uncorrected. Infrared spectra were taken as liquid films on sodium chloride plates or as KBr disks using a Perkin-Elmer Model 137 spectrophotometer and are reported in microns. Nuclear magnetic resonance spectra were obtained using a Perkin-Elmer Hitachi R-24 spectrometer with deuteriochloroform as solvent. All spectra are reported in parts per million relative to tetramethylsilane ($\delta$). Mass spectra were determined using a Du Pont 21-490 mass spectrometer at 70 eV ionization potential. Elemental analyses were performed by Galbraith Laboratories, Knoxville, Tenn.

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# Isolation and Structural Elucidation of New Potent Antileukemic Diterpenoid Esters from *Gnidia* Species[1,2]

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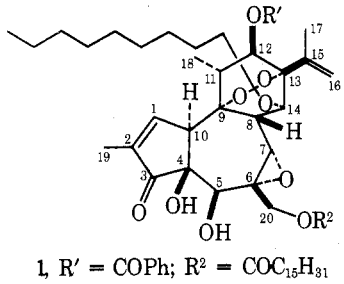
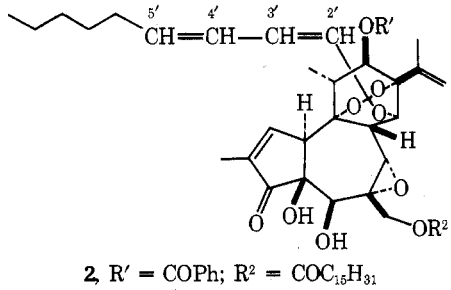
The isolation and structural elucidation of the new potent antileukemic principles, gnidilatin 20-palmitate (1), gnidilatidin 20-palmitate (2), and gnidilatin (3), and the new toxic diterpenoids, gnidilatidin (4) and gnidiglaucin (5), are reported. Esters 1 and 2 were proven to be C-20 palmitate esters of gnidilatin (3) and gnidilatidin (4), respectively, by acylation of 3 and 4 with palmitoyl chloride. Methanolysis of 1, 3, and 5 afforded the tetrol 6 as a common parent diterpenoid ortho ester. The tetrol 7 was obtained from 2 and 4. Catalytic hydrogenation of 3 and 4 gave dihydrognidilatin (8).

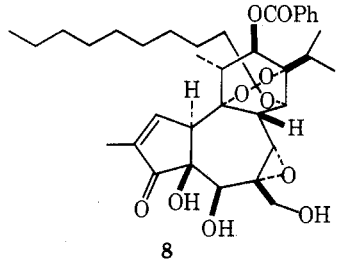
In the course of a continuing search for tumor inhibitors from plant sources, we found that alcoholic extracts of *Gnidia latifolia* Gilg.[3] and *Gnidia glaucus* Fres.[4] (Thymelaeaceae) showed significant activity in vivo against P-388 leukemia in mice.[5] We report herein the isolation and structural elucidation of the potent antileukemic principles, gnidilatin 20-palmitate (1), gnidilatidin 20-palmitate (2), and gnidilatin (3), and the companion toxic principles gnidilatidin (4) and gnidiglaucin (5).



1, R′ = COPh; R² = $COC_{15}H_{31}$
3, R′ = COPh; R² = H
5, R′ = $COCH_3$; R² = H
6, R′ = H; R² = H



2, R′ = COPh; R² = $COC_{15}H_{31}$
4, R′ = COPh; R² = H
7, R′ = H; R² = H



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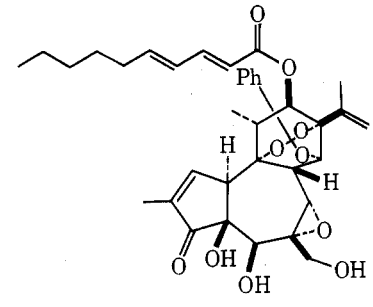

