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ACIDISSIMIN, A NEW TYRAMINE DERIVATIVE FROM THE FRUIT OF *LIMONIA ACIDISSIMA*

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ABSTRACT.—The isolation of a new tyramine derivative, acidissimin [**1**], from fruits of *Limonia acidissima* is described, and its structure elucidated. The details of the molecular structure have been established through uv, ir, ^{1}H-nmr, ^{13}C-nmr, eims, and cims measurements. Isolation of degraded material **2**, its spectral measurements, and spin decoupling experiments on **1** conclusively established the structure of acidissimin [**1**] as benzamide-*N*- {*p*-[(3,7-dimethyl-4-octadecanoyloxy-2,6-octadienyl) oxy] phenethyl }.

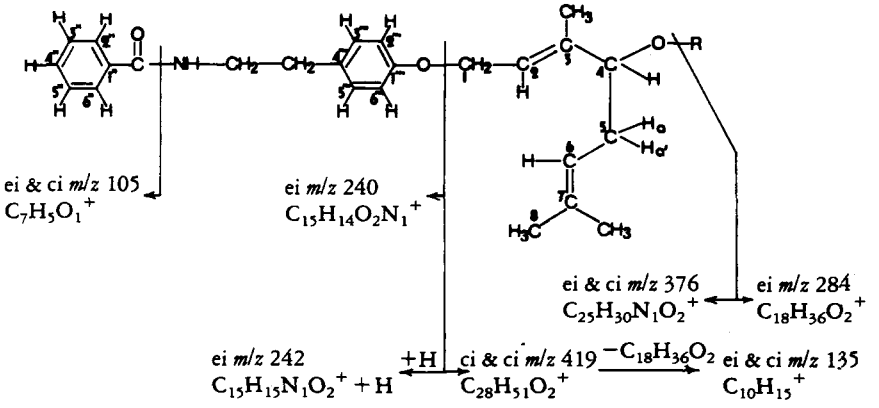
During our investigation of compounds from Indian medicinal plants (1), a new tyramine derivative, designated as acidissimin [**1**], was isolated from the fruits of *Limonia acidissima* L. (Rutaceae). The occurrence of *N*-benzoyl tyramine and its derivatives was previously reported from *Casimiroa edulis* Lex et Llave (2), *Severinia buxifolia* (Poir.) Ten. (3), *Swinglea glutinosa* (Bl.) Merr. (4), and *Pleiospermium alatum* (Wight & Arn.) Swingle (5), all belonging to the Rutaceae, and *Aniba riparia* (Nees) Mez (6) (Lauraceae). In the present communication, the structural elucidation of acidissimin [**1**] is described on the basis of the results obtained through physicochemical studies.

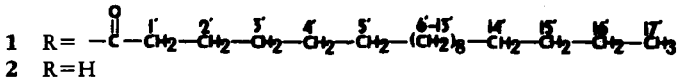
RESULTS AND DISCUSSION

The petroleum ether (60–80°) extract of the dried fruit of *L. acidissima*, on repeated chromatography over neutral alumina, furnished the white amorphous compound acidissimin [**1**], mp 65°, $[\alpha]_D \pm 0°$, $[MH]^+$ 660.4870 ($C_{43}H_{66}N_1O_4$) in cims, and the peak at highest mass as recorded in eims was observed at *m/z* 419 ($C_{28}H_{51}O_2$). From consideration of the cims and eims spectra, together with high resolution measurements, the main fragmentations for acidissimin [**1**] are set out in Scheme 1. The ir spectrum showed bands at 3342 (-NH), 2918, 2848 (C-H), 1733 (ester carbonyl), 1640 (amide carbonyl), 1531, 1509 (aromatic), 1240, 1110 (C-O-C), 809, 828 cm^{-1} (>C=C-H), and the uv spectrum registered λ max (MeOH) at 225, 277 sh, and 286 nm. The 400 MHz ^{1}H-nmr and 62.9 MHz ^{13}C-nmr (Table 1) spectral data of acidissimin revealed the presence of an ester carbonyl (δC 172.84), the benzoyl function of the amide residue [δC 128.47, 129.66, 131.26, 134.72, and 167.32; δH 7.688 (2H), 7.405 (2H), and 7.480 (1H)] and the 1,4-disubstituted aromatic ring [δC 115.03, 122.78, 130.98, and 157.45; δH 6.853 (2H) and 7.141 (2H)]. The ^{13}C nmr using the INEPT technique registered seven quarternary, eight methine, thirteen methylene, and four methyl carbon signals. The signal assignments are presented in Table 1.

