N-Isobutylamides and Butyrolactone from the Fruits of *Zanthoxylum integrifoliolum*

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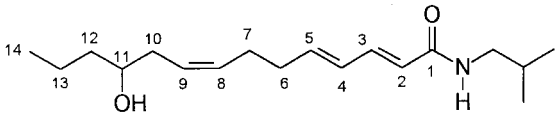
Further investigations of the $CHCl_3$-soluble fraction of the fruit of *Zanthoxylum integrifoliolum* led to the isolation of three new *N*-isobutylamides: lanyuamide IV (**1**), lanyuamide V (**2**), and lanyuamide VI (**3**), along with lanyulactone (**4**), a new butyrolactone derivative. The structures of these new compounds were elucidated by spectroscopic data.

Introduction. – *Zanthoxylum integrifoliolum* (MERR.) MERR. (Rutaceae) is an evergreen tree distributed in the northern Philippines and on Lanyu Island in Taiwan [1]. Its bark was used as a folk remedy for snake-bite by Ya-Mei aborigines of Lanyu Island and has been a good source for antiplatelet agents such as chelerythrine and avicine pseudocyanide [2]. Benzo[*c*]phenanthridines, quinolines, bishordeninyl terpene alkaloids, and triterpenoids were the major constituents of this plant (bark, root wood, and leaves), as found in previous studies [3–6]. Recently, a chemical study on the pungently tasting fruits has led to the isolation of *N*-isobutylamides, indolopyridoquinazoline, lignans, flavonoids, and other constituents [7–9]. The TLC profiles of the $CHCl_3$-soluble fraction of the fruits seems to imply that several other minor constituents still remain to be found. Careful examination of the fruits has now resulted in the characterization of four new compounds: lanyuamide IV (**1**), lanyuamide V (**2**), lanyuamide VI (**3**), and lanyulactone (**4**). In this paper, we report the isolation and structure elucidation of **1**–**4** by spectral analyses.

Results and Discussion. – Lanyuamides IV–VI (**1**–**3**) were isolated as colorless oils. The UV spectra of **1** and **3** showed absorption maxima around 259 nm, indicating the presence of a conjugated system related to *N*-isobutylamides of sorbic acid (=(2*E*,4*E*)-hexa-2,4-dienoic acid) [8]. The IR spectrum of each amide showed characteristic absorptions for the NH and CO groups of an amide moiety, indicating the presence of a (2*E*,4*E*)-2,4-dienamide skeleton [8]. The ^{1}H-NMR spectra of **1**–**3** all showed the presence of the *N*-isobutyl group, a (2*E*,4*E*)-2,4-dienamide, and two allylic methylene groups.

Lanyuamide IV (**1**) was determined to have the molecular formula $C_{18}H_{31}NO_2$ by EI- and HR-EI-MS and DEPT spectra. According to the IR, ^{1}H- and ^{13}C-NMR, DEPT, COSY, and MS data, the structure of **1** was elucidated as (2*E*,4*E*,8*Z*)-11-hydroxy-*N*-isobutyltetradeca-2,4,8-trienamide.

The presence of the OH group of **1** was established by the IR absorption at 3300 cm^{-1} (overlapped with NH) and the br. *s* in the ^{1}H-NMR at δ 1.59. The ^{1}H-NMR of **1** was similar to that of lanyuamide II [8] and



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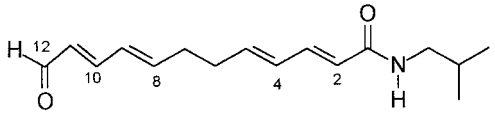

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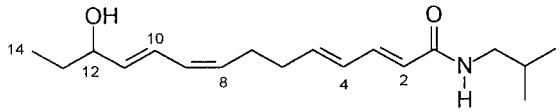

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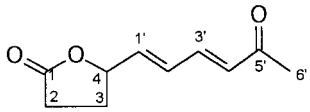

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showed also two *cis*-positioned olefinic protons at δ 5.44 and 5.53 (*m*, H–C(8), H–C(9)), as suggested by the upfield-shifted ^{13}C-NMR signal of C(7) at δ 26.6 [10]. Though the ^{13}C-NMR signal of C(10) was also shielded by the (*Z*)-double bond, it was shifted downfield to δ 39.0 due to OH–C(11). Furthermore, the ^{1}H-NMR signals of a downfield-shifted oxymethine proton at δ 3.64 (*quint*, $J = 6.0$ Hz, H–C(11)), two methylene groups at δ 1.45 (*m*, CH_2(12), CH_2(13)), and a Me group at δ 0.94 (*t*, $J = 6.8$ Hz, Me(14)) established the terminal 1-hydroxybutyl moiety of **1**. The location of the OH group at C(11) was further supported by the COSY experiment, in which CH_2(10) (δ 2.22 (*m*)) coupled with H–C(11) (δ 3.64), and by the prominent mass fragments at *m*/*z* 73 ($[C_4H_9O]^+$) and 220 ($[C_{14}H_{22}NO]^+$) arising by cleavage between C(10) and C(11).

