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CLAVUKERIN A, A NEW TRINOR-GUAIANE SESQUITERPENE FROM THE OKINAWAN SOFT CORAL CLAVULARIA KOELLIKERI

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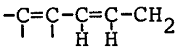
A new trinor-guaiane sesquiterpene named clavukerin A (**1**) was isolated from the Okinawan soft coral *Clavularia koellikeri* (stolonifer) and the absolute stereostructure was elucidated by the chemical and physicochemical evidence and the X-ray crystallographic analysis.

KEYWORDS —— trinor-guaiane sesquiterpene; clavukerin A; *Clavularia koellikeri*; soft coral; stolonifer; ^{1}H-NMR; X-ray analysis; CD

During the course of studies on marine natural products,[1)] we investigated the chemical constituents of the Okinawan soft coral *Clavularia koellikeri* (Class: Anthozoa; Subclass: Octocorallia; Order: Stolonifera) and isolated a fairly unstable C_{12} compound named clavukerin A. This paper deals with the evidence which is consistent with the absolute stereostructure, *i.e.* (1S,2S)-2,8-dimethyl-bicyclo[5.3.0]deca-5,7-diene (**1**) for clavukerin A.

The acetone extract of the soft coral, which was collected at Kohamajima, Okinawa Prefecture, in July, was partitioned into ethyl acetate and water. The ethyl acetate soluble portion was purified by repeated silica gel column chromatography to afford clavukerin A (**1**), colorless oil, $C_{12}H_{18}$,[2)] $[\alpha]_D^{20}$ -53° ($CHCl_3$), in 7% yield from the ethyl acetate soluble portion.

The presence of a partial structure  $-\overset{|}{C}=\overset{|}{C}-\underset{H}{C}=\underset{H}{C}-CH_2-$ in clavukerin A (**1**) was shown by the UV spectrum: λ_{max}^{MeOH} 244 nm (ε 21000), the ^{1}H-NMR spectrum (200 MHz, $CDCl_3$): δ 6.21, 1H, d, J=11.5 Hz and δ 5.54, 1H, dt, J=11.5, 5 Hz, and the ^{13}C-NMR spectrum (50 MHz, $CDCl_3$).[3)] In addition, these NMR spectra showed the presence of one olefinic methyl group (δ 1.74, 3H, s), one secondary methyl group (δ 0.78, 3H, d, J=7 Hz), two methine carbons and three methylene carbons in **1**. Thus, clavukerin A (**1**) was shown to be a bicarbocyclic hydrocarbon.

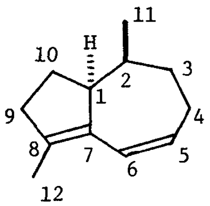
Osmium tetroxide oxidation of clavukerin A (**1**) furnished two *cis*-diols: **2** (70 %), $C_{12}H_{20}O_2$; δ 1.67 (3H, s), 4.75 (1H, d, J=2.5 Hz), 3.45 (1H, ddd, J=10.5, 6, 2.5) and **3** (5%), $C_{12}H_{20}O_2$; δ 5.45 (1H, ddd, J=12, 3, 1.5), 5.83 (1H, ddd, J=12, 6.5, 5.5), 1.25 (3H, s). Oxidation of the minor diol (**3**) with sodium periodate yielded a diketone (**4**), $C_{12}H_{18}O_2$; δ 2.13 (3H, s), 6.04 (1H, d, J=11.5), 6.77 (1H, ddd, J=11.5, 7, 4.5), 2.81 (1H, ddd, J=9.5, 5, 5). *m*-Chloroperbenzoic acid oxidation of 1 furnished a crystalline diepoxide (5), $C_{12}H_{18}O_2$, mp 94-95°C (hexane) and several other products.[3)]

The ^{1}H-NMR spectrum (500 MHz, $CDCl_3$) of the diepoxide (5) showed signals assignable to one methyl group and two protons attached to the epoxide ring (δ 1.43, 3H, s; δ 2.71, 1H, d, J=4; δ 3.23, 1H, ddd, J=4, 3, 1) and one secondary methyl group (δ 0.87, 3H, d, J=7). Furthermore, the detailed decoupling experiments led us to assign all the proton signals.[4)]