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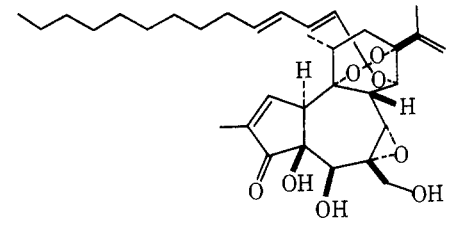

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Fractionation of the ethanol extract of *G. latifolia*, guided by a combination of an in vivo assay for antileukemic activity (P-388) and a goldfish toxicity test,[6] revealed that both the antileukemic and piscicidal activity were concentrated in the chloroform layer of a chloroform–water partition. Column chromatography on SilicAR yielded two active fractions (A and B) upon elution with ethyl acetate–benzene (1:9 and 3:7, respectively). Column chromatography of fraction A on silica gel and subsequent preparative layer chromatography on silica gel gave two closely related compounds, gnidilatin 20-palmitate (1), $C_{53}H_{78}O_{11}$, and gnidilatidin 20-palmitate (2), $C_{53}H_{74}O_{11}$. Successive column chromatography of fraction B on silica gel, then Celite, followed by preparative TLC on silica gel gave two closely related compounds, gnidilatin (3), $C_{37}H_{48}O_{10}$, and gnidilatidin (4), $C_{37}H_{44}O_{10}$.

The initial spectral data (ir, NMR) of these compounds indicated that they were structurally related to gnididin (9)[7] and huratoxin (10),[8] previously isolated from *Gnidia lamprantha* Gilg. (Thymelaeaceae) and *Hura crepitans* L. (Euphorbiaceae), respectively. The NMR spectrum of gnidilatidin (4) was almost identical with that of gnididin (9) except for the signals for the diene vinyl protons (at C-2′, 3′, 4′, and 5′),

which were identical with those of huratoxin (**10**). These data indicated that the only difference between **4** and **9** was the position of the benzoate and decadienoate esters, a conclusion supported by the uv spectra. While gnididin (**9**) showed absorption at 260 nm (characteristic of dienoate esters), gnidilatidin (**4**) showed absorption at 232 nm (characteristic of conjugated dienes). Methanolysis of **9** gave methyl decadienoate,[7] while **4** gave methyl benzoate and the tetrol **7**, $C_{30}H_{40}O_9$. Although C-5 and C-12 proton absorptions each appear as a singlet, the site of benzoate attachment in **4** can be assigned on the basis of chemical shift. In the NMR spectrum of **4** the methine resonance ($\tau$ 4.88, s, 1 H), which was shifted to $\tau$ 6.09 in the spectrum of **7**, was consistent with esterification at the C-12 position. Moreover, the C-5 methine resonance of **4** remains essentially unchanged from that of **7** and occurs downfield from the absorption which would be expected if the hydroxyl at C-12 were free. Hence gnidilatidin was assigned the structure **4**. The stereochemistry of the double bonds in the 2,4-decadienoate group remains to be established.

A comparison of the molecular formulas and spectral data of gnidilatidin 20-palmitate (**2**) and gnidilatidin (**4**) indicated that gnidilatidin 20-palmitate (**2**) was the palmitate ester of gnidilatidin (**4**). Proof was obtained by direct transformation; thus upon treatment with palmitoyl chloride in pyridine, gnidilatidin (**4**) was converted to gnidilatidin 20-palmitate (**2**). In addition, methanolysis of **2** afforded tetrol **7**. The structural problem which remained at this point was the determination of the site of attachment of the palmitate group to gnidilatidin (**4**). The NMR signals for the C-20 protons of **2** appeared at lower magnetic field by ~0.4 ppm than the corresponding signals of gnidilatidin (**4**) (see Table I), which indicated that C-20 was the point of attachment of the palmitate ester. Thus gnidilatidin 20-palmitate was shown to have the structure **2**.

