

ALKAMIDES FROM THE ROOTS OF *ECHINACEA PURPUREA*

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Key Word Index—*Echinacea purpurea*; Asteraceae; unsaturated alkylamides.

Abstract—Five new alkylamides, undeca-2E,4Z-dien-8,10-dienoic acid isobutylamide, dodeca-2E,4Z-dien-8,10-dienoic acid isobutylamide, dodeca-2E,4E,10E-trien-8-ynoic acid isobutylamide, dodeca-2E,4E,8Z-trienoic acid isobutylamide and dodeca-2E,4Z-dien-8,10-dienoic acid 2-methylbutylamide were isolated from the roots of *Echinacea purpurea*. Undeca-2E,4Z-dien-8,10-dienoic acid 2-methylbutylamide is reported for the first time in an *Echinacea* species. The stereochemistry of the previously isolated major amides was determined as dodeca-2E,4E,8Z,10E-tetraenoic acid isobutylamide and dodeca-2E,4E,8Z,10Z-tetraenoic acid isobutylamide. All structures were elucidated by means of UV, IR, MS, ¹H NMR and COSY NMR.

INTRODUCTION

Our previous investigations on *Echinacea purpurea* roots have shown that commercial drugs were frequently adulterated with the roots of *Parthenium integrifolium* L. [1]. In order to differentiate between these two species, we examined the lipophilic constituents of *Echinacea purpurea* (L.) Moench which were shown to be characteristic chromatographic markers of roots and the corresponding extracts [2]. According to previous reports [3-6] all *Echinacea* species contain unsaturated isobutylamides which are well-known for the Compositae and for their insecticidal activities [7]. Recent pharmacological studies have shown that they also might possess immunostimulatory properties [8]. We now report the structure elucidation of several new alkamides isolated from *E. purpurea* roots.

RESULTS AND DISCUSSION

Extraction of *E. purpurea* roots with hexane afforded a complex mixture of lipophilic compounds. Separation was achieved by flash column chromatography on silica gel followed by MPLC and preparative HPLC on reversed-phase material (RP-8). Nine constituents could be isolated, one of them proved to be a mixture of *Z/E*-isomers.

The IR spectra of all compounds indicated the presence of a secondary amide group (bands at 3300, 3080, 1660 and 1540 cm^{-1}) as well as olefinic double bonds (1620 and 960-1000 cm^{-1}). This together with the absorption maxima at 260 nm suggested a chromophore of conjugated dienoic acid amides for 1-5, 9 and 10. Since 8 showed absorption maxima at 211 nm only, a monoenoic unit was most likely. The mass spectra of 1-8 showed typical fragmentations of isobutylamides [$M - 72$]⁺ and [$M - 100$]⁺ which are due to cleavage between C-1 and NH, and C-1 and C-2, respectively. The presence of an isobutylamide moiety was further supported by the ¹H NMR signals at 3.18 (*dd*, H-1') 1.81 (*m*,

