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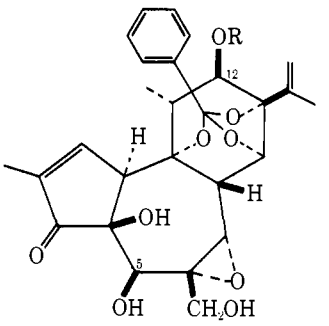
Gnididin, Gniditrin, and Gnidicin, Novel Potent Antileukemic Diterpenoid Esters from *Gnidia lamprantha*[1,2]

Sir:

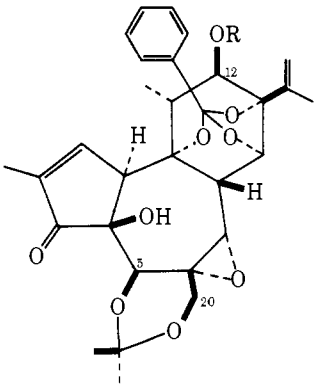
In the course of a continuing search for tumor-inhibitory natural products, we found that the ethanol extract of *Gnidia lamprantha* Gilg (Thymelaeaceae)[3] showed significant activity *in vivo* against P-388 leukemia in mice.[4] We report herein the isolation and structural elucidation of the novel and potent antileukemic principles, gnididin (**1**), gniditrin (**2**), and gnidicin (**3**). These diterpenoids are the first members of the rare daphnetoxin class which have been shown to possess antitumor activity.

Fractionation of an ethanol extract, guided initially by P-388 *in vivo* assay and later by a combination of this and goldfish toxicity tests[5] revealed that both the antileukemic and piscicidal activity were concentrated in the chloroform layer of a chloroform–water partition. Successive column chromatography on SilicAR CC-7, alumina (Merck, Act. I), alumina (Merck, Act. II), and SilicAR CC-7 followed by partition chromatography on Celite yielded two active fractions (A and B). Fraction A was shown to be a mixture of two closely related compounds which were separated by preparative thin layer chromatography on Chromar to give gnididin (**1**, 0.0002%) ($C_{37}H_{44}O_{10}$; $[\alpha]^{22}D$ +49° (*c* 0.157, $CHCl_3$); uv(max) (EtOH) 260 nm (ϵ 27,750); ir (KBr) 2.90, 5.83, 6.10, 6.18 μ; mass spec *m/e* 648.2945 (M^+ calcd 648.2932), 481, 151, 105; nmr ($CDCl_3$) τ 9.11 (3 H, br m, 10′-H), 8.62 (3 H, d, J = 7 Hz, 18-H), 7.50 (1 H, q, J = 7 Hz, 11-H), 6.37 (2 H, m), 6.08 (3 H, m), 5.73 (1 H, br s, 5-H), 4.98 (2 H, br s, 16-H), 4.89 (1 H, s, 12-H), 4.27 (1 H, d, J = 14.5 Hz, 2′-H), 3.85 (3 H, m)) and gniditrin (**2**, 0.0002%) ($C_{37}H_{42}O_{10}$; $[\alpha]^{22}D$ +51° (*c* 0.205, $CHCl_3$); uv(max) (EtOH) 306 nm (ϵ 31,800), 245 (10,400); ir (KBr) 2.89, 5.83, 6.18 μ; mass spec *m/e* 646.2776 (M^+ calcd 646.2776), 481, 149, 105; nmr ($CDCl_3$) τ 9.08 (3 H, t, J = 7.6 Hz, 10′-H), 8.63 (3 H, d, J = 7 Hz, 18-H), 7.49 (1 H, q, J = 7 Hz, 11-H), 6.37 (2 H, m), 6.08 (3 H, m), 5.73 (1 H, br s, 5-H), 4.98 (2 H, br s 16-H), 4.89 (1 H, s, 12-H), 4.6–4.0 (5 H, br m), 4.20 (1 H, d, J = 15 Hz, 2′-H).

