

INFLATENE, AN ICHTHYOTOXIC C₁₂ HYDROCARBON FROM THE STOLONIFERAN
SOFT CORAL CLAVULARIA INFLATA VAR. LUZONIANA

Richard R. Izac and William Fenical*
Institute of Marine Resources
Scripps Institution of Oceanography
La Jolla, California 92093

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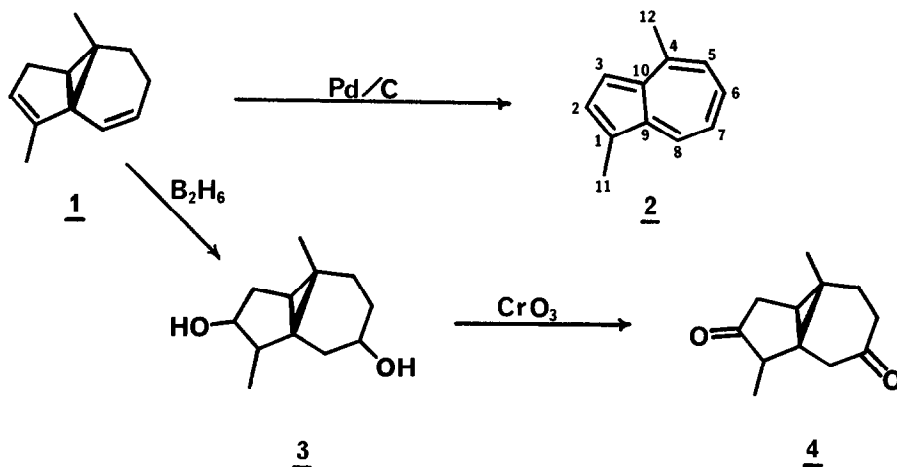
John M. Wright*
Department of Chemistry
University of California, San Diego
La Jolla, California 92093

Abstract: A new tricyclic C₁₂ hydrocarbon, inflatene (1), possessing unexpected ichthyotoxic properties, has been isolated as the major metabolite of the stoloniferan Clavularia inflata var. luzoniana. The structure of inflatene was secured by extensive FT-NMR studies with the natural product and several key derivatives.

Among the chemically-rich marine soft-corals,¹ those of the Order Stolonifera (Octocorallia, Cnidaria) represent a minor group in both species diversity and abundance. Several chemical investigations have been reported for members of this group, and in particular for Tubipora, Pachyclavularia and Clavularia species. Several sesquiterpenoids,² diterpenoids³ and novel prostanoids⁴ have been isolated, and more recently the structure of a C₁₂ diketone was reported from Clavularia koellikeri.⁵ In this communication we wish to report the isolation and structure determination of a novel tricyclic hydrocarbon, inflatene (1), from the stoloniferan coral C. inflata var. luzoniana collected in Palau, Western Caroline Islands. Inflatene possesses an unprecedented tricyclo[5.3.0^{1,6}.0]decane ring system and shows unexpected ichthyotoxicity toward the Pacific damselfish Pomacentrus coeruleus (10 µg/ml).⁶

Hexane extraction of the thawed aqueous sublimate obtained by lyophilizing frozen C. inflata var. luzoniana yielded large quantities of an odorous volatile oil (15.5 gm from 5 kg frozen animal) after careful removal of solvent. Purification of the oil by HPLC (silica gel, pentane) showed one major component which was isolated as a colorless volatile oil. The pure hydrocarbon 1 (12 g) showed $[\alpha]_D^{20} +130^{\circ}$ (c 1.63, CHCl₃) and analyzed for C₁₂H₁₆ by both mass spectral and ¹³C NMR methods. The ¹³C NMR characteristics of 1 showed the presence of two double bonds, one trisubstituted and the other disubstituted. Since inflatene possessed 5 degrees of unsaturation, the molecule was concluded to be tricyclic.⁷

After long periods of storage, inflatene samples were noted to develop faint blue colorations which suggested the conversion of 1 to azulene derivatives. Treatment of 1 with Pd/C in refluxing xylene yielded an intensely blue hydrocarbon 2, (20% overall yield) which analyzed for



$C_{12}H_{12}$. Of the numerous dimethylazulene isomers, 2 was tentatively identified as 1,4-dimethylazulene by comparison of its 1H NMR features with those reported for authentic samples.⁸

NMR Data for Inflatene (1).

