Clavularins, a New Class of Cytotoxic Compounds isolated from the Soft Coral, *Clavularia koellikeri*

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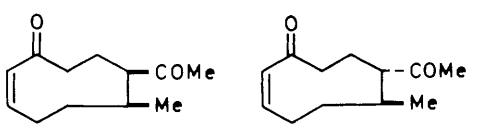
The structures of two new cytotoxic compounds, clavularins A and B have been elucidated by studies of their ^{1}H n.m.r. spectra at 360 MHz.

Here we report the structural elucidation of a new class of cytotoxic compounds,† clavularin A (**1**) and clavularin B (**2**), isolated from the soft coral, *Clavularia koellikeri* (Thomson and Dean) which lives in the shallow water of a coral reef in Ishigaki-Jima, Okinawa, Japan.

A methanol extract of the soft coral was fractionated on a silica gel column, through a TSK gel column (HW-40 coarse, Toyo Soda Co.), and by silica gel t.l.c.; the fractions were assayed against cultured cells. Two cytotoxic substances were isolated as colourless oils: clavularin A, M^+ m/z 194.1327 ($C_{12}H_{18}O_2$ requires 194.1306), λ_{max} (n-hexane) 221 nm (ϵ 9 700), ν_{max} (film) 2930, 1720, and 1679 cm^{-1} and clavularin B,



(**1**) (**2**)

† Both compounds have a strong lethal effect on PV_4 cultured cells transformed with polyoma virus; T/C 50% was observed at 0.25 μg/ml.

M^+ m/z 194.1307; λ_{max} (n-hexane) 221 nm (ϵ 10 000), ν_{max} (film) 2920, 1715, and 1675 cm^{-1}.

The skeletal structure of clavularin A was determined by intensive decoupling studies of its ^{1}H n.m.r. spectra; two-dimensional n.m.r. techniques[1,2] were applied in order to confirm the correlations and assignments.‡ The *cis*-relation of the olefinic protons was confirmed by nuclear Overhauser enhancement (n.O.e.) (23%) of 2-H (δ 5.97 in C_6D_6) caused by irradiation at 3-H (δ 6.27 in C_6D_6).

‡ N.m.r. data for clavularin A: ^{1}H n.m.r. ($CDCl_3$) δ 6.76 (1H, ddd, J 11.7, 7.2, and 4.1 Hz, 3-H), 6.02 (1H, d, J 11.7 and 2.7 Hz, 2-H), 2.81 (1H, ddd, J 9.7, 5.4, and 4.1 Hz, 7α-H), 2.49 (1H, ddd, J 17.1, 9.0, and 5.4 Hz, 9β-H), 2.42 (2H, m, 4α-H and 4β-H), 2.31 (1H, ddd, J 17.1, 8.2, and 6.6 Hz, 9α-H), 2.12 (3H, s, 7β-COMe), *ca.* 2.1 (3H, m, 5α-H, 6α-H, and 8α-H), 1.61 (1H, m, 8β-H), 1.26 (1H, m, 5β-H), and 0.83 (3H, d, J 7.2 Hz, 6β-Me); ^{13}C n.m.r. ($CDCl_3$) δ 208.2 (s), 203.2 (s), 148.1 (d), 133.5 (d), 53.7 (d), 41.5 (t), 35.4 (t), 33.5 (d), 29.5 (q), 27.4 (t), 22.1 (t), and 15.7 (q) p.p.m. N.m.r. data for clavularin B: ^{1}H n.m.r. ($CDCl_3$) δ 6.55 (1H, ddd, J 11.5, 6.2, and 3.5 Hz), 5.95 (1H, ddd, J 11.5, 2.4, and 0.9 Hz), 2.51—2.27 (5H, m), 2.11 (3H, s), 1.98—1.60 (5H, m), and 1.09 (3H, d, J 6.8 Hz); ^{13}C n.m.r. ($CDCl_3$) δ 208.3 (s), 205.9 (s), 145.5 (d), 131.8 (d), 59.3 (d), 41.5 (t), 34.6 (d), 33.3 (t), 29.9 (q), 27.7 (t), 24.6 (t), and 19.9 (q) p.p.m.

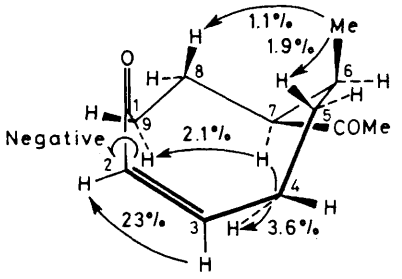


Figure 1. N.O.e.s, H (irradiated) → H (enhanced), and the negative skew of the α,β-unsaturated ketone in clavularin A.

Owing to a shielding effect of the carbonyl group, the ^{1}H n.m.r. signal of 5β-H in clavularin A appears at unusually high field (δ 1.26 in $CDCl_3$ and 0.98 in C_6D_6). The conformation from C-1 to C-6 was thus elucidated (Figure 1) and the existence of a skewed α,β-unsaturated ketone was proved by c.d. spectroscopy (see below). The β-configuration of 6-Me was indicated by n.O.e. (1.9%) of the 5β-H ^{1}H n.m.r. signal (δ 1.26 in $CDCl_3$) caused by irradiation at 6-Me (δ 0.83 in $CDCl_3$). The α-configuration of 7-H was indicated by n.O.e.s of one 4-H (3.6 %, δ 2.42 in $CDCl_3$) and one 9-H (2.1 %, δ 2.31 in $CDCl_3$) caused by irradiation at 7-H (δ 2.81 in $CDCl_3$) (7-H can be located in the vicinity of 4-H and 9-H only when it is in the α-configuration and when C-7, C-8, and C-9 are in the conformation shown in Figure 1). The 4-H and 9-H which show n.O.e.s were thus assigned as 4α-H and 9α-H. The n.O.e. difference spectrum technique[3] was applied throughout the n.O.e. studies.

The absolute structure of clavularin A was based on the c.d. Cotton effect;[4,5] a negative sign ($\Delta\epsilon$ −7.08) for the K-band (224 nm) and a positive sign ($\Delta\epsilon$ +3.89) for the R-band (339 nm) are due to the negative skew of the α,β-unsaturated ketone (Figure 1).

N.O.e. (3.6%) of 7-H (δ 2.21 in C_6D_6) in clavularin B caused by irradiation at 6-Me (δ 0.83 in C_6D_6) suggests that clavularin B is the 7-epimer of clavularin A. This was confirmed by the conversion of clavularin A into clauvularin B by hydrogen chloride in methanol.

We thank Dr. T. Noguchi (Director of Suntory Institute for Biomedical Research) for encouragement, Dr. T. Iwashita (Suntory Institute for Bioorganic Research) for the recording of n.m.r. spectra and useful suggestions, and Dr. F. M. Bayer (National Museum of Natural History, Smithsonian Institution) for the identification of the soft coral.

Received, 22nd November 1982; Com. 1328

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