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Pungent Alkamides from *Spilanthes acmella* L. var. *oleracea* Clarke

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A main pungent amide, spilanthol (1), and three alkamides, (2*E*)-*N*-(2-methylbutyl)-2-undecene-8,10-diynamide (2), (2*E*,7*Z*)-*N*-isobutyl-2,7-tridecadiene-10,12-diynamide (3), and (7*Z*)-*N*-isobutyl-7-tridecene-10,12-diynamide (4) were isolated from the flower heads of *Spilanthes acmella* L. var. *oleracea* Clarke. Their structures were established by spectroscopic methods. Compounds 2 and 4 were new and 3 was found for the first time in *Spilanthes* species. Chemotaxonomic aspects are discussed.

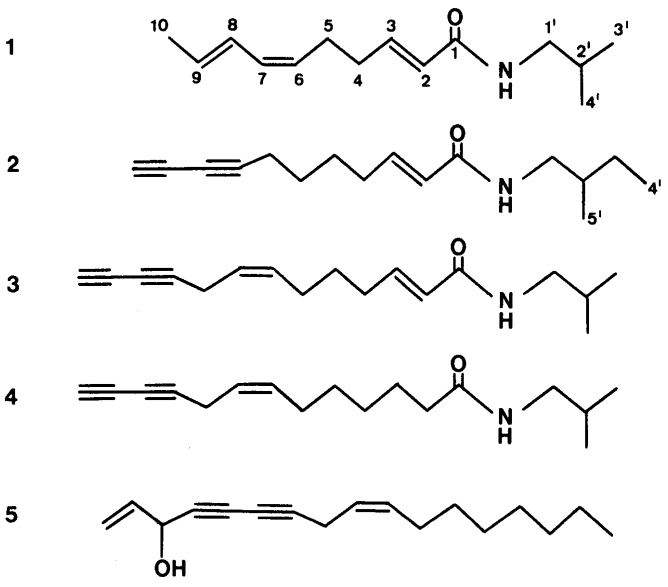
Spilanthes acmella L. var. *oleracea* Clarke is an annual herb belonging to the family Compositae, the tribe Heliantheae, and the subtribe Ecliptinae. As the flower head is yellow, this variety is distinguishable from *Spilanthes acmella* L., having violet flowers. The flowers and leaves of these species have a pungent taste accompanied by tingling and numbness, and have been used as a spice for appetizers and as folk medicine for stammering, toothache, stomatitis, and throat complaints.[1] The flower heads extract was used as a flavoring material for dentifrices and gum.[2] The active major constituent is spilanthol,[3] which was obtained first from *Spilanthes oleracea* Jacq.[4] Its structure was identical with that of affinin, which was isolated from *Heliopsis longipes*, (previously incorrectly identified as *Erigeron affinis*) as an insecticidal constituent.[5-7]

In phytochemical investigations of the genus *Spilanthes*, *Acmella ciliata*, one of the closely related species to *Spilanthes*, was found to contain 20 amides,[8,9] but only a few papers have reported on the constituents of *S. acmella*.[10-12] In this paper, we describe the isolation and structural analysis of spilanthol and three amides based on spectral evidence.

The dried flower heads, collected in Johen, Ehime, were extracted with *n*-hexane. The extract was fractionated and purified by silica gel and Sephadex LH-20 column chromatography to afford compound **1**, **4**, and a mixture of **2** and **3**, which were eventually isolated by preparative HPLC. Compound **2** has bitterness with slight astringency and **3** has a moderate pungent taste similar to that of spilanthol.

Compound **1** was a main amide constituent and had a pungent taste. The IR spectrum showed the presence of a secondary amide group (3340, 1636, and 1550 cm^{-1}), a double bond conjugated to an amide carbonyl group (1678 cm^{-1}), and a conjugated diene group with the *Z*, *E* or *E*, *Z* configuration (987 and 953 cm^{-1}). The MS spectrum had the molecular ion peak at *m/z* 221, indicative of the molecular formula $C_{14}H_{23}NO$. The ^{13}C-NMR and ^{1}H-NMR spectra showed the acid part was identified to be spilanthic acid (deca-2*E*,6*Z*,8*E*-trienoic acid). On the amine moiety, the typical signals at δ 3.15 (2H, t, H-1′), 1.78 (1H, m, H-2′), and 0.93 (6H, d, H-3′, 4′) in ^{1}H-NMR and at δ 20.1 (C-3′,4′), 28.6 (C-2′), and 46.9 (C-1′) in ^{13}C-NMR indicated the presence of an isobutylamino group. All the spectral data (MS, ^{1}H-NMR and ^{13}C-NMR) of compound **1** were identical with those of spilanthol (**1**) in the literature.[13,14]

