Phytochemistry, Vol. 26, No. 8, pp. 2289–2291, 1987.
Printed in Great Britain.

0031–9422/87 $3.00 + 0.00
Pergamon Journals Ltd.

PYRROLE AMIDES FROM *ACHILLEA AGERATIFOLIA*

H. GREGER, C. ZDERO* and F. BOHLMANN*

Institute of Botany, University of Vienna, A-1030 Vienna, Austria; * Institute of Organic Chemistry, Technical University of Berlin, D-1000 Berlin 12, FRG

(*Received* 9 *December* 1986)

Key Word Index—*Achillea ageratifolia* ssp. *serbica*; *Compositae*; *Anthemideae*; olefinic and acetylenic alkamides; pyrrolides; pyrrolidides.

Abstract—A reinvestigation of the root extract of *Achillea ageratifolia* led to the identification of nine new alkamides. Their structures and stereochemistries were elucidated by spectroscopic methods. Apart from the pyrrolidides and dehydropyrrolidides previously published for that species four further pyrrolidides together with five pyrrolides were isolated. The latter derivatives fall into a new series of alkamides. The different patterns of unsaturation of the acid residues suggest close biogenetic connections with oleic and crepenynic acid.

INTRODUCTION

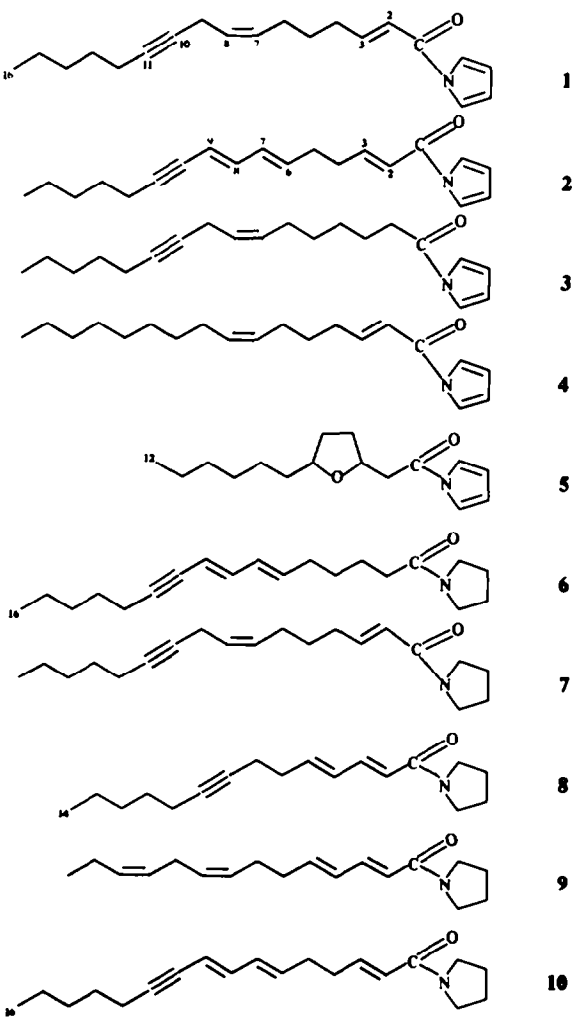
Achillea ageratifolia (Sibth. & Smith) Boiss. ssp. *serbica* (Nym.) Heimerl, distributed in the central part of Balkan peninsula, has already been investigated chemically. The underground parts were shown to accumulate unsaturated C_{16} alkamides mainly characterized by dehydropyrrolidine and pyrrolidine moieties [1]. As with the other amides known from the genus *Achillea* [2], they have been detected by comparative UV analyses and were isolated from the more polar column fractions (silica gel, petrol–ethyl ether, 1:1; and ethyl ether). In *A. ageratifolia*, however, corresponding UV spectra of the less polar fractions (petrol–ethyl ether, 9:1 and 3:1) suggested a further series of related compounds with similar chromophores. These fractions were also observed in other provenances of that species.

A re-investigation of the underground parts of a wild collection, originating from Yugoslavia, led to the identification of nine new alkamides.

