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Novel Cytotoxic Labdane Diterpenoids from *Neouvaria acuminatissima*

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Abstract: Acuminolide (**1**), a cytotoxic labdane diterpene with a new type of carbon skeleton, was isolated from the stem bark of *Neouvaria acuminatissima*, together with its congeners 17-*O*-acetylacuminolide (**2**) and an inactive derivative, spiroacuminolide (**3**). Their structures were determined on the basis of spectroscopic and chemical methods, as well as X-ray crystallography. Isolation, structure elucidation and bioassay results are described.

INTRODUCTION

Neouvaria acuminatissima (Miq.) Airy Shaw (Annonaceae) [syn.: *Uvaria acuminatissima* Miq.; *Mitrephora ferruginea* Merr. (non Boerl.); *Mitrephora merrillii* C.B. Rob.; *Mitrephora viridifolia* Elm.; *Griffithianthus merrillii* (C.B. Rob.) W.H. Brown ex Merr.] is a 10-20 m tree found in the tropical rain forests of Malaysia, the Philippines and Indonesia (Sumatra, Kalimantan).[1] As part of an ongoing drug discovery program for novel anticancer agents of plant origin, the stem bark of *N. acuminatissima* collected in the Philippines was chosen for phytochemical investigation when its EtOAc extract showed cytotoxicity for a panel of human cancer cell lines. A literature survey has shown that this plant species has not been used for any medicinal purposes. In the present communication, we report the isolation, characterization, and *in vivo* and *in vitro* bioassay evaluation of two cytotoxic labdane diterpenoids based on a novel carbon skeleton containing both an 8α,12-epoxide linkage and a butenolide moiety, namely, acuminolide [(12*S*)-8α,12-epoxy-16(*R*),17-dihydroxylabd-13(14)*Z*-en-15,16-olide] (**1**) and 17-*O*-acetylacuminolide [(12*S*)-17-acetoxy-8α,12-epoxy-16(*R*)-hydroxylabd-13(14)*Z*-en-15,16-olide] (**2**). The structure of **2** was confirmed by single-crystal

X-ray crystallography. These compounds were accompanied by a structurally related inactive analog, namely, spiroacuminolide [(12*S*)-8α,12:13,17-diepoxy-16(*R*)-hydroxylabdan-15,16-olide] (**3**), which contains a β-substituted γ-hydroxybutanolide moiety.

RESULTS AND DISCUSSION

The ethyl acetate-soluble fraction of a methanol extract of the stem bark of *N. acuminatissima* was chromatographed over silica gel, with cytotoxicity monitored using a human lung cancer cell line (Lu1), leading to the isolation of two cytotoxic diterpenes, **1** and **2**, along with a structurally related analog, **3**. Initial structural assignments of these compounds were made on the basis of ^{1}H and ^{13}C NMR spectral analysis. A series of 2D-NMR experiments was employed to provide unambiguous assignments of all the proton and carbon resonance signals, inclusive of ^{1}H-^{1}H COSY and ^{1}H-^{13}C HETCOR NMR experiments. Structural assignments have been further confirmed by selective INEPT and COLOC NMR experiments, where soft irradiation of proton resonance peaks gave signal enhancements to the long-range coupled carbon atoms. By direct comparison of the ^{1}H and ^{13}C NMR spectral data of these compounds with reported data for labdane-type[2-4] and clerodane-type[5] diterpenes bearing a β-substituted butenolide moiety as part of the structure, and further, through the comparison with labdanoids possessing an 8α,12-epoxide linkage[6-12], it was apparent that compounds **1** and **2** belonged structurally to the labdane class, having both γ-hydroxybutenolide and 8α,12-epoxide linkages.

Acuminolide (**1**), mp. 207-208°, was assigned the molecular formula $C_{20}H_{30}O_5$ from high-resolution FABMS, and showed three tertiary methyl groups in ^{1}H NMR spectrum. The presence of a β-substituted butenolide moiety was clearly indicated by NMR [δ_H 6.26 (1H, br s); δ_C 169.0 (s), 117.2 (d) and 170.9 (s)] and IR spectral data [ν_{max} 1760 (C=O), 1655 (C=C) cm^{-1}]. Hydroxyl groups in **1** were also inferred from strong absorption bands in the IR spectrum (ν_{max} 3415, 3280 cm^{-1}) as well as by a D_2O-exchange ^{1}H NMR experiment. An 8α,12-epoxide functionality was deduced from the chemical shift values of the NMR resonance signals attributed to the oxygen-bearing carbon atoms at δ_C 84.7 (s) and 74.2 (d). Selective INEPT NMR experiments further supported structural assignments of **1**, where the irradiation of H-5 enhanced the resonance signal at C-6, and irradiation at H-6, H-7, H-9, H-11, CH_3-19, and CH_3-20 enhanced the peaks at C-8/C-10, C-8, C-11, C-9, C-3, and C-9/C-10, respectively. Thus, the structure of acuminolide (**1**) was determined as (12*S*)-8α,12-epoxy-16(*R*),17-dihydroxylabd-13(14)*Z*-en-15,16-olide.

