

X-RAY CRYSTAL STRUCTURE OF ACROVESTONE, A CYTOTOXIC PRINCIPLE FROM *ACRONYCHIA PEDUNCULATA*

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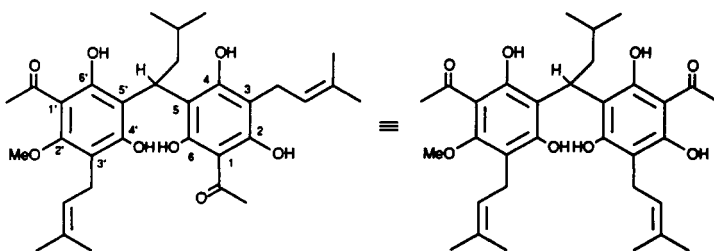
ABSTRACT.—Acrovestone [**1**] was isolated from the stem and root bark of *Acronychia pedunculata* and shown for the first time to be a cytotoxic principle. Its structure, derived from spectral data, was completely characterized by single-crystal X-ray analysis.

Acronychia pedunculata (L.) Miq. (= *Acronychia laurifolia* Blume) (Rutaceae) is a small shrub which is widely distributed in Indo-Malayan and Southern China. This plant is the only species of *Acronychia* native to the northern part of Taiwan (1). The roots, stems, leaves, and fruits of this plant have been used in folk medicine for the treatment of diarrhea, tussis, asthma, ulcers, itchy skin, scales, pain, and rheumatism, and as an antipyretic and antihemorrhagic agent as well as an aphrodisiac (2). Several compounds, including terpenoids, acetophenones, and furoquinoline alkaloids, have been isolated from the leaves, wood, stems, and roots of this plant (3–10).

As a result of our continuing search for novel bioactive natural products, the MeOH extract of the stem and root bark of *A. pedunculata* was found to show significant cytotoxicity in the human KB tissue culture assay ($ED_{50} < 20 \mu\text{g/ml}$). Bioassay-directed fractionation of the plant extract led to the isolation and characterization of acrovestone [**1**] as the cytotoxic principle from the CHCl_3 -soluble fraction.

RESULTS AND DISCUSSION

Compound **1**, isolated as pale yellow prisms, mp $142.5\text{--}143.5^\circ$, was found to be optically inactive. Exact mass measurement established the molecular formula as $\text{C}_{32}\text{H}_{42}\text{O}_8$. Strong absorption around 1608 cm^{-1} in the ir spectrum indicated that **1** possessed an acetophenone carbonyl group, and this was corroborated by the uv spectrum which revealed the presence of a 2,4,6-trioxygenated acetophenone moiety (11).



The ^1H -nmr spectrum contained signals for two prenyl [δ 1.69 (3H, s), 1.77 (6H, s), 1.84 (3H, s), 3.30 (2H, d), 3.40 (2H, d), and 5.20 (2H, t)], one 1,1-disubstituted-3-methylbutyl [δ 0.87 (6H, d), 1.41 (1H, m), 2.15 (2H, m), and 4.74 (1H, t)], two acetyl [δ 2.67 (3H, s), and 2.71 (3H, s)], one methoxy [δ 3.71 (3H, s)], and five hydroxy [δ 6.50, 9.34, 10.05, 15.65, and 15.70 (each 1H, s)] groups. On the basis of the foregoing evidence, **1** was identified as acrovestone which was isolated previously from *Acronychia vestita* (Rutaceae) and *A. laurifolia* Blume [= *A. pedunculata* (L.) Miq.] by others (6,7) who derived its constitution from chemical degradation and spectroscopic data.

