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Isobutylamides from the fruit of *Zanthoxylum integrifoliolum*

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Abstract

Investigation of the fruit of *Zanthoxylum integrifoliolum* led to the isolation of three new isobutylamides, lanyuamide I–III and six known isobutylamides, tetrahydrobungeanool, γ-sanshoöl, hydroxy γ-sanshoöl, mixture of (2*E*,4*E*,8*Z*,11*E*)- and (2*E*,4*E*,8*Z*,11Z)-2′-hydroxy-*N*-isobutyl-2,4,8,11-tetradecatetraenamide and hazaleamide which was mixed with lanyuamide III. These amides were all with a (2*E*,4*E*)-dienamide moiety and their structures were elucidated on the basis of spectral analyses.

Keywords: Zanthoxylum integrifoliolum; Rutaceae; Fruit; Alkaloids; Amides; Isobutylamides; Lanyuamide

1. Introduction

Zanthoxylum integrifoliolum (Merr.) Merr. (Rutaceae) is a large evergreen tree that grows only in the northern Philippines and on Lanyu Island in Taiwan province (Chang & Hartley, 1993). Its bark is used by Ya-Mei aborigines as a folk medicine to treat snake-bites. The fruit possesses a pungent taste, but has not been utilized as a substitute for Pericarpi Zanthoxyli. The chemical constituents of the bark (Chua, Maglaya, & Santos, 1970; Jen, Tsai, Horng, & Chen. 1993) and root wood (Ishii, Chen, Akaike, Ishikawa, & Lu, 1982) of this plant have been studied. In a preliminary study, three indolopyridoquinazoline alkaloids with anti-platelet aggregation activity were obtained from a small amount of the fruit (Sheen, Tsai, Teng, Ko, & Chen, 1996). Further examination of the chemical constituents and anti-platelet aggregation principles obtained from large amounts of fruit has resulted in the isolation of nine isobutylamides, including three new compounds: lanyuamide I–III (**1**–**3**) and six known amides, tetrahydrobungeanool (**4**) (Xiong, Shi, Yamamoto, & Mizuno, 1997), γ-sanshoöl (**5**) (Yasuda, Takeya, & Itokawa, 1981, 1982), hydroxy γ-sanshoöl (**6**) (Yasuda et al., 1981, 1982), mixture of (2*E*,4*E*,8*Z*,11*E*)- and (2*E*,4*E*,8*Z*,11*Z*)-2′-hydroxy-*N*-isobutyl-2,4,8,11-tetradecatetraenamide (**7** and **8**) (Mizutani et al., 1988) and hazaleamide (**9**) (Shibuya, Takeda, Zhang, Tong, & Kitagawa, 1992) which was mixed with **3**. Here, we describe, for the first time, the structural elucidation of these new amides.

2. Results and discussion

Nine amides (**1**–**9**) were obtained as colorless oils by chromatography on a silica gel column. The UV spectra of each amide showed maximal absorption near 259 nm, indicating the presence of a conjugated system related to sorbic acid isobutylamides (Eisner, Elvidge, & Linstead, 1953; Crombie, 1955). The IR spectra of each amide showed characteristic bands for amidoamino and amidocarbonyl groups, indicating the presence of a *trans*-2-*trans*-4-dienamide skeleton (Crombie, 1955; Dhar & Atal, 1967).

Lanyuamide I (**1**) showed an additional ketone absorption at 1700 cm^{-1} in its IR spectrum. The ^1H NMR spectrum showed an *N*-isobutyl group [δ 0.92 (6H, d, J = 6.8 Hz, H-3′, 4′), 1.79 (1H, m, H-2′), 3.16

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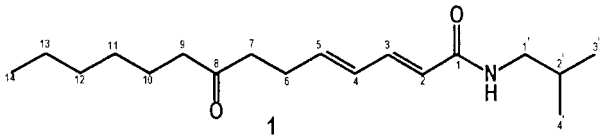

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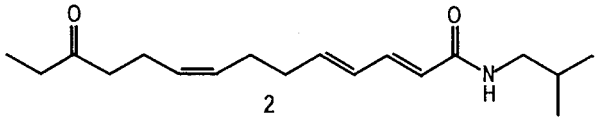

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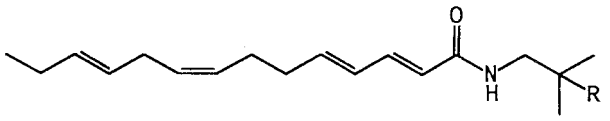

