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ANTINEOPLASTIC AGENTS, 178. ISOLATION AND STRUCTURE OF LYCHNOSTATINS 1 AND 2 FROM THE SOUTH AMERICAN *LYCHNOPHORA ANTILLANA*[1]

GEORGE R. PETTIT,* DELBERT L. HERALD, GORDON M. CRAGG, JOHN A. RIDEOUT, and PETER BROWN[2]

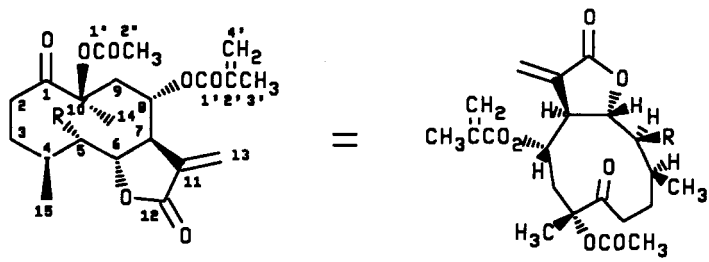
Cancer Research Institute and Department of Chemistry, Arizona State University, Tempe, Arizona 85287

ABSTRACT.—Bioassay-guided (P388 lymphocytic leukemia cell line) separation of a CH_2Cl_2/MeOH extract of *Lychnophora antillana* led to the isolation of two cytostatic (P-388, ED_{50} 2.0 and 0.19 μg/ml, respectively) germacranolides designated lychnostatins 1 [**1**] and 2 [**2**]. Structural elucidation was based initially upon high field (400 MHz) nmr and electron impact mass spectral interpretations and unequivocally completed by X-ray crystal structure determinations.

Although many species of the large plant family Compositae are well-known for a variety of reasons, including primitive medical applications, some occur in a few small and relatively unexplored tropical genera. One such genus, the *Lychnophora* of the subtribe Lychnophorinae (2,3), contain some twenty-three species indigenous primarily to Brazil. More than twenty years ago, as part of the U.S. National Cancer Institute's (NCI) world-wide exploratory programs directed (by Jonathan L. Hartwell) toward the discovery of new anticancer drugs, specimens of *Lychnophora antillana* Urb. (also known as *Piptocoma antillana*) were collected and evaluated. By 1974, an EtOH extract was found to provide 32–34% life extension against the NCI murine P-388 lymphocytic leukemia (PS system) at 4.9↦16 mg/injection. A 1979 Puerto Rican collection gave analogous biological results (including PS cell line ED_{50} 0.72 μg/ml) and led to the present study.

The plant was extracted with CH_2Cl_2-MeOH (1:1), and the extract was partitioned (4) between MeOH-H_2O (9:1↦4:1↦3:2) with hexane↦CCl_4↦CH_2Cl_2 to yield an active CH_2Cl_2-soluble fraction (PS ED_{50} 0.15 μg/ml). Separation (PS bioassay-guided) of this fraction on a Si gel column resulted in isolation of lychnostatins 1 [**1**] and 2 [**2**] as the major PS-active (ED_{50} 2.0 and 0.19 μg/ml) constituents.

Initial structural investigations revealed both cytostatic compounds to be new sesquiterpene lactones of the germacranolide type. While varied biological activity has been reported for a number of such compounds from other genera (5–18), only one example of antineoplastic activity (10) has been reported for germacranolides isolated from the *Lychnophora* (19–24). Several germacranolides distantly related to lychnostatins **1** and **2** have been isolated from Brazilian *Lychnophora* (19), *Eremanthus* (25), and



1 R=OH
2 R=H

[1]For Part 177 see Pettit and Schaufelberger (1).

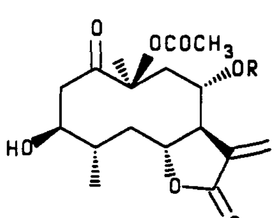
[2]Deceased March 25, 1981.

