Phytochemistry, Vol. 23, No. 8, pp. 1781–1783, 1984.
Printed in Great Britain.

0031–9422/84 $3.00 + 0.00
© 1984 Pergamon Press Ltd.

SPILANTHOL-RELATED AMIDES FROM *ACMELLA CILIATA*

RAINER MARTIN and HANS BECKER

Institut für Pharmazeutische Biologie der Universitat Heidelberg, Im Neuenheimer Feld 364, D-6900 Heidelberg, West Germany

(*Revised received* 16 *February* 1984)

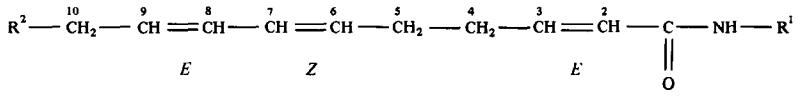
Key Word Index—*Acmella ciliata*; Compositae; amides; esterified amides; spilanthol; spilanthic acid; hydrospilanthol.

Abstract—From the flower heads of *Acmella ciliata* amides closely related to spilanthol were isolated. They possess isobutylamine, 2-phenylethylamine and 2-methylbutylamine parts. In the acidic part deca-2*E*,6*Z*,8*E*-trienoic acid, known from spilanthol, and its 2,3-dihydro derivative were found. Two compounds bear ester groups, a novelty for unsaturated amides. The structures were elucidated by means of high field ^{1}H NMR and high resolution mass spectrometry.

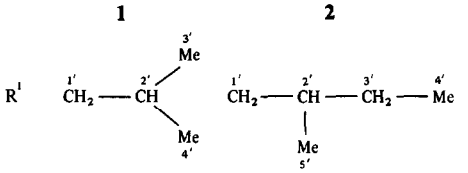
Acmella ciliata (H.B.K.) Cass. is closely related to the genus *Spilanthes*, in which three species have already been examined for amides [1–3]. Both *S. oleracea* and *S. acmella* are known to contain spilanthol (**1**) as the main amide component. Although a lot of different amides of higher plants have been described, those with a close structural relationship to spilanthol have never been found. As is already known this class of compounds very often causes problems in separation from each other [3] and unsaturated amides are rather unstable. Therefore we developed a new method for separation by using a combination of low- and high pressure liquid chromatography which yielded pure compounds with a minimum of chromatographic steps [4].

In addition to simple variations in the amine part and in the degree of unsaturation in the acid moiety, we found two esterified amides in the flower heads of *A. ciliata*. The deca-2*E*,6*Z*,8*E*-trienoic acid part of spilanthol is common to compounds **1**–**5**, therefore we propose the name 'spilanthic acid' for it. Amide **6** having one double bond less than spilanthol we name 'hydrospilanthol'.

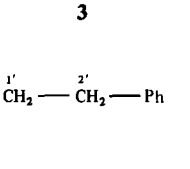
The *Z*/*E* isomerism of **1** followed from the appropriate coupling constants (Table 1), the signals were assigned by total spin decoupling. This method also gave valuable information about long range couplings in spilanthic acid. Irradiation of the appropriate signals clearly showed that the triplet sub-structure of the signal at δ5.83 (H-2) was caused by allylic coupling with H-4 while the considerable broadening of the signal of H-8 proved to be an effect of allylic coupling with both H-10 and H-6. In the mass spectrum of **1** both dominating peaks are caused by cleavage between C-4 and C-5, where *m*/*z* 81 represents



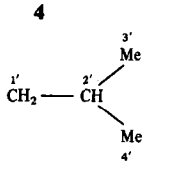
1



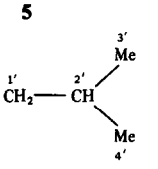
2



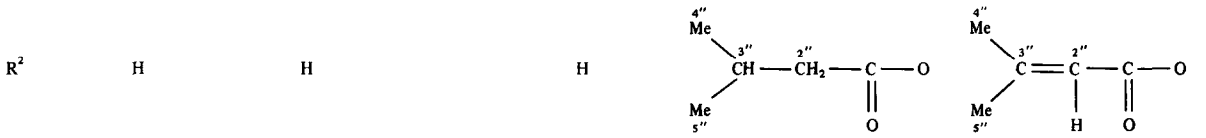
3



4



5



6

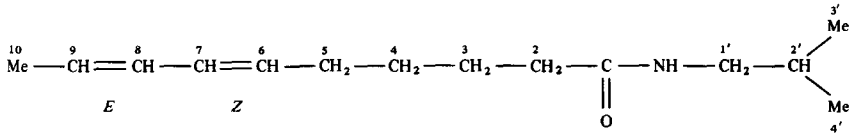


Table 1. ^{1}H NMR spectral data of compounds **1–6** (250 MHz; $CDCl_3$; TMS int. standard)