The structure of **1** was confirmed by a single-crystal X-ray analysis which provided details of the solid-state geometry. Earlier attempts by Seccombe and Kennard (12) to solve this crystal structure were unsuccessful. The crystal structure was solved by direct methods (MULTAN11/82). Full-matrix least-squares refinement of atomic positional and thermal parameters (anisotropic C, O; fixed H contributions) converged at $R = 0.060$ ($R_w = 0.084$, $\text{GOF} = 1.7$) over 2962 reflections. $R = \sum ||F_o| - |F_c|| / \sum |F_o|$; $R_w = [\sum w(|F_o| - |F_c|)^2 / \sum w|F_o|^2]^{1/2}$, GOF (goodness-of-fit) = $[\sum w\Delta^2 / (N_{\text{observations}} - N_{\text{parameters}})]^{1/2}$. Fractional coordinates for the nonhydrogen atoms are listed in Table 1. A view of the solid-state conformation of **1**, with the atom numbering scheme, is provided in Figure 1. Atom C-10 is disordered over two positions corresponding to 50% occupation of the general positions of space group *Pbca* by each enantiomer of racemic **1**. All five hydroxy groups in **1** have their hydrogen atoms lying close to the planes of the phenyl rings to which they are bonded. Hydroxy groups at C-6 and C-6' are hydrogen-bonded to the adjacent acetyl substituent of the same phenyl ring; those at C-4 and C-4' are hydrogen bonded to the oxygen atom of hydroxy substituents at C-6' and C-6, respectively, of the other phenyl rings, while the remaining hydroxy group at C-2 has its hydrogen atom directed towards the C-2'' = C-3''' double bond of the adjacent 2-methyl-2-butenyl substituent, indicating that an O-H . . . π interaction is present. Hydrogen-bonded distances (\AA) are as follows: O-14 . . . C-2'' = 2.991 (4), O-14 . . . C-3''' = 3.367 (4), O-15 . . . O-12' = 2.726 (3), O-16 . . . O-17 = 2.440 (3), O-11' . . . O-16 = 2.724 (3), O-12' . . . O-13' = 2.438 (5), H-14 . . . C-2'' = 2.22, H-14 . . . C-3''' = 2.44, H-15 . . . O-12' = 1.85, H-16 . . . O-17 = 1.58, H-11' . . . O-16 = 1.84, H-12' . . . O-13' = 1.59. There are consequently no intermolecular hydrogen bonds in crystals of **1** that contain discrete molecules separated by van der Waals distances of which the shortest for nonhydrogen atoms is 3.36 \AA between O-12' and C-5'''. Absence of strong intermolecular interactions in the solid state when coupled with the presence in **1** of a single asymmetric center which is surrounded by relatively bulky groups generates circumstances well suited for partial site occupancy by enantiomer pairs.

Average values for bond lengths associated with the phenyl ring carbon atoms are not unusual [e.g., C(ar)-C(ar) = 1.401 \AA , C(ar)-C(sp³) = 1.463 \AA , C(ar)-OH = 1.368 \AA], and other distances between ordered atoms are also close to expected values (13). In the approximately planar phenyl rings, bond angles at carbon centers bearing hydroxy or methoxy substituents [range 122.4 (3)–123.6 (3) $^\circ$, mean 123.1 $^\circ$] are considerably enlarged over those at the other carbon centers [range 116.2 (3)–117.7 (3) $^\circ$, mean 116.9 $^\circ$] and, accordingly, the rings are not regular hexagons. Similar bond angle variations have been found in symmetrically-substituted 1,3,5-trihydroxy- (14) and 1,3,5-trimethoxybenzene (15). Overcrowding of substituents and optimization of geometrical requirements for intramolecular hydrogen bonding (see above) lead to significant out-of-plane displacements of a number of the atoms bonded directly to the phenyl rings. Thus, C-7, O-14, C-1'', O-15, C-9, and O-16 are -0.039, 0.018, 0.199, 0.032, -0.048, and 0.096 \AA , respectively, from the C-1-C-6 least-squares plane, while C-8 ($\Delta = -0.179$ \AA) and O-17 ($\Delta = 0.079$ \AA) lie close to the plane. Displace-

TABLE 1. Fractional Coordinates and Equivalent Isotropic Thermal Parameters for the Non-hydrogen Atoms of Acrovestone [1], With Estimated Standard Deviations in Parentheses.

