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ALKAMIDES FROM THE ROOTS OF *ECHINACEA ANGUSTIFOLIA*

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

Abstract—The isolation and structure elucidation of 15 alkamides from the roots of *Echinacea angustifolia* is reported. In addition to several compounds previously found in *E. purpurea* the new undeca-2*Z*-en-8,10-diynoic acid isobutylamide, dodeca-2*E*-en-8,10-diynoic acid isobutylamide, undeca-2*Z*-en-8,10-diynoic acid 2-methylbutylamide, dodeca-2*E*-en-8,10-diynoic acid 2-methylbutylamide, hexadeca-2*E*,9*Z*-dien-12,14-diynoic acid isobutylamide and dodeca-2*E*,4*Z*,10*Z*-trien-8-ynoic acid isobutylamide were isolated. The isobutylamide of undeca-2*E*-en-8,10-diynoic acid was found for the first time in *Echinacea* species. All structures were established by UV, IR, NMR and mass spectroscopy.

INTRODUCTION

Preparations of *Echinacea* plants have a long tradition in the adjuvant therapy of infections and chronic inflammations and are said to stimulate unspecifically the immune system [1]. Recent pharmacological studies have shown that the lipophilic constituents might be involved in the biological activity of the drug [2]. In previous papers we reported on the lipophilic amide constituents from the aerial parts of *Echinacea purpurea*, *E. angustifolia* and *E. pallida* [3], as well as from the roots of *E. purpurea* [4], and on polyacetylenes from the roots of *E. pallida* [5]. We also found that commercial roots of *E. angustifolia* are frequently mixed with *E. pallida* [5, 6]. Therefore it is likely that in the past some investigations on *E. angustifolia* have been carried out with *E. pallida* by mistake. We have now investigated the lipophilic constituents of the underground parts of authentic *Echinacea angustifolia* DC. and report here on the isolation and structure elucidation of six new and several previously isolated alkylamides from the roots of this plant.

RESULTS AND DISCUSSION

The hexane extract of the roots was fractionated by column chromatography on silica gel. Semipreparative HPLC on reversed-phase material (RP-18) with acetonitrile–water mixtures afforded pure amide compounds **1–13**. The main constituents **14** and **15** were

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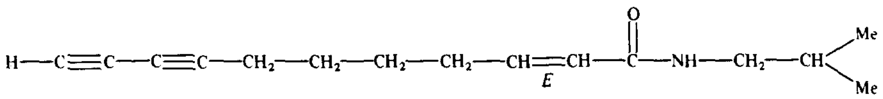

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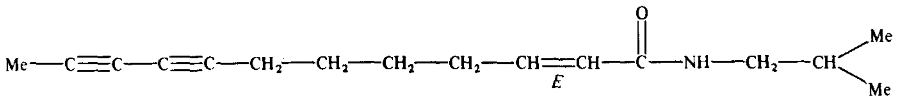

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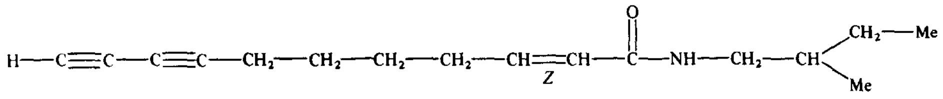

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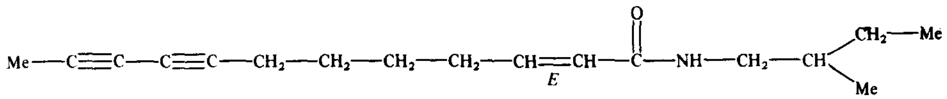

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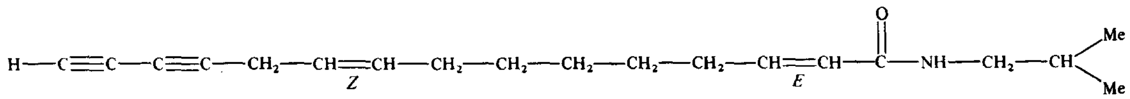

