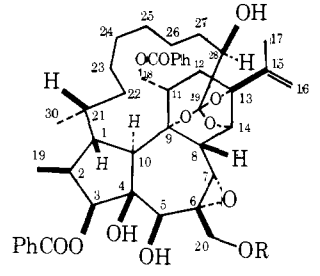
Gnidimacrin and Gnidimacrin 20-Palmitate, Novel Macrocyclic Antileukemic Diterpenoid Esters from *Gnidia subcordata*[1,2]

Sir:

In the course of a continuing search for tumor inhibitors from plant sources, we found that an ethanol extract of *Gnidia subcordata* (Meissn.) Engl. (Thymelaeaceae)[3] showed significant activity in vivo against P-388 leukemia in mice.[4] We report herein the isolation and structural elucidation of the novel and potent antileukemic principles, gnidimacrin (**1**) and gnidimacrin 20-palmitate (**2**). These appear to be the first diterpenoids which have been shown to contain a novel macrocyclic ring with one terminus at the orthoester carbon.

Fractionation of the alcohol extract, guided by a combination of P-388 in vivo assay in mice and goldfish toxicity tests,[5] revealed that both the antileukemic and piscicidal activity were concentrated, successively, in the chloroform layer of a chloroform–water partition and in the methanol layer of a 10% aqueous methanol–petroleum ether partition. Column chromatography on SilicAR CC-7 yielded two active fractions. Partition chromatography of each fraction, followed by preparative thin layer chromatography on ChromAR and silica gel, afforded gnidimacrin (**1**, 0.0005%): (mp 172–174 °C (methanol); $[\alpha]^{24}D$ −3.9° (*c* 0.51, $CHCl_3$); uv_{max} (MeOH) 229 nm (ϵ 23 000); ir ($CHCl_3$) 2.82, 5.82, 6.25, 6.32 μ. Anal. Calcd for $C_{44}H_{54}O_{12}\cdot CH_3OH$: C, 66.98, H, 7.24. Found: C, 66.80, H, 7.19. Mass spectrum (chemical ionization:methane reagent gas) *m/e* 775.3679 ($M^+ + H$, calcd 775.3691), 739, 635, 513, 123, 105; NMR ($CDCl_3$) τ 8.24 (3 H, s, 17-H), 7.03 (1 H, d, J = 2.5 Hz, 8-H), 6.71 (1 H, s, 7-H), 6.24 (2 H, s, 20-H), 6.01 (1 H, s, 5-H), 5.71 (1 H, d, J = 2.5 Hz, 14-H), 5.70 (1 H, dd, J = 10.5, 10.5 Hz, 18-H), 5.12 (1 H, br d, J = 10.5 Hz, 18-H), 5.07 (1 H, d, J = 6.0 Hz, 3-H), 5.15, 4.91 (each 1 H, br s, 16-H), 2.76–1.86 (10 H, m)) and gnidimacrin 20-palmitate (**2**, 0.000 01%): ($C_{60}H_{84}O_{13}$; $[\alpha]^{24}D$ −1.5 (*c* 0.59, $CHCl_3$); uv_{max} (MeOH) 229 nm (ϵ 25 000); ir ($CHCl_3$) 2.84, 3.42, 3.50, 5.81, 6.24, 6.30, 7.78, 7.87 μ; mass spectrum (chemical ionization:methane reagent gas) *m/e* 1013 ($M^+ + H$), 891, 775, 635, 257, 123, 105; NMR ($CDCl_3$) τ 8.18 (3 H, s, 17-H), 6.94 (1 H, d, J = 2.5 Hz, 8-H), 6.73 (1 H, s, 7-H), 6.19, 5.17 (2 H, ABq, J = 12.0 Hz, 20-H), 5.98 (1 H, s, 5-H), 5.64 (1 H, d, J = 2.5 Hz, 14-H), 5.70 (1 H, dd, J = 10.5, 10.5 Hz, 18-H), 4.97 (1 H, br d, J = 10.5 Hz, 18-H), 5.03 (1 H, d, J = 6 Hz, 3-H), 5.05, 4.83 (each 1 H, br s, 16-H), 2.66–1.79 (10 H, m)).

