Tetrahedron Letters,Vol.23,No.34,pp 3459-3462,1982 0040-4039/82/343459-04$03.00/0
Printed in Great Britain

THE ISOLATION OF NEW NORCYCLOARTENE TRITERPENOIDS FROM THE TROPICAL MARINE ALGA TYDEMANIA EXPEDITIONITIS (CHLOROPHYTA)

Valerie J. Paul and William Fenical*
Institute of Marine Resources
Scripps Institution of Oceanography
La Jolla, CA 92093

and

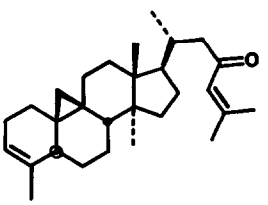
Shahin Raffii and Jon Clardy*
Department of Chemistry - Baker Laboratory
Cornell University
Ithaca, NY 14853

Summary: Three new Δ^3-norcycloartene triterpenoids, with differing side chain functionalities, have been isolated from the tropical green alga Tydemania expeditionitis collected in Guam. The structures of these new compounds were secured by x-ray crystallography and by chemical interconversion.

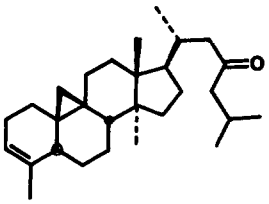
Although the triterpenes are exceptionally abundant metabolites in terrestrial organisms (over 750 compounds reported from 29 main skeletal classes[1]), few true triterpenoids have been isolated from marine sources.[2] As part of our investigations of the secondary metabolites from siphonous green algae (Chlorophyta) of the families Caulerpaceae and Udoteaceae, we investigated the tropical green alga Tydemania expeditionitis Weber-van Bosse, collected in Guam. Rather than finding the linear sesqui- and diterpenoid enol acetates more commonly produced by algae of the family Udoteaceae[3], we encountered three new Δ^3-norcycloartene derivatives $\underline{1}$-$\underline{3a}$ with different side-chain functionalities (C23-C25). These nortriterpenoids lack the toxicity and feeding deterrent properties associated with the terpenoid enol acetates usually produced by this group.

Freshly collected T. expeditionitis[4] was preserved in IPA and later repeatedly extracted with $CHCl_3$. The combined extracts were reduced to a viscous tar and the $CHCl_3$-soluble fraction (1.0 g) was fractionated over an open column of silica gel (EtOAc in isooctane). Fractions eluted with 5% EtOAc/isooctane were further purified by preparative HPLC (5 μ Si, 6% EtOAc/isooctane) to yield the three new Δ^3-norcycloartenes $\underline{1}$, $\underline{2}$ and $\underline{3a}$ as 1, 10 and 5% of the extract, respectively.

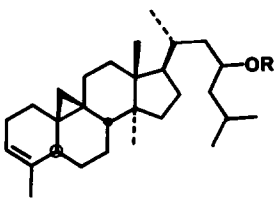
Norcycloartene $\underline{1}$[5] analyzed for $C_{29}H_{44}O$ by high-resolution mass spectrometry. The lone oxygen atom in $\underline{1}$ could be confidently assigned as part of an α,β-unsaturated ketone functionality based upon its characteristic IR ($\nu_{C=O}$ 1675 cm^{-1}, $\nu_{C=C}$ 1610 cm^{-1}) and UV absorption (λ_{max}^{MeOH} = 238 nm, ε=11,400). The presence, in $\underline{1}$, of an additional double bond and a cyclopropane ring were readily determined by ^1H NMR bands at δ 5.44 (1 H, bs) and 0.50 (1 H, d, J = 4 Hz) and -0.05 (1 H, J = 4 Hz). The eight degrees of unsaturation inherent in the formula of $\underline{1}$ were, therefore accommodated by 1 ketone, 2 olefins and 5 carbocyclic rings, one of which was cyclopropane. The complexity of this pentacyclic compound and its limited availability led to the complete solution of structure by x-ray diffraction analysis.