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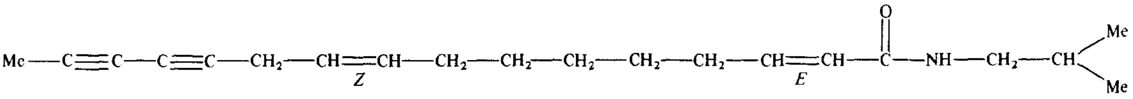

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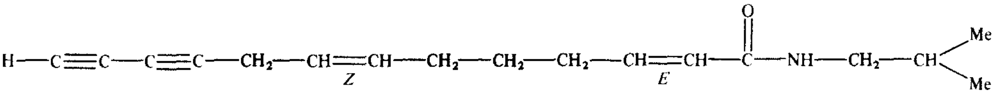

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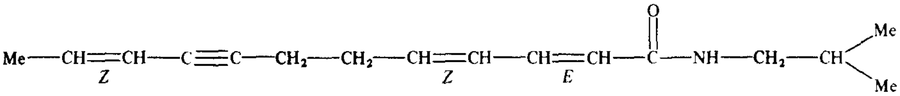

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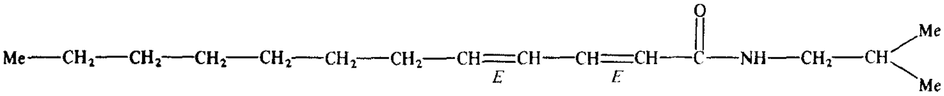

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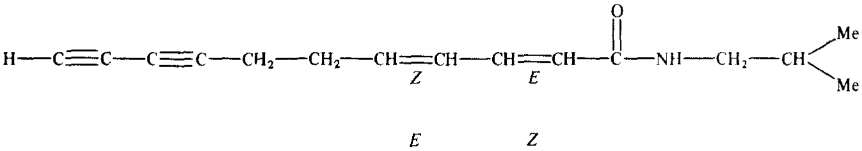

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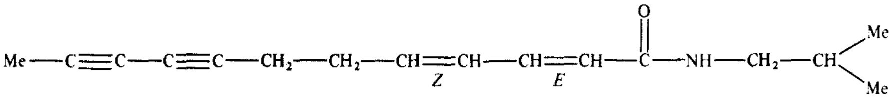

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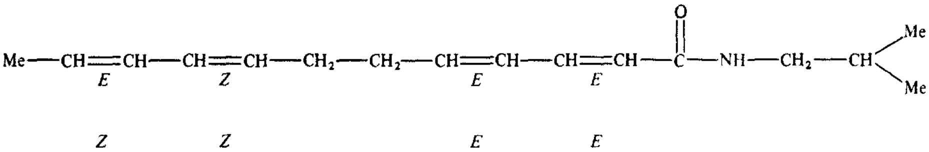

Table 1. ^{1}H NMR spectral data of compounds **1**–**7**, **9** and **10** (CDCl$_3$, 80/360 MHz, TMS as internal

