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SUBERGORGIC ACID, A NOVEL TRICYCLOPENTANOID CARDIOTOXIN FROM THE PACIFIC GORGONIAN CORAL SUBERGORGIA SUBEROSA

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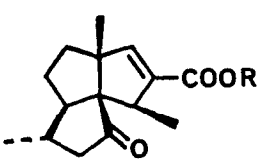
ABSTRACT: The structure of subergorgic acid (**1**), a cardiotoxic sesquiterpene with a new tricyclo[6.3.0.$0^{1,5}$]undecane (angular triquinane) skeleton, has been determined by combined spectral, chemical and x-ray crystallographic methods.

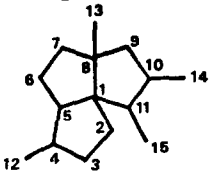
As part of our continuing research program on the isolation of novel bioactive metabolites from marine sea fans and whips (Cnidaria, Octocorallia), we found the interesting sesquiterpene acid **1** in several gorgonians from two regions of the western Pacific Ocean.[1] Acid **1** was readily purified from Guam collections of Subergorgia suberosa by initial silica gel chromatography of the crude CH_2Cl_2 extract, followed by Sephadex LH-20 chromatography (7:3 CH_2Cl_2: isooctane) to eliminate glycerides and steroids.[2] Subergorgic acid,[3] m.p. 179-180°, showed $[\alpha]_D$ -23° (c. 0.7, $CHCl_3$) and analyzed for $C_{15}H_{20}O_3$ by elemental analysis and high resolution mass spectrometry. The ^{13}C NMR spectrum of **1** had two singlet carbonyl resonances at δ 217.8 and 169.6 which, when compared with infrared absorptions at 1730, 1690, and ~3100 (broad) cm^{-1}, indicated ketone and carboxylic acid functionalities. The ^{13}C NMR spectrum also showed a trisubstituted olefinic bond which was conjugated with one of these functionalities (δ 152.1 d, and 136.6 s). This observation was reinforced by a UV absorption at 211 nm (ε=8900). Since the ^{13}C NMR resonance for the ketone carbonyl precluded conjugation (δ 217.8), subergorgic acid was formulated as an α,β-unsaturated carboxylic acid. Treatment of **1** with ethereal diazomethane produced methyl ester **2** in high yield.[4]

The molecular formula of **1** indicated six degrees of formal unsaturation. Since the ketone and α,β-unsaturated carboxylic acid functionalities accounted for three degrees of unsaturation, subergorgic acid was tricarbocyclic. Further analysis of both the ^{13}C and ^1H NMR showed a bridgehead methyl group at δ 1.22 (C-13) and two secondary methyl doublets at 1.13 (J=7.3 Hz) and 1.12 (J=6.4). Based upon the available spectral data, subergorgic acid appeared to be a tricyclic sesquiterpene acid of a new structure class.

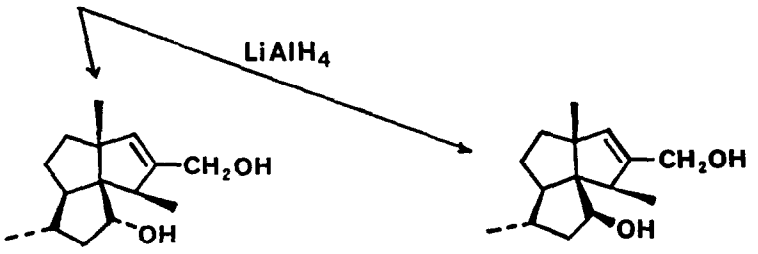
^{1}H NMR decoupling experiments were useful in defining part of the structure of **1**. Irradiation of the deshielded proton at C-9 (δ 6.43, bs) removed allylic coupling to the methine proton at C-11. Subsequent irradiation of the C-11 proton (δ 3.01, q, J=7 Hz) collapsed the doublet methyl group at 1.13 (C-15) to a sharp singlet. The lack of additional couplings with the C-9 and C-11 protons showed that the two quaternary carbons in **1** (68.5 s, 61.7 s) were adjacent to these centers. Hence the unsaturated acid and the methyl group defined by these experiments were part of one cyclopentane ring.