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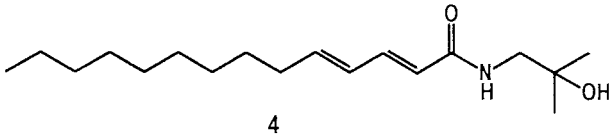

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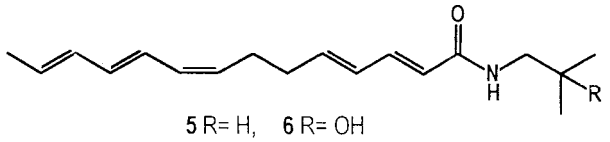

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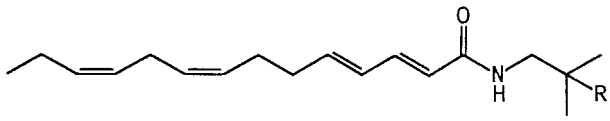

8 R= OH, 9 R= H

(2H, t, J = 6.8 Hz, H1′), 5.47 (1H, br s, NH)], four protons on a *trans*-2-*trans*-4-dienamide [δ 5.75 (1H, d, J = 14.9 Hz, H-2), 6.02 (1H, dt, J = 15.1, 6.9 Hz, H-5), 6.16 (1H, dd, J = 15.1, 10.6 Hz, H-4), 7.16 (1H, dd, J = 14.9, 10.6 Hz, H-3)], two methylenes [δ 2.42 (2H, q, J = 6.9 Hz, H-6) and 2.53 (2H, t, J = 6.9 Hz, H-7)] and an *n*-hexyl group [δ 0.88 (3H, t, J = 6.8 Hz, H-14), 1.25 (6H, br s, H-11–13), 1.56 (2H, quint, J = 7.1 Hz, H-10), 2.39 (2H, t, J = 7.1 Hz, H-9)] connected to a keto group. The molecular formula of **1** was established as $C_{18}H_{31}NO_2$ by El ($[M]^+$, m/z 293) and HR-mass spectrometry and the position of the keto group at C-8 was supported by prominent mass fragments at m/z 113 $[C_7H_{13}O]^+$ and 180 $[C_{11}H_{18}ON]^+$. According to the above data, the structure of **1** was predicted to be a (2*E*,4*E*)-8-keto-*N*-isobutyl-2,4-tetradecadienamide, which was further confirmed by COSY, HETCOR and ^{13}C NMR spectral analyses (Table 1).

Table 1
^{13}C NMR spectral data of compounds **1**–**9** (100 MHz, $CDCl_3$)

Carbon	1	2	3	4	5	6	7	8	9
1	166.1	166.2	166.3	167.5	166.3	167.3	167.4	167.4	166.3
2	122.5	122.1	122.1	121.1	122.3	121.6	121.5	121.5	122.1
3	140.6	141.0	141.1	142.0	140.8	141.7	141.8	141.8	141.1
4	129.1	128.7	128.6	128.1	128.7	128.7	128.5	128.6	128.6
5	140.7	141.9	142.0	143.8	141.6	142.3	142.7	142.6	142.0
6	23.8	32.8	32.9	32.9	32.9	33.0	32.9	32.9	32.9
7	41.5[a]	26.4	26.4	28.8	27.0	27.0	26.4	26.5	26.5
8	209.9	128.8	127.0	29.2	129.8	129.8	127.1	127.1	127.0
9	43.0[a]	129.5	128.8	29.3	129.4	129.5	128.7	129.1[a]	128.8[a]
10	28.9	21.7	30.4	29.4	125.3	125.3	30.4	25.6	25.6
11	27.0	42.0	132.6	29.5	133.3	133.4	132.6	132.1	132.0
12	31.6	211.1	129.0	31.9	131.8	131.8	128.8	128.5[a]	129.0[a]
13	22.5	36.0	25.6	22.7	129.9	130.0	25.5	20.6	20.5
14	13.9	7.76	13.8	14.1	18.3	18.3	13.8	14.2	14.2
1′	46.9	46.8	46.9	50.5	46.9	50.5	50.5	50.5	46.9
2′	28.6	28.6	28.6	71.1	28.6	71.1	71.7	71.1	28.6
3′	20.1	20.1	20.1	27.3	20.1	27.3	27.3	27.3	20.1
4′	20.1	20.1	20.1	27.3	20.1	27.3	27.3	27.3	20.1

[a] Assignments may be reversed in each column.