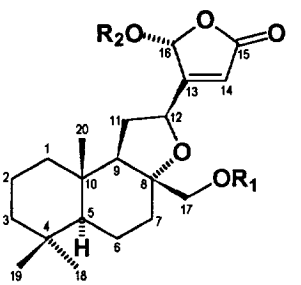
17-*O*-Acetylacuminolide (**2**), mp. 210-211°, having the molecular formula $C_{22}H_{32}O_6$ as determined by high-resolution FABMS, showed ^{1}H and ^{13}C NMR data closely resembling those of **1** except that it had an acetate functionality, as evidenced by the resonance signals at δ_H 2.10 (3H, s, OCO<u>Me</u>) and at δ_C 170.6 (s) (O<u>C</u>OMe) and 21.0 (q) (OCO<u>Me</u>). The position of acetylation was deduced from the changes in the NMR chemical shift values, i.e., two doublet protons of C-17 at δ_H 3.32 and 3.66 in **1** were shifted downfield to δ_H

3.68 and 4.49 in **2**. X-Ray analysis was performed to complete the determination of the stereochemistry of the hydroxyl group at C-16. Thus, single-crystal X-ray diffraction analysis of **2** established an α configuration for the hydroxyl group at C-16, and an *S*-configuration for the chiral carbon C-12 was also confirmed. Hence, the structure of 17-*O*-acetylacuminolide (**2**) was established as (12*S*)-17-acetoxy-8α,12-epoxy-16(*R*)-hydroxylabd-13(14)*Z*-en-15,16-olide. To further establish the structural relationship between **1** and **2**, both compounds were converted on acetylation to afford the same diacetate, **4**. Base-catalyzed hydrolysis of **2** also supported this result by producing **1**.

Table 1. ^{13}C NMR Assignments of 1-4.[a]

C #	1	2	3	4
1	39.7 (2)	39.8 (2)	40.1 (2)	39.9 (2)
2	18.4 (2)	18.4 (2)	18.7 (2)	18.3 (2)
3	42.2 (2)	42.2 (2)	42.8 (2)	42.2 (2)
4	33.1 (0)	33.1 (0)	33.5 (0)	33.1 (0)
5	57.1 (1)	57.2 (1)	57.9 (1)	57.2 (1)
6	20.3 (2)	20.4 (2)	20.8 (2)	20.4 (2)
7	34.2 (2)	34.9 (2)	35.0 (2)	35.0 (2)
8	84.7 (0)	82.2 (0)	83.0 (0)	82.4 (0)
9	61.0 (1)	61.7 (1)	60.1 (1)	61.8 (1)
10	36.4 (0)	36.3 (0)	37.1 (0)	36.4 (0)
11	29.1 (2)	28.8 (2)	25.3 (2)	29.1 (2)
12	74.2 (1)	74.7 (1)	76.4 (1)	74.1 (1)
13	169.0 (0)	170.8 (0)	80.0 (0)	168.7 (0)[b]
14	117.2 (1)	116.2 (1)	37.1 (2)	117.0 (1)
15	170.9 (0)	171.3 (0)	176.8 (0)	171.1 (0)
16	98.4 (1)	98.3 (1)	99.9 (1)	92.3 (1)
17	62.3 (2)	65.2 (2)	70.3 (2)	65.3 (2)
18	33.4 (3)	33.4 (3)	33.6 (3)	33.4 (3)
19	21.0 (3)	21.0 (3)	21.3 (3)	20.9 (3)
20	15.5 (3)	15.8 (3)	14.3 (3)	15.8 (3)
OAc	--	170.6 (0)	--	169.4 (0)[b]
				169.0 (0)[b]
	--	21.0 (3)	--	21.1 (3)[c]
				20.8 (3)[c]

[a]Chemical shifts in ppm downfield from TMS. Solvent, $CDCl_3$ for **2** and **4**, and $CDCl_3$+CD_3OD (9:1) for **1** and **3**. Number in parenthesis indicates the number of protons attached to the carbon as determined by APT experiments. [b,c]Assignments interchangeable within the same superscript.



1 $R_1 = H$, $R_2 = H$
2 $R_1 = Ac$, $R_2 = H$
4 $R_1 = Ac$, $R_2 = Ac$

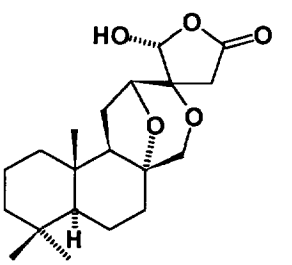


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Figure 1. Chemical structures of Compounds 1-4 (Ac = CH_3CO-).

