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ANNONACEOUS ACETOGENINS: A REVIEW

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ABSTRACT.—The Annonaceous acetogenins are a series of apparently polyketide-derived fatty acid derivatives that possess tetrahydrofuran rings and a methylated γ-lactone (sometimes rearranged to a methyl ketolactone) with various hydroxyl, acetoxyl, and/or ketoxyl groups along the hydrocarbon chain. They exhibit a broad range of potent biological activities (cytotoxicity, antitumor, antimalarial, antimicrobial, immunosuppressant, antifeedant, and pesticidal). The sources, isolation, chemistry, biogenesis, and biological actions of these compounds, published to date, are tabulated and discussed. Strategies for structural elucidation are reviewed, and structural revisions and refinements are suggested for some of the previously published compounds.

The Annonaceae is a large family of tropical and subtropical trees and shrubs comprising about 120 genera and more than 2000 species (1). The phytochemistry of the Annonaceae through 1982 has been reviewed by Leboeuf *et al.* (2). Their review details the various alkaloids, carbohydrates, lipids, amino acids, proteins, polyphenols, essential oils, terpenes, and aromatic compounds typically found in these plants. Many species in this family are used in traditional medicine for various purposes; however, most of the previous phytochemical studies have not attempted to explain these folkloric uses through testing of biological activities. Bioactivity-directed isolation studies are strongly suggested in the search for potentially useful bioactive secondary metabolites in all medicinal plants.

Such an activity-directed fractionation by Jolad *et al.* (3), using 3PS in vivo murine leukemia testing of an EtOH extract of the roots of *Uvaria accuminata*, led to the isolation and structural elucidation of uvaricin, an unusual antitumor compound. Uvaricin was thus the first example of a new class of extremely bioactive compounds that are now referred to as the Annonaceous acetogenins. Chemically, these are C_{35}–C_{38} compounds, apparently of polyketide origin, possessing one or two tetrahydrofuran rings and a γ-lactone (either saturated or unsaturated), usually involving a three-carbon chain attached to a long aliphatic chain, and having long unbranched aliphatic regions that are variously hydroxylated, acetoxylated, or ketonized. We predict that additional chemical variations exist and will be found in the future.

The purpose of this review is to present the sources, isolation, chemistry, possible biogenesis, and biological activities of this rapidly growing class of natural compounds. Some helpful structural elucidation strategies and techniques for the determination of the stereochemistries of these typically waxy and amorphous acetogenins, via spectroscopic means, are presented. Also, a number of structural revisions and refinements of previously published compounds are suggested.

STRUCTURAL CLASSES

Three structural classes are currently evident when considering the number and arrangement of the tetrahydrofuran rings. For purposes of this review, the discussion is organized on the basis of these structural classes. It is quite possible that new classes exist and are awaiting discovery. The structures, physical and spectral data, bioactivities, sources, and references are presented in tabular form; ^{13}C-nmr assignments, if available, are provided around the structural illustrations of each compound. Explanations of the tabular footnotes and abbrevations used for the biological activities are pre-

sented at the bottom of Table 1. Within the tables, compounds are generally presented in chronological order of discovery.

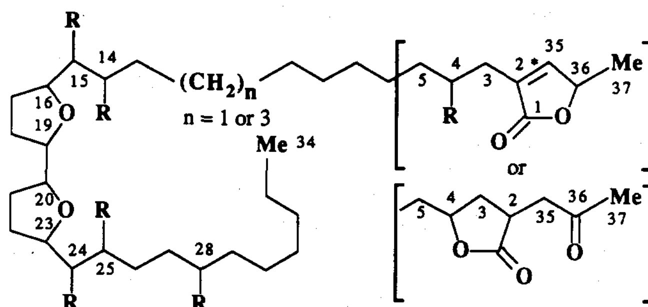
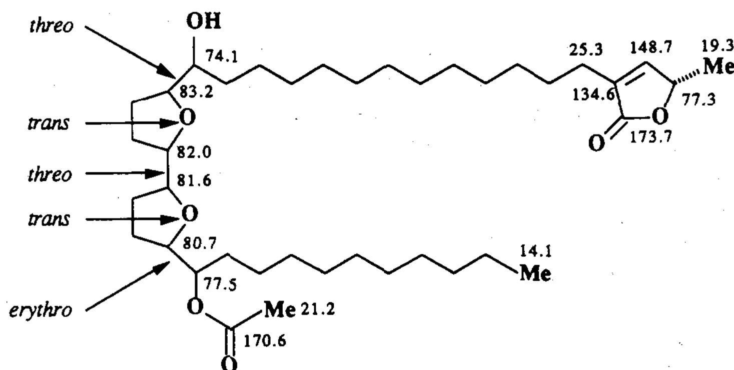


FIGURE 1. The adjacent bis-tetrahydrofuran ring acetogenin carbon skeleton. R represents either H, OH, ketone carbonyl, or acetoxyl. The * indicates that the lactone ring may be saturated.

ADJACENT BIS-TETRAHYDROFURAN RING ACETOGENINS.—So far, acetogenins with adjacent bis-tetrahydrofuran rings [**1–19**] are the largest class of this compound type (Table 1). Uvaricin is the prototype of compounds in this class (3). All of the reported compounds of this class possess the same basic carbon skeleton (Figure 1), with the exceptions of the proposed structures, as initially reported, for cherimoline (4) and dihydrocherimoline (4).

TABLE 1. Acetogenins with Adjacent Bis-tetrahydrofuran Rings[a].

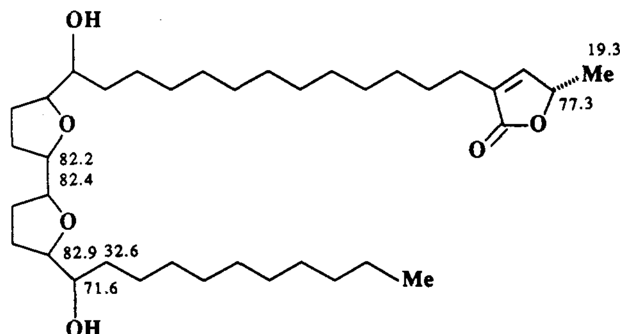
1. UVARICIN (3,5) $C_{39}H_{68}O_7$ MW 648



MP: Just above room temperature
$[\alpha]^{25}D$: +11.3 (MeOH)
MS: 630 $[M-H_2O]^+$, 588, 570, 435, 283, 365, 353, 295, 111.
UV: (EtOH) 207 (ϵ 12,730)
IR: (CCl_4) 3590, 2940, 2860, 1768, 1745, 1650, 1465, 1370, 1317, 1240, 1195, 1115, 1065, 1023, 945, 875, 850.
^1H NMR: ($CDCl_3$) 0.88 (3H, t, H-34), 1.25 (m, H-5–13, -26–33), 1.28 (2H, m, H-14), 1.41 (3H, d, H-37), 1.55 (3H, m, H-4, -25), 1.6–2.0 (8H, m, H-17, -18, -21, -22), 2.05 (3H, s, H-2′), 2.26 (2H, t, H-3), 3.38 (1H, m, H-15), 3.81 (1H, q, H-16), 3.87 (1H, m, H-19), 3.88 (1H, m, H-20), 4.01 (1H, q, H-23), 4.99 (1H, qq, H-36), 6.99 (1H, q, H-35).
(C_6D_6) 0.86 (J=7.0 Hz, H-37), 0.89 (J=6.4 Hz, H-34), 1.25 (H-5–13, -26–33), 1.3–1.7 (H-17, -18, -14, -4), 1.6–1.9 (H-21, -22, -25), 1.77 (H-2′), 2.13 (J=6.9, 4.4, 1.5, 1.5 Hz, H-3), 3.38 (H-15), 3.70 (H-19), 3.78 (H-20), 3.83 (J=6.3, 6.3 Hz, H-16), 4.03 (J=6.3, 6.3, 6.3 Hz, H-23), 4.30 (J=7.0, 1.5 Hz, H-36), 5.20 (H-24), 6.10 (H-35).

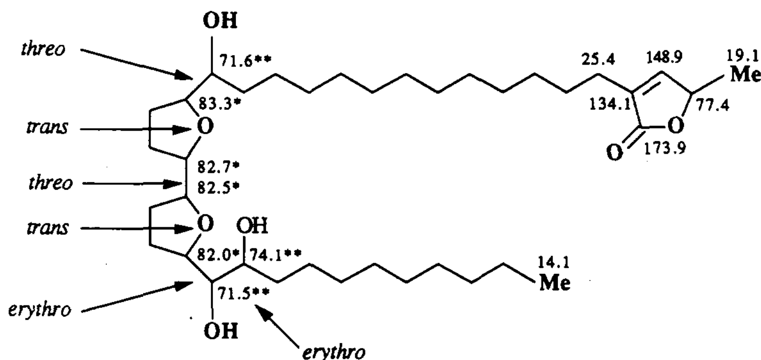
^{13}C NMR: ($CDCl_3$)
BIOLOGICAL ACTIVITY: In vivo 3PS 157% T/C at 1.4 mg/kg
DERIVATIVES: Uvaricinone (oxidation product of uvaricin)
SOURCES: *Uvaria acuminata* Oliv., roots (3)

2. DESACETYLUVARICIN (5) $C_{37}H_{66}O_6$ MW 606



MP: 63–65°
$[\alpha]^{25}D$: +9.30 (c = 1.41, MeOH)
MS: 606 $[M]^+$, 588, 570, 552
IR: ($CHCl_3$) 1775 cm^{-1}
^{1}H NMR: ($CDCl_3$) 3.8–4.0 (5H, H-16, -19, -20, -23, -24), 1.28 (H-25).
^{13}C NMR: ($CDCl_3$)
BIOLOGICAL ACTIVITY: Not reported
DERIVATIVES: Diacetate equivalent to uvaricin acetate
SOURCES: *U. acuminata*, roots (5)

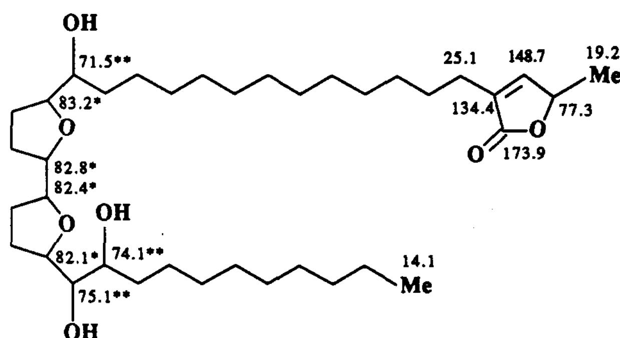
3. ROLLINICIN (6, 18) (see revised structure **32**) $C_{37}H_{66}O_7$ MW 622



The *, ** indicate that the assignments are interchangeable.

MP: 30–32°
$[\alpha]^{25}D$: +6.8 (c = 0.2411, $CHCl_3$)
CIMS: 623 (4.9), 605 (6.5), 587 (14.2), 569 (15.3), 399 (17.0), 347 (64.9), 329 (14.6), 295 (100), 265 (5.7), 251 (3.6), 239 (20.6), 237 (3.1), 223 (3.2), 209 (3.5), 195 (8.7), 181 (3.9), 169 (15.6), 167 (5.0), 153 (7.1), 141 (11.8), 139 (7.4), 127 (1.2), 125 (10.4), 111 (9.6), 99 (14.9), 97 (29.0), 96 (7.6), 85 (9.9), 71 (6.9)
UV: (CH_2Cl_2) 231 nm (log ε 4.31)
IR: (CCl_4) 3590, 3464, 2930, 1775, 1463, 1375, 1318, 1090, 710
^{1}H NMR: ($CDCl_3$, 89.56 or 360 MHz) 0.88 (3H, t, J = 7 Hz, H-34), 1.26 (br s, H-5–13, -27–33), 1.28 (m, H-14), 1.40 (3H, d, J = 6.8 Hz, H-37), 1.54 (3H, m, H-4, -26), 1.85–1.98 (8H, m, H-17, -18, -21, -22), 2.26 (2H, t, H-3), 3.40 (1H, m, H-15), 3.60 (1H, m, H-25), 3.86–3.93 (5H, m, H-16, -19, -20, -23, -24), 4.99 (1H, dq, J = 1.5, 6.8 Hz, H-36), 6.98 (1H, q, J = 1.5 Hz, H-35)
^{13}C NMR: ($CDCl_3$, 22.5 MHz)
BIOLOGICAL ACTIVITY: Cytotoxicity: 9PS $ED_{50} = 2.9 \times 10^{-8}$ μg/ml
DERIVATIVES: Rollinicin triacetate (9PS $ED_{50} = 2.6 \times 10^{-4}$ μg/ml)
Trimethylsilyl rollinicin
SOURCES: *Rollinia papilionella* Diels., roots (6)

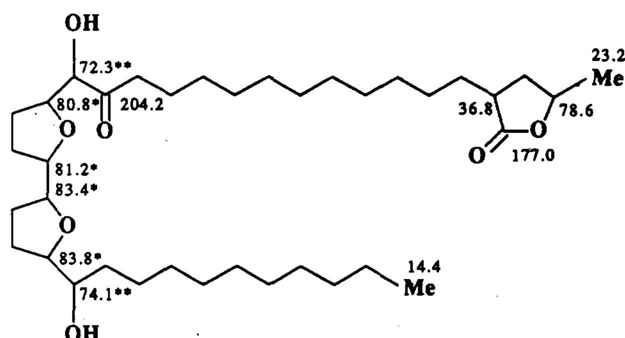
4. ISOROLLINICIN (6) (see revised structure 32) $C_{37}H_{66}O_7$ MW 622



The *, ** indicate that the assignments are interchangeable.

MP: 66–68°
CIMS: 623 (1.5), 605 (9.8), 587 (9.8), 569 (7.8), 347 (43.3), 329 (9.8), 311 (14.1), 295 (100), 265 (9.5), 251 (7.1), 239 (5.9), 237 (6.2), 223 (4.0), 209 (4.3), 195 (4.6), 181 (5.0), 169 (4.4), 141 (9.5), 111 (12.5), 85 (10.9), 71 (17.4)
UV: (CH_2Cl_2) 231 nm (log ε 4.30)
IR: (CCl_4) 3589, 3460, 2929, 1768, 1468, 1375, 1319, 1074, 716
^{1}H NMR: ($CDCl_3$, 89.56 or 360 MHz) 0.88 (3H, t, $J=7$ Hz, H-34), 1.26 (br s, H-5–13, -27–33), 1.36 (2H, m, H-14), 1.40 (3H, d, $J=6.8$ Hz, H-37), 1.54 (3H, m, H-4, -26), 1.81–2.01 (8H, m, H-17, -18, -21, -22), 2.26 (2H, t, H-3), 3.40 (1H, m, H-15), 3.82 (1H, m, H-25), 3.86–3.93 (5H, m, H-16, -19, -20, -23, -24), 4.99 (1H, dq, $J=1.5$, 6.8 Hz, H-36), 6.98 (1H, q, $J=1.5$ Hz, H-35)
^{13}C NMR: ($CDCl_3$, 22.5 MHz)
BIOLOGICAL ACTIVITY: Cytotoxicity: 9PS $ED_{50}=10^{-2}$ μg/ml
DERIVATIVES: None reported
SOURCES: *R. papilionella*, roots (6)

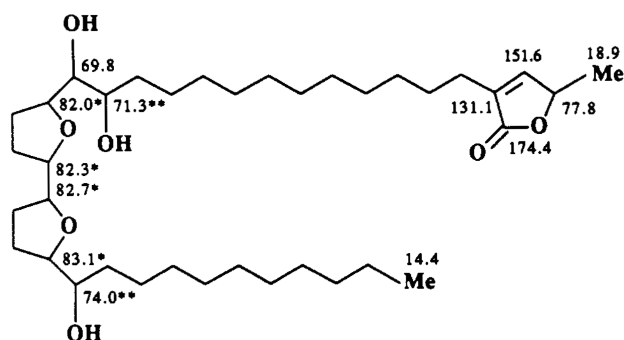
5. ROLLINONE (7) (see revised structure 33) $C_{37}H_{66}O_7$ MW 622



The *, ** indicate that the assignments are interchangeable.

MP: 54–55°
$[\alpha]_{25}$: +25.0° ($c=0.1371$, $CHCl_3$)
CIMS: 623 $[M+H]^+$ (10.8), 622 (0.4), 605 (9), 587 (9), 569 (7), 451 (2.5), 381 (5), 363 (28), 341 (1.7), 312 (21), 311 (100), 293 (13), 283 (0.5), 265 (5), 253 (1), 241 (10), 223 (3), 171 (1), 141 (9), 113 (7), 99 (5.1), 85 (5), 71 (7.6)
UV: (CH_2Cl_2) 231, 280 nm (ε 15199)
IR: (CCl_4) 3486, 2930, 1780, 1726, 1466, 1410, 1356, 1050, 715
^{1}H NMR: ($CDCl_3$, 89.56 or 360 MHz) 0.88 (3H, t, $J=7$ Hz, H-34), 1.26 (34H, br s), 1.47 (3H, m), 1.7–2.0 (10H, m), 2.18 (3H, d, $J=9$ Hz, H-37), 2.6 (2H, m, H-35), 3.05 (1H, m), 3.08 (1H, dt, $J=18.3$, H-16), 3.42 (1H, m), 3.8–3.95 (7H, m), 4.40 (1H, m, H-36)
^{13}C NMR: (C_6D_6, 22.5 MHz)
BIOLOGICAL ACTIVITY: Cytotoxicity: 9PS $ED_{50}<10^{-5}$ μg/ml in vivo 3PS 147% T/C at 1.4 mg/kg
DERIVATIVES: Rollinone diacetate
SOURCES: *R. papilionella*, roots (7)

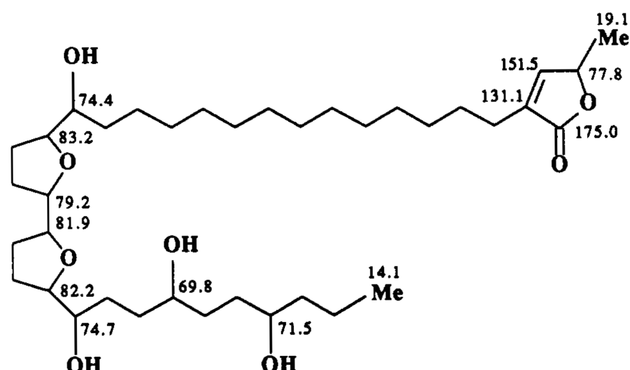
6. 14-HYDROXY-25-DESOXYROLLINICIN (8) $C_{37}H_{66}O_7$ MW 622
(see revised structure **34**)



The *, ** indicate that the assignments are interchangeable.