The structures of **1** and **2** were determined by a combination of spectral and chemical evidence. Methanolysis of gnididin (**1**) yielded the tetrol **5** and a methyl ester which was identified as methyl *n*-deca-*trans*-2-*trans*-4-dienoate by gc–mass spectral (M^+, *m/e* 182) and by catalytic reduction to methyl *n*-decanoate. The positions and trans nature of the double bonds in the ester side chain were indicated by the intense absorption at 260 nm (ϵ 27,750) in the uv spectrum of **1**.[6] The tetrol **5** was identified as 12-hydroxydaphnetoxin by comparison (ir, uv, mass spectral, nmr, and tlc) with an authentic sample obtained by methanolysis of mezerein (**4**).[7,8] In the nmr spectrum of **1** the resonance (τ 4.89, s, 1 H) attributed to the methine at the esterified position (shifted to τ 6.00 in the spectrum of **5**) would be consistent with esterification at either C-5 or C-12, since neither this signal nor that assigned to the methine under the secondary hydroxyl (τ 5.73) showed discernible coupling. However, on treatment with acetone and *p*-toluenesulfonic acid,[9] gnididin (**1**) readily afforded an acetonide (**7**, mass spec M^+ at *m/e* 688; nmr τ 8.49, 8.54, 2s, 6 H) in the nmr spectrum of which the signal attributed to the proton of the secondary hydroxyl methine (τ 5.86, s, 1 H) was observed at higher field than in the spectrum of **1**, indicating acetonide formation at C-5 and C-20.[10] Hence gnididin could be assigned the structure **1**.



1, R = COCH=CHCH=CH(CH$_2$)$_4$CH$_3$
2, R = COCH=CH(CH=CH)$_2$(CH$_2$)$_2$CH$_3$
3, R = COCH=CHC$_6$H$_5$
4, R = COCH=CHCH=CHC$_6$H$_5$
5, R = H
6, R = COC$_6$H$_5$



7, R = COCH=CHCH=CH(CH$_2$)$_4$CH$_3$
8, R = COCH=CH(CH=CH)$_2$(CH$_2$)$_2$CH$_3$
9, R = COCH=CHC$_6$H$_5$
10, R = COCH=CHCH=CHC$_6$H$_5$
11, R = H
12, R = COC$_6$H$_5$

The structure of gniditrin (**2**) was established in essentially the same manner as for gnididin (**1**). Thus methanolysis of **2** afforded 12-hydroxydaphnetoxin (**5**) and methyl *n*-decatrienoate and treatment of **2** with acetone and *p*-toluenesulfonic acid yielded the corresponding acetonide **8** [mass spec *m/e* 686.3080 (M^+ calcd for $C_{40}H_{46}O_{10}$, 686.3089); nmr τ 8.51, 8.54, 2s, 6 H].

Preparative thin layer chromatography of fraction B on Chromar yielded a third antileukemic compound, gnidicin (**3**, 0.0002%): $C_{36}H_{36}O_{10}$; $[\alpha]^{22}D$ +86.5° (*c*, 0.155, $CHCl_3$); uv(max) (EtOH) 280 nm (ϵ 21,000), 224 (17,700); ir (KBr) 2.90, 5.83, 6.10 μ; mass spec *m/e* 628.2295 (M^+, calcd 628.2309), 610, 597, 481, 105; nmr ($CDCl_3$) τ 8.60 (3 H, d, J = 7.5 Hz, 18-H), 7.43 (1 H, q, J = 7.5 Hz, 11-H), 6.33 (2 H, m), 6.09 (3 H, m), 5.74 (1 H,

s, 5-H), 4.94 (2 H, br s, 16-H), 4.82 (1 H, s, 12-H), 3.61 (1 H, d, J = 16 Hz, 2′-H), 2.34 (1 H, d, J = 16 Hz, 3′-H). Gnidicin (**3**) afforded 12-hydroxydaphnetoxin (**5**) and methyl cinnamate on methanolysis and an acetonide **9** (mass spec, M^+ at m/e 668; nmr τ 8.53, br s, 6 H) under similar conditions to those used above. Gnidicin (**3**) may be the same as the compound isolated by Coetzer and Pieterse[11] from *Lasiosyphon burchellii* and characterized by its hydrolysis products.

It is noteworthy that 12-hydroxydaphnetoxin (**5**), which bears no ester function at C-12, shows no antileukemic activity. In contrast, the benzoate ester **6** [$C_{34}H_{34}O_{10}$; $[\alpha]^{26}D$ +64° (c 0.24, $CHCl_3$); uv(max) (EtOH) 231 nm (ϵ 18,400); mass spec m/e 602.2152 (M^+, calcd 602.2152), prepared by benzoylation of 12-hydroxydaphnetoxin 5,20-acetonide (**11**) followed by acid hydrolysis of **12**] shows antileukemic activity of the same order as the naturally occurring esters **1**, **2**, and **3**. The results to date support the hypothesis that the ester affixed at C-12 may act as a carrier moiety (*e.g.*, in processes concerned with cell penetration or selective molecular complex formation).[12] Investigations are in progress to determine the significance of the epoxide, the cyclopentenone, the orthoester, and of other structural features for the antileukemic activity of these diterpenoid esters and related compounds.