Spiroacuminolide (**3**), mp. 222-223°, exhibited the molecular formula $C_{20}H_{30}O_5$ by high-resolution FABMS, which is identical with that of **1**, suggesting a structural similarity. The absence of a double bond in the lactone ring was suggested from the IR (absence of C=C absorption maximum at 1650-1655 cm^{-1}) and ^{13}C NMR [downfield shift of C-15 to δ_C 176.8 (s); upfield shifts of C-13 and C-14 to δ_C 80.0 (s) and 37.1 (t),

respectively, due to the ring saturation] data. In ^{1}H NMR spectrum, two new doublets at δ_H 2.21 and 2.66, assigned to protons attached to C-14 [δ_C 37.1 (t)] as confirmed by a ^{1}H-^{13}C HETCOR experiment, were attributed to isolated geminal coupling (J = 17.3 Hz), indicative of the quaternary nature of C-13. An epoxide linkage was inferred as being formed between C-13 and C-17, affecting the downfield shift (8 ppm) of C-17. The multiplicity and coupling pattern (br d, J = 7.4 Hz) of the H-12 ^{1}H NMR resonance, resulting from the spin-spin interaction of H-12 with H-11α (J = 7.4 Hz) and H-11β (J < 0.5 Hz), was attributed to changes in dihedral angle resulting from the formation of a new dioxane ring, a notion also supported by molecular models. The structure of spiroacuminolide (**3**), a putative biogenetic congener of **1** and **2**, thus was assigned as (12*S*)-8α,12:13,17-diepoxy-16(*R*)-hydroxylabdan-15,16-olide.

Compounds **1-3** were evaluated against a panel of human cancer cell lines and cultured P388 cells. Compounds **1** and **2** were broadly cytotoxic, exhibiting ED_{50} values, ranging from 10^{-1} to 10^0 μg/ml in several cell lines. With the human cell lines, the most potent activity was observed with melanoma (Mel2) (ED_{50}: 0.7 μg/ml) and prostate (LNCaP) (ED_{50}: 0.8 μg/ml) cells for compounds **1** and **2**, respectively. Compound **3** was not significantly active for any of the cancer cell lines tested. Acuminolide (**1**) was inactive when tested *in vivo* against a HT-29 human colorectal xenograft model in nude mice at 40-60 mg/kg (maximum tolerated dose 70 mg/kg). 17-*O*-Acetylacuminolide (**2**) showed no significant activity when tested *in vivo* against a KB human epidermoid carcinoma murine model at 110 mg/kg.

EXPERIMENTAL

General procedures: Mps: uncorr.; UV: EtOH; IR: film; ^{1}H and ^{13}C NMR spectra were recorded on 300 or 360 MHz instruments with TMS as int. standard; Low- and high resolution data were obtained on a Finnigan MAT-90 instrument.

Plant material: An initial sample of the stem bark of *Neouvaria acuminatissima* (Miq.) Airy Shaw (Annonaceae) was collected in a tropical rain forest on the lower slopes of Mt. Natib near Morong, Bataan, Philippines, in February, 1991. A larger recollection of the stem bark sample was made in January, 1993, in a limestone forest at Loquilokon, Philippines. Voucher specimens (Soejarto and Madulid 7541; Soejarto *et al.* 7906) have been deposited at the National Herbarium of the Philippines (PNH) in Manila, Philippines, and at the John G. Searle Herbarium, Field Museum of Natural History, Chicago, Illinois, U.S.A.

Extraction and isolation: The dried, powdered stem bark of *N. acuminatissima* (542 g) was extracted with MeOH (2 x 1.8 lit.) at room temperature. After filtration and evaporation of the solvent, the dried MeOH extract was defatted with *n*-hexane (2 x 300 ml), and then partitioned between EtOAc and H_2O, with the EtOAc extract evaporated to dryness to yield a brown powder (3.2 g). The EtOAc extract exhibited significant cytotoxic activity with a human lung cancer cell line (Lu1; ED_{50} 9.0 μg/ml). This residue was subjected to CC over Si gel (250 g), using $CHCl_3$-MeOH gradient solvent mixtures as eluents to give 24 bulked fractions. Cytotoxic fraction 4 (0.91 g), eluted with $CHCl_3$-MeOH (99:1), showed two major spots on tlc, and gave a major active compound, **2** (86 mg, 0.016% w/w) following purification using active charcoal and repeated recrystallization in MeOH. The mother liquor obtained from above was evaporated and chromatographed over Si gel using *n*-hexane-EtOAc-MeOH (75:25:0.4) as eluent to yield an inactive compound, **3** (21 mg, 0.004% w/w). Cytotoxic fraction 7 (0.71 g) was further purified over Si gel by sequentially eluting with *n*-hexane-EtOAc (3:2); *n*-hexane-EtOAc (1:1); *n*-hexane-EtOAc (1:4) to afford, following MeOH crystallization, a minor active compound, **1** (72 mg, 0.013% w/w).