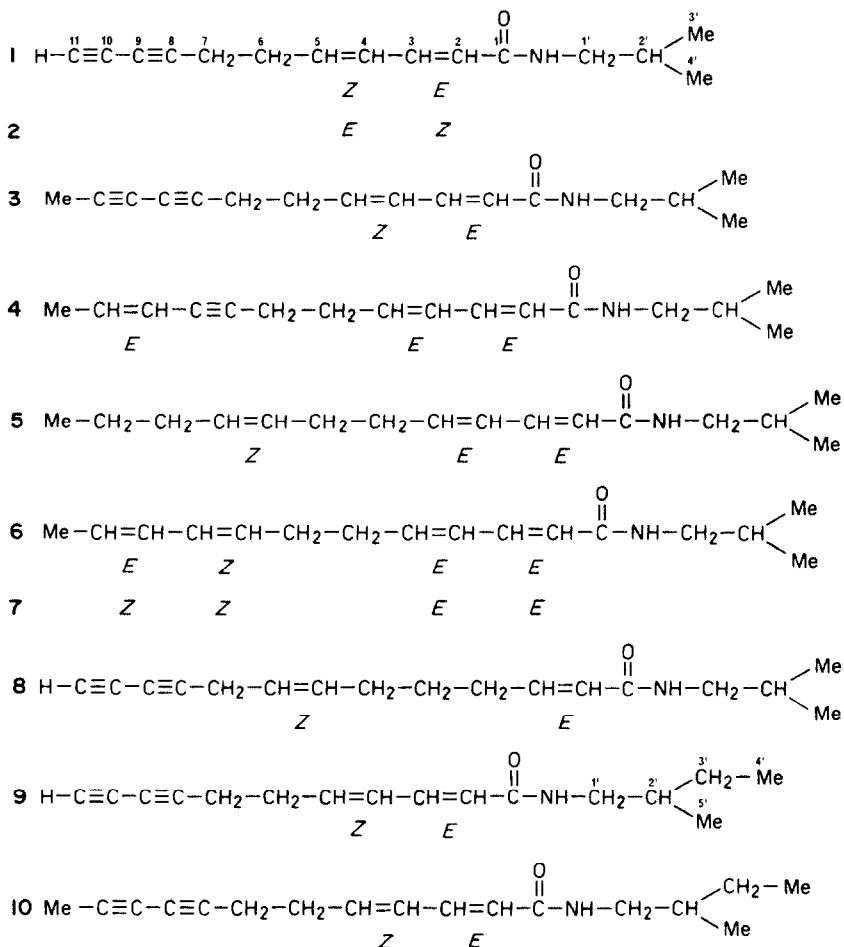
H-2') and 0.92 ppm (*d*, H-3', H-4'). The olefinic protons of the dienoic acid could be assigned to the signals at 5.8 ppm (*d*, H-2), 7.5 ppm (*dd*, H-3), 6.2 ppm (*dd*, H-4) and 6.0 ppm (*dt*, H-5). The *Z/E*-stereochemistry of the double bonds was determined by the coupling constants, 11 Hz (*Z*) or 15 Hz (*E*). In case of overlapping signals the patterns were simplified by spin decoupling experiments. Due to the deshielding effect of the carbonyl group, the β -proton (H-3) is the signal shifted farthest downfield. The assignments of the proton signals were confirmed by spin decoupling experiments and thus structures 1-5 and 8 were established.

In the ¹H NMR spectrum of 2 the olefinic proton signals differed from the others because of the *Z*-configuration of the α,β -double bond. Irradiation experiments clearly showed that the signal shifted furthest downfield in 2 at 7.5 ppm resulted from the γ -proton, and not from the β -proton as in the case of compounds 1, 3-7 and 9, 10. This difference is due to the effect of anisotropic electron regions of the carbonyl group towards the α,β -*Z*-double bond. In the ¹H NMR spectrum of 2 taken in CDCl_3 , the signals of H-6 and H-7 appeared as a broad singlet. Addition of C_6D_6 resolved the signal into a multiplet and a triplet, which enabled us unambiguously to assign the structure of 2.

The main constituents 6 and 7 of the amide fraction in *Echinacea purpurea* roots crystallized from hexane as a 1:1 mixture. The absorption maxima at 231 and 261 nm indicated a second chromophore in addition to the dienamide moiety. COSY NMR and spin decoupling experiments clearly showed the presence of the *Z/E*-isomers 6 and 7. Due to the different stereochemistry at the C-10 double bond, the signals of H-8 through H-11 of the two compounds were not identical. In the 10*Z*-isomer 7 the protons H-8 and H-9 showed a downfield shift, while H-10 and H-11 were shifted upfield compared with the 10*E*-isomer 6. Irradiation experiments afforded the coupling constants between H-10 and H-11 in 6 ($J_{10,11} = 15$, *trans*) and 7 ($J_{10,11} = 11$, *cis*) and confirmed the structures of the two isomers. They have already been

Table 1. ^1H NMR spectral data of compounds 1–10 (CDCl₃, 360/500 MHz, TMS as int.)

