perimental results confirm the presence of N-3-methylbutylacetamide in the venom of the southern yellowjacket and demonstrate alarm pheromone activity of this compound similar to that obtained with venom extracts. This is only the second alarm pheromone of a social wasp identified to date. The alarm pheromone of *Vespa crabro* L. is reported to be 2-methyl-3-butene-2-ol[7].

N-3-Methylbutylacetamide is reportedly a volatile constituent of tobacco[11], wines and cheeses[13]. Although the amounts of this chemical in these products are not known, the possibility exists of eliciting an attack from a yellowjacket colony with these or other natural materials containing N-3-methylbutylacetamide. This compound also has been found in the rectal glands of the Queensland fruit fly, *Dacus tryoni* (Froggatt), although its function in gland secretions is unknown[10].

1 The authors thank E. Adamak, R. Murphy and F. Takken for technical assistance. This article reports the results of research only. Mention of a proprietary product does not constitute an endorsement or the recommendation for its use by USDA.

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0014-4754/88/010082-02$1.50 + 0.20/0

Goniothalamicin and annonacin: Bioactive acetogenins from *Goniothalamus giganteus* (Annonaceae)

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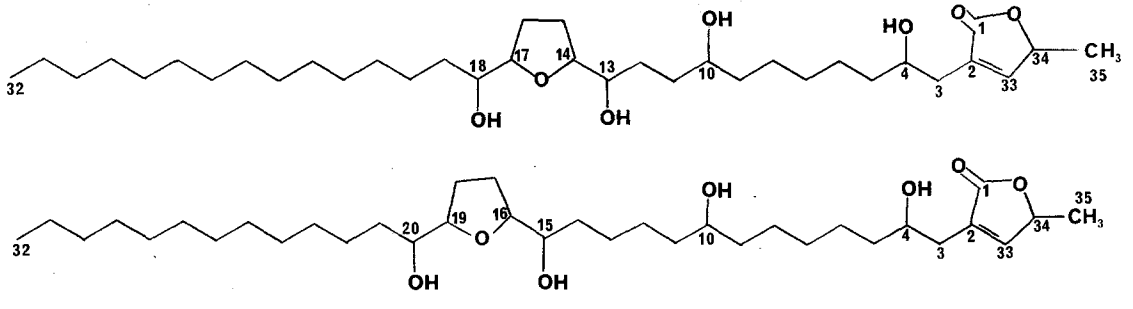
Summary. Using brine shrimp lethality for activity-directed fractionation, goniothalamicin (**I**), a new tetrahydroxy-mono-tetrahydrofuran fatty acid γ-lactone (acetogenin), has been isolated from ethanolic extracts of the stem bark of *Goniothalamus giganteus* Hook. f., Thomas (Annonaceae). This novel compound was found to be cytotoxic and insecticidal and inhibited the formation of crown gall tumors on potato discs. Annonacin (**II**), the only other reported mono-tetrahydrofuran acetogenin, was also isolated; the previously reported 9ASK (astrocytoma reversal) activity of **II** was confirmed, and **II** is now also found to be weakly active against 3PS murine leukemia.

Key words. Goniothalamicin; annonacin; acetogenins; *Goniothalamus giganteus*; Annonaceae; brine shrimp; insecticidal; cytotoxic; antileukemic activity.

Previous phytochemical studies of the genus *Goniothalamus* have yielded the bioactive compounds altholactone (goniothalenol)[1], a furano-2-pyrone, and goniothalamin[1, 2], a styrylpyrone, as well as pinocembrin[1] (5,7-dihydroxyflavone) and a number of 5,6-dihydro-2-pyrones[3]. During our continuing investigation of higher plants as sources of novel biologically active secondary metabolites, we noted that ethanolic extracts of the stem bark of *Goniothalamus giganteus* Hook. f., Thomas (Annonaceae) exhibited significant murine toxicity in the 3PS lymphocytic leukemia system[4]. The fractionation of the ethanolic extract was guided by a convenient bioassay involving brine shrimp lethality[5]. Through multiple solvent partitionings and chromatographic steps, monitoring the fractions with thin-layer chromatography (TLC) and brine shrimp lethality, goniothalamicin (**I**), a novel acetogenin and the recently reported mono-tetrahydrofuran acetogenin, annonacin[6] (**II**), were isolated and subsequently characterized.