RESULTS AND DISCUSSION

From the less polar column fractions of the petrol–ethyl ether (2:1) extract five derivatives were isolated which belong to a new type of alkamide. All are characterized by a pyrrole moiety. Whereas the acid residues of the major compounds **1** and **2** have already been described for corresponding dehydropyrrolidides (pyrrolideides), pyrrolidides and isobutylamides ([1], compare also **7** and **10**), those of the amides **3**, **4** and **5** are reported here for the first time.

The analysis of the more polar fractions revealed in addition to the compounds reported previously [1], (+)sesamin, sitosterol, stigmasterol as well as four new pyrrolidides (**6**–**9**). Comparing the chain length of the acid residues, in *A. ageratifolia* obviously the C_{16} amides dominate, whereas the C_{12} (**5**) and C_{14} (**8**, **9**) derivatives occur only in small amounts. The different patterns of unsaturation resemble those of some C_{18} alkamides which have recently been isolated from *A. lycaonica* and *A. chamaemelifolia* and have led to some general conclusions about the biogenetic connections of the acid moieties [3]. Taking into account a loss of two carbon atoms by β-oxidation, the *Z*-orientated double bond at position C-7/C-8 (in **1**, **3**, **4**, **7**) may be directly derived



from oleic acid. The 6*E*-, 8*E*-orientated double bonds (in **2**, **6**, **10**) are most likely formed by allylic rearrangement [3, 4]. Finally, the co-occurrence of pyrrolidides and corresponding mono- and bis dehydro derivatives may be an indication for the ability to dehydrogenate pyrrolidides.

In the ^{1}H NMR spectra of the pyrrolide series (**1**–**5**) (Table 1) a pair of triplets around δ7.3 and 6.3 was visible. Furthermore, the base peak in the mass spectra corresponded to C_4H_5N. These data required the presence of pyrrolides. This was also supported in the ^{13}C NMR spectrum of **1** (Experimental) by a pair of doublets at δ112.2 and 113.1. The nature of the unsaturated acid chains followed from the ^{1}H NMR spectral data. In the spectrum of **1** all signals could be assigned by spin decoupling. Starting with the H-2 signal (δ6.58 *dt*) the protons H-3–H-9 could be assigned. As the signal of H-9 showed a long range coupling with H-12 the remaining signals could be assigned, though those of H-14 and H-15 were overlapped. However, since the corresponding multiplet was coupled with H-13 and H-16 no other interpretation is possible. The signals in the spectra of **2**–**4** could be similarly assigned. The configurations of the double bonds followed from the observed couplings.

The ^{1}H NMR spectrum of the pyrrolide **5** showed a pronounced difference from those of **1**–**4** (Table 1). Whilst the presence of a pyrrolide was obvious again, the signals of olefinic protons were missing. The presence of a pyrrolide with an oxygen function at C-3 was indicated by a pair of double doublets at δ3.20 and 2.91 which were coupled with a fourfold doublet at δ4.50. Spin decoupling allowed the assignment of the remaining signals. The resulting sequence required the presence of a tetrahydrofuran ring. This assumption was supported by the ^{13}C NMR spectrum (see Experimental).

It has already been pointed out in previous papers [3, 5] that, in the IR spectra of alkamides, characteristic absorption bands show regularities which allow a rapid determination of the amine parts. Accordingly, all pyrrolides described in the present paper are characterized by a strong, sharp band at 1463–1462 cm^{-1} accompanied by two middle sized bands at 690 and 590 cm^{-1} (see Experimental). Together with the pronounced bands at 1700 and 1638 cm^{-1} ($\geq$N–C=O/C=C stretching region) they may be easily distinguished from corresponding pyrrolidides or pyrrolideides.

The ^{1}H NMR spectra of the amides **6**–**9** (Table 1) showed the typical signals of pyrrolidides [1, 6]. Again the nature of the acid chains could be deduced from the results of spin decoupling. In the spectrum of **9** in deuteriochloroform the signals of the olefinic protons were an overlapped multiplet. However, after addition of deuteriobenzene these signals were separated sufficiently for spin decoupling and the configuration of the double bonds could then be deduced from the couplings.