The presence of an *N*-benzoyl-*O*-substituted tyramine residue in acidissimin [**1**] is clearly evident from the appearance of the secondary amide proton signal as a broad triplet at δ 6.109 and the signals at δ 2.873 and 3.688 as a triplet and a slightly broadened quartet for the methylene protons of Ar-CH_2 and HN-CH_2, respectively. This partial structure was further supported from mass spectral peaks at *m/z* 242 $[C_{15}H_{15}N_1O_2+H]^+$ and 105 $[C_6H_5CO]^+$.

Furthermore, the peak at *m/z* 419 $[C_{28}H_{51}O_2]^+$ and the base peak at *m/z* 135





SCHEME 1. Structure of acidissimin [**1**], its ms fragmentation peaks, and ^{13}C-nmr chemical shift values.

TABLE 1. ^{1}H-nmr (400 MHz) and ^{13}C-nmr (62.9 MHz) Data of Acidissimin [**1**], in ppm with $CDCl_3$ as Solvent and TMS as Internal Reference.

Position	δC	δH	Multiplicity	J (Hz)
1	64.58	4.557 (2H)	d	6.05
2	126.77	5.712 (1H)	tt	6.05, 1.1
3	137.65	—	—	—
3-Me	13.09	1.723 (3H)	d	1.1
4	77.50	5.151 (1H)	t	6.9
5a	31.74	2.308 (1H)	ddd	6.6, 12.2, 13.0
5a′		2.393 (1H)	dd	7.1, 14.6
6	118.93	5.010 (1H)	tt	7.1, 1.4
7	134.29	—	—	—
7-Me	25.72	1.664 (3H)	d	1.1
8	17.91	1.606 (3H)	d	0.8
4-O-C(=O)-	172.84	—	—	—
1′	34.60	2.283 (2H)	dd or t	7.4, 7.6
2′–16′	[a]	1.250 (30H)	s (broad)	—
17′	14.08	0.878 (3H)	t	6.9
1″	134.72	—	—	—
2″,6″	129.66	7.688 (2H)	dt	6.9, 1.6
3″,5″	128.47	7.405 (2H)	tt	7.4, 1.5
4″	131.26	7.480 (1H)	tt	7.4, 1.4
CO-NH	167.32	6.109 (1H)	t (broad)	6.0
N-CH_2	41.28	3.688 (2H)	br q	7.1, 1.4
Ar-CH_2	34.84	2.873 (2H)	t	6.9
1‴	157.45	—	—	—
2‴,6‴	122.78	6.853 (2H)	dt	8.8, 2.5
3‴,5‴	115.03	7.141 (2H)	dt	8.8, 2.5
4‴	130.98	—	—	—

[a] δC of 2′, 3′, 4′, 5′, 6′–13′, 14′, 15′, and 16′ are at 25.05, 29.15, 29.30, 29.33, 29.68, 29.49, 31.91, and 22.67, respectively.

$[C_{10}H_{15}]^+$, arising from the loss of octadecanoic acid, i.e., $[419-C_{18}H_{36}O_2]^+$, and the peak at *m/z* 284 confirmed the presence of a monoterpenoid stearate residue in acidissimin [**1**].

The ^{1}H-nmr spectrum clearly indicated the appearance of three signals for protons at C-8, 7-Me, and 3-Me at δ 1.606, 1.664, and 1.723, respectively, as three sets of doublets because of the long-range coupling with allylic protons at C-6 and C-2. The two-proton doublet at δ 4.557 and one proton triplet at δ 5.151 disclosed the environments of the C-1 and C-4 protons as O-CH_2-CH=C< and -CH(CH_2)-OR, respectively. The vinylic protons at C-2 and C-6 appeared as an apparent tt pattern at δ 5.712 and δ 5.010, respectively, whereas the C-5 protons showed the expected ddd and dd pattern for a 4-substituted geranyloxy residue. The ^{1}H-nmr signal at δ 5.712 (H-2) might also be considered to be arising from the neryloxy residue (4), but the ^{13}C-nmr chemical shift value of the methyl carbon ($^{\delta}$C 13.09) attached to the olefinic double bond appears to be more diagnostic (7–10) in judging the stereoisomerism in geranyl-neryl systems. Based on this, the 4-substituted geranyloxy moiety is proposed for the acidissimin molecule. An upfield three-proton triplet at δ 0.878 (H_3-17′), a broad singlet at δ 1.250 (H_2-2′ to H_2-16′) for thirty methylene protons, and a dd or t pattern at δ 2.283 for H_2-1′ for an O-CO-CH_2- environment definitely suggest an octadecanoyloxy function that can only be attached to the geranyl residue at its 4 position in order to explain the nmr pattern observed for acidissimin [**1**].

