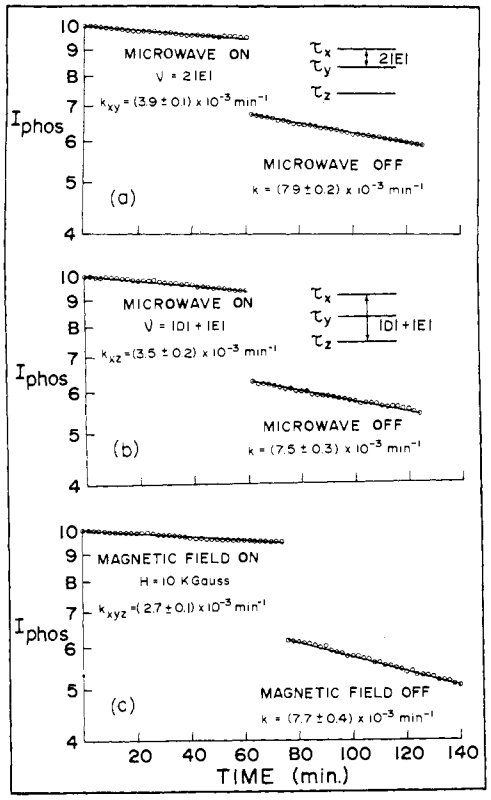

Figure 1. The effect of microwaves in resonance with zero-field transitions or 10 kG static magnetic field on the rate of photochemical reaction of the triplet state of solid pyrimidine in benzene at 1.6°K. At this temperature, the spin–lattice relaxation between the zf levels is slow and the system exists in a state of spin alignment.

$$\frac{dS_0}{dt} = \sum_{i=x,y,z} (\beta_i T_i - \alpha_i I S_0) \qquad (2)$$

where x, y, and z are the three orthogonal zf states. The dependence on the intensity of the exciting light (I) is included explicitly in the ISC ($\alpha_i I$) as well as the photochemical reaction ($\gamma_i I$) rates. β_i is the decay rate of the sublevel i to the ground state.

Since the reaction is slow compared with photophysical processes, these rate equations can be solved under a pseudo-steady-state approximation. The rate constant for this photoreaction at certain light level, $k(I)$, is found to be

$$k(I) = \frac{YI^2}{1 + XI} \qquad (3)$$

where $Y = \Sigma_i n_i \gamma_i$, $X = \Sigma_i n_i = \Sigma_i \alpha_i/\beta_i$.

If a microwave field in resonance with the $x \leftrightarrow y$ zf transition is applied, the population of the two levels tend to equalize. Under this condition, the quantities k_{xy}, Y_{xy}, and X_{xy} are obtained by substituting the new steady-state population[7] $n_{xy} = (\alpha_x + \alpha_y)/\beta_x + \beta_y)$ for n_x and n_y in eq 3. Notice that resonant microwaves could affect the rate of the photochemical reaction if either the populations or the photoreactivities (measured by γ_i) of its zf levels are unequal or both. The question most interesting to chemists would be whether the rate of the photochemistry of a triplet state depends on the initial direction of the unpaired spins in the molecular framework, *i.e.*, $\gamma_x \neq \gamma_y \neq \gamma_z$.

The relative population of the zf levels (known for pyrimidine)[8] is related to the γ_i's as follows.

$$\frac{Y_{xy}}{Y} = \frac{k_{xy}(1 + X_{xy}I)}{k(1 + XI)} = \frac{n_{xy}(\gamma_x + \gamma_y) + n_z\gamma_z}{n_x\gamma_x + n_y\gamma_y + n_z\gamma_z} \qquad (4)$$

The relative values of γ_i can be determined by simultaneously solving this and a similar equation involving, *e.g.*, Y_{xz}/Y, if I is calculated by determining k at two different light intensities.

In five different experiments, using different exciting light intensities, the above method gave $\gamma_x:\gamma_y:\gamma_z$ the ratio 1:0.70 ± 0.13:0.5 ± 0.1; *i.e.*, the rate of the photochemistry induced by an absorption of a second photon by the lowest triplet state depends on the orientation of the spins in the molecular framework.

γ contains the product of the probabilities of both the absorption of the second photon and the nonradiative process(es) leading to the observed photochemistry. Spin selectivity could arise if the different zf levels have different probabilities for either or both of these two processes. For example, the second photon could excite the molecule to a higher energy triplet state which, *via* spin-orbital coupling, radiationlessly and spin selectively crosses to a photochemically active singlet state or to a singlet state of product. It should be emphasized that this is a tentative explanation. However, in any mechanism postulated, the coupling between the spin motion and the orbital motion (responsible for the chemical reaction) must be invoked.

