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New Amides from *Spilanthes oleracea*

Short Communication

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In addition to the well known affinin [= spilanthol, (2*E*,6*Z*,8*E*)-deca-2,6,8-trienoic acid isobutylamide (**1**)], the corresponding 2-methyl-butylamide (**2**), and two new acetylenic alkamides were isolated from *Spilanthes oleracea* L. by reversed phase medium pressure chromatography: (*Z*)-non-2-en-6,8-diynoic acid isobutylamide (**3**) and (*Z*)-dec-2-en-6,8-diynoic acid isobutylamide (**4**). The structures and their stereochemistries were elucidated by ^{1}H-NMR, ^{13}C-NMR (**2** and **3**), MS, UV, IR, and CD (**2**). The chemotaxonomic significance of the distribution of alkamides within the *Compositae* tribe *Heliantheae* is briefly discussed.

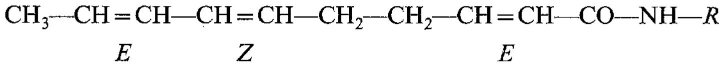
(Keywords: Acetylenic isobutylamides; Alkamides; Compositae-Heliantheae; ^{13}C-NMR; Reversed phase medium pressure liquid chromatography; Spilanthes oleracea)

Neue Amide aus Spilanthes oleracea (Kurze Mitteilung)

Aus *Spilanthes oleracea* wurden neben dem seit langem bekannten Affinin [= Spilanthol, (2*E*,6*Z*,8*E*)-Deca-2,6,8-triensäureisobutylamid (**1**)] durch Umkehrphasen-Mitteldruckchromatographie das entsprechende 2-Methylbutylamid (**2**) und zwei neue acetylenische Alkamide isoliert: (*Z*)-Non-2-en-6,8-diinsäure-isobutylamid (**3**) und (*Z*)-Dec-2-en-6,8-diinsäure-isobutylamid (**4**). Die Strukturen und deren Stereochemie wurden mittels ^{1}H-NMR, ^{13}C-NMR (**2** und **3**), MS, UV, IR und CD (**2**) aufgeklärt. Die chemotaxonomische Bedeutung der Verbreitung von Alkamiden innerhalb der Compositen-Tribus *Heliantheae* wird kurz diskutiert.

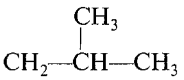
Spilanthes oleracea L. (*Compositae-Heliantheae*) is well known both for its insecticidal activity and for its pungent taste, producing formication and local anaesthesia[1]. The active material was first obtained in the crude state from the flower heads by *Gerber*[2] (1903), who designated it "spilanthol". Later it was shown that spilanthol (for which various structures have been proposed) was identical with affinin (**1**) (Ref.[3]). The correct stereochemistry of **1** was established by *Crombie* et al.[4].

Continuing our current studies on naturally occurring alkamides (for Ref. see [5]) we investigated the flower heads and roots of *S. oleracea* in order to obtain more detailed information on the complete amide pattern of this species. Besides the dominating affinin (**1**) both extracts afforded a second tlc-fraction, which could be separated further by medium pressure liquid chromatography on a reversed phase column (RP-8 material, *Me*OH—H_2O). The analysis of 50 g roots afforded 65 mg **1**, 9 mg **2**, 6 mg **3**, and 1 mg **4**.



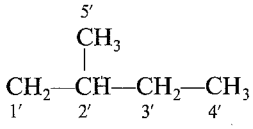
$$CH_3{-}\underset{E}{CH{=}CH}{-}\underset{Z}{CH{=}CH}{-}CH_2{-}CH_2{-}\underset{E}{CH{=}CH}{-}CO{-}NH{-}R$$