H	1	2	3	4	5
2	5.79 *dt* (15; 1)	5.70 *d* (11)	5.79 *dt* 15; 1)	5.70 *dt* (11; 1)	5.77 *dt* (15; 1)
3	6.83 *dt* (15; 7)	5.97 *dt* (11; 7)	6.83 *dt* (15; 7)	5.97 *dt* (11; 7)	6.81 *dt* (15; 7)
4	2.30 *m*	2.68 *dt* (11; 7)	2.25 *m*	2.68 *dt* (7; 7)	2.26 *m*
5	1.60 *m*	1.60 *m*	1.62 *m*	1.58 *m*	1.56 *m*
6	1.60 *m*	1.60 *m*	1.62 *m*	1.58 *m*	1.56 *m*
7	2.30 *m*	2.26 *m*	2.25 *m*	2.29 *t* (7)	2.28 *t* (6)
8				—	—
9				—	—
10				—	—
11	1.98 *t* (1)	1.95 *t* (1)	—	1.96 *s*	—
12	—	—	1.90 *t* (1)	—	1.91 *s*
15				—	—
16					
N-H	5.45 *br s*	5.55 *br s*	5.45 *br s*	5.43 *br s*	5.63 *br s*
1′	3.18 *dd* (7; 7)	3.14 *dd* (7; 7)	3.18 *dd* (7; 7)	3.17 *m*	3.20 *m*
2′	1.80 *m*	1.80 *m*	1.80 *m*	1.58 *m*	1.56 *m*
3′	0.93 *d* (7)	0.93 *d* (7)	0.93 *d* (7)	1.41/1.16 *m*	1.41/1.15 *m*
4′	0.93 *d* (7)	0.93 *d* (7)	0.93 *d* (7)	0.91 *t* (7)	0.91 *t* (7)
5′				0.91 *d* (7)	0.91 *d* (7)

identified by chromatographic comparison with authentic samples as the isomeric dodeca-2*E*,4*E*,8*Z*,10*E*/*Z*-tetraenoic acid isobutylamides, which were also found to be the main constituents in *E. purpurea* roots [4]. The alkylamide moiety of the isolated compounds could clearly be deduced from the IR spectrum. Characteristic bands at 3300, 3080, 1660 and 1540 cm^{-1} were due to a secondary amide group. A monoenoic structure –CH=CH–CO– was indicated by a band at 980 cm^{-1}, whereas an absorption at 990–1000 cm^{-1} suggested a dienoic moiety –CH=CH–CH=CH–CO–. Bands at 2220–2300 cm^{-1} could be assigned to a terminal acetylenic group H–C≡C–. In the UV spectrum an absorption maximum at 260 nm indicated a dienoic acid amide moiety for compounds **9–15**, while a sharp absorption maximum at 211 nm was due to monoenoic structures in compounds **1–5**. In the UV spectra of the monoenoic amides **6–8** the absorption at 211 nm was overlapped by a maximum at 199 nm, which is caused by the presence of an additional double bond at C-9. The amide moiety could further be established by the mass spectrum. While the peak matching $[\mathrm{M}-15]^+$, $[\mathrm{M}-43]^+$, $[\mathrm{M}-57]^+$, $[\mathrm{M}-72]^+$ and $[\mathrm{M}-100]^+$ was characteristic for an isobutylamide structure, fragments at $[\mathrm{M}-15]^+$, $[\mathrm{M}-29]^+$, $[\mathrm{M}-43]^+$, $[\mathrm{M}-86]^+$ and $[\mathrm{M}-114]^+$ could be assigned to a 2-methyl-butylamide moiety.

In the ^{1}H NMR spectrum characteristic signals at δ 3.18 (*dd*, H-1′), 1.80 (*m*, H-2′) and 0.92 (*d*, H-3′ and H-4′) indicated the isobutylamide moiety in compounds **1–3** and **6–13**. Compounds **4** and **5** showed different signals caused by the amine part. Methylation at C-3′ resulted in an asymmetric carbon at C-2′ which split the proton signals of C-1′ into two symmetric multiplets centred at δ 3.2 and the proton signals of C-3′ into two multiplets at 1.41 and 1.15. These findings suggested the presence of a 2-methyl-butylamide structure. The protons of the acid moiety could be assigned by comparison with previously isolated alkamides [4]. The stereochemistry of the double bonds was deduced from the observed coupling constants. The different stereochemistry of compounds with an *α,β-Z*-double bond (e.g. **2** and **4**) caused an upfield shift of about 1 ppm for the doublet triplet signal of proton H-3 compared with the other amides with *α,β-E*-configuration.