The appearance of a peak at *m/z* 376 $[C_{25}H_{30}N_1O_2]^+$, representing loss of octadecanoic acid from the molecular ion, indicates strongly that in the fragment bearing *m/z* 376 the tyramine and geranyl moieties are linked through an ether bond (Scheme 1).

Selective spin decoupling studies have also been conducted on different nmr signals, and the variations observed in the related signal positions are summarized in Table 2. The observed changes conclusively prove structure **1** for acidissimin.

Base saponification of acidissimin [**1**] with 5% EtOH/KOH at room temperature overnight furnished octadecanoic acid and a compound **2** having $[MH]^+$ 394.2371 ($C_{25}H_{32}N_1O_3$). The structure of octadecanoic acid was confirmed through direct comparison with its spectral data, and the structure for **2** received confirmation through its ir (3336 cm^{-1}) and 400 MHz ^{1}H-nmr spectral measurements.

EXPERIMENTAL

GENERAL EXPERIMENTAL PROCEDURES.—Mp's are uncorrected. ^{1}H-nmr (400 MHz and 250 MHz) and ^{13}C-nmr (62.9 MHz) spectra were taken in $CDCl_3$ using TMS as internal standard

TABLE 2. Results of Selective Decoupling on Acidissimin [**1**] (250 MHz, $CDCl_3$).

Signal irradiated at δ	Changes observed at δ
0.878	No Change
2.308, 2.393 (2.283)	5.010 → broad singlet
	5.151 → singlet
2.873	3.688 → doublet
3.688	2.873 → simplified
	(6.109 → changed the shape)
4.577	5.712 → singlet
5.010	2.308, 2.393 → simplified
5.151	2.308, 2.393 → simplified
5.712	4.557 → singlet
6.109	3.688 → triplet

on JNMR-GX 400 NMR and Hitachi R-250 NMR spectrometers; ms were measured at 100 eV (ci in beam) and 70 eV (ei) on a Hitachi M-80B double focusing gas chromatograph-mass spectrometer; ir spectra were measured on a Nihon Bunko IR-810 infrared spectrophotometer, and the uv spectrum was obtained on a Beckman 26 spectrophotometer. Si gel was used for tlc and neutral Al_2O_3 for cc.

PLANT MATERIAL.—Plant material for the investigation was collected locally, and voucher specimens (No. 386 and 388) are kept at the Herbarium of the Department of Botany, University of Burdwan.

EXTRACTION AND ISOLATION PROCEDURE.—Air-dried fruits of *L. acidissima* (2 kg) were exhaustively extracted with petroleum ether (60–80°) for 48 h in a Soxhlet extractor. The solvent was removed under reduced pressure, and the crude extract (43 g) was subjected to cc over neutral Al_2O_3. Elution with solvent and solvent mixtures [petroleum ether, petroleum ether-C_6H_6 (9:1), petroleum ether-C_6H_6 (7:3), petroleum ether-C_6H_6 (1:1), C_6H_6, C_6H_6-$CHCl_3$ (3:1), C_6H_6-$CHCl_3$ (1:1), $CHCl_3$, $CHCl_3$-MeOH (99:1), $CHCl_3$-MeOH (95:5), $CHCl_3$-MeOH (90:10)] of increasing polarity resulted in the isolation of a crude brownish material from the petroleum ether-C_6H_6 (9:1) eluate: crystallization from a hexane/EtOAc mixture furnished a white amorphous compound, acidissimin [**1**] (58 mg), mp 65°, $[\alpha]_D \pm 0°$ ($CHCl_3$).

ACIDISSIMIN [**1**].—Cims *m/z* (rel. int.) $[MH]^+$ 660 (2), 419 (18), 376 (69), 242 (42), 135 (100), 105 (46); eims *m/z* 419 (17), 376 (3), 284 (5), 240 (5), 135 (100), 105 (39).

SAPONIFICATION OF ACIDISSIMIN [**1**].—Acidissimin [**1**] (20 mg) was hydrolyzed with 5% EtOH/KOH (25 ml) at room temperature overnight. After usual processing, the crude hydrolysates were purified through preparative tlc over Si gel. The neutral fraction on two developments using hexane-EtOAc (1:1) afforded purified **2** (5.3 mg, R_f 0.31), and the acidic fraction on three developments using hexane-EtOAc (2:1) furnished octadecanoic acid (2 mg, R_f 0.16).

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