1 *R* =

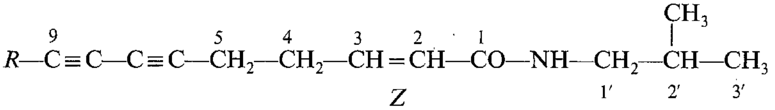


$$CH_2{-}\overset{\overset{\textstyle CH_3}{|}}{CH}{-}CH_3$$

2 *R* =



$$\underset{1'}{CH_2}{-}\overset{\overset{\overset{5'}{CH_3}}{|}}{\underset{2'}{CH}}{-}\underset{3'}{CH_2}{-}\underset{4'}{CH_3}$$



$$R{-}\overset{9}{C}{\equiv}C{-}C{\equiv}\overset{5}{C}{-}\overset{4}{CH_2}{-}CH_2{-}\underset{Z}{\overset{3}{CH}{=}\overset{2}{CH}}{-}\overset{1}{CO}{-}NH{-}\underset{1'}{CH_2}{-}\overset{\overset{\textstyle CH_3}{|}}{\underset{2'}{CH}}{-}\underset{3'}{CH_3}$$

3 *R* = H **4** *R* = CH_3

Compound **2** was very recently isolated from *S. ciliata* H.B.K. [as *Acmella ciliata* (H.B.K.) Cass.][6] and was shown to be the 2-methyl-butyl homologue to affinin (**1**). The acetylenic compounds **3** and **4** were detected for the first time, however, the acid moieties have proved to be the same as already described for some aromatic alkamides from *S. alba* L'Hérit[7].

The co-occurrence of affinin and the rare 2-methyl-butylamide **2** together with the acetylenic amides characterized by a 2-(*Z*)-orientated double bond appears to be typical for some *Heliantheae* genera[5] which are grouped together in the subtribe *Ecliptinae*[8].

The data of **2** agree with the ones presented in Ref.[6], however, additional spectroscopic data were recorded. **2** is expected to be optically active (C-2′ is a chiral centre; for $[\alpha]_D$ and circular dichroism see Exp.). IR and UV spectra, and a detailed analysis of the ^{1}H-NMR resonance of the amine part of **2** (especially for the well separated diastereotopic CH_2-1′ and CH_2-3′ protons) are reported in the Exp. part. The structure of **2** was additionally confirmed by ^{13}C-NMR, the chemical shifts of the acid moiety being almost identical with the ones described for affinin (**1**)[9].

The two new alkamide components were both of the acetylenic acid-isobutylamide type. This was revealed immediately by the ^{1}H-NMR spectra. The signals at 3.14 (t, 2 H), 1.81 (m, 1 H), and 0.95 ppm (d, 6 H) indicate clearly the isobutylamide moiety, the broad singlet at 1.99 (1 H) for **3** and at 1.91 ppm (3 H) for **4** are typical for terminal acetylenic $HC\equiv$ (**3**, also indicated by bands at 3 306 and 614 cm^{-1} in the IR) and $CH_3—C\equiv$ (**4**), respectively. The two olefinic protons show a *cis*-coupling of 11 Hz (therefore *Z*-configuration of the double bond). The conjugated position of the double bond with respect to CO may be seen from the coupling pattern and the chemical shifts (^{1}H- and ^{13}C-NMR). The carbon chain is continued with two methylene groups: CH_2-4 is represented by a broad pseudo-quartet, CH_2-5 by a somewhat broadened triplet. The number of $—C\equiv C—$ elements follows from the mass spectra which decide clearly in favor of a C-9 chain for **3** and a C-10 chain for **4**.

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Experimental

Instruments: Optical rotation: Perkin-Elmer 241 polarimeter; Circular dichroism: Jobin-Yvon Mark III; IR: Perkin-Elmer 398; UV: Perkin-Elmer Lambda 5; MS: Varian MAT CH-7 (EI-MS) and Varian MAT 311 A (field desorption and high resolution); NMR: Bruker WM 250 (250 MHz).

Plant Material: S. oleracea was cultivated under field conditions in the Botanical Garden of the University of Vienna. Voucher specimens are deposited at the herbarium of the Institute of Botany, University of Vienna (WU).