Compounds **6, 8** and **10** have already been identified as constituents of *Echinacea* [7, 8]. Compounds **2–5, 7** and **9** have been isolated for the first time, while compound **1** has previously been found in *Acmella ciliata* [9]. The alkamides **11–15** were recently also identified as constituents of *E. purpurea* [4]. While **14** and **15** are major constituents both in *E. angustifolia* and *E. purpurea*, compounds **11–13** occur in *E. angustifolia* in much smaller quantities than in *E. purpurea*.

EXPERIMENTAL

Air-dried and ground roots (290 g) of *E. angustifolia*, cultivated by Spagyros SA, Boncourt, Switzerland (voucher specimen deposited at the Inst. of Pharm. Biology, Munich) were extracted in a Soxhlet for 3 days with *n*-hexane. The yellow extract was subsequently fractionated by CC (30 × 4 cm) on silica gel (particle size < 0.063 mm; eluent EtOAc–hexane 1:5). Final purification by semiprep. HPLC (LiChrosorb RP-18, 7 μm, 250 × 10 mm) with appropriate MeCN–H_2O mixtures as eluent (40–80%) afforded 9 mg **1**, 30 mg **2**, 7 mg **3**, 2 mg **4**, 2 mg **5**, 4 mg **6**, 2 mg **7**, 2 mg **8**, 4 mg **9**, 30 mg **10**, 3 mg **11**, 4 mg **12** and 2 mg **13** as pure compounds. Analytical HPLC was carried out with LiChrospher 100-CH 18, Hibar 125 × 4 mm, particle size 5 μm, Merck; gradient system 40–80% MeCN in 30 min. Analytical TLC: silica gel 60 F_{254}, 0.25 mm, Merck; mobile phase *n*-hexane–EtOAc (2 : 1). UV spectra were recorded online by diode array detection (Hewlett Packard 1040A) in MeCN–H_2O mixtures. IR: KBr. ^{1}H NMR: TMS.

Undeca-2E-en-8,10-diynoic acid isobutylamide (**1**). Colourless crystals from *n*-hexane, mp 69°; UV $\lambda_{\max}$ nm: 211 (ε = 10 600); IR $\nu_{\max}$ cm^{-1}: 3300, 3080, 1670, 1550 (–CO–NH–); 2950 (CH–); 2300, 2220 (H–C≡C–); 1630, 980 (–C=C–); EIMS *m/z* (rel. int.): 231 (1) $[\mathrm{M}]^+$, 216 (1) $[\mathrm{M}-\mathrm{Me}]^+$, 202 (5), 188 (3) $[\mathrm{M}-\mathrm{C_3H_7}]^+$,

standard; chemical shifts in δ-values; coupling constants in Hz in parenthesis)

6	**7**	**9**	**10**
5.78 *d* (15)	5.76 *dt* (15; 1)	5.87 *d* (15)	5.75 *d* (15)
6.83 *dt* (15; 7)	6.83 *dt* (15; 7)	7.55 *dd* (15; 11)	7.19 *dd* (15; 11)
2.18 *dt* (7; 7)	2.17 *dt* (7; 7)	6.17 *dd* (11; 11)	6.13 *dd* (15; 11)
1.47 *m*	1.40 *m*	5.88 *dt* (11; 7)	6.06 *dt* (15; 7)
1.36 *m*	1.40 *m*	2.57 *dt* (7; 7)	2.14 *dt* (7; 7)
1.36 *m*	1.40 *m*	2.47 *dt* (7; 1)	1.26 *m*
2.05 *dt* (7; 7)	2.02 *dt* (7; 7)	—	1.26 *m*
5.40 *dt* (11; 7)	5.40 *dt* (11; 7)	—	1.26 *m*
5.50 *dt* (11; 7)	5.46 *dt* (11; 7)	5.45 *dq* (11; 1)	1.26 *m*
3.02 *d* (7)	2.98 *d* (7)	5.90 *m*	1.26 *m*
—	—	1.84 *dd* (7; 1)	0.88 *t* (7)
2.01 s			
	1.90 *s*	—	—
5.50 *br s*	5.50 *br s*	5.57 *br s*	5.55 *br s*
3.18 *dd* (7; 7)	3.15 *dd* (7; 7)	3.18 *dd* (7; 7)	3.16 *dd* (7; 7)
1.82 *m*	1.80 *m*	1.80 *m*	1.80 *m*
0.93 *d* (7)	0.93 *d* (7)	0.93 *d* (7)	0.93 *d* (7)
0.93 *d* (7)	0.93 *d* (7)	0.93 *d* (7)	0.93 *d* (7)
—			