The molecular formula of gnidilatin (**3**) indicated that it was a tetrahydro derivative of gnidilatidin (**4**), and, as expected, the NMR spectrum of **3** did not have signals corresponding to decadienoate vinyl protons. Catalytic hydrogenation of gnidilatin (**3**) and gnidilatidin (**4**) over 10% palladium–carbon gave dihydro and hexahydro derivatives, respectively. Spectral data and TLC comparisons indicated the identity of these compounds.

Gnidilatin 20-palmitate (**1**) was shown to be the 20-palmitate of gnidilatin (**3**) by the same method as that used for the structural elucidation of gnidilatidin 20-palmitate (**2**).

By an isolation procedure very similar to that described above, gnidiglaucin (**5**) was isolated from an alcoholic extract of *Gnidia glaucus* Fres.

The molecular formula $C_{32}H_{46}O_{10}$ was advanced for gnidiglaucin (**5**) on the basis of high-resolution mass spectrometry. The presence of an acetate ester was indicated by the loss of 42 and 43 amu in the mass spectrum. Furthermore, there appeared in the NMR spectrum a signal for the acetate group at $\tau$ 8.16 (3 H, s). The other signals of gnidiglaucin (**5**) were very similar to that of gnidilatin (**3**) except for the absence of signals corresponding to a benzoate group, which indicated that gnidiglaucin (**5**) was the 12-acetate of the tetrol **6**. Methanolysis of gnidiglaucin (**5**) gave a deacetyl derivative which was identical with the tetrol **6** obtained from gnidilatin (**3**). A comparison of the NMR spectrum of gnidiglaucin (**5**) with that of tetrol **6** led to the conclusion that the position of the acetate group was at C-12.

Gnidilatin 20-palmitate (**1**) and gnidilatidin 20-palmitate (**2**) exhibited substantial inhibitory activity at optimal doses of 0.5–2 mg/kg of body weight against the P-388 leukemia in mice, and gnidilatin (**3**) showed moderate inhibitory activity at about 80 $\mu$g/kg. On the other hand, gnidilatidin (**4**) and

Table I. NMR Spectra of Diterpenoid Esters and Derivatives[a]

| | 1 | 5 | 7 | 8 | 10 | 11 | 12 | 14 | 16 | 17 | 18 | 19 | 20 |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| Gnidilatin 20-palmitate (**1**) | 2.54 br s | 5.88 s | 6.60 s | 6.49 d $J$ = 2 | 6.30 br s | 7.53 q $J$ = 7 | 4.90 s | 5.29 d $J$ = 2 | 5.10 s | 8.22 s | 8.69 d $J$ = 7 | 8.30 br s | 5.27, 6.23 AB q $J$ = 12 |
| Gnidilatidin 20-palmitate (**2**) | 2.41 br s | 5.76 s | 6.36 s | 6.29 d $J$ = 2 | 6.07 br s | 7.50 q $J$ = 7 | 4.77 s | 5.10 d $J$ = 2 | 4.99 s | 8.12 s | 8.68 d $J$ = 7 | 8.23 br s | 5.28, 6.13 AB q $J$ = 12 |
| Gnidilatin (**3**) | 2.54 br s | 5.89 s | 6.47 s | 6.47 d $J$ = 2 | 6.22 br s | 7.52 q $J$ = 7 | 4.90 s | 5.26 d $J$ = 2 | 5.10 br s | 8.20 s | 8.67 d $J$ = 7 | 8.28 br s | 6.27, 6.45 AB q $J$ = 11 |
| Gnidilatidin (**4**) | 2.50 br s | 5.87 s | 6.44 s | 6.44 d $J$ = 2 | 6.20[b] | 7.50 q $J$ = 7 | 4.88 s | 5.20 d $J$ = 2 | 5.08 br s | 8.18 s | 8.66 d $J$ = 7 | 8.28 br s | 6.20[b] |
| Gnidiglaucin (**5**) | 2.53 br s | 5.83 s | 6.53 s | 6.61 d $J$ = 2 | 6.30 br s | 7.72 q $J$ = 7 | 5.15 s | 5.39 d $J$ = 2 | 5.11 br s | 8.23 br s | 8.77 d $J$ = 7 | 8.23 br s | 6.16, 6.30 AB q $J$ = 12 |
| (**6**) | 2.45 br s | 5.75 s | 6.50 s | 6.30 d $J$ = 2.5 | 6.25 br s | 7.52 q $J$ = 7 | 6.16 s | 5.37 d $J$ = 2.5 | 4.95, 4.90 each s | 8.14 s | 8.78 d $J$ = 7 | 8.17 br s | 6.11, 6.29 AB q $J$ = 12 |
| (**7**) | 2.41 br s | 5.76 s | 6.47 s | 6.25 d $J$ = 2 | 5.93 ~6.16[b] | 7.51 q $J$ = 7 | 6.09 s | 5.28 d $J$ = 2 | 4.90 s | 8.12 s | 8.77 d $J$ = 7 | 8.19 br s | 5.93 ~6.16[b] |
| Gnididin (**9**) | | 5.73 s | 6.37[b] | 6.37[b] | 6.08[b] | 7.50 q $J$ = 7 | 4.89 s | | 4.98 s | 8.12 s | 8.62 d $J$ = 7 | 8.21 br s | 6.08[b] |
| Huratoxin (**10**) | 2.39 | 5.77 | 6.54 | 7.06 d $J$ = 2.5 | | | | 5.55 d $J$ = 2.5 | 5.08, 4.96 | 8.20 s | | 8.20 br s | 6.16 |