Atom	x	y	z	$B_{eq}(\text{\AA}^2)$
C-1	0.5657 (2)	0.5372 (2)	0.0684 (2)	4.10 (6)
C-2	0.5630 (2)	0.5940 (2)	0.0110 (2)	4.33 (7)
C-3	0.6132 (2)	0.6039 (2)	-0.0459 (2)	4.51 (7)
C-4	0.6687 (2)	0.5573 (2)	-0.0433 (2)	4.19 (7)
C-5	0.6759 (2)	0.5007 (2)	0.0120 (2)	3.95 (6)
C-6	0.6231 (2)	0.4909 (2)	0.0655 (2)	4.03 (6)
C-7	0.5148 (2)	0.5249 (2)	0.1293 (2)	4.79 (7)
C-8	0.4553 (2)	0.5738 (3)	0.1424 (3)	7.0 (1)
C-9	0.7382 (2)	0.4511 (2)	0.0135 (2)	5.44 (8)
C-10A ^a	0.7997 (3)	0.4858 (4)	0.0401 (4)	4.4 (1)
C-10B ^a	0.7760 (4)	0.4328 (4)	0.0817 (4)	4.9 (2)
C-11	0.7966 (2)	0.5086 (3)	0.1320 (2)	8.3 (1)
C-12	0.8097 (3)	0.4660 (3)	0.2031 (4)	10.4 (2)
C-13	0.8527 (5)	0.5624 (5)	0.1233 (5)	14.8 (3)
O-14	0.5073 (1)	0.6375 (1)	0.0105 (2)	6.21 (6)
O-15	0.7155 (1)	0.5698 (1)	-0.1006 (2)	5.51 (6)
O-16	0.6270 (1)	0.4335 (1)	0.1159 (1)	5.17 (5)
O-17	0.5190 (1)	0.4697 (2)	0.1733 (1)	6.15 (6)
C-1'	0.7747 (2)	0.3419 (2)	-0.1794 (2)	4.78 (7)
C-2'	0.7369 (2)	0.2776 (2)	-0.1636 (2)	4.57 (7)
C-3'	0.6997 (2)	0.2688 (2)	-0.0947 (2)	4.40 (7)
C-4'	0.6992 (2)	0.3270 (2)	-0.0402 (2)	4.26 (7)
C-5'	0.7363 (2)	0.3912 (2)	-0.0504 (2)	4.19 (7)
C-6'	0.7722 (2)	0.3975 (2)	-0.1211 (2)	4.57 (7)
C-7'	0.8178 (2)	0.3536 (2)	-0.2493 (2)	6.25 (9)
C-8'	0.8264 (3)	0.2971 (4)	-0.3145 (3)	10.4 (2)
O-9'	0.7382 (1)	0.2213 (1)	-0.2178 (1)	5.95 (6)
C-10'	0.6796 (3)	0.2160 (3)	-0.2660 (3)	8.6 (1)
O-11'	0.6607 (1)	0.3150 (1)	0.0256 (1)	5.27 (5)
O-12'	0.8062 (1)	0.4614 (1)	-0.1328 (2)	5.90 (6)
O-13'	0.8498 (2)	0.4113 (2)	-0.2569 (2)	7.90 (8)
C-1''	0.6035 (2)	0.6588 (2)	-0.1133 (2)	5.85 (9)
C-2''	0.5934 (2)	0.7362 (2)	-0.0888 (2)	5.70 (9)
C-3''	0.5427 (2)	0.7804 (2)	-0.1092 (2)	5.72 (9)
C-4''	0.5436 (3)	0.8596 (3)	-0.0828 (3)	7.7 (1)
C-5''	0.4828 (3)	0.7588 (3)	-0.1583 (3)	8.4 (1)
C-1'''	0.6601 (2)	0.1999 (2)	-0.0771 (2)	5.33 (8)
C-2'''	0.5855 (2)	0.2080 (2)	-0.0918 (3)	5.55 (9)
C-3'''	0.5438 (2)	0.1569 (2)	-0.1166 (2)	6.06 (9)
C-4'''	0.4697 (2)	0.1733 (3)	-0.1317 (3)	8.8 (1)
C-5'''	0.5630 (3)	0.0784 (3)	-0.1289 (4)	9.5 (1)

^aOccupancy factor = 0.50.

ments of directly bonded C-7', O-9', C-1''', O-11', C-9, and O-13' from the C-1'-C-6' least-squares plane are, respectively, -0.057, -0.025, -0.029, 0.030, -0.089, and -0.075 Å. Acetyl group atoms C-8' ($\Delta = -0.112$ Å) and O-13' ($\Delta = -0.075$ Å) again lie close to the ring plane, but the methoxy carbon atom, C-10', is displaced very significantly ($\Delta = 1.226$ Å) as the usually preferred co-planar arrangement (15-20) for aryl methoxy groups is prohibited by the substituents at C-1' and C-3'. The greater displacement of C-1'' ($\Delta = 0.199$ Å) vs. C-1''' ($\Delta = -0.029$ Å) from their respective ring planes may be due, in part at least, to the geometrical demands associated with the interaction between the hydroxy group at C-2 and the C-2'' = C-3'' double bond.