The high-resolution mass spectrum (HRMS) of **2** showed a molecular ion peak at *m/z* 245.1752, indicative of the molecular formula $C_{16}H_{23}NO$. The IR spectrum showed absorption bands attributable to a triple bond (2225 cm^{-1}) in addition to a secondary amide group (3299, 1627, and 1554 cm^{-1}), and a double bond conjugated with an amide carbonyl group (1669 cm^{-1}). In the ^{1}H-NMR spectrum, the signals at δ 5.77 (d, J=15 Hz) and 6.80 (dt, J=15 Hz and 7 Hz) were attributed to olefinic protons, H-2 and H-3, respectively, which were conjugated with an amide carbonyl and assigned to be *E*-configuration. By a spin decoupling experiment, four methylenes were deduced to be located between a double bond and a triple bond. A broad singlet at δ 1.96 showed the presence of a terminal methine proton with long range coupling to methylene. On the amine moiety, a pair of 1H ddq signals at δ 1.17 and 1.41 were attributed to methylene protons of C-3′ and another pair of ddd signals at δ 3.14 and 3.27 were attributed to methylene protons of C-1′ due to the presence of an asymmetric carbon at C-2′. Furthermore, the finding of five carbons at δ 45.2, 35.1, 27.1, 11.3, and 17.2 in the ^{13}C-NMR spectrum supported a 2-methylbutylamine moiety[15] in the structure of **2**, whose optical rotation was observed as $[\alpha]_D$=4.9°. The ^{13}C-NMR spectrum showed four carbons of conjugated terminal triple bonds at δ 77.9, 65.0, 68.5,



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Table I. ^{1}H-NMR Spectral Data for Compounds **1**—**4**
(δ-values, at 400 MHz, in $CDCl_3$, TMS as internal standard)

No.	1		2		3		4	
H-2	5.79 br. d	(15)	5.77 br. d	(15)	5.78 br. d	(15)	2.17 t	(7)
3	6.83 dt	(15; 7)	6.80 dt	(15; 7)	6.80 dt	(15; 7)	1.63 m	
4	2.23—2.35 m		2.20 m		2.19 br. dt	(7; 7)	1.37 m	
5	2.23—2.35 m		1.57 m		1.55 tt	(7; 7)	1.37 m	
6	5.26 dt	(11; 7)	1.57 m		2.07 br. dt	(7; 7)	2.04 br. dt	(7; 7)
7	5.97 dd	(11; 11)	2.27 m		5.48 dt	(10; 7)	5.51 dt	(10; 7)
8	6.29 br. dd	(11; 15)			5.42 dt	(10; 7)	5.39 dt	(10; 7)
9	5.70 dq	(15; 7)			2.99 br. d	(7)	3.00 br. d	(7)
10	1.78 d	(7)						
11			1.96 br. s					
13					1.98 br. s		1.98 br. s	
$1'_a$	3.15 dd	(7; 6)	3.14 ddd	(14; 7; 6)	3.15 dd	(7; 7)	3.08 dd	(7; 7)
$1'_b$			3.27 ddd	(14; 7; 6)				
2′	1.78 m		1.57 m		1.80 tqq	(7; 7; 7)	1.76 tqq	(7; 7; 7)
$3'_a$	0.93 d	(7)	1.17 ddq	(14; 7; 7)	0.93 d	(7)	0.93 d	(7)
$3'_b$			1.41 ddq	(14; 7; 7)				
4′	0.93 d	(7)	0.91 t	(7)	0.93 d	(7)	0.93 d	(7)
5′			0.91 d	(7)				
H-N	5.47 br. s		5.38 br. s		5.45 br. s		5.46 br. s	

Coupling constants in Hz in parenthesis.