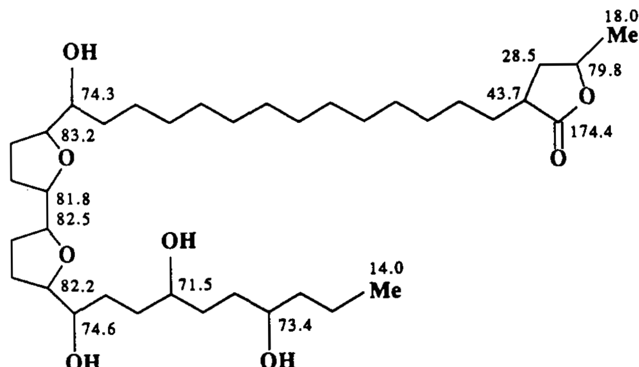
MP: 68–70°
[α]^{25}D: +15° (c = 0.06, MeOH)
MS: 451 (5), 433 (5), 415 (5), 381 (17), 363 (42), 345 (14), 341 (0.5), 317 (3), 311 (100), 309 (0.5), 293 (5), 282 (2), 275 (1), 247 (1), 241 (11), 223 (3), 169 (1), 141 (9), 139 (26), 125 (6), 111 (1), 97 (1), 95 (21), 83 (11), 71 (33), 69 (62)
UV: 218, 245 sh, 285, 293 sh, 332
IR: 3400, 3080, 2900, 1740, 1715, 1640, 1060
^{1}H NMR: ($CDCl_3$, 250 MHz) 0.87 (3H, t, J = 6.5 Hz, H-34), 1.20–1.30 (34H, br s), 1.44 (3H, d, J = 7 Hz, H-37), 1.36–1.58 (4H, m, H-4, -25), 1.80–2.00 (8H, m, H-17, -18, -21, -22), 2.43 (2H, m, H-3), 3.40 (2H, m, H-14, -24), 3.80–3.97 (5H, m, H-15, -16, -19, -20, -23), 5.06 (1H, dq, J = 7, 1.5 Hz, H-36), 7.19 (1H, d, J = 1.5 Hz, H-35)
^{13}C NMR: ($CDCl_3$, 90.56 MHz)
BIOLOGICAL ACTIVITY: None reported
DERIVATIVES: 14-Hydroxy-25-desoxyrollinicin triacetate
SOURCES: *Annona reticulata* L., stem bark (8)

7. CHERIMOLINE (4) (see revised structure **35**) $C_{37}H_{66}O_8$ MW 638



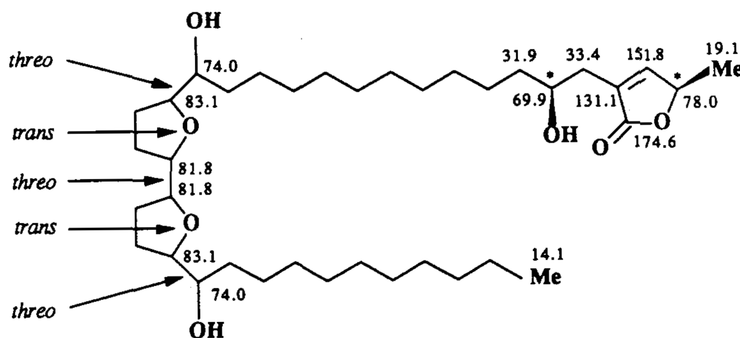
ıot reported
+64° (c = 0.3, MeOH)
638, 620, 602, 584, 449, 431, 413, 95, 379, 361, 329, 309, 291, 281, 259, :41, 223, 205
.750 cm^{-1}
ıR: ($CDCl_3$, 400 MHz) 0.88 (3H, t, H-34), 1.26 (m, H-5–14, -33), 1.30 (m, H-15, -26, -27, -29, -30, -32), 1.43 (3H, d, H-37), 1.69 (m, H-4, -18, -19, -22, -23), 2.40 (2H, t, H-3), 3.40 (m, H-16, -25, -28, -31), 3.80 (m, H-17, -20, -21, -24), 5.06 (1H, dq, H-36), 7.20 (d, H-35)
^{13}C NMR: ($CDCl_3$, 25.5 MHz)
BIOLOGICAL ACTIVITY: Antimicrobial activity
BST[b] LC_{50} = 1.24 ppm
Cytotoxicity[b] 9KB ED_{50} = 6 × 10^{-12} μg/ml; 9PS ED_{50} < 10^{-2} μg/ml; A549 ED_{50} = 10^{-3} μg/ml; HT-29 ED_{50} > 10^{-3} μg/ml; 9ASK inactive at 100 μg/ml
DERIVATIVES: Tetraacetylcherimoline, tetramethylcherimoline, tetraketocherimoline
SOURCES: *Annona cherimolia* Mill., seeds (4, 13)

8. DIHYDROCHERIMOLINE (4) (see section on Structural Revisions and Refinements) $C_{37}H_{64}O_8$ MW 636



MP: Not reported
[α]D: Not reported
CIMS: 640, 622, 604, 586, 451, 433, 415, 397, 381, 363, 345, 329, 311, 293, 283, 275, 259, 241, 223, 205
UV: 210
IR: 1770
^{1}H NMR: ($CDCl_3$, 90 MHz) 0.86 (t, H-34), 1.24 (m, H-3–15, -26, -27, -29, -30, -32, -33), 1.32 (d, H-37), 1.60 (m, H-18, -19, -22, -23), 2.33 (m, H-35), 2.54 (m, H-2), 3.39 (m, H-16, -25, -28, -31), 3.78 (m, H-17, -20, -21, -24), 4.46 (m, H-36)
^{13}C NMR: ($CDCl_3$, 25.5 MHz)
BIOLOGICAL ACTIVITY: Antimicrobial Activity
BST[b]: LC_{50} = 4.02 ppm
Cytotoxicity[b]: 9KB ED_{50} = 2×10^{-8} μg/ml; 9PS ED_{50} < 10^{-2} μg/ml; A549 ED_{50} = 10^{-3} μg/ml; HT-29 ED_{50} = 10^{-3} μg/ml; 9ASK inactive at 100 μg/ml
DERIVATIVES: Tetraacetyldihydrocherimoline
SOURCES: *An. cherimolia* seeds (4, 13)

9. ASIMICIN (9) $C_{37}H_{66}O_7$ MW 622



*This illustration is meant to show only the relative configurations at C-4 and C-36.

MP: 68–69°
$[\alpha]^{25}$D: Not reported
FABMS: 623 $[MH]^+$, 451, 381, 311, 293, 275, 241, 171, 153, 141
UV: (MeOH) 208 (ε 14300)
IR: 1750
^{1}H NMR: ($CDCl_3$, 470 MHz) 0.86 (3H, t, H-34), 1.25 (m, H-6–13, -26–33), 1.41 (3H, d, H-37), 1.55 (6H, m, H-5, -14, -25), 1.6–2.0 (8H, m, H-17, -18, -21, -22), 2.38 (1H, ddt, H-3b), 2.51 (1H, dddd, H-3a), 3.37 (2H, br q, H-15, -25), 3.86 (1H, m, H-4), 3.79–3.89 (4H, m, H-16, -19, -20, -23), 5.06 (1H, qq, H-36), 7.17 (1H, q, H-35)
(C_6D_6, 470 MHz) 0.86 (H-37), 0.92 (H-34), 1.35 (H-6–13, -26–33), 1.55 (H-14, -25), 1.4–1.8 (H-5, -17, -18, -21, -22), 2.27 (H-3b), 2.35 (H-3a), 3.45 (H-15, -24), 3.73 (H-19, -20), 3.77 (H-4), 3.86 (H-16, -23), 4.30 (H-36), 6.35 (H-35)
^{13}C NMR: ($CDCl_3$, 50 MHz) (9)
CD: (c = 0.025; EtOH) $[\theta]_{265}$ 0.00°, $[\theta]_{260}$ −199.04°, $[\theta]_{250}$ −995.20°, $[\theta]_{246}$

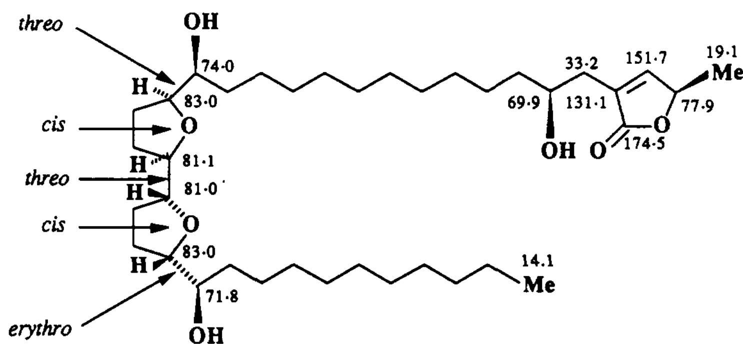
$-2288.95°$, $[\theta]_{234}$ $-2687.04°$, $[\theta]_{230}$ $-2388.48°$, $[\theta]_{225}$ $-59.712°$, $[\theta]_{224}$ $0.00°$, $[\theta]_{220}$ $4080.32°$, $[\theta]_{218}$ $7862.08°$ (10)

BIOLOGICAL ACTIVITY: BST $LC_{50} = 0.03$ ppm; PD[c] 70% tumor inhibition.
Cytotoxicity: 9KB $ED_{50} < 10^{-5}$ μg/ml; 9PS $ED_{50} < 10^{-12}$ μg/ml; A549 $ED_{50} = 10^{-3}$ μg/ml; HT-29 $ED_{50} = 3.3 \times 10^{-11}$ μg/ml; 9ASK cytotoxic at 10 μg/ml. NCI Human Tumor Cell Panel av. molar log $IC_{50} = -6.0$, delta 2.3, range 3.6
In vivo 3PS 124% T/C at 0.025 mg/kg and toxic at 0.22 mg/kg; L1210 131% T/C at 200 μg/kg[d]
Pesticidal Activity: Active against MBB 70% mortalities at 10 ppm, 100% at 50 ppm; MA 20% at 100 ppm, 100% at 500 ppm; ML 100% at 1 ppm; NE 100% at 0.1 ppm; BFL 50% at 0.5%; SCB 50% at 0.5% (11)
Antifeedant Activity: SCB 86% at 0.1% and 100% at 0.5% (11)
Antimalarial Activity[e]: Indochina W-2 $IC_{50} = 4767.50$ ng/ml, $IC_{90} = 8220.90$ ng/ml; Sierra Leone D-6 $IC_{50} = 3776.90$ ng/ml, $IC_{90} = 7767.40$ ng/ml.
Immunosuppressant Activities[f]: In vitro 95% T-cell suppression at 0.1 μg/ml; in vivo (mice) 49% T-cell suppression at 5.0 mg/kg

DERIVATIVES: Asimicin triacetate

SOURCES: *Asimina triloba* Dunal., bark, seeds (9); *Annona squamosa* L., seeds (12); *An. cherimolia*, seeds (13); *Rollinia mucosa* Jacq., seeds (14)

10. ROLLINIASTATIN 1 (15) $C_{37}H_{66}O_7$ MW 622



Relative stereochemistry determined by X-ray; the absolute configuration may be the mirror image of the illustration.

MP: 81–83°

[α]: $[\alpha]_{589}$ +25.2°, $[\alpha]_{578}$ +26.2°, $[\alpha]_{546}$ +30.1°, $[\alpha]_{436}$ +48.5°, $[\alpha]_{365}$ +76.7° ($c = 1.03$, CH_2Cl_2)
UV: (MeOH) 224 (log ε 3.93)
IR: ($CHCl_3$) 3580, 3460, 2928, 2855, 1748

EIMS: 381, 341, 311, 241, 171, 141

^{1}H NMR: ($CDCl_3$, 300 MHz) 0.85 (3H, t, H-34), 1.25 (H-6–13, -26–33), 1.41 (3H, d, H-37), 1.45 (m, H-5, -25), 1.50 (m, H-14), 1.7–1.9 (m, H-17, -18, -21, -22), 2.36 (1H, dddd, $J = 15.1$, 8.1, 1.2, 1.4 Hz, H-3b), 2.50 (1H, dddd, $J = 15.1$, 3.5, 1.5, 1.6 Hz, H-3a), 3.38 (1H, m, H-15), 3.85 (6H, m, H-4, -16, -19, -20, -23, -24), 5.02 (1H, dddq, $J = 1.5$ Hz, H-36), 7.16 (1H, ddd, $J = 1.5$ Hz, H-35)

^{13}C NMR: ($CDCl_3$)

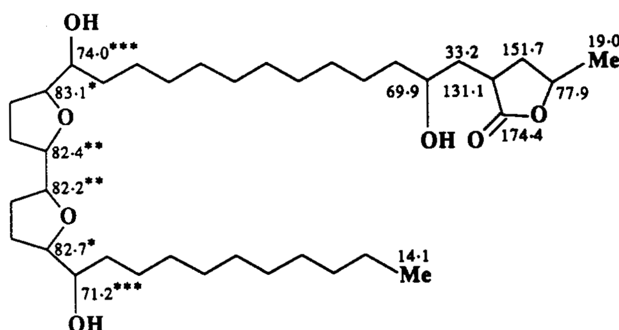
CD: ($c = 0.025$; abs. EtOH) $[\theta]_{265}$ 0.00°, $[\theta]_{260}$ $-199.04°$, $[\theta]_{250}$ $-1393.28°$, $[\theta]_{240}$ $-2587.52°$, $[\theta]_{235}$ $-2786.56°$, $[\theta]_{230}$ $-2089.92°$, $[\theta]_{225}$ 0.00°, $[\theta]_{220}$ 6369.28° (10)

BIOLOGICAL ACTIVITY: Cytotoxicity: 9PS $ED_{50} = 4.5 \times 10^{-5}$ μg/ml; in vivo 3PS 128% T/C at 0.25 mg/kg

DERIVATIVES: Rolliniastatin 1 triacetate (28)

SOURCES: *R. mucosa*, seeds (15)

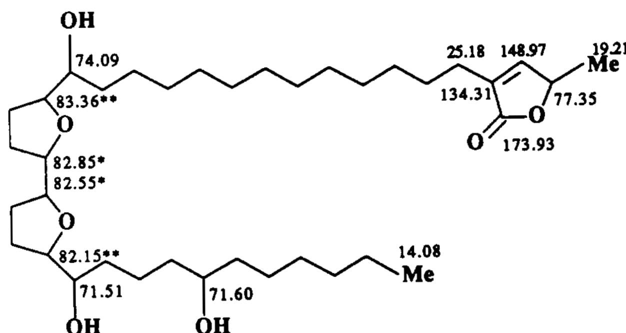
11. ROLLINIASTATIN 2 (14) $C_{37}H_{66}O_7$ MW 622



The *, **, *** indicate that the assignments are interchangeable.

MP: 73–76°
$[\alpha]^{27}D$: +5.30 (c = 0.23, $CHCl_3$)
UV: (MeOH) 210 nm (log ε 3.85)
IR: ($CHCl_3$) 3450, 2925, 2855, 1755, 1720 sh, 1615, 1465, 1320
HREIMS: 604.4661 $[M-H_2O]^+$, 451.3109 (0.4), 433.2956 (6), 415.2860 (11), 381.2633 (3), 363.2560 (43), 345.2463 (19), 312.2296 (19), 311.2254 (100), 293.2252 (19)
^1H NMR: ($CDCl_3$, 400 MHz) 0.87 (3H, t, J = 6.8 Hz, H-34), 1.25 (32H, m, H-6–13, -26–33), 1.42 (3H, d, J = 6.8 Hz, H-37), 1.46 (2H, m, H-5), 1.50 (2H, m, H-14), 1.7–2.0 (8H, m, H-17, -18, -21, -22), 2.39 (1H, dddd, J = 15.1, 3.5, 1.6, 1.5 Hz, H-3b), 2.52 (1H, dddd, J = 15.1, 3.5, 1.6, 1.5 Hz, H-3a), 3.38 (1H, m, H-15), 5.05 (1H, ddd, J = 6.8, 1.5, 1.5 Hz, H-36), 7.16 (1H, ddd, J = 1.5, 1.5, 1.2 Hz, H-35)
^{13}C NMR: ($CDCl_3$, 100 MHz)
BIOLOGICAL ACTIVITY: Cytotoxicity: 9PS $ED_{50} = 2.3 \times 10^{-4}$ μg/ml
DERIVATIVES: None
SOURCES: *R. mucosa*, seeds (14)

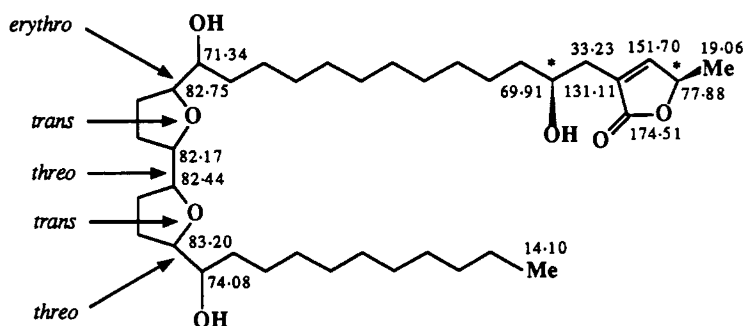
12. SQUAMOCIN (16) $C_{37}H_{66}O_7$ MW 622



The *, ** indicate that the assignments are interchangeable.

MP: <30°
$[\alpha]^{20}D$: +15.0 (c = 1.7, MeOH)
UV: (MeOH) 215 nm (log ε 3.5)
IR: ($CHCl_3$) 3680, 3585, 3460, 3015, 2940, 2855, 1755
EIMS: 604, 586, 568, 519, 435, 417, 399, 365, 347, 329, 317, 295, 267, 239, 221
^1H NMR: ($CDCl_3$, 400 MHz) 0.83 (3H, t, J = 7 Hz, H-34), 1.20–1.27 (m, H-5–13, -31–33), 1.3 (m, H-14, -25, -26a, -30), 1.36 (3H, d, J = 6.8 Hz, H-37), 1.35–1.40 (m, H-27, -29), 1.50 (m, H-4, -17a, -18a, -22a), 1.6 (1H, m, H-26b), 1.76 (m, H-21a), 1.87 (m, H-21b), 1.90 (m, H-17b, -18b, -22b), 2.21 (2H, tt, J = 7.7, 1.4 Hz, H-3), 3.33 (1H, dt, J = 11, 7.5 Hz, H-15), 3.52 (1H, m, H-28), 3.76 (m, H-16, -23, -24), 3.86 (2H, m, H-19, -20), 4.95 (1H, qq, J = 6.8, 1.4, H-36), 6.96 (1H, q, J = 1.4 Hz, H-35)
^{13}C NMR: ($CDCl_3$, 100 MHz)
BIOLOGICAL ACTIVITY: Cytotoxicity: L1210 ED_{50} = 0.58 μg/ml (16)
Pesticidal Activity: strong ovicidal and larvicidal activity in tests at 125–140 μg/2 g of diet for *Drosophila melanogaster* (17)
DERIVATIVES: Jones oxidation products **a**,**a′** and their methyl esters **b** and **b′** (see Figure 9)
SOURCES: *An. squamosa*, seeds (12,16,17)

13. BULLATACIN (10) $C_{37}H_{66}O_7$ MW 622



*This illustration is meant to show only the relative configurations at C-4 and C-36.

