Uvaricin, a New Antitumor Agent from *Uvaria accuminata* (Annonaceae)

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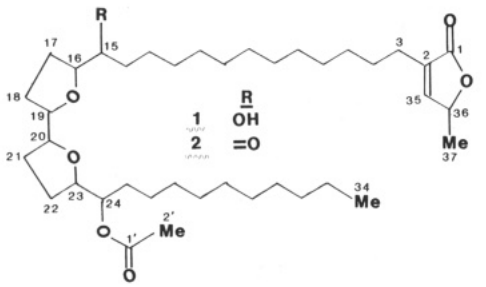
The isolation and spectral characterization of the novel antitumor agent uvaricin (1), a bis(tetrahydrofuranoid) fatty acid lactone from the roots of *Uvaria accuminata* (Annonaceae), are described, and its biogenesis is discussed.

During thhe course of our continuing search for plants having tumor inhibitory constituents, an ethanol extract of the roots of the title plant yielded a waxy substance, named uvaricin, which demonstratd antitumor properties in the in vivo PS system (P-388 lymphocytic leukemia in mice).[1]

Uvaricin (1) melted just above room temperature and was optically active with $[\alpha]^{25}_D$ +11.3° (MeOH). The electron-impact (70 eV) mass spectrum of 1 did not display a recognizable molecular ion peak but a peak at m/z 630 ($M^{+\cdot} - H_2O$) was clearly observed. The molecular weight was conclusively established from its chemical ionization (CH_4) and desorption chemical ionization (NH_3) mass spectra as 648 and its molecular formula as $C_{39}H_{68}O_7$.

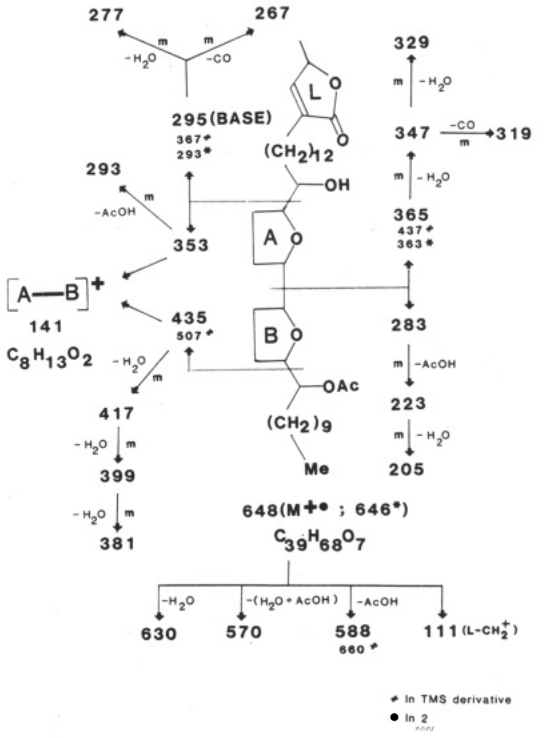


The IR spectrum of 1 showed bands typical of hydroxyl, olefin, α,β-unsaturated and ester carbonyls, *n*-alkyl chain, and methyl groups.

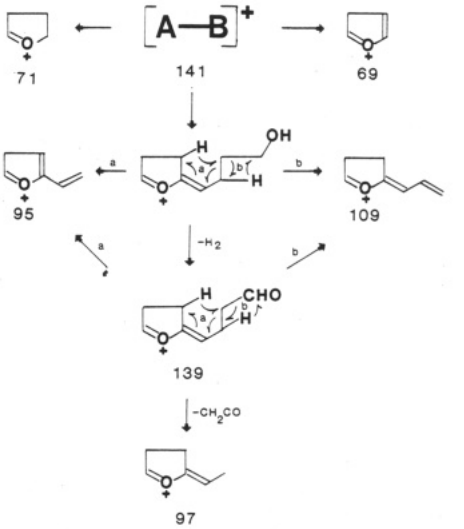
From the many similar methylene absorptions in its ^1H and ^{13}C NMR spectra (Table I) 1 was clearly a long-chain fatty acid derivative. An *n*-butyl group was clearly present from the ^1H NMR spectrum, with the other end occupied by the lactone grouping (^1H NMR, IR, and UV data are consistent with literature data[2] for this grouping).