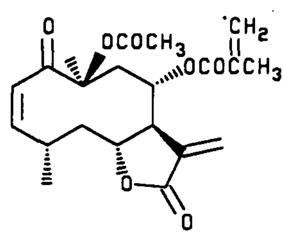
Piptolepis (21,26) species. One of these, isolated from *Lychnophora blanchetii* (19), was assigned structure **3** a structural isomer of lychnostatin 1.

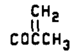
Ir, ^{1}H-, ^{13}C-nmr, and mass spectral analyses suggested the presence of an α-methylene lactone, as well as methacrylate, acetate, and ketone groups. From mass spectral data, it was determined that lychnostatin 1 [**1**] differed from lychnostatin 2 [**2**] only by having an additional oxygen atom. In addition, eims exhibited significant peaks corresponding to $[M - HOAc]^{+}$ and $[M - HOAc - CH_2 = C(CH_3)CO_2H]^{+}$ fragment ions, thereby confirming the presence of the ester groups. The spectral data and molecular formula were also consistent with a ten-membered carbon ring bearing the substituents just noted. Extensive ^{1}H-nmr and ^{13}C-nmr decoupling experiments provided sufficient additional information to allow assignment of the α-methacrylate unit adjacent to the lactone. The nmr data also seemed to suggest that a hydroxyl group in lychnostatin 1 was adjacent to the lactone ring. From empirical formula data, the presence of macrocyclic ring unsaturation seemed to be excluded for both compounds. Because neither the complete regio nor stereo relationships of the macrocyclic ring substituents could be definitively ascertained from the above information alone, a number of structural possibilities remained.

In order to establish unambiguously the complete structures of lychnostatins 1 and 2, single crystal X-ray diffraction analyses were undertaken (Table 1). Cell parameters for both lychnostatins were nearly identical, suggesting that each of the compounds had similar cell packing characteristics and conformations. Indeed, this assumption proved to be correct. An X-ray-analysis-derived structure for lychnostatin 1 is shown in Figure 1. The absence of unsaturation in the 10-membered macrocyclic rings for both lychnostatins was thereby established. Although unusual, this result was not without precedent (19,25,27,28). Also established were the orientation of the macrocyclic ring and the relative stereochemistry of the ring substituents for both compounds. The β disposition of the C-4 and C-7 substituents, as well as the C-10 methyl, was readily apparent for the lychnostatins.

For lychnostatin 1, the additional oxygen atom was found to be present as a β-oriented C-5 hydroxy group. The two ester substituents attached to the C-8 and C-10 ring atoms of both compounds, as well as the C-6 oxygen atom (which forms part of the *trans*-fused α-methylene lactone ring) were all α-oriented with respect to the 10-membered ring. The more stable *trans*-fusion of the lactone ring to the 10-membered ring is a feature commonly observed for a majority of germacranolide sesquiterpene lactones. The α-methylene–γ-lactone rings of both lychnostatins 1 and 2 exhibited some nonplanarity (endocyclic torsion angle moduli sum of 49° and 55°, respectively). Examples





3 R= 

5 2,3-*trans*

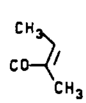
4 R= 

TABLE 1. Crystal Data Experimental and Refinement Parameters for Lychnostatin 1 [**1**] and Lychnostatin 2 [**2**].

