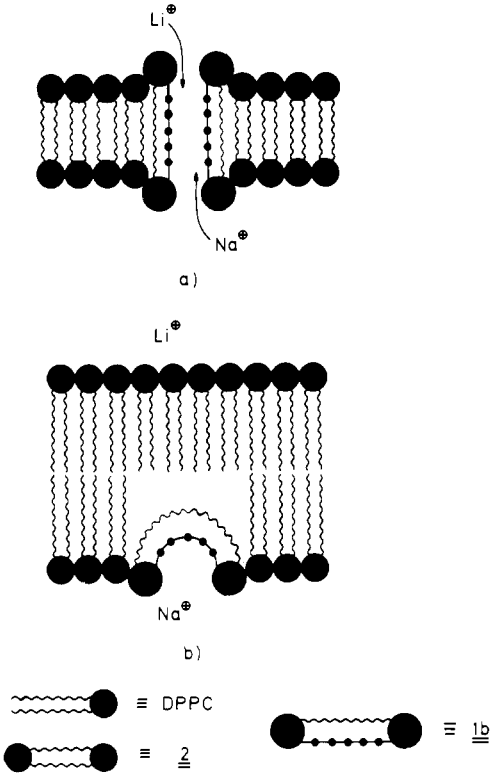


Figure 1. (a) Model of a monolayered membrane with an α,ω-double-charged channel former **1b**. The channel is presumably formed by several molecules of **1b**. (b) **1b** is too short to cross a bilayer membrane.

formation and forms complexes with alkali ions.[3] We prepared the monopyromellitic ester **1b** with pyromellitic dianhydride in pyridine.[4] In this derivative the negative charges at both ends of the molecule should favor a stretched conformation. **1b** is soluble in water at pH 7 and precipitates below pH 4.

Monolayer membrane vesicles were prepared by sonication of the symmetric bolaamphiphile **2**.[5] Lithium ions were entrapped within the inner aqueous volume, when **2** was sonicated in the presence of 0.03 M lithium chloride in the solution. Gel filtration on Sephacryl S 1000 with 0.03 M NaCl removed the external lithium ions. The chromatographed vesicles were tested for lithium ions by atomic emission spectroscopy.[6] The monolayered vesicles reproducibly entrapped the expected amounts of lithium ions. Addition of the monensin derivative **1b** to the aqueous solution *after* sonication did not change this figure. This experiment proves, that **1b** does *not* act as an ionophore for lithium ions.

If, however, the amphiphile **2** was cosonicated with 1.0, 0.1, or 0.01 mol % of **1b** no lithium was entrapped (Table I). 0.01 mol % corresponds to 1 molecule of **1b** per 10^4 molecules of **2** or in the order of 1–10 molecules of **1b** per vesicle.[7] This would indicate that less than 10 molecules of **1b** are sufficient to make a monolayer vesicle membrane penetrable to lithium ions. At 0.001 mol % of **1b** no lithium was released from the vesicles (Table I). From these experiments we conclude the formation of discrete channels (Figure 1a).

The same experiments were performed with dipalmitoylphosphatidylcholine (DPPC) bilayer membrane vesicles and 1 mol % of **1b**. No leakage of lithium occurred (Table I). The bolaamphiphile **1b** is too short to cross the bilayer lipid membrane (Figure 1b).

Table I. Lithium Concentration[a] of Gel Filtrated[b] Vesicles Containing the Channel Former **1b**

[**1b**], mol %	C_{Li^+} vesicle **2**,[c] ppb	C_{Li^+} vesicle DPPC,[c] ppb
0	29	25
0.001	28	
0.01	0	
0.1	0	
1.0	0	23

[a] ppb values from atomic emission spectra (AES) are given. [b] 1 mL of the vesicle dispersion (1.0×10^{-3} M) was applied to a Sephacryl S 1000 column (1 × 25 cm, 0.03 mol NaCl). Vesicles eluted in the 4–6-mL fraction, which was used for AES. [c] Cosonication of **1b** with **2** or DPPC at 50 °C.

It was also observed that compound **1b** itself forms vesicles on sonication. These vesicles entrapped the water-soluble dye pyranine (8-hydroxy-1,3,6-pyrenetrisulfonate),[8] but no lithium.

