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TYRAMINE DERIVATIVES FROM THE FRUIT OF *LIMONIA ACIDISSIMA*

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ABSTRACT.—The isolation of two new tyramine derivatives, acidissiminol [**1**] and acidissiminin epoxide [**2**], and *N*-benzoyltyramine [**3**] from the fruits of *Limonia acidissima* is described. Structures were elucidated by uv, ir, nmr, eims, and/or cims measurements. The comparison of spectral data of **1** with those of an authentic sample prepared from acidissiminin [**4**], previously isolated from the same plant, conclusively established the structure of **1** as benzamide-*N*-{*p*-[(3,7-dimethyl-4-hydroxy-2,6-octadienyl)oxy]phenethyl}. Spin decoupling experiments on **2** and structure elucidation of degraded material **5** obtained through hydrolytic cleavage of **2**, established the structure of acidissiminin epoxide [**2**] as benzamide-*N*-{*p*-[(3,7-dimethyl-6,7-epoxy-4-octadecanoyloxy-2-octenyl)oxy]phenethyl}.

The family Rutaceae is known for economically important secondary metabolites (1). *Limonia acidissima* L. is well known for its medicinal properties (2). Several coumarins, a triterpene, steroids, limonoids, benzoquinone and alkaloids (3–7), acids (8), and a tyramine derivative, acidissiminin (9), have already been reported from different parts of this plant. The occurrence of *N*-benzoyltyramine and its derivatives was pre-

viously reported from a few species of Rutaceae (10–15) and from one species of Lauraceae (16). We now report the structure elucidation of two new tyramine derivatives, acidissiminol [**1**] and acidissiminin epoxide [**2**], along with the isolation of *N*-benzoyltyramine [**3**] from the fruits of *L. acidissima*.

RESULTS AND DISCUSSION

The MeOH extracts of the defatted

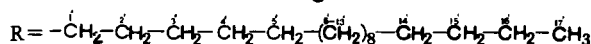
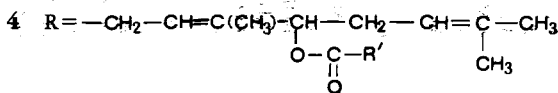
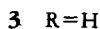
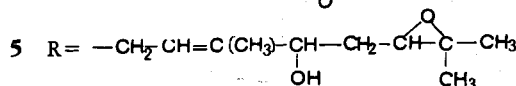
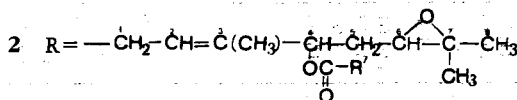
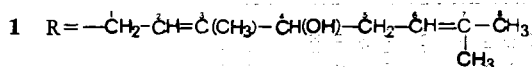
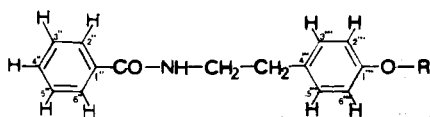


TABLE 1. ^1H -nmr and ^{13}C -nmr Data of Acidissiminol [1], Acidissimin Epoxide [2], and N-Benzoyltyramine [3] (ppm, CDCl_3 as solvent, TMS as internal standard).

Position	Compound											
	Compound 1		Compound 2				Compound 3					
	δ_{C}	δ_{H}	Multiplicity	J (Hz)	δ_{H}	Multiplicity	J (Hz)	δ_{H}	Multiplicity	J (Hz)		
1	64.59	4.584 (2H)	d	6.6	4.566 (2H)	d	6.3					
2	126.79	5.754 (1H)	t (br)	6.4	5.783 (1H)	tt	6.0, 1.1					
3	141.72											
3-Me	12.59	1.717 (3H)	d	1.1	1.750 (3H)	d	1.1					
4	76.21	4.063 (1H)	t (br)	7.3, 6.2	5.369 (1H)	t	6.3					
5a	34.8	2.285 (1H)	t (br)	7.3, 6.2	1.962 (1H)	ddd	6.3, 7.3, 14.3					
5a'	32.69				1.860 (1H)	rd	5.8, 14.6					
6	119.61	5.104 (1H)	t (br)	7.3, 1.2	2.706 (1H)	t	6.0					
7	134.67											
7-Me	25.93	1.639 (3H)	s	—	1.259 (3H)	s	—					
8	18.03	1.739 (3H)	s	—	1.275 (3H)	s	—					
4-OH		1.779 (1H)	s (br)	—				5.42 (1H)	s			
$\text{C}_6\text{H}_4\text{-OH}$												
4-O-C=O	172.72											
1'	34.52											
2'-16'												
17'	14.09											
1''	135.40											
2''-6''	129.77	7.750 (2H)	d (br)	8.6	2.329 (2H)	t	7.6					
3''-5''	128.57	7.439	m		1.249 (3OH)	s (br)	—					
4''	131.41				0.879 (3H)	t	6.9					
CO-NH	167.47							7.72 (2H)	m			
N-CH ₂	41.29	6.217 (1H)	q	6.7	7.692 (2H)	dt	6.9, 1.4					
Ar-CH ₂	34.79	3.679 (2H)	t	6.7	7.407 (2H)	tt	7.3, 1.1					
1'''	157.48	2.868 (2H)	t	6.7	7.482 (1H)	tt	7.4, 1.4					
2'''-6'''	120.89	6.873 (2H)	d	8.6	6.124 (1H)	t (br)	6.0					
3'''-5'''	114.93	7.148 (2H)	d	8.6	3.684 (2H)	q (br)	6.6					
4'''	130.95				2.873 (2H)	t	6.9					
								7.35-7.5 (3H)	m			
								6.15 (1H)	br peak	6.2		
								3.69 (2H)	q	6.2		
								2.86 (2H)	t			
								6.80 (2H)	d	8.2		
								7.15 (2H)	d	8.2		