MP: 69–70°

[α]: $[\alpha]_{589}^{23}$ +13.00, $[\alpha]_{578}$ +14.70, $[\alpha]_{546}$ +19.04, $[\alpha]_{436}$ +36.63, $[\alpha]_{365}$ = +66.9 (c = 0.004, $CHCl_3$)

UV: (EtOH) 215.2 (ε = 7974)

IR: (KBr) 3430, 1750

CIMS: (isobutane) 623 $[MH]^+$

CIMS: (ammonia) 622 $[M + NH_4 - H_2O]^+$, 640 $[M + NH_4]^+$

EIMS: 622 $[M]^+$, 451, 381, 311, 243, 241, 141; ms of Ac, Ac-d_3, TMSi, TMSi-d_9 derivatives

^{1}H NMR: ($CDCl_3$, 470 MHz) 0.85 (3H, t, J = 6.81 Hz, H-34), 1.25 (br s, H-6–13, -26–33), 1.3–2.0 (m, H-5, -17, -18, -21, -22, -25, -14), 1.41 (3H, d, J = 6.83 Hz, H-37), 2.36 (1H, ddt, J = 15.0, 8.0, 1.4 Hz, H-3b), 2.50 (1H, dddd, J = 15.0, 4.0, 1.5, 1.1 Hz, H-3a), 3.38 (1H, tt, J = 8.0, 2.0 Hz, H-24), 3.80 (m, H-4), 3.83 (m, H-15, -20, -23), 3.92 (m, H-16, -19), 5.05 (1H, ddq, J = 6.83 Hz, H-36), 7.17 (1H, d, J = 1.70 Hz, H-35); (C_6D_6, 470 MHz) 0.80 (3H, d, J = 6.83 Hz, H-37), 0.9 (3H, t, J = 6.81 Hz, H-34), 1.25 (br s, H-6–13, -26–33), 1.3–2.1 (H-5, -14, -17, -18, -21, -22, -25), 2.20 (1H, ddt, J = 15.0, 8.0, 1.4 Hz, H-3b), 2.30 (1H, dddd, J = 15.0, 4.0, 1.5, 1.1 Hz, H-3a), 3.45 (1H, ttt, J = 8.0, 2.0, 1.0, H-24), 3.67 (1H, ddd, J = 6.0, 1.0 15.0 Hz, H-20), 3.71 (br tt, H-4), 3.85 (1H, ddd, J = 8.0, 6.9 Hz, H-23), 3.89 (2H, m, H-15, -16), 3.99 (1H, dt, J = 8.5, 2.5 Hz, H-16), 4.23 (1H, ddq, J = 6.83 Hz, H-36), 6.25 (1H, d, J = 1.70 Hz, H-35)

^{13}C NMR: ($CDCl_3$, 50 MHz)

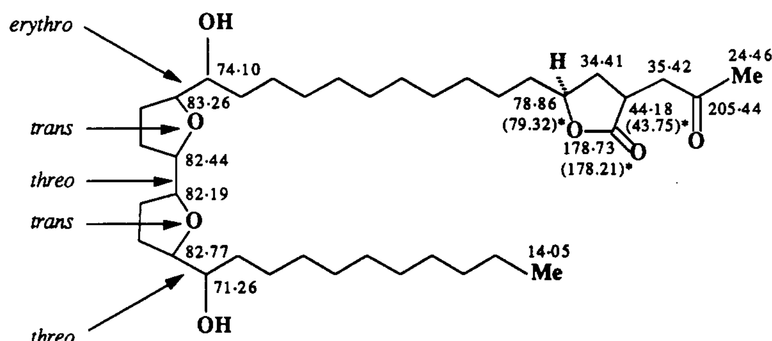
CD: (c = 0.025, abs. EtOH) $[\theta]_{265}$ 0.00°, $[\theta]_{260}$ −298.56°, $[\theta]_{250}$ −995.20°, $[\theta]_{240}$ −2189.44°, $[\theta]_{233}$ −2587.52°, $[\theta]_{230}$ −2348.67°, $[\theta]_{225}$ 0.00°, $[\theta]_{220}$ 4378.88°, $[\theta]_{218}$ 7961.60°

BIOLOGICAL ACTIVITY: BST LC_{50} = 0.00159 μg/ml; PD 53% tumor inhibition
Cytotoxicity: 9PS $ED_{50} = 10^{-15}$–10^{-16} μg/ml; 9KB $ED_{50} = 6.188 \times 10^{-14}$ μg/ml; A546 $ED_{50} = 1.25 \times 10^{-13}$ μg/ml; MCF-7 $ED_{50} > 10$ μg/ml; HT-29 $ED_{50} = 10^{-12}$ μg/ml. NCI Human Tumor Cell Panel (without dilution) ranged from 10° to 10^{-6}; av. molar log $IC_{50} = -1.0$, delta 2.0, range 4.0; in vivo L1210 leukemia 138% T/C at 50 μg/kg[d]
Pesticidal Activity: active against MA 80% at 1 ppm, ML 80% at 10 ppm, CRW 80% at 24 ppm, 2SSM 20% at 10 ppm

DERIVATIVES: Bullatacin triacetate (BST LC_{50} = 5.7 ppm; 9PS $ED_{50} = 3.89 \times 10^{-3}$ μg/ml; 9KB $ED_{50} = 6.85 \times 10^{-7}$ μg/ml; A549 $ED_{50} = 2 \times 10^{-3}$ μg/ml; HT-29 $ED_{50} = > 10^{-1}$ μg/ml), dihydrobullatacin (BST LD_{50} = 0.0145 ppm; A549 $ED_{50} < 10^{-6}$ μg/ml; HT-29 $ED_{50} = 3.33 \times 10^{-5}$ μg/ml)

SOURCES: *Annona bullata* Rich., bark (10); *An. squamosa*, bark (18)

14. BULLATACINONE (10) $C_{37}H_{66}O_7$ MW 622



*Numbers in parentheses indicate the corresponding chemical shifts of the bullatacinone minor diastereomers.

MP: 90.5–90.7°

[α]: $[\alpha]_{589}$ +12.00, $[\alpha]_{578}$ +12.50, $[\alpha]_{546}$ +14.50, $[\alpha]_{436}$ +29.75, $[\alpha]_{365}$ +51.25 (c = 0.400, $CHCl_3$)

UV: (EtOH) 203.5 nm (ε 3799)

IR: (KBr) 1770, 1715

CIMS: (isobutane) 623 $[MH]^+$, 661 $[MH+38]^+$

CIMS: (ammonia) 623 $[MH]^+$, 640 $[MNH_4]^+$

EIMS: 622 $[M]^+$, 451, 381, 311, 241, 141; ms of Ac, Ac-d_3, TMSi, TMSi-d_9 derivatives

^{1}H NMR: (C_6D_6, 470 MHz) 0.90 (3H, t, J = 7.05 Hz, H-34), 1.25 (br s, H-6–13, -26–33), 1.3–2.0 (1H, H-5), 1.35, 1.65 (H-17, -18), 1.40 (ddd, H-3b, -14a), 1.42 (H-21, -22), 1.52 (H-21, -22), 1.55 (H-14b, -37), 1.60 (H-25b), 1.70 (1H, ddd, J = 12.82, 3.38, 9.34 Hz, H-3a), 1.93 (1H, dd, J = 18.31, 9.34 Hz, H-35a) [1.89, dd, H-35a],* 2.10 (H-25a), 2.53 (1H, dd, J = 3.42 Hz, H-35b) [2.64, dd, H-35b],* 2.71 (1H, dddd, J = 9.34, 9.34, 9.34, 3.42 Hz, H-2), [2.62, dddd, H-2],* 3.49 (1H, tt, J = 8.06, 2.38 Hz, H-15), 3.66 (1H, ddd, J = 5.96, 1.00, 14.93 Hz, H-20), 3.85 (1H, ddd, J = 8.06, 6.96 Hz, H-16), 3.89 (2H, m, H-19, -24), 3.95 (1H, dd, J = 8.4, 2.5 Hz, H-23), 4.05 (m, H-4) [3.72, m, H-4]*

*numbers in brackets indicate the corresponding chemical shifts of the bullatacinone minor diastereomer

^{13}C NMR: ($CDCl_3$, 50 MHz)

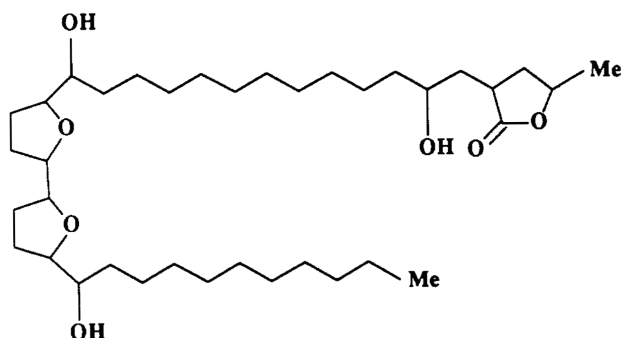
CD: (c = 0.1, abs. EtOH) $[\theta]_{250}$ 0.00°, $[\theta]_{240}$ −124.4°, $[\theta]_{235}$ −248.8°, $[\theta]_{230}$ −497.6°, $[\theta]_{225}$ −870.8°, $[\theta]_{220}$ −1555°, $[\theta]_{215}$ −1990.4°, $[\theta]_{212}$ −2177°, $[\theta]_{210}$ −1492.8°, $[\theta]_{207}$ −746.4°, $[\theta]_{205}$ 0.00°

BIOLOGICAL ACTIVITY: BST LC_{50} = 0.003 μg/ml; PD 15% tumor inhibition
Cytotoxicity: 9PS $ED_{50} = 4.23 \times 10^{-3}$ μg/ml; 9KB $ED_{50} < 10^{-12}$ μg/ml; A549 $ED_{50} = 10^{-3}$ μg/ml; HT-29 $ED_{50} = 5 \times 10^{-12}$ μg/ml; NCI Human Tumor Cell Panel av. molar log IC_{50} = −1.3, delta 2, range 2.2
In vivo L1210 144% T/C at 400 μg/kg[d]
Pesticidal Activity: not active in ML at 10 ppm, MA at 400 ppm, SAW at 400 ppm, CRW at 24 ppm, or 2SSM at 400 ppm

DERIVATIVES: Bullatacinone diacetate (BST LC_{50} > 10 ppm; 9PS $ED_{50} = 4.22 \times 10^{-2}$ μg/ml; 9KB $ED_{50} = 5 \times 10^{-3}$ μg/ml; A549 $ED_{50} = 2.8 \times 10^{-2}$ μg/ml; HT-29 $ED_{50} = 10^{-1}$ μg/ml)

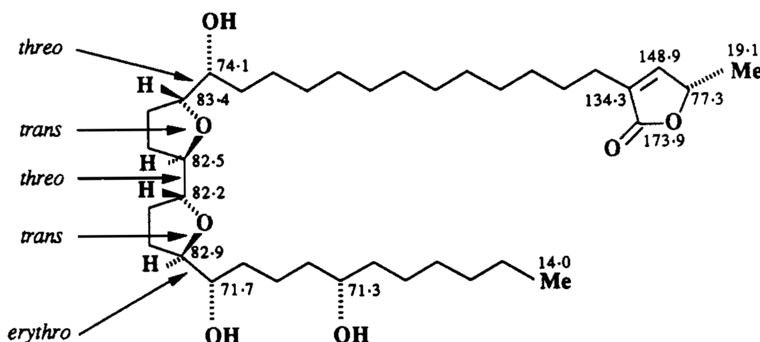
SOURCES: *An. bullata*, bark (10); *An. squamosa*, bark (18).

15. LAHERRADURINE (13) $C_{37}H_{68}O_7$ MW 624



MP: 85–86°
CIMS: 624 $[M]^+$, 453, 435, 417, 399, 383, 365, 347, 331, 313, 295, 285, 241, 223, 203
EIMS: 453 (4), 435 (11), 417 (15), 399 (11), 383 (7), 365 (42), 347 (69), 313 (98), 311 (17), 295 (100), 285 (5), 267 (10), 241 (22), 223 (6), 203 (5), 141 (14)
IR: ($CHCl_3$) 3590, 2930, 2860, 1765, 1450, 1355, 1300, 1055, 955, 865
^{1}H NMR: ($CDCl_3$, 250 MHz) 0.90 (3H, t, $J=$ 6.8 Hz, H-34), 2.08–2.17 (46H, m), 1.36 (3H, d, $J=6.7$ Hz, H-37), 2.62–2.25 (5H, m, H-2, -3, -35), 4.21–3.45 (7H, m, H-4, -15, -16, -19, -20, -23, -24), 4.53 (1H, dd, $J=6.7$, 1.7 Hz, H-36)
BIOLOGICAL ACTIVITY: None reported
DERIVATIVES: Triacetyllaherradurine
SOURCES: *An. cherimolia*, seeds (13)

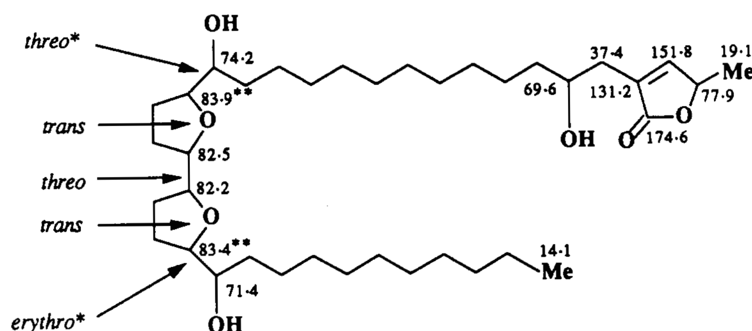
16. ANNONIN I (12,19,20) $C_{37}H_{66}O_7$ MW 622



The relative stereochemistry determined by X-ray; the absolute configuration may be the mirror image of the illustration.

MP: 51–54°
$[\alpha]^{25}D$: +21.5 ($c=0.15$, CH_2Cl_2)
UV: (MeCN) 209 nm (log ε 3.98)
IR: (KBr) 3440, 3380, 2920, 2850, 1745, 1655, 1465, 1320, 1120, 1070, 1020, 960, 930, 910, 860, 840, 740, 630
MS: 604 (<1) $[M-H_2O]^+$, 586 (<1), 568 (<1), 519 (1), 501 (3), 483 (3), 465 (2), 417 (7), 399 (17), 347 (63), 329 (15), 319 (15), 295 (100), 267 (19), 239 (19), 203 (8), 195 (14), 169 (18), 135 (22), 121 (33), 109 (39), 97 (49), 95 (66), 83 (34), 81 (60), 71 (44), 69 (39), 67 (46), 57 (27), 55 (56), 43 (35), 41 (35)
^{1}H NMR: ($CDCl_3$, 360 MHz) 0.878 (3H, t, $J=6.6$ Hz), 1.405 (3H, d, $J=6.5$ Hz), 2.05–1.18 (46H, m), 2.263 (2H, tt), 3.400 (1H, q), 3.6 (1H, m), 3.77–3.97 (5H, m), 4.995 (1H, qq), 6.988 (1H, q)
^{13}C NMR: ($CDCl_3$, 50.31 or 90.56 MHz)
BIOLOGICAL ACTIVITY: Cytotoxicity reported
DERIVATIVES: Dihydroannonin, potassium salt of a substituted 4-hydroxypentanoic acid of annonin I triacetate
SOURCES: *An. squamosa*, seeds (12) (17,19,20)

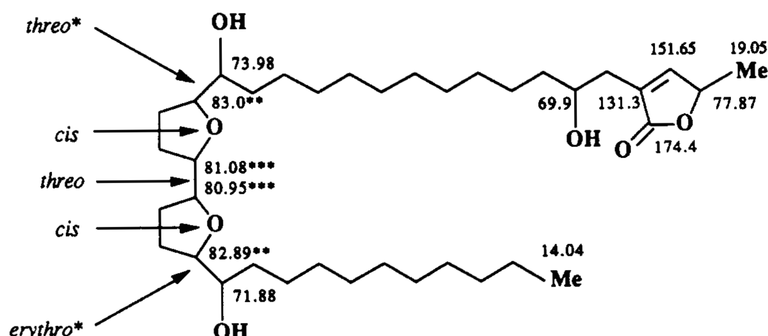
17. ANNONIN VI (12) $C_{37}H_{66}O_7$ MW 622



The *, ** indicate that the assignments are interchangeable.

MP: Not reported
$[\alpha]^{25}D$: +15.3 (c=0.4, CH_2Cl_2)
MS: 604 (<1) $[M-H_2O]^+$, 586 (<1), 569 (<1), 463 (1), 451 (2), 445 (1), 433 (5), 415 (8), 381 (1), 363 (26), 345 (16), 323 (1), 311 (100), 305 (2), 293 (18), 281 (1), 275 (8), 263 (2), 171 (2), 153 (4), 141 (8), 123 (12), 111 (8), 109 (2), 97 (26), 95 (31), 81 (38), 71 (30), 67 (26), 57 (28), 55 (36), 43 (78), 41 (18)
IR: (KBr) 3418, 2920, 2850, 1747, 1644, 1472, 1324, 1146, 1099, 1026, 960, 928, 912, 858, 719
^1H NMR: Not reported
^{13}C NMR: ($CDCl_3$, 50.31 or 90.56 MHz)
BIOLOGICAL ACTIVITY: Cytotoxicity and anthelmintic against NE
DERIVATIVES: Annonin VI triacetate
SOURCES: *An. squamosa*, seeds (12)

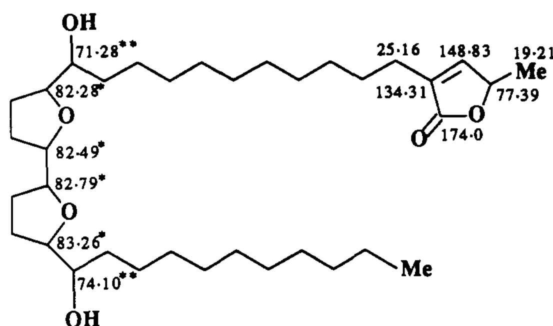
18. 4-HYDROXY-25-DESOXYNEOROLLINICIN (21) $C_{37}H_{66}O_7$ MW 622



The *, ** indicate that the assignments are interchangeable.

MP: 25°
IR: (CCl_4) 3478, 2928, 2856, 1763, 1465, 1318, 1074
^1H NMR: ($CDCl_3$, 300 MHz) 0.88 (3H, t, J= 6.8 Hz, H-34), 1.25 (m, H-6–13, -26–33), 1.43 (3H, d, J=6.8 Hz, H-37), 1.5 (6H, m, H-5, -14, -25), 1.7–2.0 (8H, m, H-17, -18, -21, -22), 2.40 (1H, dd, J= 8, 15 Hz, H-3a), 2.53 (1H, ddd, J=3, 15, 1.5 Hz, H-3b), 3.41 (1H, m, H-15), 3.80–3.92 (5H, m, H-16, -19, -20, -23, -24), 3.88 (1H, m, H-4), 5.06 (1H, q, J= 6.8 Hz, H-36), 7.20 (1H, br s, H-35)
^{13}C NMR: ($CDCl_3$, 75 MHz)
BIOLOGICAL ACTIVITY: Cytotoxicity: A549 ED_{560}< 10^{-3} μg/ml; MCF-7 ED_{50}< 10^{-3} μg/ml; HT-29 ED_{50} = 1.26 μg/ml
DERIVATIVES: 4-Hydroxy-25-desoxyneorollinicin triacetate
SOURCES: *R. papilionella*, roots (21)

19. NEOANNONIN (17) $C_{35}H_{62}O_6$ MW 578



The *, ** indicate that the assignments are interchangeable.

MP: Not reported
[α]^{22}D: +18.8 (c = 1.35, MeOH)
UV: (MeOH) 3425, 3375, 2925, 2850, 1760, 1470, 1390, 1320, 1120, 1080, 1020, 960, 910, 790, 720, 630
CIMS: (isobutane) 579 [MH]$^+$, 561, 543, 525, 407, 337, 311, 267, 241
EIMS: 524 (12.3), 506 (7.5), 437 (3.5), 409 (2.4), 407 (4.7), 389 (6.4), 371 (13.2), 337 (9.5), 319 (37.7), 301 (16.0), 267 (100)
^{1}H NMR: ($CDCl_3$, 500 MHz) 0.85 (3H, t, J = 7.0 Hz), 1.15–1.34 (28H, br s), 1.35–1.37 (2H, m), 1.38 (3H, d, J = 6.8 Hz), 1.40–1.55 (2H, m), 1.56–1.66 (2H, m), 1.74–1.81 (2H, m), 1.82–1.91 (6H, m), 1.92–2.0 (2H, m), 2.23 (2H, ddt, J = 1.7, 1.7, 7.0 Hz), 3.37 (1H, dt, J = 7.1, 4.1 Hz), 3.80–3.87 (3H, m), 3.88–3.93 (2H, m), 4.97 (1H, dtq, J = 1.7, 1.7, 6.8 Hz), 6.96 (1H, dt, J = 1.7, 1.7 Hz)
^{13}C NMR: ($CDCl_3$, 125 MHz)
BIOLOGICAL ACTIVITY: Pesticidal activity: strong ovicidal and larvicidal activity in feeding tests at 125–140 μg/2 g of diet for *D. melanogaster*
DERIVATIVES: Diacetylneoannonin
SOURCES: *An. squamosa*, seeds (17)

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[a]BST: Brine shrimp lethality, *Artemia salina*
PD: Inhibition of crown gall tumors on potato discs
9KB: Human nasopharyngeal carcinoma
9PS: Methylcholanthrene-induced murine lymphocytic leukemia
A549: Human lung carcinoma
MCF-7: Human breast carcinoma
HT-29: Human colon adenocarcinoma
9ASK: Astrocytoma reversal via tubulin inhibition
3PS: In vivo murine lymphocytic leukemia
L1210: In vivo or in vitro murine leukemia
CRW: Corn root worm, *Diabrotica undecimpunctata*
2SSM: Two-spotted spider mite, *Tetranychus urticae*
MBB: Mexican bean beetle, *Epilachna varvestis*
MA: Melon aphid or cotton aphid, *Aphis gossypii*
ML: Mosquito larvae, *Aedes aegypti*
NE: Nematode, *Caenorhabditis elegans*
BFL: Blowfly larvae, *Colliphora vicina*
SCB: Striped cucumber beetle, *Acalymma vittatum*

[b]A sample was provided by Dr. Diego Cortes. BST was performed at Purdue University. Cytotoxicity testing was provided by the Purdue University Cell Culture Laboratory.