Table II. ^{13}C-NMR Spectral Data for Compounds **1**—**4**
(δ-values, at 100 MHz, in $CDCl_3$)

No.	1	2	3	4
C-1	166.0	165.9	165.9	173.0
2	124.2	124.3	124.3	36.9
3	143.5	143.6	143.9	28.9*
4	32.1	31.3	31.3	28.8*
5	26.4	27.4*	27.7	27.0
6	127.7	27.5*	26.5	25.7
7	129.5	18.9	132.2	132.9
8	126.7	77.9	122.1	122.0
9	130.0	65.0	17.6	17.5
10	18.3	68.5	76.3	76.4
11		64.7	64.7	64.5
12			68.4	68.5
13			65.0	64.9
1′	46.9	45.2	46.9	46.8
2′	28.6	35.1	28.6	28.6
3′	20.1	27.1	20.1	20.1
4′	20.1	11.3	20.1	20.1
5′		17.2		

* Assignments interchangeable.

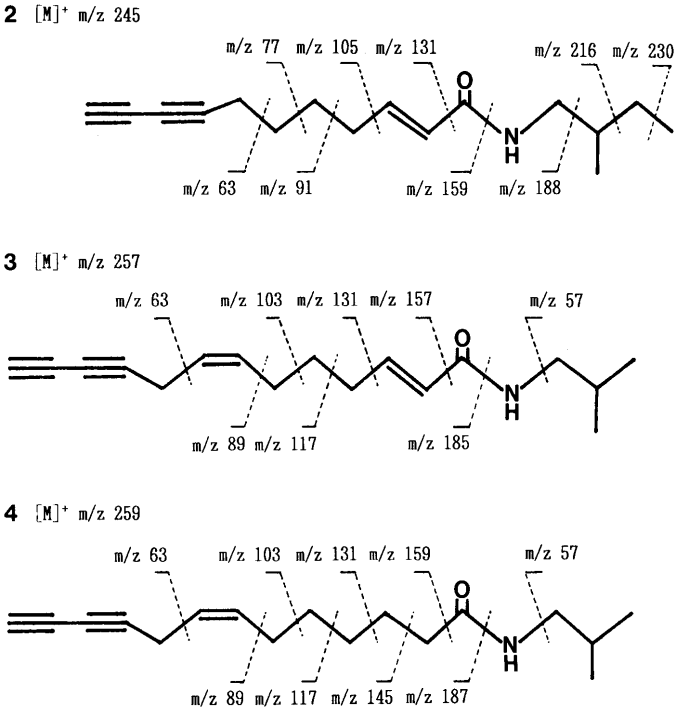


Fig. 1. Mass Fragmentation Patterns of Compounds **2**—**4**.

and 64.7, and a methylene carbon adjacent to a triple bond at δ 18.9, indicative of upfield shift by the anisotropy of the diamagnetic susceptibility of the triple bond.[16] The assignment of *sp*-carbon was decided on the basis of the data of penta-1,3-diyne.[16] Thus compound **2** was concluded to be (2*E*)-*N*-(2-methylbutyl)-2-undecene-8,10-diynamide. In the mass spectrum, besides the base ion peak at *m/z* 131 due to cleavage between C-1 and C-2, the occurrence of fragment ions at *m/z* 230 $[M-CH_3]^+$, 216 $[M-C_2H_5]^+$, and 188 $[M-C_4H_9]^+$ can be explained as depicted on the structural formula (Fig. 1). In particular the fragments at *m/z* 63 $[C_5H_3]^+$ and 91 $[C_7H_7]^+$ supported the presence of a partial structure $HC\equiv C-C\equiv C-CH_2-CH_2-CH_2-$. This compound was new, although the 2*Z*-isomer was isolated from *Echinacea angustifolia*.[17]

The HRMS spectrum of **3** had a molecular ion peak at *m/z* 257.1746, indicative of the molecular formula $C_{17}H_{23}NO$. The IR spectrum was similar to that of **2**. In the ^{1}H-NMR spectrum, the signals at δ 3.15 (2H, dd), 1.80 (1H, tqq), and 0.93 (6H, d) showed a typical pattern for an isobutylamino group. Besides the characteristic signals of *E*-olefinic protons conjugated with the amide carbonyl group, the signals for another olefinic proton appeared around δ 5.45. When the methylene signal at δ 2.99 (br. d) was irradiated, one proton multiplet at δ 5.45 collapsed to a doublet at δ 5.42 (1H) with a coupling constant of $J=10$ Hz, while irradiated at δ 2.07, a doublet of another proton appeared at δ 5.48 (1H, $J=10$ Hz). This showed the