H	1	2	3	4	5
2	5.88 <i>d</i> (15)	5.53 <i>d</i> (11)	5.86 <i>d</i> (15)	5.79 <i>d</i> (15)	5.76 <i>d</i> (15)
3	7.49 <i>dd</i> (15; 11)	6.38 <i>dd</i> (11; 11)	7.50 <i>dd</i> (15; 11)	7.20 <i>dd</i> (15; 11)	7.19 <i>dd</i> (15; 10)
4	6.17 <i>dd</i> (11; 11)	7.51 <i>dd</i> (15; 11)	6.15 <i>dd</i> (11; 11)	6.22 <i>dd</i> (15; 11)	6.15 <i>dd</i> (15; 11)
5	5.84 <i>dt</i> (11; 8)	5.98 <i>dt</i> (15; 7)	5.85 <i>dt</i> (11; 8)	6.15 <i>dt</i> (15; 7)	6.10 <i>dt</i> (15; 7)
6	2.55 <i>dt</i> (7; 7)	2.40 <i>m</i>	2.52 <i>dt</i> (7; 7)	2.50 <i>dt</i> (7; 7)	2.18 <i>m</i>
7	2.38 <i>dt</i> (7; 1)	2.40 <i>m</i>	2.36 <i>t</i> (7)	2.42 <i>dt</i> (7; 7)	2.18 <i>m</i>
8					5.35 <i>m</i>
9					5.35 <i>m</i>
10				5.66 <i>d</i> (15)	2.00 <i>dt</i> (7; 7)
11	1.98 <i>t</i> (1)	1.98 <i>s</i>		5.88 <i>m</i>	1.37 <i>m</i>
12			1.90 <i>s</i>	1.83 <i>dd</i> (6; 1)	0.90 <i>t</i> (7)
13					
NH	5.60 <i>br s</i>	5.60 <i>br s</i>	5.56 <i>br s</i>	5.50 <i>br s</i>	5.50 <i>br s</i>
1'	3.18 <i>dd</i> (7; 7)	3.14 <i>dd</i> (7; 7)	3.18 <i>dd</i> (7; 7)	3.18 <i>dd</i> (7; 7)	3.17 <i>dd</i> (7; 7)
2'	1.81 <i>m</i>	1.80 <i>m</i>	1.81 <i>m</i>	1.81 <i>m</i>	1.78 <i>m</i>
3'	0.93 <i>d</i> (7)	0.93 <i>d</i> (7)	0.93 <i>d</i> (7)	0.93 <i>d</i> (7)	0.92 <i>d</i> (7)
4'	0.93 <i>d</i> (7)	0.93 <i>d</i> (7)	0.93 <i>d</i> (7)	0.93 <i>d</i> (7)	0.92 <i>d</i> (7)
5'					



standard; chemical shifts in δ -values (ppm); coupling constants in Hz in parentheses)

6	7	8	9	10
5.76 d (15)	5.76 d (15)	5.79 d (15)	5.88 d (15)	5.90 d (15)
7.18 dd (15; 10)	7.18 dd (15; 10)	6.81 dt (15; 7)	7.49 dd (15; 11)	7.48 dd (15; 11)
6.17 dd (15; 11)	6.17 dd (15; 11)	2.19 dt (7; 7)	6.17 dd (11; 11)	6.15 dd (11; 11)
6.10 dt (15; 7)	6.10 dt (15; 7)	1.55 m	5.82 dt (11; 8)	5.82 dt (11; 8)
2.28 m	2.28 m	2.06 dt (7; 7)	2.55 dt (7; 7)	2.52 dt (7; 7)
2.28 m	2.28 m	5.49 dt (11; 7)	2.37 t (7)	2.35 t (7)
5.25 dt (11; 7)	5.43 dt (11; 7)	5.43 dt (11; 7)		
5.97 dd (11; 11)	6.30 dd (11; 11)	3.00 d (6)		
6.32 dd (15; 11)	6.26 dd (11; 7)			
5.69 m	5.55 m		1.97 s	
1.76 dd (7; 1)	1.75 dd (7; 1)			1.90 t (1)
		1.99 s		
5.50 br s	5.50 br s	5.50 br s	5.56 br s	5.71 br s
3.16 dd (7; 7)	3.16 dd (7; 7)	3.16 dd (7; 7)	3.23 m	3.23 m
1.76 m	1.76 m	1.81 m	1.58 m	1.58 m
0.92 d (7)	0.92 d (7)	0.92 d (7)	1.17/1.41 m	1.17/1.41 m
0.92 d (7)	0.92 d (7)	0.92 d (7)	0.92 t (7)	0.92 t (7)
			0.92 d (7)	0.92 d (7)

