## Communications to the Editor

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# CLAVUKERIN C, A NEW TRINOR-GUAIANE SESQUITERPENE HAVING A HYDROPEROXY FUNCTION, FROM THE OKINAWAN SOFT CORAL *CLAVULARIA KOELLIKERI*

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A new trinor-guaiane sesquiterpene named clavukerin C (2), having a hydroperoxy function, was isolated together with clavukerin A (1), clavukerin B (3), and bicyclogermacrene (4) from the Okinawan soft coral (stolonifer) *Clavularia koellikeri*. The absolute stereostructure of clavukerin C (2) was elucidated. Clavukerin B (3) was found to be identical with or antipodal to trinoranastreptene, which was previously reported as a liverwort metabolite.

KEYWORDS —— trinor-guaiane sesquiterpene; clavukerin B; clavukerin C; bicyclogermacrene; *Clavularia koellikeri*; soft coral; stolonifer; hydroperoxy containing trinorsesquiterpene

In search of bioactive constituents from marine organisms,[1] we isolated a new trinor-guaiane sesquiterpene named clavukerin A (1) from the Okinawan soft coral (stolonifer) *Clavularia koellikeri* and elucidated the absolute stereostructure.[2,3] In a continuing study of the chemical constituents of the same soft coral, we recently isolated a new trinor-sesquiterpene named clavukerin C (2), which was characteristic by possessing a hydroperoxy function, together with clavukerin B (3) which was identical with or antipodal to trinoranastreptene,[5] and bicyclogermacrene (4).[6] Elucidation of the absolute stereostructure of clavukerin C (2) is the major subject of this communication.[7]

An acetone extract of the fresh soft coral (collected in July at Kohama-jima, Okinawa Prefecture) was partitioned in an AcOEt-$H_2O$ mixture and the AcOEt-soluble portion was subjected to Toyo Pearl (HW-40 Super Fine) column chromatography (MeOH). The fraction containing clavukerin C was further partitioned in a hexane-MeOH mixture and the hexane soluble portion was purified by HPLC (μPorasil, hexane-AcOEt) to furnish clavukerin C (2) (1% from the AcOEt extract).[8]

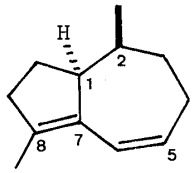
Clavukerin C (2), unstable colorless oil, $C_{12}H_{18}O_2$,[9] $[\alpha]_D^{20}$ -61° ($CHCl_3$), was a conjugated diene as shown by its UV absorption spectrum [$\lambda_{max}^{MeOH}$ nm (ε): 255 (8500), 247 (sh)(8300)]. The presence of the hydroperoxy function in 2 was suggested by the positive reactions with the N,N-dimethyl-*p*-phenylenediammonium

dichloride reagent[10] and the ferrous thiocyanate reagent.[11] The $^{13}$C-NMR spectrum (22.5 MHz, $CDCl_3$) of 2 showed signals ascribable to four olefinic carbons [ δc 151.1 (s), 131.8 (s), 133.9 (d), 121.0 (d)] and a quaternary carbon bearing a hydroperoxy function [δc 97.1 (s)], while the $^1$H-NMR spectrum (500 MHz, $CDCl_3$) showed signals due to 2-$CH_3$ (δ 1.05, 3H, d, J=7.5 Hz), 8-$CH_3$ (δ 1.28, 3H, s), and two olefinic protons at C-5 (δ 5.91, 1H, ddd, J=11.5, 5.5, 5.5 Hz) and at C-6 (δ 5.81, 1H, d, J=11.5 Hz). Dehydrogenation of 2 over 10% Pd-C in diglyme yielded 1,4-dimethylazulene (5)[4,12] as experienced for clavukerin A (1).[2] This suggested a 2,8-dimethyl-bicyclo[5.3.0]decane skeleton for 2. In addition, the pyridine-induced solvent shift[13] was observed for the 6-H signal [δ ($CDCl_3$) - δ ($CDCl_3$-$d_5$-pyridine=2:1)=-0.15 ppm]. These findings supported the presence of the 1(7),5-diene-8-hydroperoxy structure in clavukerin C (2). Finally, photosensitized oxygenation[14] of clavukerin A (1) in MeOH-pyridine (10:1) containing Rose Bengal quantitatively furnished clavukerin C (2). Consequently, the absolute stereostructure of clavukerin C was determined to be (2S,8R)-2,8-dimethyl-8-hydroperoxybicyclo[3.5.0]deca-1(7),5-diene (2). Clavukerin C (2) is a rare example of a naturally occurring hydroperoxy compound isolated from marine organisms. A few other examples have been found, *e.g.* from the red alga *Laurencia snyderiae*[15] and from two kinds of tunicates, *Phallusia mamillata*[16] and *Ciona intestinalis*.[16] The physiological function of these hydroperoxy compounds is an interesting subject for investigation.