Goniothalamicin (**I**) was found to be cytotoxic in the 9KB (human nasopharyngeal carcinoma, $ED_{50} < 10^{-2}$ μg/ml) and the 9PS (murine lymphocytic leukemia, $ED_{50} < 10^{-1}$ μg/ml)[4] systems. This cytotoxic activity paralleled the brine shrimp lethality throughout the fractionation of the stem bark. Goniothalamicin was toxic to the brine shrimp [LC_{50} 37 ppm, 95% confidence intervals: 13–295 ppm] and also toxic to blowfly larvae[7] (100% mortality at 1% conc.). In a plant tumor system[8, 9], **I** significantly inhibited the formation of crown gall tumors on potato discs (32% and 28%); this plant tumor system[8, 9] has shown a positive correlation with the 3PS murine lymphocytic leukemia system[4] in vivo, indicating that **I** might possess antitumor activity; however, no 3PS activity was observed at



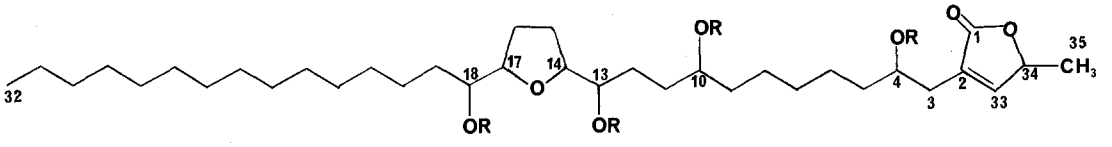


Figure 1. Derivatives of goniothalamicin analyzed by MS.

I R = H
III R = Ac
IV R = TMS
V R = Ac-d_3
VI R = TMS-d_9
VII R = TMS, 2,33-dihydro

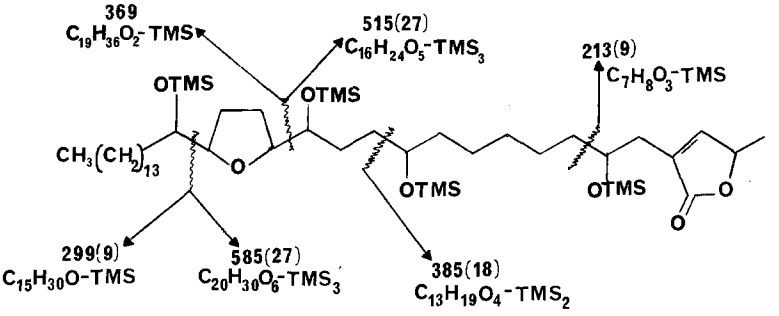


Figure 2. Diagnostic MS fragment ions of **IV** and **VI**.

doses up to 2.5 mg/kg; higher doses exhibited murine toxicity. In the 9ASK (astrocytoma reversal) assay [10], goniothalamicin was inactive. Annonacin (**II**) was ten times as active against brine shrimp (LC_{50} 3.3 ppm); it also was insecticidal against blowfly larvae (100% mortality at 1% conc.) and mosquito larvae (70% mortality at 10 ppm) and was active in the potato disc crown gall antitumor system (45% inhibition). The potato disc activity of annonacin was correctly indicative of weak 3PS in vivo murine antileukemic activity (T/C 124% at 0.95 mg/kg). Also, the previously reported 9ASK activity of annonacin [6] was confirmed (15–30% reversal at 100 µg/ml).

Goniothalamicin (**I**) was isolated as a whitish wax (m.p. 86–88 °C, $[\alpha]_D$ + 1.6 °). The high resolution chemical ionization (isobutane) mass spectra (CIMS) gave an MH^+ at m/z 597.4732 (calc. 597.4734) corresponding to the molecular formula $C_{35}H_{64}O_7$. The presence of four hydroxyl moieties was suggested by four successive losses of water (m/z 18) from the molecular ion in the CIMS. In addition, the IR spectrum contained a broad absorption at 3450 cm^{-1}, consistant with the presence of hydroxyl groups. The CIMS of the acetate derivative (**III**) and the trimethylsilyl derivative (TMS) (**IV**) demonstrated that **I** had four exchangeable protons as evidenced by MH^+ ions corresponding to $MH^+ + 4\,Ac$ and $MH^+ + 4\,TMS$, thus, confirming the presence of four hydroxyl groups.