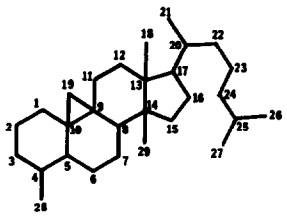
1



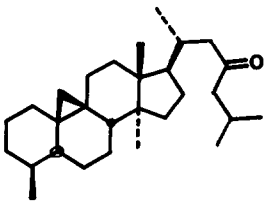
2



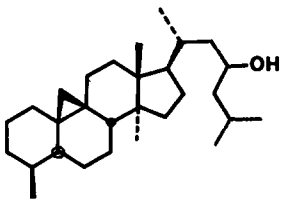
3a, R=H
3b, R=Ac



4



5



6

Suitable crystals of norcycloartene 1 were obtained from hexane. Preliminary x-ray photographs showed monoclinic symmetry and accurate lattice constants, determined by a least-squares fit of fifteen diffractometer measured 2θ-values, were $\underset{\sim}{a}$ = 12.896(2), $\underset{\sim}{b}$ = 7.601(1), $\underset{\sim}{c}$ = 12.479(2)Å and $\underset{\sim}{\beta}$ = 93.93(2)°. Systematic extinctions (0k0 absent if k = 2n+1), crystal density (1.12g/cm^3), and the presence of chirality were uniquely accommodated by the common chiral space group $P2_1$ with one molecule of composition $C_{29}H_{44}O$ forming the asymmetric unit. All unique diffraction maxima with 2θ ≤ 114° were collected on a computer controlled four-circle diffractometer using graphite monochromated CuKα radiation (1.54178Å) and a variable speed ω-scan technique. Of the 1820 reflections surveyed in this fashion, 1583 (87%) were judged observed ($|F_o| \geq 3\sigma(F_o)$) after correction for Lorentz, polarization and background effects. No crystal decomposition was detected by periodically monitoring check reflections and no absorption corrections were used.

A phasing model was achieved using a multisolution weighted tangent formula approach.[6] The E-synthesis from the phase set with the highest figure of merit showed all of the nonhydrogen atoms. Partial refinement followed by a difference electron density synthesis revealed all of the hydrogen atoms. Final refinement was done using a block diagonal least squares procedure with anisotopic nonhydrogen atoms and fixed isotropic hydrogens have converged to a conventional crystallographic discrepancy index of 0.045 for the observed reflections.[7] Figure 1 illustrates the final computer-generated perspective drawing for norcycloartene 1. The absolute configuration was not determined and that shown in the Figure was based only upon biogenic precedents.

Norcycloartene 2[8] analyzed for $C_{29}H_{46}O$ and lacked the UV absorption recognized in 1. The presence of a non-conjugated ketone IR absorption at 1710 cm^{-1}, and the fact that 2 lacked one

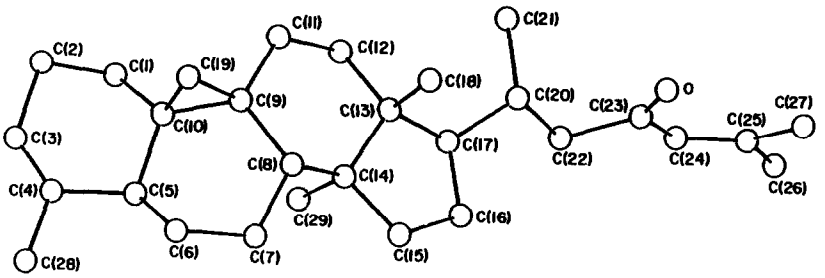


Figure 1

degree of unsaturation, suggested that 2 was the C24-C25 saturated analog of 1. To confirm this assignment, both 1 and 2 were converted to the saturated ketone 5 by catalytic hydrogenation (Pt/Et_2O/RT/20 min).[9] Compound 5 from 1 showed $[\alpha]_D$ +25.8° (c 0.7, $CHCl_3$). The comparable optical rotations of 5, as produced from both 1 and 2, confirmed 2 to possess the same absolute stereochemistry as 1 (not defined however).

