nae, and to describe the behavioral significance of these compounds.

This is the first time that a long chain epoxide, such as (Z,Z)-3,6-cis-9S,10R-epoxyheneicosadiene, has been identified in a noctuid moth sex pheromone. Nevertheless, it has been found by EAG screening[19] that other neotropical Catocalinae males have either polyunsaturated hydrocarbons or epoxiderivatives as key compounds. The 9S, 10R enantiomeres always give the best EAG responses, except within the genus *Zale* where the opposite one; 9R,10S, is the key compound. In the field it has been found possible to attract males of *Zale duplicata* by (Z,Z)-3,6-cis-9R,10S epoxyheneicosadiene[20].

Great similarities exist between the sex pheromones of Catocalinae and Arctiidae moths. Pheromones identified in all the Arctiidae sub-families, including Ctenuchinae, are also blends of polyunsaturated hydrocarbons[10,21] or unsaturated epoxides[13,17], or mixtures of the two[15]. More experimental data are required before it can be concluded that there is a close relationship between Catocalinae and Arctiidae within the super-family Noctuoidea; in particular, studies of biochemical pathways of pheromone synthesis within Catocalinae that might be similar to that described for Arctiid moths[16,22].

1 Heath, R. R., Tumlinson, J. H., Leppla, N. C., McLaughlin, J. R., Dueben, B. D., Dundulis, E., and Guy, R. H. J. chem. Ecol. *9* (1983) 645.
2 Underhill, E. W., Palaniswamy, P., Abrams, S. R., Bailey, B. K., Steck, W. F., and Chisholm, M. D., J. chem. Ecol. *9* (1983) 1413.
3 Descoins, C., Lalanne-Cassou, B., Malosse, C., and Milat, M. L., C. r. Acad. Sci. Paris, Série III, *302* (1986) 509.
4 Landolt, P. J., Heath, R. R., and Leppla, N. C., Envir. Ent. *15* (1986) 1272.
5 Heath, R. R., Landolt, P. J., Leppla, N. C., and Dueben, B. D., J. chem. Ecol. *14* (1988) 1121.
6 Renou, M., Thesis, Université Pierre et Marie Curie, Paris 1979.
7 Mori, K., and Edata, T., Tetrahedron *42* (1986) 3471.
8 Rollin, P., and Pougny, J. R., Tetrahedron *42* (1986) 3479.
9 Barth, R., Mems Inst. Oswaldo Cruz *52* (1954) 296.
10 Einhorn, J., Boniface, B., Renou, M., and Milat, M. L., C. r. Acad. Sci. Paris, Serie III, *298* (1984) 573.
11 Braumer, A., Budzikiewicz, H., and Boland, W., Org. Mass Spectrom. *17* (1982) 161.
12 Einhorn, J., Malosse, C., and Wirsta, P., Adv. Mass Spectrom. (1985) 1367.
13 Hill, A. S., and Roelofs, W. L., J. chem. Ecol. *7* (1981) 655.
14 Hill, A. S., Kovalev, B. B., Nikolaeva, L. N., and Roelofs, W. L., J. chem. Ecol. *8* (1982) 383.
15 Bell, W. T., and Meinwald, J., J. chem. Ecol. *12* (1986) 385.
16 Frerot, B., Renou, M., Malosse, C., and Descoins, C., Ent. exp. appl. *46* (1988) 281.
17 Frerot, B., Pougny, J. R., Milat, M. L., Rollin, P., and Malosse, C., C. r. Acad. Sci. Paris, Serie III, *306* (1988) 157.
18 Bestmann, H. J., Vostrowsky, O., and Platz, H., Experientia *33* (1977) 874.
19 Renou, M., Lalanne-Cassou, B., Dore, J. C., and Milat, M. L., J. Insect Physiol. *34* (1988) 481.
20 Wong, J. W., Underhill, E. W., Mackensie, J. L., and Chisholm, M. D., J. chem. Ecol. *11* (1985) 727.
21 Jain, S. C., Dussourd, D. E., Conner, W. E., Eisner, T., Guerrero, A., and Meinwald, J., J. org. Chem. *48* (1983) 2266.
22 Roelofs, W. L., and Bjostad, L., Bio-org. Chem. *12* (1984) 279.