EXPERIMENTAL

A. ageratifolia ssp. *serbica* was collected near Višegrad, SR Bosna i Hercegovina, Yugoslavia. Voucher specimens have been

Table 1. ^{1}H NMR spectral data of compounds **1**–**9** (400 MHz, $CDCl_3$, TMS as internal standard)

	1	**2**	**3**	**4**	**5**	**6**	**7**	**8**	**9**
H-2	6.58 *dt*	6.56 *dt*	2.81 *t*	6.56 *dt*	3.20 *dd* 2.91 *dd*	2.25 *t*	6.11 *dt*	6.11 *br d*	6.00 *d*
H-3	7.25 *dt*	7.24 *dt*	1.79 *tt*	7.28 *dt*	4.50 *dddd*	1.66 *tt*	6.90 *dt*	7.27 *dd*	7.26 *dd*
H-4	2.35 *ddt*	2.44 *br dt*	1.43 *m*	2.36 *ddt*	2.27 *m* 1.60 *m*	1.45 *tt*	2.21 *br dt*	6.23 *br dd*	6.20 *br dd*
H-5	1.62 *tt*	2.36 *dt*	1.33 *m*	1.60 *tt*	2.05 *m* 1.55 *m*	2.12 *br dt*	1.53 *tt*	6.11 *dt*	6.10 *dt*
H-6	2.11 *br dt*	5.73 *dt*	2.07 *m*	2.10 *br dt*	3.96 *ddt*	5.73 *dt*	2.08 *br dt*	2.34 *br dt*	2.21 *m*
H-7	5.43 *m*	6.12 *br dd*	5.43 *m*	5.34 *m*	1.40 *m*	6.07 *dd*	5.45 *m*	2.28 *m*	
H-8	5.50 *m*	6.47 *dd*		5.42 *m*		6.46 *dd*		—	5.38 *m**
H-9	2.90 *br d*	5.52 *dt*	2.90 *br s*	2.02 *br dt*		5.48 *br d*	2.89 *br dt*	—	
H-10	—	—	—	1.32 *m*	1.28 *m*	—	—	2.13 *tt*	2.78 *br dd*
H-11	—	—	—			—	—	1.47 *tt*	5.38 *m*
H-12	2.11 *br t*	2.31 *dt*	2.13 *br t*		0.88 *t*	2.31 *dt*	2.13 *tt*	1.33 *m*	
H-13	1.48 *tt*	1.53 *tt*	1.48 *tt*	1.27 *m*	—	1.52 *tt*	1.48 *tt*		2.07 *dq*
H-14	1.32 *m*	1.35 *m*	1.33 *m*		—	1.34 *m*	1.33 *m*	0.90 *t*	0.98 *t*
H-15					—				
H-16	0.89 *t*	0.90 *t*	0.89 *t*	0.89 *t*	—	0.90 *t*	0.90 *t*	—	—
H-1′	7.38 *t*	7.36 *t*	7.31 *br s*	7.38 *t*	7.32 *t*	3.46 *t*	3.52 *t*	3.54 *t*	3.54 *t*
						3.40 *t*	3.50 *t*	3.52 *t*	3.92 *t*†
H-2′	6.32 *t*	6.32 *t*	6.29 *t*	6.32 *t*	6.23 *t*	1.95 *tt*	1.96 *tt*	1.96 *tt*	1.97 *tt*
						1.86 *tt*	1.86 *tt*	1.87 *tt*	1.87 *tt*

*$CDCl_3/C_6D_6$, 2:1; H-9 5.30 *dt* (J = 10, 7 Hz), H-12 5.18 *dt* (J = 10, Hz).