174 (3) $[M-C_4H_9]^+$, 160 (6), 159 (4) $[M-C_4H_{10}N]^+$. 149 (7), 131 (20) $[M-C_5H_{10}NO]^+$, 116 (28), 103 (30), 91 (100) $[C_7H_7]^+$, 54 (58).

Undeca-2Z-*en*-8,10-*diynoic acid isobutylamide* (**2**). Colourless oil; UV λ_{max} nm: 211 ($\varepsilon = 13\,600$); IR ν_{max} cm^{-1}: 3300, 3080, 1660, 1540 (–CO–NH–); 2950 (CH–); 2300, 2220 (H–C≡C–); 1630, 970 (–C=C–); EIMS *m/z* (rel. int.): 231 (1) $[M]^+$, 216 (1) $[M-Me]^+$, 203 (4), 188 (3) $[M-C_3H_7]^+$, 174 (3) $[M-C_4H_9]^+$, 168 (2) $[M-C_5H_3]^+$, 159 (10) $[M-C_4H_{10}N]^+$, 146 (10), 141 (9), 131 (73) $[M-C_5H_{10}NO]^+$. 117 (76), 103 (27), 91 (95) $[C_7H_7]^+$. 57 (100) $[C_4H_9]^+$.

Dodeca-2E-*en*-8,10-*diynoic acid isobutylamide* (**3**). Colourless crystals from *n*-hexane, mp 104°; UV λ_{max} nm: 211; IR ν_{max} cm^{-1}: 3300, 3080, 1670, 1550 (–CO–NH–); 2960 (CH–); 1620, 980 (–C=C–); EIMS *m/z* (rel. int): 245 (3) $[M]^+$, 230 (2), $[M-Me]^+$, 217 (10), 202 (5) $[M-C_3H_7]^+$, 188 (5) $[M-C_4H_9]^+$, 173 (10) $[M-C_4H_{10}N]^+$, 169 (11), 149 (19), 145 (39) $[M-C_5H_{10}NO]^+$, 130 (30), 117 (46), 105 (30) $[C_8H_9]^+$, 91 (46) $[C_7H_7]^+$, 77 (47) $[C_6H_5]^+$, 57 (100) $[C_4H_9]^+$.

Undeca-2Z-*en*-8,10-*diynoic acid* 2-*methylbutylamide* (**4**). Colourless oil; UV λ_{max} nm: 211; IR ν_{max} cm^{-1}: 3300, 3080, 1660, 1540 (–CO–NH–); 2960 (CH–); 2220, 2300 (H–C≡C–); 1630 (–C=C–); EIMS *m/z* (rel. int.): 246 (100) $[M+H]^+$, 230 (13) $[M-Me]^+$, 216 (38) $[M-C_2H_5]^+$, 202 (10) $[M-C_3H_7]^+$, 188 (40) $[M-C_4H_9]^+$, 159 (52) $[M-C_5H_{12}N]^+$, 131 (86) $[M-C_6H_{12}NO]^+$, 117 (78), 104 (43), 91 (76) $[C_7H_7]^+$, 71 (63), 63 (48), 55 (81), 43 (97).