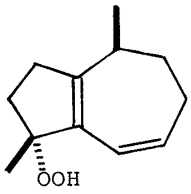
Purification of the above-mentioned AcOEt soluble portion by $SiO_2$ column chromatography (hexane), then by preparative GLC (15% PEGS on Chromosorb WAW) and HPLC (Cosmosil 5$C_{18}$, MeOH-$H_2O$), furnished clavukerin A (1)[2] as the major component and clavukerin B (3) and bicyclogermacrene (4)[6,17] as minor constituents.[8]

Clavukerin B (3), colorless oil, $C_{12}H_{16}$, $[\alpha]_D^{24}$ +172° ($CHCl_3$), was shown to be a tricyclic trinor-sesquiterpene by the $^{13}$C-NMR spectrum showing four olefinic carbon signals [δc 140.5 (s), 128.2 (d), 124.1 (d), 122.4 (d)], and by the $^1$H-NMR spectrum exhibiting signals due to two methyl groups [δ 0.93 (3H, s) for 2-$CH_3$, δ 1.73 (3H, dd-like, J=*ca.* 2, 2 Hz) for 8-$CH_3$] and three olefinic protons [δ 5.49 (1H, ddd, J=10.0, 6.5, 2.5 Hz) for 5-H; δ 6.11 (1H, dd, J=10.0, 2.5 Hz) for 6-H; δ 5.16 (1H, br s) for 9-H]. Catalytic hydrogenation of clavukerin B (3) over 10% Pd-C provided the 5,6-dihydro derivative (6), colorless oil, $C_{12}H_{18}$, $[\theta]_{214}$ +24000 (pos. max.), $^1$H-NMR (δ): 0.81 (3H, s, 2-$CH_3$), 1.64 (3H, dd-like, J=*ca.* 2, 2 Hz, 8-$CH_3$), 1.15 (1H, d, J=7.5 Hz, 1-H), 1.97 (1H, br d, J=17.5 Hz, 10β-H), 2.41 (1H, ddq-like, J=*ca.* 17.5, 7.5, 2.5 Hz, 10α-H), and 5.10 (1H, br s, 9-H). Hydroboration-oxidation of 6 yielded the 9α-hydroxy derivative (7), colorless oil, $C_{12}H_{20}O$. The $^1$H-NMR spectrum of 7 showed signals due to 8-$CH_3$ (δ 1.08, 3H, d, J=7.0 Hz), 2-$CH_3$ (δ 1.01, 3H, s), 1-H (δ 0.89, 1H, d, J=6.0 Hz), 8-H (δ 1.91, 1H, dq, J=7.0, 7.0 Hz), 10α-H (δ 1.84, 1H, m), 10β-H (δ 2.19, 1H, dd, J=14.0, 8.0 Hz), and 9-H (δ 3.76, 1H, ddd, J=8.0, 7.0, 6.5 Hz). Furthermore, the irradiation of the 2-$CH_3$ signal resulted in a 6% NOE enhancement of the 9β-H signal.

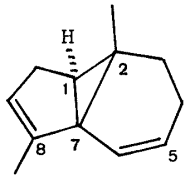
Based on the above-described evidence, clavukerin B (3) was found to be identical with or antipodal to trinoranastreptene, which was recently isolated from the cultured cells of the liverwort *Calypogeia granulata*. The relative stereostructure of trinoranastreptene was demonstrated as 3 by Takeda and Katoh, but



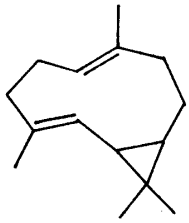
clavukerin A (1)



clavukerin C (2)



clavukerin B (3)
(without absolute configuration)



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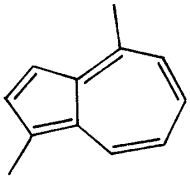

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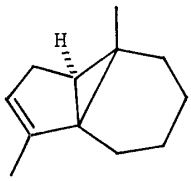

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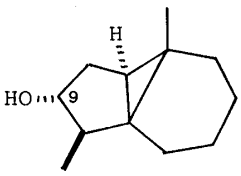

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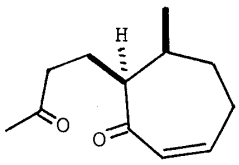

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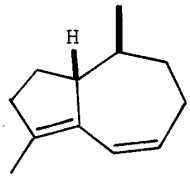