Acuminolide [(12S)-8α,12-epoxy-16(R),17-dihydroxylabd-13(14)Z-en-15,16-olide] (1). Colorless needles, mp. 207-208°; $[\alpha]_D^{20}$ +36.2° (*c* 1.34, $CHCl_3$); UV λ_{max}^{EtOH} nm (log ε): 212.5 (5.87); IR ν_{max} (film) cm^{-1}: 3415 (OH), 3280 (OH), 2955, 2920, 2870, 1760 (C=O), 1655 (C=C), 1465, 1130, 1050, 945; ^1H NMR [300 MHz, $CDCl_3$+CD_3OD (9:1)]: δ 0.80 (3H, s, Me-20), 0.83 (3H, s, Me-19), 0.88 (3H, s, Me-18), 1.01 (1H, dd, *J* = 12.3, 2.4 Hz, H-5), 1.11 (1H, ddd, *J* = 13.0, 13.0, 3.4 Hz, H-1α), 1.19 (1H, ddd, *J* = 13.3, 13.3, 3.6 Hz, H-3α), 1.26 (1H, m, H-7α), 1.29 (1H, m, H-6β), 1.43 (1H, m, H-3β), 1.47 (1H, m, H-2α), 1.51 (1H, m, H-1β), 1.67 (1H, ddddd, *J* = 13.5, 13.5, 13.5, 2.4, 2.4 Hz, H-2β), 1.80 (1H, m, H-9), 1.82 (1H, m, H-6α), 1.89 (1H, m, H-11α), 2.22 (1H, m, H-11β), 2.39 (1H, br d, *J* = 9.2 Hz, H-7β), 3.32 (1H, d, *J* = 11.0 Hz, H-17), 3.66 (1H, d, *J* = 11.0 Hz, H-17), 4.93 (1H, dd, *J* = 7.6, 7.6 Hz, H-12), 6.02 (1H, s, H-14), 6.26 (1H, br s, H-16); ^{13}C NMR (75.6 MHz, $CDCl_3$+CD_3OD (9:1)]: see Table 1; CIMS *m/z* (rel. int.): 351 (M^+ +1; 3.7), 333 (100), 319 (35), 315 (88), 297 (51), 287 (27), 269 (8), 219 (11), 189 (14), 137 (13); HR-FABMS: calcd for $C_{20}H_{30}O_5$ 350.2093, found 350.2093. Acetylation of **1**: Compound **1** (5 mg) in pyridine (1 ml) was treated with Ac_2O (1 ml) and left at room temp. for 30 min. to afford the diacetate **4**, Colorless needles, mp. 185-187°; IR ν_{max} (film) cm^{-1}: 2950, 2870, 2850, 1800 (C=O), 1770 (C=O), 1740 (C=O), 1655 (C=C), 1460, 1370, 1240, 1210, 1150, 1030, 945, 875, 760; ^1H NMR (300 MHz, $CDCl_3$): δ 0.84 (3H, s, Me-19), 0.87 (3H, s, Me-20), 0.89 (3H, s, Me-18), 1.02 (1H, m, H-5), 2.11 (3H, s, OCOMe), 2.17 (3H, s, OCOMe), 3.62 (1H, d, *J* = 11.9 Hz, H-17), 4.54 (1H, d, *J* = 11.9 Hz, H-17), 4.83 (1H, dd, *J* = 7.5, 7.5 Hz, H-12), 6.11 (1H, s, H-14), 6.21 (1H, s, H-16); ^{13}C NMR (75.6 MHz, $CDCl_3$): see Table 1; CIMS: 435 (M^+ +1).

17-O-Acetylacuminolide [(12S)-17-acetoxy-8α,12-epoxy-16(R)-hydroxylabd-13(14)Z-en-15,16-olide] (2). Colorless needles, mp. 210-211°; $[\alpha]_D^{20}$ +62.5°(*c* 1.79, $CHCl_3$); UV λ_{max}^{EtOH} nm (log ε): 208.5 (5.91); IR ν_{max} (film) cm^{-1}: 3370 (OH), 2965, 2930, 2870, 1745 (C=O), 1650 (C=C), 1460, 1390, 1225, 1155, 1125, 950; ^1H NMR (300 MHz, $CDCl_3$): δ 0.83 (3H, s, Me-19), 0.86 (3H, s, Me-20), 0.88 (3H, s, Me-18), 1.04 (1H, br d, *J* = 12.4 Hz, H-5), 1.10 (1H, ddd, *J* = 12.0, 12.0, 3.3 Hz, H-1α), 1.20 (1H, ddd, *J* = 14.1, 14.1, 3.8 Hz, H-3α), 1.35 (1H, m, H-6β), 1.39 (1H, m, H-7α), 1.44 (1H, m, H-3β), 1.48 (1H, m, H-2α), 1.51 (1H, m, H-1β), 1.68 (1H, ddddd, *J* = 13.9, 13.9, 13.9, 2.0, 2.0 Hz, H-2β), 1.82 (1H, m, H-6α), 1.85 (1H, m, H-9), 1.86 (1H, m, H-11α), 2.10 (3H, s, OCOMe), 2.21 (1H, m, H-7β), 2.23 (1H, m, H-11β), 3.68 (1H, d, *J* = 11.7 Hz, H-17), 4.49 (1H, d, *J* = 11.7 Hz, H-17), 4.94 (1H, dd, *J* = 7.5, 7.5 Hz, H-12), 6.04 (1H, s, H-14), 6.12 (1H, br s, H-16); ^{13}C NMR (75.6 MHz, $CDCl_3$): see Table 1; CIMS *m/z* (rel. int.): 393 (M^+ +1; 17), 375 (10), 357 (5), 333 (51), 319 (100), 315 (46), 297 (30), 269 (7), 219 (3), 189 (14), 137 (6); HR-FABMS: calcd for $C_{22}H_{32}O_6$ 392.2212, found 392.2199. Base-catalyzed hydrolysis of **2**: Compound **2** (5 mg) in MeOH (1 ml) was treated with 0.1 M KOH (1 ml) and left at room temp. for 2 hr. to give the hydrolysis product identical with **1** based on FABMS (*m/z* 349, M^+ -1; negative ion mode) and co-tlc. Acetylation of **2**: Compound **2** (5 mg) in pyridine (1 ml) was treated with Ac_2O (1 ml) and left at room temp. for 30 min. to afford the same diacetate **4**, as obtained from **1**.