C#	$^1H^a$			$^{13}C^b$		
	δ	m	J(Hz)	δ	m^c	J_{C-H}^d
1	--	--	--	140.3	s	--
2	5.16	m		124.0	d^f	160
3	2.45 ~1.9	ddd m	(17.6, 7.5, 1.0)	31.6 ^e	t	129
4	--	--	--	27.9 ^e	s^g	--
5	1.60	m		22.2	t	133
6	2.05	m		29.1	t	139
7	5.49	ddd	(9.8, 8.0, 2.0)	122.1	d^f	160
8	6.12	ddd	(9.8, 2.5, 1.0)	128.1	d^f	154
9	--	--	--	42.4	s^g	--
10	1.90	d	(10) ^h	31.7	d	163
11	1.72	dd	(2.0, 1.0)	14.9 ^e	q	126 ^e
12	0.93	s		13.3 ^e	q	125 ^e

a. Run in $CDCl_3$ solution at 360 MHz with internal TMS. b. Run in $CDCl_3$ solution at 50.3 MHz with internal TMS. c. Multiplicities were determined by single frequency off-resonance decoupling. d. Natural coupling constants were determined by gated-decoupling methods. e. Values may be interchanged. f. Conclusive assignments made by 1H - ^{13}C Cross Correlation Spectroscopy. g. Assignments made by $^{13}C[^1H]$ NOE measurements. h. Assignment made by T1 Inversion-Recovery measurements.

The 1H and ^{13}C NMR spectra of 1 initially provided little insight into the structure of 1. The compound contained two methyl groups, one of which was a bridgehead methyl and the other a highly coupled (1H spectrum) olefinic methyl. The disubstituted olefin was assigned as Z on the

basis of a vicinal 9.8 Hz coupling, and the lack of additional coupling to the δ 6.12 proton (C8) suggested an adjacent quaternary center (C9). A gated-decoupling ^{13}C NMR experiment with 1, which provided natural J values, gave significant insight into the structure of inflatene. Although there were no high-field carbon or proton resonances, the unusually large $J_{\text{C-H}}$ value of 163 Hz for the ^{13}C doublet at 31.7 ppm, in conjunction with two higher-field singlet resonances (27.9 and 42.4 ppm), could be confidently interpreted to show the presence of a pentasubstituted cyclopropane ring.

Of the numerous structural possibilities which incorporated these data, structure 1 was confidently assigned based upon extensive ^1H and ^{13}C NMR experimentation. Conventional ^1H NMR single frequency decoupling, in combination with ^1H - ^1H 2D Cross-Correlation Spectroscopy (COSY)⁹ defined the constellations C11-C1-C2-C3-C10 and C5-C6-C7-C8. However, since C4 and C9 isolated this latter four carbon unit, the olefin could be placed at either C5-C6 or C7-C8. The solution to this problem was found using selective $^{13}\text{C}[^1\text{H}]$ Difference NOE measurements.¹⁰ Irradiation (at ^1H freq.) of the bridgehead methyl (C12, δ 0.93) yielded a 50% enhancement of the ^{13}C singlet at 27.9 ppm, thus establishing this resonance from C4. Subsequent irradiation of the olefin proton at δ 6.12 (the proton known to be adjacent to the quaternary center) produced a 45% enhancement of the other ^{13}C singlet at 42.4 ppm. Since these irradiations enhanced different quaternary carbons, the Z disubstituted olefin must be at C7-C8 rather than C5-C6. Molecular models further showed that the C11 methyl and the C2 and C8 olefin protons were in close proximity in this arrangement. Irradiation of the C11 methyl (δ 1.72) showed proton NOE enhancements only to those protons (δ 5.17 and 6.12).