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multiplet signals at δ 5.45 were attributed to two protons on the isolated *Z*-olefinic bond at δ 5.42 and 5.48 (1H, each), and the broad doublet at δ 2.99 must be assigned to the methylene of C-9 between an isolated double bond and a triple bond, while a double triplet signal at δ 2.07 was identified as C-6 methylene. Furthermore, when the methylene signal at δ 2.99 was irradiated, the singlet of the terminal methine proton at δ 1.98 was sharpened. The spin decoupling experiment allowed the assignment of olefinic signals and the other three methylene signals. The ^{13}C-NMR spectrum agreed with the olefinic carbons conjugated with amide carbonyl at δ 124.3 (C-2) and 143.9 (C-3), and the isolated olefinic carbons at δ 132.9 (C-7) and 122.0 (C-8). The assignment of C-7 and C-8 was decided in comparison with the ^{13}C-NMR data of falcarinol (**5**).[18] Four *sp*-carbon signals at δ 64—77 in the ^{13}C-NMR and a fragment at *m/z* 63 in the mass spectrum showed the presence of $HC\equiv C-C\equiv C-CH_2-$. In the mass spectrum, a fragment at *m/z* 89 instead of 91 found in **2** supported the presence of a double bond at the 7-position. Thus compound **3** was identified as (2*E*,7*Z*)-*N*-isobutyl-2,7-tridecadiene-10,12-diynamide, the ^{1}H-NMR data of which were identical with those of the compound isolated from *E. angustifolia*[17] and *E. purpurea*,[19] but was found for the first time in *Spilanthes* species.

The molecular formula of compound **4** was identified as $C_{17}H_{25}NO$ on the basis of HRMS ($[M]^+$ at *m/z* 259.1946). This formula suggested two more protons added to the molecular of **3**. The IR spectrum showed an absorption band of amide carbonyl at 1647 cm^{-1}. In the ^{1}H-NMR spectrum the signals at δ 5.45 (2H, m) showed the presence of an isolated double bond with *Z*-configuration as **3**. The position of the isolated double bond was presumed to be the 3-position or the 7-position, because one methylene signal was observed at δ 3.00 as a doublet. When this doublet signal was irradiated, the singlet of a terminal methine proton at δ 1.98 was sharpened. So the isolated double bond was identified as being in the 7-position. This was further confirmed by comparison of the mass fragmentation patterns of **3** and **4** (Fig. 1). That is, the common fragment ion peaks at *m/z* 63 $[C_5H_3]^+$, 89 $[C_7H_5]^+$, 103 $[C_8H_7]^+$, 117 $[C_9H_9]^+$, 131 $[C_{10}H_{11}]^+$, and 57 $[C_4H_9]^+$ (base) appeared, except the fragments of $[M-C_4H_{10}N]^+$ and $[M-C_5H_{10}NO]^+$. Therefore compound **4** was established to be (7*Z*)-*N*-isobutyl-7-tridecene-10,12-diynamide, which is a new compound.

In the family Compositae (Asteraceae), the tribes Heliantheae and Anthemideae are known to contain not only purely olefinic alkamides but also olefinic and acetylenic alkamides. Within Heliantheae alkamides were found from only a few genera, which were placed in the subtribe Ecliptinae by Robinson.[20] Many alkamides were reported from the plants belonging to the subtribe Ecliptinae such as *Spilanthes alba*,[21] *S. oleracea*,[15] *Echinacea purpurea*,[19,22] *E. angustifolia*[17] and *Acmella ciliata*.[8,9] Structure elucidation is useful in chemotaxonomic evaluations.[23,24] In this study, three alkamides obtained from *S. acmella* L. var. *oleracea* Clarke were acetylenic amides, with a common partial structure $HC\equiv C-C\equiv C-CH_2-$. As C_{13}-amides have been isolated only from *E. purpurea* and *E. angustifolia*, the occurrence of C_{13}-amides, **3** and **4**, in this study indicated a close relationship between the genera *Spilanthes* and *Echinacea*. The unsaturated pattern observed in **3** and **4** was the same with those of C_{18}- and C_{15}-amides isolated from *Achillea* species (Anthemideae) and C_{15}- and C_{13}-amides from *Echinacea* species. It was reported by Greger *et al.*[25] that C_{18}-amides isolated from *Achillea lycaonica* were derived from oleic acid→crepenynic acid→dehydro crepenynic acid and C_{15}-amide might be directly derived from C_{18}-amide through β-oxidation and oxidative decarboxylation. Bohlmann *et al.* also reported[19] that C_{13}-amide in *Echinacea* was most likely formed by chain shortening from C_{16}-amide. Consequently, compounds **3** and **4** might be formed by the same pathway ($C_{16}\rightarrow C_{13}$) in *Echinacea*.