^a δ_{C} of 2', 3', 4', 5', 6' to 13', 14', 15', and 16' at 24.99, 29.18, 29.29, 29.35, 29.68, 29.49, 31.93, and 22.68, respectively.

fruit on chromatography over neutral Al_2O_3 afforded three compounds. Acidissiminol [**1**]: mp 85–87°; hrcims m/z 394.2371 [MH]⁺ (calcd for $\text{C}_{25}\text{H}_{32}\text{N}_1\text{O}_3$, 394.2382). Acidissiminin epoxide [**2**], mp 105–106°; hrcims m/z 676.4875 [MH]⁺ (calcd for $\text{C}_{43}\text{H}_{66}\text{N}_1\text{O}_5$, 676.4941), highest mass peak in hrcims m/z 435.3873 (calcd for $\text{C}_{28}\text{H}_{51}\text{O}_3$, 435.3838). *N*-Benzoyltyramine [**3**]: mp 159–160°, hrcims m/z 242.1156 [MH]⁺ (calcd for $\text{C}_{15}\text{H}_{16}\text{N}_1\text{O}_2$, 242.1181). The spectral assignments for ¹H nmr of **1–3** and ¹³C nmr using DEPT and INEPT techniques on **1** and **2**, respectively, as presented in Table 1, are in conformity with the spectral data (1) for acidissiminin [**4**].

The ¹³C nmr (100.62 MHz) of **1** registered six quaternary, eight methine, four methylene, and three methyl carbon signals using the DEPT technique. The hydroxyl function (δ_{H} 1.779, broadened singlet) can only be attached to the geranyl residue at its C-4 position in order to explain the observed chemical shift values δ_{C} 76.21 and δ_{H} 4.063 (1H, broadened triplet) for C-4 and methine proton, respectively, at position 4. Comparison of spectral characteristics of **1** with those of an authentic compound, prepared through alkaline hydrolysis of acidissiminin [**4**], conclusively established the structure of acidissiminol [**1**] as benzamide-*N*-{*p*-[(3,7-dimethyl-4-hydroxy-2,6-octadienyl)oxy]phenethyl}.

Acidissiminin epoxide [**2**] showed seven quaternary, eight methine, thirteen methylene, and four methyl carbon signals in ¹³C nmr (62.9 MHz) using the INEPT technique. The presence of an epoxy function at the C-6, C-7 position of the geranyl residue in acidissiminin [**4**] was reflected through observed changes in the chemical shift values of C-6 and C-7 from δ_{C} 118.93 and 134.29 in **4** to δ_{C} 60.68 and 57.78 in **2**. The ¹H nmr also indicated expected changes in chemical shift values from δ_{H} 5.010 for H-6 in **4** to that of δ_{H} 2.706 in **2**. The results of spin decoupling studies on **2** at room temperature (Table 2) corroborated the proposed structure of **2** as benzamide-*N*-{*p*-[(3,7-dimethyl-6,7-epoxy-4-octadecanoyloxy-2-octenyl)oxy]phenethyl}. Alkaline hydrolysis of **2** at room temperature overnight resulted in isolation of **5** and octadecanoic acid.

The third tyramine derivative was identified as *N*-benzoyltyramine [**3**] [benzamide-*N*-(*p*-hydroxy phenethyl)] through spectroscopic analyses.

EXPERIMENTAL

GENERAL EXPERIMENTAL PROCEDURES.—Mp's are uncorrected. ¹H-nmr (400 MHz, 250 MHz, 200 MHz, 100 MHz) and ¹³C-nmr (62.9 MHz and 100.62 MHz) spectra were taken in CDCl_3 using TMS as internal standard on JNM-R-GX 400, Bruker AM 400, Hitachi R-250, Varian XL 200, and JEOL 100 NMR spectrometers. Mass spectral analyses were performed at 100 eV (ci in beam) and 70 eV (ei) on a Hitachi M-80B double focussing gas chromatograph-hrms. Ir spectra were measured on a Nihon

TABLE 2. Results of Selective Decoupling on Acidissiminin Epoxide [**2**] (250 MHz, CDCl_3).