References and Notes

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(3) Roots were collected in Kenya in June, 1972. We thank Dr. R. E. Perdue, Jr., U.S.D.A., Beltsville, Md., for supplying the plant material.
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Consideration of a Report[1] on the Formulation of Monomeric Cobalt–Dioxygen Adducts. Continued Support for $Co(III)-O_2^-$

Sir:

In recent years there has been considerable interest in the 1:1 binding of dioxygen, O_2, to cobalt(II) complexes. The thermodynamics of the binding reaction have been explored, not only with solution complexes[2] but with cobalt porphyrin incorporated into hemoglobin and myoglobin.[3]

On the basis of the frozen solution epr spectra of dioxygen adducts of low-spin cobalt(II) Schiff base-complexes, it was concluded[4] that the majority (> 80%) of the spin density from the parent compound is transferred to dioxygen upon complexation. This fact, coupled with an observed O–O stretching frequency of ~1130 cm^{-1}, similar to that of O_2^-,[5] led to the formulation of the complexes as $Co(III)-O_2^-$; that is, that the best formal description is that of a superoxo complex of low-spin cobalt(III).[4] It was further suggested that the dioxygen is singly coordinate and exhibits a bent geometry (Pauling structure).[6] Studies[7] of oxygenated cobalt porphyrin systems, coboglobins, and other cobalt complexes give quite similar epr spectra, suggesting similar electronic and steric properties of all monomeric cobalt–dioxygen complexes.

This view of the properties of $Co-O_2$ was supported by the X-ray study[8] of a cobalt(II) dioxygen adduct, in which Co–O–O is nonlinear and with an O–O bond length similar to that of a superoxide anion. Further confirmation was obtained in studies[9,10] employing ^{17}O labeled O_2, where the spin density on oxygen is found to be close to 100%. The formulation $Co(III)-O_2^-$ is also in accord with the observation[2d] that the oxygen uptake of cobalt(II) Schiff base chelates increases with the ease of oxidation of the chelates and with increasing solvent polarity.

Carbon monoxide and alkyl isocyanide complexes of cobalt(II) porphyrins have also been reported.[11] Carbon monoxide binds weakly, and will not bind to a five-coordinate Co(II) porphyrin;[7d] however, CO will act as a fifth ligand to a four-coordinate Co(II) porphyrin. The epr spectra obtained are characteristic of a low-spin Co(II) adduct, quite different from spectra of corresponding $Co-O_2$ complexes.[11]

In view of this large body of accumulated data, we were surprised to read a recent report[1] that the high-spin, five-coordinate cobalt(II) Schiff base Co^{II}(salMeDPT)[12] bound O_2, CO, and CH_3NC and that the CO adduct gave identical and the CH_3NC adduct similar epr spectra to that of the O_2 adduct. Previous studies[7a,13] of high-spin Co^{II}(salHDPT), the unmethylated analog of Co(Me), had not explored CO binding. Furthermore, it was reported[1] that for Co(Me) binding either O_2 or CO produced similar changes in the nmr spectra. It seemed to us highly improbable that CO should bind to the five-coordinate Co(Me) and even more improbable that an adduct, if formed, should show an *identical* epr and similar nmr spectrum to that of the O_2 adduct. Admittedly Co(Me) is high spin, unlike most systems previously studied, and so it was not possible to dismiss this recent report *a priori*. We have therefore examined the question of O_2, CO, and RNC binding to both Co(Me) and Co(H).

The chelate compounds Co(Me) and Co(H) were prepared following published procedures[14] and showed satisfactory elemental analyses. Solvents (toluene and CH_2Cl_2) were distilled from appropriate drying agents and stored under N_2. The CO was passed through an aminated silica-gel trap[15a,b] (−78°) to remove CO_2, Ridox[15c] (Fisher Scientific) to remove O_2, and 4A molecular sieves to remove H_2O. The chelate Co(H) was studied in either toluene or CH_2Cl_2; Co(Me) was examined in CH_2Cl_2 and in a 50/50 (v/v) toluene–CH_2Cl_2 mixture in order to reproduce the conditions of ref 1. Epr samples were prepared under rigorously anaerobic conditions, and the absence of oxygen contamination was verified by epr. Neither Co(Me) nor Co(H) in degassed, dilute solution shows a signal at room temperature; both show a broad high-spin signal (with g_1 ~4.3) when frozen to 77°K (Figure 1A).

To these solutions was then added CO at a pressure of 960 Torr with the sample either (a) in a toluene slush