Treatment of gnidimacrin (**1**) at room temperature with *p*-iodobenzoyl chloride afforded gnidimacrin 20-*p*-iodobenzoate (**3**): $C_{51}H_{57}IO_{13}$; mp 176–178 °C; uv_{max} (MeOH) 229 nm (ϵ 26 000), 255 (ϵ 17 500); ir 2.84, 5.81, 6.24, 6.29, 7.84 μ.



1, R = H
2, R = $COC_{15}H_{31}$
3, R = COC_6H_4I

The molecular structure and absolute stereochemistry of gnidimacrin were established by a single-crystal x-ray analysis, conducted at liquid nitrogen temperature, of the 20-*p*-iodobenzoate (**3**). Crystals of the derivative conform to space group $P2_12_12_1$ with a = 38.895 (7), b = 18.518 (3), and c = 13.446 (2) Å (λ 1.5418 Å, T = 113 K). The observed density at room temperature, by flotation, is 1.360 g cm^{-3}, clearly consistent with two molecules of the complex in the asymmetric unit. The analysis has also established the presence of eight molecules of water of hydration in the unit cell yielding a calculated room temperature density of 1.361 g cm^{-3}, in excellent agreement with that observed. The analysis thus required the location of 132 non-hydrogen atoms.

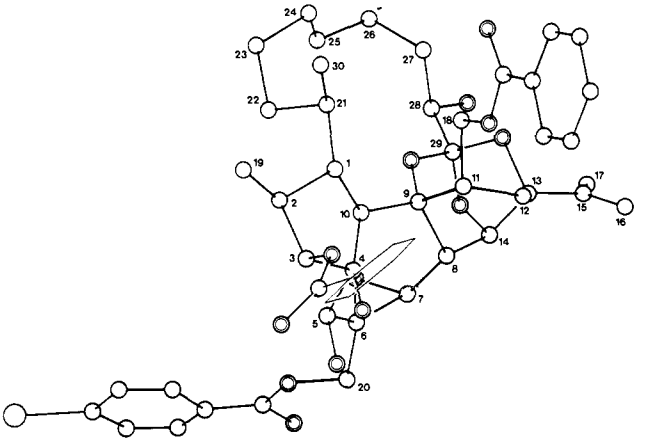


Figure 1. Diagrammatic view of one of the two crystallographically independent molecules in the asymmetric unit seen in the conformation adopted in the crystal. Single circles represent carbon, double circles oxygen, and the larger circle iodine. Carbon atoms are numbered to correspond with the scheme adopted in the structural formula of **1**.

Scattered intensity significantly above background [$I > 2\sigma(I)$] was measured by diffractometry at 5645 of the 6972 independent reciprocal lattice points examined to sin θ/λ = 0.532. The structure was solved by the heavy atom method and refined by Fourier and least-squares methods to a current R of 0.119 with anisotropic thermal parameters adopted for I, O, and C. Most of the hydrogen atoms are visible in a three-dimensional difference electron-density map but have not yet been included in the calculations.

The absolute configuration of the *p*-iodobenzoate derivative was established at an early stage in the refinement by application of Hamilton's R ratio test.[6] A convincing difference in R (0.236 vs. 0.250) emerged for the two possible enantiomer structures when the anomalous dispersion terms for iodine[7] were included in separate structure factor calculations. Refinement of the preferred enantiomer included allowance for these terms and at the present stage of refinement R for the opposite enantiomer is 0.155. Independent confirmation of the absolute configuration has been obtained through measurement of intensity differences in 22 selected Bijvoet pairs of reflections having pronounced differences in F_c for the two structures.

A view of the molecular structure of one of the two independent molecules of **3** is given in Figure 1. Both molecules in the asymmetric unit are chemically identical and show only slight differences in conformation as defined by their endocyclic torsion angles.

A comparison of the molecular formulas and spectral data of **1** and **2** led to the inference that **2** was the palmitate ester of **1**. Proof was obtained by direct transformation; thus upon treatment with palmitoyl chloride in pyridine **1** yielded a less polar compound, which was shown to be identical with **2**. The NMR signals for the C-20 protons of **2** appeared at lower magnetic field than the corresponding signals of **1**, which indicated that C-20 was the point of attachment of the palmitate ester.