†$CDCl_3/C_6D_6$, 2:1; 3.34 *t*, 3.07 *t*, 1.52 *tt*, 1.47 *tt*. J(Hz): 2,3 = 15; 2,4 = 1.5; 12,13 = 13,14 = 15,16 = 7; 1′, 2′$_1$ = 1′,2′$_2$ = 2; 3,4 = 7; compound **1**: 4,5 = 5,6 = 6,7 = 8,9 = 7; compound **2**: 4,5 = 5,6 = 7; 6,7 = 8,9 = 15; 7,8 = 11; 9,12 = 2; compound **3**: 2,3 = 3,4 = 7; compound **4**: 4,5 = 5,6 = 6,7 = 8,9 = 9,10 = 7; compound **5**: 2,2′ = 15; 2,5 = 5.5; 2,3′ = 7; 3,4 = 3′,4 = 5,6 = 5′,6 = 6,7 = 7; 11,12 = 6.5; compound **6**: 2,3 = 3,4 = 4,5 = 12,13 = 15,16 ~ 7; 6,7 = 8,9 = 15; 7,8 = 10; 9,12 = 1.8; compound **7**: 2,3 = 15; 2,4 = 1.5; 4,5 = 5,6 = 6,7 = 12,13 = 13,14 = 15,16 = 7; 8,9 = 5; 9,12 = 2; compound **8**: 2,3 = 4,5 = 15; 3,4 = 10; 5,6 = 6,7 = 10,11 = 13,14 ≃ 7; 7,11 = 2; compound **9**: 2,3 = 4,5 = 15; 3,4 = 10; 5,6 = 13,14 = 7; 9,10 = 10,11 = 6.

deposited at the Herbarium of the Institute of Botany, University of Vienna (WU).

Fresh air-dried underground parts (160 g) were cut into small pieces and extracted with petrol (60–80°)–Et_2O (2:1) for several days at room temp. The resulting extract was roughly fractionated by CC (silica gel). The CC fractions were taken as follows. A: petrol and petrol–Et_2O (19:1), B: petrol–Et_2O (9:1), C: petrol–Et_2O (3:1), D: petrol–Et_2O (1:1), E: Et_2O, F: Et_2O–MeOH (9:1). Fraction A gave nothing of interest. Prep. TLC of fraction B (silica gel, PF 254, petrol–Et_2O, 19:1, two developments) gave 20 mg **1** (R_f 0.5), 15 mg **2** (R_f 0.35) and a mixture which gave by repeated prep. TLC 5 mg **4** (R_f 0.7) and 5 mg **3** (R_f 0.58). Repeated prep. TLC of fraction C (petrol–Et_2O, 3:1) gave 2 mg **5** (R_f 0.6). Prep. TLC of fraction D (Et_2O–petrol, 1:1) gave 5 mg (+)sesamin, 15 mg coniferyl-dec-2*E*-en-4-ynoate, 5 mg sitosterol and 5 mg stigmasterol. Prep. TLC of fraction E gave 20 mg hexadeca-2*E*,6*E*,8*E*-trien-10-ynoic acid dehydropyrrolidide, 2 mg hexadeca-2*E*,7*Z*,12*Z*-trien-10-ynoic acid dehydropyrrolidide and 0.5 mg hexadeca-2*E*, 6*E*, 8*E*-trien-10-ynoic acid isobutylamide [1]. Prep. TLC of fraction F (Et_2O–petrol, 3:1, two developments) gave 30 mg **10** and a mixture which gave by HPLC (RP 8, MeOH–H_2O, 4:1, *ca* 100 bar) 2 mg **8** (R_t 4.7 min.), 1 mg **9** (R_t 5.7 min.), purified by prep. TLC (Et_2O–petrol, 3:1, five developments) 6 mg **7** (R_t 8.6 min.) and 2 mg **6** (R_t 10.3 min.).

Hexadeca-2E, 7Z-dien-10-ynoic acid pyrrolide (**1**). Colourless oil; UV $\lambda_{max}^{Et_2O}$ nm: 249, 218; IR $\nu_{max}^{CCl_4}$ cm^{-1}: 3013, 2925 (*s*), 2854, 1698 (*s*), 1638 (*s*), 1462 (*s*), 1403, 1341 (*s*), 1284 (*s*), 1258, 1122 (*s*), 1070 (*s*), 968, 935 (*s*), 864, 690, 590; MS *m/z* (rel. int.): 297.209 $[M]^+$ (7) (calc. for $C_{20}H_{27}NO$: 297.209), 296 $[M-1]^+$ (2), 254 $[M-C_3H_7]^+$ (10), 240 $[M-C_4H_9]^+$ (12), 226 $[M-C_5H_{11}]^+$ (64), 67 $[C_4H_5N]^+$ (100); ^{13}CNMR ($CDCl_3$, C-1–C-16): 162.7 *s*, 119.6 *d*, 152.4 *d*, 31.1 *t*, 26.5 *t*, 27.6 *t*, 126.1 *d*, 129.9 *d*, 17.3 *t*, 80.4 *s*, 78.0 *s*, 18.7 *t*, 28.7 *t*, 32.3 *t*, 22.2 *t*, 14.0 *q*; C-1′ 119.2 *d*; C-2′ 113.1 *d* (a few signals may be interchangeable).