Isolation of the Compounds: 50 g of fresh air dried roots were cut into small pieces and extracted with petrol (60—80°)—Et_2O (1 : 1) for two days at room temperature. The concentrated extract containing a mixture of **1**–**4** was further chromatographed by repeated tlc (SiO_2 GF 254 Merck, petrol-Et_2O 3 : 7 or CH_2Cl_2-Et_2O 19 : 1). Affinin (**1**, lower *Rf* relative to **2**–**4**) could be separated completely from **2**–**4**. For the separation of **2**–**4** a reversed phase column with homemade RP-8 material was used (Merck LiChroprep Si 60, 25–40 μm, modified with octylmethyldichlorosilane; 12.8% C). Column parameters: 35 × 200 mm, 2 000 theoretical plates (tested with benzene, 70% aqu. *Me*OH). The eluent was $MeOH—H_2O$ with a flow rate of 1–2 cm min^{-1}; detection by UV at 254 nm; k'(**2**) = 8.5, k'(**3**) = 1.8, k'(**4**) = 3.1.

(2E,6Z,8E)-Deca-2,6,8-trienoic acid 2-methyl-butylamide (**2**)

$[\alpha]_D^{20} = +5.1°$, $[\alpha]_{436}^{20} = +10.5°$, $[\alpha]_{365}^{20} = +20°$ ($c = 0.8$, $CHCl_3$); CD (*Et*OH, λ/nm): 260 ($\Delta\varepsilon = —0.12$), 248 ($\Delta\varepsilon = \pm 0.0$), 238 ($\Delta\varepsilon = —0.5$), < 230 (pos. slope);

UV (Et_2O, λ/nm): 229 (ε = 31 000), 215 (sh., ε = 25 500); IR (CCl_4, cm^{-1}): 3 446 (m), 3 316 (m), 3 013 (m), 2 956 (s), 2 922 (m), 2 869 (w), 1 676 (s), 1 641 (s), 1 502 (s), 1 458 (m), 1 446 (m), 1 432 (w), 1 373 (m), 1 343 (m), 1 288 (w), 1 277 (m), 1 238 (w), 1 203 (m), 1 167 (m), 1 085 (w), 978 (s), 947 (s), 926 (w), 841 (w); ^{1}H-NMR for the amino moiety ($CDCl_3$. δ/ppm): 3.27 (ddd, 1 H, J = 14, 7 and 7 Hz, pseudoquintet, CH_2-1′), 3.14 (ddd, 1 H, J = 14, 7 and 7 Hz, pseudoquintet, CH_2-1′), 1.58 (octet, 1 H, J = 7 Hz, CH-2′), 1.42 (ddq, 1 H, J = 14, 7 and 7 Hz, pseudo-septet, CH_2-3′), 1.16 (ddq, 1 H, J = 14, 7 and 7 Hz, pseudo-septet, CH_2-3′), 0.93 (t, 3 H, *Me*-4′), 0.92 (d, 3 H, *Me*-5′) [J (gem. 1′, 1′) = J (gem. 3′-3′) = 14 Hz and J(NH, 1′) = J (1′, 2′) = J (2′, 3′) = J (2′, 5′) = J (3′, 4′) = 7 Hz, proved by decoupling exp. with irrad. at 5.46 (NH), 3.20 (average of 3.27 and 3.14), 1.58, 1.42, 1.16, and 0.92 ppm]; ^{13}C-NMR ($CDCl_3$, δ/ppm, J-modulated): 166.0 (C 1), 143.4 (C 3), 129.9 (C 9), 129.5 (C 7), 127.7 (C 6), 126.8 (C 8), 124.3 (C 2), 45.2 (C 1′), 35.1 (C 2′), 32.1 (C 4), 27.1 (C 3′), 26.4 (C 5), 18.3 (C 10), 17.2 (C 5′), 11.3 (C 4′), for the assignments of the resonances see Ref.[9]; for further ^{1}H-NMR data and MS see Ref.[6].