Experimental

UV absorption spectra were taken with a Hitachi 220 spectrophotometer and IR spectra with a Jasco IR-S and a Perkin Elmer 1720X. ^{1}H-NMR (400 MHz) and ^{13}C-NMR (100 MHz) spectra were run on a JEOL GX-400 using tetramethylsilane (TMS) as an internal standard. HRMS and EIMS were obtained on a Hitachi M-2000. Optical rotation was measured with an Union PM-101. Column chromatographies were done using Pharmacia Sephadex LH-20 and Merck silica gel 60 (70—230 mesh), and TLC was done using silica gel GF-254. HPLC was done with a Shimadzu LC-4A for analysis and a Shimadzu LC-6A for preparation.

Fractionation and isolation. The *n*-hexane extract (200 g) of air-dried flower heads of *S. acmella* L. var. *oleracea* Clarke was chromatographed on a silica gel column to separate 36 fractions. The combined fractions 14—25 eluted with 10% EtOAc in CH_2Cl_2 were rechromatographed on a silica gel column. The column was eluted with hexane of increasing polarity with acetone to give 60 fractions (frs. 1—60). Fraction 26 eluted with 10% acetone–hexane was successively chromatographed on a Sephadex LH-20 column with isopropyl alcohol (IPA) and a silica gel column with *n*-hexane–CH_2Cl_2–EtOAc as the eluent to give compound **1** (spilanthol) as oil. The combined fractions 42—43, eluted with 20% acetone–hexane, were successively chromatographed on a Sephadex LH-20 column with IPA and silica gel column with *n*-hexane–acetone and CH_2Cl_2–EtOAc to give compound **4** and a mixture of **2** and **3**. Separation of **2** and **3** was achieved by HPLC (Develosil ODS-10/20, 20$\varnothing$ × 250 mm, solvent system, MeOH–H_2O (75:25); flow rate, 6 ml/min; detector, UV 230 nm). Finally, 2.5 g **1**, 10 mg **2**, 5 mg **3**, and 5 mg **4** were obtained. The purity of each compound was examined by TLC and HPLC.

(*2E,6Z,8E*)-*N-isobutyl-2,6,8-decatrienamide* (**1**) (spilanthol). Colorless oil. UV λ_{max} (MeOH) nm (log ε): 229 (3.62). IR ν_{max} (film) cm^{-1}: 3340, 3150, 3080, 3020, 1678, 1636, 1550, 1240, 1160, 987, 953. MS *m/z* (%): 221 (M^+, $C_{14}H_{23}NO$, 9), 206 (3), 192 (4), 141 (92), 126 (37), 98 (30), 81 (base), 79 (23), 41 (10).

(*2E*)-*N*-(*2-methylbutyl*)-*2-undecene-8,10-diynamide* (**2**). Colorless oil. UV λ_{max} (MeOH) nm (log ε): 212.5 (4.21). $[\alpha]_D$ +4.9° (*c* = 0.41, $CHCl_3$). IR ν_{max} (film) cm^{-1}: 3299, 3084, 2225, 1669, 1627, 1554, 1274, 1230, 978. HRMS *m/z*: 245.1752 (M^+, Calcd. for $C_{16}H_{23}NO$: 245.1779). EIMS *m/z* (%): 245 (M^+, 13), 230 (12), 216 (39), 188 (44), 159 (28), 131 (base), 105 (28), 91 (91), 77 (47), 63 (68).