Parameters	Compound	
	1	**2**
Crystal data		
Molecular formula	$C_{21}H_{28}O_8$	$C_{21}H_{28}O_7$
F.W.	408.45	392.45
F(000)	872	840
Space group	$P2_12_12_1$	$P2_12_12_1$
Crystal dimensions (mm)	0.07 × 0.10 × 0.32	0.08 × 0.10 × 0.45
Radiation, Å	CuKα, λ = 1.54184	CuKα, λ = 1.54184
Temperature, °C	26 ± 1	26 ± 1
Cell constants		
a, Å	5.876 (2)	5.785 (4)
b, Å	8.865 (1)	8.902 (3)
c, Å	40.057 (8)	40.457 (6)
V, Å^3	2089.3	2083.4
Z	4	4
ρo, g/cm^3	1.289	1.245
ρc, g/cm^3	1.298	1.251
μ, cm^{-1}	7.9	7.4
Collection Parameters		
Instrument	Enraf-Nonius CAD4 diffractometer	Enraf-Nonius CAD4
Monochromator	Graphite crystal, incident beam	Graphite crystal, incident beam
Attenuator	Ni foil, factor 11.9	Ni foil, factor 11.9
Take-off angle, deg	2.8	2.8
Detector aperature, mm	4.0 to 5.9 horizontal, 4.0 vertical	4.0 to 5.9 horizontal, 4.0 vertical
Crystal-detector dist.	21 cm	21 cm
Scan type	ω–2θ	ω–2θ
Scan rate, °/min (in ω)	1 to 5	1 to 5
Scan width, deg	0.9 + 0.140 tan θ	0.8 + 0.140 tan θ
Maximum 2θ, deg	150.0	150.0
No. of refl. measured	2676 total, 2537 unique	2602, 2532 unique
Corrections made	Lorentz-polarization Linear decay, (0.815 to 1.185 on I)	Lorentz-polarization Linear decay, (0.985 to 1.129 on I) Empirical absorption, (0.88 to 0.99 on I)
Solution and Refinement Parameters		
Solution method	Direct Methods	Direct Methods
Hydrogen atoms	Refined, Uiso = 0.06 Å^2, restrained to ride	Refined, Uiso = 0.06 Å^2, restrained to ride
Refinement	Full matrix least-squares	Full matrix least-squares
Minimization function	$\Sigma w(\|Fo\|-\|Fc\|)^2$	$\Sigma w(\|Fo\|-\|Fc\|)^2$
Least-squares weights	$1/\sigma^2(Fo)$	$1/\sigma^2(Fo)$
Anomalous dispersion	All non-hydrogen atoms	All non-hydrogen atoms
Reflections included	2178 with $Fo^2 > 3.0\sigma(Fo^2)$	1297 with $Fo^2 > 3.0\sigma(Fo^2)$
Parameters refined	262	254
Unweighted R factor	0.045	0.049
Weighted R factor	0.044	0.038
EDS of obs. of unit wt.	2.30	2.74
Convergence, Largest shift, Å	0.07	0.06
High peak in final diff. map, e/Å^3	0.20 (4)	0.20 (5)
Computer hardware	PDP-11/23, MicroVax II	—
Computer software	SDP-PLUS (Enraf-Nonius & B.A. Frenz and Assoc., Inc.) CRYSTALS (CCL, Univ. of Oxford)	—

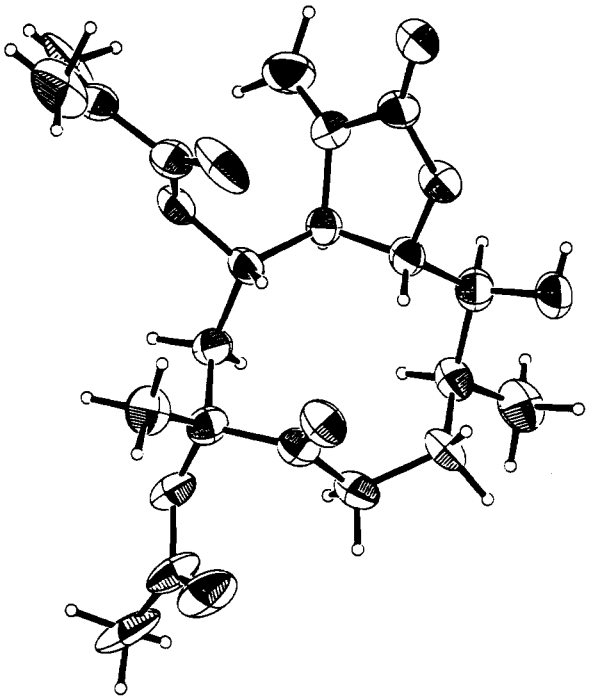


FIGURE 1. Crystal structure (ORTEP representation) of lychnostatin 1.

covering the range of nearly complete planarity to pronounced non-planarity of the *trans*-fused lactone ring have been reported (10, 27, 29–32).