From the central portion of the molecule, six methinyl carbons bearing oxygen were visible in the NMR; the ^1H absorptions of several of these overlapped in $CDCl_3$, but all were separated in C_6D_6.[3] One of these was in a secondary alcohol grouping and another was in a secondary acetate, leaving four in two ether groupings. A ^1H–^1H decoupling study showed the alcoholic methinyl to be attached to an ether methinyl, the acetate methinyl to be attached to a second ether methinyl, and the remaining two ether methinyls to be attached to one another. From

Scheme I. *m/z* **Ratios of Significant Fragment Ions in the Mass Spectrum of 1**



Scheme II. *m/z* **Ratios and Possible Structures of Fragment Ions from the *m/z* 141 Ion in the Mass Spectrum of 1**



the molecular formula and the lack of further unsaturated carbons, two additional rings were needed. The observation that the vicinal ^1H–^1H coupling constants involving

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(3) The effects of changing solvent from $CDCl_3$ to C_6D_6 on the ^1H chemical shifts in the vicinities of the π systems of 1 were striking and suggest strong interaction between these π systems and that of C_6D_6. The protons on carbons 2′, 35, 36, and especially 37 were shifted *upfield*, and the proton on carbon 24 was shifted *downfield*.

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Table I. ^1H and ^{13}C Chemical Shifts (δ) and ^1H-^1H Coupling Constants (J, Hz) in 1 and 2

	1				1	
atom	$\delta(^{13}C)^a$	$\delta(^1H)^a$	$\delta(^1H)^b$	2, $\delta(^1H)^a$	atoms	J^b
1	173.7 s				3, 4	6.9, 4.4
2	134.6 s				3, 35	1.5, 1.5
3	25.3 t	2.26 ~t	2.13	2.27		
4	27.5 t	~1.55 m	~1.4, ~1.5	~1.55	15, 16	6.3
5-13	~29.6 t[c]	~1.25 m	~1.25	~1.25	16, 17	6.3
14	33.6 t	~1.28 m	~1.34	2.54	19, 20	5.0
15	74.1 d	3.38 m	3.38			
16	83.2 d	3.81 q	3.83	4.40 t (J = 7.0)	22, 23	6.3, 6.3
17-18	27.8-29.8 t[c]	1.6-2.0 m	1.3-1.7	1.6-2.2	23, 24	6.3
					24, 25	8.3, 4.2
19	82.0 d,	3.87 m,	3.70	~4.04 m	33, 34	6.4
20	81.6 d	3.88 m	3.78	3.92 q	35, 36	1.5
21-22	27.8-29.8 t[c]	1.6-2.0 m	1.6-1.9	1.6-2.0	36, 37	7.0
23	80.7 d	4.01 q	4.03	~4.01		
24	75.5 or 77.3 d	4.94 ddd	5.20	4.94		
25	31.3 t	~1.55 m	~1.66	~1.55		
26-30	~29.6 t[c]					
31	29.4 t	~1.25 m	~1.25	~1.25		
32	32.0 t					
33	22.7 t					
34	14.1 q	0.88 t	0.89	0.88		
35	148.7 d	6.99 q	6.10	7.00		
36	75.5 or 77.3 d	4.99 qq	4.30	5.00		
37	19.3 q	1.41 d	0.86	1.41		
1'	170.6 s					
2'	21.2 q	2.05 s	1.77	2.05		

[a] In $CDCl_3$. [b] In C_6D_6 for better peak separation. [c] The CH_2 peaks in the δ 27.8-29.8 region are arranged as follows: single peaks at δ 27.8, 28.3, 28.5, 28.8, 29.2, and 29.8; a peak for 2 CH_2's at δ 29.4; a peak for 9 CH_2's at δ 29.6.

the oxygen-bearing methinyls were all about 6 Hz suggested the presence of five-membered rings as shown.