In summary we have presented the first ion channel, which is selective for thin membranes (≤20 Å), and proof for the thinness of the thinnest membrane reported so far. The number of channels per vesicle can be varied from, presumably, one to several thousand. Several applications of the perforated vesicle membranes can be imagined, if efficient stopcocks can be developed to close and reopen the holes. This possibility is investigated currently. Head groups different from pyromellitic acid, e.g., positively charged ammonium groups, will also be investigated.

Acknowledgment. This work was supported by the Deutsche Forschungsgemeinschaft, the Fonds der Chemischen Industrie, and the Förderungskommission für Forschung und wissenschaftlichen Nachwuchs der Freien Universität Berlin (FGS "Biomembranen").

Registry No. 1b, 91003-04-2; lithium, 7439-93-2.

(3) Anteunis, M. J. O.; Rodius, N. A. *Bioorg. Chem.* **1978**, *7*, 47.
(4) Apell, H.-H.; Bamberg, E.; Alpes, H.; Läuger, P. *J. Membr. Biol.* **1977**, *31*, 171.
(5) Mathieu, J., Dissertation Freie Universität Berlin, in preparation.
(6) Stengelin, S.; Beress, L.; Lauffer, L.; Hucho, F. In "Toxins as Tools in Neurochemistry"; Hucho, F., Ouchinnikov, Y.A., Eds.; W. de Gruyter: Berlin, New York, 1983; p 101.
(7) An average number of 1.2×10^5 molecules in the monolayer vesicle membrane was assumed. The average diameter from electron microscopy was 1000 Å.
(8) Kano, K.; Fendler, J. H. *Biochim. Biophys. Acta* **1978**, *509*, 289.

Kalihinol-A, a Highly Functionalized Diisocyano Diterpenoid Antibiotic from a Sponge

Clifford W. J. Chang,[1] Amarendra Patra,[2] Deborah M. Roll, and Paul J. Scheuer*

Department of Chemistry
University of Hawaii at Manoa
Honolulu, Hawaii 96822

Gayle K. Matsumoto and Jon Clardy*

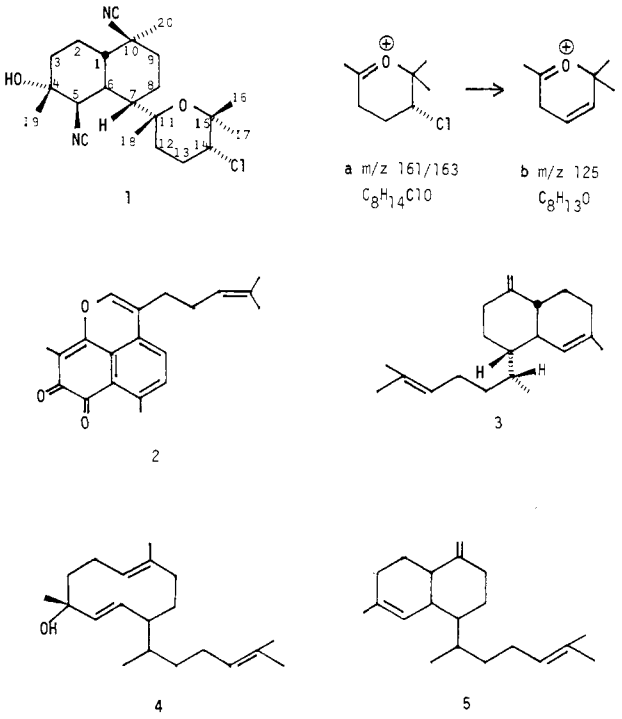
Department of Chemistry, Cornell University
Ithaca, New York 14850

Received April 27, 1984

The isocyano function occurs naturally in compounds isolated from terrestrial microorganisms and from marine sponges. A majority of the sponge metabolites have been sesquiterpenes. Isocyanoterpenes have generally been monofunctionalized and have often been accompanied by corresponding isothiocyano and formamido derivatives. We report here the structure of a new richly functionalized tricyclic diterpene, which bears isocyano, hydroxyl, tetrahydropyranyl, and chlorine functions. Compound **1**, which we have named kalihinol-A,[3] exhibits in vitro activity against *Bacillis subtilis*, *Staphylococcus aureus*, and *Candida albicans*

(1) On sabbatical leave from the University of West Florida, Pensacola, FL, Fall 1983.
(2) UNESCO Fellow from the University of Calcutta, Spring 1983.
(3) Kalihi is a residential neighborhood in Honolulu and the first author's early home. The numbering follows that of biflorin.[10]
(4) Kupchan, S. M.; Britton, R. W.; Ziegler, M. F.; Sigel, C. W. *J. Org. Chem.* **1973**, *38*, 178–179.