[a] Spectra were measured as $CDCl_3$ solutions at 100 MHz. Coupling constants are in hertz and shift values are in units of $\tau$. Multiplicity: d, doublet; q, quartet; s, singlet; br s, broadened singlet; AB q, AB quartet. [b] Inclusive of the other indicated protons.

gnidiglaucin (**5**) showed no inhibitory activity. These data indicate that the 12-benzoate and 20-palmitate esters may act as important carrier moieties (e.g., in processes concerned with cell penetration or selective molecular complex formation).[9] Investigations are in progress to determine the significance of the epoxide, the cyclopentenone, the ortho esters, and of the other structural features which may be important for the antileukemic activity of these diterpenoid esters.

## Experimental Section

**General.** Uv spectra were measured on a Beckman Model DK-2A recording spectrophotometer. Infrared spectra were determined on a Perkin-Elmer Model 257 recording spectrophotometer. NMR spectra were determined on a Varian HA-100 spectrometer or a JEOL PS-100 pulsed FT NMR spectrometer interfaced to a Texas Instrument JEOL 980 A computer, and chemical shifts are reported using the $\tau$ scale. Mass spectra were determined on Hitachi Perkin-Elmer Model RMU-6E or AEI Model MS-902 spectrometers. Values of $[\alpha]_D$ were determined on a Perkin-Elmer Model 141 automatic polarimeter. All thin layer chromatography was carried out on commercially prepared plates; silica gel refers to silica gel 60 F-254 (E. Merck) and ChromAR to ChromAR 7GF (Mallinckrodt). For column chromatography silica gel refers to silica gel 60 (E. Merck), SilicAR to SilicAR CC-7 (Mallinkrodt), and Celite to Celite 545 (distributed by Sargent-Welch). Visualization of TLC was effected with vanillin spray (2.5 g vanillin–10 ml ethanol–50 ml concentrated sulfuric acid).

