ASIMICIN, A NEW CYTOTOXIC AND PESTICIDAL ACETOGENIN FROM THE PAWPAW, ASIMINA TRILOBA (ANNONACEAE)

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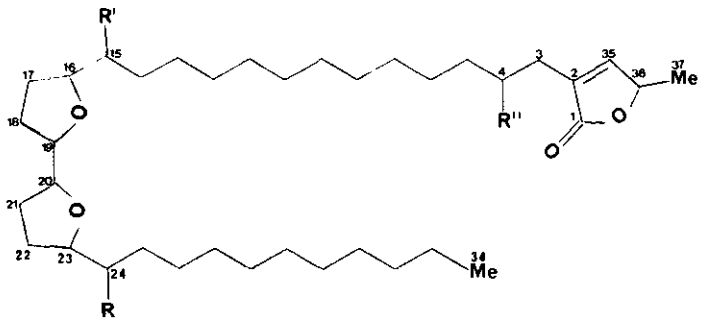
Abstract - Asimicin, a new trihydroxy-bistetrahydrofuran fatty acid γ-lactone has been isolated, using brine shrimp lethality for activity-directed fractionation, from extracts of the bark and the seeds of the pawpaw tree, Asimina triloba Dunal. (Annonaceae). This compound is extremely cytotoxic and shows promising pesticidal activities.

A 95% ethanol extract of the leaves and twigs of the pawpaw, Asimina triloba Dunal. (Annonaceae)[1], was toxic to mice at 6.25 mg/kg in the 3PS lymphocytic leukemia system.[2] Testing of the seeds, wood, bark, leaves and twigs, in a convenient bioassay involving brine shrimp lethality,[3] determined the bark to be the most potent plant part. Through multiple partitionings and chromatographic steps, monitoring the fractionations with thin-layer chromatography and brine shrimp lethality, asimicin (**1**), a novel acetogenin, was isolated as a major bioactive component of the bark and the seeds.

Previously reported linear Annonaceous acetogenins include the cytotoxic/antitumor compounds, uvaricin[4] (**2**) and desacetyluvaricin[5] from Uvaria accuminata and rollinicin, isorollinicin,[6] and rollinone[7] from Rollinia papillionella, and the antimicrobial compounds, cherimoline and dihydrocherimoline from Annona cherimolia.[8] Asimicin (**1**) was extremely cytotoxic in the 9KB (human nasopharyngeal carcinoma, ED_{50} $<10^{-5}$ ug/ml) and the 9PS (murine lymphocytic leukemia, ED_{50} $<10^{-7}$ ug/ml) systems.[2] Promising pesticidal activity[9] paralleled the brine shrimp lethality throughout the fractionation of the bark and included significant activity against the stripped cucumber beetle, Mexican bean beetle, mosquito larvae, blowfly larvae, melon aphid, two spotted spider mite, and the free-living nematode, Caenorabditis elegans. These pesticidal actions of asimicin further expand the spectrum of biological activities of this new class of natural compounds.

Asimicin (1) was isolated as a whitish wax (mp 68-69°C). The high resolution fast atom bombardment (FAB) MS gave an MH^+ at m/z 623.4863 (calc. 623.4863) corresponding to the molecular formula $C_{37}H_{66}O_7$. The presence of three hydroxyl moieties was obvious by three successive losses of water (m/z 18) from the molecular ion (Figure 1). The formation of a triacetate (3) (Ac_2O/pyridine) and broad IR absorption at 3420 cm^{-1} confirmed the presence of three hydroxyls.