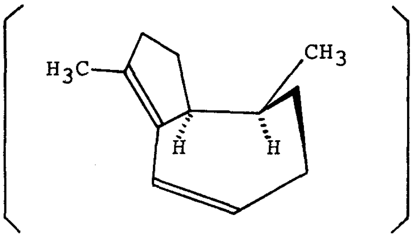
Based on the above evidence, clavukerin A (1) was shown to possess either a 2,8-dimethyl-bicyclo[5.3.0]decane skeleton or (less likely) a strained 1,10-dimethyl-bicyclo[5.2.1]decane skeleton. In order to investigate this matter, the diepoxide (5) was subjected to the X-ray crystallographic analysis. Data were collected on a Syntex R3 diffractometer system by the ω scanning technique within 2θ less than 45° using a variable-scan speed and graphite-monochromated Mo-Kα radiation. Crystal data: $C_{12}H_{18}O_2$, orthorhombic, space group $P2_12_12_1$, a=5.959(3), b=6.950(3), c=25.925(11) Å, Dx=1.20 g.cm^{-3}, Z=4, and μ(MoKα)=0.9 cm^{-1}. The structure was solved by the direct method using MULTAN on a Syntex XTL program. All the hydrogen atoms were found on a difference Fourier map. The refinements of atomic parameters were carried out by block-diagonal least-squares calculations. The final R-value was 0.080 for 576 reflections [$I>1.96\sigma(I)$]. The molecular structure of the diepoxide (5) and the numbering of atoms are shown in Fig. 1. Thus, the stereostructure of the diepoxide has been clarified as 5 (except for the absolute configuration), which possesses a 2,8-dimethyl-bicyclo[5.3.0]decane skeleton.

In order to establish the absolute stereostructure of clavukerin A (1), the CD spectra of the benzoyl derivatives of the *cis*-diol (2) were investigated. *p*-Bromobenzoylation of 2 furnished the monobromobenzoate (2a), $C_{19}H_{23}O_3Br$ [δ 4.97, 1H, br s, 6-H; δ 4.81, 1H, ddd, J=11, 6, 2, 5-H), in which the less hindered 5α-equatorial hydroxyl group was esterified. On the other hand, benzoylation of 2 furnished the dibenzoate (2b), $C_{26}H_{28}O_4$ [δ 6.31, 1H, br s, 6β-H; δ 4.97, 1H, ddd, J=9.5, 7, 2, 5β-H), which on alkaline hydrolysis (0.1 M K_2CO_3-aq. MeOH) yielded two monobenzoates: 2c, $C_{19}H_{24}O_3$ (δ 6.07, 1H, br s, 6β-H; δ 3.63, 1H, ddd, J=11, 5.5, 2.5, 5β-H) and 2d, $C_{19}H_{24}O_3$ (δ 4.99, 1H, s, 6β-H; δ 4.84, 1H, ddd, J=11, 6, 2, 5β-H). In order to clarify the free hydroxyl configuration of the both monobenzoates (2c, 2d), the pyridine-induced shifts in the ^{1}H-NMR[5)] of 2c and 2d were examined. As shown in Table I, the 6β-equatorial proton signal of 2c was significantly shifted lower due to the presence of the 5α-equatorial hydroxyl group, whereas the 2α-axial proton signal of 2d was similarly shifted lower due to the 6α-axial hydroxyl group. Furthermore, the ^{13}C-NMR spectra of 2b, 2c, and 2d showed similar chemical shift values for the respective carbons[3)] except those for C_3, C_4, C_5, and C_6, which were affected by the esterification shift. Thus, 2b, 2c, and 2d have been shown to possess similar stereostructures, as depicted in Chart 1 (2b', 2c', and 2d').

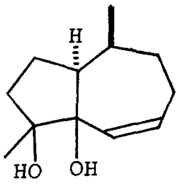
Finally, the benzoate chirality method[6)] has been applied to 2b, 2c, and 2d to establish their absolute stereostructures. It has been demonstrated that, in the CD spectrum of a compound having more than one CD exciton in its molecule, an additivity relation exists in the amplitudes of individual CD excitons.[6)] Thus, the CD Cotton curve due to the coupling of the 5,6-dibenzoate excitons of 2b can



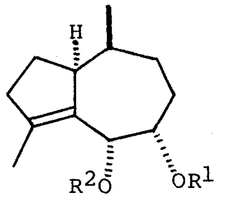
1 clavukerin A

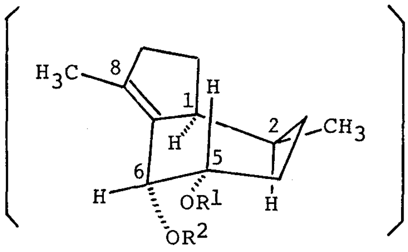


1′

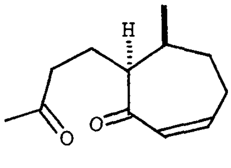


3



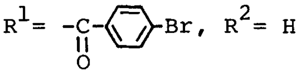


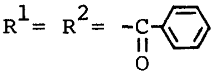
2′ ~ 2d′

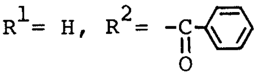


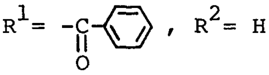
4

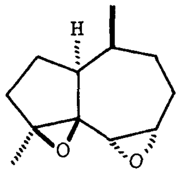
2 $R^1 = R^2 = H$

2a


2b


2c


2d




5

Chart 1

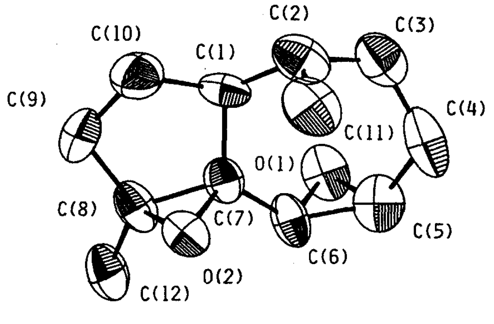


Fig. 1

Table I. ^{1}H-NMR Data for 2c and 2d (200 MHz, δ)