(Z)-Non-2-en-6,8-diynoic acid isobutylamide (**3**)

Colourless oil; UV (Et_2O, λ/nm): 239 (sh, ε = 11 000), 216 (ε = 21 000); IR (CCl_4, cm^{-1}): 3 447 (m, NH), 3 306 (s, C≡C—H), 3 013 (w), 2 956 (s), 2 922 (m), 2 867 (w), 2 223 (m, C≡C), 1 725 (w), 1 674 (s, C=O), 1 637 (m, *cis*-C=C), 1 498 (s), 1 463 (m), 1 424 (w), 1 384 (w), 1 366 (w), 1 322 (w), 1 257 (s), 1 210 (m), 1 194 (m), 1 170 (w), 1 093 (m), 1 017 (m), 614 (s, C≡C—H); ^{1}H-NMR ($CDCl_3$, δ/ppm): 6.06 (dt, 1 H, J = 11 and 7 Hz, H-3), 5.80 (br. d, 1 H, J = 11 Hz, H-2), 5.60 (very br., 1 H, NH), 3.14 (t, 2 H, J = 7 Hz, H-1′), 2.91 (dt, 2 H, J = 7 and 7 Hz, pseudo-q, H-4), 2.45 (br. t, 2 H, J = 7 Hz, H-5), 1.99 (br. s, 1 H, ≡CH-9), 1.81 (m, 1 H, H-2′), 0.95 (d, 6 H, 2 *Me*-3′); ^{13}C-NMR ($CDCl_3$, δ/ppm, J-modulated): 166.2 (C 1), 141.7 (C 3), 124.2 (C 2), 64.9 (C 9), 46.8 (C 1′), 28.6 (C 2′), 27.0 (C 4), 20.2 (C 3′), 19.0 (C 5), quaternary acetylenic C-resonances too week for detection; MS [EI, 70 eV, 70 °C, m/z (rel. Int.)]: 203 (16, M^+, $C_{13}H_{17}NO$ requires 203.1310, high resolution 203.131), 131 (52, M^+-$C_4H_{10}N$), 104 (52), 103 (48, 131-CO), 77 (53), 63 (36, $C_5H_3{}^+$), 57 (83), 41 (100).

(Z)-Dec-2-en-6,8-diynoic acid isobutylamide (**4**)

Colourless oil; UV (Et_2O, λ/nm): 237 (weak sh., ε = 13 000), 216 (ε = 21 500); IR (CCl_4, cm^{-1}): 3 445 (m), 2 953 (m), 2 920 (s), 2 848 (m), 1 733 (w), 1 673 (s), 1 638 (m), 1 497 (s), 1 463 (m), 1 365 (w), 1 326 (m), 1 264 (m), 1 193 (m), 1 169 (m); ^{1}H-NMR ($CDCl_3$, δ/ppm): 6.05 (dt, 1 H, J = 11 and 7 Hz, H-3), 5.80 (br. d, 1 H, J = 11 Hz, H-2), 5.62 (very br., 1 H, NH), 3.13 (t, 2 H, J = 7 Hz, H-1′), 2.86 (dt, 2 H, J = 7 and 7 Hz, pseudo-q, H-4), 2.43 (br. t, 2 H, J = 7 Hz, H-5), 1.91 (br. s, 3 H, ≡C—*Me*-10), 1.81 (m, 1 H, H-2′), 0.95 (d, 6 H, J = 7 Hz, 2 *Me*-3′); MS [field desorption, *m*/z (rel. Int.)]: 217 (100, M^+); MS (EI, 70 eV, 70 °C, *m*/z (rel. Int.)]: 217 (5, M^+, $C_{14}H_{19}NO$ requires 217.1467, high resolution 217.147), 145 (31, M^+-$C_4H_{10}N$), 117 (28, 145-CO).

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