No abnormalities were observed for the bond distances and angles of either compound, the values being in good agreement with those reported for similar substances (27, 29, 30, 33–35). Conformational analysis of the data for lychnostatins 1 and 2, with respect to the 10-membered macrocyclic ring, revealed a conformational deviation from that previously proposed and/or observed for cyclodecane/cyclodecanone rings (36–39). Among these are two boat-boat conformations, referred to as the "O-inside" and the "O-outside" conformations, as depicted in Figure 2. In the "O-inside" conformation, the oxygen is approximately perpendicular to the imagined plane of the cyclodecane ring, whereas in the "O-outside" conformation, the oxygen lies approximately in the plane of the ring. The "O-inside" is conformationally favored over the "O-outside," primarily due to the decreased number of destabilizing intra-annular hydrogen atom interactions present in this conformation (36). The conformation assumed by lychnostatins 1 and 2 is depicted in Figure 3 as a twist chair-boat conformation. Although subtly different from the boat-boat "O-inside" conformer, it still maintains one essential distinguishing feature of that conformer, i.e., positioning of the carbonyl oxygen in a perpendicular orientation to the plane of the 10-membered ring.

With lychnostatins 1 and 2, significant intra-annular hydrogen interactions (interatomic bond distance $<2 \times$ H van der Waals radii or ca. 2.30 Å) occur on both the α and β faces, as signified by arrows in Figure 3. Table 2 summarizes the intra-annular interatomic distances occurring in the lychnostatins. In each case the carbonyl oxygen, O-1, does not seem to participate in any significant intra-annular interactions. All intra-annular atomic distances involving O-1 were found to be 2.40 Å or greater. On the other hand, a greater number of hydrogen-hydrogen intra-annular interactions occur in the conformer adopted by lychnostatins 1 and 2, as compared to the "normal" boat-boat

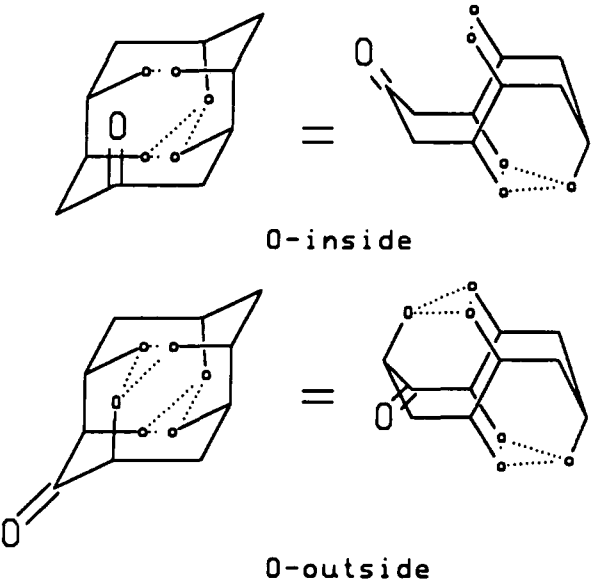


FIGURE 2. Two possible cyclodecanone conformers.

O-inside conformer. Presumably, the *trans*-fusion of the α-methylene–γ-lactone ring to the 6,7 position of the cyclodecanone ring, as well as the α orientation of the ester side chains, provides steric factors contributing to this conformational modification or deviation.