A positive response to Kedde's reagent[10] suggested the presence of an α, β-unsaturated lactone. An IR carbonyl absorption band at 1750 cm^{-1} and a UV (MeOH) λ max at 208 nm (ε = 14,300)



		R	R'	R''
1	Asimicin	OH	OH	OH
2	Uvaricin	OAc	OH	H
3	Asimicin triacetate	OAc	OAc	OAc

also indicated an α, β-unsaturated lactone. In addition, selective ^1H-^1H decouplings in the ^1H NMR ($CDCl_3$) showed associated signals at δ 7.17 [1H, H(35), q, J = 1.5, 1.5], 5.06 [1H, H(36), qq, J = 6.8, 1.5, 1.5], and 1.41 [3H, H(37), d, J = 6.8], specifically indicating an α, β-unsaturated γ-lactone[11] (Table 1). These assignments were confirmed in the ^{13}C NMR spectrum by resonances at δ 174.6 [1C, s, C(1)], 151.8 [1C,d, C(35)], 131.1 [1C, s, C(2)], 78.0 [1C, d, C(36)] and 19.1 [1C, q, C(37)].

Furthermore, the ^{13}C NMR spectrum of asimicin (1) showed signals due to oxygen-bearing carbons at δ 74.0 [2C, d, C(15) and C(24)] and 69.9 [1C, d, C(4)], suggesting three secondary hydroxyl moieties, two of which were equivalent. In the ^1H NMR ($CDCl_3$) spectrum, a signal at δ 3.37 (2H, brq) was attributed to the hydrogens at C(15) and C(24), and a signal at δ 3.86 (1H, m) was attributed to the proton at C(4). These assignments were substantiated by the ^1H NMR spectrum of the triacetate in which signals for the C(15) and C(24) protons were shifted downfield to δ 4.85, and the C(4) proton signal was shifted downfield to δ 5.10.

The bistetrahydrofuran heterocyclic moiety was indicated by multiple resonances for five protons between δ 3.79-3.89 in the ^1H NMR ($CDCl_3$) spectrum; these were resolved in C_6D_6 into signals at δ 3.86 (2H, m), 3.77 (1H, m), and 3.73 (2H, m). These proton resonances and signals in

the ^{13}C NMR ($CDCl_3$) at δ 81.8 [2C, d, C(19) and C(20)] and 83.1 [2C, d, C(23) and C(16)] were directly analogous to similar signals in uvaricin[4]. The saturated alkyl chain, typical of the acetogenins, was suggested by multiple CH_2-resonances between δ 29.7-25.5 in the ^{13}C NMR spectrum and signals at δ 0.86 [3H, C(34), t] and 1.25 (ca. 32H, broad) in the ^1H NMR ($CDCl_3$).

Confirmation of the structural placement of the three hydroxyl groups was based on selective ^1H-^1H decoupling experiments and the FAB MS fragmentation pattern. Decoupling of the two equivalent oxygen-bearing methine protons at δ 3.45 in C_6D_6 showed association with the protons of C(23) and C(16) at δ3.86, suggesting the presence of hydroxyl groups at C(15) and C(24). In the FAB MS, α-cleavage between C(24) and C(23) gave fragments at m/z 171 and m/z 153 (171-H_2O). α-Cleavage between C(16) and C(15) produced a fragment at m/z 311 followed by sequential loss of two moles of water; such a fragmentation suggested that the remaining hydroxyl group must be attached at one of the carbons between C(3-14) (see Figure 1). Decoupling of the oxygen-bearing methine proton at δ 3.77 showed association with signals at 2.51 (1H, dddd) and 2.38 (1H, ddt). The signals at δ 2.51 and 2.38 were attributed to nonequivalent methylene protons at C(3), with the nonequivalence being a result of the hydroxyl group at C(4). The methylene protons at C(3) exhibited geminal coupling (J = 15); the proton at δ 2.51 (3a) was coupled to the proton of C(4) (J = 3.5); and the proton at δ 2.38 (3b) was coupled to the same proton (J = 8).[12] Proton 3a also exhibited long-range coupling with the proton at C(35) (J = 1.2) and C(5a) (J=1.7), and proton 3b showed long range coupling to the proton on C(36) (J = 1.5) and C(35) (J=1.5). Thus, the third hydroxyl assignment was concluded to be at C(4), a novel feature for the acetogenins. Configurations at the chiral centers remain unresolved.

