Communications to the Editor

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STRUCTURE AND STEREOCHEMISTRY OF BRYOPHYLLIN-A, A NOVEL POTENT CYTOTOXIC BUFADIENOLIDE ORTHOACETATE FROM BRYOPHYLLUM PINNATUM

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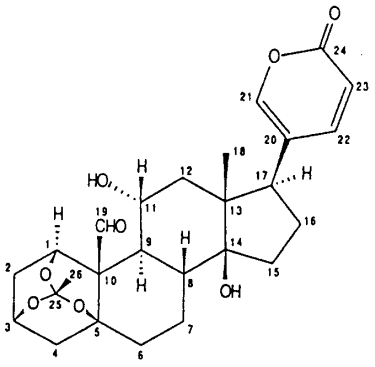
Bryophyllin-A, a novel bufadienolide 1,3,5-orthoacetate with potent cytotoxicity, and the known bersaldegenin-3-acetate have been isolated from Bryophyllum pinnatum and their structures have been established from spectral data and single-crystal X-ray analyses.

KEYWORDS—— bryophyllin-A; bufadienolide; Bryophyllum pinnatum; Crassulaceae; cytotoxicity; antitumor activity; X-ray analysis

As a result of our continuing searches among Chinese medicinal plants for novel potent antitumor agents,[1)2)] the methanolic extract of the whole plant of Bryophyllum pinnatum (Crassulaceae),[3)] known as "Luo Di Sheng Ken" in Taiwan, was found to show potent cytotoxicity in vitro against tumor culture cells.[4)] Bioassay-directed fractionation of the aforementioned cytotoxic extract led to the isolation of bryophyllin-A (**1**; 0.0001% yield) and the known bersaldegenin-3-acetate (**2**; 0.000026% yield) after purification by repeated silica gel



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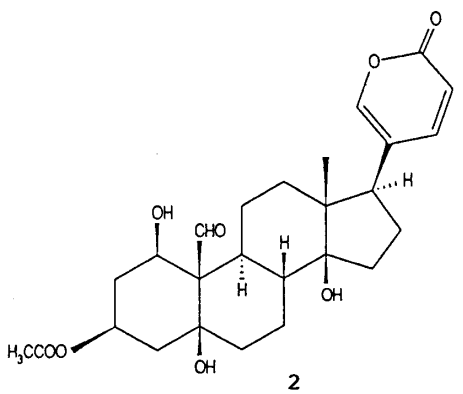

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column chromatography ($CHCl_3$-MeOH) and reversed phase HPLC (Nucleosil $7C_{18}$ 10 X 300 mm, MeOH : H_2O= 4 : 1). Bersaldegenin-3-acetate was isolated from Bersama abyssinica (Melianthaceae).[5] The ^{1}H-NMR and physical data for **2** were in accord with those reported in ref. 5.

Bryophyllin-A(**1**) was crystallized from methanol as colorless rhombic prisms, $C_{26}H_{32}O_8$, m/z 472.2119 (M^+), mp 267-270°C (dec.), $[\alpha]_D^{20}$ -14.4° (c 0.50, $CHCl_3$), UV λ_{max} (MeOH): 298nm (5800), IR ν_{max}($CHCl_3$): 3450(OH), 1705(C=O) and 1120(C-O) cm^{-1}. The ^{1}H-NMR spectrum (400 MHz, $CDCl_3$-CD_3OD=10:1) revealed the presence of a α-pyrone [δ 7.72(1H, dd, J=9.7 and 2.5Hz, H-22), 7.22 (1H, d, J=2.5Hz, H-21), 6.27 (1H, d, J= 9.7Hz, H-23)], a orthoacetate [δ 5.13 (1H, d, J=4Hz, H-1), 4.36 (1H, br.s, H-3), and 1.42(3H, s, Me-25)], an aldehyde [δ10.31(1H, s, H-19)], and one secondary hydroxy [δ4.21 (1H, m, $W_{1/2}$=25 Hz, H-11)] groups.