Spiroacuminolide [(12S)-8α,12:13,17-diepoxy-16(R)-hydroxylabdan-15,16-olide] (3). Colorless needles, mp. 222-223°; $[\alpha]_D^{20}$ +6.8°(*c* 1.03, $CHCl_3$); UV λ_{max}^{EtOH} nm (log ε): 207.5 (6.15); IR ν_{max} (film) cm^{-1}: 3440 (OH), 3005, 2955, 2870, 1785 (C=O), 1450, 1125, 1080, 925; ^1H NMR [(300 MHz, $CDCl_3$+CD_3OD (9:1)]: δ 0.86 (3H, s, Me-19), 0.89 (3H, s, Me-18), 1.02 (1H, m, H-5), 1.05 (3H, s, Me-20), 1.14 (1H, ddd, *J* = 12.3, 12.3, 3.2 Hz, H-1α), 1.22 (1H, ddd, *J* = 13.8, 13.8, 3.2 Hz, H-3α), 1.24 (1H, dddd, *J* = 13.6, 13.6, 13.6, 3.4 Hz, H-6β), 1.41 (1H, m, H-7α), 1.44 (1H, m, H-3β), 1.50 (1H, m, H-2α), 1.55 (1H, m, H-1β), 1.69 (1H, m, H-9), 1.74 (1H, m, H-2β), 1.75 (1H, m, H-11α), 1.82 (1H, m, H-6α), 1.83 (1H, m, H-7β), 2.03 (1H, m, H-11β), 2.21 (1H, d, *J* = 17.3 Hz, H-14), 2.66 (1H, d, *J* = 17.3 Hz, H-14), 3.76 (1H, d, *J* = 12.6 Hz, H-17), 4.05 (1H, d, *J* = 12.6 Hz, H-17), 4.65 (1H, br d, *J* = 7.4 Hz, H-12), 5.90 (1H, s, H-16); ^{13}C NMR (75.6 MHz, $CDCl_3$+CD_3OD (9:1)]: see Table 1; CIMS *m/z* (rel. int.): 351 (M^+ +1; 48), 333 (100), 315 (63), 302 (64), 287 (19), 233 (37), 215 (10), 189 (47), 137 (5); HR-FABMS: calcd for $C_{20}H_{30}O_5$ 350.2093, found 350.2084.

X-ray experimental data and structure analysis of 2: *Crystal Data.* $C_{22}H_{32}O_6$, M = 392.49, Monoclinic, $P2_1$, a = 6.519(3), b = 9.582(2), c = 16.809(5) Å, β = 92.33(3)°, V = 1049(1) Å^3, (by least-squares refinement on diffractometer angles for 12 automatically centered reflections), λ = 1.54178 Å, Z = 2, D_c = 1.24g cm^{-3},

Table 2. Atomic coordinates (x10^4) and equivalent isotropic displacement coefficients (Å^{2}x10^3) [Equivalent isotropic U defined as one third of the trace of the orthogonalized Uij tensor].

Atom	x	y	z	U (eq)
O (23)	-581 (5)	-270	-3713 (2)	44 (1)
C (10)	-600 (7)	2487 (7)	-2120 (3)	39 (2)
C (8)	-128 (8)	159 (7)	-2896 (3)	38 (2)
O (24)	3656 (7)	1101 (6)	-5801 (2)	76 (2)
O (8)	2615 (6)	-1517 (5)	-2915 (2)	48 (1)
C (9)	-813 (7)	1689 (7)	-2914 (3)	38 (2)
C (1)	-1620 (9)	3930 (7)	-2222 (3)	52 (2)
C (5)	-1876 (8)	1602 (7)	-1526 (3)	42 (2)
C (2)	-1846 (10)	4645 (8)	-1420 (3)	62 (2)
C (7)	-1388 (8)	-638 (7)	-2313 (3)	45 (2)
C (14)	2793 (8)	-382 (8)	-4807 (3)	55 (2)
C (17)	2179 (8)	-72 (7)	-2745 (3)	44 (2)
C (4)	-2159 (8)	2271 (7)	-699 (3)	50 (2)
O (16)	289 (7)	1857 (6)	-5921 (2)	67 (2)
C (11)	77 (8)	2216 (6)	-3691 (3)	44 (2)
O (21)	3696 (9)	-1872 (7)	-1652 (3)	90 (2)
C (19)	-168 (10)	2340 (9)	-159 (3)	62 (2)
C (6)	-1246 (9)	62 (6)	-1495 (3)	47 (2)
C (13)	1498 (8)	665 (7)	-4765 (3)	43 (2)
C (3)	-3021 (9)	3764 (8)	-841 (3)	61 (2)
O (15)	5509 (8)	-857 (8)	-5704 (3)	114 (3)
C (12)	-263 (9)	915 (7)	-4232 (3)	45 (2)
C (20)	1674 (8)	2713 (7)	-1857 (3)	49 (2)
C (16)	1950 (9)	1703 (7)	-5396 (3)	53 (2)
C (21)	3336 (8)	-2326 (7)	-2300 (3)	51 (2)
C (18)	-3779 (10)	1456 (10)	-248 (4)	73 (3)
C (22)	3565 (12)	-3794 (7)	-2559 (5)	71 (3)
C (15)	4129 (10)	-143 (9)	-5467 (4)	70 (2)