The most polar norcycloartene derivative isolated, 3a,[10] analyzed for $C_{29}H_{48}O$ and showed characteristic IR alcohol absorption (ν_{OH} 3480 cm^{-1}). Acetylation (Ac_2O/py) yielded a monoacetate 3b which confirmed this latter assignment. Since 3a was a dihydro derivative of 2, it was initially assigned as the simple ketone reduction product (alcohol at C23). Oxidation of 3a with pyridinium chlorochromate (PCC) yielded the natural product 2, in high yield [$[\alpha]_D$ +16.6° (c 0.3, $CHCl_3$)]. In addition, hydrogenation of 3a (Pt/Et_2O) yielded compound 6, which when oxidized with PCC yielded the saturated ketone 5. Compound 5 via 3a showed $[\alpha]_D$ +15° (c 0.2, $CHCl_3$). This latter transformation yielded a direct comparison of 3a with the x-ray structure 1, which also established the absolute stereochemistry of 3a to be identical with 1.

The carbon skeleton of 1-3a (31-norcycloartane) has previously been observed from the terrestrial plants *Smilax aspera*[11] and *Polypodium vulgare*[12]. Since the cycloartane skeleton has been implicated in the direct biosynthesis of phytosterols,[12,13] we analyzed for the presence of more conventional sterols in this alga. The total 3β-hydroxysterol content of the extract was 0.72%, and the sterols were composed of 73% β-sitosterol, 13% cholesterol, 10% mixture of 24-methyl-24-methylenecholesterol and 4% 24-methyl-22-dehydrocholesterol.

Acknowledgements. We wish to thank Dr. Wil Kokke, Stanford University for providing sterol analyses. We gratefully acknowledge the financial support provided by the National Science Foundation (grant OCE80-14167 to WF) and the National Institutes of Health (grant CA24487 to JC).

