Isobutylamides of Unsaturated Fatty Acids from *Chrysanthemum morifolium* Associated with Host-Plant Resistance against the Western Flower Thrips

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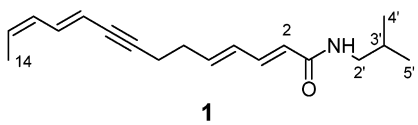
Three unsaturated fatty acid isobutylamides, *N*-isobutyl-2*E*,4*E*,10*E*,12*Z*-tetradecatetraen-8-ynamide (**1**, new), *N*-isobutyl-2*E*,4*E*,12*Z*-tetradecatrien-8,10-diynamide (**2**), and *N*-isobutyl-2*E*,4*E*,12*E*-tetradecatrien-8,10-diynamide (**3**), were isolated from the leaves and flowers of *Chrysanthemum morifolium*. The structure of **1** was determined by spectral data interpretation. The concentration of **1** in chrysanthemum varieties was previously positively correlated with host-plant resistance against the western flower thrips, *Frankliniella occidentalis*.

The western flower thrips (WFT), *Frankliniella occidentalis* (Pergande), has been the major insect pest facing the greenhouse industry in Canada since the late 1980s.[1] Many factors have been investigated with respect to their effect on the interaction between chrysanthemum plants, *Chrysanthemum morifolium* (Ramat) (Compositae), and the WFT. While morphological characteristics of chrysanthemum,[2] variation in pollen production,[3] and the difference in color of blooms[4] were found to have little impact on this interaction, chemical ecology has been suggested to play a major role in the resistance of chrysanthemum against the WFT.[5,6] De Jager et al.[5] found that at least 76% of the variation in resistance of chrysanthemum cultivars could be attributed to the leaf sap. A more recent study by Fung et al.[7] suggested the role of secondary metabolites in the chrysanthemum−thrips interaction. To date, however, no specific compounds have been identified as being associated with resistance against the WFT.

A study of 27 chrysanthemum varieties at our research center revealed significant differences in susceptibility to damage by the WFT.[8] A project was undertaken to investigate possible biochemical mechanisms of resistance against the WFT in the chrysanthemum. A compound was identified whose concentration in chrysanthemum varieties correlated with the degree of resistance against the WFT exhibited by those varieties.[9] We report here the structure of this new compound (**1**) and two related compounds (**2** and **3**) isolated from *C. morifolium*.



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Leaves of the WFT-resistant chrysanthemum variety Super Yellow were extracted in acetone. The identified compounds were partitioned into ethyl acetate and further purified by TLC and semipreparative HPLC. Under the HPLC conditions used, the retention time of **1** was 31.7 min. The UV spectrum of this compound (λ_{max} 270 nm) provided evidence of a highly conjugated molecule. The EIMS suggested a molecular mass of 271. This deduction was confirmed by CIMS (methane), which exhibited an intense peak at *m*/*z* 272. The accurate mass determination by HRESIMS (*m*/*z* 272.2003, $[M + H]^+$) established the molecular formula as $C_{18}H_{25}NO$ (calculated for $C_{18}H_{26}NO$, 272.2014). The vapor-phase FT-IR spectrum suggested the presence of an amide group (1698, 1495 cm^{-1}) and at least one *cis* carbon−carbon double bond (3030 cm^{-1}).[10]

^{1}H NMR and DQF-COSY (double-quantum filtered correlation spectroscopy) experiments revealed an isobutyl group attached to nitrogen [δ 4.53 (H-1′, 1H), 2.98 (H-2′, 2H), 1.52 (H-3′, 1H), and 0.68 (H-4′,5′, 6H)]. Also apparent were signals of a conjugated diene moiety [δ 5.33 (H-2, 1H), 7.44 (H-3, 1H), 6.01 (H-4, 1H), and 5.65 (H-5, 1H)], which showed coupling to a methylene group [δ 2.05 (H-6, 2H)], which in turn coupled to another methylene group [δ 2.14 (H-7, 2H)]. An HMBC experiment revealed a carbonyl carbon (δ 165.5) and two acetylenic carbons (δ 91.8 and 81.3). HMBC correlation between H-2 and C-1 placed the conjugated diene adjacent to the carbonyl carbon. HMBC correlation between H-2′ and C-1 linked the isobutyl group to the carbonyl through the nitrogen. The DQF-COSY revealed yet another conjugated diene moiety [δ 5.62 (H-10, 1H), 6.99 (H-11, 1H), 5.88 (H-12, 1H), and 5.27 (H-13, 1H)] coupled to a methyl group [δ 1.40 (H-14, 3H)]. A weak correlation was observed in the DQF-COSY spectrum between H-7 and H-10. In addition, HMBC correlations were observed between H-7 and the acetylenic carbons C-8 and C-9. These findings placed the acetylene group between C-7 and C-10. Further HMBC correlations between C-8 and H-6 and between C-9 and H-11 allowed assignment of the chemical shift of C-8 as δ 91.8 and that of C-9 as δ 81.3. The $J_{12,13}$ coupling constant (10.4 Hz) confirmed *cis* stereochemistry at this double bond. The coupling constants for the remaining double bonds ($J_{10,11}$ = 15.5 Hz, $J_{4,5}$ = 15.0 Hz, $J_{2,3}$ = 15.0 Hz) indicated *trans* stereochemistry. HMBC and HMQC (heteronuclear multiple quantum co-