A positive response to Kedde's [11] reagent suggested the presence of an α,β-unsaturated γ-lactone. A prominant IR carbonyl absorption at 1745 cm^{-1} and a UV(MeOH) λ max at 215 nm ($\varepsilon = 3.86$) supported the presence of this functionality. Expected resonances in the ^1H NMR and signals in ^{13}C-NMR [6, 12] confirmed the presence of the α,β-unsaturated-γ-lactone. Subsequent ^1H-^1H decoupling experiments showed coupling between the protons on C(3), H(3a) and H(3b), to the single proton at C(4), establishing the presence of an hydroxyl group at C(4) as in asimicin [12] and annonacin [6].

In addition to the resonances due to the oxygenated carbons of the lactone and the four hydroxylated carbons at δ74.3 (d), δ74.1 (d), δ71.4 (d) and δ69.8 (d), the ^{13}C NMR showed two resonances at δ82.7 (d) and δ82.4 (d) also due to oxygen bearing carbons. These ^{13}C NMR resonances and their corresponding ^1H NMR resonances at δ3.83 were directly analogous to similar signals in annonacin (**II**), indicating the presence of a single tetrahydrofuran moiety.

The placement of the hydroxyl groups alpha to the tetrahydrofuran functionality was established by ^1H-^1H decoupling experiments that linked the two-proton signal at δ3.81 to one-proton signals at δ3.43 and δ3.38. The subsequent downfield shift of these signals to δ4.79 (m) in the ^1H NMR of the acetate derivative (**III**) confirmed this assignment.

The single remaining hydroxyl group gave a one-proton signal at δ3.61 (m) in the ^1H NMR; irradiation of this resonance indicated that this hydroxyl group was isolated along the alkyl chain.

To determine the placement of the tetrahydrofuran ring and the isolated hydroxyl group along the hydrocarbon chain, mass spectral studies were undertaken. The mass spectra of the underivatized goniothalamicin (**I**) often gave irreproducible results due to pyrolysis products and thermal rearrangements. This necessitated the synthesis of several derivatives of **I** (fig. 1), of which the TMS derivative [bis(trimethylsilyl)acetamide in pyridine] (**IV**) and the perdeuterotrimethylsilyl derivative (**VI**) [TMS-d_9, bis(perdeuterotrimethylsilyl)acetamide in pyridine] gave the most reproducible mass spectra. Figure 2 shows the key fragments of derivatives **IV** and **VI** and their corresponding elemental compositions as determined by exact mass measurements. The ion at m/z 213 in the EI spectrum of **IV** increased by 9 µm in the spectrum of **VI** and 2 µm in the spectrum of **VII**, the TMS 2,33-dihydro derivative (10% Pd/C in EtOH), providing further evidence that the C(4) was hydroxylated. The ion at m/z 385, which shifted by 18 µm in the spectrum of **VI** and 2 µm in the spectrum of **VII**, indicated that a hydroxyl group is located at C(10). The number of carbons between the two rings was established by the ions at m/z 515 and 585.

The length of the hydrocarbon chain attached to the tetrahydrofuran ring was indicated by ions at m/z 299 and 369 in the mass spectrum of **IV** and the ion at m/z 308 in the mass spectrum of **VI**. Homologous ions observed in the EI spectrum of **III**, **V**, **VI**, and **VII** supported these assignments.

Annonacin (**II**) was obtained as a whitish wax with m.p. 57 °C. The molecular formula of annonacin was established by high resolution CIMS, and the IR, ^{13}C NMR and ^{1}H NMR spectra were identical to the published values for annonacin[6]. Co-TLC with an authentic sample of annonacin in five different TLC systems and optical rotations (α_D + 1.4 °) showed complete homogeneity, indicating that the two isolates were identical.