Similarly, the structures of lanyuamides V and VI and lanyulactone were established by spectroscopic means as being (2*E*,4*E*,8*E*,10*E*)-*N*-isobutyl-12-oxododeca-2,4,8,10-tetraenamide (**2**), (2*E*,4*E*,8*Z*,10*E*)-12-hydroxy-*N*-isobutyltetradeca-2,4,8,10-tetraenamide (**3**), and γ-[(1*E*,3*E*)-5-oxohexa-1,3-dienyl]butyrolactone (**4**), respectively. The molecular formula of lanyuamide V (**2**) was established as $C_{16}H_{23}NO_2$ by EI- and HR-EI-MS and DEPT spectra. The presence of an aldehyde group was suggested by the IR absorption at 1680 cm^{-1} and the NMR signals at δ(H) 9.54 (*d*, $J = 7.6$ Hz, CHO–C(12)) and δ (C) 193.7. The UV spectrum of **2** showed maxima at 259 (sh) and 275 nm, indicating the presence of a highly conjugated system with an aldehyde group, related to *N*-isobutylamides of sorbic acid. In the ^{1}H-NMR spectrum, this (*E*,*E*)-dienal moiety was observed at δ 6.25 (*m*, H–C(8)), 6.34 (*dd*, $J = 15.2$, 10.4 Hz, H–C(9)), 7.07 (*dd*, $J = 15.2$, 10.8 Hz, H–C(10)), and 6.10 (*dd*, $J =$ 15.2, 7.6 Hz, H–C(11)) and confirmed by COSY data. Furthermore, the ^{13}C-NMR spectrum showed the downfield-shifted chemical shifts of C(7) at δ 31.8 and C(6) at δ 32.3, indicating that **2** is an amide with (*all-E*)-configuration [10].

By the EI- and HR-EI-MS as well as the ^{13}C-NMR spectra, a molecular formula $C_{18}H_{29}NO_2$ was deduced for lanyuamide VI (**3**). The ^{1}H-NMR spectrum of **3** was similar to that of **1**, except that **3** showed the signals of two additional *trans*-positioned olefinic protons at δ 5.68 (*dd*, $J = 15.2$, 6.6 Hz, H–C(11)) and 6.46 (*dd*, $J = 15.2$, 10.0 Hz, H–C(10)) (^{13}C-NMR: δ 136.2 (C(11)), 125.5 (C(10))). A downfield-shifted oxymethine proton at δ 4.10 (*q*, $J = 6.6$ Hz, H–C(12)), a CH_2 group at δ 1.55 (*m*, CH_2(13)), and a Me signal at δ 0.93 (*t*, $J = 7.6$ Hz, Me(14)) indicated the 12-position for the OH group present in **3**. This was confirmed by the COSY experiment, in which H–C(11) (δ 5.68) was correlated with H–C(12) (δ 4.10) and by the mass fragment at *m*/*z* 57 (100, $[C_3H_5O]^+$) in the MS.

The molecular formula $C_{10}H_{12}O_3$ of lanyulactone (**4**) was suggested by its EI- and HR-EI-MS data. The ^{1}H-NMR spectrum of **4** revealed two sets of *trans*-positioned olefinic protons at δ 6.14 (*dd*, $J = 15.2$, 6.1 Hz) and 6.43 (*dd*, $J = 15.2$, 11.2 Hz) and at δ 7.10 (*d*, $J = 15.8$, 11.2 Hz), and 6.21 (*d*, $J = 15.8$ Hz), which were assigned to H–C(1′)/H–C(2′) and H–C(3′)/H–C(4′), respectively. An acetyl group at δ 2.29 (*s*, Me (6′)) was located at the terminal of the side chain. In addition, the presence of a butyrolactone moiety was suggested by the signals of two CH_2 groups at δ 2.04 (*m*, H_a–C(3)), 2.49 (*dq*, $J = 19.2$, 7.2 Hz, H_b–C(3)), and 2.56 (*t*, $J = 7.2$ Hz, 2 H–C(2)) and a downfield-shifted oxymethine proton at δ 5.07 (br. *q*, $J = 6.1$ Hz, H–C(4)).