[c]PD or BST was performed at Purdue University.

[d]In vivo or in vitro L1210 murine leukemia testing was provided by the Upjohn Co.

[e]Antimalarial testing against drug resistant strains of *Plasmodium falciparum* was provided by the Walter Reed Army Hospital.

[f]In vivo and in vitro T-cell suppression testing was provided by Eli Lilly and Co.

[g]Previously unreported data.

MONOTETRAHYDROFURAN ANNONACEOUS ACETOGENINS.—McCloud *et al.* (22) reported annonacin as the first Annonaceous acetogenin containing a single tetrahydrofuran ring. Since this report several other monotetrahydrofuran acetogenins have been described (18, 22–24) (Table 2). Figure 2 illustrates the distinguishing features and the basic carbon skeleton of this structural subclass.

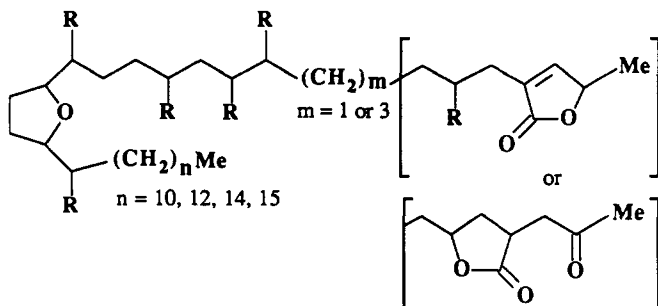
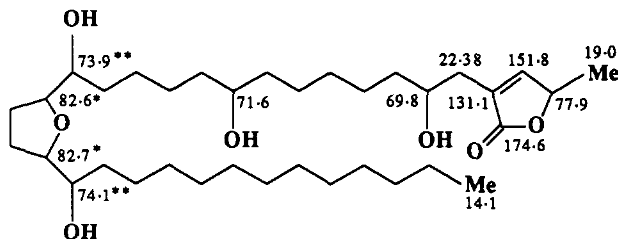


FIGURE 2. The monotetrahydrofuran ring acetogenin carbon skeleton. R represents either H, OH, or ketone carbonyl.

TABLE 2. Acetogenins With a Monotetrahydrofuran Ring.

20. ANNONACIN (22–26) (see refined structure **36**) $C_{35}H_{64}O_7$ MW 596



The *, ** indicate that assignments are interchangeable.

MP: 57°

[α]: +1.40

UV: 207 (ε 15390)

IR: 3440, 1750

EIMS of TMSi derivative: 213.0961 [$C_7H_8O_3$ (TMSi)], 271.2446 [$C_{13}H_{26}O$ (TMSi)], 341.2859 [$C_{17}H_{32}O_2$ (TMSi)], 385.2228 [$C_{13}H_{19}O_4$ $(TMSi)_2$], 543.3346 [$C_{18}H_{28}O_5$ $(TMSi)_3$], 614.3796 [$C_{22}H_{35}O_6$ $(TMSi)_3$], 885 [$C_{35}H_{61}O_7$ $(TMSi)_4$] (22)

^{1}H NMR: ($CDCl_3$, 470 MHz) 0.85 (3H, t, J= 6.9 Hz, H-32), 1.2–1.7 (m, H-5–9, -11–14, -21–31), 1.40 (3H, d, J= 6.8 Hz, H-35), 1.67 and 1.97 (m, H-17, -18), 2.38 (1H, dddd, J= 14, 8, 1, 0.5 Hz, H-3a), 2.51 (1H, dddd, J= 14, 3.4, 0.5, 0.5 Hz, H-3b), 3.38 (2H, dt, J= 11.6, 5.8 Hz, H-15, -20), 3.56 (1H, m, H-10), 3.77 (2H, dt, J= 11.6, 6.9 Hz, H-16, -19), 3.81 (1H, tt, J= 8, 4.5 Hz, H-4), 5.04 (1H, dq, J= 6.8, 1.4 Hz, H-34), 7.16 (1H, d, J= 1.4, 0.5 Hz, H-33) (22)

^{13}C NMR: ($CDCl_3$, 50 MHz) (22)

BIOLOGICAL ACTIVITY: BST LC_{50} = 3.3 ppm; PD 45% and 72% tumor inhibition (23)

Cytotoxicity: 9KB $ED_{50} = 10^{-3}$ μg/ml; 9PS $ED_{50} = 10^{-5}$ μg/ml (22); A549 $ED_{50} = 10^{-3}$ μg/ml; HT-29 ED_{50} = 3 μg/ml (26).

Antimitotic Activity: 9ASK 51% reversal (19); 15–30% reversal at 100 μg/ml (23).

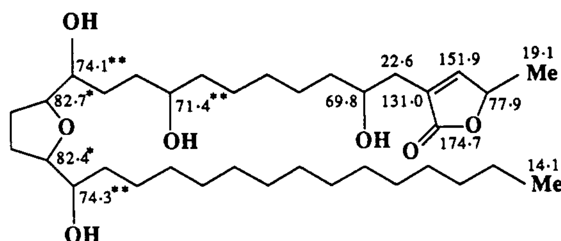
NCI Human Tumor Cell Panel av. molar log IC_{50} = −0.75, delta 2.0, range 3.7; in vivo 3PS 124% T/C at 0.95 mg/kg (23)

Pesticidal Activity: BFL 100% mortality at 1%, ML 70% at 10 ppm (23)

Antimalarial Activity[a]: Indochina W-2 IC_{50} = 1511.60 ng/ml, IC_{90} = 74673.00 ng/ml; Sierra Leone D-6 IC_{50} = 1300.70 ng/ml, IC_{90} = 12448.00 ng/ml.

DERIVATIVES: Annonacin triacetate, trimethylsilylannonacin, trimethylsilyldihydroannonacin
SOURCES: *Annona densicoma* Mart., stem bark (22)
Goniothalamus giganteus Hook. f., Thomas, stem bark (23)
An. squamosa, seeds (24).

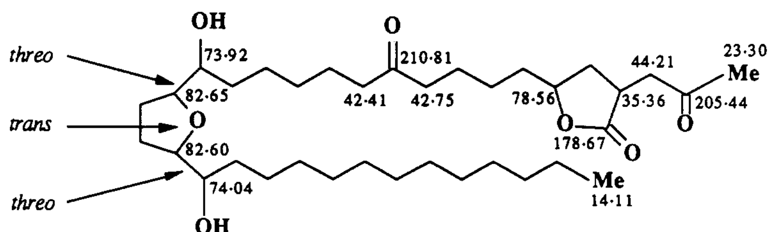
21. GONIOTHALAMICIN (23) $C_{35}H_{64}O_7$ MW 596
(see refined structure **37**)



The *, ** indicate that assignments may be interchangeable.

MP: 86–88°
[α]D: +1.6°
UV: (MeOH) 215 ([log ε 3.86)
IR: 3450, 1745
CIMS: 597.4732 $[MH]^+$ eims of Ac and TMSi derivatives, deuterated Ac and TMSi derivatives
^{1}H NMR[b]: ($CDCl_3$, 470 MHz) 0.88 (3H, d, J = 6.9 Hz, H-32), 1.2–2.0 (m, H-5–9, -11–12, -19–31), 1.41 (3H, d, J = 6.8 Hz, H-35), 2.38 (1H, dddd, H-3a), 2.51 (1H, dddd, H-3b), 3.38 (1H, m, H-13*), 3.43 (1H, m, H-18*), 3.61 (1H, m, H-10), 3.83 (3H, m, H-4, -14, -17), 5.06 (1H, qd, H-34), 7.19 (1H, d, H-33) (*indicates that the assignments are interchangeable)
^{13}C NMR: ($CDCl_3$, 50 MHz)
BIOLOGICAL ACTIVITY: BST LC_{50} = 37 ppm (13/295); PD 32%, 28%, and 68% tumor inhibition
Cytotoxicity: 9KB $ED_{50} < 10^{-2}$ μg/ml; 9PS $ED_{50} < 10^{-1}$ μg/ml; 9ASK inactive at 100 μg/ml. NCI Human Tumor Cell Panel av. molar log $IC_{50} = -0.9$, delta 1.9, range 3.5
In vivo 3 PS: inactive up to 2.5 mg/kg, higher dose exhibited toxicity
Pesticidal Activity: BFL 100% mortality at 1%
Antimalarial Activity[a]: Indochina W-2 IC_{50} = 1590.00 ng/ml, IC_{90} = 23631.00 ng/ml; Sierra Leone D-6 IC_{50} = 1084.50 ng/ml, IC_{90} = 8229.90 ng/ml.
DERIVATIVES: Goniothalamicin tetraacetate, tetra(trimethylsilyl)goniothalamicin, tetra-(trimethylsilyl)dihydrogoniothalamicin, goniothalamicin Ac-d_3 derivative and goniothalamicin TMSi-d_9 derivative
SOURCE: *G. giganteus*, stem bark (23)

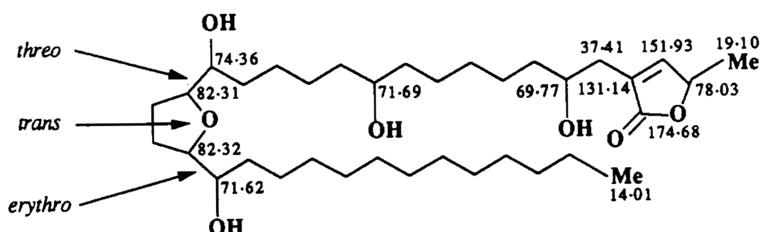
22. SQUAMONE (18) $C_{35}H_{62}O_7$ MW 594



MP: 88°
UV: (MeOH) 204 (log ε 3.12)
IR: (KBr) 3518, 2912, 2851, 1743, 1707, 1702, 1466, 1405, 1369, 1287, 1164, 1067, 1000, 953, 723
^{1}H NMR: (C_6D_6, 500 MHz) 0.90 (3H, t, H-32), 1.2–1.7 (H-5–7, -11–14, -21–23), 1.45–1.70 (H-17, -18), 1.54 (3H, m, H-35), 1.65 (2H, m, H-3), 1.912 (1H, dd, J = 18.4, 9.15 Hz, H-33a), 1.94 (d, J =

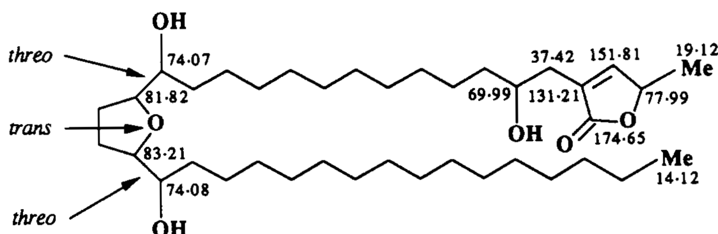
7.24 Hz, H-8), 2.07 (1H, t, $J=7.24$ Hz, H-10), 2.510 (1H, dd, $J=18.4$, 3.47 Hz, H-33b), 2.69 (1H, dddd, $J=9.15$, 9.15, 9.15, 3.47 Hz, H-2), 3.33 (2H, m, H-15, -20), 3.62 (2H, dt, $J=6.32$, 2.11 Hz, H-16, -19), 3.995 (1H, dddd, $J=$ 8.24, 8.24, 4.85, 3.38 Hz, H-4)
^{13}C NMR: ($CDCl_3$, 50 MHz)
EIMS of squamone TMSi derivative: 541, 467, 397, 341, 271
BIOLOGICAL ACTIVITY: BST $LC_{50}=2.1$ ppm
Cytotoxicity: A549 $ED_{50}=1.34$ µg/ml; MCF-7 $ED_{50}=2.14$ µg/ml; HT-29 $ED_{50}=$ 1.5 µg/ml
DERIVATIVES: Squamone diacetate, squamone TMSi and TMSi-d_9 derivatives, "tetrahydrosquamone" (BST $LC_{50}=2.7$ ppm; A549 $ED_{50}=1.41\times10^{-1}$ µg/ml; MCF-7 $ED_{50}=9.89\times10^{-3}$ µg/ml; HT-29 $ED_{50}=2.98\times10^{-1}$ µg/ml)
SOURCES: *An. squamosa*, bark (18)

23. ANNONACIN A (24) $C_{35}H_{64}O_7$ MW 596



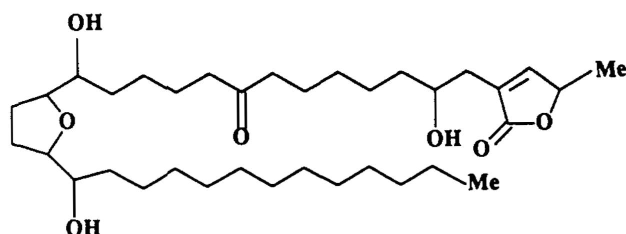
MP: Not reported
$[\alpha]^{25}D$: $+23.8°$ ($c=0.4$, CH_2Cl_2)
CIMS: 597 $[MH]^+$ (100), 579 (77), 561 (82), 543 (38), 523 (2), 397 (1), 379 (7), 361 (11), 343 (2), 309 (45), 297 (4), 291 (12), 273 (8), 269 (17), 253 (8), 241 (8), 235 (4), 223 (3), 199 (16), 141 (5), 123 (6), 111 (20), 109 (7), 97 (13), 83 (13), 81 (13), 71 (19)
IR: (film) 3420, 2920, 2850, 1740, 1650, 1460, 1310, 1200, 1110, 1075, 1025, 950, 850
^{1}H NMR: ($CDCl_3$, 360 MHz) 0.88 (H-32), 1.42 (H-35), 3.40 (H-15), 3.60 (H-10), 3.82 (H-4, -16, -19, -20), 5.02 (H-34), 7.17 (H-33)
^{13}C NMR: ($CDCl_3$, 50.31 or 90.56 MHz)
BIOLOGICAL ACTIVITY: None reported
DERIVATIVES: Annonacin A triacetate
SOURCES: *An. squamosa*, seeds (24)

24. ANNONASTATIN (24) $C_{38}H_{70}O_6$ MW 622



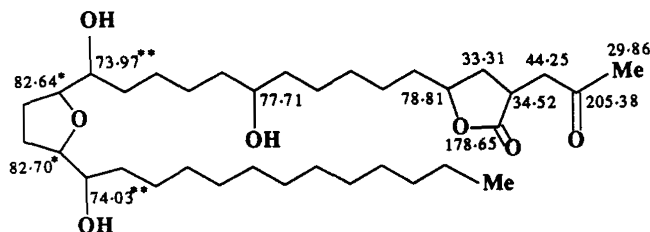
MP: Not reported
$[\alpha]^{25}D$: $+15.0°$ ($c=1.1$, CH_2Cl_2)
IR: (film) 3410, 2920, 2850, 1750, 1650, 1460, 1310, 1195, 1115, 1065, 1020, 950, 850
CIMS: 623 $[MH]^+$ (64), 605 (100), 587 (26), 569 (8), 493 (3), 475 (1), 381 (4), 363 (3), 345 (2), 311 (24), 293 (4), 275 (1), 241 (3), 223 (1), 141 (2), 123 (2), 111 (2), 91 (8), 81 (4), 69 (6), 67 (4)
^{1}H NMR: ($CDCl_3$, 360 MHz) 0.88 (H-35), 1.42 (H-38), 3.40 (H-15, -20), 3.84 (H-4, -16, -19), 5.05 (H-37), 7.17 (H-36)
^{13}C NMR: ($CDCl_3$, 50.31, 90.56 MHz)
SOURCES: *An. squamosa*, seeds (24)

25. ANNONACIN-10-ONE (25,26) $C_{35}H_{62}O_7$ MW 594



MP: 73–75°
$[\alpha]^{20}D$: +31.1 (c=0.06, MeOH)
UV: (MeOH) 210 nm (log ϵ 4.03)
IR: 1700, 1749
MS: 377 (10), 359 (18), 325 (55), 307 (60), 289 (63), 293 (13), 221 (45), 211 (8), 199 (1), 141 (13), 123 (23)
^{1}H NMR: ($CDCl_3$, 470 MHz) 0.88 (3H, t, J= 6.8 Hz, H-32), 1.44 (3H, d, J=6.8 Hz, H-35), 1.26–1.60 (m, H-5–8, -12–14, -21–31), 1.68, 1.99 (m, H-17, -18), 2.39 (t, J=7.5, H-11), 2.41 (1H, dd, J= 14, 7.5 Hz, H-3a), 2.42 (t, J=7.5 Hz, H-9), 2.52 (1H, ddt, J=14, 3.2, 1.0 Hz, H-3b), 3.40 (2H, m, H-15, -20), 3.79 (2H, dd, J=12, 6.5 Hz, H-16, -19), 3.83 (1H, m, H-4), 5.06 (1H, qd, J=6.8, 1.4 Hz, H-34), 7.18 (1H, d, J=1.4 Hz, H-33)
^{13}C NMR: Not reported
BIOLOGICAL ACTIVITY: Cytotoxicity: 9PS $ED_{50}=10^{-6}$ μg/ml; A549 $ED_{50}=10^{-1}$ μg/ml; HT-29 ED_{50}=1 μg/ml
DERIVATIVES: Annonacin-10-one triacetate
SOURCES: *An. densicoma*, stem bark (25,26)

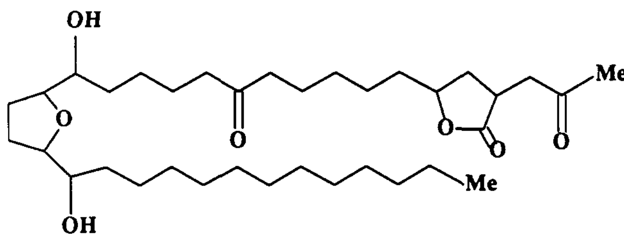
26. ISOANNONACIN (25,26) $C_{35}H_{64}O_7$ MW 596



The *, ** indicate that the assignments are interchangeable.

MP: 96–98°
$[\alpha]^{20}D$: +24.8 (c=0.12, MeOH)
IR: 1700, 1760
MS: 397 (2), 379 (6), 361 (32), 327 (1), 309 (75), 291 (45), 241 (15), 199 (5), 141 (9)
^{1}H NMR: ($CDCl_3$, 470 MHz) 0.88 (3H, t, J= 6.8 Hz, H-32), 1.25–1.60 (m, H-5–8, -12–14, -21–31), 1.43 (m, H-9, -11), 1.66, 1.98 (m, H-17, -18), 1.99 (1H, m, H-3b), 2.20 (3H, s, H-35), 2.23 (1H, ddd, J=9.5, 9.0, 3.6 Hz, H-3a), 2.66 (1H, dd, J=17, 9.5 Hz, H-33a), 3.01 (1H, dd, J=9.5, 9.0 Hz, H-33b), 3.02 (1H, dddd, J=17, 9.5, 9.0, 3.6 Hz, H-2), 3.41 (ddd, J=12.2, 6.4, 5.2 Hz, H-15, -20), 3.59 (m, H-10), 3.80 (dd, J=12.2, 6.4 Hz, H-16, -19), 4.54 (1H, tdd, J= 7.0, 4.3, 3.6 Hz, H-4)
^{13}C NMR: ($CDCl_3$, 67.5 MHz)
BIOLOGICAL ACTIVITY: Cytotoxicity: 9PS ED_{50}=3 μg/ml; A549 $ED_{50}=2\times10^{-2}$ μg/ml; HT-29 $ED_{50}=2\times10^{-3}$ μg/ml
DERIVATIVES: Isoannonacin triacetate
SOURCES: *An. densicoma*, stem bark (25,26)

27. ISOANNONACIN-10-ONE (25,26) $C_{35}H_{62}O_7$ MW 594



MP: 103.5°
$[\alpha]^{20}D$: +19.8 (c=0.05, MeOH)
IR: 1740, 1715, 1695
MS: 395 (2), 377 (8), 359 (18), 325 (20), 307 (32), 289 (40), 239 (14), 221 (34), 199 (3), 141 (10)
^{1}H NMR: ($CDCl_3$, 470 MHz) 0.89 (3H, t, J=7.0 Hz, H-32), 1.25–1.60 (m, H-5–8, -12–14, -21–31), 1.68 (m, H-17), 1.99 (m, H-3b, -18), 2.20 (3H, s, H-35), 2.23 (ddd, J=9.5, 9.0, 3.6 Hz, H-3a), 2.41 (t, J=7.5 Hz, H-11), 2.42 (t, J=7.5 Hz, H-9), 2.68 (1H, dd, J=17, 9.0 Hz, H-33a), 3.01 (1H, dd, J=9.5, 9.0 Hz, H-33b), 3.03 (1H, dddd, J=17, 9.5, 9.0, 3.6 Hz, H-2), 3.41 (m, H-15, -20), 3.79 (dd, J=12, 6.5 Hz, H-16, -19), 4.55 (1H, tdd, J=7.0, 4.3, 3.6 Hz, H-4)
^{13}C NMR: Not reported
BIOLOGICAL ACTIVITY: Cytotoxicity: 9PS ED_{50}=0.5 μg/ml; A549 $ED_{50}=7\times10^{-2}$ μg/ml; HT-29 $ED_{50}=9\times10^{-3}$ μg/ml
DERIVATIVES: Isoannonacin-10-one diacetate
SOURCES: *An. densicoma*, stem bark (25,26)

[a]Antimalarial testing against drug resistant strains of *Plasmodium falciparum* was provided by the Walter Reed Army Hospital.
[b]Previously unreported data.