Acknowledgments. This work was supported by grant No. CA 30909 from the National Cancer Institute, NIH. The 470 MHz NMR instrument was available through the Purdue University Biochemical Magnetic Resonance Laboratory which is supported by NIH grant No. RR1077 from the Biotechnology Resources Program of the Division of Research Resources. Special thanks are due to the Purdue Cell Culture Laboratory for cytotoxicity results, Mr Barnard A. Scott at Eli Lilly and Co. (Greenfield) for pesticide testing, Dr John M. Cassady of Purdue University for a sample of annonacin, and Mr Thomas G. McCloud for helpful discussions.

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0014-4754/88/010083-03$1.50 + 0.20/0

1,1-Dimethyl-5,6-dihydroxyindolinium chloride from a deep water marine sponge, *Dercitus* sp.

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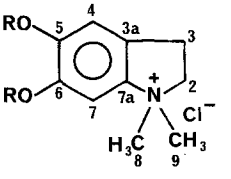
11 June 1987

Summary. 1,1-Dimethyl-5,6-dihydroxyindolinium chloride (**1a**) was identified from a deep water sample of the marine sponge, *Dercitus* sp., and its structure was elucidated by spectral methods.
Key words. 1,1-Dimethyl-5,6-dihydroxyindolinium chloride; marine natural products; *Dercitus*; marine sponge.

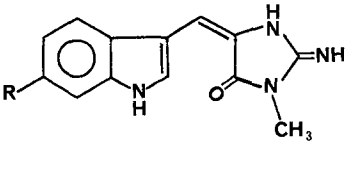
Numerous nitrogen-containing metabolites have been isolated from marine sponges[3–5], but only a small percentage of these metabolites contain a quaternary ammonium functionality. In this note, we report the isolation and identification of 1,1-dimethyl-5,6-dihydroxyindolinium chloride (**1a**), a new marine natural product from a deep water sponge, *Dercitus* sp. Gray, 1867[6]. Two tryptophan derivatives, 2'-de-N-methyl-aplysinopsin (**2a**) and 6-bromo-2'-de-N-methyl-aplysinopsin (**2b**), have been reported from a shallow water sample of *Dercitus* sp.[7].

The sponge was collected northwest of Goulding Cay, Bahamas, in August, 1985, at a depth of 215 m using the Harbor Branch Oceanographic Institution's submersible, the Johnson Sea-Link II. Sequential solvent extraction of the fresh frozen sponge (97 g) with EtOAc and MeOH yielded crude extracts (0.15 g and 2.7 g, respectively). From a portion of the MeOH extract (2 g), **1a** (118 mg) was purified with multilayer planetary coil CCC[8] using a solvent system of $CHCl_3$–MeOH–H_2O (5/10/6), followed by recrystallization from MeOH–$CHCl_3$ (m.p. 244 °C).

The molecular formula of **1a** was deduced as $C_{10}H_{14}NO_2Cl$ from elemental analysis of the monohydrate of the chloride salt (calculated for $C_{10}H_{16}NO_3Cl$: C, 51.5; H, 6.86; N, 6.00; Cl, 15.0; found: C, 51.58; H, 6.96; N, 6.02; Cl, 15.62) and high resolution FABMS (m/z of $C_{10}H_{14}NO_2$, 180.1021, Δ 0.4 nm). The presence of a 1,2,4,5-tetrasubstituted benzene ring in **1a** was suggested by the ^{1}H NMR singlets (d_4-MeOH) at δ 6.81 (H-4) and 7.09 (H-7), the chemical shifts and multiplicities of the sp^2 carbons (from proton decoupled and DEPT ^{13}C NMR experiments in d_4-MeOH: δ 124.9 (C-3a, s), 112.5 (C-4, d), 149.5 (C-5, s), 147.7 (C-6, s), 104.4 (C-7, d), and 139.5 (C-7a, s), and the relationship of these ^{13}C NMR doublets with the ^{1}H NMR singlets (from a C–H correlation experiment[9]). The presence of two phenolic hydroxyls in **1a** was suggested by the ^{13}C NMR singlets with chemical shifts of δ 147.7 and 149.5, IR bands at 3360 and 3140 cm^{-1}, the absence of a carbonyl band in the IR spectrum, and the formation of a diacetate (**1b**) upon treatment of **1a** with pyridine and acetic anhydride (**1b**:



1a, R = H
1b, R = Ac



2a, R = H
2b, R = Br