It is noteworthy that the aliphatic unsaturated amides isolated from the pericarps of *Z. bungeanum* [11][12] (Huaziao) and *Z. piperitum* [13] (Sanziao) as the pungent foodstuff predominantly had a hydroxy-substituted *N*-isobutyl group (*i.e.*, a 1-hydroxy-1,1-dimethylethyl group), while the aliphatic unsaturated amides isolated from the fruits of *Z. integrifoliolum* [8] carried predominantly an unsubstituted *N*-isobutyl group. Moreover, the common constituent α-sanshool found in the former two *Zanthoxylum* species was lacking in the fruit extract from *Z. integrifoliolum*.

Experimental Part

General. TLC: silica gel *60* F_{254} precoated plates (*Merck*). Column chromatography (CC): silica gel *60* (*Merck* 70–230 mesh, 230–400 mesh, ASTM)). M.p.: *Yanaco* micro-melting-point apparatus; uncorrected. Optical rotation: *Jasco DIP-370* polarimeter; in $CHCl_3$. IR Spectra: *Hitachi 260-30* spectrophotometer; neat at 25°; $\tilde{\nu}$ in cm^{-1}. UV Spectra: *Jasco-UV-240* spectrophotometer; in EtOH; λ_{max} (log ε) in nm. EI-MS Spectra: *VG-Biotech Quattro-5022* spectrometer; *m*/*z* (rel. %). HR-EI-MS: *Jeol JMX-HX-110* mass spectrometer. ^{1}H- and ^{13}C-NMR Spectra: *Varian Gemini-200* or *Varian Unity Plus-400* spectrometer; δ in ppm rel. to $SiMe_4$, *J* in Hz.

Plant Material. Fruits of *Z. integrifoliolum* were collected on Lanyu Island, Taitung County, Taiwan, in August 1995. A voucher sample (no. Chen 5528) was deposited in the Herbarium of the School of Pharmacy, College of Pharmacy, Kaohsiung Medical University, Kaohsiung, Taiwan, R.O.C.

Extraction and Isolation. Dried fruits (16.5 kg) were extracted and isolated as previously described [8] to afford H_2O (*Fr. D*, 620 g), BuOH (*Fr. B*, 130 g), hexane (*Fr. A*, 420 g), and $CHCl_3$ (*Fr. C*, 220 g) fractions. Another part of the $CHCl_3$-soluble *Fr. C* (54.6 g) was submitted to CC (silica gel, CH_2Cl_2, then gradient AcOEt/$CHCl_3$) to give 16 fractions (*C1* to *C16*). *Fr. C3* (4.13 g) was re-subjected to CC (silica gel, Me_2CO/hexane 1:10, then gradient Me_2CO/hexane) to give 40 fractions (*C3-1* to *C3-40*). *Fr. C3-20* (43.2 mg) was purified by prep. TLC (CH_2Cl_2/AcOEt 15:1) to give three bands (*C3-20-1* to *C3-20-3*). The crude extract of band *C3-20-1* was purified again by prep. TLC (AcOEt/hexane 1:1): **4** (2.9 mg). Band *C3-20-2* was further purified by prep. TLC (AcOEt/C_6H_6 1:2): **2** (1.1 mg). Band *C3-20-3* was also further purified by prep. TLC (AcOEt/C_6H_6 1:2): **3** (2.6 mg). *Fr. C9* (AcOEt/CH_2Cl_2 1:5; 1.55 g) was subjected to CC (silica gel, Me_2CO/$CHCl_3$ 1:10, then gradient Me_2CO/$CHCl_3$) to provide 14 fractions (*C9-1* to *C9-14*). *Fr. C9-6* (Me_2CO/$CHCl_3$ 1:5; 197.2 mg) was re-subjected to CC (silica gel, Me_2CO/$CHCl_3$ 1:10, then gradient Me_2CO/$CHCl_3$) to give 12 fractions (*C9-6-1* to *C9-6-12*). *Fr. C9-6-9* (Me_2CO/$CHCl_3$ 1:3; 29.7 mg) was re-subjected to CC (silica gel, AcOEt/CH_2Cl_2 1:10, then gradient AcOEt/CH_2Cl_2) to give 6 fractions (*C9-6-9-1* to *C9-6-9-6*). *Fr. C9-6-9-6* (AcOEt, 4.7 mg) was further purified by prep. TLC (AcOEt/CH_2Cl_2 1:5): **1** (2.5 mg).

