

ACIDISSIMIN, A NEW LIMONOID FROM *LIMONIA ACIDISSIMA*

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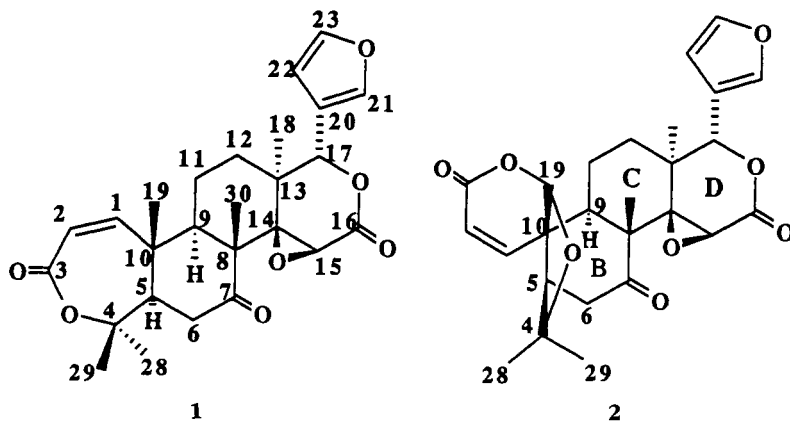
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ABSTRACT.—Two limonoids, obacunone [**1**] and acidissimin [**2**] have been isolated from the stem bark and root bark, respectively, of *Limonia acidissima*. Acidissimin is a new compound whose structure has been elucidated by spectroscopic means.

*Limonia acidissima* L. (Rutaceae), commonly known as wood apple, is distributed in dry warm regions of Burma, India, Malaya, and Sri Lanka (1,2). Various parts of the plant are employed in indigenous medicine (3), and the ripe fruit is used as a dessert and a source of beverages, cream, and jellies (4). Several coumarins, some of which show antifungal (5,6) and insecticidal<sup>1</sup> properties, have been isolated from the roots (6–9), stem-bark (6,10), leaves (10–12), and the fruit pericarp (13). Other natural products reported to be present in the plant include alkaloids (13), a benzoquinone (6), flavone glycosides (11,14), sterols (10,12,13), and triterpenoids (12,14). The present study describes the isolation and structure elucidation of a new limonoid, acidissimin, from the stem

bark and a known limonoid, obacunone, from the root bark of *L. acidissima*.

The first of two crystalline compounds isolated from *L. acidissima* gave a protonated molecular ion at 455 [MH]<sup>+</sup>, corresponding to a molecular formula of C<sub>26</sub>H<sub>30</sub>O<sub>7</sub> and consistent with its being a tetranortriterpene (limonoid). The mp, optical rotation, ir and ms of this compound, as well as its <sup>1</sup>H- and <sup>13</sup>C-nmr spectra, were identical to those reported for obacunone [**1**] (15,16). However, the <sup>13</sup>C-nmr chemical shifts have been completely and unambiguously reassigned through the use of heteronuclear 2D correlated (HETCOR) spectroscopy, in conjunction with a phase-sensitive double-quantum filtered COSY, and nOe measurements (Table 1).



<sup>1</sup>B.M.R. Bandara, A.A.L. Gunatilaka, M.A.S.K. Ranasinghe, S. Sotheeswaran, and E.M.K. Wijeratne, manuscript in preparation.

The second of these compounds, acidissimin [**2**], yielded a molecular formula of C<sub>26</sub>H<sub>28</sub>O<sub>8</sub> from high resolution

TABLE 1.  $^{13}\text{C}$ -nmr ( $\text{CDCl}_3$ ) Assignments for Obacunone [1] and Acidissimin [2].