NONADJACENT BIS-TETRAHYDROFURAN ANNONACEOUS ACETOGENINS.—To date, there are three reported compounds of this structural subclass: gigantecin (27), bullatalicin (28), and sylvaticin (29). All of these compounds have been found in our laboratory and are characterized by the general structure illustrated in Figure 3. Stereochemistries at the junction of the methylene group and the tetrahydrofuran rings remain unsolved. All structures described so far are 4-hydroxy derivatives. From the spectral data published, almunequine (13) appears to be in this structural subclass, although the compound is not fully characterized.

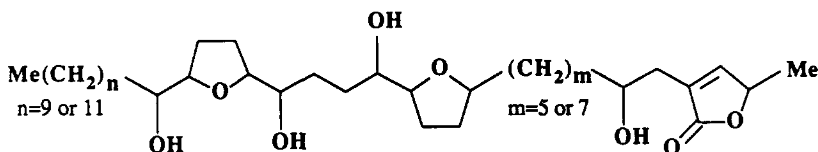
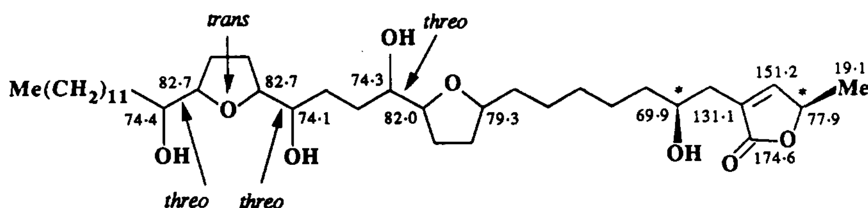


FIGURE 3. The non-adjacent tetrahydrofuran ring acetogenin carbon skeleton.

TABLE 3. Acetogenins With Non-adjacent Bis-Tetrahydrofuran Rings.

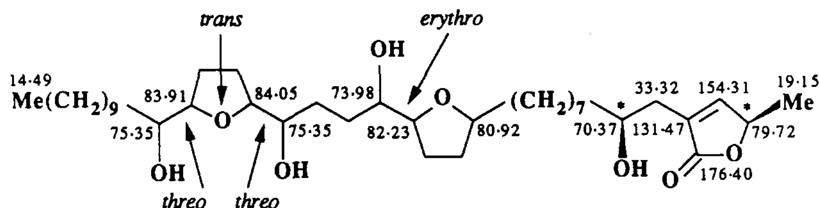
28. GIGANTECIN (27) $C_{37}H_{66}O_8$ MW 638



*This illustration is meant to show only the relative configurations at C-4 and C-36.

MP: 96–98°
[α]D: +3.15
UV: 220 (ε 1250)
IR: 1745
CIMS: 639.9470 $[MH]^+$, eims of gigantecin TMSi, 2,35-dihydro TMSi, TMSi-d_9 derivative
^1H NMR[a]: $CDCl_3$ (200 MHz) 0.88 (3H, t, H-34), 1.25 (ca. 26H, br s), 1.25–1.59 (4H, m), 1.41 (3H, d, H-37), 1.59 (4H, m), 1.80 (4H, m), 2.38 (1H, dddd, H-3a), 2.51 (1H, dddd, H-3b), 3.40 (3H, m, H-14, -17, -22), 3.87 (5H, m, H-4, -10, -13, -18, -21), 5.06 (1H, dq, H-36), 7.17 (1H, d, H-35)
^{13}C NMR: ($CDCl_3$, 50 MHz)
CD: Identical to rolliniastatin, asimicin, bullatacin
BIOLOGICAL ACTIVITY: BST $LC_{50} = 222$ ppm; PD 83% tumor inhibition
Cytotoxicity: 9KB $ED_{50} < 10^{-5}$ μg/ml; 9PS $ED_{50} < 10^{-2}$ μg/ml; A549 $ED_{50} = 2.19 \times 10^{-7}$ μg/ml; MCF-7 $ED_{50} = 4.11 \times 10^{-9}$ μg/ml; HT-29 $ED_{50} = 2.68 \times 10^{-4}$ μg/ml
Antimitotic Activity: 9ASK 31–50% reversal at 10 μg/ml
DERIVATIVES: Gigantecin TMSi, TMSi-d_9, 2,35 dihydro TMSi derivatives, gigantecin triacetate
SOURCES: *G. giganteus*, bark (27)

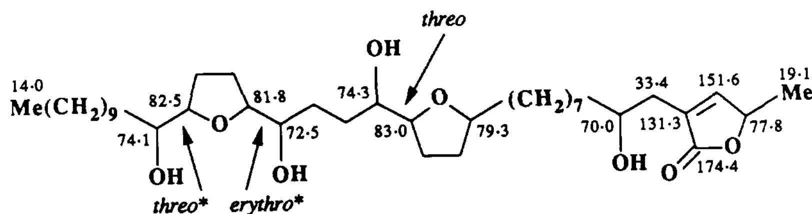
29. BULLATALICIN (28) $C_{37}H_{67}O_8$ MW 638



*This illustration is meant to show only the relative configurations at C-4 and C-36.

MP: 120–121°
[α]D: +13.25 ($c = 0.004$ g/ml, EtOH)
UV: (EtOH) 212 (ε 7270)
IR: (KBr) 3430, 1748, 1726
MS: 527, 469, 397, 339, 329, 309, 241, 171, 141, 111; ms of Ac and TMSi derivatives
^1H NMR: (C_6D_6, 500 MHz) 0.808 (3H, d, J = 6.8 Hz, H-37), 0.905 (3H, t, J = 7.1, H-34), 1.230 (1H, m, H-22a), 1.29 (m, H-6–10, -26–33), 1.375 (1H, H-14a), 1.390 (1H, m, H-5b), 1.500 (m, H-11a, -13a, -21a), 1.525 (1H, m, H-17b), 1.600 (H-18, -25), 1.625 (1H, H-14a), 1.630 (1H, H-5a), 1.680 (4H, m, H-11b, -13b, -21b, -22b), 1.773 (1H, H-17a), 2.200 (1H, ddt, J = 14.7, 8.2, 1.2 Hz, H-3b), 2.300 (1H, dddd, J = 14.7, 3.7, 1.8, 1.2 Hz, H-3a), 3.387 (1H, t, J = 7.3 Hz, H-24), 3.437 (1H, t, J = 7.7 Hz, H-19), 3.64 (1H, H-16), 3.708 (3H, m, H-4, -15, -23), 3.800 (2H, H-12, -20), 4.235 (1H, qq, J = 6.7, 1.3 Hz, H-36), 6.240 (1H, d, J = 1.3 Hz, H-35)
^{13}C NMR: (CD_3OD, 50 MHz)
CD: ($c = 0.023$ mg/ml, EtOH) $[\theta]_{247.0}$ 0.0, $[\theta]_{243.0}$ −100.64, $[\theta]_{235.2}$ 5.54, $[\theta]_{227.0}$ 771.15, $[\theta]_{218.0}$ 2124.82, $[\theta]_{215.0}$ 2729.5, $[\theta]_{213.3}$ 2327.31, $[\theta]_{211.6}$ 2171.97 (molecular ellipticities are shown here, rather than ellipticities as given in reference 28).
BIOLOGICAL ACTIVITY: BST $LC_{50} = 0.154$ ppm; PD 63% tumor inhibition
Cytotoxicity: 9KB $ED_{50} > 10$ μg/ml; A549 $ED_{50} = 2.34 \times 10^{-7}$ μg/ml; MCF-7 $ED_{50} = 2.34$ μg/ml; HT-29 $ED_{50} = 8.8 \times 10^{-6}$ μg/ml
Antimitotic Activity: toxic with slight reversal at 100 μg/ml
DERIVATIVES: Bullatalicin tetraacetate (A549 $ED_{50} < 10^{-3}$ μg/ml; MCF-7 $ED_{50} < 10^{-3}$ μg/ml; HT-29 $ED_{50} = 1.35$ μg/ml), bullatalicin TMSi derivative
SOURCES: *An. bullata*, bark (28)

30. SYLVATICIN (29) $C_{37}H_{66}O_8$ MW 638



The * indicates that the assignments are interchangeable.

MP: 48–50°
$[\alpha]^{23}D$: +5.9 (c = 0.542, $CHCl_3$)
IR: (CH_2Cl_2) 3700, 3300, 1750
FABMS: 639.4836 $[MH]^+$, eims of sylvaticin, its TMSi, TMSi-d_9 derivatives see Figure 8
^{1}H NMR: ($CDCl_3$, 300 MHz) 0.86 (3H, t, H-34), 1.25 (br s, H-6–10, -26–33), 1.4–1.6 (H-5, -11, -17, -18, -25), 1.41 (3H, d, H-37), 1.6–2.0 (H-13, -14, -21, -22), 2.38 (1H, ddt, H-3b), 2.500 (1H, dddd, H-3a), 3.40 (1H, m, H-24), 3.48 (m, H-16), 3.73 (m, H-4), 3.78–3.93 (H-12, -15, -20, -23), 3.88 (m, H-19), 5.04 (1H, qq, H-36), 7.17 (1H, d, H-35)
^{13}C NMR: ($CDCl_3$, 50 MHz)
BIOLOGICAL ACTIVITY: BST LC_{50}=26 ppm
Cytotoxicity: 9KB $ED_{50} < 10^{-3}$ µg/ml; A549 $ED_{50} < 10^{-3}$ µg/ml; HT-29 $ED_{50} < 10^{-3}$ µg/ml
Pesticidal Activity: protection of cantalope leaves from SCB
DERIVATIVES: Sylvaticin tetraacetate, sylvaticin TMSi and TMSi-d_9 derivatives
SOURCES: *Rollinia sylvatica* St. Hill., dried fruits (29)

31. ALMUNEQUINE (13) $C_{37}H_{66}O_8$ MW 638

STRUCTURE NOT DETERMINED

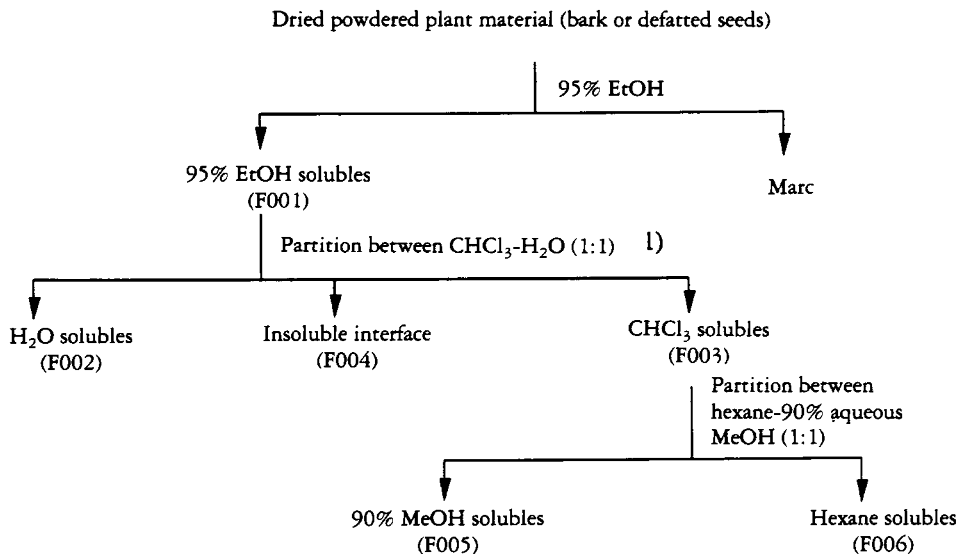
MP: 106–108°
FABMS: 639 $[M+1]^+$
CIMS: 639 $[M+1]^+$, (100), 622 (21), 621 (41), 603 (27), 585 (18), 567 (6), 535 (4), 433 (13), 415 (10), 397 (5), 379 (5), 363 (54), 361 (5), 345 (25), 327 (15), 293 (27), 275 (14), 265 (4), 257 (2), 239 (13), 221 (3), 141 (6), 111 (12)
IR: ($CHCl_3$) 3580, 2940, 2870, 1755, 1460, 1370, 1325, 1060, 1030, 960
^{1}H NMR: ($CDCl_3$, 250 MHz) 0.89 (3H, m), 1.26 (32H, m), 1.41 (3H, d, J=7.12 Hz), 1.70 (4H, m), 1.92 (8H, m), 2.26 (2H, m), 3.83–3.43 (8H, m), 5.01 (1H, dd, J= 1.42, 7.12 Hz), 7.00 (1H, dd, J= 1.42 Hz)
^{13}C NMR: ($CDCl_3$, 25.5 MHz) 173.9, 148.9, 134.6, 83.5, 82.4, 82.1, 79.5, 77.5, 75.0, 74.6, 72.0, 71.9, 37.7, 37.5, 35.8, 32.8, 32.0, 29.5, 28.7, 28.6, 27.6, 26.3, 25.8, 25.3, 22.7, 19.4, 14.2
BIOLOGICAL ACTIVITY: Antimicrobial activity reported
DERIVATIVES: None
SOURCES: *An. cherimolia*, seeds (13)

[a]Previously unreported data.

ISOLATION AND DETECTION

The Annonaceous acetogenins have been isolated, so far, from the genera *Annona, Asimina, Goniothalamus, Rollinia,* and *Uvaria*. Extracts of the seeds, bark, and roots of these genera have yielded the compounds. Typically two extraction schemes have been employed. When the seeds are used, they are first defatted with hexane or petroleum ether. The acetogenins are extracted by the defatting solvent which is then subjected to further solvent partitioning (4, 13–15). However, we have found that EtOH extracts of the defatted seeds yield greater biological activity and additional acetogenins. EtOH extraction, by either cold percolation or Soxhlet extractor, is preferred for the bark and roots; this is then followed by solvent partitioning. Scheme 1 summarizes this typical method, which concentrates the activity in the aqueous MeOH fraction (F005). Brine shrimp lethality (BST) (30) is the most expedient bioassay for monitoring the fractionations and gives better, more statistically comparable, results than cytotoxicity or pesticidal tests.

Once the concentrated Annonaceous acetogenin partition fraction has been obtained, a variety of chromatographic techniques can be utilized to isolate the pure compounds. These include gradient elution cc (Si gel or reversed-phase) (3, 10), mplc (Michelle-Miller columns) (10), rotary chromatography (Chromatotron) (9, 10, 23, 27), steric exclusion (Sephadex) (14, 15, 21), high speed counter-current distribution (hsccd) (14), hplc (3, 12, 14, 20, 24), and tlc (3–29).



SCHEME 1. Standard flow sheet of extraction and initial partitioning. Bioactivities are usually concentrated in F005.

The spectrometric detection of the Annonaceous acetogenins during their chromatographic separation is difficult due to very weak uv absorption. Therefore, the best method for the detection of these compounds during fractionation involves the monitoring of each fraction by tlc followed by the development of the chromatograph with a variety of spray reagents. Tlc systems such as $CHCl_3$-MeOH (9:1), CH_2Cl_2-MeOH (19:1), C_6H_6-EtOAc (4:6), and C_6H_6-EtOAc-MeOH (5:4:1) have been successfully utilized. The Annonaceous acetogenins typically give R_f values between 0.2 and 0.7 in these tlc systems (9, 10, 11, 18, 23, 27–29). Two nonspecific spray reagents, 5% phosphomolybdic acid in MeOH (10) and 0.5% tetrazolium blue in MeOH-5 N NaOH (1:1) (9), give grayish-blue and purple chromophores, respectively, for these compounds. Kedde's reagent (31), 2% 3,5-dinitrobenzoic acid-2 N methanolic KOH (1:1), is diagnostic for the α,β-unsaturated γ-lactone subunit; this reagent makes it possible to differentiate acetogenins with a saturated γ-lactone from those possessing an unsaturated γ-lactone. Faint red-pink chromophores which fade quickly are indicative of a positive reaction to Kedde's reagent. Legal's reagent (26), 1% sodium nitroprusside in absolute EtOH-1 N NaOH (1:1), reacts with α,β-unsaturated γ-lactones to give a red-violet chromophore. No chromophore is seen with saturated γ-lactones or α,β-unsaturated δ-lactones.

STRUCTURAL ELUCIDATION STRATEGIES

PRIMARY STRUCTURE.—The complete structural elucidation of the Annonaceous acetogenins is not a trivial matter. Classical methods such as uv, ir, ^{1}H-nmr and ^{13}C-nmr spectroscopy, and mass spectrometry are essential in identifying the structural subunits (γ-lactone, tetrahydrofuran rings). However, the determination of the placement of substitutents along the carbon skeleton involves innovative mass spectrometric strategies. Furthermore, the waxy, amorphous nature of these compounds makes them unsuitable for direct X-ray diffraction studies, and because the acetogenins are difficult to convert to crystalline derivatives that are suitable for X-ray analysis, other methods have been used to predict the stereochemistry.

The linear acetogenins all possess a characteristic γ-lactone subunit (**A1**–**A5**, Figure 4), which can be differentiated by uv, ir, ^{1}H-nmr, and ^{13}C-nmr data as well as chemical conversion. As mentioned above, positive reactions to Kedde's reagent (31) and/or Legal's reagent (32) are indicative of the α,β-unsaturated γ-lactone with compounds possessing subunits **A1** or **A2**. Beacuse conjugation is minimal, uv spectroscopy is not very useful; the ir spectrum, however, affords helpful information for distinguishing between the saturated (1770 cm^{-1}) and the unsaturated (1745 cm^{-1}) lactone moieties.

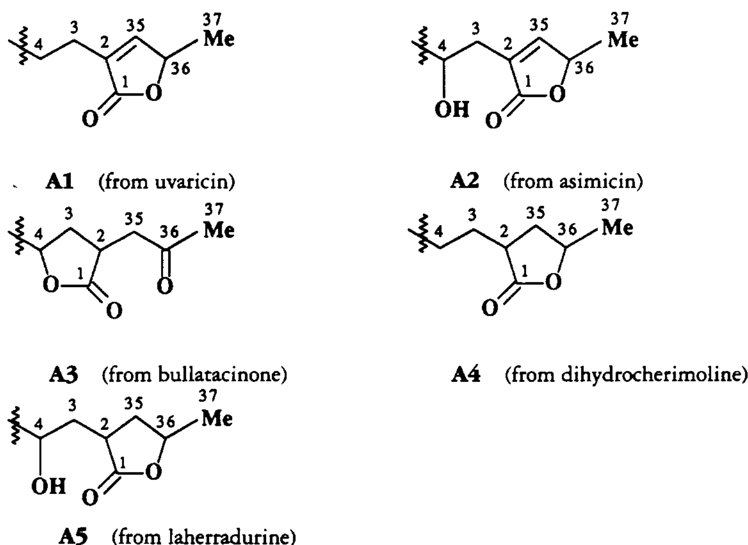


FIGURE 4. Annonaceous acetogenin γ-lactone subunit types.