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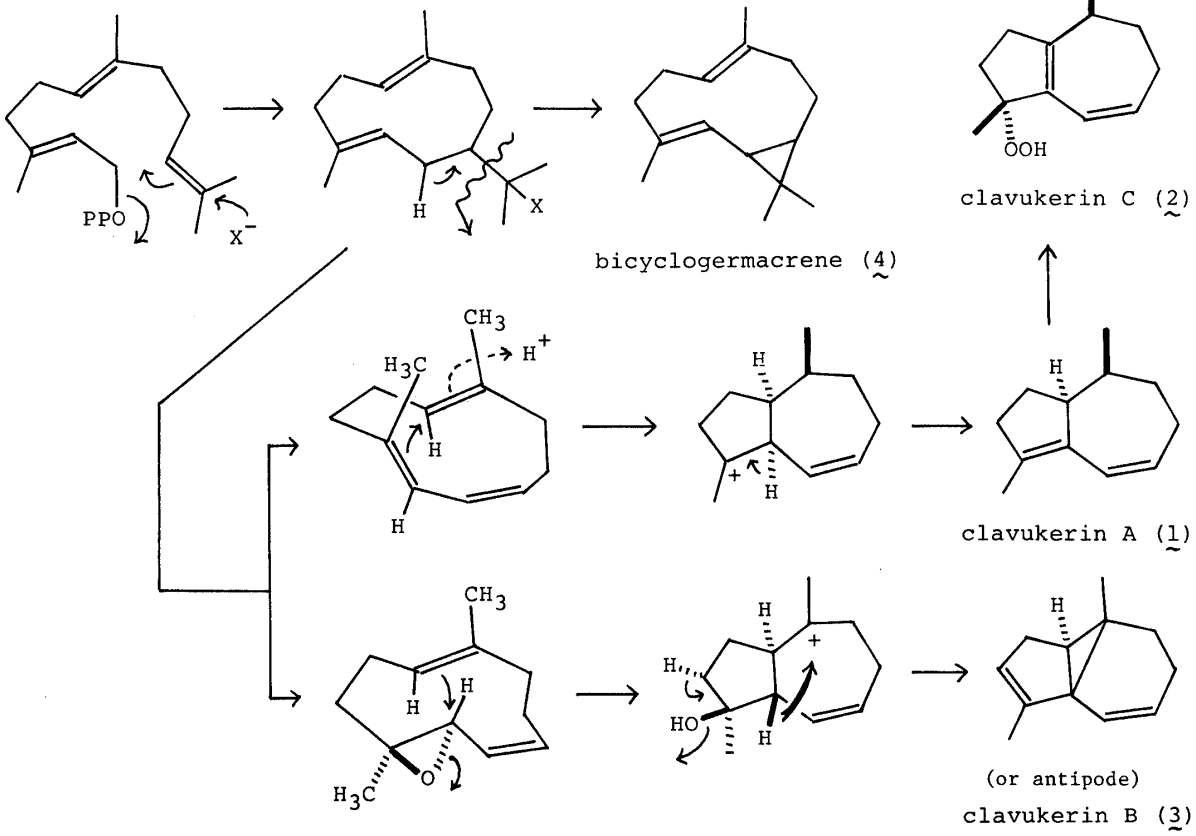
Hypothetical Biogenetic Pathway



the optical property was unknown.[5]

The approximate biogenetic pathways for these clavukerins are assumed to be as shown here. Considering the quantitative photooxidative conversion from clavukerin A (1) to clavukerin C (2), 2 is presumably biosynthesized from 1. Recently, Endo and his group reported the isolation of cytotoxic clavularin A (8), which was identical with one of the degradation products of clavukerin A (1),[2] from the same kind of soft coral.[18] We assume that clavularin A (8) might be either biogenetically or secondarily (during the isolation procedure) formed from clavukerin C (2).[19]

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3) A trinor-sesquiterpene has been recently isolated from an Australian soft coral *Cespitularia* sp. and the structure 9 has been proposed.[4] However, we have noticed that this trinor-sesquiterpene reported by the Australian group might be identical with our clavukerin A (1) from the comparison of reported physical data: $[\alpha]_D$, UV, $^1$H-NMR, $^{13}$C-NMR, so that the revision of the structure from 9 to 1 seems to be necessary.
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8) It should be mentioned here that the composition of trinor-sesquiterpenes described in this paper varied depending upon the year collected, although the soft corals investigated were collected in the same waters and in the same season.
9) The molecular compositions of compounds with the chemical formulae were determined by high resolution mass spectrometry.
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17) Bicyclogermacrene (4), $C_{15}H_{24}$, $[\alpha]_D^{17}$ -83° ($CHCl_3$), $^1$H-NMR (500 MHz, $d_6$-acetone ,δ): 4.85 (1H, dd-like, J=*ca.* 11.0, 5.0), 4.39 (1H, d, J=11.5), 1.33 (1H, dd, J= 11.5, 9.0), 0.64 (1H, ddd-like, J=12.0, *ca.* 9.0, *ca.* 3.0), 1.64 (3H, d, J=1.0), 1.48 (3H, dd-like, J=*ca.* 1.0, 1.0), 1.08, 1.02 (both 3H, s). Based on these physical and optical properties, bicyclogermacrene (4) isolated here was considered to be antipodal to the one reported by Nishimura, et al.[6]
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19) Very recently, we have become aware of a similar suggestion which was made for the biogenesis of a 4,5-secoeudesmanolide: G. Appendino, P. Gariboldi, M. Calleri, G. Chiari, and D. Viterbo, J. Chem. Soc. Perkin I, 1983, 2705.

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