Carbon	Compound	
	1	2
C-1	156.9	150.5
C-2	122.8	118.8
C-3	166.5	160.1
C-4	83.9	86.2
C-5	57.2 <sup>a</sup>	53.1
C-6	39.8	37.5
C-7	207.2	208.7
C-8	53.2	49.2
C-9	49.1	41.7
C-10	43.0 <sup>a</sup>	49.1
C-11	16.9 <sup>a</sup>	19.0
C-12	32.7 <sup>a</sup>	29.0
C-13	37.3 <sup>a</sup>	38.3
C-14	64.9	67.1
C-15	52.8 <sup>a</sup>	54.1
C-16	166.7	166.5
C-17	77.9	77.7
C-18	19.8 <sup>a</sup>	24.8
C-19	21.0 <sup>a</sup>	103.8
C-20	119.9	119.7
C-21	140.8	141.0
C-22	109.8	109.5
C-23	143.0	143.1
C-28	32.0 <sup>a</sup>	20.1
C-29	16.4 <sup>a</sup>	15.6
C-30	26.7 <sup>a</sup>	31.5

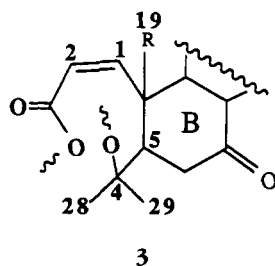
<sup>a</sup>Carbons that have been assigned or reassigned in this study.

mass measurement and elemental analysis. This was consistent with a limonoid skeleton containing an extra degree of unsaturation and an additional oxygen atom compared with obacunone [1]. The  $^1\text{H}$ - and  $^{13}\text{C}$ -nmr spectra revealed that much of the skeleton of **2** bore similarities to the co-occurring compound **1**, as evidenced by the characteristic protons of the furan ring, the diagnostic C-15 methine proton, and the carbon chemical shifts indicative of an epoxide moiety (Table 1). The  $^{13}\text{C}$  APT spectrum of **2** in conjunction with its HETCOR and COSY spectrum permitted direct assignment of the protons and carbons of the B, C, D, and furan rings. This left the placement of three oxygen, seven carbon, and ten hydrogen atoms in

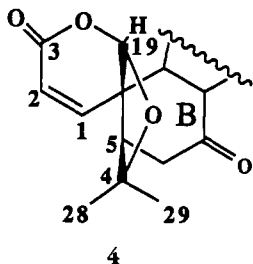
such a manner as to account for the remaining four degrees of unsaturation.

The structurally unassigned carbon atoms were composed of one ester carbonyl carbon (160.1), two olefinic methines (150.5, 118.8), two C-methyl groups (15.6, 20.1), one methine (103.8), and one  $\text{sp}^3$ -hybridized quaternary carbon attached to an oxygen atom (86.2). The two singlet methyls were geminal ( $^4J_{\text{HH}}$  coupled in the long range COSY) and must be attached to the oxygenated quaternary carbon at 86.2, which could therefore be assigned to C-4. The long-range COSY experiment optimized for  $^4J_{\text{HH}}$  coupling also demonstrated coupling of the protons on the C-29 methyl to the C-5 methine, thus establishing the C-4/C-5 carbon-carbon bond. The two olefinic methines formed part of an  $\alpha,\beta$ -unsaturated ester system, as evidenced by their  $^{13}\text{C}$  chemical shifts and their corresponding  $^1\text{H}$ -nmr chemical shifts and coupling constants (6.53, d,  $J = 10$  Hz; 6.13, d,  $J = 10$  Hz). The  $\beta$  proton of this system exhibits no other coupling and thus must be bound to the quaternary carbon C-10. In the absence of an O-H stretching frequency in the infrared of **2**, the remaining oxygen attached to C-4 must form an ether linkage, leaving the remaining two degrees of unsaturation to be made up by two rings. The known structural features to this point are depicted in substructure **3**.