Table 3. Bond lengths (Å) with estimated standard deviations in parentheses.

Atoms	Bond length (Å)
O (23) - C (8)	1.453 (6)
O (23) - C (12)	1.452 (6)
C (10) - C (9)	1.540 (7)
C (10) - C (1)	1.540 (9)
C (10) - C (5)	1.574 (8)
C (10) - C (20)	1.545 (7)
C (8) - C (9)	1.533 (9)
C (8) - C (7)	1.510 (8)
C (8) - C (17)	1.531 (7)
O (24) - C (16)	1.447 (8)
O (24) - C (15)	1.348 (10)
O (8) - C (17)	1.444 (8)
O (8) - C (21)	1.361 (7)
C (9) - C (11)	1.535 (7)
C (1) - C (2)	1.523 (9)
C (5) - C (4)	1.549 (7)
C (5) - C (6)	1.532 (9)
C (2) - C (3)	1.520 (9)
C (7) - C (6)	1.529 (7)
C (14) - C (13)	1.314 (9)
C (14) - C (15)	1.456 (9)
C (4) - C (19)	1.554 (8)
C (4) - C (3)	1.551 (10)
C (4) - C (18)	1.537 (9)
O (16) - C (16)	1.377 (7)
C (11) - C (12)	1.553 (8)
O (21) - C (21)	1.186 (8)
C (13) - C (12)	1.504 (8)
C (13) - C (16)	1.492 (8)
O (15) - C (15)	1.211 (10)
C (21) - C (22)	1.483 (10)

Table 4. Bond angles (°) with estimated standard deviations in parentheses.

Atoms	Angle (°)
C (8) - O (23) - C (12)	108.5 (3)
C (9) - C (10) - C (1)	109.0 (4)
C (9) - C (10) - C (5)	104.5 (4)
C (1) - C (10) - C (5)	108.5 (4)
C (9) - C (10) - C (20)	111.7 (4)
C (1) - C (10) - C (20)	108.1 (5)
C (5) - C (10) - C (20)	114.8 (4)
O (23) - C (8) - C (9)	101.7 (4)
O (23) - C (8) - C (7)	112.0 (4)
C (9) - C (8) - C (7)	109.3 (4)
O (23) - C (8) - C (17)	106.0 (4)
C (9) - C (8) - C (17)	115.1 (5)
C (7) - C (8) - C (17)	112.1 (4)
C (16) - O (24) - C (15)	108.9 (5)
C (17) - O (8) - C (21)	117.5 (4)
C (10) - C (9) - C (8)	116.2 (4)
C (10) - C (9) - C (11)	123.3 (5)
C (8) - C (9) - C (11)	102.3 (4)
C (10) - C (1) - C (2)	111.2 (5)
C (10) - C (5) - C (4)	115.6 (5)
C (10) - C (5) - C (6)	113.1 (4)
C (4) - C (5) - C (6)	114.2 (4)
C (1) - C (2) - C (3)	112.7 (6)
C (8) - C (7) - C (6)	110.3 (5)
C (13) - C (14) - C (15)	109.1 (6)
C (8) - C (17) - O (8)	107.8 (4)
C (5) - C (4) - C (19)	114.5 (5)
C (5) - C (4) - C (3)	107.4 (4)
C (19) - C (4) - C (3)	109.8 (5)
C (5) - C (4) - C (18)	109.9 (5)
C (19) - C (4) - C (18)	107.9 (5)
C (3) - C (4) - C (18)	107.0 (5)
C (9) - C (11) - C (12)	100.6 (5)
C (5) - C (6) - C (7)	112.7 (4)
C (14) - C (13) - C (12)	131.4 (6)
C (14) - C (13) - C (16)	108.9 (5)
C (12) - C (13) - C (16)	119.7 (5)
C (2) - C (3) - C (4)	114.9 (5)
O (23) - C (12) - C (11)	107.3 (4)
O (23) - C (12) - C (13)	111.3 (5)
C (11) - C (12) - C (13)	112.5 (5)
O (24) - C (16) - O (16)	109.9 (4)
O (24) - C (16) - C (13)	124.3 (5)
O (16) - C (16) - C (13)	125.8 (5)
O (8) - C (21) - O (21)	122.5 (6)
O (8) - C (21) - C (22)	110.7 (5)
O (21) - C (21) - C (22)	126.8 (6)
O (24) - C (15) - C (14)	108.8 (6)
O (24) - C (15) - O (15)	121.5 (6)
C (14) - C (15) - O (15)	129.6 (7)