Signal irradiated at (δ)	Change observed at (δ)
0.879	No change
1.960	2.706→singlet
2.329	No Change
2.706	1.860; 1.962→simplified
2.873	3.684→doublet
3.684	2.873→singlet
4.566	5.783→singlet
5.369	1.860; 1.962→simplified
5.783	4.566→singlet
6.124	3.684→triplet

Bunko IR-810 and Shimadzu IR-408 ir spectrometers. The uv spectra were obtained on a Beckman 26 spectrophotometer. Si gel was used for tlc and neutral Al_2O_3 for cc.

PLANT MATERIAL.—The fruits of *L. acidissima* for the investigation were collected in Burdwan during March and April, and voucher specimens (No. 386 and 388) are kept at the Herbarium of the Department of Botany, University of Burdwan.

EXTRACTION AND ISOLATION PROCEDURE.—Defatted fruits (petroleum ether 60–80°) (2 kg) were exhaustively extracted with MeOH for 48 h in a Soxhlet extractor. The solvent was removed under reduced pressure, and the crude extract (38 g) was subjected to cc over neutral Al_2O_3 . Elution with solvent mixtures of increasing polarity resulted in the isolation of compounds 1–3.

ACIDISSIMINOL [1].—A white amorphous material (36 mg) was isolated from the C_6H_6 - $CHCl_3$ (4:1) eluate, which on crystallization from hexane-EtOAc mixture showed mp 85–87°; uv λ max (MeOH) nm 268, 275, 283; ir ν max (KBr) cm^{-1} 3336 (-OH and -NH stretch), 1637 (amide carbonyl), 1577, 1543, 1509 (aromatic C-C stretch), 827, 809 (>C=C-H); hrcims m/z (rel. int.) [MH]⁺ 394.2371 (4), 324.1556 (20), 294.1461 (64), 242.1666 (61), 153.1246 (10), 135.1168 (60), 120.0563 (100).

ACIDISSIMININ EPOXIDE [2].—Petroleum ether (60–80°)- C_6H_6 (1:1) elution afforded 2 as a white amorphous material (20 mg). This on crystallization from hexane- C_6H_6 mixture showed mp 105–106°; uv λ max (MeOH) nm 225, 227, 286; ir ν max (KBr) cm^{-1} 3342 (-NH stretch), 2918, 2848 (C-H stretch), 1733 (ester carbonyl), 1531, 1509 (aromatic), 1240, 1110 (C-O-C), 828, 809 (>C=C-H); hrcims m/z (rel. int.) [MH]⁺ 676.4875 (5), 435.3806 (2), 392.2193 (25), 285.2873 (32), 242.1222 (61), 151.1123 (100), 105.0341 (50); hrcims m/z (rel. int.) 435.3873 (5), 284.2712 (8), 240.1072 (10), 151.1097 (100), 105.0326 (18).

SAPONIFICATION OF ACIDISSIMININ EPOXIDE [2].—Acidissiminin epoxide [2] (20 mg) was hydrolyzed with 5% KOH/EtOH (25 ml) at room temperature overnight. The reaction mixture was added to H_2O (30 ml) and extracted twice with Et_2O . The combined Et_2O layer was washed with H_2O , dried over anhydrous Na_2SO_4 , and evaporated under reduced pressure to give 5 (11 mg). This was purified over Si gel tlc [hexane-EtOAc (1:1) 2 developments] which gave purified 5 (2.9 mg, R_f 0.05). The aqueous layer of the hydrolysis product was acidified with dilute HCl and extracted with Et_2O . Usual workup afforded octadecanoic acid (5 mg), which on preparative Si

gel tlc [hexane-EtOAc (2:1), 3 developments] afforded purified octadecanoic acid (2 mg, R_f 0.16, co-chromatographed with authentic octadecanoic acid).

COMPOUND 5.—Hrcims (in beam) 100 eV, m/z (rel. int.) [MH]⁺ 410.2345 (27), 392.2241 (13), 376.2233 (3), 242.1162 (100), 169.1243 (53), 155.1453 (10), 151.1154 (13), 120.0583 (58), 105.0350 (46); ir ν max (KBr) cm^{-1} 3420 (-OH), 3334 (-NH), 1640 (-CONH), 1578, 1534, 1510 (aromatic C-C stretch), 1110 (C-O-C), 826, 804 (>C=C-H).

OCTADECANOIC ACID.—Eims (70 eV) m/z (rel. int.) 284 (33), 256 (3), 241 (9), 185 (8), 129 (22), 73 (75), 43 (100).

N-BENZOYLTYRAMINE [3].—Compound 3 isolated from C_6H_6 - $CHCl_3$ (3:1) eluate and crystallized from a hexane/EtOAc mixture furnished a white amorphous compound (73 mg), mp 159–160°; uv λ max (MeOH) nm 226, 274; ir ν max (KBr) cm^{-1} 3340 (-OH and -NH stretch), 1655 (amide carbonyl), 1565, 1530, 1500 (aromatic C-C stretch); hrcims m/z (rel. int.) [MH]⁺ 242.1156 (100), 129.0918 (4), 120.0569 (30), 105.0349 (16).

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