0002-7863/84/1506-4644$01.50/0



1 2 3 4 5

but is inactive against *Escherichia coli*.

The sponge was collected in May 1981 in Apra Harbor, Guam, and identified as an *Acanthella* sp. The freeze-dried sponge (30 g) was subjected to a Kupchan partition scheme by successive extraction with hexane, carbon tetrachloride, chloroform, and methanol. CCl_4 extract (0.47 g) had strong in vitro activity against *C. albicans*. It was further purified in 100-mg batches on Sephadex LH 20 ($CHCl_3$/MeOH, 1:1). Activity was concentrated in fraction 2, which contained the bulk of the material. Chromatography of this fraction (61 mg) on BioSil A ($CHCl_3/CCl_4$, 3:1) yielded two bioactive components, 11.5 mg of **1** and 28.1 mg of a fraction that needed HPLC purification and is not yet identified. Slow evaporation of a hexane–acetone solution of **1** yielded rectangular plates, mp 233 °C, $[\alpha]_D$ + 16° (*c* 1.0, $CHCl_3$).

Spectral characterization of kalihinol-A (**1**)[5] suggested the presence of 3° hydroxyl (no reaction with Ac_2O/pyridine), isocyano, and ether functions on the basis of IR bands at 3595, 3390, 2135, 2100 sh, and 1105 cm^{-1}. Its diterpenoid nature was revealed by methyl singlets at δ 1.40 (*gem*-OH), 1.33 (6 H), and a 3-H triplet at δ 1.29 ($J \sim 2$ Hz, thus, *gem*-NC),[6] in the ^1H NMR spectrum. A signal at δ 3.72 (1 H, dd, J = 12, 5 Hz) was first assigned to a proton at a carbon bearing an isocyano group. A broad one-proton singlet at δ 4.51 remained unchanged in D_2O/MeOD and could not be assigned.[7]

Electron impact mass spectrometry showed no molecular ion. The highest peak at m/z 357.2561 corresponded to $C_{22}H_{33}N_2O_2$ (calcd 357.2542). Significant fragments *a* at m/z 163 (71 rel %), 161 (94)[8] and *b* at 125 (94) were correctly interpreted only after chlorine was revealed by X-ray diffraction, which also clarified the one-proton signal at δ 3.72 (H-14).

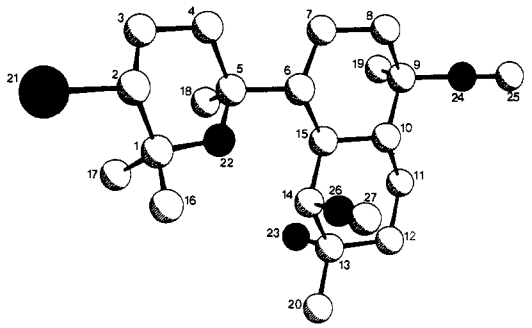


Figure 1. Computer-generated perspective drawing of kalihinol-A. Hydrogens are omitted for clarity and no absolute configuration is implied.

Preliminary X-ray photographs of kalihinol-A displayed orthorhombic symmetry. Accurate lattice constants, determined by a least-squares fit of 15 diffractometer measured 2θ values, were a = 6.899 (7) Å, b = 16.3897 (18) Å, and c = 19.3295 (15) Å. Systematic extinctions, an estimated density, and the presence of chirality was uniquely accommodated by space group $P2_12_12_1$ with one molecule of composition $C_{22}H_{33}O_2N_2Cl$ forming the asymmetric unit. All unique diffraction maxima with $2\theta \leq 114°$ were collected on a computer-controlled four-circle diffractometer using a variable-speed 1° ω-scan and graphite monochromated Cu Kα radiation (1.541 78 Å). Of the 1703 reflections surveyed, 1451 (85%) were judged observed ($|F_o| \geq 3\sigma(F_o)$) after correction for Lorentz, polarization, and background effects. A phasing model was found routinely.[9] Block diagonal least-squares refinements with an isotropic heavy atom and fixed, isotropic hydrogens have converged to a standard crystallographic residual of 0.0643 for the observed reflections. Additional crystallographic details are available and are described in the supplementary material.