The diagnostic ^{1}H-nmr and ^{13}C-nmr chemical shifts for the γ-lactone subunits are presented in Table 4. The structural subunits **A1**, **A2**, and **A3** have all been verified by homonuclear decoupling experiments (9, 10) and/or 2D-nmr techniques (COSY, HETCOR) (10, 14, 15). The **A3** subunit was further confirmed by chemical conversion of bullatacin (**A2** type) to bullatacinone (**A3** type) (Figure 5) (10); similar conversions were made for annonacin to isoannonacin and annonacin-10-one to isoannonacin-10-one. This in vitro conversion is important because it gives some hint for the in vivo synthetic relationships of different acetogenins. The **A2** type is easily distinguished from the **A1** type by its coupling pattern in the ^{1}H-nmr for the C-3 protons which are at δ 2.38 (dddd, H_a-3) and δ 2.51 (ddt, H_b-3) rather than at δ 2.26 (t, 2H), as well as a diagnostic ^{13}C-nmr resonance for C-4 at δ 69.9 (d). The **A3** subunit is readily identified by its C-37 methyl ketone resonance at δ 2.20 (s) in the ^{1}H nmr and its C-36 carbonyl resonance in the ^{13}C nmr at δ 205.44 (s). The stereochemistries at C-2 in the **A3** and **A4** subunits currently remain undefined, and bullatacinone [**14**] seems to be a 2:1 mixture of diastereomers at this position (10).

The existence of tetrahydrofuran rings with adjacent hydroxyl groups within this class of compounds is apparent by the presence of ^{13}C-nmr resonances due to hydroxylated carbons in the region from δ 71 to 75 and other oxygenated carbons in the range of δ 79–84 corresponding to the tetrahydrofuran oxygenated carbons. These signals and their corresponding ^{1}H-nmr resonances from δ 3.3 to 4.1 are also indicative of the dihydroxyl tetrahydrofuran moieties. The Annonaceous acetogenins possess three different types of tetrahydrofuran subunits (**B1**–**B3**, Figure 6).

TABLE 4. Diagnostic ^{1}H-nmr and ^{13}C-nmr Chemical Shifts for the γ-lactone Moiety.[a] (subunits **A1**–**A5**)

Carbon	**A1**(from uvaricin [**1**])		**A2**(from asimicin [**9**])		**A3**(from bullatacinone [**14**])		**A4**(from dihydrocherimoline [**8**])		**A5**(from laherradurine [**15**])	
	^{1}H nmr	^{13}C nmr	^{1}H nmr	^{13}C nmr	^{1}H nmr	^{13}C nmr	^{1}H nmr	^{13}C nmr	^{1}H nmr	^{13}C nmr
C-1	—	173.7 s	—	174.6 s	—	178.73 s	—	175.0	—	—
C-2	—	134.6 s	—	131.1	2.71 dddd	44.18 d	—	131.1	2.62–2.25	—
C-3	2.26	25.3 t	2.51 dddd 2.38 ddt	37.4 t	1.70 ddd 1.40 ddd	34.41 t	2.40 t	25.2	2.62–2.25	—
C-4	≤1.55 m	27.5 t	3.86 m	69.9 d	4.05 m	78.86 d	1.69 m	25.5	4.21–3.45	—
C-35	6.99 q	148.7 d	7.17 d	151.8 d	1.93 dd 2.53 dd	35.42 t	7.20 d	151.5	2.62–2.25	—
C-36	4.99 qq	77.3 d	5.06 qq	78.0 d	—	205.44 s	5.06 dq	77.8	4.53 dd	—
C-37	1.41 d	19.3 q	1.41 d	19.1 q	1.55 s	24.46 q	1.43 d	19.1	1.36 d	—

[a]All values were recorded in $CDCl_3$, with the exception of the ^{1}H-nmr spectrum of bullatacinone which was recorded in C_6D_6.

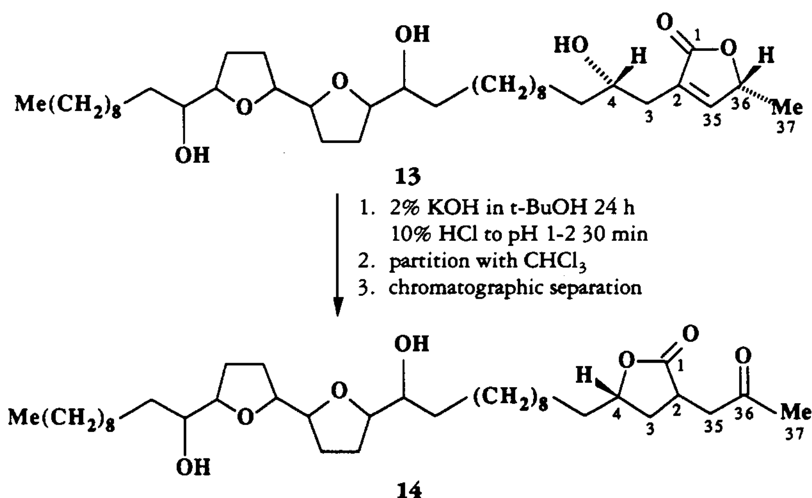


FIGURE 5. Chemical conversion of bullatacin [**13**] (**A2** subtype) to bullatacinone [**14**] (**A3** subtype) (10).

These subunits are easily differentiated by their ^{13}C-nmr signals; those of the **B1** type have four resonances between δ 81 and 83. The **B2** type have only three resonances in this range as well as a fourth resonance, upfield at ca. δ 79, which is indicative of the oxygenated tetrahydrofuran carbon lacking an adjacent hydroxyl group. The **B3** types have only two ^{13}C-nmr signals in the range δ 81–83.

To determine the placement of the tetrahydrofuran rings and any isolated functional groups (hydroxyl, keto, acetoxyl) along the hydrocarbon chain, careful mass spectral fragmentation analysis is necessary. The mass spectra of underivatized acetogenins often give irreproducible results due to the formation of pyrolysis and thermal rearrangement products (23,29). Therefore, a mass spectrometry strategy involving synthetic derivatives is preferable. For example, in the case of bullatacin [**13**] (10), five derivatives (TMSi, TMSi-d_9, Ac, Ac-d_3, and 2,35-dihydro) were prepared and subjected to mass spectral fragmentation analysis (Figure 7). The elemental composition (hreims) of certain diagnostic peaks is also determined to confirm peak assignments.

The fragment ions with their added TMSi groups frequently undergo the loss of TMSiOH forming new ions which undergo further fragmentation. This fragmentation can be verified by linked scan ms (ms/ms) from the daughter ion spectra. This procedure

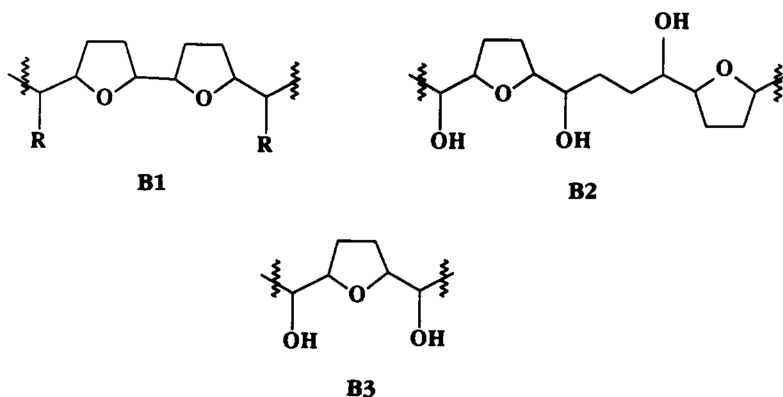


FIGURE 6. Annonaceous acetogenin tetrahydrofuran subunits **B1**–**B3**.

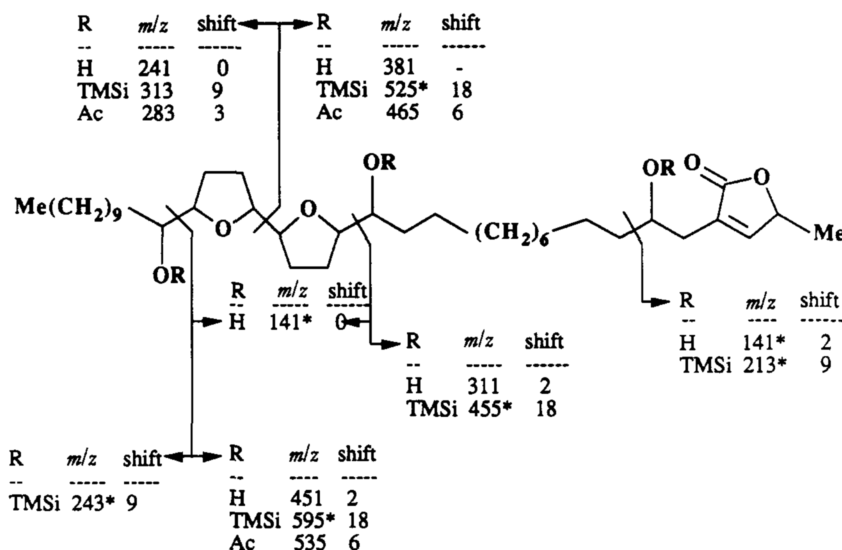


FIGURE 7. Eims data (*m/z*) for bullatacin [**13**] and its derivatives (10). "R" designates the underivatized material (H), the acetyl derivative (Ac), or the trimethylsilyl derivative (TMSi). Shifts indicate the change in mass observed for hydrogenation of the lactone in the underivatized material, for the d_9 TMSi derivative, or for the d_3 acetyl derivative. Exact mass measurements (within 3 mmu) confirmed the elemental compositions of the proposed fragments marked with *.

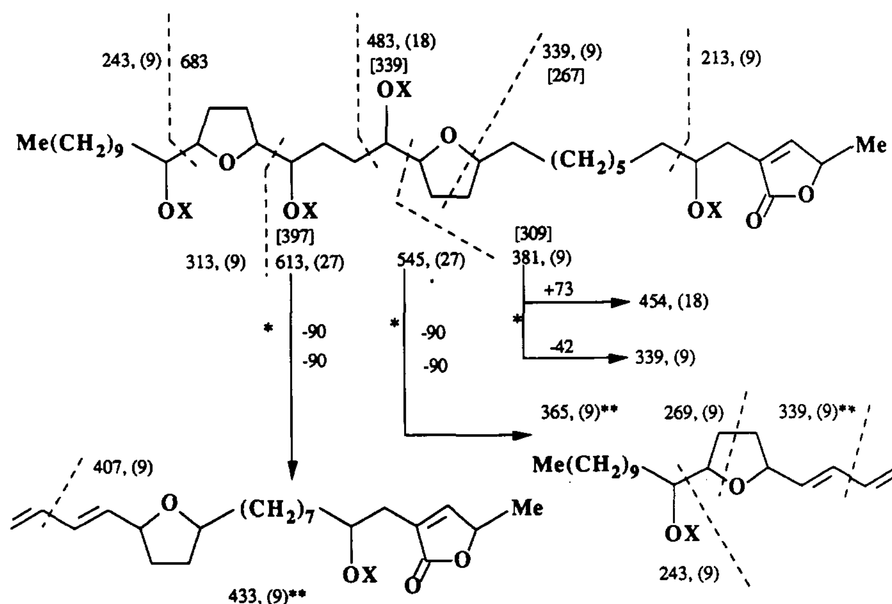


FIGURE 8. Diagnostic eims fragment ions (*m/z*) of sylvaticin [**30**], sylvaticin-TMSi derivative, and sylvaticin-TMSi-d_9 derivative (29). The * refers to fragments verified by link-scan ms/ms. Hrms (within 3 mmu) confirmed each fragment composition (with the exception of the fragments marked with **). The numbers 9, 18, or 27 in parentheses refer to mass shifts of the TMSi-d_9 derivative. The numbers in brackets refer to the mass fragments of the underivatized sylvaticin.

was helpful in the structural elucidation of sylvaticin [**30**] (Figure 8) (29). These and other mass spectrometric procedures applied to the acetogenins will be described in more detail in a future paper.[1]

Another helpful method of analysis involves degradation with Jones oxidation (Figure 9) followed by mass spectral analysis of the resultant products and their methyl esters (Figure 10). However, this methodology is applicable only if a large quantity of the compound in question has been isolated. Such cases are rare. This procedure was helpful with the structural elucidation of squamocin [**12**] (16).

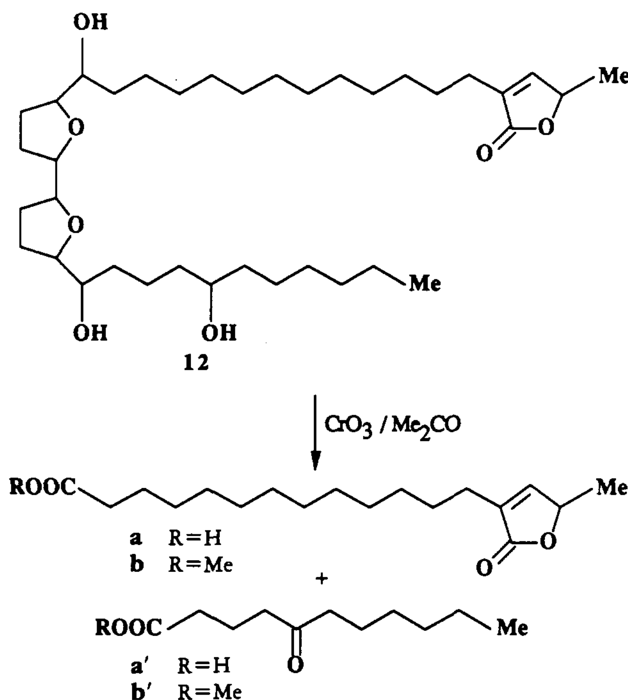


FIGURE 9. Structures of Jones oxidation products (**a** and **a′**), and their methyl esters (**b** and **b′**) [adapted from Fujimoto *et al.* (16)].

STEREOCHEMISTRY.—Once the primary structure has been determined, it is important to define the stereochemistry of the existing chiral centers in order to distinguish the numerous diastereomeric acetogenins. For example, asimicin, rolliniastatin 1, rolliniastatin 2, bullatacin, and 4-hydroxy-25-deoxyneorollinicin all have the same pattern of contiguous atoms and are diastereomers. A glance at the cytotoxicites (Table 1 and Table 6) reveals that the relative structure-activity relationships seem to be quite dependent on the stereochemistries. The waxy, amorphous, or microcrystalline nature of these compounds has, thus far, proven to be unsuitable for direct X-ray crystallographic studies.

The region of greatest stereochemical variation is the tetrahydrofuran-containing moieties (up to six chiral centers). A unique methodology for determining the relative stereochemical relationships among the six stereogenic carbon atoms in the adjacent

[1] Y.-M. Lui, K.V. Wood, and D.L. Smith, unpublished results.

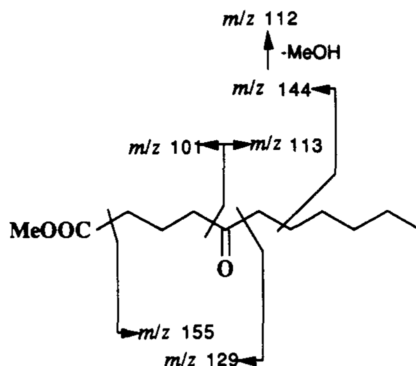


FIGURE 10. Eims fragmentation pattern of Jones oxidation product methyl ester **b′** [adapted from Fujimoto *et al.* (16)].

bis-tetrahydrofuran dihydroxy moiety [**B1**] has been recently described by Hoye and co-workers (33,34). This method involved the laborious synthesis of a group of twelve diastereomeric dibutyl diacetylated bis-tetrahydrofuran models; the relative stereochemistries were known because of the methods used in their syntheses (35,36). Careful comparison of the ^{1}H-nmr spectra of these compounds indicated that stereochemical information could be extracted from iterative and synergistic analysis of the very small differences in their high-field proton chemical shifts. The application of this technique was first successfully applied to the acetogenins uvaricin (33), asimicin, and rolliniastatin 1 (34). The comparison of the ^{1}H-nmr shifts of the diagnostic protons of the twelve isomeric model compounds and those of uvaricin [**1**], uvaricin acetate (33), asimicin [**9**] triacetate, and rolliniastatin 1 [**10**] triacetate (34) allowed the assignment of the relative stereochemistries of this portion of these compounds (Figure 11).

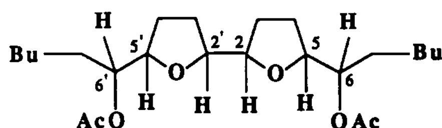
This approach has been validated by the agreement of the assignments so determined with those of X-ray crystallographic data for the 15-*O*-*p*-bromophenylurethane derivative of rolliniastatin 1 (15). The relative stereochemistries are identical.

A second technique for determining the relative stereochemistry of this portion of the acetogenins has been described by Born *et al.* (12). In this technique, minor differences in the ^{1}H-nmr and ^{13}C-nmr chemical shifts of two models (A and B) are compared with the corresponding signals of the acetogenin annonin I [**16**] (Table 5). Stereochemical information can be abstracted from the comparison data.

The conclusion from this application of methodology agrees exactly with the X-ray crystallographic data for the determination of annonin I (12). Further validation of this methodology is obtained by comparison of the results of this method with those obtained from the technique of Hoye and co-workers (33,34). The agreement of results from these three methods confirms their usefulness in helping to solve the stereochemical questions of the tetrahydrofuran regions of the Annonaceous acetogenins (24).

These nmr techniques are also applicable to the monotetrahydrofuran and the non-adjacent-tetrahydrofuran classes of acetogenins. The successful stereochemical assignments for monotetrahydrofuran acetogenins, such as annonacin (24), goniothalamicin (23), squamone (18), annonacin A (24), annonastatin (24), and the non-adjacent tetrahydrofuran acetogenins, giganticin (27), bullatalicin (28), and sylvaticin (29), have shown the helpfulness of these methods.

Another stereochemical region of concern is the portion containing the γ-lactone ring. This portion can have one chiral center (**A1** type), two chiral centers (**A2**, **A3**, and



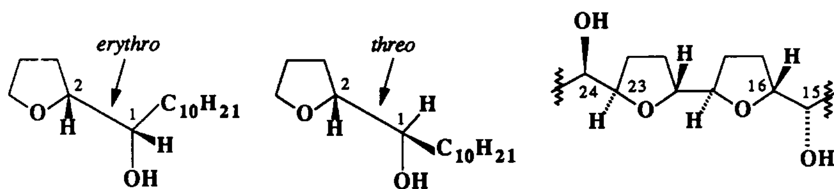
6':5'/3':2'/2':2/2:5/5:6 stereorelationships

	5.0–4.9	4.9–4.8	4.8 ~ 4.1	4.1–4.0	4.0–3.9	3.9–3.8	3.8–3.7	3.7 ~ 2.08	2.08–2.06	2.06–2.04
er/c/th/c/er a	6				5	2				Ac
er/t/th/c/er b	6'				5 5'	2	2'			Ac
er/t/th/t/er c	6			5	2					Ac
er/c/er/c/er d	6				5		2			Ac
er/t/er/c/er e	6 6'				5 5'		2'			Ac
er/t/er/t/er f	6				5	2				Ac
th/c/th/c/th g	6				5	2			Ac	
th/t/th/c/th h		6'		5	2 5'	2'			Ac	
th/t/th/t/th i		6			5	2			Ac	
th/c/er/c/th j		6			5		2		Ac	
th/t/er/tc/th k		6'			5 5'	2'			Ac Ac	
th/t/er/t/th l		6			5	2			Ac	
δ(ppm)	5.0	4.9	4.8 ~ 4.1		4.0	3.9	3.8	3.7 ~ 2.08	2.06	2.04
uvaricin	24			23		19/20 16				Ac
uvaricin acetate	24	15			16/23	19/20			Ac	Ac
asimicin triacetate		24/15			16/23	19/20			Ac's	
rolliniastatin triacetate	24	15			16 23	20/19			Ac	Ac

FIGURE 11. ¹H-nmr chemical shifts of the diagnostic protons in the 12 isomeric dibutylated diacetates (a–l) and of uvaricin, uvaricin acetate (33), asimicin triacetate, and rolliniastatin 1 triacetate (34); see structures **1**, **9**, and **10** [adapted from Hoye and Suhadolnik (33) and Hoye and Zhuang (34)]. Dots above the numbers indicate the chemical shifts of the corresponding protons.