In substructure **3** only the methine at 103.8 ppm has not been accounted for. This must be C-19 (R = CH in **3**) bound to the non-oxygenated  $\text{sp}^3$ -hybridized quaternary carbon C-10 (49.1). From this point the two remaining degrees of



unsaturation can only be accounted for by connecting both the ester and the C-4 oxygen to C-19 thus forming two rings. The typical acetal  $^{13}\text{C}$  chemical shift of C-19 (103.8) substantiates this. Substructure **4** depicts this novel spiro-ketal moiety, demonstrating an unusual oxidation level for limonoids at C-19.



All that remained was to establish the relative stereochemistry at C-19 and to assign unambiguously the  $^{13}\text{C}$  chemical shifts for each of the four methyl groups. Two of the methyl groups could be placed through the use of long-range COSY spectra. This yielded a relatively strong  $^4J_{\text{HH}}$  coupling from the proton on C-17 to the protons corresponding to the C-18 methyl. Subsequent analysis of the HETCOR spectrum yielded the C-18 chemical shift as 24.8 ppm. C-29 was established in the same manner via its long range proton-proton coupling to C-5. The third methyl was established by difference nOe measurements. A 6.4% nOe enhancement of the proton on C-19 was observed when decoupling the  $^1\text{H}$  methyl singlet at  $\delta$  1.36. This peak correlated with the  $^{13}\text{C}$  methyl signal at 31.5 ppm and corresponds to C-30, thus also establishing the  $\beta$  stereochemistry at C-19. The remaining methyl must by default be assigned to C-28. C-29 and C-28 could be differentiated on the grounds that C-29 experiences a strong  $\gamma$ -gauche shielding effect from C-6.

Both obacunone [**1**] and acidissimin [**2**] are obviously derived from a common modified tetranortriterpene precursor in which the C-3–C-4 bond has been

oxidatively cleaved as represented by **3**. In **1**, C-19 remains as a methyl group (R = Me), although in **2**, C-19 has been oxidized to an aldehyde (R = CHO). Acetal formation in **2** to give both a six-membered lactone and a tetrahydrofuran takes preference over formation of the seven-membered lactone observed in **1**.

## EXPERIMENTAL

**GENERAL EXPERIMENTAL PROCEDURES.**—Melting points were determined on a Kofler hot-stage microscope and are uncorrected. Optical rotations were measured with either a Perkin-Elmer Model 241 or Model 121 polarimeter at 21°. Uv spectra were recorded on a Varian DMS90 spectrophotometer and ir spectra on either a Shimadzu Model IR-408 or a Perkin-Elmer Model 683 instrument. Low resolution eims and cims ( $\text{NH}_3$ ) were obtained using a VG Micromass 7070F spectrometer operating at 70 eV. Exact mass measurements were carried out on the same instrument using perfluorokerosene as reference.  $^1\text{H}$ -nmr homonuclear correlated 2D (COSY) spectra were recorded on a Varian VXR 500 spectrometer;  $^1\text{H}$ - and  $^{13}\text{C}$ -nmr 1D, heteronuclear 2D (HETCOR) spectra, as well as nOe measurements, were recorded on a Varian VXR 300S spectrometer. Chemical shift values are given in  $\delta$  (ppm) in  $\text{CDCl}_3$  with TMS as the internal standard (s, singlet; d, doublet; t, triplet; q, quartet; m, multiplet; br, broad).

**PLANT MATERIAL.**—The stem bark and root bark of *L. acidissima* were collected from a reservation park at Mihintale, North-Central Province of Sri Lanka in February 1984. A herbarium sample representative of the collection is deposited in the herbarium of the Department of Botany, University of Peradeniya, Sri Lanka.