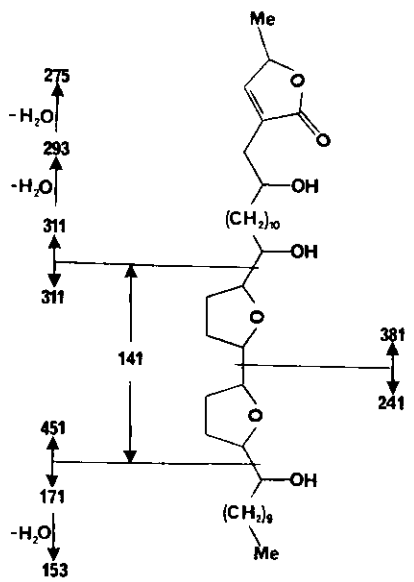


Figure 1. FAB MS of Asimicin (MH^+, m/z 623.4863, calc. 623.4863).

TABLE I. ^{1}H and ^{13}C Chemical Shifts (δ) and ^{1}H-H Coupling Constants (J, Hz) of Asimicin (1) and Asimicin Triacetate (3).

Atom	(^{13}C)[a], 50 MHz	(^{1}H)[a], 470 MHz	(^{1}H)[b], 470 MHz	(^{1}H)[a], 300 MHz	Atoms	J[c]
1	174.6 (s)	--	--	--	3a, 3b	15
2	131.1 (s)	--	--	--	3a, 4	3.5
3a	37.4 (t)[d]	2.51 (dddd)	2.35	2.53	3b, 4	8.0
3b		2.38 (ddt)	2.27		3a, 35	1.5
4	69.9 (d)	3.86 (m)	3.77	5.09	3b, 35	1.5
5	31.9 (t)[d]	1.55 (m)	1.4-1.5	1.5-1.9	3a, 5a	1.7
6	28.9 (t)[d]	1.25 (m)	1.35	1.25	3b, 36	1.5
7-13	29.5 (t)[d]				35, 36	1.5
14	33.4 (t)[d]	1.55 (m)	1.55	1.5-1.9	36, 37	6.8
15	74.0 (d)	3.37 (brq)	3.45	4.85		
16	83.1 (d)	3.79-3.89 (m)	3.86	3.98 (brq)		
17	28.4 (t)[d]	1.6-2.0 (m)	1.4-1.8	1.5-1.9		
18	25.5 (t)[d]	1.6-2.0 (m)	1.4-1.8	1.5-1.9		
19	81.8 (d)	3.79-3.89 (m)	3.73	3.90		
20	81.8 (d)	3.79-3.89 (m)	3.73	3.90		
21	25.6 (t)[d]	1.6-2.0 (m)	1.4-1.8	1.5-1.9		
22	25.6 (t)[d]	1.6-2.0 (m)	1.4-1.8	1.5-1.9		
23	83.1 (d)	3.79-3.89 (m)	3.86	3.98 (brq)		
24	74.0 (d)	3.37 (brq)	3.45	4.85		
25	33.3 (t)[d]	1.55 (m)	1.55	1.5-1.9		
26-30	29.6 (t)[d]					
31	29.3 (t)[d]	1.25 (m)	1.35	1.25		
32	29.7 (t)[d]					
33	22.7 (t)					
34	14.1 (q)	0.86 (t)	0.92	0.87		
35	151.8 (d)	7.17 (q)	6.35	7.06		
36	78.0 (d)	5.06 (qq)	4.30	5.01		
37	19.1 (q)	1.41 (d)	0.86	1.41		
4 OAc	--	--	--	2.01		
15 OAc	--	--	--	2.06		
24 OAc	--	--	--	2.06		

a) In $CDCl_3$ b) In C_6D_6 c) In $CDCl_3$, recorded at 200 MHz d) These methylene carbon assignments are tentative, and may be interchanged.

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