Dodeca-2E-*en*-8,10-*diynoic acid* 2-*methylbutylamide* (**5**). Colourless oil; UV λ_{max} nm: 211; IR ν_{max} cm^{-1}: 3300, 3080, 1665, 1540 (–CO–NH–); 2940 (CH–); 1620, 980 (–C=C–); EIMS *m/z* (rel. int.): 259 (3) $[M]^+$, 258 (5), 244 (3) $[M-Me]^+$, 230 (10) $[M-C_2H_5]^+$, 216 (5), 202 (7) $[M-C_4H_9]^+$, 188 (8), 173 (10) $[M-C_5H_{12}N]^+$, 160 (16), 145 (46) $[C_{11}H_{13}]^+$, 130 (37) $[C_{10}H_{10}]^+$, 117 (77), 105 (57) $[C_8H_9]^+$, 91 (82) $[C_7H_7]^+$, 77 (84) $[C_6H_5]^+$, 55 (100).

Pentadeca-2E,9Z-*dien*-12,14-*diynoic acid isobutylamide* (**6**). Colourless oil; UV λ_{max} nm: 199, 211 sh, IR ν_{max} cm^{-1}: 3300, 3080, 1670, 1550 (–CO–NH–); 2940 (CH–); 2220 (H–C≡C–); 1635, 980 (–C=C–); CIMS (NH_3) *m/z* (rel. int.): 286 (100) $[M+1]^+$.

Hexadeca-2E,9Z-*dien*-12,14-*diynoic acid isobutylamide* (**7**). Colourless oil; UV λ_{max} nm: 199, 211 sh; IR ν_{max} cm^{-1}: 3300, 3080, 1670, 1550 (–CO–NH–); 2950 (CH–); 1630, 980 (–C=C–); EIMS *m/z* (rel. int.): 300 (45) $[M+H]^+$, 284 (16) $[M-CH_3]^+$, 256 (45) $[M-C_3H_7]^+$, 242 (34) $[M-C_4H_9]^+$, 216 (39), 199 (26) $[M-C_5H_{10}NO]^+$, 154 (43), 149 (53), 177 (52), 91 (49), 83 (84), 57 (100), 43 (55).

Dodeca-2E,4Z,10Z-*trien*-8-*ynoic acid isobutylamide* (**9**). Colourless oil; UV λ_{max} nm: 231 sh, 261; IR ν_{max} cm^{-1}: 3300, 3080, 1660, 1550 (–CO–NH–); 2960 (CH–); 1620, 990, 950 (–C=C–); EIMS *m/z* (rel. int.): 246 (100) $[M+H]^+$, 230 (54) $[M-Me]^+$, 202 (37) $[M-C_3H_7]^+$, 188 (44) $[M-C_4H_9]^+$, 173 (57) $[M-C_4H_{10}N]^+$, 145 (76) $[M-C_5H_{10}NO]^+$, 131 (58), 177 (55), 105 (43), 91 (58), 77 (67), 66 (61), 57 (77), 41 (63).

Dodeca-2E-4E-*dienoic acid isobutylamide* (**10**). Colourless oil; UV λ_{max} nm: 260; IR ν_{max} cm^{-1}: 3300, 3080, 1650, 1545 (–CO–NH–); 2950 (CH–); 1620, 990 (–C=C–); EIMS *m/z* (rel. int.): 251 (82) $[M]^+$, 236 (25) $[M-Me]^+$, 208 (10) $[M-C_3H_7]^+$, 195 (13), 179 (100) $[M-C_4H_{10}N]^+$. 152 (62), 113 (42), 96 (90), 81 (95), 67 (38), 55 (52), 41 (46).

Trideca-2E,7Z-dien-10,12-diynoic acid isobutylamide (**8**), undeca-2E,4Z-dien-8,10-diynoic acid isobutylamide (**11**), undeca-2Z,4E-dien-8,10-diynoic acid isobutylamide (**12**), dodeca-2E,4Z-dien-8,10-diynoic acid isobutylamide (**13**), dodeca-2E,4E,8Z,10E-tetraenoic acid isobutylamide (**14**) and dodeca-2E,4E,8Z,10Z-tetraenoic acid isobutylamide (**15**) were identical in their spectroscopic data with the compounds isolated previously from *E. purpurea* [4].

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