Lanyuamide II (**2**) showed an $[M+1]^+$ ion at m/z 292 by FAB mass spectroscopy, which is 2 amu less than that of **1** and suggests one more disubstituted double bond in **2**.The ^1H NMR spectrum of **2** indicated that the two allylic methylenes at δ 2.19 (4H, m, H-6 and H-7) were coupled with H-5 of a 1,3-dienamide and one of the olefinic protons (2H, m), which also coupled with the third allylic methylene at δ 2.30 (2H, q, J = 6.8 Hz, H-10). The additional double bond was assigned at C-8 and C-9 by a subsequent COSY experiment. The geometry of the C-8 and C-9 double bond was determined to be the *Z*-form by the chemical shift of allylic C-7 at δ 26.4 ppm and C-10 at δ 21.7 ppm (Xiong et al., 1997). Two methylene groups, [(δ 2.41, 2H, q, J = 7.2 Hz, H-13) and 2.44 (2H, t, J=7.6 Hz)], neighboring a keto group, were found in the ^1H NMR spectrum. The former was only coupled with a primary methyl group at δ 1.05 (3H, t, J = 7.2 Hz, H-14) and the latter was coupled with H-10 at δ 2.30 (2H, q, J = 6.8 Hz, H-11). Thus, this keto group was unambiguously assigned at C-12, which appeared at δ 211.1 ppm in the ^{13}C NMR spectrum and showed maximal absorption at 1720 cm^{-1} in the IR spectrum. According to the above data, the structure of **2** was elucidated as (2*E*,4*E*,8*Z*)-12-keto-*N*-isobutyl-2,4,8-tetradecatrienamide, and fully confirmed by COSY, HETCOR, DEPT and ^{13}C NMR spectral analyses (Table 1).

Lanyuamide III (**3**) was isolated as an oil, admixed with a structurally-similar amide in a 1:3 ratio. The mixture showed a molecular ion at m/z 275 and a molecular formula as $C_{18}H_{29}NO$ by El- and HR mass

spectrometry. All major signals in the ^{1}H NMR spectrum were completely identical with those of hazaleamide (**9**) (Shibuya et al., 1992). However, the chemical shifts of minor signals at δ 0.93 (6H, d, J = 6.8 Hz), 0.96 (3H, t, J = 7.4 Hz), 2.00 (2H, m), 2.74 (2H, t, J = 6.0 Hz) and 5.31 (2H, m) of **3** were all close to H-3′ and H-4′, H-14, H-13, H-10 and H-11, H-12 of **9**. Thus, these protons in **3** could be assigned to the same location as those in **9**. The other signals of **3** completely overlapped with the respective signals of **9** as measured by their integration. From the above analyses of the ^{1}H NMR spectrum, the major **9** and the minor **3** components of the mixture were deduced to be a pair of geometric isomers which differed only at one double bond. Thus, the presence of allylic carbons, C-10 at δ 30.4 and C-13 at δ 25.6 as minor signals in **3** substantially differed from C-10 at δ 25.6 and C-13 at δ 20.5 in **9**. This fact supports the geometry of C-11 and C-12 in **3** to be in the *E*-form (Xiong et al., 1997). From the above data, the structure of **3** was elucidated as (2*E*,4*E*,8*Z*,11*E*)-*N*-isobutyl-tetradecatetraenamide, which was further confirmed by COSY, HETCOR, DEPT and ^{13}C NMR spectral analyses (Table 1).

3. Experimental

M.p.'s are uncorr. ^{1}H (400 MHz) and ^{13}C (100 MHz) NMR were taken in $CDCl_3$. Chemical shifts were given in δ with TMS as int. standard. MS were measured using a direct inlet system. IR spectra were determined neat, and UV spectra were measured with EtOH as solvent. Silica gel (60–230 mesh, 230–400 mesh) (Merck) was used for CC and silica gel 60 F-254 for TLC.

3.1. Plant material

Fruits of *Z. integrifoliolum* were collected at Lanyu Island, Tai-tung County, Taiwan, on August 12th, 1995. A voucher specimen was deposited in the Herbarium of the School of Pharmacy, Kaohsiung Medical College, Kaohsiung, Taiwan.

