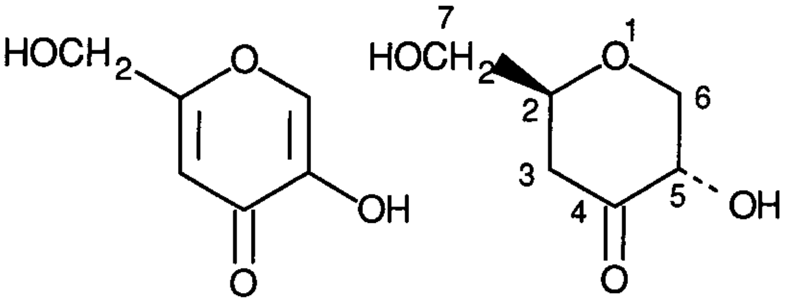
HALICLONOL, A NEW TETRAHYDROPYRANONE FROM THE CARIBBEAN SPONGE *HALICLONA HOGARTHI*

Patrizia Ciminiello, Valeria Costantino, Ernesto Fattorusso,*Silvana Magno, and Alfonso Mangoni
Dipartimento di Chimica delle Sostanze Naturali, Via D. Montesano 49, I-80131, Napoli, Italy

Abstract- Haliclonol (**2**), a unique hydroxylated tetrahydropyranone, was isolated from the Caribbean sponge *Haliclona hogarthi,* and its structure, including absolute stereochemistry, was elucidated by spectroscopic (ir, nmr, ms and cd) data.

Kojic acid (**1**) is an antibiotic compound produced when various moulds (mainly *Aspergillus* species) are grown on glucose or xylose. Since 1907, when it was first isolated by Saito,[1] it was extensively investigated on account of its very interesting chemical and biological properties. In our continuing search for bioactive marine compounds, from the sponge *Haliclona hogarthi* we have isolated haliclonol [(2*S*, 5*S*)-2-hydroxymethyl-5-hydroxytetrahydropyr-4-one, **2**], a tetrahydro derivative of kojic acid, which represents a quite unusual marine metabolite, the closest structural analogue being bissetone, a pyranone isolated from the gorgonian *Briareum polyanthes.*[2]

Haliclona hogarthi is a brownish-olive, soft and compressible demospongia, which was collected at -2 m in the lagoon of Little S. Salvador (Bahamas) during Summer 1990. Freshly collected animals were stored frozen and then extracted first with MeOH-toluene 3:1 and then with $CHCl_3$. The EtOAc soluble material from the extracts (4.0 g) was repeatedly chromatographed by normal and reversed-phase hplc to give 2.7 mg of pure **2** as a viscous oil ($[\alpha]_D$ -21.4°).



1 **2**

Compound (2) was analyzed for $C_6H_{10}O_4$ by hrms and ^{13}C-nmr spectroscopy. The carbonyl signal at δ 207.4 in the ^{13}C- nmr spectrum and the corresponding band at 1721 cm^{-1} in the ir spectrum established the presence of a carbonyl group. This functionality accounted for one of the two unsaturations implied by the molecular formula, the second being a cycle owing to the lack of additional resonances in the sp^2 region of the ^{13}C-nmr spectrum. The presence in the ^1H-nmr spectrum of a doublet at δ 3.49 (1H, J = 3.7 Hz) and a double doublet at δ 1.95 (1H, J = 6.2 and 6.6 Hz), both D_2O exchangeable, together with the strong infrared absorption observed at 3400 cm^{-1}, showed **2** to possess one primary and one secondary hydroxyl function.

Decisive information on the structure of **2** was gained from interpretation of the 500 MHz ^1H and COSY nmr spectra, where a good resolution of the resonances of each methylene, methine and hydroxyl was obtained. Couplings of H2 with H3ax, H3eq and both H7's, and those between H7's and the OH proton resonating at δ 1.95 were evidenced by COSY spectrum, and were also confirmed by conventional decoupling. This defined the first isolated spin system of **2**. Analogously couplings between H6ax, H6eq, H5, and the OH proton resonating as a doublet at δ 3.49 identified the second spin system. With the help of direct carbon-proton nmr correlation the corresponding partial structures (C3-C2-C7 and C5-C6) were confidently established. A long range carbon-proton correlation (COLOC) nmr experiment allowed the partial structures to be combined: the correlation spots observed between C4 and H3ax, H3eq and H2 located the carbonyl function on the carbon chain, while the coupling detected between H6ax and C2 established an ether linkage between C2 and C6.

Table 1. NMR Spectral Data of Compound **2** ($CDCl_3$).