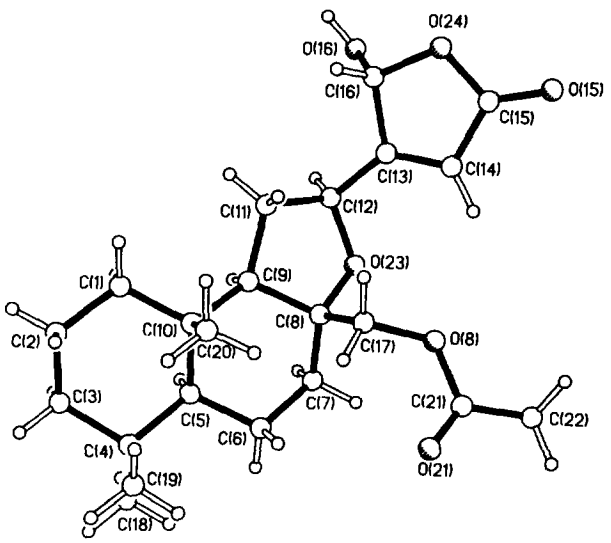


Figure 2. Perspective view of the molecular structure of 17-*O*-acetylacuminolide (2) as determined by X-ray crystallography.

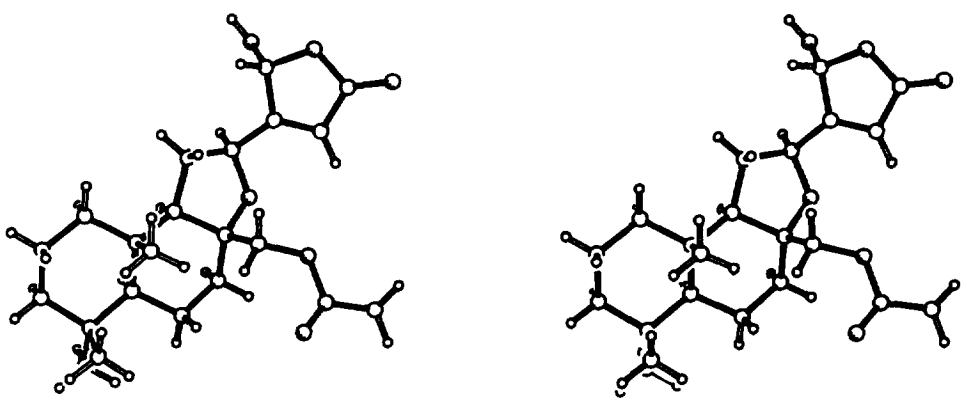
Table 5. Anisotropic displacement coefficients ($Å^2x10^3$) of the form exp $\{-2\pi^2(h^2a^{*2}U_{11} + \cdots + 2hka^*b^*U_{12})\}$.

Atom	U_{11}	U_{22}	U_{33}	U_{12}	U_{13}	U_{23}
O (23)	59 (2)	38 (2)	35 (2)	-5 (2)	0 (2)	1 (2)
C (10)	36 (3)	37 (3)	42 (3)	5 (2)	-4 (2)	0 (2)
C (8)	44 (3)	37 (3)	33 (3)	-4 (2)	-6 (2)	-4 (2)
O (24)	85 (3)	81 (3)	63 (2)	20 (3)	31 (2)	25 (3)
O (8)	65 (2)	34 (2)	44 (2)	11 (2)	-3 (2)	-1 (2)
C (9)	38 (3)	39 (3)	37 (3)	3 (2)	-2 (2)	1 (2)
C (1)	56 (3)	43 (3)	55 (3)	10 (3)	-9 (3)	-8 (3)
C (5)	41 (3)	48 (3)	38 (3)	-1 (3)	-4 (2)	-4 (2)
C (2)	71 (4)	50 (4)	63 (4)	16 (3)	-9 (3)	-19 (3)
C (7)	53 (3)	37 (3)	46 (3)	-4 (3)	8 (3)	0 (3)
C (14)	57 (4)	62 (4)	46 (3)	13 (3)	4 (3)	10 (3)
C (17)	46 (3)	42 (3)	43 (3)	4 (3)	-2 (2)	-1 (3)
C (4)	53 (3)	59 (4)	39 (3)	3 (3)	0 (2)	-10 (3)
O (16)	90 (3)	53 (3)	56 (2)	14 (2)	-1 (2)	11 (2)
C (11)	57 (3)	34 (3)	42 (3)	-2 (3)	-2 (2)	6 (3)
O (21)	143 (5)	79 (3)	46 (3)	28 (3)	-11 (3)	10 (2)
C (19)	65 (4)	75 (5)	44 (3)	7 (4)	-13 (3)	-7 (3)
C (6)	55 (3)	44 (3)	41 (3)	-1 (3)	5 (3)	6 (3)
C (13)	53 (3)	44 (3)	30 (2)	-8 (3)	-4 (2)	1 (2)
C (3)	62 (4)	70 (4)	52 (3)	16 (3)	-2 (3)	-20 (3)
O (15)	111 (4)	132 (5)	104 (4)	66 (4)	55 (3)	41 (4)
C (12)	57 (3)	45 (3)	34 (3)	2 (3)	1 (2)	3 (3)
C (20)	49 (3)	46 (3)	51 (3)	1 (3)	-2 (2)	-7 (3)
C (16)	63 (4)	54 (4)	41 (3)	5 (3)	7 (3)	-1 (3)
C (21)	49 (3)	56 (4)	50 (4)	8 (3)	11 (3)	16 (3)
C (18)	65 (4)	101 (6)	55 (4)	-10 (4)	21 (3)	-13 (4)
C (22)	76 (5)	45 (4)	92 (5)	13 (3)	1 (4)	7 (4)
C (15)	72 (4)	73 (5)	65 (4)	19 (4)	16 (3)	14 (4)