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Gigantecin: A novel antimitotic and cytotoxic acetogenin, with nonadjacent tetrahydrofuran rings, from *Goniothalamus giganteus* (Annonaceae)

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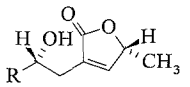
Summary. Gigantecin (**I**), a novel tetrahydroxy-di-tetrahydrofuran fatty acid γ-lactone (acetogenin), was isolated from an ethanolic extract of the stem bark of *Goniothalamus giganteus* Hook. f., Thomas (Annonaceae), by means of activity-directed fractionation (brine shrimp lethality test). This new compound is extremely cytotoxic to human tumor cells, inhibits crown gall tumors on potato discs, and is active in an assay designed to detect antimitotic agents (9 ASK).
Key words. Gigantecin; acetogenins; *Goniothalamus giganteus*; Annonaceae; brine shrimp; antimitotic; cytotoxic; crown gall tumors; potato disc assay; 9 ASK.

Tetrahydrofuranoid acetogenins represent a new class[1] of bioactive compounds occurring in certain genera of the plant family Annonaceae. In recent years, our research has focused on the isolation and characterization of these diversely bioactive (antitumor, cytotoxic, pesticidal)[2-5] compounds. Extracts of the stem bark of *Goniothalamus giganteus* Hook. f., Thomas (Annonaceae) were pesticidal against four test organisms and exhibited significant murine toxicity in the 3 PS lymphocytic leukemia system[6] (toxic at 6.25 mg/kg). Our previous bioactivity-directed studies of the bark of *Goniothalamus giganteus* have yielded the bioactive compounds altholactone (syn.: goniothalenol, a furano-2-pyrone)[7], goniothalamin (a styrylpyrone)[7], and pinocembrin (5,7-dihydroxy

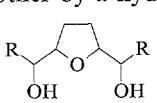
flavone)[7] and two C-35 tetrahydroxy monotetrahydrofuran acetogenins (goniothalamicin and annonacin)[4]. The continued fractionation of the ethanolic extract was guided by a convenient bioassay involving brine shrimp lethality[8]. Through multiple partitionings and chromatographic steps, monitoring the fractions with thin layer chromatography (TLC) and the brine shrimp bioassay, gigantecin (**I**), a novel bioactive acetogenin, was isolated.

Gigantecin (**I**) was obtained as a whitish wax (m.p. 96–98 °C, $[\alpha]_D^+$ 3.15, MeOH, c. 0.025 mg/ml). The high resolution chemical ionization (isobutane) mass spectra (CIMS) gave an MH^+ at m/z 639.4830 (calc. 639.4836) corresponding to the molecular formula $C_{37}H_{66}O_8$. The presence of four hydroxyl moieties was suggested by four successive losses of water (18 amu each) from the molecular ion in the CIMS. The presence of an α,β-unsaturated γ-lactone was suggested by a prominent IR absorption at 1745 cm^{-1} and a UV λ maximum at 220 nm ($\varepsilon = 1250$). ^{1}H-NMR peaks at δ 7.17 (1H, d, C-35), 5.06 (1H, dq, C-36), 2.51 (1H, dddd, H_b-C-3), 2.38 (1H, dddd, H_a-C-3), and 1.41 (3H, d, C-37) indicated that gigantecin (**I**) contained the structural subunit A which is also present in the structure of the acetogenin, asimicin[2]. ^{13}C-NMR absorptions at δ 174.6 (s, C-1), 151.2 (d, C-35) 131.1 (s, C-2), 77.9 (d, C-36) 69.9 (d, C-4), and 19.1 (q, C-37) confirmed the presence of this subunit. Essentially identical cd curves for rolliniastatin[5,9] (structure determined by X-ray), asimicin[5], bullatacin[5], and gigantecin suggest that the absolute configuration at C-4 and C-36, respectively, is the same in all four cases.

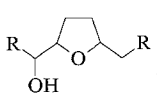


(A)

In addition to resonances due to the oxygenated carbons of subunit A and the three secondary hydroxy-bearing carbons at δ 74.4 (d, C-22), 74.3 (d, C-17) and 74.1 (d, C-14), (these assignments are tentative), the ^{13}C-NMR showed three resonances at δ 82.7 (d, C-21, C-18), 82.0 (d, C-13), and 79.3 (d, C-10) also due to oxygen-bearing carbons. These ^{13}C-NMR resonances and their corresponding ^{1}H-NMR resonances at δ 3.87 (5H, m, C-4, C-10, C-13, C-18, C-21), and 3.40 (3H, m, C-14, C-17, C-22) resemble analogous signals in goniothalamicin[4]. However, it was apparent that gigantecin possesses two tetrahydrofuran rings (subunits B and C), separated from each other by a hydrocarbon chain.