The absolute configuration of lychnostatins 1 and 2 could not be ascertained from the X-ray data, only the relative configuration; nearly identical R values were obtained for both enantiomers. Thus, either the structures depicted by **1** and **2** or their mirror images are equally plausible. A less reliable method (27, 29–31, 40, 41) for affixing absolute stereochemistry about the ring juncture of the α-methylene–γ-lactone and the cyclodecanone ring, based upon cd data, also failed due to interference by the methacrylate moiety with the diagnostic $n\rightarrow\pi^*$ transition curve of the lactone. Finally, utilization of information based solely on the possible biosynthetic pathway previously proposed for the generation of germacranolides must also be excluded, as there are no carbon-carbon double bonds in the 10-membered ring that might indicate its mode of origin. The aforementioned problems concerning absolute configurational assignments and correct classification (12↦6 or 12↦8 lactonization) have been encountered earlier (27,29).

As previously mentioned, Bohlmann *et al.* (19) have investigated *L. blanchetii* collected in northeast Brazil and assigned structures **3**, **4**, and **5** to three of the con-

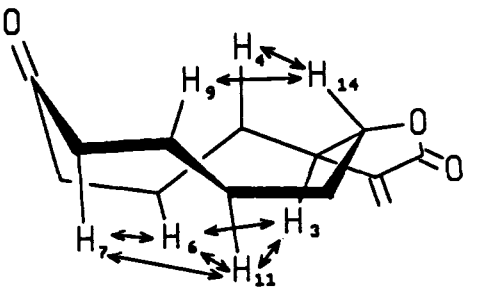


FIGURE 3. Conformation assumed by the lychnostatins.

TABLE 2. Intra-annular Atomic Distances in Lychnostatin 1 [**1**] and Lychnostatin 2 [**2**] (<3.00 Å).

Atoms	Lychnostatin 1		Lychnostatin 2	
	α-face	β-face	α-face	β-face
H-3–H-6	2.09 Å		2.10 Å	
H-3–H-11	2.36 Å		2.43 Å	
H-3–H-7	>3.00 Å		>3.00 Å	
H-4–H-9		>3.00 Å		>3.00 Å
H-4–H-14		2.12 Å		2.13 Å
H-4–O-1		2.54 Å		2.58 Å
H-6–H-7	2.31 Å		2.26 Å	
H-6–H-11	2.30 Å		2.29 Å	
H-7–H-11	2.19 Å		2.20 Å	
H-9–H-14		2.07 Å		2.07 Å
H-9–O-1		2.50 Å		2.49 Å
H-14–O-1		2.40 Å		2.40 Å

stituents. In addition to these germacranolides, they also identified the two pentacyclic triterpenes, lupeol and lupenone. In the present study, we found betulinic and ursolic acids as representatives of the latter group. More importantly, germacranolide **3** appears to be a structural isomer of lychnostatin 1. From biosynthetic considerations it seems likely that structure **3** may need further refinement.

Lychnostatins 1 [**1**] and 2 [**2**] now augment the small number of germacranolides known to exhibit cell growth inhibitory and/or antineoplastic activity (6, 10, 12, 13, 15–18). The lychnostatins are also unusual in that they don't completely satisfy the postulate proposed by Manchand and Blount (17) that antitumor activity requires, in addition to the α-methylene–γ-lactone, an oxygen function or double bond at C-4. Further experiments directed at biological evaluation and unambiguously defining absolute configuration of the lychnostatins are under way.

EXPERIMENTAL

Solvents used for chromatography were redistilled. Ambient cc procedures employed Si gel (70–230 mesh), supplied by E. Merck, Darmstadt. Cc under pressure was carried out using prepacked Lobar Lichro Prep Si gel 60 (40–63 um). Fraction collection was partially automated, using a Gilson microfractionator. Tlc was performed with Si gel GHLF from Analtech, Inc. The tlc plates were developed by uv light and/or a ceric sulfate spray reagent.