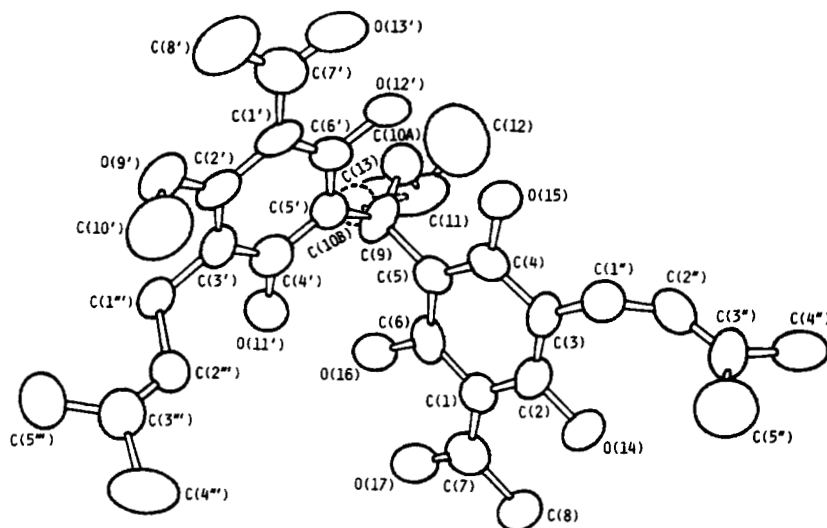


FIGURE 1. Atom numbering scheme and solid-state conformation of acrovestone [1]; hydrogen atoms have been omitted for clarity.

Acrovestone [1] demonstrated potent cytotoxicity (100% inhibition at 0.5 $\mu\text{g}/\text{ml}$) in the KB tissue culture assay (21). It also showed significant cytotoxicity against A-549, P-388, and L-1210 cells with ED_{50} values of 0.98, 3.28, and 2.95 $\mu\text{g}/\text{ml}$, respectively.

EXPERIMENTAL

PLANT MATERIALS.—*A. pedunculata* was collected in Taipei Hsien, Taiwan, in April 1986. The plant was identified by Professor C.S. Kuoh of the National Cheng-Kung University, Taiwan, and a specimen has been deposited in the herbarium of Cheng-Kung University.

EXTRACTION AND SEPARATION.—The air-dried and powdered root and stem bark of *A. pedunculata* (4.54 kg) were extracted exhaustively with hot MeOH. The extract was filtered and concentrated under reduced pressure to furnish a brown syrup (534 g), which was then partitioned between H_2O and CHCl_3 , EtOAc, and *n*-BuOH. Extraction of the CHCl_3 layer with 5% HCl solution followed by neutralization of the acidic layer with NH_4OH and further extraction with CHCl_3 yielded a basic residue (105 mg) after concentration. The CHCl_3 layer, after removal of the basic portions, was chromatographed on Si gel and eluted exhaustively with *n*- C_6H_{14} -EtOAc (4:1) to afford 13 fractions. Pale yellow crystals of acrovestone [1] (6.5 g) were isolated from fraction 1 by recrystallization.