References

1. T.K. Devon and A.I. Scott, Handbook of Naturally Occurring Compounds, Vol. II, 1972, Acad. Press, N.Y., p. 281.
2. a) B.N. Ravi, R.J. Wells, and K.D. Croft, J. Org. Chem. 46, 1998 (1981); b) E. Jegov, J. Polonsky, E. Lederer, K.H. Shulte-Elte, B. Egger and G. Ohloff, Nouveau Journal de Chimie, Vol. 1, No. 6, 529 (1977); c) U. Shmueli, S. Carmely, A. Groweiss and Y. Kashman, Tetrahedron Lett. 709 (1981).
3. a) V.J. Paul, H.H. Sun, and W. Fenical, Phytochem. 21, 468 (1982); b) T. Nakatsu, B.N. Ravi, and D.J. Faulkner, J. Org. Chem. 46, 2435 (1981); c) H.H. Sun and W. Fenical, Tetrahedron Lett., 685 (1979); d) R.J. Wells and K.D. Barrow, Experientia 35, 1544 (1979).
4. We wish to thank Ms. Jeanine Stojkovich, Department of Botany, UC-Berkeley, for the collection and identification of T. expeditionitis in Guam.
5. For norcycloartene 1: $[\alpha]_D$ +5.6° (c 1.0, $CHCl_3$); UV: λ_{max}^{MeOH}=238 nm, ε = 11,400; IR ($CHCl_3$): 2940, 1675, 1610, 1450, 1380 cm^{-1}; HRMS: M^+ m/z 408.3397 for $C_{29}H_{44}O$; ^1H NMR (360 MHz, CCl_4): δ 6.09 (1 H, bs), 5.44 (1 H, bs), 2.56 (1 H, d, J = 14 Hz), 2.44 (1 H, d, J = 14 Hz), 2.28 (3 H, s), 2.17 (6 H, m), 2.04 (3 H, s), 1.73 (6 H, bs), 1.47 (4 H, m), 1.42 (4 H, m), 1.18 (3 H, s), 1.07 (6 H, s), 1.02 (3 H, d, J = 7 Hz), 0.50 (1 H, d, J = 4 Hz), -0.05 (1 H, d, J = 4 Hz).
6. All crystallographic calculations were done on a PRIME 750 computer operated by the Department of Chemistry, Cornell University and funded in part by NSF 80-26-27. The principal programs used were REDUCE and UNIQUE, data reduction programs, Leonowicz, M.E., Cornell University, 1978; BLS78A, anisotropic block-diagonal least squares refinement, Hirotsu, K. and Arnold, E., Cornell University, 1980; XRAY76, the X-ray System of Crystallographic Programs, edited by Stewart, J.M., University of Maryland, Technical Report TR-455, March, 1976: ORTEP, crystallographic illustration program, Johnson, C.K., Oak Ridge, ORNL-3794; BOND, molecular metrics program, Hirotsu, K., Cornell University, 1978; MULTAN-68, "A system of Computer Programs for the Automatic Solution of Crystal Structures from X-ray Diffraction Data". University of York, England. Principal author P. Main. For literature description of MULTAN see: Germain, G.; Main, P.; Woolfson, M.M.; Acta Crystallogr. Sect. B. 1970, 26, 274-285 and Woolfson, M.M. Acta Crystallogr. Sect. B., 1977, 33, 219-225.
7. Further details (supplementary data) for norcycloartene 1 have been deposited in the Cambridge Crystallographic Data Center.
8. For norcycloartene 2: $[\alpha]_D$ +16.1° (c 1.4, $CHCl_3$); HRMS M^+ m/z = 410.3534 for $C_{29}H_{46}O$; IR ($CHCl_3$): 2960, 1710, 1460, 1375 and 1215 cm^{-1}; ^{13}C NMR (50 MHz, $CDCl_3$): 137.9 s, 121.8 d, 52.6 z, 51.9 d, 50.8 z, 49.5 s, 45.5 s, 42.9 d, 40.6 d, 34.2 z, 32.9 s, 32.6 z, 29.4 s, 28.1 d, 27.3 t, 25.4 t, 24.5 d, 23.5 z, 23.2 t, 22.7 q, 22.5 q, 22.2 t, 20.9 q, 19.5 q, 18.3 q, 16.4 q, 12.6 z, 28 c observed; ^1H NMR (360 MHz, CCl_4): δ 5.27 (1 H, s), 2.31 (1 H, d, J = 14 Hz), 2.14 (2 H, m), 1.09 (1 H, m), 2.00 (4 H, m), 1.72 (1 H, m), 1.5 (4 H, m), 1.34 (1 H, m), 1.24 (4 H, m), 0.99 (3 H, s), 0.91 (3 H, d, J = 6 Hz), 0.89 (6 H, s), 0.88 (3 H, s), 0.83 (3 H, d, J = 7 Hz), 0.50 (1 H, d, J = 4 Hz), -0.10 (1 H, d, J = 4 Hz).
9. For the perhydroketone 5: IR ($CHCl_3$): 2960, 2930, 1700, 1460 and 1375 cm^{-1}; ^1H NMR ($CDCl_3$): δ 2.32 (1 H, d, J = 14 Hz), 2.14 (3 H, m), 2.05 (m), 1.95 (m), 1.57 (3 H, m), 1.24 (4 H, m), 1.00 (3 H, bs), 0.90 (3 H, s), 0.89 (6 H, s), 0.88 (3 H, s), 0.82 (3 H, d, J = 7 Hz), 0.55 (1 H, d, J = 4.3 Hz), 0.28 (1 H, d, J = 4.3 Hz). The stereochemistry of the C4 methyl group in 5 was assigned β based upon the predicted hydrogenation from the less hindered α face of these molecules, see: E. Kho, D.K. Imagawa, M. Rohmer, J. Kashman and C. Djerassi, J. Org. Chem. 46, 1836 (1981).
10. For norcycloartene 3a: [α] +12.6° (c 1.7, $CHCl_3$), HRMS: M^+ m/z = 412.3720 for $C_{29}H_{48}O$; IR ($CHCl_3$): 3480, 2950, 1450, 1375 cm^{-1}; ^1H NMR (360 MHz, CCl_4): δ 5.27 (1 H, bs), 3.67 (1 H, m), 2.32 (1 H, d, J = 14 Hz), 2.00 (2 H, m), 1.70 (1 H, m), 1.56 (4 H, bs), ∿1.3 (4 H, m), 1.12 (1 H, m), 0.98 (3 H, s), 0.90 (3 H, s), 0.89 (6 H, s), 0.88 (3 H), 0.48 (1 H, d, J = 4 Hz), 0.10 (1 H, d, J = 4 Hz).
11. K.N.N. Ayengar and S. Rangaswami, Tetrahedron Lett. 3567 (1967).
12. G. Berte, F. Bottari, A. Marsili, I. Morelli, M. Povani and A. Mandelbaum. Tetrahedron Lett. 125 (1967).
13. P. Benveniste, L. Hirth and G. Ourisson, Phytochem. 5, 45 (1966).

(Received in USA 21 May 1982)