Figure 1 is a computer-generated perspective drawing of the final X-ray model of kalihinol-A. Hydrogens are omitted for clarity, and since the X-ray experiment defined only the relative stereostructure, the enantiomer shown is an arbitrary choice. There are three six-membered rings in the structure: two cyclohexane rings in the *trans*-decalin and a tetrahydropyran. The conformations of each of these rings is best described as a chair. There are two isonitriles: one in an axial position at C-14 and one equatorial at C-9. There is an equatorial chlorine substituent at C-2. In general bond distances and angles agree well with generally accepted values.

Although kalihinol-A (**1**) has an unrearranged isoprenoid skeleton, it represents an uncommon bicyclic diterpenoid type. This carbon skeleton was first encountered by Prelog and coworkers[10] in the *o*-quinone biflorin (**2**) from the terrestrial plant *Capraria biflora*. A second example, biflora-4,10(19), 15-triene (**3**) was reported by Meinwald and co-workers[11] from a termite soldier. Very recently, Kashman et al.[12] isolated the diterpenoid

(5) IR ($CHCl_3$) ν_{max} 3595, 3390, 2135, 2100 sh, 1385, 1378, 1105 cm^{-1}; UV (MeOH) end absorption; ^1H NMR ($CDCl_3$, 300 MHz) δ 4.51 (1 H br s, H-5), 3.72 (1 H dd, J = 12, 5 Hz, H-14), 2.04-1.96 (3 H m), 1.72–1.44 (7 H m), 1.40 (3 H s, Me-19), 1.33 (6H, s, Me-16, Me-17), 1.29 (3 H, br t, $J \sim 2$ Hz, Me-20), 1.24 (1 H, m, H-9), 1.15 (3 H, s, Me-18), 0.98 (1 H, ddd, J = 14, 14, 3 Hz, H-7); EIHRMS m/z, M^+ – Cl, 357.2561 (calcd for $C_{22}H_{33}N_2O_2$, 357.2542), M^+ – Cl – HCN, 330.2424 (calcd for $C_{21}H_{32}NO_2$, 330.2433); EILRMS m/z, 357 (4%), 330 (7), 216 (9), 202 (8), 163 (71), 162 (18), 161 (94), 107 (58), 105 (22), 93 (34), 81 (41), 79 (25), 71 (36), 67 (53), 59 (45), 55 (40), 53 (31), 43 (58), 41 (100).

(6) *gem*-Isocyano protons are split by ^{14}N: Kuntz, I. D., Jr.; Schleyer, P. v. R.; Allerhand, A. *J. Chem. Phys.* **1961**, *35*, 1533–1534.

(7) The chemical shift of known HCNC ranges from δ 3.03 for 2-isocyanopupukeanane (Hagadone, M. R.; Burreson, B. J.; Scheuer, P. J.; Finer, J. S.; Clardy, J. *Helv. Chim. Acta* **1979**, *62*, 2484–2494) to δ 3.27 for acanthellin-1 (Minale, L.; Riccio, R.; Sodano, G. *Tetrahedron* **1974**, *30*, 1341–1343). The δ 4.51 signal therefore seemed to be at too low a field to be assigned to a methine carbon bearing an isocyano group. We thank one of the referees for questioning the original assignment.

(8) Hollenbeak, K. H.; Schmitz, F. J.; Houssain, M. B.; van der Helm, D. *Tetrahedron* **1979**, *35*, 541–545. Observed an analogous fragment.