isolated from *Echinacea* species [4] but the stereochemistry of the double bonds was not established. Our results now show that their structures are consistent with the amides, which were previously found in *Asiasarum heterotropoides* Maek. [9].

The structures of **9** and **10** differed from the previous amides in the amine moiety, which could be deduced from the MS-fragmentation pattern ($[M - 86]^+$ instead of $[M - 72]^+$) and the ^1H NMR spectra. An asymmetric carbon at C-2' was revealed by two symmetrical multiplets centered at 3.2 ppm, which also caused splitting of the signal of the protons at C3' in the methylene area. From these findings and literature data [10, 11] the presence of a 2-methylbutylamide moiety was deduced. Compounds **1**, **3**–**5** and **10** have been isolated for the first time. Compounds **2** and **8** have been reported as *Echinacea* constituents by Bohlmann [4, 5], while **9** has previously been isolated from *Acemella ciliata* Cass. [12]. Pharmacological studies are in progress to show whether these compounds are involved in the immunostimulatory activity of *Echinacea* preparations.

EXPERIMENTAL

1.8 kg air-dried and ground roots of *E. purpurea* cultivated by the Bayer. Landesanstalt für Bodenkultur und Pflanzenbau (voucher specimen deposited at the Inst. of Pharm. Biology, Munich) were extracted in a Soxhlet with *n*-hexane for 48 hr. The extract (12.4 g, crude gum) was fractionated by low pressure CC (45 \times 5 cm) on silica gel (particle size <0.063 mm) with *n*-hexane–EtOAc (3:1) as eluent. The fractions were chromatographed by TLC (silica gel 60 F₂₅₄, 0.25 mm, Merck; mobile phase *n*-hexane–EtOAc 2:1) and HPLC (LiChrospher 100–CH 18, Hibar 125 \times 4 mm, particle size 5 μm , Merck; gradient system acetonitrile–water, 40–60% in 30 min). Purification was achieved by medium pressure LC (40 \times 3 cm) on RP-8 material with acetonitrile–water mixtures as eluent. Where necessary, final purification was carried out by semipreparative HPLC (LiChrosorb RP-18, 7 μm , 250 \times 7 mm; 40% acetonitrile as eluent).

Finally 10 mg **1**, 23 mg **2**, 8 mg **3**, 2 mg **4**, 5 mg **5**, 60 mg of a mixture of **6** and **7**, 8 mg **8**, 3 mg **9** and 5 mg **10** were obtained. All compounds were colourless.

UV spectra were recorded online by diode array detection (Hewlett Packard 1040 A) in acetonitrile–water mixtures. IR spectra: KBr. ^1H NMR spectra: TMS as int. standard.

Undeca-2E,4Z-dien-8,10-dynoic acid isobutylamide (**1**). Crystals from *n*-hexane, mp 61°; UV λ_{max} nm: 259; IR ν_{max} cm^{-1} : 3300, 3080, 1670, 1540 (–CO–NH–); 2900 (CH); 2220 (H–C=C–); 1630, 1000 (–C=C–); EIMS m/z (rel. int.): 229 (29) $[M]^+$, 214 (5) $[M - \text{Me}]^+$, 186 (5) $[M - \text{C}_3\text{H}_7]^+$, 167 (38) $[\text{C}_{10}\text{H}_{11}\text{NO}]^+$, 157 (100) $[M - \text{C}_4\text{H}_{10}\text{N}]^+$, 128 (22) $[M - \text{C}_5\text{H}_{11}\text{NO}]^+$, 95 (33), 66 (55), 57 (92) $[\text{C}_4\text{H}_9]^+$, 43 (91) $[\text{C}_5\text{H}_7]^+$.