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Table 1. ^{1}H and ^{13}C NMR Spectral Data for **1**

position	δ_H[a]	δ_C[b]	HMBC[c]
1		165.5	
2	5.33 (1H, d, J = 15.0)	123.3	C-1, C-4
3	7.44 (1H, dd, J = 15.0, 10.8)	140.2	C-1, C-2, C-4, C-5
4	6.01 (1H, dd, J = 15.0, 10.8)	129.6	C-3, C-6
5	5.65 (1H, dt, J = 15.0, 7.0)	139.4	C-3, C-6
6	2.05 (2H, q, J = 7.0)	32.2	C-4, C-5, C-7, C-8
7	2.14 (2H, td, J = 7.0, 2.1)	19.3	C-5, C-6, C-8, C-9
8		91.8	
9		81.3	
10	5.62 (1H, dt, J = 15.5, 2.1)	111.4	C-12
11	6.99 (1H, dd, J = 15.5, 11.3)	136.0	C-9, C-12
12	5.88 (1H, ddq, J = 11.3, 10.4, 1.7)	128.9	C-10
13	5.27 (1H, dq, J = 10.4, 6.5)	128.2	
14	1.40 (3H, dd, J = 6.5, 1.7)	12.7	C-12, C-13
1′	4.53 (1H, br t, J = 6.5)		
2′	2.98 (2H, dd, J = 6.7, 6.5)	46.5	C-1, C-3′, C-4′/5′
3′	1.52 (1H, nonet, J = 6.7)	28.7	C-2′, C-4′/5′
4′,5′	0.68 (6H, d, J = 6.7)	19.8	C-2′, C-3′

[a] Benzene-d_6, 500 MHz, J in Hz. [b] $CDCl_3$, from HMBC and HMQC spectra, 500 MHz. [c] $CDCl_3$, 500 MHz, carbons correlating with proton resonance.

herence) experiments allowed assignment of all carbon and proton resonances (Table 1). The above data confirmed the identity of the compound to be *N*-isobutyl-2*E*,4*E*,10*E*,12*Z*-tetradecatetraen-8-ynamide (**1**), a compound that has not been previously reported.

Two other compounds (**2** and **3**) with identical UV spectra (λ_{max} 252, 266, and 280 nm) were eluted by reversed-phase HPLC at 30.0 and 30.3 min, respectively. Their mass spectra (indistinguishable from each other and similar to that of **1**) indicated a molecular mass of 269. One isomer was identified by ^{1}H NMR[11] as *N*-isobutyl-2*E*,4*E*,12*Z*-tetradecatrien-8,10-diynamide (**2**). The vapor-phase FT-IR spectrum of the other compound indicated the absence of *cis* stereochemistry, and it was identified as *N*-isobutyl-2*E*,4*E*,12*E*-tetradecatrien-8,10-diynamide (**3**), an isomer of **2**. These compounds have been reported in *Achillea* spp.[11,12] and *Anthemis fuscata*,[13] but not previously in the genus *Chrysanthemum*.

Experimental Section

General Experimental Procedures. UV spectra were obtained with the diode array detector of a Hewlett-Packard 1100 HPLC using a Sphericlone ODS-2 column (15 cm × 4.6 mm, Phenomenex) and an acetonitrile/water mobile phase gradient (90:10 to 75:25, v/v over 37.5 min). Under these conditions, the retention times of compounds **1**, **2**, and **3** were 31.7, 30.0, and 30.3 min, respectively. Vapor-phase IR spectra were recorded using GC−FTIR on a Hewlett-Packard 5965A GC−IRD (HP-5 25 m × 0.32 mm column, 0.52 μm film thickness, Agilent Technologies). ^{1}H spectra and HMBC and HMQC experiments were performed on a Varian Unity 500 MHz spectrometer in deuterated benzene or deuterated chloroform. EIMS and CIMS (methane) were obtained using a Fisons MD800 GC−MS on a DB-5 20 m × 0.25 mm column (film thickness 0.1 μm, J&W Scientific) with an ionizing energy of 70 eV. HRMS were performed on a Micromass Autospec instrument using electrospray ionization (ESI).

Plant Material. Chrysanthemum cuttings of the WFT-resistant variety Super Yellow were supplied by Yoder Canada (Leamington, ON) and grown in the greenhouse at the Agriculture and Agri-Food Canada research center in Vineland Station, ON, and sampled in August 1999. A voucher specimen (accession number 791548) was deposited in the Vascular Plant Herbarium, Agriculture and Agri-Food Canada, Ottawa.