H No.	1	2	3	4	5	6
2	5.83 *dt* (15; 1.5)	5.79 *dt* (15; 1.5)	5.71 *dt* (15; 1.5)	5.79 *br d* (15)		2.18 *t* (8)
3	6.82 *dt* (15; 6.5)	6.83 *dt* (15; 6.5)	6.82 *dt* (15; 6.5)	6.84 *dt* (15; 6.5)		1.66 *tt* (8; 7.5)
4	2.28 *m*	2.29 *m*	2.28 *m*	2.32 *m*		1.43 *tt* (7.5; 7.5)
5						2.18 *dt* (7.5; 7.5)
6	5.26 *dt* (11; 7)	5.27 *dt* (11; 7)	5.25 *dt* (11; 7)	5.47 *dt* (11; 7)		5.27 *dt* (10.5; 7.5)
7	5.97 *dd* (11; 11)	5.98 *dd* (11; 11)	5.97 *dd* (11; 11)	6.02 *dd* (11; 11)		5.96 *dd* (10.5; 11)
8	6.29 *br dd* (11; 15)	6.30 *br dd* (11; 15)	6.28 *br dd* (11; 15)	6.54 *br dd* (11; 15)	6.55 *br dd* (11; 15)	6.31 *br dd* (11; 15)
9	5.70 *dq* (15; 7)	5.70 *dq* (15; 7)	5.70 *dq* (15; 7)	5.75 *dt* (15; 6.5)	5.75*	5.68 *dq* (15; 6.5)
10	1.78 *br d* (7)	1.78 *br d* (7)	1.78 *br d* (7)	4.63 *br d* (6.5)	4.65 *br d* (6.5)	1.78 *br d* (6.5)
1′	3.14 *dd* (7; 6.5)	3.20 *m*	3.59 *dt* (6.5; 7)	3.16 *dd* (6.5; 6.5)		3.08 *dd* (6.5; 6)
2′	1.80 *tqq* (6.5; 7; 7)	1.57 *m*	2.85 *t* (7)	1.80 *tqq* (6.5; 7; 7)		1.78 *tqq* (6.5; 6.5; 6.5)
3′	0.93 *d* (7)	1.18/1.40 *m*	Phe: 7.25 *m*	0.93 *d* (7)		0.91 *d* (6.5)
4′		0.91 *dd* (7; 7)	—			
5′	—	0.91 *d* (6.5)	—	—		—
2″	—	—	—	2.22 *d* (7)	5.71 *br s*	—
3″	—	—	—	2.11 *m*	—	—
4″	—	—	—	0.96 *d* (6.5)	2.18 *d* (1)	—
5″	—	—	—		1.91 *d* (1)	—

Chemical shifts in δ-values (ppm); numbers in parentheses are coupling constants in Hz; NH always between δ5 and 6, caused one coupling constant given for H-1′.
*Partially obscured by H-2 and H-2″.

the molecule part C-5 to C-10 while *m/z* 141 corresponds to fragment C-4 to C-3′/C-4′ after transfer of one hydrogen. Since cleavage at this point is very easy, *m/z* 81 is a valuable indicator for the corresponding molecule part.

The structure of the acid part of **2** followed by comparing the ^{1}H NMR signals with those of **1**. The amine part exhibited a very characteristic multiplet centered at δ3.2. This signal of two protons was exactly symmetrical, each part consisting of five lines. This proved, together with the multiplets at 1.18 and 1.4 and the other signals of H-4′ and H-5′ the presence of 2-methylbutylamine, which might be derived biogenetically from isoleucine. The multiplets mentioned above are an effect of the asymmetrical C-2′ and therefore expected for those prochiral methylene groups. These findings are not consistent with the literature data given for the same amine part [5] but they are supported by the mass spectrum. The latter showed loss of an ethyl group which could only be eliminated by the amine moiety. The peak at *m/z* 155 is analogous to that at *m/z* 141 in **1**.