**Extraction and Preliminary Fractionation of *Gnidia latifolia*.** The dried ground wood of stems and stem bark (30 kg) was extracted at room temperature by stirring with 95% ethanol (216 l.) for 24 h. The extraction mixture was filtered and concentrated below 30 °C in vacuo to a syrupy residue (~900 ml). The residue was partitioned between chloroform (3 × 8 l.) and water (10 l.) and the combined chloroform layers were concentrated to give a brown tar (440 g), which was chromatographed on a SilicAR CC-7 column (5 kg) by eluting with benzene followed by benzene containing increasing amounts of ethyl acetate. Elution with 10% ethyl acetate in benzene gave fraction A (35 g), which was found to be active against P-388 lymphocytic leukemia in the mouse (PS). Elution with 30% ethyl acetate in benzene gave fraction B (58 g), which showed activity against PS and toxicity against goldfish.

**Isolation of Gnidilatin 20-Palmitate (1) and Gnidilatidin 20-Palmitate (2).** Careful column chromatography of fraction A on silica gel (600 g) with hexane containing increasing amounts of acetone gave a fraction which was shown to contain two closely related compounds. Preparative thin layer chromatography of this fraction on silica gel gave gnidilatin 20-palmitate (**1**, 62 mg, 0.0002%) [$[\alpha]^{23}D$ +45° (*c* 0.58, $CHCl_3$); uv (MeOH) $\lambda_{max}$ 231 nm ($\epsilon$ 18 000); ir ($CHCl_3$) 2.83, 3.41, 3.50, 5.79, 6.12, 6.24, 6.29, 7.90 $\mu$; mass spectrum *m/e* 890.5539 ($M^+$, calcd for $C_{53}H_{78}O_{11}$ 890.5544)], and gnidilatidin 20-palmitate (**2**, 45 mg, 0.00015%) [$[\alpha]^{23}D$ +27° (*c* 0.15, $CHCl_3$); uv (MeOH) $\lambda_{max}$ 232 nm ($\epsilon$ 41 000); ir ($CHCl_3$) 2.85, 3.42, 3.52, 5.80, 5.91, 6.04, 6.13, 6.24, 6.30, 7.90 $\mu$; mass spectrum *m/e* 886.5237 ($M^+$, calcd for $C_{53}H_{74}O_{11}$, 886.5231)].

**Isolation of Gnidilatin (3) and Gnidilatidin (4).** Column chromatography of fraction B on SilicAR (700 g) with chloroform containing increasing amounts of methanol gave an active fraction (7 g). Partition chromatography on Celite (10% $CHCl_3$–heptane:17% aqueous methanol) followed by preparative TLC on silica gel gave gnidilatin (**3**, 37 mg, 0.00012%) [$[\alpha]^{23}D$ +52° (*c* 0.24, $CHCl_3$); uv (MeOH) $\lambda_{max}$ 231 nm ($\epsilon$ 15 000); ir ($CHCl_3$) 2.83, 3.41, 3.49, 5.79, 5.89, 6.12, 6.24, 6.28, 7.89 $\mu$; mass spectrum *m/e* 652.3240 ($M^+$ calcd for $C_{37}H_{48}O_{10}$, 652.3248)] and gnidilatidin (**4**, 33 mg, 0.00011%) [$[\alpha]^{23}D$ +28° (*c* 0.16, $CHCl_3$); uv (MeOH) $\lambda_{max}$ 232 nm ($\epsilon$ 36 000); ir ($CHCl_3$) 2.84, 3.42, 3.50, 5.85, 5.90, 6.14, 6.25, 6.28, 7.87 $\mu$; mass spectrum *m/e* 648.2927 ($M^+$, calcd for $C_{37}H_{44}O_{10}$, 648.2935)].

**Methanolysis of Gnidilatidin (4).** To 0.5 ml of 0.1 N sodium methoxide in methanol was added gnidilatidin (**4**, 3 mg). The mixture was stirred at room temperature for 18 h. The reaction mixture was dried with a stream of nitrogen, and separated by preparative TLC on ChromAR to give methyl benzoate and the tetrol **7** (2.3 mg): uv (MeOH) $\lambda_{max}$ 231 nm ($\epsilon$ 30 000); ir ($CHCl_3$) 2.90, 5.89, 6.05, 6.14, 6.24 $\mu$; mass spectrum *m/e* 544.2672 ($M^+$, calcd for $C_{30}H_{40}O_9$, 544.2672).