A4 types), or three chiral centers (**A5** type) (Figure 4). The first stereochemistry solved for this structural portion was the absolute configuration of uvaricin and desacetyluvaricin at C-36 by Jolad *et al.* (3). They ozonized uvaricin [**1**] with an oxidative workup in basic solution. The acidic fraction, which contained lactic acid and a long chain acid,

TABLE 5. ¹H- and ¹³C-nmr Chemical Shifts of the Diagnostic Protons for Diastereomers **A** and **B** and Annonin I [adapted from Born *et al.* (12)].



Parameter	A	B	Parameter	Annonin I
H-1	3.84	3.40	H-15, H-24	3.40, 3.87
H-2	3.84	3.79	H-16, H-23	3.88, 3.87
C-1	71.83	73.87	C-24, C-15	71.7, 74.1
C-2	82.29	82.47	C-16, C-23	83.4, 82.9

was derivatized and subjected to glc on a column with a chiral and nonracemic stationary phase that had been shown to resolve the derivatives of *R*- and *S*-lactic acid. A peak coinciding with that of the derivative of *S*-lactic acid indicated that uvaricin has the *S* configuration at C-36 (3). There is a good chance that all these acetogenins have the same *S* configuration, but this is, of course, not a certainty. Nature has provided too many exceptions.

To suggest the absolute configuration at C-4 of the **A3** type, cd spectra have been used. This approach was followed to study the chiral center at C-4 of bullatacinone [**14**] (10). Comparison of cd curves of bullatacinone with those of two model compounds, rubrenolide and rubrynolide (two 2,4-disubstituted-γ-butyrolactones) (37) of known chirality, enabled the assignment of the *S* configuration to the chiral center at C-4 (Figure 12) by the application of the modified Hudson lactone rule (38).

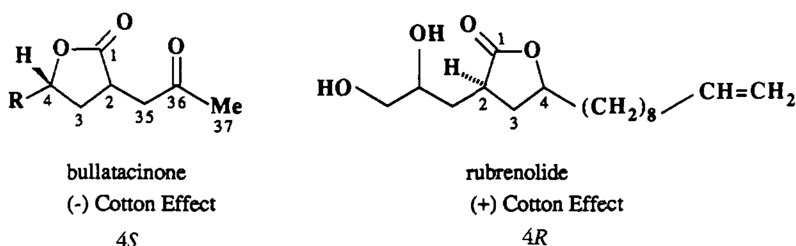
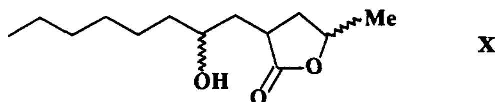


FIGURE 12. Cd spectral comparison between bullatacinone and rubrenolide.

The relative stereochemistry of the **A2** portion in asimicin [**9**], bullatacin [**13**], gigantecin [**24**], and bullatalicin [**25**] has been proposed based on the similarities of nmr and cd data with those of rolliniastatin 1. However, in a personal communication, Hoye's group has prepared both diastereomers of the model hydroxybutanoids represented by **X** and find their ^{1}H-nmr and ^{13}C-nmr spectra to be virtually indistinguishable. It would be prudent to raise a word of caution about basing the stereochemical assignment of the **A2** portion solely on such comparison of nmr and cd data.

There are, of course, some other problems associated with the above techniques in solving the stereochemistry of acetogenins. It is difficult, using Hoye's technique, to determine if the relative stereochemistry goes from C-15 to C-24 or from C-24 to C-15 if this portion of the molecule is unsymmetrical. The same problem exists for Born's technique. It is also impossible to determine the relative configuration for isolated single functional groups such as hydroxyls, and it is also impossible to determine the connection between the chiral portions, such as the stereorelations between tetrahydrofuran rings and γ-lactone rings and isolated hydroxyl groups. For this reason, many people have tried to perform X-ray crystallography using acetogenin derivatives. The first successful X-ray structure was obtained for rolliniastatin 1 [**10**] by Pettit *et al.* (15) for the crystalline 15-*O*-*p*-bromophenylurethane derivative of **10**. The only other X-ray crystallographic study reported to date was done by Born *et al.* (12) and was performed by reduction of annonin I [**16**] followed by hydrolysis to obtain a crystalline potassium salt (Figure 13).



X

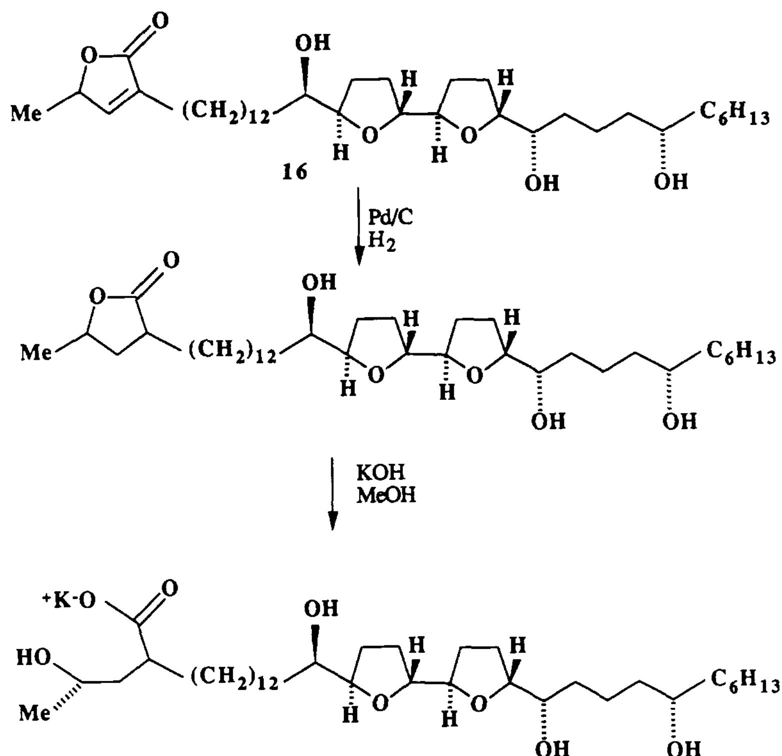


FIGURE 13. Reduction and hydrolysis of annonin I [**16**] to form a crystalline salt for X-ray [adapted from Born *et al.* (12)].

BIOLOGICAL ACTIVITIES

Annonaceous plants or their extracts have been employed extensively in folklore as pesticides, antitumor agents, emetics, etc. Undoubtedly, many of these previously unexplained folkloric uses can be attributed to this new acetogenin class of very potent bioactive compounds, and their extended biological evaluation and exact mechanism of action are a future challenge for biologists.

ANTITUMOR ACTIVITY AND CYTOTOXICITY.—Many plant members in this family have been used in folklore as anticancer medicines (13, 15, 16). The acetogenin components isolated from these plants that have shown significant cytotoxicity and antitumor in vivo activity are listed in Table 6.

In order to test further the selective specificities of the cytotoxic activity, asimicin, annonacin, goniothalamicin, bullatacin, and bullatacinone were sent to the NIH, NCI at Bethesda, Maryland, for human tumor cell tests in a panel including cells of leukemia, non-small cell lung cancer, small cell lung cancer, colon cancer, breast cancer, CNS cancer, melanoma, ovarian cancer, and renal cancer. Highly potent cytotoxicities with the range of log IC_{50}'s of over 5 orders of magnitude show that good selectivities are evident; the data for asimicin [**9**] and bullatacin [**13**] have been published (39, 10). Bullatacinone, in unreported data, has the best selectivity, with one cell line being highly selective. In view of these actions, a U.S. patent has been granted for the composition of matter of asimicin (40), and another is pending for bullatacin and bullatacinone (41).

Annonacin (22–26) and gigantecin (27) are reported to have antimitotic (9ASK) activities, suggesting tubulin inhibition although annonacin does not seem to bind di-

TABLE 6. Cytotoxicity and Antitumor Activity of Annonaceous Acetogenins.[a]

Compound	In vitro							In vivo	
	9PS	9KB	A549	MCF-7	HT-29	9ASK	L1210	3PS	L1210
	ED_{50} (μg/ml)							% T/C (mg/kg)	
Uvaricin [1]	—	—	—	—	—	—	—	157% (1.4)	—
Rollinicin [3]	2.9×10^{-8}	—	—	—	—	—	—	—	—
Rollinicin triacetate	2.6×10^{-4}	—	—	—	—	—	—	—	—
Isorollinicin [4]	10^{-2}	—	—	—	—	—	—	—	—
Rollinone [5]	$<10^{-5}$	—	—	—	—	—	—	147% (1.4)	—
Cherimoline[b] [7]	$<10^{-2}$	6×10^{-12}	10^{-3}	—	$>10^{-3}$	inact.	—	—	—
Dihydrocherimoline[b] [3]	$<10^{-2}$	2×10^{-8}	10^{-3}	—	10^{-3}	inact.	—	—	—
Asimicin [9]	$<10^{-12}$	$<10^{-5}$	10^{-3}	—	3.3×10^{-11}	inact.	—	124% (0.025)	131% (0.2)
Rolliniastatin 1 [10]	4.5×10^{-5}	—	—	—	—	—	—	128% (0.25)	—
Rolliniastatin 2 [11]	2.3×10^{-4}	—	—	—	—	—	—	—	—
Squamocin [12]	—	—	—	—	—	—	0.58	—	—
Bullatacin [13]	10^{-15}	6.2×10^{-14}	1.3×10^{-13}	>10	10^{-12}	inact.	—	—	138% (0.05)
Bullatacin triacetate	3.9×10^{-3}	6.9×10^{-7}	2×10^{-3}	—	$>10^{-1}$	—	—	—	—
Dihydrobullatacin	—	—	$<10^{-6}$	—	3.3×10^{-5}	—	—	—	—
Bullatacinone [14]	4.2×10^{-3}	$<10^{-12}$	10^{-3}	—	5×10^{-12}	inact.	—	—	144% (0.4)
Bullatacinone diacetate	4.2×10^{-2}	5×10^{-3}	2.8×10^{-2}	—	10^{-1}	—	—	—	—
4-Hydroxy-25-desoxyneo-rollinicin [18]	—	—	$<10^{-3}$	$<10^{-3}$	1.26	—	—	—	—
Annonacin [20]	10^{-5}	10^{-3}	10^{-3}	—	3	51% 15–30%	—	124% (0.95)	—
Goniothalamicin [21]	$<10^{-1}$	$<10^{-2}$	—	—	—	—	—	—	—

TABLE 6. Continued.

Compound	In vitro							In vivo	
	9PS	9KB	A549	MCF-7	HT-29	9ASK	L1210	3PS	L1210
	ED_{50} (μg/ml)							% T/C (mg/kg)	
Squamone [22]	—	—	1.34	2.14	1.5	—	—	—	—
Tetrahydrosquamone	—	—	1.4×10^{-1}	9.9×10^{-3}	3.0×10^{-1}	—	—	—	—
Annonacin-10-one [25]	10^{-6}	—	10^{-1}	—	1	—	—	—	—
Isoannonacin [26]	3	—	2×10^{-2}	—	2×10^{-3}	—	—	—	—
Isoannonacin-10-one [27]	5×10^{-1}	—	7×10^{-2}	—	9×10^{-3}	—	—	—	—
Gigantecin [28]	$<10^{-2}$	$<10^{-5}$	2.2×10^{-7}	4.1×10^{-9}	2.9×10^{-4}	31–50%	—	—	—
Bullatalicin [29]	—	>10	2.3×10^{-7}	2.34	8.8×10^{-6}	—	—	—	—
Bullatalicin tetraacetate	—	—	$<10^{-3}$	$<10^{-3}$	1.35	—	—	—	—
Sylvaticin [30]	—	$<10^{-3}$	$<10^{-3}$	—	$<10^{-3}$	—	—	—	—

[a] For definitions of abbreviations see footnote to Table 1.
[b] Compounds obtained from Dr. Diego Cortes and tested at Purdue University.

rectly to tubulin (25). Bullatacin is inactive in phorbol receptor and protein kinase tests (10). The action of the acetogenins in multiple-drug-resistant cells, mediated by an intracellular glycoprotein "pump," suggests a possible extracellular action on cell membranes. The tetrahydrofurans are reminiscent of the ionophore antibiotics such as monensin.

PESTICIDAL ACTIVITY OF ANNONACEOUS ACETOGENINS.—Moeschler *et al.* patented annonin, for which the structure was unknown and which was not defined as an acetogenin, as an insecticidal compound in 1984 (20) and 1987 (19). In 1988, Mikolajczak *et al.* patented the entire group of Annonaceous acetogenins as pesticides (11). In this latter patent, asimicin was claimed as an example for the first structurally defined pesticidal acetogenin. A divisional patent then protected the composition of matter of asimicin (40). Other acetogenins have been subsequently reported to have pesticidal activity; those with specific results reported include bullatacin (10), annonin VI (12), annonacin (22–26), goniothalamicin (23), and sylvaticin (29) (Table 7). Annonin (squamocin) and neoannonin showed strong ovicidal and larvicidal activity in the *Drosophila* feeding test at 125–140 μg/2 g of diet for *Drosophila melanogaster* (17). Evaluation of a standardized crude extract (F020) (11,42) of the bark of *As. triloba* shows promise as a garden pesticide (43), and commercial firms are being sought to finance the necessary tests required by the U.S. Environmental Protection Agency.

MISCELLANEOUS ACTIVITIES.—Cherimoline and dihydrocherimoline have been reported to have antimicrobial activity (4). Asimicin, annonacin, and goniothalamicin are reported to have antimalarial activity equal or superior to tetracycline (Table 8). Furthermore, asimicin shows T-cell inhibition in both in vitro (95% inhibition at 0.1 μg/ml) and in vivo (rats) (40% inhibition at 5.0 mg/kg) systems. All of the acetogenins from our laboratory were isolated by testing for brine shrimp lethality activity (30) and

TABLE 7. Pesticidal Activity of Tested Acetogenins.[a,b]

Compound	Concentration (ppm)	MBB	MA	ML	NE	BFL	SCB	SAW	CRW	2SSM
		% mortality								
Asimicin [**9**]	5000	—	—	—	—	—	50	—	—	—
	1000	—	—	—	—	100	—	—	—	—
	500	100	100	—	—	—	—	—	—	—
	100	100	20	100	100	0	—	—	—	—
	50	100	0	—	—	—	—	—	—	—
	10	70	0	100	100	0	—	—	—	—
	1	—	—	100	100	0	—	—	—	—
	0.1	—	—	75	100	—	—	—	—	—
Bullatacin [**13**]	400	—	90	—	—	—	—	0	—	20
	100	—	80	—	—	—	—	0	—	30
	24	—	—	—	—	—	—	—	80	—
	10	—	80	80	—	—	—	0	—	20
	6	—	—	—	—	—	—	—	20	—
	1	—	80	0	—	—	—	—	—	—
	0.5	—	—	0	—	—	—	—	—	—
Annonacin [**20**]	10000	—	—	—	—	100	—	—	—	—
	10	—	—	70	—	—	—	—	—	—
Goniothalamicin [**21**]	10000	—	—	—	—	100	—	—	—	—
Sylvaticin [**30**]		—	—	—	—	—	active	—	—	—
Annonin VI [**17**]		—	—	—	active	—	—	—	—	—

[a]Several methodologies are summarized in the references (11,42).
[b]For definitions of abbreviations see footnote to Table 1.

inhibition of crown gall tumors on potato discs (44). The former is predictive of cytotoxicity and pesticidal activity; the latter is predictive of 3PS (P388) in vivo murine antileukemic activity. The results are shown in Table 9. These two bench top bioassays have proven to be very useful in our work with activity-directed fractionations leading to these and other classes of compounds (39).

TABLE 8. Antimalarial Activity of Tested Acetogenins and Tetracycline.[a]

Compound	Drug-Resistant Clones of *Plasmodium falciparum*	
	Indochina W-2 IC_{50} (ng/ml)	Sierra Leone D-6 IC_{50} (ng/ml)
Asimicin [9]	4767.50	3776.90
Annonacin [20]	1511.60	1300.70
Goniothalamicin [21]	1590.00	1084.50
Tetracycline	5385.40	3364.50

[a]Results obtained from the Walter Reed Army Hospital.

The tabulated cytotoxicity data in Table 6 illustrate a number of structure-activity relationships among the Annonaceous acetogenins. Generally, the data indicate that the most potent cytotoxic compounds possess an adjacent bis-tetrahydrofuran ring subunit; the nonadjacent bis-tetrahydrofuran ring acetogenins show lesser cytotoxicity, with the monotetrahydrofuran compounds being the least cytotoxic. The hydroxyl moieties are extremely important in regard to the bioactivities of these compounds. Acetylation of the hydroxyl groups of rollinicin [**3**], bullatacin [**13**], bullatacinone [**14**], and bullatalicin [**29**] led to a decrease in their cytotoxicity. Furthermore, reduction of the ketone moieties of squamone [**22**] resulted in a substantially increased level of cytotoxicity. This suggests that oxidation of the hydroxyl groups to ketones would decrease activity and, conversely, reduction of the natural keto groups to hydroxyls could increase activity.

The relative importance of the γ-lactone subunit type is illustrated by the

TABLE 9. Summary of Brine Shrimp Assay (BST) and Potato Disc (PD) Antitumor Assay Results for Tested Acetogenins.

Compound	BST LC_{50} (ppm)	PD % inhibition (T/C)
Bullatacin [**13**]	0.00159	53
Bullatacinone [**14**]	0.003	15
Asimicin [**9**]	0.03	70
Bullatalicin [**29**]	0.154	63
Cherimoline [**7**][b]	1.24	—[a]
Squamone [**22**]	2.1	—
Annonacin [**20**]	3.3	45, 72
Dihydrocherimoline [**8**][b]	4.02	—
Sylvaticin [**30**]	26	—
Goniothalamicin [**21**]	37	32, 28, 68
Gigantecin [**28**]	222	83

[a]—indicates the compound was not tested.

[b]Compounds obtained from Dr. Diego Cortes and tested at Purdue University.

cytotoxicities of bullatacin [**13**], bullatacinone [**14**], and dihydrobullatacin (10). The α,β-unsaturated γ-lactone compound, bullatacin, is much more active than its reduction product (dihydrobullatacin), and bullatacin is much more active than the keto-lactone compound, bullatacinone (10). Although both were selective, bullatacin displayed greater cytotoxic potencies than bullatacinone, and bullatacinone showed much greater selectivity among the various cell lines tested at the NCI.

The importance of stereochemistry is obvious. So far, there are five reported diastereomers of asimicin, and each has a different spectrum of activity and potency. A change in the stereochemistry of a single chiral center can result in a cytotoxicity change from 1×10^{-3} to 1×10^{-13} (asimicin to bullatacin), which is an increase in activity of over a billionfold.

Of the acetogenins that have been tested for pesticidal activity (Table 7), several have selective activity against only certain pests. However, the keto-lactone compound, bullatacinone, does not show any activity at the same dose levels. This suggests that the terminal γ-lactone ring, not present in bullatacinone, might play an important role in the pesticidal activity of acetogenins. Furthermore, a mixture of these compounds, as found in many crude extracts, should show useful additive and possibly synergistic effects that would extend the spectrum of pests affected. Thus, it may be possible, and even advisable and more economical, to employ mixtures of acetogenins in crude extracts as biodegradable pesticides.