The details of the photochemistry involved are now being worked out and the complete work will be published at a later date.

Acknowledgment. The authors wish to thank Dr. Peter Esherick of this group for his critical comments throughout this work. The financial support of the Office of Naval Research is also greatly acknowledged.

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Modification of the Cavity of β-Cyclodextrin by Flexible Capping

Sir:

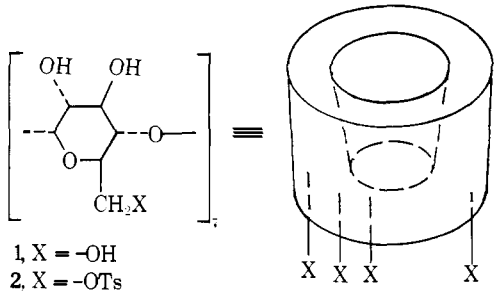
The cyclodextrins (cycloamyloses) have generated great interest[1] because of their ability to bind substrates into their cavity utilizing hydrophobic interactions in aqueous solution. In this respect they mimic binding sites in enzymes, and cyclodextrins have been utilized accordingly in a number of enzyme models. Simple cyclodextrins themselves are

reagents[2] or catalysts[3] for some reactions occurring within the cyclodextrin-substrate complex, and a variety of functional groups[4] have also been attached to the cyclodextrin system in order to provide a catalytic group for intracomplex processes.

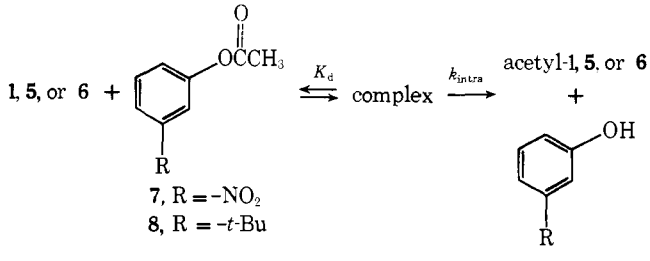
The chief problem with these models has to do with the nature of the binding by the cyclodextrin cavity itself. Thus, the interior of the cavity, with ether oxygens, is not quite nonpolar, and in addition both ends of the cavity are open to the solvent. The result is that this cylindrical cavity with open ends does not really immobilize a bound substrate, so the cyclodextrin-substrate complex does not have the well-defined geometry required if truly large intracomplex rates are to result from well-placed functional groups. A second problem is that with most substrates binding constants are relatively weak, and high concentrations of substrate are required to saturate the cavity.

To overcome these problems, we have attached groups on the bottom, or primary, side of β-cyclodextrin (cycloheptaamylose (**1**)) which would be expected to cluster so as to form a hydrophobic "floor" on the cavity. We find that in such modified cyclodextrins one substrate at least is indeed bound more strongly. Perhaps more important, rates of intracomplex reactions are increased as expected if the complexes have a more rigid well-defined geometry.

Reaction of cyclohepta(amylose 6-toluenesulfonate) (**2**)[5] with methylamine or with ethylamine affords the corresponding cyclohepta(*N*-methylamylose-6-amine) (**3**)[6] and cyclohepta(*N*-ethylamylose-6-amine) (**4**).[6] In common with other charged derivatives of the cycloamyloses,[7] the binding and reactions of these derivatives are heavily influenced by their positive charges in ordinary pH regions. However, with formic acetic anhydride they can be converted to the *N*-formyl derivatives (**5**)[6] and (**6**),[6] which are now neutral but still highly water soluble. Molecular models show that in **5** and **6** the alkyl groups can cluster to close the bottom of the cavity; this is the expected geometry in aqueous solution, in which the polar formyl groups will project into the water. The properties of **5** and **6** confirm the prediction from the models that, relative to **1**, the cavity is more hydrophobic but shallower and that substrates have a more fixed position in their complexes with **5** and **6**.