(9) 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 Leonwicz, M. E. Cornell University, 1978), MULTAN 78, a system 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) (Main, P.; Hull, S. E.; Lessinger, L.; Germain, G.; Declercq, J. P.; Woolfson, M. M. University of York, England, 1978), BLS78A, an anisotropic block diagonal least-squares refinement (Hirotsu, K.; Arnold, E. Cornell University, 1980), PLUTO78, a crystallographic Data Centre, 1978), and BOND, a program to calculate molecular parameters and prepare tables (Hirotsu, K. Cornell University, 1978).

(10) Comin, J.; Gonsalves de Lima, O.; Grant, H. N.; Jackman, E. M.; Keller-Schierlein, W.; Prelog, V. *Helv. Chim. Acta* **1963**, *46*, 409–415.

(11) Wiemer, D. F.; Meinwald, J.; Prestwich, G. D.; Solheim, B. A.; Clardy, J. *J. Org. Chem.* **1980**, *45*, 191–192.

(12) Kashman, Y.; Groweiss, A.; Carmely, S.; Kinamoni, Z.; Czarkie, D.; Rotem, M. *Pure Appl. Chem.* **1982**, *54*, 1995–2010.

obscuronatin (**4**) and its rearrangement product **5** from soft corals of the genus *Xenia*. Kalihinol-A (**1**) is the most richly functionalized example of this skeletal type.

Acknowledgment. We thank Drs. C. Ireland and G. R. Schulte for collection of the animal, Professor P. Bergquist for identification, and the National Science Foundation and the Sea Grant College Program for financial support. C.W.J.C. thanks the University of West Florida for a Faculty Development Award.

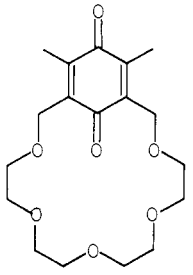
Supplementary Material Available: IR and ^{1}H NMR (300 MHz) spectra and tables of fractional coordinates, equivalent isotropic thermal parameters, bond distances, bond angles, torsional angles, and observed and calculated structure factors (20 pages). Ordering information is given on any current masthead page.

Redox-Active Crown Ethers: Molecules Designed to Couple Ion Binding with a Redox Reaction

Robert E. Wolf, Jr., and Stephen R. Cooper*†

Department of Chemistry, Harvard University
Cambridge, Massachusetts 02138
Received March 19, 1984

Coupled reactions, i.e., reactions that mutually influence each other, play a fundamental role in biological processes such as ion transport and oxidative phosphorylation.[1] To gain insight into coupled reactions we have begun exploration of the behavior of redox-active crown ethers[2] such as

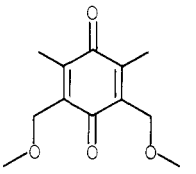


I

a class of molecules in which the proximity of the electroactive quinone group to the ion-binding crown moiety results in coupling of redox reactions of the quinone with ion bonding by the crown.[7]

Cyclic voltammetric studies of I in DMF provide strong evidence for the desired coupling between complexation and redox reactions of this molecule: the presence of alkali metal salts makes the quinone easier to reduce.[3] Replacement of 0.1 M $Et_4N^+ClO_4^-$ as supporting electrolyte with 0.1 M $M^+ClO_4^-$ (M = Li, Na) $M^+BF_4^-$ (M = K) changes the Q/SQ^- formal potential (E_f) from −0.60 V vs. SCE to −0.55, −0.48, and −0.47 V vs. SCE, respectively, shifts of +50, +120, and +130 mV. These shifts reflect metal ion binding by the crown group (with I–SQ binding Na^+ and K^+ 100 times more strongly than I[4]) and indicate that redox reactions of the quinone and ion binding by the crown influence each other, i.e., these reactions are now coupled.

Both the magnitude and cation dependence of the potential shifts establish that they arise from complex formation, and not from simple ion pairing. For example, E_f of I shifts anodically by 120 mV upon replacement of 0.1 M $Et_4N^+ClO_4^-$ with 0.1 M $NaClO_4$ as supporting electrolyte; that of benzoquinone changes by only 10 mV under the same conditions, and that of II



II

undergoes only a 20-mV shift. Clearly ion pairing between Na^+ or K^+ and the semiquinones cannot account quantiatively for the observed shifts. Moreover, anodic shifts from ion pairing should decrease with the charge/radius ratio of the cation, and therefore decline in the order $Li^+ > Na^+ > K^+$ (as observed for, e.g., benzoquinone); the shifts observed for I, however, decrease in the order $K^+ > Na^+ > Li^+$ and are more consistent with progressively worse "fit" between metal ion and crown. Thus the shifts in E_f caused by alkali metal cations *neither qualitatively nor quantitatively obey those expected for simple ion pairing*; they are, however, consistent with complexation of the metal cations, and as such they indicate coupling between complexation and redox reactions.