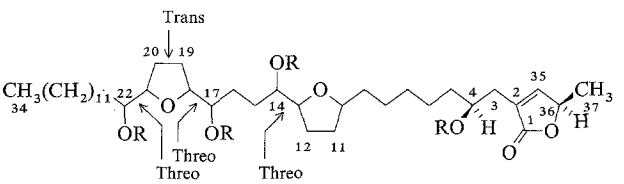


(B)



(C)

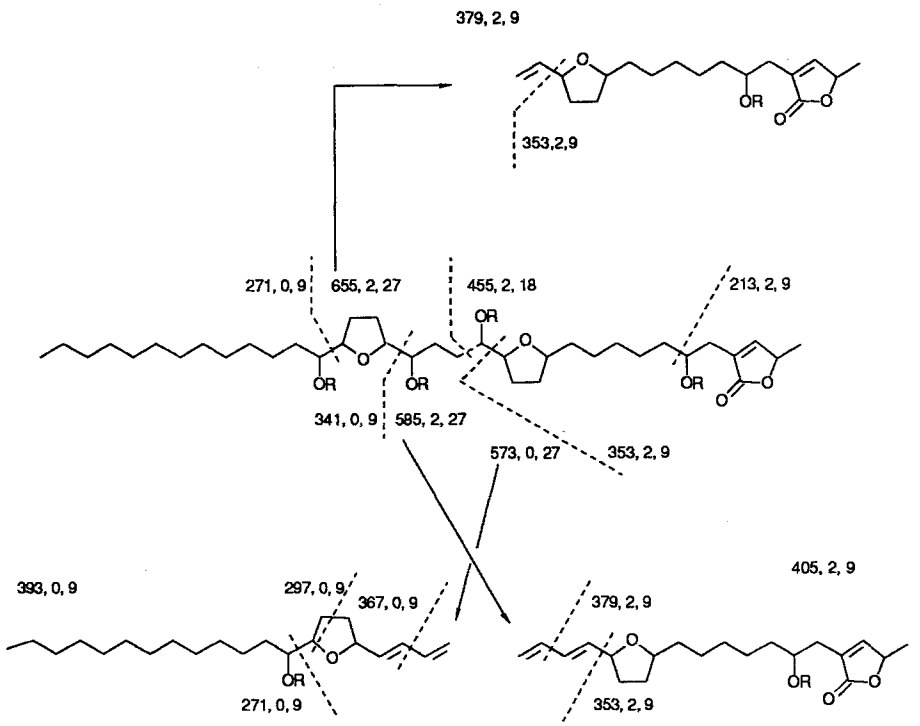
To determine the placement of the tetrahydrofuran rings (subunits B and C) and the length of the hydrocarbon chains attached to the subunits (A, B, and C), mass spectral studies were undertaken. The mass spectra of underivatized gigantecin (**I**) often gave irreproducible results due to formation of pyrolysis products and thermal rearrangements. This necessitated the synthesis of several derivatives including the TMS derivative **II**, [bis(trimethylsilyl) acetamide in pyridine], the 2,35-dihydro TMS derivative **III** (**I** was reduced in abs. EtOH/H_2/10% Pd on carbon followed by TMS derivatization), and the perdeutero trimethyl silyl derivative **IV**. A fragmentation scheme which was derived from the EIMS of compounds **I**, **II**, **III**, and **IV** is presented in the figure. The mass shift for the deuterated derivative **IV** was used with exact mass information to determine the elemental compositions of the fragments illustrated. The mass shift (9, 18, or 27) registered when recording the MS of **IV** differentiates ions which bear one, two, or three hydroxyl groups, respectively. The mass shift (0 or 2 amu) found upon hydrogenating the double bond in the lactone differentiates ions which contained the lactone subunit. The fragment ions with TMS groups frequently undergo loss of TMSOH, forming new ions which undergo further fragmentation. This was verified from the daughter ion spectra (E/B linked scans) of several structurally diagnostic fragments, as indicated by the arrows in the figure. For example, the daughter ion spectrum of the fragment at m/z 573 shows two consecutive losses of TMSOH to give the ion at m/z 393, which fragments further to give ions at m/z 367, 297, and 271.



I R = H
II R = TMS
III R = TMS and 2,35 dihydro
IV R = TMS-d_9

The relative stereochemistries within the carbon centers C-21/C-22, C-17/C-18, and C-13/C-14 were determined by comparing the ^{13}C NMR signals for the hydroxylated carbons (C-22, C-17, and C-14) with model compounds of known relative stereochemistry[10]. This comparison indicated that the relative configurations within the above couples are all of the threo type. The substitution pattern of the tetrahydrofuran ring of subunit B was established as trans by comparing the ^{1}H-NMR of the tetra-acetate derivative of gigantecin with a group of diacetyl dibutylated bis-tetrahydrofurans of known stereochemistry[11]. Identical ^{1}H-NMR shifts for the protons of C-21 and C-18 (δ 3.98) confirmed the trans assignment[11].