1, X = -OH
2, X = -OTs
3, X = -NHCH3
4, X = -NHCH2CH3
5, X = -NCH=O (CH3)
6, X = -N(CH2CH3)-CH=O



7, R = -NO2
8, R = -t-Bu

Table I. Rate and Dissociation Constants for Reactions of Cycloheptaamylose Derivatives with *m*-Nitro and *m-tert*-Butylphenyl Acetate[a]

Substrate	Reagent	$10^3 k_{intra}$ (sec^{-1})	k_{intra}/k_{un}[d]	$10^4 K_d$ (M)
7	1	11.9 ± 0.05	64	53 ± 7[b]
7	5	123 ± 5	660	51 ± 7
7	6	210 ± 40	1140	260 ± 50
8	1	4.13 ± 0.25	365	1.95 ± 0.17[c]
8	5	37 ± 5	3300	4.6 ± 0.9

[a] In pH 9.00 (I = 0.2) $NaHCO_3$–Na_2CO_3 buffer, 25.0°, with 0.50–0.75% (v/v) CH_3CN added. [b] Reported (ref 2): 80 × 10^{-4} M. [c] Reported (ref 2): 1.3 × 10^{-4} M. [d] k_{un} is the hydrolysis rate for **7** or **8** in the absence of a cyclodextrin.

The reactions examined were acetyl transfers from *m*-nitrophenyl acetate and *m-tert*-butylphenyl acetate to the secondary hydroxyls of **4** and **5**, a process already known[2] for **1**. These reactions showed Michaelis-Menten kinetics; the measured rate constants, k_{intra}, and dissociation constants for reagent-substrate complexes, K_d, were derived in the standard way and are listed in Table I.

As these data show, the methyl floor in **5** produces a tenfold increase in the intracomplex rate for substrates **7** and **8**, which we ascribe to better fixed geometry. The ethyl floor in **6** has an even larger effect. However, overall binding is either unaffected (**5** with **7**) or weakened (**6** with **7**, **5** with **8**) despite the new hydrophobic surface. Models suggest that this is because the cavity is now a little too *shallow* for the substrates; we have confirmed this by examining a molecule which will fit well into the new shallower cavity.

1-Adamantanecarboxylic acid (**9**) is an excellent competitive inhibitor of cyclodextrin complex formation with substrates, since **9** itself is strongly bound.[2] We have studied the reactions of **1**, **5**, and **6** with **7** in the presence of the inhibitor **9**, and determined the dissociation constant for the complex of **9** with these cyclodextrins by standard techniques. With **1** the dissociation constant is 1.60 × 10^{-3} M (reported,[2] 0.7 × 10^{-3} M) but with **5** it is 6.7 × 10^{-5} M. Thus with this inhibitor the effect of the new hydrophobic floor in **5** or **6** is to increase binding by more than 20-fold.

Another observation confirms these interpretations. We find[7] that the inhibition data for **9** with unsubstituted **1** fit only if the **1**–**9** complex binds a second molecule of **9** at higher **9** concentrations. The formation of a 2:1 complex is also demonstrated by a plot[7] of the optical rotation change in **1** as a function of added **9**. The second binding constant is ca. 10^2 times the first. Apparently **9** goes partially into the cavity of **1** forming a *floor*. This now leads to improved binding of a second molecule of **9**. No such behavior is found for **5** or **6**, which show evidence for binding of only one molecule of **9**. They already have a floor and can bind **9** only in the top.

Thus the simple modification of cyclodextrin described here markedly improves its potential for use in enzyme models. The incorporation of additional *catalytic* groups is the obvious next step.

Acknowledgment. Financial support of this work by a grant from the National Institutes of Health is gratefully acknowledged.

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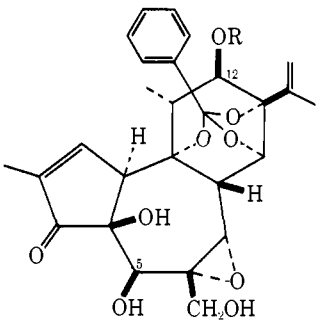
Gnididin, Gniditrin, and Gnidicin, Novel Potent Antileukemic Diterpenoid Esters from *Gnidia lamprantha*[1,2]

Sir:

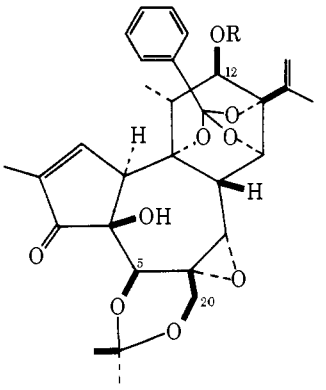
In the course of a continuing search for tumor-inhibitory natural products, we found that the ethanol extract of *Gnidia lamprantha* Gilg (Thymelaeaceae)[3] showed significant activity *in vivo* against P-388 leukemia in mice.[4] We report herein the isolation and structural elucidation of the novel 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,