ACROVESTONE [1].—Pale yellow prisms (MeOH); mp 142.5–143.5 $^\circ$; $[\alpha]_D^{25} \pm 0^\circ$ ($c = 1.0$, CHCl_3); hrms calcd for $\text{C}_{32}\text{H}_{42}\text{O}_8$ m/z $[\text{M}]^+$ 554.2880, found 554.2840; uv (MeOH) λ max nm (log ϵ) 230.1 (4.4), 296.6 (4.29), 338.7 (4.11); ir (KBr) ν max cm^{-1} 3267, 2959, 2919, 2867, 1608, 1450, 1368, 1320; $^1\text{H-nmr}$ (CDCl_3) δ 0.87 (6H, d, $J = 6.4$ Hz, 2 \times Me), 1.41 (1H, m), 1.69 (3H, s, Me), 1.77 (6H, s, 2 \times Me), 1.84 (3H, s, Me), 2.15 (2H, m), 2.67 (3H, s, -Ac), 2.71 (3H, s, -Ac), 3.30 (2H, d, $J = 6.6$ Hz), 3.40 (2H, d, $J = 6.6$ Hz), 3.71 (3H, s, OMe), 4.74 (1H, t, $\tau, J = 7.7$ Hz), 5.20 (2H, t, $J = 6.6$ Hz), 6.50 (1H, br, OH), 9.34 (1H, br, OH), 10.05 (1H, br, OH), 15.65 (1H, s, OH), 15.70 (1H, br, OH); m/z (%) $[\text{M}]^+$ 554 (0.4), 479 (0.1), 318 (29.6), 303 (22.5), 263 (12.3), 250 (16.7), 248 (19.3), 247 (100), 236 (30), 235 (22.6), 233 (18), 219 (61), 221 (19), 207 (28), 205 (16), 195 (13), 193 (23), 181 (49), 165 (23); $^{13}\text{C-nmr}$ (CDCl_3) δ 17.9 (q), 18.0 (q), 22.2 (t), 22.5 (q), 22.7 (q), 23.1 (t), 25.7 (q), 25.8 (q), 27.0 (d), 28.6 (d), 30.7 (q), 32.7 (q), 39.4 (t), 62.6 (q), 99.8 (s), 104.7 (s), 106.2 (s), 108.2 (s), 108.6 (s), 113.0 (s), 116.7 (s), 121.6 (d), 123.1 (d), 131.8 (s), 136.7 (s), 158.3 (s), 160.2 (s), 160.8 (s), 162.6 (s), 204.2 (s), 204.3 (s).

X-RAY CRYSTAL STRUCTURE ANALYSIS OF ACROVESTONE [1].—Crystal data: $\text{C}_{32}\text{H}_{42}\text{O}_8$, MW = 554.69, orthorhombic, space group $Pbca(D_{2h}^{12})$ -No. 61, $a = 19.824$ (2), $b = 18.230$ (2), $c = 16.829$ (2) \AA [lit. (7) $a = 19.611$ (12), $b = 18.190$ (10), $c = 16.790$ (9) \AA with axes rotated to cab], $V = 6082$ (2) \AA^3 (from 25 orientation reflections, $40^\circ < \theta < 48^\circ$), $Z = 8$, $D_{\text{calcd}} = 1.211$ $\text{g}\cdot\text{cm}^{-3}$, $\mu(\text{CuK}\alpha$ radiation, $\lambda =$

1.5418 Å) = 6.7 cm⁻¹; crystal dimensions 0.26 × 0.26 × 0.40 mm. Intensity data (+*b*, +*k*, +*l*, θ_{max} = 67°, 5404 reflections) were recorded on an Enraf-Nonius CAD-4 diffractometer [CuKα radiation, incident-beam graphite monochromator; ω-2θ scans, scanwidth (1.10 + 0.14 tan θ)°]. The data were corrected for the usual Lorentz and polarization effects, but only those 2962 reflections with I > 3.0σ(I) were retained for the analysis.

The crystal structure was solved by direct methods (MULTAN11/82). Approximate C and O atom positions, except for atoms C-10-C-13 of the 1,1-disubstituted-3-methylbutyl moiety, were obtained from an initial *E* map based on phases that yielded the highest combined figure-of-merit. Several rounds of full-matrix least-squares adjustment of atomic positional and isotropic thermal parameters followed. A difference Fourier synthesis was then evaluated, and it not only yielded positions for the remaining C atoms but also revealed that C-10 was disordered over two sites ca. 1.3 Å apart and corresponding to 50% occupation of the general positions of space group *Pcba* by enantiomer pairs. Positional and anisotropic temperature factor parameters were next adjusted by several further rounds of full-matrix least-squares calculations. A series of difference Fourier syntheses verified that calculated hydrogen atom positions, save for those at C-9 which were masked by disordered C-10A and C-10B, coincided with positive regions, and these atoms were included at their calculated positions in the subsequent least-squares iterations which converged at R = 0.060 (R_w = 0.084, GOF = 1.7). Final nonhydrogen atom coordinates are listed in Table 1.¹

Neutral atom scattering factors used in the structure-factor calculations were taken from International Tables for X-ray Crystallography (22). Crystallographic calculations were performed on PDP11/44 and MicroVAX computers by use of the Enraf-Nonius Structure Determination Package. In the least-squares iterations, ΣwΔ² [w = 1/σ²(|F_o|), Δ = (|F_o| - |F_c|)] was minimized.

BIOLOGICAL ASSAY.—The *in vitro* cytotoxicity assay was carried out according to a National Cancer Institute protocol (21).

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¹Atomic coordinates for this structure 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 1EW, UK.

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