All melting points are uncorrected and were observed using a Koeffler-type melting point apparatus. Each substance was colorless. Ir spectra were recorded with a Perkin-Elmer Model 299 spectrophotometer. Optical rotations were determined with a Perkin-Elmer model 241 Automatic Polarimeter. The 100 MHz ^{1}H-nmr spectra were recorded with a Varian XL-100 instrument and the 400 MHz with a Bruker WH-400 nmr spectrophotometer. The ^{13}C spectra were obtained employing a Bruker WH-90 at 22.63 MHz. TMS was used as an internal reference, and δ values are reported in ppm. Mass spectra were obtained using an MAT 312 spectrophotometer.

PLANT MATERIAL.—*L. antillana* (stems and leaves, herbarium specimens NSC B 507 14 maintained by the USDA) was recollected in Puerto Rico in 1979, under auspices of the Economic Botany Laboratory, Agricultural Research Center East, USDA, Beltsville, Maryland, as part of a joint NCI-USDA program directed by Drs. M.I. Suffness and J.A. Duke.

EXTRACTION AND SOLVENT PARTITIONING.—The stems and leaves of *L. antillana* (54 kg) were extracted with CH_2Cl_2-MeOH (1:1) (320 liters) at ambient temperature for 4 days. Decantation of solvent and subsequent dilution with H_2O (25% by volume) allowed the chlorocarbon phase to separate. The CH_2Cl_2 was removed to yield a viscous, brown gum (1145 g) which was further purified by partitioning (4) employing the sequence MeOH-H_2O (9:1↦4:1↦3:2) against, respectively, hexane↦CCl_4↦CH_2Cl_2. An aliquot (40 g) of the CH_2Cl_2 fraction (total weight, 315 g) was chromatographed on a column of Si gel (800 g). Elution with hexane-Me_2CO (9:1) (6.0 liters to 10.0 liters) yielded fraction A (0.18 g) as a color-

less solid. Further elution (13.0 to 18.0 liters) resulted in isolation of lychnostatin 1 [**1**] as a colorless solid (0.31 g, 5.7×10^{-5}% yield).

Rechromatography of fraction A (see above) on a column of Si gel (18 g, dry packed column) and elution with CH_2Cl_2 (200 ml) afforded a single product, lychnostatin 2 [**2**] (20 mg, 3.7×10^{-6}% yield). Further elution with CH_2Cl_2-MeOH (44:1) gave a mixture (0.10 g) which was rechromatographed on Si gel (Lobar B column). Gradient elution with CH_2Cl_2-MeOH (99:1→44:1) (400 ml total) led to a pure compound (34 mg) that recrystallized from MeOH/CH_2Cl_2 to give betulinic acid (20 mg), mp 304–307°. Further elution with the same solvents yielded another minor product (22 mg), which proved to be ursolic acid, mp 260–265°. Both triterpene carboxylic acids were identified by comparison (tlc, ir, ^{1}H nmr) with authentic specimens.