Hexadeca-2E, 6E, 8E-*trien*-10-*ynoic acid pyrrolide* (**2**). Colourless crystals, mp 34°; UV $\lambda_{max}^{Et_2O}$ nm: 277 sh, 266; IR $\nu_{max}^{CCl_4}$ cm^{-1}: 3015, 2925 (*s*), 2853, 2206, 1700 (*s*), 1638 (*s*), 1462 (*s*), 1403, 1346 (*s*), 1292 (*s*), 1261 (*s*), 1121 (*s*), 1070 (*s*), 980 (*s*), 930, 690, 591; MS *m/z* (rel. int.): 295.194 $[M]^+$ (10) (calc. for $C_{20}H_{25}NO$: 295.194), 161 $[C_5H_{11}C{\equiv}C(CH{=}CH)_2CH_2]^+$ (34), 105 (46), 91 (100), 67 (62).

Hexadeca-7*Z*-*en*-10-*ynoic acid pyrrolide* (**3**). Colourless oil; UV $\lambda_{max}^{Et_2O}$ nm: 236; IR $\nu_{max}^{CCl_4}$ cm^{-1}: 3010, 2925 (*s*), 2852, 1720 (*s*), 1638, 1463 (*s*), 1402, 1331, 1277 (*s*), 1116 (*s*), 1070 (*s*), 921, 692, 610; MS *m/z* (rel. int.): 299.225 $[M]^+$ (calc. for $C_{20}H_{29}NO$: 299.225), 95 (26), 81 (36), 68 (68), 67 (100).

Hexadeca-2E, 7*Z*-*dienoic acid pyrrolide* (**4**). Colourless oil; UV $\lambda_{max}^{Et_2O}$ nm: 247, 219; IR $\nu_{max}^{CCl_4}$ cm^{-1}: 2999, 2922 (*s*), 2849, 1698 (*s*), 1638 (*s*), 1462 (*s*), 1403, 1341 (*s*), 1286 (*s*), 1257, 1119 (*s*), 1070 (*s*), 969, 935 (*s*), 864, 690, 591; MS *m/z* (rel. int.) 301.241 $[M]^+$ (4.5) (calc. for $C_{20}H_{31}NO$: 301.241), 235 $[M-C_4H_4N]^+$ (8), 93 (42), 81 (66), 67 (100).

3,6-*Epoxydodecanoic acid pyrrolide* (**5**). Colourless oil; UV $\lambda_{max}^{Et_2O}$ nm: 237; IR $\nu_{max}^{CCl_4}$ cm^{-1}: 2950, 2922 (*s*), 2850, 1716 (*s*), 1463 (*s*), 1402, 1332 (*s*), 1306, 1250, 1064 (*s*), 923 (*s*), 692, 610; MS *m/z* (rel. int.): 263.188 $[M]^+$ (8) (calc. for $C_{16}H_{25}NO_2$: 263.188), 197 $[M-C_4H_4N]^+$ (3), 178 $[M-C_6H_{13}]^+$ (5), 155 $[M-CH_2CONC_4H_4]^+$ (3), 95 (20), 67 (100); ^{13}C NMR ($CDCl_3$, C-1–C-12): 168.5 *s*, 41.2 *t*, 74.5 *d*, 31.8 *t*, 32.2 *t*, 79.3 *d*, 35.8 *t*, 26.1 *t*, 29.4 *t*, 31.8 *t*, 22.6 *t*, 14.1 *q*; C-1′ 119.2 *d*; C-2′ 113.1 *d*.

