_3$), δ 4.32 (1H dd, J= 6.0, 1.5 Hz, 2-H), 9.63 (1H d, J=1.5 Hz, 3-H), 2.46 (1H, X in ABX, ddd, J=6.5, 3.5, 3.0 Hz, 15-H), 3.56, 3.75 (1H each, AB in ABX, $J_{AB}$=9.0, $J_{AX}$=3.5, $J_{BX}$=3.0 Hz, 16-$H_2$), 2.18 (3H s, 12-$CH_3$), was obtained by ozone oxidation of 18 and also from kericembrenolide A (7) by deacetylation and subsequent ozone oxidation. The *trans* configuration between 1-H and 15-H in 19 was evidenced by the coupling constants of $J_{1,2}$=6.0 Hz and $J_{1,15}$=6.5 Hz.

Based on the above evidence, kericembrenolides A (7), B (8), C (9), and E (11) were determined respectively to have 6-acetoxy-, 9-acetoxy-, 6,14-diacetoxy-, and 6,14-dihydroxy-cembra-3*E*,7*E*,11*E*,15-tetraen-17,2-olide structures.

The absolute configurations of these cembrenolides were determined by applying Horeau's method [12] to kericembrenolide D (10) and 18 to show the 14*S* and 9*R* configurations: $[\alpha]_D$ of recovered α-phenylbutyric acid were -13° from 10 and +2.5° from 18. Consequently, the 1*S*, 2*S*, 9*R*, and 14*S* configurations in 10 and 8 were determined. Deacetylation of kericembrenolides A (7) and D (10) and subsequent benzoylation and ozone oxidation of the products provided 20, $^1H$ NMR (500 MHz, $CD_3OD$): δ 5.86 (1H dddd, J=9.0, 9.0, 4.5, 3.5 Hz, 6-H), 5.27 (1H d, J=9.0 Hz, 7-H) and 21, $^1H$ NMR (500 MHz, $CD_3OD$): δ 5.95 (1H m, 6-H), 5.30 (2H br d, J=9.0 Hz, 3,7-H). The CD spectra of 20: $[\theta]_{225}$ +20000 (pos. max.) and 21: $[\theta]_{225}$ +23000 (pos. max.), showed their 6*S* configurations ($J_{6,7}$=9.0 Hz in 20 and 21).[13]

Consequently, the absolute stereostructures of kericembrenolides A (7), B (8), C (9), D (10), and E (11) were determined as shown. These cembrenolides were shown to exhibit growth inhibitory effect against B-16 Melanoma cells ($IC_{50}$ μg/ml): A 3.8, B 2.5, C 1.3, D 1.2, and E 1.8.

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