Pos.		δ_H (mult., J in Hz)	δ_C
2		3.76 (dddd, 12, 5.5, 3, 2.2)	79.4 (CH)
3	ax	2.72 (dd, 14, 12)	42.0 (CH_2)
	eq	2.53 (dd, 14, 2.2)	
4			207.4 (C)
5		4.27 (ddd, 11, 7.5, 3.7)	73.0 (CH)
6	ax	3.33 (dd, 11, 11)	72.2 (CH_2)
	eq	4.46 (dd, 11, 7.5)	
7	a	3.81 (ddd, 11.5, 6.6, 3)	64.9 (CH_2)
	b	3.62 (ddd, 11.5, 6.2, 5.5)	
5-OH		3.49 (d, 3.7)	
7-OH		1.95 (dd, 6.6, 6.2)	

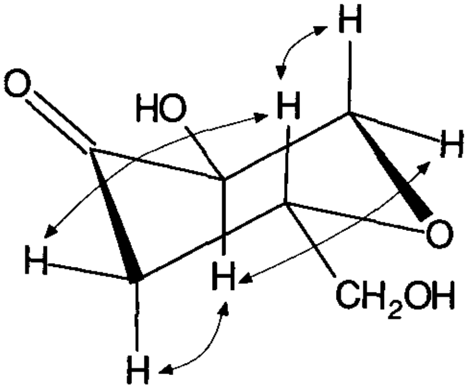


Figure 1. Nuclear Overhauser effects shown by compound (**2**).

The relative configuration of the two asymmetric carbon atoms (C2 and C5) in **2** was established by nuclear Overhauser effect (nOe) difference experiments and coupling constant measurements. The 1,3-diaxial relationship of H6ax and H3ax with H2 and H5, respectively, evidenced by the nOe's reported in Figure, indicates a chair-like conformation for the molecule.The axial orientation of H2 and H5 is also supported by their coupling constants (11 and 7.5 Hz for H5 and 12 and 2.2 Hz for H2); the unusually large H6eq-H5 coupling constant is consistent with the reported values for analogous compounds.[3] All the above data point to a (2*R*, 5*R*) or (2*S*, 5*S*) stereochemistry for compound (**2**).

The absolute configuration of C2, and therefore of the whole molecule, was established on the basis of the cd spectrum, using the octant rule and referring to the the chair conformation determined above for **2.** The equatorial hydroxyl group at position 5 has no significative effect on the cd spectrum, being located in the plane between two octants. Hence, the observed negative ellypticity ($\Delta\varepsilon_{289}$ = - 0.060) must be exclusively due to the asymmetric C2, which therefore possesses an *S* configuration. Compound (**2**) can be therefore formulated as (2*S*, 5*S*)-2-hydroxy-methyl-5-hydroxytetrahydropyr-4-one.

As a part of an investigation on the chemical properties of kojic acid, Ichimoto and Tatsumi reported in 1964 a study on its catalytic hydrogenation.[3] By using palladium catalyst they obtained hexahydrokojic acid as the major compound, and minor percentages of allomaltol and of a tetrahydroderivative identified on the basis of the elementary analysis and the ir spectrum. A comparison of the naturally occurring haliclonol and the synthetic tetrahydrokojic acid, obtained in very low yield (1%) according to Ichimoto, showed that the two products possess coincident spectral properties, the only difference being the optically inactivity of the synthetic product, which therefore resulted to be a racemic mixture of **2** and its enantiomer.

As far as the biogenetic origin of compound (**2**), glucose could be reasonably hypothesized as a precursor of haliclonol. The role of glucose as a direct precursor has been established for kojic acid,[4] which is apparently formed without cleavage of any of the original carbon-carbon bonds. An analogous biosynthesis could occur in the marine sponge *H. hogarthi*, taking into account that the asymmetric carbons in **2** have the same configuration as the corresponding carbons of the hypothesized precursor, *d*-glucose.

EXPERIMENTAL

General methods. Electron impact mass spectra were obtained at 70 eV on a Kratos MS50 mass spectrometer. Fourier transform ir spectra were recorded on a Bruker IFS-48 spectrophotometer . Optical rotations were measured on a Perkin Elmer Model 141 polarimeter, using a 10-cm microcell. Circular dichroism spectra were performed on a Jasco J500A spectrophotometer in MeOH solution. ^{1}H- and ^{13}C-nmr spectra were determined on a Bruker AMX-500 spectrometer in $CDCl_3$. Proton chemical shifts were referenced to the residual chloroform signal (δ 7.26). ^{13}C-Nmr spectra were referenced to the center peak of $CDCl_3$ at 77.0 ppm. The multiplicities of ^{13}C resonances were determined by DEPT experiments, which were performed using polarization transfer pulses of 90° and 135°, obtaining in the first case only signals for -CH groups and in the other case positive signals for -CH and -CH_3 and negative ones for -CH_2 groups. Coherence transfer delays were adjusted to an average C-H coupling of 135 Hz. Nuclear Overhauser effect (nOe) measurements were performed on samples previously degassed by bubbling Ar through the solution for 15 min.