Gnidimacrin and gnidimacrin 20-palmitate are members

of the daphnetoxin class of diterpenoid esters which includes such compounds as gnididin,[8] gnidilatin 20-palmitate,[9] and mezerein.[10] It is noteworthy that gnidimacrin and gnidimacrin 20-palmitate exhibit the most potent antileukemic activity of any members of the daphnetoxin class reported to date, and are the first such compounds found to contain the novel macrocyclic orthoester structural feature. Investigations are in progress to determine the potential significance of the macrocyclic orthoester, the epoxide, and other structural features in relation to the biological activity of gnidimacrin and gnidimacrin 20-palmitate.

References and Notes

(1) Tumor Inhibitors. 116. Part 115: S. M. Kupchan, J. A. Lacadie, G. A. Howie, and B. R. Sickles, *J. Med. Chem.*, in press.

(2) Supported by research grants from the National Cancer Institute (N.C.I.) (CA-11718, CA-11760, CA-17562, and CA-12059) and the American Cancer Society (CI-102K), and contracts with the Division of Cancer Treatment, N.C.I., National Institutes of Health (N01-CM-12099 and N01-CM-67002).

(3) Leaves were collected in Kenya in February, 1974. The authors acknowledge with thanks receipt of the dried plant material from Dr. R. E. Perdue, Jr., U.S.D.A., Beltsville, Md., in accordance with the program developed by the National Cancer Institute.
(4) Antileukemic activity was assayed under the auspices of the National Cancer Institute, by the procedures described by R. I. Geran, N. H. Greenberg, M. M. McDonald, A. M. Schumacher, and B. J. Abbott, *Cancer Chemother. Rep., Part 3*, **3**, 1 (1972). Gnidimacrin (**1**) showed optimal values of T/C of about 180 at dosage levels of 12–16 μg/kg, and gnidimacrin 20-palmitate (**2**) showed optimal values of T/C of about 190 at the 30–50 μg/kg level.
(5) Piscicidal activity was assayed using a procedure similar to that described by W. A. Gersdorff, *J. Am. Chem. Soc.*, **52**, 3440 (1930). Gnidimacrin showed toxicity at a concentration of 100 μg/l.
(6) W. C. Hamilton, *Acta Crystallogr., Sect. A*, **25**, 165 (1969).
(7) "International Tables for X-Ray Crystallography", Vol. IV, Kynoch Press, Birmingham, England, 1974, pp 148–151.
(8) S. M. Kupchan, J. G. Sweeny, R. L. Baxter, T. Murae, V. A. Zimmerly, and B. R. Sickles, *J. Am. Chem. Soc.*, **97**, 672 (1975).
(9) S. M. Kupchan, Y. Shizuri, W. C. Sumner, Jr., H. R. Haynes, A. P. Leighton, and B. R. Sickles, *J. Org. Chem.*, in press.
(10) S. M. Kupchan and R. L. Baxter, *Science*, **187**, 652 (1975).

S. Morris Kupchan,* Yoshikazu Shizuri, Tatsushi Murae
James G. Sweeny, H. Richard Haynes, Ming-Shing Shen
James C. Barrick, Robert F. Bryan*
Department of Chemistry, University of Virginia
Charlottesville, Virginia 22901

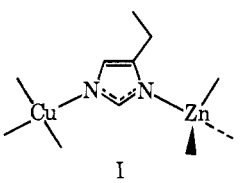
Dick van der Helm, Kang K. Wu
Department of Chemistry, University of Oklahoma
Norman, Oklahoma 73069

Received May 18, 1976

Imidazolate-Bridged Complexes of Copper(II)

Sir:

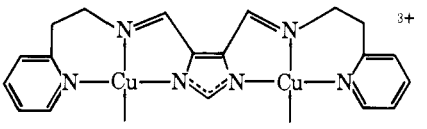
Recent studies[1] of bovine superoxide dismutase (SOD)[2] have revealed the presence of a bimetallic copper(II)–zinc(II) center at the active site. X-Ray crystallographic studies[3] of the enzyme at 3.0 Å resolution showed the metal atoms to be about 6 Å apart, with His 61 occupying the intervening space. This result is consistent with the presence of a bridging imidazolate ion, I. Although imidazolate (im) bridged metals are known