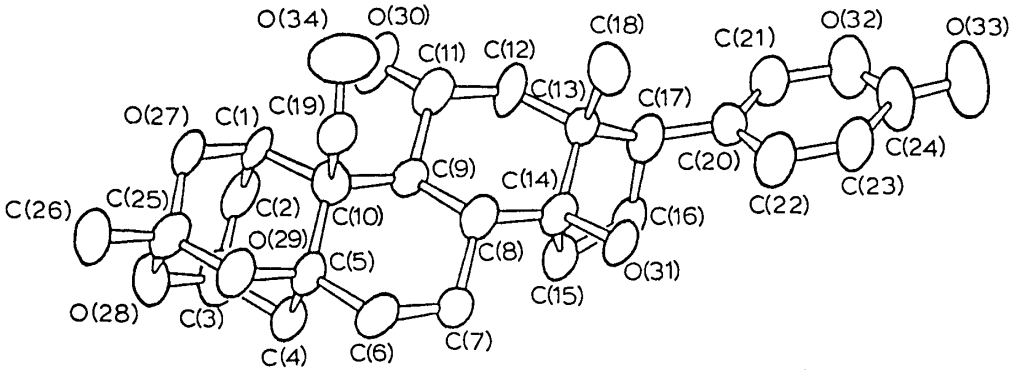

Fig. 1. Structure and Solid-State Conformation of Bryophyllin-A(**1**)
Hydrogen atoms have been omitted for clarity.

Single-crystal X-ray analyses established the complete structures and stereochemistries of **1** and **2**.[9] A view of the solid-state conformation of **1** is provided in Fig. 1.

Other related bufadienolides, such as hellebrigenin-3-acetate and -3,5-diacetate from Bersama abyssinica by Kupchan et al., also demonstrated cytotoxic (KB cells) and antitumor (Walker 256 carcinosarcoma) activity.[7] Daigremontianin and bersaldegenin-1,3,5-orthoacetate isolated from Kalanchoe daigremontiana,[6] was found to have positive inotropic and sedative activity.[8] Bryophyllin-A demonstrated remarkable cytotoxicity in KB cells (ED_{50}= 14ng/ml) and potent cytotoxicity in human lung carcinoma A-549 (ED_{50}= 10ng/ml) and colon HCT-8 tumor (ED_{50}= 30ng/ml) cells.

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3) The sample was collected in the spring of 1987 in Taipei, Taiwan. A voucher specimen of this plant is kept at the Institute of Botany, Academia Sinica, Taipei, Taiwan.
4) In vitro activity was assayed by Dr. Y. C. Cheng and Mr. M. Fisher of the Cancer Research Center, and Dr. J. J. Chang of the School of Medicine, UNC-CH, according to literature methods (R.I. Geran, N. H. Greenberg, M. M. MacDonald, A.M.Schumacher,and B.J.Abbott, Cancer Chemother. Rep., Part 3, 1 (1972); K. H. Lee, Y. M. Lin, T. S. Wu, D. C. Zhang, T. Yamagishi, T. Hayashi, I. H. Hall, J. J. Chang, R. Y. Wu, and T. H. Yang, Planta Medica, in press.)
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9) Crystal data (**1**), $C_{26}H_{32}O_8$, M=472.54, orthorhombic, space group $P2_12_12_1$, a= 14.778(7), b=16.362(5), c = 9.092(4) Å, U = 2198. 4Å^3, Z = 4, $D_{calc.}$ = 1.428 g cm^{-3}, μ(Cu-Kα) = 8.3 cm^{-1}, sample dimensions: 0.18 χ0.24χ 0.05 mm; (**2**) $C_{26}H_{34}O_8$, M=474.56, orthorhombic, space group $P2_12_12_1$, a = 14.307(2), b = 14.794(1), c = 11.014(3)Å , U = 2331.2Å^3, Z=4, $D_{calc.}$=1.352g cm^{-3}, μ(Cu-Kα) = 7.8 cm^{-1}, sample dimensions: 0.16 χ 0.18 χ 0.40 mm. One octant of intensity data for each crystal [1731(**1**) and2360(**2**) reflections to 57° and 67°, respectively] was recorded on an Enraf-Nonius CAD-4 diffractometer (Cu-K α radiation, incident-beam graphite monochromator; ω-2 θ scans). Data were corrected for the usual Lorentz and polarization effects. Both crystal structures were solved by direct methods [MULTAN 11/82 for (**1**); RANTAN for (**2**). Full-matrix least-squares refinement (Enraf-Nonius SDP) of non-hydrogen atom positional and thermal parameters, with hydrogen atoms included at their calculated positions, converged to R= 0.060 (R_w=0.073) and R= 0.044 (R_w= 0.057), respectively, over 1044 (**1**) and 1286 (**2**) reflections with $I>3.0\sigma$ (I) and $w= 1/\sigma^2([F_o])$.

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