The COSY spectrum was collected as 1024×512 points, and processed using a sinusoidal multiplication prior to both Fourier transformations with zero filling prior to the second, to afford the final 1024×1024 data matrix, which was symmetrized prior to plotting.

The heteronuclear chemical shift correlation (HETCOR) spectrum[4] was recorded by taking 256 blocks of 1024 points each, with interpulse delays optimized for a $^1J_{CH}$ of 150 Hz. The spectrum was zero-filled to 512 points in F_1, and subjected to square sine multiplication and to sinusoidal multiplication in F_2 and F_1 domains, respectively, before the Fourier transformations.

The long range heteronuclear correlation (COLOC) spectrum was recorded as 2048×128 points was processed using an exponential and a trapezoidal multiplication in F_2 and F_1 domains, respectively. Zero filling in F_1 prior the Fourier transformations led to the final data matrix of 2048 × 256 points. The interpulse delays were adjusted for an average $^{2,3}J_{CH}$ of 8 Hz.

Medium pressure liquid chromatographies (mplc) were performed on a Büchi 861 apparatus using an SiO_2 (230-400 mesh) column. High performance liquid chromatographies (hplc) were performed on a Varian 5020 apparatus equipped with an RI-3 refractive index detector, using Hibar columns.

Collection and extraction. Specimens of *H. hogarthi* were collected in the lagoon of Little San Salvador (Bahamas), during the Summer 1990, and identified by dr. M. Pansini (University of Genoa). They were frozen when still alive at -18° C and then dispatched to the laboratory. Reference specimens are deposited at the Dipartimento di Chimica delle Sostanze Naturali, University of Naples. The collected animals (96 g, dry weight after extraction) were homogenized and extracted with MeOH/toluene 3:1 (500 ml x 5) at room temperature for 24 h. The extracts were evaporated *in vacuo* to give an aqueous phase, which was extracted with EtOAc. Evaporation of the combined EtOAc extracts afforded 4.0 g of an oily residue.

Isolation of haliclonol [(2*S*, 5*S*)-2-hydroxymethyl-5-hydroxytetrahydropyr-4-one, 2]: Fractionation of this oil was accomplished by medium pressure liquid chromatography (mplc) on an SiO_2 column, eluted using a step gradient of polarity with *n*-hexane/EtOAc and EtOAc/MeOH mixtures. Fractions (30.3 mg) eluted with EtOAc/MeOH (9:1) afforded a mixture containing compound(**2**), which was rechromatographed by high-performance liquid chromatography (hplc) using a Hibar LiChrosorb Si60 (10x250 mm) column with a mobile phase of EtOAc. Final separation was achieved by hplc on a Hibar Superspher RP-18 column with $MeOH:H_2O$ (7:3) as eluent, leading to2.7mg of pure compound (**2**) as an oil : $[\alpha]_D^{25°}$ (MeOH) -21.4°; hrms, *m/z* (assignment, relative intensities) 146.3568 (M^+, $C_6H_{10}O_4$, 3.3%), 128.0475 (M^+-H_2O, $C_6H_8O_3$, 1.2%), 116.0470 (M^+-H_2CO, $C_5H_8O_3$, 37.9%), 103.0341 ($C_4H_7O_3$, 60.1%), 98.0376 (M^+-H_2CO-H_2O, $C_5H_6O_2$, 16.3%), 85.0225 ($C_4H_5O_2$, 50.6%), 73 (18.9%), 70 (19.9%), 69 (19.6%), 61 (21.1%), 58 (21.9%), 57 (39.1%), 44 (72.1%), 43 (100%), 31 (55.6%); ir, ν_{max}(KBr) 3400, 1721 cm^{-1}; ^1H- and ^{13}C-nmr data are reported in Table 1.

Syntesis of *rac*-haliclonol. Catalytic hydrogenation of kojic acid (1.7 g) was performed on colloidal palladium as described.[2] After being filtered and dried, the reaction mixture was chromatographed by mplc on a silica gel column with mixtures of increasing polarity of EtOAc/MeOH, and fractions eluted with EtOAc/MeOH 98:2 were shown to contain **2** by tlc. The chloroform-soluble part of these fractions was subjected to a final purification by hplc (column: Hibar Superspher Si60; eluent: $CHCl_3$-MeOH 93:7) to yield 19.8 mg of an optically inactive compound, otherwise identical to compound(**2**).

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