**ISOLATION OF OBACUNONE [1].**—Air-dried and powdered root bark (2 kg) was exhaustively extracted with petroleum ether (bp 60–80°) under reflux conditions. Evaporation of solvent on a rotavapor yielded the petroleum ether extract as a brown semi-solid (40 g). A part of the extract (30 g) was subjected to cc on Si gel (mesh 35–75, 600 g) using solvent combinations of petroleum ether, EtOAc, and MeOH with increasing polarity. The fraction eluted with petroleum ether-EtOAc (3:2) was further partitioned by flash chromatography on two consecutive Si gel 60 columns. The fraction eluted with  $\text{CH}_2\text{Cl}_2$ -MeOH (99:1) from the first column was placed on a second column. The fraction eluted with  $\text{CH}_2\text{Cl}_2$  from this column was further purified on preparative tlc plates using  $\text{Et}_2\text{O}$ -MeOH (49:1) as the eluent to obtain obacunone [**1**] as a white crystalline solid (27 mg). This compound was identified

by comparison of its physical and spectral properties (mp, optical rotation, uv, ir,  $^1\text{H}$  nmr, ms) with those reported previously (15, 16). The  $^{13}\text{C}$ -nmr chemical shifts of **1** have been unambiguously reassigned using HETCOR techniques (Table 1).

**ISOLATION OF ACIDISSIMIN [2].**—Air-dried and powdered stem bark (4 kg) was successively and exhaustively extracted with petroleum ether and  $\text{CH}_2\text{Cl}_2$  under reflux conditions. Removal of the solvent on a rotavapor gave the extract as a brown semi-solid (30 g). A part of the extract (27 g) was fractionated on a Si gel (mesh 35–75, 600 g) column using solvent combinations of petroleum ether,  $\text{CHCl}_3$ , and MeOH with increasing polarity. The fraction eluted with  $\text{CHCl}_3$  was partitioned by flash chromatography on a Si gel 60 column. The fraction eluted from this column with  $\text{CH}_2\text{Cl}_2$  was further purified on preparative tlc plates using  $\text{Et}_2\text{O}$  as the eluent to obtain a white crystalline solid (30 mg) which has been named acidissimin [**2**]: mp  $284^\circ$ ;  $[\alpha]_{\text{D}} +22^\circ$  ( $c = 0.5$ ,  $\text{Me}_2\text{CO}$ ); uv  $\lambda$  max (MeOH) (log  $\epsilon$ ) 206 nm (8.8); ir  $\nu$  max ( $\text{CHCl}_3$ ) 1740, 1720, 1030,  $975\text{ cm}^{-1}$ ; hreims  $m/z$   $[\text{M} - \text{Me}]^+$  453.1551, calcd for  $\text{C}_{25}\text{H}_{25}\text{O}_8$ , 453.1549;  $m/z$   $[\text{M} - \text{C}_6\text{H}_3\text{O}_3]^+$  345.1703, calcd for  $\text{C}_{20}\text{H}_{25}\text{O}_5$ , 345.1702; eims (70 eV)  $m/z$  (rel. int.) 453 (0.5), 411 (0.5), 397 (0.5), 381 (0.5), 345 (65), 241 (2), 229 (3.5), 201 (6), 193 (7), 175 (9), 174 (10), 135 (12), 123 (12), 121 (15), 119 (12), 115 (10), 109 (13), 107 (17), 105 (20), 95 (70), 91 (45), 77 (41), 69 (33), 59 (54), 43 (100); cims ( $\text{NH}_3$ )  $m/z$  (rel. int.)  $[\text{MH}]^+$  469, (54), 345 (100), 171 (27), 169 (26);  $^1\text{H}$  nmr ( $\text{CDCl}_3$ )  $\delta$  7.41 (1H, br s, H-21), 7.40 (1H, br s, H-23), 6.53 (1H, d,  $J = 10$  Hz, H-1), 6.32 (1H, br s, H-22), 6.13 (1H, d,  $J = 10$ , H-2), 6.06 (1H, s, H-19), 5.53 (1H, s, H-17), 3.99 (1H, s, H-15), 2.60–2.75 (4H, m, H-6, H-9, H-5), 1.50–1.87 (4H, m, H-11, H-12), 1.36 (3H, s, H-30), 1.28 (6H, s, H-18, H-29), 1.16 (3H, s, H-28);  $^{13}\text{C}$  nmr see Table 1.

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