LYCHNOSTATIN 1 [**1**].—Recrystallization from Me_2CO/hexane afforded crystals melting at 228–230°: tlc R_f 0.85 in $CHCl_3$-MeOH (9:1); $[\alpha]^{24}D$ +89° (c = 1.0, $CHCl_3$); ir (KBr) ν max 3580, 3430, 1780, 1732, 1710, 1702 (sh), 1660, 1633, 1460, 1380, 1308, 1276, 1267, 1253, 1154, 1120, 1095, 1073, 1060, 1020, 993, 960, 816, 805, 763, 700, 600 cm^{-1}; ^{1}H nmr (400 MHz, $CDCl_3$) δ 1.08 (d, 3H, J = 8 Hz, Me-15), 1.62 (1H, br s, -OH), 1.74 (3H, s, Me-14), 1.94 (3H, s, Me-3′), 1.70–2.10 (3H, m, $-CH_2$-), 2.03 (3H, s, Me-2″), 2.32 (1H, dd, J = 16, 2 Hz, $-CH_2$), 2.66–2.74 (3H, m, -CH-), 3.07 (1H, d, J = 10 Hz, H-7), 3.41 (1H, dd, J = 8, 7 Hz, H-5), 4.35 (1H, d, J = 8 Hz, H-6), 4.84 (1H, m, H-8), 5.65 (2H, br s, H-13a or H-13b, H-4′a or H-4′b), 6.16 (1H, br s, H-4′a or H-4′b), 6.24 (1H, br s, H-13a or H-13b); ^{13}C nmr (22.63 MHz, $CDCl_3$), 207.61 (s, C-1), 169.56 (s, C-12), 168.45 (s, C-1″), 165.82 (s, C-1′), 135.93 (s, C-11 or C-2′), 134.92 (s, C-11 or C-2′), 126.31 [t (2C), C-13 and C-4′], 84.91 (s, C-10), 82.44 (d, C-6), 77.50 (d, C-5), 70.77 (d, C-8), 44.29 (d, C-7), 41.07 (t, C-2 or C-9), 35.32 (t, C-2 or -9), 32.07 (d, C-4), 24.05 (t, C-3), 22.42 (q, C-14), 21.38 (q, C-2″), 20.24 (q, C-3′), 18.23 (q, C-15); eims m/z $[M]^+$ 408, $[M - HOAc]^+$ 348, $[M - H_2O - C_4H_5O_2]^+$ 305, $[M - HOAc - C_4H_6O_2]^+$ 262, $[M - H_2O - HOAc - C_4H_5O_2]^+$ 245. *Anal.* calcd for $C_{21}H_{28}O_8$, C 61.75, H 6.91; found C 61.66, H 6.67%.

LYCHNOSTATIN 2 [**2**].—Recrystallization of lychnostatin 2 [**2**] from Me_2CO/hexane provided fine needles: mp 190–193°; $[\alpha]^{30}D$ +20.9° (c = 0.67, $CHCl_3$); ir (KBr) ν max 2950, 1780, 1740, 1712, 1645, 1460, 1385, 1312, 1300, 1275, 1178, 1156, 1126, 1105, 1065, 1022, 955, 878, 810, 741, 614 cm^{-1}; ^{1}H nmr (100 MHz, $CDCl_3$) δ 1.04 (3H, d, J = 6 Hz, Me-15), 1.80 (3H, s, Me-14), 1.96 (3H, s, Me-3′), 1.4–2.2 (4H, m, $-CH_2$-), 2.06 (3H, s, Me-2″), 2.23 (1H, dd, J = 15, 2 Hz, $-CH_2$-), 2.70 (3H, m, -CH-), 3.05 (2H, m), 4.37 (1H, m, H-6), 4.96 (1H, dd, J = 8, 2 Hz, H-5), 5.73 (2H, d, J = 2 Hz, H-13a or H-13b, H-4′a or H-4′b), 6.18 (1H, br s, H-4′a or H-4′b), 6.35 (1H, br s, H-13a or H-13b); ^{13}C-nmr (22.63 MHz, $CDCl_3$) δ 208.29 (s, C-1), 169.62 (s, C-12), 168.97 (s, C-1″), 165.92 (s, C-1′), 135.86 (s, C-11 or C-2′), 134.69 (s, C-11 or C-2′), 126.53 (t, C-13 or C-4′), 124.88 (t, C-13 or C-4′), 84.26 (s, C-10), 77.86 (d, C-6), 68.24 (d, C-8), 46.99 (d, C-7), 43.44 (t, C-5), 30.09 (t, C-2 or C-9), 35.91 (t, C-2 or C-9), 29.70 (d, C-4), 27.13 (t, C-3), 23.98 (q, C-14), 21.51 (q, C-2″), 21.25 (q, C-3′), 18.20 (q, C-15); spsims m/z $[M+Na]^+$ 415; eims m/z $[M]^+$ 392, $[M-CH_2CO]^+$350, $[M - HOAc]^+$ 332, $[M - C_5H_5O_4]^+$ 263, $[M - HOAc - C_4H_6O_2]^+$ 246, hrfabms m/z $[M + {}^7Li]^+$ 399.1996 (calcd $C_{21}H_{28}O_7 + {}^7Li$, 399.19952).