Hexadeca-6E, 8E-*dien*-10-*ynoic acid pyrrolidide* (**6**). Colourless oil; UV $\lambda_{max}^{Et_2O}$ nm: 277, 266; IR $\nu_{max}^{CCl_4}$ cm^{-1}: 3010, 2924 (*s*), 2852, 1641 (*s*), 1422 (*s*), 1339, 1319, 1223, 1189, 1164, 1085, 980 (*s*); MS *m/z* (rel. int.): 301.241 $[M]^+$ (12) (calc. for $C_{20}H_{31}NO$: 301.241), 232 $[M-C_4H_7N]^+$ (8), 202 (38), 113 (100), 98 (72), 70 $[C_4H_8N]^+$ (62).

Hexadeca-2E, 7*Z*-*dien*-10-*ynoic acid pyrrolidide* (**7**). Colourless oil; UV $\lambda_{max}^{Et_2O}$ nm: 246, 219; IR $\nu_{max}^{CCl_4}$ cm^{-1}: 3009, 2923 (*s*), 2865, 1657(*s*), 1615 (*s*), 1419 (*s*), 1338, 1300, 1286, 1222, 1188, 1164, 1093, 1037, 970, 912, 857, 688; MS *m/z* (rel. int.): 301.241 $[M]^+$ (2) (calc. for $C_{20}H_{31}NO$: 301.241), 300 $[M-1]^+$ (3), 258 $[M-C_3H_7]^+$ (12), 244 $[M-C_4H_9]^+$ (100), 232 $[M-C_4H_7N]^+$ (28), 98 (75), 70 $[C_4H_8N]^+$ (68), 55 (100).

Tetradeca-2E,4E-*dien*-8-*ynoic acid pyrrolidide* (**8**). Colourless oil; UV $\lambda_{max}^{Et_2O}$ nm: 256; IR $\nu_{max}^{CCl_4}$ cm^{-1}: 2946, 2923 (*s*), 2865, 1649 (*s*), 1623 (*s*), 1598 (*s*), 1414 (*s*), 1341, 1332, 1297, 1222, 1189, 1165, 1113, 1037, 994 (*s*), 913, 862; MS *m/z* (rel. int.): 273.209 $[M]^+$ (16) (calc. for $C_{18}H_{27}NO$: 273.209), 272 $[M-H]^+$ (37), 244 $[M-Et]^+$ (6), 230 $[M-C_3H_7]^+$ (18), 216 $[M-C_4H_9]^+$ (22), 202 $[M-C_5H_9]^+$ (40), 164 (84), 98 (96), 70 $[C_4H_8N]^+$ (82), 55 (100).

Tetradeca-2E,4E,8*Z*,11*Z*-*tetraenoic acid pyrrolidide* (**9**). Colourless oil; UV $\lambda_{max}^{Et_2O}$ nm: 256; IR $\nu_{max}^{CCl_4}$ cm^{-1}: 3003, 2959 (*s*), 2923 (*s*), 2865, 1649 (*s*), 1621 (*s*), 1598 (*s*), 1413 (*s*), 1339, 1331, 1298, 1222, 1188, 1163, 1112, 1037, 994 (*s*), 913, 862, 693; MS *m/z* (rel. int.): 273.209 $[M]^+$ (9) (calc. for $C_{18}H_{27}NO$: 273.209), 272 $[M-1]^+$ (6), 246 (4), 232 (7), 218 (8), 204 (20), 165 (26), 164 (32), 98 (94), 67 (94), 55 (100).

Acknowledgements—We are grateful to Dr K. Valant-Vetschera for plant collection. Support by the Österreichischer Fonds zur Förderung der wissenschaftlichen Forschung (Project 4837) is gratefully acknowledged.

REFERENCES

1. Greger, H., Zdero, C. and Bohlmann, F. (1983) *Liebigs Ann. Chem.* 1194.
2. Greger, H. (1984) *Planta Med.* **50**, 366.
3. Greger, H., Hofer, O. and Werner, A. (1987) *Phytochemistry* **26**, 2235.
4. Bohlmann, F., Burkhardt, T. and Zdero, C. (1973) *Naturally Occurring Acetylenes*. Academic Press, London.
5. Greger, H. (1985) *Pl. Syst. Evol.* **150**, 1.
6. Greger, H., Zdero, C. and Bohlmann, F. (1984) *Phytochemistry* **23**, 1503.