**Acylation of Gnidilatidin (4) with Palmitoyl Chloride.** To a solution of gnidilatidin (**4**, 5 mg) in 0.5 ml of pyridine was added palmitoyl chloride (50 $\mu$l) and the reaction mixture was kept at room temperature for 2 h. The reaction mixture was evaporated to dryness with a stream of nitrogen and separated by preparative TLC on ChromAR to give a palmitate (5.1 mg), which was found to be identical with gnidilatidin 20-palmitate (**2**) by comparison of spectral data.

**Methanolysis of Gnidilatidin 20-Palmitate (2).** Methanolysis of gnidilatidin 20-palmitate (**2**, 15 mg), as described above for gnidilatidin (**4**), gave the tetrol **7** (2.5 mg), methyl benzoate, and methyl palmitate, identified by comparison with authentic samples.

**Catalytic Hydrogenation of Gnidilatin (3).** Gnidilatin (**3**, 5 mg) was subjected to atmospheric pressure hydrogenation in absolute ethanol (5 ml) using 10% palladium on charcoal (5 mg) as catalyst. After 2 h the catalyst was removed by filtration and the solvent was evaporated to afford a colorless glass (5 mg). Preparative TLC on ChromAR with 3% methanol in chloroform gave dihydrognidilatin (**8**, 4.2 mg): uv (MeOH) $\lambda_{max}$ 231 nm ($\epsilon$ 17 000); ir ($CHCl_3$) 2.83, 5.80, 5.83, 6.13, 6.25, 6.29, 7.89 $\mu$; NMR ($CDCl_3$) $\tau$ 9.16–9.04 (9 H, m, 16-, 17-, and 10′-H), 8.78 (~16 H), 8.73 (3 H, d, $J$ = 7 Hz, 18-H), 8.29 (3 H, br s, 19-H), 7.57 (1 H, q, $J$ = 7 Hz, 11-H), 6.60 (1 H, d, $J$ = 2 Hz, 8-H), 6.50 (1 H, s, 7-H), 6.32 (1 H, br s, 10-H), 6.31, 6.15 (2 H, AB q, $J$ = 12 Hz, 20-H), 5.90 (1 H, s, 5-H), 5.50 (1 H, d, $J$ = 2 Hz, 14-H), 4.90 (1 H, s, 12-H), 2.56 (1 H, br s, 1-H), 2.64–2.07 (5 H, PhCOO); mass spectrum *m/e* 654.3408 ($M^+$, calcd for $C_{37}H_{50}O_{10}$, 654.3405).

**Catalytic Hydrogenation of Gnidilatidin (4).** Gnidilatidin (**4**, 5 mg) was hydrogenated as described above for gnidilatin (**3**) to give hexahydrognidilatidin (4.3 mg), which was characterized by direct spectral comparison with **8** described above.

**Acylation of Gnidilatin (3) with Palmitoyl Chloride.** Acylation of gnidilatin (**3**, 5 mg) with palmitoyl chloride, as described above for gnidilatidin (**4**), gave the palmitate (4.8 mg), characterized as gnidilatin 20-palmitate (**1**) by direct spectral comparison with the authentic sample.

**Methanolysis of Gnidilatin (3).** Methanolysis of gnidilatin (**3**, 3 mg), as described above for gnidilatidin (**4**), gave the tetrol **6** (2.2 mg): uv (MeOH) $\lambda_{max}$ 241 nm ($\epsilon$ 6000); ir ($CHCl_3$) 2.90, 5.89, 6.12 $\mu$; mass spectrum *m/e* 548.2980 ($M^+$, calcd for $C_{30}H_{44}O_9$, 548.2985).