3.2. Extraction and isolation

Dried fruits (16.5 kg) were brayed, extracted with MeOH and concd in vacuo to leave a brownish fluid. The MeOH ext. was partitioned with $CHCl_3$–H_2O (1:1). The H_2O soluble fr. was partitioned between H_2O: *n*-BuOH (1:1) to afford a H_2O fr. (620 g) and *n*-BuOH fr. (130 g). The $CHCl_3$ soluble fr. was extracted with 90% MeOH: *n*-hexane (1:1), yielding an *n*-hexane fr. (420 g). The 90% MeOH ext. was first treated with $CHCl_3$ to produce yellowish crystal I (2.54 g), then a second batch of yellowish crystal II (0.58 g) was obtained from the filtrate. The filtrate was concd under red. pres. to obtain a $CHCl_3$ fr. (220 g). Part of the $CHCl_3$ soluble fr. (99 g) was chromatographed over silica gel (2,500 g), eluting with CH_2Cl_2 gradually enriched with EtOAc to give 12 frs (C1–C12) [Cl (3.75 g, CH_2Cl_2), C2 (0.88 g, CH_2Cl_2), C3 (2.92 g, CH_2Cl_2), C4 (11.4 g, CH_2Cl_2), C5 (1.63 g, CH_2Cl_2), C6 (0.15 g, CH_2Cl_2–EtOAc, 8:2), C7 (1.63 g, EtOAc), C8 (19.1 g, EtOAc), C9 (18.1 g, EtOAc), C10 (1.36 g, $CHCl_3$–MeOH, 19:1), C11 (8.0 g, $CHCl_3$–MeOH, 9:1), C12 (5.1 g, $CHCl_3$–MeOH, 8:2)]. Fr. C5 (1.63 g) was rechromatographed on silica gel (49 g) using *n*-hexane–acetone (2:1) to yield 12 frs (C5-1–C5-12). Fr. C5-1 (64.5 mg) was purified by prep. TLC (*n*-hexane–acetone, 5:1) to yield a mixture of **3** and **9** (23.5 mg). Fr. C5-2 (40 mg) was purified with prep. TLC (CH_2Cl_2: EtOAc, 15:1) to afford **5** (24.7 mg). Fr. C8 (19.1 g) was rechromatographed on silica gel (480 g) and eluted with $CHCl_3$–MeOH mixts. to yield 13 frs (C8-1–C8-13). Fr. C8-5 (4.1 g) was separated by silica gel CC (125 g) and eluted with $CHCl_3$–acetone to obtain 10 frs (C8-5-1–C8-5-10). Fr. C8-5-1 (68 mg) was rechromatographed by silica gel (2 g), eluting with *n*-hexane–EtOAc (1:1), gradually enriched with EtOAc, to obtain 7 frs (C8-5-1-1–C8-5-1-7). Then, fr. C8-5-1-2 (44.8 mg) was purified by prep. TLC (*n*-hexane–EtOAc, 1:1) to give 2 (27.8 mg). Fr. C8-5-2 (340 mg) was rechromatographed on silica gel (40 g) and eluted with $CHCl_3$–acetone to afford 6 frs (C8-5-2-1–C8-5-2-6). Fr. C8-5-2-3 (77.3 mg) was rechromatographed on silica gel (2.3 g) and eluted with *n*-hexane–EtOAc mixts to yield 9 frs (C8-5-2-3-1–C8-5-2-3-9). Fr. C8-5-2-3-2 (47 mg) was further purified by prep. TLC (*n*-hexane–EtOAc, 1:1) to obtain 1 (30 mg). Part (2.01 g) of fr. C9 (18.06 g) was rechromatographed on silica gel (60 g) and eluted with CH_2Cl_2–MeOH mixts and 13 frs (C9-1–C9-13) were collected. Part (195 mg) of fr. C9-4 (1.12 g) was purified by prep. TLC (*n*-hexane–acetone, 2:1) to afford 4 (21.1 mg), 6 (77.8 mg) and a mixture of **7** and **8** (37 mg).