BIOSYNTHESIS OF ANNONACEOUS ACETOGENINS

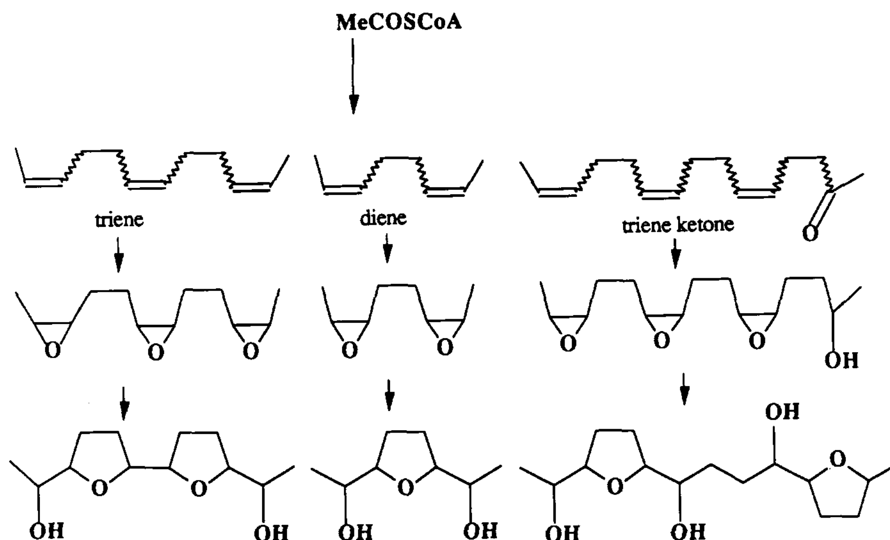
Because the Annonaceous acetogenins are a new and relatively unknown class of compounds, no experimental work on the biosynthesis of this class of compounds has been reported, although several proposals have been made for the natural origin of the adjacent bis-tetrahydrofuran ring acetogenins.

Acetogenins are a group of long chain compounds headed by a γ-lactone moiety (sometimes rearranged to methyl ketone); they have one or more tetrahydrofuran rings incorporated in the chain. This suggests clearly that they are derived from the polyketide pathway. Retrosynthetic analysis of known acetogenins reveals that their precursor could have been assembled by the linear combination of two and three carbon units (acetic acid and propanoic acid) via acetyl-CoA, malonyl-CoA, and propanyl-CoA, through mechanisms analogous to the well-known pathway for fatty acid biosynthesis.

All three classes of the tetrahydrofuran rings can be obtained by epoxidation of triene, diene, or triene ketone intermediates followed by ring openings and closures. Stereochemistries of the tetrahydrofuran rings and adjacent hydroxyl groups depend on the regiochemistry of double bonds, the face of epoxidation, and the way to open and close the epoxide rings (end-to-end or inside-out) as hypothesized in Figure 14.

Jolad *et al.* (3) proposed that uvaricin is probably biosynthesized from tetratriaconta-15,19,23-trienoic acid via triepoxidation followed by addition of HOAc. Precedents for this are the conversion of polybutadiene to the corresponding polyepoxide and then to a polytetrahydrofuran (45) and a similar study starting with the 1,5-diene geranyl acetate (46). The lactone ring, presumbly formed by an aldol-type condensation involving a three-carbon compound, is of a type long known to be used in biosynthesis of fatty acid derivatives (47). Cortes *et al.* (4) also stated that cherimoline and 2,35-dihydrocherimoline may be regarded as two new metabolites of fatty acids.

Abreo and Sneden (21) proposed that isolation from the same plant of two related acetogenins, rollinicin and isorollinicin, with a diastereomeric relationship between the bis-tetrahydrofuran moieties, is not surprising if these compounds are biogeneti-



Triene: (Z,Z,E)-uvaricin, rollinicin, rolliniastatin 1, annonin I, annonin VI, 4-hydroxy-25-desoxy-neorollinicin
(Z,Z,Z)-asimicin
(E,Z,Z)-bullatacin, bullatacinone
Diene: (Z,Z)-annonacin, goniothalamicin, squamone, annonastatin
(Z,E)-annonacin A
Triene ketone: (Z,Z,Z)-gigantecin
(Z,Z,E)-bullatalicin
(Z,E,Z) or (E,Z,Z)-sylvaticin

FIGURE 14. Hypothesis for the biosynthesis of tetrahydrofuran rings of Annonaceous acetogenins.

cally derived from triepoxidation of a triene intermediate followed by a zipper-like epoxide ring opening and closure to the subsequent epoxide to form the bis-tetrahydrofuran and adjacent hydroxyl groups. Rollinicin and 4-hydroxy-25-desoxyneorollinicin, both of which were isolated from *R. papilionella*, could be derived from a common triene, i.e., a derivative of tetratriaconta-(15Z,19Z,23E)-trienoic acid. If this intermediate is visualized in a standard staggered conformation, then epoxidation of all three double bonds from the same face followed by ring opening and closure would give a compound with the same stereochemistry, proposed as 4-hydroxy-25-desoxyneorollinicin. If the 19(Z) double bond were epoxided from the face opposite the one from which the 15(Z) and 23 (E) double bonds were, then the relative stereochemistry postulated for rollinicin would result from the zipper reaction. Obviously, acetogenins from other plants, e.g., asimicin, can have different intermediate precursors (21). McCloud *et al.* (22) used the name "polyketide" for annonacin, which implied the polyketide pathway as the biosynthetic pathway.

Nair *et al.* (48) developed haploid plants from in vitro anther callus of *An. squamosa* on a Nitsch basal medium supplemented with 6-benzyl-aminopurine and naphthalene acetic acid. They also induced multiple shoot formation from excised leaf explant seedlings on a Murashige and Skoog basal medium containing benzylaminopurine and kinetin (49). These cell culture systems may benefit future biosynthetic studies of the Annonaceous acetogenins.

STRUCTURAL REVISIONS AND REFINEMENTS

The close examination of the published spectral data of the reported Annonaceous

acetogenins reveals a number of structural inconsistencies. The evaluation of these inconsistencies has resulted in the following suggested structural revisions. Some structural refinements in terms of relative stereochemistry are also suggested.

Examination of the diagnostic ^{13}C-nmr signals for rollinicin [**3**], isorollinicin [**4**], annonin I [**16**], and squamocin [**12**] indicates a great deal of homogeneity (Table 10). The difference between these reported structures is the placement of a single hydroxyl group at C-28 for annonin I and squamocin, and C-25 for rollinicin and isorollinicin. The structure of annonin I has been proven by X-ray crystallographic studies (Figure 13), and the structure of squamocin has been determined by degradative chemical methods followed by ms analysis (Figure 9). The placement of the hydroxyl at C-25 in rollinicin and isorollinicin was made on the basis of ms analysis, in particular the hrcims of rollinicin and rollinicin triacetate. The hrcims of rollinicin led the investigators to state that "the position of the third hydroxyl group was limited to C-25 through C-28" (6). The specific placement of this hydroxyl group at C-25 was based on the hrcims of rollinicin triacetate. However, the key proposed daughter ion in the hrcims of the rollinicin triacetate, which led to the placement of the remaining hydroxyl group at C-25, was not confirmed by ms/ms. Furthermore, the COSY spectra of rollinicin[2] shows no coupling between the methine protons of the hydroxylated carbons C-15, C-24, and C-25, thus proving that two adjacent hydroxyl moieties at C-24 and C-25 are not present. We suggest it is quite probable that rollinicin and isorollinicin are diastereomers of annonin I [**16**] and squamocin [**12**] (see revised structure **32**). Furthermore, the ^{13}C-nmr (Table 10) and melting point data for squamocin and rollinicin suggest that these compounds are probably identical.

It is readily apparent from the ^{1}H-nmr spectra of rollinone[3] [**5**] that it is a diastereomer of bullatacinone [**14**]. The ^{1}H-nmr signal at δ 2.18 (d, Me) in rollinone is at-

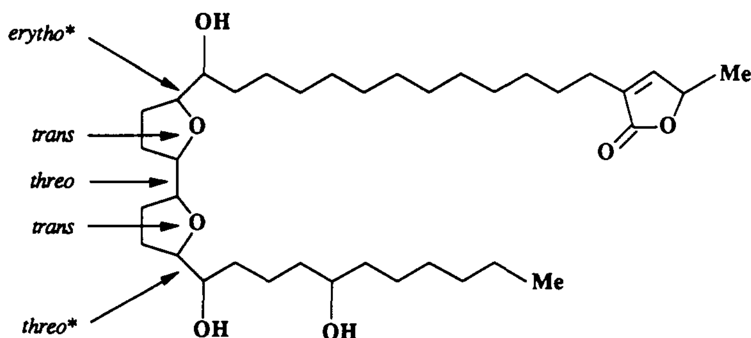
TABLE 10. The Diagnostic ^{13}C-nmr Signals of Isorollinicin, Rollinicin, Annonin I, and Squamocin.

Carbon	Compound			
	Isorollinicin [**4**]	Rollinicin [**3**]	Annonin I [**16**]	Squamocin [**12**]
C-1	173.9	173.9	173.9	173.93
C-35	148.7	148.9	148.9	148.97
C-2	134.4	134.1	134.3	134.31
C-16[a]	83.2	83.3	83.4	83.36
C-19[a]	82.8	82.7	82.9	82.85
C-20[a]	82.4	82.5	82.5	82.55
C-23[a]	82.1	82.0	82.2	82.15
C-36	77.3	77.4	77.4	77.35
C-24[b]	75.1	74.1	74.1	74.09
C-25/28[b]	74.1	71.6	71.7	71.60
C-15[b]	71.5	71.5	71.3	71.51
	—[c]	37.5	37.4	37.48
	—	37.1	—	37.23
	33.4	—	33.1	33.12
	32.5	—	32.4	32.49
	31.9	31.8	31.8	31.88

[a,b]Assignments are interchangeable.
[c]—indicates that no ^{13}C-nmr signal corresponding to this resonance was reported.

[2]A copy of the COSY spectra of rollinicin was provided by Dr. Albert T. Sneden.

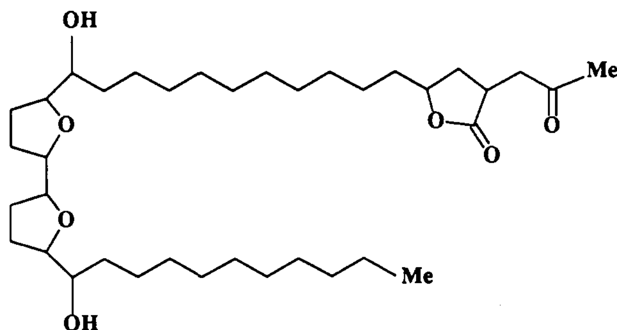
[3]A copy of the ^{1}H-nmr spectra of rollinone was provided by Dr. Albert T. Sneden through Dr. John M. Cassady. Dr. Sneden has communicated similar observations in a recent paper submitted to *J. Nat. Prod.*



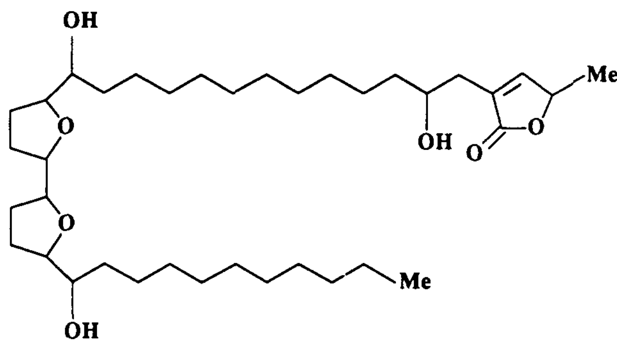
32. Revised structure of rollinicin; isorollinicin would be a diastereomer of the same contiguous atoms. * indicates that the assignments are interchangeable.

tributed to the C-37 methyl group of the saturated γ-lactone. This assignment is clearly incorrect; a methyl group of this type will display a chemical shift of approximately δ 1.35 (10), which is upfield from the α,β-unsaturated γ-lactone methyl group at δ 1.41. This resonance is obviously due to the methyl ketone group of the type **A3** lactone. The reported multiplicity (doublet) is likely the result of two separate methyl ketone signals of a racemic mixture, rather than a true doublet. Close examination of the relative intensities of the two resonances of this reported doublet show an intensity ratio of 3 to 1, indicative of a mixture. This same situation was also seen in the ^{1}H-nmr of bullatacinone, where the nonsymmetrical abundance of the two methyl signals indicated a racemic mixture. Therefore, we suggest the structural revision **33** for rollinone, it having the same contiguous atom sequence as bullatacinone. The relative stereochemistry of the tetrahydrofuran subunit of rollinone cannot be determined from the published spectral data. It appears, however, that rollinone (mp 54–56°) is not identical to bullatacinone (mp 90.5–90.7°) based on a comparison of their melting points.

In the previous section on the primary structural elucidation of the γ-lactone subunit (**A1–A5**), a comparison of the diagnostic ^{1}H-nmr and ^{13}C-nmr chemical shifts was made. The chemical shift of δ 69.8 in the ^{13}C-nmr spectra of 14-hydroxy-25-desoxyrollinicin [**6**] clearly indicates that this compound is of the **A2** γ-lactone type and possesses a hydroxyl group at the C-4 position instead of the C-14 position. Therefore, 14-hydroxy-25-desoxyrollinicin is another diastereomer of asimicin [**9**]. Furthermore, it has been suggested (14) that 14-hydroxy-25-desoxyrollinicin is identical to rolliniastatin 2 [**11**]. This, however, has not been confirmed due to the lack of a specimen of 14-hydroxy-25-desoxyrollinicin for direct comparison with rolliniastatin 2.



33. Revised structure of rollinone



34. Revised structure of 14-hydroxy-25-desoxyrollinicin

Examination of the C-3 protons of cherimoline [7] clearly shows the complex coupling pattern associated with a hydroxyl group at the C-4 position.[4] This assertion is substantiated by a ^{13}C-nmr resonance at δ 69.8 which is indicative of a hydroxyl moiety at the C-4 position. Further examination of the ^{13}C-nmr spectral data suggests that cherimoline possesses a non-adjacent bis-tetrahydrofuran ring subunit. This suggested structural revision has been substantiated by the eims analysis of the TMSi and TMSi-d_9 derivatives of cherimoline (Figure 15).[5,6] The fragmentation patterns of the TMSi and TMSi-d_9 derivatives of cherimoline, illustrated in Figure 15, are virtually identical to

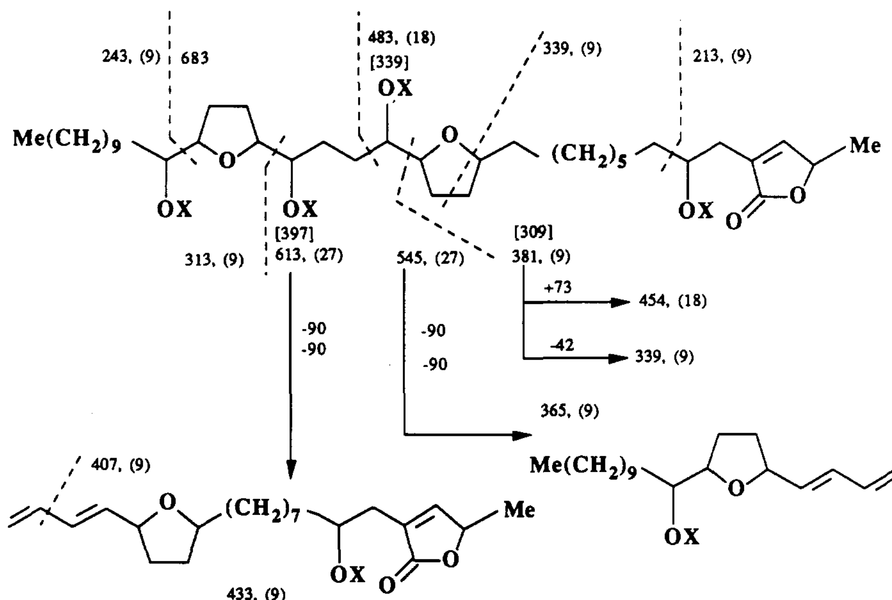


FIGURE 15. Diagnostic eims fragment ions *m/z* of cherimoline [**35**], chermoline-TMSi derivative, and cherimoline-TMSi-d_9 derivative. The numbers 9, 18, 27 in parentheses refer to mass shifts of the TMSi-d_9 derivative. The numbers in brackets refer to mass fragments of the underivatized cherimoline.

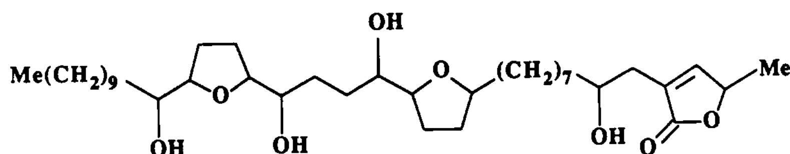
[4]A copy of the 400 MHz ^{1}H-nmr of cherimoline was provided by Dr. Diego Cortes.

[5]A sample of cherimoline was provided by Dr. Diego Cortes.

[6]Additional eims of the TMSi and TMSi-d_9 derivatives of cherimoline were performed at Purdue University by Dr. David L. Smith and Ya-Mei Liu, who are preparing a separate manuscript on the ms of the acetogenins.

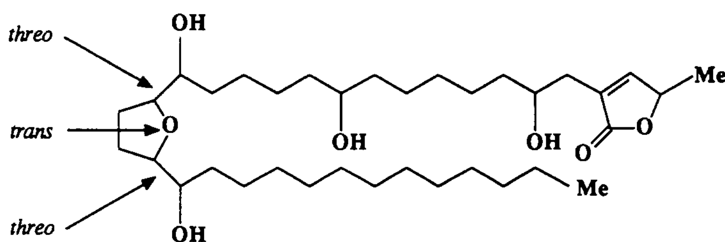
those of sylvaticin [**30**] (Figure 8). Therefore, based on these new data we propose the structural revision **35** for cherimoline. Furthermore, the ^{13}C-nmr spectral data for the diagnostic tetrahydrofuran ring carbons of dihydrocherimoline [**8**] are not in agreement with those of cherimoline [**7**], indicating that nomenclature describing **8** is a misnomer and should be changed.

Although the structure of almunequine [**31**] has not been determined, the published ^{1}H-nmr and ^{13}C-nmr spectral data suggest several structural subunits. The ^{13}C-nmr signals at δ 83.5, 82.4, 82.1, and 79.5 are indicative of the **B2** type tetrahydrofuran subunit. The ^{1}H-nmr and ^{13}C-nmr data also show that **31** possesses an **A1** type α,β-unsaturated γ-lactone subunit.

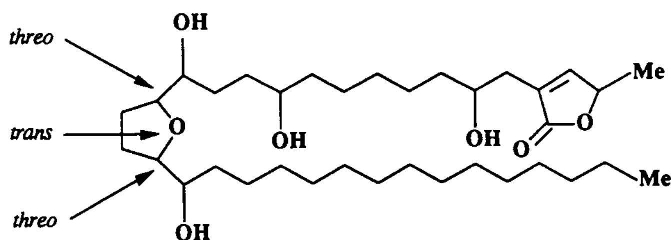


35. Revised structure of cherimoline

Utilizing the previously described methods of Hoye and co-workers (33–36) and Born *et al.* (12) for establishing the relative stereochemistry of the tetrahydrofuran ring systems, we have now assigned the relative stereochemistry of this structural subunit for the compounds annonacin [**20**] and goniothalamicin [**21**]. The suggested structural refinements for annonacin and goniothalamicin are illustrated by structures **36** and **37**, respectively. This refinement of annonacin is in agreement with the stereochemistry of annonacin as originally reported by Lieb *et al.* (24).



36. Refined structure of annonacin



37. Refined structure of goniothalamicin

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Laboratory, Purdue University Cancer Center, for cytotoxicity testing; to D.L. Smith and Y.-M. Liu, for eims spectra of cherimoline; to D. Cortes, for samples of cherimoline and dihydrocherimoline; to A.T. Sneden for the spectra of rollinicin and rollinone; to the Upjohn Co., for the in vivo L1210 results; to the Eli Lilly Co., for the immunosuppressant results; and to the Walter Reed Army Hospital, for the antimalarial test results.

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