Confirmation of the structure of 1 was also obtained by the synthesis and spectral analysis of two derivatives. Standard hydroboration ($\text{B}_2\text{H}_6/\text{THF}/\text{H}_2\text{O}_2$) yielded the diol 3, which via Jones oxidation was converted to the diketone 4. ^1H NMR analyses of these derivatives fully supported their structures.¹² As would be expected, the cyclopentanone and cyclohexanone carbonyl groups in 4 were found to result in IR absorptions at 1751 and 1720 cm^{-1} .

Inflatene was by far the major metabolite of this variety of C. inflata, which was devoid of the clavularane and rearranged dolabellane diterpenoids reported earlier from this source. Two very minor metabolites, a cembrene derivative¹³ and batyl alcohol, were also observed. Inflatene is obviously related biogenetically to a bicyclic C_{12} diene hydrocarbon recently isolated from an undescribed species of the soft coral Cespitularia.¹⁴

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6. The tricyclo[5.3.0^{1,6}]decane component of inflatene has been observed in the hydrocarbon anastreptene, a sesquiterpenoid isolated from liverworts; see Andersen, N.H., Y. Ohta, A. Moore and C.-L.W. Tseng. Tetrahedron, 34, 41 (1978).
7. Inflatene showed the following additional spectral features: IR (film): 3070, 2930, 1640, 1430, 1370, 1180, 1155, 1115, 1074, 1042, 950, 849, 782 and 730 cm⁻¹; UV (hex): 218 nm ($\epsilon = 6,600$); MS (70 ev): M⁺ m/z 160 (C₁₂H₁₆⁺).
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11. The C10 cyclopropane proton was not resolved in any of the ¹H NMR spectra taken of 1. A T1 Inversion-Recovery experiment, with Tau values of 2.3 sec nulled all methyl and methylene protons and left only the methine protons displayed in the dispersion mode.
12. For diol 3: mp 117-118^o; IR (CHCl₃): 3590, 3430, 3000, 2920, 2860, 1500, 1370, 1040 and 1020 cm⁻¹; MS (70 ev): M⁺ m/z = 196 (C₁₂H₂₀O₂⁺); ¹H NMR (360 MHz, CDCl₃): δ 3.78 (1 H, ddd, J = 7.7, 7.0, 5.9 Hz), 3.68 (1 H, br m), 2.21 (1 H, dd, J = 13.9, 7.7 Hz), 2.05 (1 H, dd, J = 13.8, 5.0 Hz), 1.96 (1 H, dd, J = 7.0, 6.9 Hz), 1.85 (1 H, m), 1.74 (1 H, dd, J = 13.8, 6.6 Hz), 1.52 (2 H, m), 1.42 (1 H, m), 1.35 (1 H, m), 1.11 (3 H, d, J = 6.9 Hz), 1.04 (3 H, s), and 0.86 (1 H, d, J = 5.9 Hz). For diketone 4: oil; IR (CHCl₃): 2941, 1751, 1720, 1449, 1408, 1379, 1156 cm⁻¹; MS (70 ev): M⁺ m/z = 192 (C₁₂H₁₆O₂⁺), M⁺-CH₃ m/z = 177, M⁺-CO m/z = 164; ¹H NMR (360 MHz, CDCl₃): δ 2.86 (1 H, d, J = 19 Hz), 2.78 (1 H, ddd, J = 19, 6, 2 Hz), 2.63 (1 H, d, J = 19 Hz), 1.9-2.4 (6 H, m), 1.53 (1 H, d, J = 6 Hz), 1.12 (3 H, d, J = 6.9 Hz), 1.02 (3 H, s).
13. The cembrane derivative observed in extracts of this soft coral was tentatively identified as 19(or 20)-acetoxy-4,5,8,9-diepoxycebra-1,3-diene.
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