		2-Me	8-Me	1α-H	2α-H	5β-H	6β-H
2c	$CDCl_3$	0.89	1.81	2.92	2.27	3.63	6.07
	$CDCl_3$-d_5-pyr. (1:1)	0.87	1.72	2.89	2.39	3.80	6.36
2d	$CDCl_3$	0.89	1.75	3.08	2.30	4.84	4.99
	$CDCl_3$-d_5-pyr. (1:1)	0.89	1.76	3.17	2.62	4.92	5.24

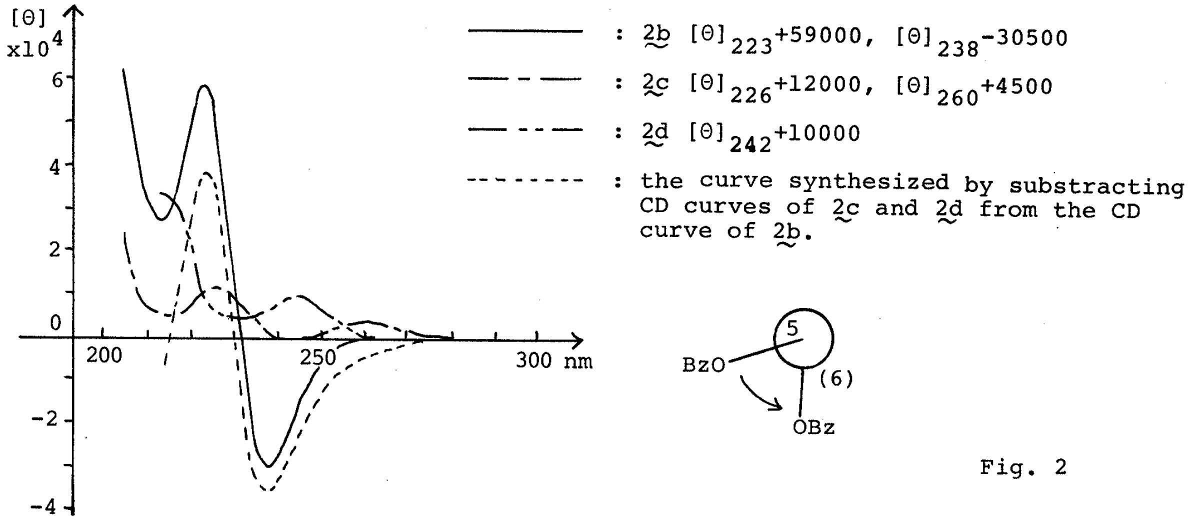


Fig. 2

be obtained by substracting the CD curves of 2c and 2d from the CD curve of 2b. As shown in Fig. 2, the CD curve, which was synthesized for the 5,6-dibenzoate excitons of 2b, showed a negative first Cotton effect. Thus, the 5S and 6R configurations of 2b have been clarified, and, consequently, the 1S and 2S configurations of 2b have also been established.

Furthermore, since the ^{1}H-NMR signal assignable to 5-H in clavukerin A (1) was observed at δ 5.54 (1H, dt, J=11.5, 5), and the CD spectrum of 1 showed a negative CD maximum: $[\theta]_{242}$ -24000 due to the heteroannular diene chromophore (*cf.* 1'), the absolute stereostructure of clavukerin A (1) has been determined. Clavukerin A (1) seems to be a first example of the naturally occurring 2,8-dimethyl-hexahydroazulene derivative and is presumed to be a trinor-sesquiterpene rather than an acetogenin.[7]

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2) The molecular compositions of compounds with the chemical formulae were determined by high resolution mass spectrometry.
3) The details will be reported in our full paper.
4) δ 2.23 (ddd, J=4, 1.5, 1, 4β-H), 2.25 (dd, J=4, 3, 4α-H), 1.65 (dddd, J=14.5, 4, 4, 1.5, 3β-H), 1.54 (ddd, J=14.5, 5, 4, 3α-H), 1.82 (m, 2α-H), 2.15 (ddd, J=11, 8, 3, 1α-H), 1.29, 1.36 (both 1H, m, 10-H_2), 1.95 (dd, J=13.5, 8, 9β-H), 1.67 (ddd, J=13.5, 8, 4, 9α-H).
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7) Very recently, we became aware of the characterization of two new cytotoxic C_{12} compounds clavularins A and B isolated from the same species of the Okinawan soft coral by Endo et al.: M. Endo, M. Nakagawa, Y. Hamamoto, and T. Nakanishi, J. Chem. Soc. Chem. Commun., 1983, 322.

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