**Methanolysis of Gnidilatin 20-Palmitate (1).** Methanolysis of gnidilatin 20-palmitate (**1**, 5 mg) by the same method used for gnidilatidin (**4**) afforded methyl benzoate, methyl palmitate, and the tetrol **6** (2.8 mg), which was identified by spectral comparison with **6** described above.

**Isolation of Gnidiglaucin (5).** The dried ground roots (30 kg) of *Gnidia glaucus* Fres. were extracted by the same method described for *G. latifolia* to give a brown tar (1.45 kg), which was partitioned between chloroform (4 + 2 + 2 l.) and water (4 l.). The combined chloroform layers were concentrated to give a brown tar (335 g), which was further partitioned between 10% aqueous methanol (2 l.) and petroleum ether (2 + 1 l.). The combined petroleum ether layers were concentrated to give a brown oil (135 g), which was chromatographed on a SilicAR column (1.5 kg) by eluting with chloroform containing increasing amounts of methanol. Elution with 2% methanol in chloroform gave a fish-toxic fraction (2.5 g), preparative TLC of which on silica gel yielded gnidiglaucin (**5**, 30 mg, 0.0001%): $[\alpha]^{24}D$ +36° (*c* 0.50, $CHCl_3$); uv (MeOH) $\lambda_{max}$ 241 nm ($\epsilon$ 7600); ir ($CHCl_3$) 2.82, 5.72, 5.90, 6.14, 8.00 $\mu$; mass spectrum (chemical ionization: methane reagent gas) *m/e* 591.3140 ($M^+ + H$, calcd for $C_{32}H_{47}O_{10}$, 591.3166).

**Methanolysis of Gnidiglaucin (5).** Methanolysis of gnidiglaucin (**5**, 5 mg), as described above for gnidilatidin (**4**), gave the tetrol **6** (3.8 mg), identified by spectral comparison with **6** obtained from gnidilatin (**3**).

**Registry No.**—**1**, 60195-67-7; **2**, 60195-68-8; **3**, 60195-69-9; **4**, 60195-70-2; **5**, 60209-66-7; **6**, 60209-62-3; **7**, 60195-71-3; **8**, 60195-72-4; **9**, 55306-11-1; **10**, 33465-16-6; palmitoyl chloride, 112-67-4.

## References and Notes

(1) Tumor Inhibitors. 117. Part 116: S. M. Kupchan, Y. Shizuri, T. Murae, J. G. Sweeny, H. R. Haynes, M.-S. Shen, J. C. Barrick, R. F. Bryan, D. van der Helm, and K. K. Wu, submitted for publication.

(2) This investigation was supported by grants from the National Cancer Institute (CA-11718) and the American Cancer Society (CI-102K), and by contracts with the Division of Cancer Treatment, National Cancer Institute (NO1-CM-12099 and NO1-CM-67002).

(3) The wood of stems and stem bark were collected in Kenya in Nov 1972.

(4) The roots were collected in Tanzania in Oct 1973. The authors acknowledge with thanks receipt of both dried plant materials from Dr. Robert E. Perdue, Jr., U.S. Department of Agriculture, in accordance with the program developed by the National Cancer Institute.

(5) 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). Gnidilatin 20-palmitate and gnidilatidin 20-palmitate showed T/C ca. 170 at dosage levels between 2 and 0.5 mg/kg. In addition, these compounds showed significant inhibitory activity (i.e., T/C >125) at doses down to 20 $\mu$g/kg. Gnidilatin showed T/C 140–130 in the 80–20 $\mu$g/kg

dosage range.
(6) Piscicidal activity was assayed using a procedure similar to that described by W. A. Gersdorff, *J. Am. Chem. Soc.*, **52**, 3440 (1930). Gnidilatin, gnidilatidin, and gnidiglaucin showed toxicity at concentrations of 50 μg/l.
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(8) K. Sakata, K. Kawazu, and T. Mitsui, *Agric. Biol. Chem.*, **35**, 2113 (1971).
(9) A similar proposal has been advanced for the role of the long aliphatic chain in the piscicidal activity of huratoxin; cf. ref 8.