While gigantecin (**I**) was only moderately active in the brine shrimp test[8] (LC_{50} = 222 ppm), it strongly inhibited (83%) development of crown gall tumors on potato discs which is predictive of 3PS (P388) in vivo murine antileukemic activity[12]. In the older cytotoxicity tests[6], it was very active 9KB (human nasopharyngeal car-



Diagnostic EIMS fragment ions of **I**, **II**, **III**, and **IV**. High resolution MS (within 3×10^{-3} amu) confirmed each fragment composition. Numbers 0 or 2 following the ion mass refer to mass shifts in the 2,35-dihydro TMS derivative (**III**). Numbers 9, 18, or 27 refer to mass shifts in the TMS-d_9 derivative (**IV**).

cinoma, $ED_{50} < 10^{-5}$ µg/ml; 9PS chemically-induced murine leukemia, $ED_{50} < 10^{-2}$ µg/ml). In our panel of human tumor cell lines it was highly active: A-549, lung carcinoma, $ED_{50} = 2.19 \times 10^{-7}$ µg/ml; breast carcinoma, $ED_{50} = 4.11 \times 10^{-9}$ µg/ml; HT-29, colon adenocarcinoma, $ED_{50} = 2.68 \times 10^{-4}$ µg/ml. In the 9ASK test (astrocytoma reversal)[13], which is an indication of tubulin inhibition and antimitotic action, it was active (31–50% reversal at 10 µg/ml). Sufficient material was not available for pesticidal testing, but as an acetogenin[3], it very likely contributes to the pesticidal activity seen in the crude extracts and early column fractions[4]. Gigantecin represents the first acetogenin having nonadjacent tetrahydrofuranoid rings.

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1 Jolad, S. D., Hoffmann, J. J., Schram, K. H., and Cole, J. R., J. org. Chem. *47* (1982) 3151.
2 Rupprecht, J. K., Chang, C.-J., Cassady, J. M., McLaughlin, J. L., Mikolaczak, K. L., and Weisleder, D., Heterocycles *24* (1986) 1197.
3 Mikolaczak, K. L., McLaughlin, J. L., and Rupprecht, J. K., U.S. Patent No. 4,721,727 (issued Jan. 26, 1988).
4 Alkofahi, A., Rupprecht, J. K., Smith, D. L., Chang, C.-J., and McLaughlin, J. L., Experientia *44* (1988) 83.
5 Hui, Y.-H., Rupprecht, J. K., Liu, Y.-M., Anderson, J. E., Smith, D. L., Chang, C.-J., and McLaughlin, J. L., J. nat. Prod. *53* (1989) 463.
6 Geran, R. I., Greenberg, N. H., McDonald, M. M., Schumacher, A. M., and Abbott, J. B., Cancer Chemother. Rep. *3* (1972) protocols 16 and 13.
7 ElZayat, A. A. E., Ferrigni, N. R., McCloud, T. G., McKenzie, A. T., Byrn, S. R., Cassady, J. M., Chang, C.-J., and McLaughlin, J. L., Tetrahedron Lett. *26* (1984) 955.
8 Meyer, B. M., Ferrigni, N. R., Putnam, J. E., Jacobsen, L. B., Nichols, D. E., and McLaughlin, J. L., Planta med. *45* (1982) 31.
9 Pettit, G. R., Craig, G. M., Polonsky, J., Herald, D. L., Goswami, A., Smith, C. R., Moretti, C., Schmidt, J. M., and Weisleder, D., Can. J. Chem. *65* (1987) 1433.
10 Born, L., Lieb, F., Lorentzen, J. P., Moeschler, H., Nonfon, M., Sollner, R., and Wendish, D., Planta med. (in press).
11 Hoye, T. R., and Suhadolnik, J. C., J. Am. chem. Soc. *109* (1987) 4402.
12 Ferrigni, N. R., Putnam, J. E., Anderson, B., Jacobsen, L. B., Nichols, D. E., Moore, D. S., and McLaughlin, J. L., J. nat. Prod. *45* (1982) 679.
13 Lgarashi, K., Lkeyama, S., Takeuchi, T., and Sugino, Y., Cell Struct. Funct. *3* (1978) 103.

0014-4754/90/050539-03$1.50 + 0.20/0