X-RAY CRYSTAL STRUCTURE DETERMINATIONS OF LYCHNOSTATIN 1 [**1**] AND LYCHNOSTATIN 2 [**2**].—Preliminary examinations and data collections for lychnostatins 1 and 2 were performed at room temperature by the moving-crystal, moving-counter technique with background measurements made on both sides of the peak using an Enraf-Nonius CAD-4 automatic diffractometer. Crystal data, collection, and refinement parameters for the two compounds are summarized in Table 1. In each case, data was corrected for Lorentz and polarization effects. For lychnostatin 2, an additional semi-empirical absorption correction was also applied [the absorption correction being based on a series of psi scans (42)]. Space group assignments for each compound were derived on the basis of Laue symmetry and observed systematic extinctions. Cell dimensions were determined from least-squares refinement, using the setting angles of 25 carefully measured reflections. The structures were solved by direct methods (43). Scattering factors were taken from Cromer and Waber (44). Initial stages of refinement were performed using the SDP-PLUS (45) software package; final refinements were done with CRYSTALS (46). Anomalous dispersion corrections were made in Fc (47) for both compounds; the values of $\Delta F'$ and $\Delta F''$ were those of Cromer (48); extinction coefficients were refined on both compounds.[3] A perspective view (49) displaying all essential conformational and configurational features for lychnostatins 1 and 2 appears in Figure 1.

Colorless crystals of lychnostatin 1, arising from MeOH-H_2O solution, were used in mass spectral,

[3]Atomic coordinates for these structures have been deposited with the Cambridge Crystallographic Data Centre and can be obtained on request from Dr. Olga Kennard, University Chemical Laboratory, Lensfield Road, Cambridge CB2 1 EW, UK.

density and X-ray data collections. The observed density and mass spectral data indicated a single molecule of lychnostatin 1 per asymmetric unit in space group $P2_12_12_1$. All unique reflections (one octant) were collected. Structural solution proceeded without incident, the nonhydrogen atoms being located readily. The remaining hydrogen atom coordinates were calculated at ideal positions and assigned fixed coordinates and isothermal parameters during subsequent structure-factor full-matrix least-squares refinements. Here the function minimized for least-squares was $\Sigma w(|F| - |Fc|)^2$ with the weight w defined as $1/\sigma^2(FO)$. Refinement was continued until convergence to a residual of $R = 0.045$ and $R_w = 0.044$.

The crystal structure of lychnostatin 2 was performed on a fine needle-shaped crystal obtained from Me_2CO/heptane solution. Observed density measurements again indicated one molecule per asymmetric unit corresponding to the $P2_12_12_1$ space group. Solution by direct methods proceeded with some difficulty. After a number of unsuccessful preliminary attempts, a starting set of seven reflections was used (from 400 reflections) with the largest E's (minimum E of 1.29) in order to generate 12,659 relationships. In addition, the lower limit of probability of acceptance of phases determined by the sigma 1 formula being included in the starting set was extremely low (i.e., 0.650). In this manner, a total of 200 possible phase sets were generated; the phase set with the highest overall figure of merit (2.99) provided an E map which revealed all 28 nonhydrogen atoms. Hydrogen atom coordinates again were calculated, fixed, and assigned isotropic thermal parameters in subsequent least-squares refinements. Anisotropic refinement was done on all nonhydrogen atoms by full matrix least-squares methods. Refinement converged to a residual of $R = 0.049$ and $R_w = 0.038$.

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