Undeca-2Z,4E-dien-8,10-dynoic acid isobutylamide (**2**). Crystals from *n*-hexane, mp 59–60° (Lit. 60–61° [4]); UV λ_{max} nm: 257; IR ν_{max} cm^{-1} : 3300, 3080, 1650, 1550 (–CO–NH–); 2900 (CH); 2220 (H–C=C–); 1620, 1005, 970 (–C=C–); EIMS m/z (rel. int.): 229 (52) $[M]^+$, 214 (9) $[M - \text{Me}]^+$, 186 (13) $[M - \text{C}_3\text{H}_7]^+$, 166 (82) $[\text{C}_{10}\text{H}_{16}\text{NO}]^+$, 157 (65) $[M - \text{C}_4\text{H}_{10}\text{N}]^+$, 152 (83) $[\text{C}_5\text{H}_{14}\text{NO}]^+$, 128 (100) $[M - \text{C}_5\text{H}_{11}\text{NO}]^+$, 115 (52) $[M - \text{C}_6\text{H}_5]^+$, 110 (55), 96 (47), 77 (35), 66 (80), 57 (78) $[\text{C}_4\text{H}_9]^+$, 41 (76).

Dodeca-2E,4Z-dien-8,10-dynoic acid isobutylamide (**3**). Crystals from *n*-hexane, mp 74°; UV λ_{max} nm: 260; IR ν_{max} cm^{-1} : 3300, 3080, 1660, 1550 (–CO–NH–); 2900 (CH); 1620, 990 (–C=C–); EIMS m/z (rel. int.): 243 (100) $[M]^+$, 228 (13) $[M - \text{Me}]^+$, 215 (17), 200 (13) $[M - \text{C}_3\text{H}_7]^+$, 186 (20) $[M - \text{C}_4\text{H}_9]^+$, 171 (23) $[M - \text{C}_4\text{H}_{10}\text{N}]^+$, 143 (27) $[M - \text{C}_5\text{H}_{10}\text{NO}]^+$, 128 (37) $[M - \text{C}_6\text{H}_{13}\text{NO}]^+$, 115 (30) $[M - \text{C}_{10}\text{H}_8]^+$, 77 (18), 67 (18), 57 (22) $[\text{C}_4\text{H}_9]^+$, 43 (17) $[\text{C}_3\text{H}_7]^+$, 41 (19).

Dodeca-2E,4E,10E-trien-8-ynoic acid isobutylamide (**4**). Oil; UV λ_{max} nm: 232, 261; IR ν_{max} cm^{-1} : 3300, 3080, 1660, 1550 (–CO–NH–); 2900 (CH); 1630, 995 (–C=C–); EIMS m/z (rel. int.): 245 (37) $[M]^+$, 230 (12) $[M - \text{Me}]^+$, 202 (7) $[M - \text{C}_3\text{H}_7]^+$, 188 (15) $[M - \text{C}_4\text{H}_9]^+$, 173 (30) $[M - \text{C}_4\text{H}_{10}\text{N}]^+$, 167 (38) $[\text{C}_{10}\text{H}_{11}\text{NO}]^+$, 145 (100) $[M - \text{C}_5\text{H}_{10}\text{NO}]^+$, 129 (53), 115 (83) $[M - \text{C}_{10}\text{H}_{10}]^+$, 110 (45), 104 (57).