The mass spectral fragmentation of 1 (Scheme I) was used to establish the remaining structural features. α-Fission between oxygenated methinyl-oxygenated methinyl bonds gave pairs of peaks adding up to the molecular weight: m/z 295 ($C_{18}H_{31}O_3$) - 353 ($C_{21}H_{37}O_4$) and m/z 365 ($C_{22}H_{37}O_4$) - 283 ($C_{17}H_{31}O_3$); m/z 435 ($C_{26}H_{43}O_5$) was also found. These fragments show the central portion to consist of two adjacent C_4H_6O units; this unit is seen more directly as a peak at m/z 141 ($C_8H_{13}O_2$) and its fragmentation peaks (Scheme II). The locations of the hydroxyl and acetate groupings and the numbers of methylene groups in each chain were suggested from the molecular formulas of the primary α-fission products and were strongly supported by their further fragmentations (Scheme I; m = metastable peak observed) and those of the trimethylsilyl (Me_3Si) derivative: the peaks at m/z 295, 365, and 435 for hydroxyl-containing fragments were replaced by peaks 72 mass units higher in the Me_3Si derivative; the ions at m/z 283 and 353 containing acetate readily lose acetic acid. The lactone grouping shows as a peak at m/z 111 ($C_6H_7O_2$) and as a loss of 112 ($C_6H_8O_2$) from the ions at m/z 295, 365, and 435 via McLafferty rearrangement involving the alkene linkage. The elemental compositions of all peaks shown in Scheme I were verified by high-resolution exact mass measurements.

As can be seen from Table I and Scheme I, the ^1H NMR and mass spectral changes occurring on oxidation to uvaricinone (2), the corresponding ketone, supported structure 1 for uvaricin.

While the lactone ring, presumably formed by an aldol-type condensation involving a three-carbon compound, is of a type found previously in fatty acid derivatives,[2] the central portion of the molecule is apparently novel. Uvaricin (1) is probably biosynthesized from tetratriaconta-15,19,23-trienoic acid via triepoxidation followed by addition of acetic acid. Precedents for this are the conversion of polybutadiene to the corresponding polyepoxide and then to a polytetrahydrofuran[4] and a similar study starting with the 1,5-diene geranyl acetate.[5] Bis(tetrahydrofurans) have been found in nature before, e.g., monensin[6] and septamycin[7] and its relatives,[8] but not in an all acetate-derived chain and not flanked by a hydroxyl and an acetate as occurs in 1.

Experimental Section[9]

Isolation of Uvaricin (1). Dried roots of *U. accuminata* were powdered in a Wiley mill and stored at -10 °C prior to extraction. The powdered material (14 lb) was extracted in a Lloyd extractor with 95% EtOH for 96 h. The EtOH extract, after removal of the solvent, was repeatedly triturated with ether. The combined ether-soluble extract was filtered and dried under vacuum. The ether-insoluble resinous material was treated likewise with ethyl acetate, filtered, and vacuum dried. The ether-soluble (123 g) and ethyl acetate soluble (42 g) extracts were then subjected to three-funnel partitioning between cyclohexane, methanol, and water (7:5:1). Layers showing 1 on TLC were combined, and the solvent was evaporated off. The resulting residue (59 g) was subjected to EM SiO_2-60 chromatography, eluting the column initially with methylene chloride (100%) and then with methylene chloride containing gradually increasing amounts of ether. Fractions containing 1 as judged from TLC were combined, freed from solvent by evaporation, and subjected to normal phase preparative HPLC [Waters Prep LC/SYSTEM 500A liquid chromatograph equipped with a refractive index detector: Waters Prep PAK-500/SILICA cartridge column; *n*-hexane/EtOAc (70:30)] followed by preparative TLC [SiO_2-60 PF-254; methylene chloride/EtOAc (85:15)]. Repetition of the preparative HPLC

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procedure, using reversed phase Waters Prep PAK-500/C_{18} cartridge column and 7% aqueous MeOH as eluting solvent, gave pure 1 as judged from analytical reversed phase HPLC [Spectra Physics Model 3500B liquid chromatograph equipped with a UV detector set at 254 nm: Whatman Partisil PXS 10/25 ODS 2 C_{18} (25 cm × 4.6 mm i.d.) column; 7% aqueous MeOH].