3.3. Lanyuamide I (*1*)

Colorless oil. UV λ_{max}^{EtOH} nm ($\log \varepsilon$) 259 (4.00). IR ν_{max}^{Neat} cm^{-1} 3300 (NH), 1700 (CO), 1650 (C=C), 1625 (CO). EI-MS *m*/*z* (rel. int.): 293 [M]$^+$ (18), 180 (97), 165 (82), 152 (100), 113 (63), 107 (36), 81 (38), 79 (48), 57 (54), 43 (58), 41 (47). HR-MS: $C_{18}H_{31}NO_2$, found: 293.2350, calcd: 293.2355. ^{1}H NMR: δ 0.88 (3H, t, J = 6.8 Hz, H-14), 0.92 (6H, d, J = 6.8 Hz, H-3′, 4′), 1.25 (6H, br s, H-11–13), 1.56 (2H, quint, J = 7.1 Hz, H-10), 1.79 (1H, m, H-2′), 2.39 (2H, t, J = 7.1 Hz, H-9), 2.42 (2H, q, J=6.9 Hz, H-6), 2.53 (2H, t, J = 6.9 Hz, H-7), 3.16 (2H, t, J=6.8 Hz, H-1′), 5.47 (1H, br s, NH, exchangeable with D_2O), 5.76 (1H, d, J = 14.9 Hz, H-2), 6.02 (1H, dt, J=15.1, 6.9 Hz, H-5), 6.16 (1

H, dd, J = 15.1, 10.6 Hz, H-4), 7.16 (1 H, dd, J = 14.9, 10.6 Hz, H-3). ^{13}C NMR spectral analyses: see Table 1.

3.4. Lanyuamide II (**2**)

Colorless oil. UV λ_{max}^{EtOH} nm (log ε): 259 (3.58). IR ν_{max}^{Neat} cm^{-1}: 3320 (NH), 1720 (CO), 1648 (C=C), 1630 (CO). FAB-MS (positive): 292 $[M+H]^+$. ^{1}H NMR: δ 0.93 (6H, d, J = 6.8 Hz, H-3′, 4′), 1.05 (3H, t, J = 7.2 Hz, H-14), 1.79 (1 H, m, H-2′), 2.19 (4H, m, H-6, H-7), 2.30 (2H, q, J = 6.8 Hz, H-10), 2.41 (2H, q, J = 7.2 Hz, H-13), 2.44 (2H, t, J = 7.6 Hz, H-11), 3.16 (2H, t, J = 6.8 Hz, H-1′), 5.35 (2H, m, H-8, H-9), 5.59 (1H, br s, NH, exchangeable with D_2O), 5.77 (1H, d, J = 15.2 Hz, H-2), 6.05 (1H, dt, J = 15.0, 6.4 Hz, H-5), 6.15 (1H, dd, J = 15.0, 10.6 Hz, H-4), 7.16 (1H, dd, J = 15.2, 10.6 Hz, H-3). ^{13}C NMR spectral analyses: see Table 1.

3.5. Lanyuamide III (**3**)

Colorless oil. UV λ_{max}^{EtOH} nm (log ε): 259 (4.23). IR ν_{max}^{Neat} cm^{-1}: 3300 (NH), 1650 (C=C), 1625 (CO). EI-MS m/z (rel. int.): 275 $[M]^+$ (1), 154 (13), 152 (11), 95 (13), 94 (12), 81 (19), 79 (20), 69 (16), 68 (15), 67 (79), 66 (30), 65 (13), 57 (53), 55 (57), 44 (18). HR-MS: $C_{18}H_{29}NO$, found: 275.2255, calcd: 275.2234. ^{1}H NMR: 0.93 (6H, d, J = 6,8 Hz, H-3′ 4′), 0.96 (3H, t, J = 7.4 Hz, H-14), 1.79 (1H, m, H-2′), 2.00 (2H, m, H-13), 2.21 (4H, m, H-6, H-7), 2.74 (2H, t, J = 6.0 Hz, H-10), 3.17 (2H, t, J = 6.8 Hz, H-1′), 5.31 (2H, m, H-11, H-12), 5.38 (2H, m, H-8, H-9), 5.48 (1 H, br s, NH, exchangeable with D_2O), 5.76 (1H, d, J = 15.2 Hz, H-2), 6.06 (1H, dt, J = 15.2, 6.4 Hz, H-5), 6,15 (1H, dd, J = 15.2, 10.2 Hz, H-4), 7.18 (1H, dd, J = 15.2, 10.2 Hz, H-3). ^{13}C NMR spectral analyses: see Table 1.

Acknowledgements

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