1 R=H; **2** R=Me



5



3 **4**

In an attempt to produce derivatives for ^{1}H NMR analysis, acid **1** was reduced with $LiAlH_4$ in cold ether to yield the epimeric diols **3** and **4**.[5] Although these derivatives were separable and readily analyzed by NMR methods, no additional structural information could be obtained. Since the structure of **1** was inadequately defined, a single crystal x-ray diffraction study was performed on subergorgic acid. Preliminary x-ray photographs displayed orthorhombic symmetry, and accurate lattice constants of a=12.899(3), b=13.201(4), c=16.165(3) Å were determined from a least squares fit of fifteen diffractometer measured 2θ-values. Systematic extinctions, crystal density, and the observed optical rotation were uniquely accomodated by space group $P2_12_12_1$ with <u>two</u> molecules of **1** forming the asymmetric unit. All unique diffraction maxima with $2\theta \leq 114°$ were recorded on a computer controlled four-circle diffractometer using variable speed, 1° ω-scans and graphite monochromated Cu Kᾱ radiation (1.54178 Å). After correction for Lorentz, polarization, and background effects, 1697(80%) reflections were judged observed ($|F_o| \geq 3\sigma(F_o)$).[6] A phasing model was found using MULTAN, and block diagonal least squares refinement with anisotropic nonhydrogen atoms and isotropic hydrogens have converged to a standard crystallographic residual of 0.0743 for the observed reflections.[7]

Figure 1 is a computer generated perspective drawing of one of the two identical subergorgic acids making up the asymmetric unit. The acid forms a hydrogen bonded dimer with a noncrystallographic two-fold axis at approximately 0.00, 0.375, 0.00. Subergorgic acid (**1**) represents a new example in the modest body of tricyclo[6.3.0.$0^{1,5}$]undecanes (triquinanes).

The basic ring system is identical to several other sesquiterpenes such as isocomene[8], silphinene[9], and senoxydene[9]. However, the substitution pattern is novel, and we suggest the name "subergane" for this new skeleton and the numbering shown in **5**.

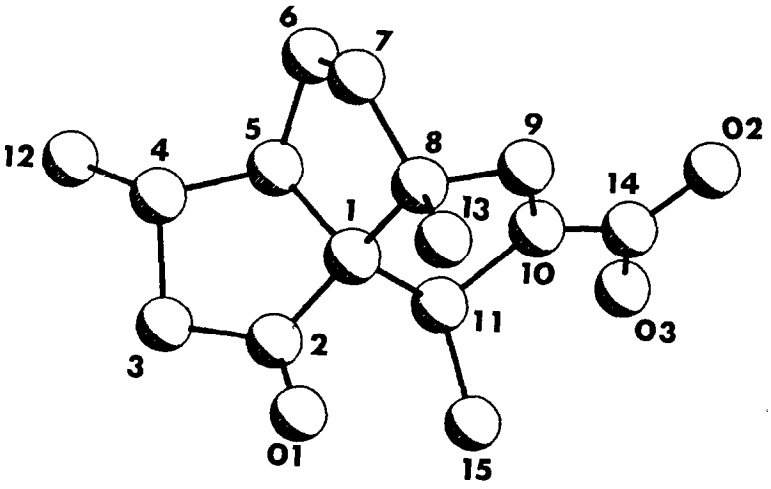


Figure 1. A computer generated perspective drawing of the final x-ray model of subergorgic acid (**1**). Hydrogens are omitted for clarity, and no absolute configuration is implied.

One of the most interesting features of subergorgic acid is its unexpected cardiotoxic properties. In the isolated guinea pig heart assay, acid **1** inhibits neuromuscular transmission at threshold levels as low as 0.16 μg/ml.[10]

ACKNOWLEDGMENTS. The Scripps research was supported by NOAA, Office of Sea Grant, Department of Commerce, under Grant #NA80AA-D-00120 project, R/MP-32, through the California Sea Grant Program, and in part by the California State Resources Agency. The Cornell research was supported by the New York State Sea Grant Program and NSF INT14133. The U.S. government is authorized to reproduce and distribute copies for governmental purposes. We wish to thank Valerie J. Paul for her valuable assistance in the collection and identification of S. suberosa in Guam.

NOTES AND REFERENCES

1. The Canton group has encountered this metabolite in an unidentified gorgonian from the South China Sea. An initial report of their work has already appeared in Zhongshan Daxue Xuebao Ziran Kexueban **1982**, 3, 69-71 (Chinese); CA 98:68827d (English). In this preliminary report, elements of stereochemistry were inaccurately assigned. This note serves to correct this situation.

2. In addition, the pregnane steroids Δ^1-pregnene-3βol-20-one, Δ^4-pregnene-3,20-dione, Δ^4-pregnene-3βol-20-one, and $\Delta^{1,4}$-pregnadiene-3,20-dione were isolated.