Extraction and Isolation. Fresh leaves (520 g fresh weight) of mature plants (six weeks after transplanting) were collected and submerged in 2 L of acetone overnight at room temperature. The extract was then decanted and filtered through a Whatman No. 1 filter paper and concentrated to 1/5 of its original volume at <40 °C. The extract was then partitioned into an equal volume of ethyl acetate (EtOAc) three times and evaporated to dryness, leaving a residue weighing 4.65 g. The sample was redissolved in EtOH, and 5% aqueous lead acetate was added to precipitate pigments. The sample was centrifuged and decanted, ethanol was evaporated on a rotary evaporator at <45 °C, and the sample was partitioned into EtOAc.

The extract was further cleaned up by TLC (2 mm layer, silica gel GF, Analtech, Newark, DE) using a solvent system of hexane/ethyl acetate (1:1) and UV light (254 nm) for detection. The band between R_f = 0.30−0.65 was extracted with EtOAc/hexane (1:1) and evaporated, leaving a residue of 18 mg. This was further purified by TLC using hexane/EtOAc/CH_2Cl_2 (3:2:1). Compounds **1**−**3** were recovered from a band between R_f = 0.60−0.66 (2.5 mg).

Final purification of **1** was by semipreparative HPLC using a Hewlett-Packard 1050 HPLC equipped with a diode-array UV detector on a Supelcosil SPLC-18 column (25 cm × 10 mm i.d., 5 μm, Supelco) and an acetonitrile/water mobile phase gradient (50:50 v/v for 1.0 min, then changed linearly to 80:20 over 10 min, then back to 50:50 over 5.0 min, flow rate 2.5 mL/min). Under these conditions, the retention time of **1** was 15.7 min. Final purification of **2** was achieved using an isocratic mobile phase consisting of acetonitrile/water (65:35) at a flow rate of 2.5 mL/min. The retention time of **2** was 11.8 min. Collected fractions were evaporated on a rotary evaporator and redissolved in EtOAc. Purified samples were protected from light in foil-wrapped vials.

***N*-Isobutyl-2*E*,4*E*,10*E*,12*Z*-tetradecatetraen-8-ynamide (1):** colorless oil; UV (H_2O/CH_3CN) λ_{max} 270 nm; IR (vapor) ν_{max} 3474, 3367, 3031, 2968, 2930, 2884, 1698, 1644, 1618, 1495, 1375, 1325, 1247, 1208, 1167, 1136, 1075, 1039, 988, 852, 802 cm^{-1}; ^{1}H and ^{13}C NMR data, see Table 1; EIMS (70 eV) *m*/*z* 271 $[M]^+$ (11), 270 (18),167 (40), 143 (21), 129 (29), 105 (75), 103 (57), 79 (100), 77 (99), 66 (59), 41 (30); CI-MS *m*/*z* 272 ($[M + H]^+$, 100), 300 ($[M + C_2H_5]^+$, 25); ESIMS *m*/*z* 272 ($[M + H]^+$, 100), 167 (58), 145 (22), 143 (12), 131 (37), 129 (16) 105 (23), 100 (38), 57 (33); HRESIMS *m*/*z* 272.2003 ($M + H^+$, calcd for $C_{18}H_{26}NO$ 272.2014).

***N*-Isobutyl-2*E*,4*E*,12*Z*-tetradecatrien-8,10-diynamide (2):** colorless oil; UV consistent with literature values;[14] IR (vapor) ν_{max} 3470, 3038, 2968, 2934, 2884, 1699, 1645, 1618, 1496, 1370, 1324, 1247, 1168, 988, 862 cm^{-1}; ^{1}H NMR consistent with literature values;[11] EIMS *m*/*z* 269 $[M]^+$ (44), 197 (10), 167 (34), 154 (26), 141 (37), 129 (20), 103 (59), 77 (100), 66 (53), 57 (53), 41 (31).

***N*-Isobutyl-2*E*,4*E*,12*E*-tetradecatrien-8,10-diynamide (3):** UV consistent with literature values;[14] IR (vapor) 3468, 2968, 2934, 1699, 1646, 1619, 1496, 1263, 1217, 1187, 1166, 1108, 1083, 989, 786 cm^{-1}; EIMS *m*/*z* 269 $[M]^+$ (41), 197 (9), 167 (25), 154 (31), 141 (29), 129 (18), 103 (68), 77 (100), 66 (43), 57 (45), 41 (27).

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Supporting Information Available: Structure, UV, and NMR data for *N*-isobutyl-2*E*,4*E*,12*Z*-tetradecatrien-8,10-diynamide (**2**); structure and UV data for *N*-isobutyl-2*E*,4*E*,12*E*-tetradecatrien-8,10-diynamide (**3**). This material is available free of charge via the Internet at http://pubs.acs.org.

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