(*2E,7Z*)-*N-isobutyl-2,7-tridecadiene-10,12-diynamide* (**3**) Colorless oil. UV λ_{max} (MeOH) nm (log ε): 211.5 (4.26). IR ν_{max} (film) cm^{-1}: 3300, 3094, 2224, 1668, 1628, 1554, 1274, 1234, 980. HRMS *m/z*: 257.1746 (M^+, Calcd. for $C_{17}H_{23}NO$: 257.1778). EIMS *m/z* (%): 257 (M^+, 12). 242 (6), 185 (23), 157 (32), 131 (33), 117 (45), 103 (28), 89 (13), 63 (25), 57 (base).

(*7Z*)-*N-isobutyl-7-tridecene-10,12-diynamide* (**4**). Colorless oil. IR ν_{max} (film) cm^{-1}: 3306, 2223, 1647, 1563, 1542, 1272. HRMS *m/z*: 259.1946 (M^+, Calcd. for $C_{17}H_{25}NO$: 259.1935). EIMS *m/z* (%): 259 (M^+, 2), 244 (3), 216 (11), 187 (6), 159 (27), 145 (29), 131 (27), 117 (42), 103 (22), 89 (13), 63 (24), 57 (base).

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References

1) M. Jacobson, in "Naturally Occurring Insecticides," ed. by M. Jacobson and D. Crosby, Marcel Dekker, New York, 1971, pp. 149—156.
2) Lion Corp., Japan Kokai Tokkyo Koho, 85/75424 (Apr. 27, 1985).
3) H. Mitsuhashi, "Illustrated Medicinal Plants of the World in Colour," Hokuryukan, Tokyo, 1988, p. 576.
4) E. Gerber, *Arch. Pharm.*, **241**, 270—289 (1903).
5) M. Jacobson, *Chem. Ind.*, 50—51 (1957).
6) M. Jacobson, Jr. F. Acree, and H. L. Haller, *J. Org. Chem.*, **12**, 731—732 (1947).
7) Jr. F. Acree, M. Jacobson, and H. L. Haller, *J. Org. Chem.*, **10**, 236—242 (1945).
8) R. Martin and H. Becker, *Phytochemistry*, **23**, 1781—1783 (1984).
9) R. Martin and H. Becker, *Phytochemistry*, **24**, 2295—2300 (1985).
10) N. R. Krishnaswamy, S. Prasanna, T. R. Seshadri, and N. C. Vedantham, *Phytochemistry*, **14**, 1666—1667 (1975).
11) D. K. Mukharya and A. H. Ansari, *Indian J. Chem.*, **26B**, 87 (1987).
12) A. H. Ansari, K. Mukharya, and V. K. Saxena, *Indian J. Pharm. Sci.*, **50**, 106 (1988).
13) J. Correa, S. Roquet, and E. Diaz, *Organic Magnetic Resonance*, **3**, 1—5 (1971).
14) I. Yasuda, K. Takeya, and H. Itokawa, *Chem. Pharm. Bull.*, **28**, 2251—2253 (1980).
15) H. Greger, O. Hofer, and A. Werner, *Monatsh. Chem.*, **116**, 273—277 (1985).
16) H. O. Kalinowski, S. Berger, and S. Braun, "Carbon-13 NMR Spectroscopy," John Wiley and Sons, Chichester, 1988, pp. 147—148.
17) R. Bauer, P. Remiger, and H. Wagner, *Phytochemistry*, **28**, 505—508 (1989).
18) F. Gafner, G. W. Reynolds, and E. Rodriguez, *Phytochemistry*, **28**, 1256—1257 (1989).
19) F. Bohlmann and H. Hoffmann, *Phytochemistry*, **22**, 1173—1175 (1983).
20) H. Robinson, "Smithsonian Contrib. Botany," Vol. 51, Smithsonian Press, Washington D. C., 1981, pp. 1—102.
21) F. Bohlmann, J. Ziesche, H. Robinson, and R. M. King, *Phytochemistry*, **19**, 1535—1537 (1980).
22) R. Bauer, P. Remiger, and H. Wagner, *Phytochemistry*, **27**, 2339—2342 (1988).
23) H. Greger, in "Chemistry and Biology of Naturally-Occurring Acetylenes and Related Compounds (NOARC)," ed. by J. Lam. H. Breteler, T. Armason, and L. Hansen, Elsevier, Amsterdam, 1988, pp. 159—178.
24) L. P. Christensen and J. Lam, *Phytochemistry*, **30**, 11—49 (1991).
25) H. Greger, O. Hofer, and A. Werner, *Phytochemistry*, **26**, 2235—2242 (1987).

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