3. For subergorgic acid (**1**): m.p. 179-180°; $[\alpha]_D$ -23° (c. 0.7, $CHCl_3$); ^1H NMR (360 MHz, $CDCl_3$): δ 6.43 (1H, bs, C-9), 3.01 (1H, q, J=7 Hz, C-11), 2.37 (1H, dd, J=16.7, 6.7, C-3), 2.09 (1H, dd, J=8.8, 6.7, C-5), 2.01 (1H, dd, J=16.7, 12.6, C-3), 1.22 (3H, s, C-13), 1.13 (3H, d, J=7.3, C-15), 1.12 (3H, d, J=6.4, C-12); ^{13}C NMR (50 MHz, $CDCl_3$): δ217.8 (s, C-2), 169.6 (s, C-14), 152.1 (d, C-9), 136.6 (s, C-10), 68.5 (s, C-1), 61.7 (s, C-8), 62.6, 51.6, 33.3 (all d's, C-4, C-5, and C-11), 49.9, 38.2, 28.3 (all t's, C-3, C-6, and C-7), 23.4, 19.9, 17.7 (all q's, C-12, C-13, and C-15); HRMS (EI): M^+ m/z=248.1405 (26%), M^+-H_2O 230.1332 (100%), 215.1118 (29%), M^+-COOH 203.1443 (27%), 202 (30%); IR (CH_2Cl_2): 1730, 1690, and 1645 cm^{-1}.

4. For methyl ester **2**: ^1H NMR ($CDCl_3$): δ 6.28 (1H, s, C-9), 3.73 (3H, s, OMe), 3.02 (1H, q, J=7.1, C-11), 2.35 (1H, dd, J=16.6, 6.8, C-3), 2.08 (1H, dd, J=8.9, 6.8, C-5), 2.00 (1H, dd, J=16.6, 12.6, C-3), 1.20 (3H, s, C-13), 1.12 (3H, d, J=6.4, C-12), 1.11 (3H, d, J=7.1, C-15); IR (CCl_4): 1730, 1710, 1650, 1540, 1520, 1490, and 1460 cm^{-1}.

5. For alcohol **3**: ^1H NMR (360 MHz, $CDCl_3$): δ 5.14 (1H, s, C-9), 4.18 (3H, m, C-2 and C-14), 2.76 (1H, q, J=7, C-11), 1.93 (1H, ddd, J=15, 10, 4, C-3), 1.30 (3H, d, J=7, C-15), 1.15 (3H, s, C-13), 1.13 (3H, d, J=6.6, C-12); IR (CCl_4): 3600, 1640, 1540, 1520, 1500, and 1450 cm^{-1}. For alcohol **4**: ^1H NMR ($CDCl_3$): δ 5.25 (1H, s, C-9), 4.36 (1H, bs, C-2), 4.15 (2H, AB q, J=13.4, C-14), 2.36 (1H, q, J=7, C-11), 1.33 (3H, s, C-13), 1.05 (3H, d, J=7, C-15), 1.04 (3H, d, J=6.4, C-12); IR (CCl_4): 3600, 1640, 1540, 1510, 1490, and 1450 cm^{-1}. The stereochemistries of **3** and **4** were based on the downfield shift of the ^1H NMR signal of C-13 in **4** and the similar shift of C-15 in **3**.

6. All crystallographic calculations were done on a PRIME 850 computer operated by the Cornell Chemistry Computing Facility. Principal programs employed were: REDUCE and UNIQUE, data reduction programs by M. E. Leonowicz, Cornell University, 1978; MULTAN 78 and MULTAN 80, systems of computer programs for the automatic solution of crystal structures from x-ray diffraction data (locally modified to perform all Fourier calculations including Patterson syntheses) written by P. Main, S. E. Hull, L. Lessinger, G. Germain, J. P. Declercq and M. M. Woolfson, University of York, England, 1978 and 1980; BLS78A, an anisotropic block diagonal least squares refinement written by K. Hirotsu and E. Arnold, Cornell University, 1980; PLUTO78, a crystallographic illustration program by W. D. S. Motherwell, Cambridge Crystallographic Data Centre, 1978; and BOND, a program to calculate molecular parameters and prepare tables written by K. Hirotsu, Cornell University, 1978.

7. Crystallographic data have been deposited with the Cambridge Crystallographic Data Centre, University Chemical Laboratory, Lensfield Road, Cambridge, ENGLAND CB2 1EW and are available from them. Please include a complete literature citation when ordering.

8. Zalkow, L.H.; Harris, R.N.; Van Derveer, D.; Bertrand, J.A. J. C. S. Chem. Commun. **1977**, 456.

9. Paquette, L.A.; Leone-Bay, A. J. Am. Chem. Soc. **1983**, 105, 7352 and Paquette, L.A.; Annis, G.D. J. Am. Chem. Soc. **1983**, 105, 7358 and references cited therein.

10. These data were provided via a collaborative research program with Professor Robert Jacobs, University of California, Santa Barbara. A complete assessment of the biological properties of this new terpene acid will be provided by Professor Jacobs and his students.

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