Uvaricin (1). The IR [(CCl_4) 3590, 2940, 2860, 1768, 1745, 1650, 1465, 1370, 1317, 1240, 1195, 1115, 1065, 1023, 945, 875, 850, 715 cm^{-1}], UV [λ_{max} (EtOH) 207 nm (ϵ 12730)], ^1H and ^{13}C NMR (Table I), and mass (Schemes I and II) spectra were in accord with structure 1.

Anal. Calcd for $C_{39}H_{68}O_7$: C, 72.2; H, 10.4. Found: C, 71.8; H, 10.9.

Uvaricinone (2), prepared from CrO_3-pyridine oxidation followed by purification by preparative TLC [SiO_2-60 PF-254; methylene chloride/EtOAc (90:10)], an oil. Its IR [(CCl_4) 1765, 1742, 1718, 1235, 710 cm^{-1}], ^1H NMR (Table I), and mass (Scheme I) spectra were in accord with structure 2.

Uvaricin (1) demonstrated an activity of 157% test/control (T/C) at 1.4 mg/kg in the PS test system. Activity in the PS system is defined as an increase in the survival of treated animals over that of controls resulting in a T/C ≥ 125%.[1] Uvaricin has been selected by the NCI for tumor panel testing.

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Selective Reductions. 29. A Simple Technique To Achieve an Enhanced Rate of Reduction of Representative Organic Compounds by Borane-Dimethyl Sulfide

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A dramatic increase in the rate of reduction of esters by borane–dimethyl sulfide (BMS) is observed when dimethyl sulfide is removed from the reaction mixture. On the basis of this observation, a new, improved procedure has been developed for the reduction by BMS of respresentative organic functional groups, such as esters, nitriles, and amides. The procedure involves addition of BMS to the substrate in refluxing tetrahydrofuran, allowing the liberated dimethyl sulfide to distill off during the reaction. Stoichiometric studies established the minimum amount of BMS required for the complete reduction of these functional groups. Thus, esters require 2 equiv of hydride (HB<) for the reduction of >C=O to >CH_2. Employing this stoichiometry, the reduction of aliphatic esters is quite rapid, complete in 0.5 h, while the reduction of aromatic esters is slower, requiring 4–16 h. The corresponding alcohols are produced in excellent yields. On the other hand, nitriles require 3 equiv of hydride (one borane unit/nitrile) and are reduced rapidly in 0.25 h to the corresponding borazine complex, readily hydrolyzed to the corresponding amines. On the other hand, amides require different equivalents of hydride, depending on the particular type of amide undergoing reduction. Thus, tertiary amides require 5 equiv of hydride and form the amine–borane adducts in 0.25 h. Secondary amides liberate hydrogen prior to forming the amine–borane complex, utilizing 6 equiv of hydride in 0.25–1.0 h. However, primary amides require only 4 equiv of hydride, 2 for hydrogen liberation and 2 for reduction, producing in 1.0–2.0 h the amine dibora derivatives, which are sufficiently weakly basic as not to complex with BMS. The ease of reduction of amides follows the order tertiary ≥ secondary > primary. A simple procedure has been described for the reduction of tertiary and secondary amides using decreased amounts of BMS in the presence of boron trifluoride etherate. Unlike lithium aluminum hydride, super hydride, etc., the tendency for C–N bond cleavage to produce the alcohol is completely absent in these reductions of BMS. The reagent permits the presence of many common substituents, such as nitro, chloro, methoxy, etc. The reaction is not significantly susceptible to electronic and steric effects. Simple procedures have been developed for isolating the products. This study establishes a convenient synthetic route for the selective reduction of various organic functional groups with BMS where this transformation is desired in synthetic operations.

Since the discovery of sodium borohydride,[2] it has been utilized as an excellent reagent for the reduction of aldehydes and ketones.[3] However, it is generally too slow for the convenient reduction of carboxylic esters.[3] The reduction of such esters by diborane,[4] borane–tetrahydrofuran,[5] or by borane–dimethyl sulfide[6,7] is also relatively slow. Accordingly, lithium aluminum hydride,[8,9] lithium borohydride,[10,11] or calcium borohydride[12] have been the preferred reagents for such reductions.[13] On the

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