Dodeca-2E,4E,8Z-trienoic acid isobutylamide (**5**). Oil; UV λ_{max} nm: 259; IR ν_{max} cm^{-1} : 3300, 3080, 1660, 1550 (–CO–NH–);

2920 (CH); 1630, 995 ($-C=C-$); EIMS m/z (rel. int.): 249 (32) [$M]^+$, 234 (5) [$M-Me]^+$, 220 (6), 206 (9) [$M-C_3H_7]^+$, 181 (32), 177 (100) [$M-C_4H_{10}N]^+$, 167 (95) [$C_{10}H_{17}NO]^+$, 152 (57), 149 (60) [$M-C_5H_{10}]^+$, 115 (38) [$M-C_{10}H_{14}]^+$, 110 (92).

Dodeca-2E,4E,8Z,10E-tetraenoic acid isobutylamide (6) and dodeca-2E,4E,8Z,10Z-tetraenoic acid isobutylamide (7). Mixture crystallized as needles from *n*-hexane, mp 70° (Lit. 69° [9]); UV λ_{max} nm: 231, 260 ($\epsilon=24\,000$, 28 500); IR ν_{max} cm⁻¹: 3300, 3100, 1660, 1550 ($-CO-NH-$); 2930 (CH); 1635, 1000, 950 ($-C=C-$); EIMS m/z (rel. int.): 247 (45) [$M]^+$, 232 (5) [$M-Me]^+$, 175 (12) [$M-C_4H_{10}N]^+$, 167 (90) [$C_{10}H_{17}NO]^+$, 152 (33), 147 (13) [$M-C_5H_{10}NO]^+$, 128 (44) [$C_7H_{14}NO]^+$, 115 (63) [$M-C_{10}H_{12}]^+$, 94 (44), 81 (100) [$C_6H_9]^+$, 66 (75), 57 (76) [$C_4H_9]^+$, 41 (77).

Trideca-2E,7Z-dien-10,12-dienoic acid isobutylamide (8). Oil; UV λ_{max} nm: 211; IR ν_{max} cm⁻¹: 3300, 3080, 1670, 1550 ($-CO-NH-$); 2930 (CH); 2220, 2300 (H-C≡C-); 1635, 980 ($-C=C-$); EIMS m/z (rel. int.): 257 (24) [$M]^+$, 242 (13) [$M-Me]^+$, 228 (37), 214 (32) [$M-C_3H_7]^+$, 200 (11) [$M-C_4H_9]^+$, 185 (31) [$M-C_4H_{10}N]^+$, 157 (51) [$M-C_5H_{10}NO]^+$, 149 (56), 129 (85), 115 (75) [$M-C_{11}H_{10}]^+$, 91 (59), 81 (76) [$C_6H_9]^+$, 69 (68), 57 (100) [$C_4H_9]^+$.

Undeca-2E,4Z-dien-8,10-dienoic acid 2-methylbutylamide (9). Oil; UV λ_{max} nm: 261; IR ν_{max} cm⁻¹: 3300, 3080, 1660, 1550 ($-CO-NH-$); 2950 (CH); 2290, 2220 (H-C≡C-); 1620, 990, 960 ($-C=C-$); EIMS m/z (rel. int.): 243 (15) [$M]^+$, 242 (21), 228 (8) [$M-Me]^+$, 214 (4), 186 (10) [$M-C_4H_9]^+$, 172 (13) [$M-C_5H_{11}]^+$, 157 (33) [$M-C_5H_{12}N]^+$, 128 (100) [$M-C_6H_{13}NO]^+$, 115 (14), 110 (14).

Dodeca-2E,4Z-dien-8,10-dienoic acid 2-methylbutylamide (10). Oil; UV λ_{max} nm: 261; IR ν_{max} cm⁻¹: 3300, 3080, 1660, 1550 ($-CO-NH-$); 2920 (CH); 1640, 990, 960 ($-C=C-$); EIMS m/z (rel.

int.): 256 (100) [$M-1]^+$, 242 (20) [$M-Me]^+$, 228 (16), 213 (25), 199 (15), 185 (28), 171 (23) [$M-C_5H_{12}N]^+$, 149 (48), 129 (71), 112 (90), 100 (76).

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