# Synthesis of α-Dehydrobiotin[1]

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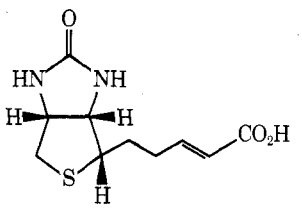
α-Dehydrobiotin has been synthesized from the bicyclic sulfonium salt **7**. The five-carbon acid side chain was elaborated by cleavage of the sulfonium ring with acetate ion, hydrolysis to a hydroxypropyl side chain, oxidation to a propionaldehyde side chain, and coupling with triethyl phosphonoacetate. A new reagent, orthophosphoric acid, was used to effect debenzylation of the urea moiety.

α-Dehydrobiotin (**1**) is an extremely effective antagonist of biotin that has been isolated from a natural source as a



**1**

consequence of its antibiotic activity against a variety of bacteria and fungi.[2] Subsequently, it was reported that α-dehydrobiotin is accompanied by two other biotin antagonists[3] and that it is a product of the catabolism of biotin.[4]

We undertook synthesis of this biologically interesting molecule since we had available experience and intermediates derivingfrom the synthesis of biotin itself.[5] The approach we followed was to divert the biotin synthesis cited above at a stage in which the difficult stereochemical problems have been solved and in which a reactive center is present where the double bond is to be formed.

**Debenzylated Series.** The intermediate which was expected to be the most useful in this kind of approach is the cyclic sulfonium salt **2**;[5c] however, as will be seen, an unexpected difficulty developed. The first step is oxidation of the terminal carbon atom of the latent side chain in **2** to the oxidation level of an aldehyde. Analogy from the biotin synthesis[5] suggests that sulfonium salt **2** can react as if it were a covalent bromide with the appropriate side chain. Accordingly **2c**[6] was reacted with the sodium salt of 2-nitropropane,[7] to give a compound assigned structure **3c**, a hemiacetal form of desired aldehyde. Since a priori sulfonium salt **2** has two other points where attack of the nitronate anion could have occurred, hemiacetal **3c** was submitted to x-ray crystallographic analysis for confirmation of its structure. Two stereoscopic views of the result are shown in Figure 1. Additional confirmation was obtained by reaction of **3c** with methanol or aniline under acidic conditions to give **4c** or **5c**, respectively. Reaction with hydroxylamine gave oxime **6c**, the only compound of the series with an actual rather than a latent side chain.

The next operation to be carried out is addition of the remaining two carbon atoms of the side chain. Reaction of **3c** with triethyl phosphonoacetate[8] should have been feasible, but we could obtain no product. Perhaps the hemiacetal ring is so stable that there is no appreciable concentration of the aldehyde form. Reaction of **3c** with malonic acid and piperidine gave a product whose elemental analysis and mass spectrum are consistent with a dimer of a dehydration product of **3c**. This propensity of **3c** to self-condense was also evident when treatment of **3c** with acetic anhydride gave a similar "dimer" acetate. Since these products did not appear to have any synthetic utility, their structures were not investigated further. The NMR spectrum of the "dimer" is not readily interpretable, but is clearly not consistent with any symmetrical dimer.

**Racemic Series (a).** These unproductive results forced a retreat to the precursor of **3**, the dibenzyl derivative **7** (X = Br). Here we chose a lengthier reaction sequence. The ring was cleaved with acetate ion to give **8a** whose basic hydrolysis gave alcohol **9a**. The next operation was to specifically oxidize the alcohol function to an aldehyde without affecting the thioether function. This was achieved with dimethyl sulfoxide/dicy-

Scheme I