Lanyuamide IV (=*(2*E*,4*E*,8*Z*)-11-Hydroxy*-N*-(2-methylpropyl)tetradeca-2,4,8-trienamide*; **1**). Colorless oil. $[\alpha]_D^{25} = -42.9$ ($c = 0.11$, $CHCl_3$). UV: 260 (3.85). IR: 3300 (NH, OH), 1650 (C=C), 1630 (CONH). ^{1}H-NMR ($CDCl_3$, 400 MHz): 0.92 (*d*, $J = 6.8$, Me_2CHCH_2); 0.94 (*t*, $J = 6.8$, Me(14)); 1.45 (*m*, CH_2(12), CH_2(13)); 1.59 (br. *s*, OH–C(11), D_2O exchangeable); 1.79 (*sept.*, $J = 6.8$, Me_2CHCH_2); 2.19 (*m*, CH_2(6), CH_2(7)); 2.22 (*m*, CH_2(10)); 3.16 (*t*, $J = 6.4$, Me_2CHCH_2); 3.64 (*quint.*, $J = 6.0$, H–C(11)); 5.44 (*m*, H–C(8));

5.47 (br. *s*, NH, D_2O exchangeable); 5.53 (*m*, H−C(9)); 5.76 (*d*, *J* = 15.2, H−C(2)); 6.06 (*dt*, *J* = 15.2, 6.2, H−C(5)); 6.15 (*dd*, *J* = 15.2, 10.4, H−C(4)); 7.18 (*d*, *J* = 15.2, 10.4, H−C(3)). ^{13}C-NMR ($CDCl_3$, 100 MHz): 14.0 (C(14)); 18.9 (C(13)); 20.1 (Me_2CHCH_2); 26.6 (C(7)); 28.6 (Me_2CHCH_2); 32.8 (C(6)); 35.4 (C(12)); 39.0 (C(10)); 46.9 (Me_2CHCH_2); 71.1 (C(11)); 122.1 (C(2)); 126.2 (C(8)); 128.7 (C(4)); 131.7 (C(9)); 140.9 (C(3)); 141.8 (C(5)); 166.2 (C(1)). EI-MS: 293 (7.87, M^+), 250 (17), 220 (10), 180 (9), 167 (70), 166 (22), 152 (16), 127 (5), 113 (5), 73 (52), 57 (72), 55 (100), 43 (50). HR-EI-MS: 293.2354 ($C_{18}H_{32}NO_2^+$; calc. 293.2353).

Lanyuamide V (= *(2*E*,4*E*,8*E*,10*E*)*-N-*(2-Methylpropyl)-12-oxododeca-2,4,8,10-tetraenamide*; **2**). Colorless oil. UV: 275 (4.30), 259 (sh, 4.23). IR: 3400 (NH), 1680 (CHO), 1640 (CONH). ^{1}H-NMR ($CDCl_3$, 400 MHz): 0.93 (*d*, *J* = 6.8, Me_2CHCH_2); 1.79 (*sept.*, *J* = 6.8, Me_2CHCH_2); 2.35 (*m*, CH_2(6), CH_2(7)); 3.17 (*t*, *J* = 6.4, Me_2CHCH_2); 5.48 (br. *s*, NH−C(1), D_2O exchangeable); 5.78 (*d*, *J* = 14.8, H−C(2)); 6.05 (*m*, H−C(5)); 6.10 (*dd*, *J* = 15.2, 7.6, H−C(11)); 6.18 (*m*, H−C(4)); 6.25 (*m*, H−C(8)); 6.34 (*dd*, *J* = 15.2, 10.4, H−C(9)); 7.07 (*dd*, *J* = 15.2, 10.4, H−C(10)); 7.19 (*d*, *J* = 14.8, 10.8, H−C(3)); 9.54 (*d*, *J* = 7.6, CHO−C(12)). ^{13}C-NMR ($CDCl_3$, 100 MHz): 20.1 (Me_2CHCH_2); 28.6 (Me_2CHCH_2); 31.8 (C(7)); 32.3 (C(6)); 46.9 (Me_2CHCH_2); 122.6 (C(2)); 129.2 (C(4)); 129.3 (C(9)); 130.5 (C(11)); 140.4 (C(8)); 140.6 (C(3)); 145.0 (C(5)); 152.1 (C(10)); 166.1 (C(1)); 193.7 (C(12)). EI-MS: 261 (1.9, M^+), 189 (38.8), 166 (63.9), 110 (35.6), 94 (28.1), 67 (100), 66 (73.1), 57 (27.5). HR-EI-MS: 261.1725 ($C_{16}H_{23}NO_2^+$; calc. 261.1721).