Table 6. Hydrogen atom coordinates ($x10^4$) and isotropic displacement coefficients ($Å^2x10^3$).

Atom	x	y	z	U
H (9A)	-2265	1648	-3032	37 (13)
H (1A)	-798	4488	-2560	128 (33)
H (1B)	-2957	3832	-2477	54 (16)
H (5A)	-3242	1582	-1762	36 (13)
H (2A)	-503	4840	-1192	55 (16)
H (2B)	-2546	5517	-1505	79 (21)
H (7A)	-2797	-666	-2502	72 (19)
H (7B)	-895	-1580	-2273	53 (16)
H (14A)	2825	-1189	-4469	68 (18)
H (17A)	2927	524	-3090	132 (33)
H (17B)	2571	142	-2203	36 (13)
H (016)	288	2697	-6084	65 (21)
H (11A)	-644	3018	-3900	31 (12)
H (11B)	1510	2429	-3615	41 (13)
H (19A)	-442	2778	339	114 (28)
H (19B)	870	2858	-421	56 (17)
H (19C)	302	1403	-64	71 (20)
H (6A)	139	-5	-1282	50 (15)
H (6B)	-2119	-422	-1141	47 (15)
H (3A)	-3037	4233	-336	43 (13)
H (3B)	-4416	3681	-1040	63 (17)
H (12A)	-1490	1053	-4557	77 (20)
H (20A)	2318	3273	-2249	175 (44)
H (20B)	2354	1825	-1814	108 (27)
H (20C)	1769	3179	-1351	87 (22)
H (16A)	2331	2582	-5159	50 (15)
H (18A)	-3947	1874	264	157 (40)
H (18B)	-3306	513	-180	104 (30)
H (18C)	-5071	1457	-543	140 (36)
H (22A)	4079	-4354	-2122	214 (51)
H (22B)	4518	-3822	-2980	153 (41)
H (22C)	2262	-4154	-2751	148 (40)

Figure 3. Stereoscopic view of the compound 2 (17-*O*-acetylacuminolide).



F(000) = 424, μ(Cu-Kα) = 6.9 cm^{-1}. Data crystal (colourless prism) had approximate dimensions 0.41 x 0.34 x 0.23 mm. *Data Collection and Processing.* Three-dimensional, room temperature (295K) X-ray data was collected on a Siemens R3m/V diffractometer with monochromatised Cu-Kα X-radiation. 2Θ/ω mode with scan range (ω) 1.14° plus Kα separation and a variable scan speed (4.88-14.65° min^{-1}). 1602 reflections measured (3<2Θ<115°, min. hkl -8 0 0, max. hkl 8 11 19); 1529 unique reflections [R(σ) = 0.047, Friedel opposites merged] of which 1365 reflections had I > 2.0σ (I). No absorption correction was applied. 1 control reflection monitored every 99 reflections showed no appreciable decay during 13.6 hours of exposure of the crystal to X-rays. *Structure Analysis and Refinement.* Direct methods resulted in the location of all the non-hydrogen atoms. Full matrix least-squares refinement with anisotropic thermal parameters was used for all non-hydrogen atoms. Hydrogen atoms were refined in riding mode. Individual weights were applied according to the scheme $w = [\sigma^2(F_o) + 0.0007 |F_o|^2]^{-1}$ and refinement converged at R = 0.048, R_w = 0.051. Maximum and mean shift/error in the final cycle of refinement were 0.024 and 0.002, respectively. The final electron-density difference synthesis showed no peaks > 0.19 or < -0.20 $eÅ^{-3}$. All computations were carried out using the SHELXTL PLUS (μ-VAX II) system of programs.[13]

Bioassay evaluations: Compounds **1-3** were screened for cytotoxicity against a panel of human cancer cell lines and murine P388 cells, according to established protocols.[14] ED_{50} values of >4 μg/ml were regarded as negative. Among the cell lines represented, a human lung cancer cell line (Lu1) was used to guide the fractionations of **1-3** from the crude MeOH extracts of *N. acuminatissima.* Compounds **1** and **2** were tested *in vivo* at their maximum tolerated dose against the HT-29 human colorectal and KB human epidermoid carcinoma models, respectively, implanted s.c. Doxorubicin was run as a positive control. Samples were administered i.p. on days 1, 5, 9 in 10% DMSO, 1% Tween. Groups of 6-8 animals were used in each study.

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