I

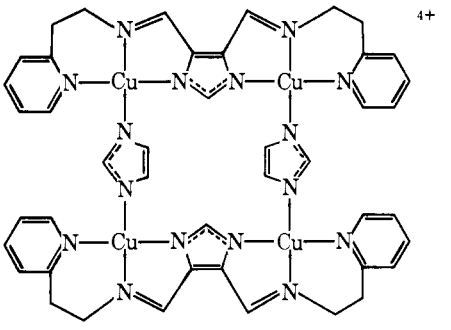
to exist in crystalline solids such as $Cu_3(imH)_8(im)_2(ClO_4)_4$,[4] $M(im)_2$, M = Cu, Zn,[5] and $Cu(imH)_2(im)Cl$,[6] no soluble binuclear or small polynuclear complexes containing this ion as a bridging ligand have apparently been characterized.[7] Such complexes would be interesting to study in conjunction with investigations of the chemical and physical properties of I in bovine SOD. A program to prepare and characterize soluble, imidazolate bridged metal complexes has therefore been undertaken. Here we report preliminary results describing the synthesis of copper(II) complexes in this class, the x-ray crystal structure of one such derivative, the effect of pH on the stability of the imidazolate bridged dicopper(II) moiety, and the magnetic exchange promoted by the bridging imidazolate ligand.

Imidazole-4,5-dicarboxylic acid was converted to the methyl diester, reduced with $LiAlH_4$ to the dialcohol, and then oxidized with MnO_2 to the dicarboxaldehyde.[8] A solution of 0.5 mmol of the imidazole-4,5-dicarboxyaldehyde and 0.5 mmol of aqueous NaOH was added to an aqueous methanolic solution containing 1 mmol each of 2-(2-aminoethyl)pyridine and cupric nitrate. After stirring the mixture at 35 °C for 18 h, the solvent was removed from the resulting blue-green solution in vacuo. The residue was dissolved in methanol and filtered. Upon standing, the filtrate yielded blue microcrystals (λ_{max}^{MeOH} 637 nm; 650 nm in H_2O, pH 4) which analyzed as the dicopper complex of the ligand 4,5-bis[2-(2-pyridyl)-ethyliminomethyl]imidazolate (bpim), $Cu_2(bpim)(NO_3)_3 \cdot 2H_2O$,[9] containing II. Addition of 1 mmol of imidazole to the



II

same blue-green solution before removal of solvent gave an immediate color change to blue. Workup in a similar manner produced blue needles ($\lambda_{max}^{CH_3OH}$ 608 nm) of a nitrate salt of $[Cu_2(bpim)(im)]_2^{4+}$ which, upon recrystallization from water, gave beautiful blue tetragonal prisms of $[Cu_2(bpim)(im)]_2(NO_3)_4 \cdot 4H_2O$,[10] containing III.



III

This compound crystallizes in the tetragonal system, space group $I4_1/a$, with eight formulas in a unit cell of dimensions $a = b = 27.204(10)$ Å, $c = 14.704(6)$ Å, ρ_{calcd}[10] = 1.674 g/cm^3, $\rho_{obsd} = 1.650(2)$ g/cm^3. The structure, shown in Figure 1, was determined by heavy atom methods using 2821 independent reflections ($2\theta \leq 50°$, $F_o^2 > 2\sigma(F_o^2)$) collected on a computer controlled diffractometer at 23° using monochromatized Mo Kα radiation. The value for the conventional agreement factor, $R_1 = \sum \| F_o | - | F_c \| / \sum | F_o |$, at the present stage of refinement is 0.066. Details will be reported later.

The structure determination confirms that the $Cu_2(bpim)^{3+}$ moiety (II) contains an imidazolate bridge. In III, two of these units are linked by two additional bridging imidazolate ligands. The resulting dimer has crystallographically required twofold symmetry. Selected geometric details are given in the caption to Figure 1. Water and nitrate oxygen atoms are weakly coordinated to the remaining, axial sites on copper in the crystal.