Lanyuamide VI (= *(2*E*,4*E*,8*Z*,10*E*)-12-Hydroxy*-N-*(2-methylpropyl)tetradeca-2,4,8,10-tetraenamide*; **3**). Colorless oil. $[\alpha]_D^{25} = -20.9$ (*c* = 0.13, $CHCl_3$). UV: 259 (4.27), 237 (4.23). IR: 3350 (NH, OH), 1660, 997 (C=C), 1630 (CONH). ^{1}H-NMR ($CDCl_3$, 400 MHz): 0.92 (*d*, *J* = 6.8, Me_2CHCH_2); 0.93 (*t*, *J* = 7.6, Me(14)); 1.55 (*m*, CH_2(13)); 1.79 (*sept.*, *J* = 6.8, Me_2CHCH_2); 2.26 (*m*, CH_2(6)); 2.31 (*m*, CH_2(7)); 3.16 (*t*, *J* = 6.4, Me_2CHCH_2); 4.10 (*q*, *J* = 6.6, H−C(12)); 5.41 (*dt*, *J* = 10.0, 7.2, H−C(8)); 5.49 (br. *s*, NH), D_2O exchangeable); 5.68 (*dd*, *J* = 15.2, 6.6, H−C(11)); 5.76 (*d*, *J* = 15.2, H−C(2)); 6.03 (*t*, *J* = 10.0, H−C(9)); 6.07 (*dt*, *J* = 15.0, 6.4, H−C(5)); 6.15 (*dd*, *J* = 15.0, 10.4, H−C(4)); 6.46 (*dd*, *J* = 15.2, 10.0, H−C(10)); 7.18 (*dd*, *J* = 15.2, 10.4, H−C(3)). ^{13}C-NMR ($CDCl_3$, 100 MHz): 9.6 (C(14)); 20.1 (Me_2CHCH_2); 27.0 (C(7)); 28.6 (Me_2CHCH_2); 30.1 (C(13)); 32.7 (C(6)); 46.9 (Me_2CHCH_2); 74.0 (C(12)); 122.2 (C(2)); 125.5 (C(10)); 128.6 (C(8)); 128.8 (C(4)); 130.8 (C(9)); 136.2 (C(11)); 141.0 (C(3)); 141.7 (C(5)); 166.2 (C(1)). EI-MS: 291 (7.87, M^+), 262 (23), 167 (54), 152 (13), 66 (32), 67 (36), 57 (100). HR-EI-MS: 291.2180 ($C_{18}H_{29}NO_2^+$; calc. 291.2198).

Lanyulactone (= *4,5-Dihydro-5-[(1*E*,3*E*)-5-Oxohexa-1,3-dienyl]furan-2(3*H*)-one*; **4**). Colorless oil. UV: 267 (4.12). IR: 1770 (CO, lactone), 1670 (conjugated CO). ^{1}H-NMR ($CDCl_3$, 400 MHz): 2.04 (*m*, H_a−C(3)); 2.29 (*s*, Me(6′)); 2.49 (*dq*, *J* = 19.2, 7.2, H_b−C(3)); 2.56 (*t*, *J* = 7.2, CH_2(2)); 5.07 (br. *q*, *J* = 6.1, H−C(4)); 6.14 (*dd*, *J* = 15.2, 6.1, H−C(1′)); 6.21 (*d*, *J* = 15.8, H−C(4′)); 6.43 (*dd*, *J* = 15.2, 11.2, H−C(2′)); 7.10 (*d*, *J* = 15.8, 11.2, H−C(3′)). ^{13}C-NMR ($CDCl_3$, 100 MHz): 27.6 (C(6′)); 28.1 (C(3)); 28.4 (C(2)); 78.9 (C(4)); 129.9 (C(4′)); 132.0 (C(2′)); 138.6 (C(3′)); 140.8 (C(1′)); 176.2 (C(1)); 198.1 (C(5′)). EI-MS: 180 (25, M^+), 138 (27), 137 (22), 121 (19), 109 (36), 95 (66), 81 (83), 77 (50), 55 (56), 43 (100). HR-EI-MS: 180.0782 ($C_{10}H_{12}O_3^+$; calc. 180.0777).

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