Parallel EPR studies of the corresponding crown semiquinone I-SQ [(I-SQ^-] = 2.5 mM, generated in DMF by reduction of the quinone crown at −0.8 V vs. SCE) provide further evidence supporting complex formation with alkali metal ions, and also yield insight into the structure of the complex formed. In the presence of 0.1 M $Et_4N^+ClO_4^-$ the EPR spectrum of I-SQ has $a(CH_3)$ = 2.05 G and $a(CH_2)$ = 0.83 G (Figure 1a). Introduction of 0.1 M $NaClO_4$ changes the spectrum strikingly to one with $a(CH_3)$ = 2.5 G and $a(CH_2)$ < 0.2 G, as well as ^{23}Na superhyperfine splitting of 0.9 G (Figure 1b); that of II-SQ is unaffected. The appearance of ^{23}Na shfs reflects the strength of complexation, while the changes in the CH_3 and CH_2 hfs for I-SQ indicate that complexation perturbs that $1b_{2u}$ (in D_{2h}) HOMO of the semiquinone, causing greater localization of electron density at the methylene end and concomitantly greater localization of spin density at the methyl end. We tentatively suggest that the semiquinone partially cants toward the plane of the complexed crown ring[5] such that the oxygen atom in the 1-position of the

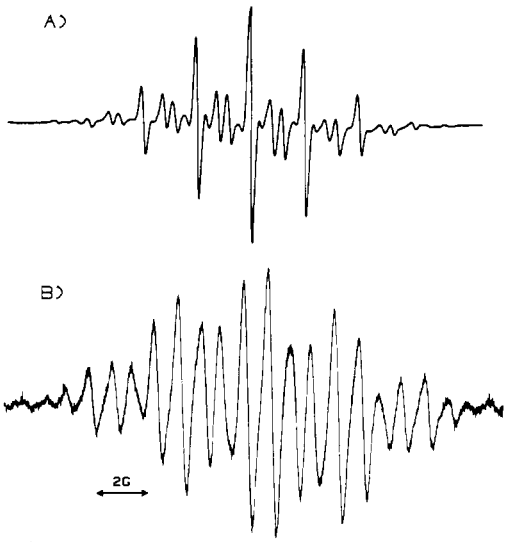


Figure 1. EPR spectra of I-SQ (a) in the absence of $NaClO_4$ and (b) in the presence of 0.2 M $NaClO_4$. Quinone crown concentration 1 mM in DMF containing 0.1 M $Et_4N^+ClO_4^-$. Modulation amplitude 0.05 G; microwave power 0.8 mW.

† Address correspondence to this author at the Inorganic Chemistry Laboratory, University of Oxford, Oxford OX1 3QR England.

(1) Racker, E. *Acc. Chem. Res.* **1979**, *12*, 338–44.

(2) Synthesis and characterization of I will be described subsequently. Wolf, R. E., Jr.; Cooper, S. R., manuscript in preparation.

(3) Only one cathodic and one anodic wave are observed for the Q/SQ^- couple (with ΔE_{pp} = 60–70 mV), consistent with complexation/decomplexation of the alkali metal ion that is rapid on the cyclic voltammetric time scale. The SQ^-/HQ^{2-} potential also shifts, but no more than does benzoquinone itself. These shifts probably derive largely from simple ion pairing and hence are uninteresting.

(4) This difference in stability constants is calculated from $E_f^{complex} - E_f^{free} = -RT/nF\ln(K_Q/K_{SQ})$.

(5) Seiler, P.; Dobler, M.; Dunitz, J. D., *Acta Crystallogr., Sect. A* **1974**, *B30*, 2744–5.

0002-7863/84/1506-4646$01.50/0