In the ^{1}H NMR of **3** the signals of a 2-phenylethylamine part in addition to those of spilanthic acid were visible. In the mass spectrum, *m/z* 189 represented the analogous fragment to *m/z* 141 in **1** and *m/z* 104 is due to McLafferty rearrangement of the amine part.

The ^{1}H NMR of **4** and **5** differed from that of **1** as far as the doublet of 10-Me in **1** was replaced by a doublet of only two protons at δ4.63 and 4.65, respectively. In addition to this, the spectrum of **4** showed the same signals as an authentic sample of isovaleric acid. This was confirmed by a peak at *m/z* 85 in the mass spectrum of **4**, representing the appropriate acyl-cation. In the mass spectrum of **5** this fragment was replaced by one at *m/z* 83, the analogous dehydroion. The structure of the latter undoubtedly followed from the doublet structure of H-4″ and H-5″ caused by allylic coupling with H-2″ and from the chemical shift of both methyl groups, which is in accordance to literature data [6]. The attachment of the ester group at C-10 in **4** was corroborated by spin decoupling.

Structure **6** followed from the lack of two olefinic signals of spilanthic acid in the ^{1}H NMR of **6** together with the mass spectrum. The peak at *m/z* 115 is explainable by McLafferty rearrangement.

All amides reported here may be of future chemotaxonomic importance. A special emphasis is implied by the two esterified amides which seem to be rare. Hydrospilanthol might be of certain interest for the biosynthesis of such unsaturated acid derivatives, which, as far as we know, is unknown for amides that only contain double bonds.

EXPERIMENTAL

Plants were grown in a field near Karlsruhe (W. Germany). A specimen voucher is deposited in the Herbarium of the Institut f. System. Botanik and Pflanzengeographie der Universität Heidelberg.

Fresh flower heads (180 g) were homogenized with Me_2CO in a blender and allowed to stand overnight at room temp. Extraction was repeated twice, the amides separated from other compounds by CC (silica gel, CH_2Cl_2–EtOAc gradient). Group separation of the amides was achieved by low pressure CC first on silica gel with CH_2Cl_2–EtOAc gradients, then on RP-8 with MeOH–H_2O gradients. Final purification was by means of HPLC (RP-8, MeOH–H_2O, range 3:2 to 17:3, depending on sample). Compounds **2** and **3** could only be separated from each other on a CN-phase (*n*-hexane–$CHCl_3$ 4:1). R_f values on TLC (silica gel, CH_2Cl_2–EtOAc 9:1): 0.25 for **4** and **5**, 0.35–0.6 for **1**–**3** and **6**. Columns: Lobar silica size C, Lobar RP-8 size B, Hibar LiChrosorb RP-8 (250 × 4 mm, particle size 7 μm), Hibar LiChrosorb CN (250 × 10 mm, particle size 7 μm), Merck. For flow sheet and details see ref. [4]. In addition to other amides 210 mg **1**, 2 mg **2** and **6**, 1 mg **3** and less than 1 mg **4** and **5** were obtained. MS was by EI at 100 eV, direct inlet.

Spilanthol (**1**). MS *m/z* (rel. int.): 221.1775 $[M]^+$ (15) ($C_{14}H_{23}NO$, requires: 221.1780), 141.1153 $[C_8H_{15}NO]^+$ (86) (requires: 141.1153), 81.0703 $[C_6H_9]^+$ (100) (requires: 81.0703).

Spilanthic acid 2-methylbutylamide (**2**). MS *m/z* (rel. int.): 235.1947 $[M]^+$ (15) ($C_{15}H_{25}NO$, requires: 235.1936), 206.1537 $[M-Et]^+$ (2) ($C_{13}H_{20}NO$, requires: 206.1545), 155.1318 $[C_9H_{17}NO]^+$ (85) (requires: 155.1310), 86 $[NHR_1]^+$ (16), 81 (100).

Spilanthic acid 2-phenylethylamide (**3**). MS *m/z* (rel. int.): 269.1789 $[M]^+$ (6) ($C_{18}H_{23}NO$, requires 269.1780), 189 (30), 104 (31), 81 (100).

10-Hydroxyspilantholisovalerate (**4**). MS *m/z* (rel. int.): 321.2311 $[M]^+$ (4) ($C_{19}H_{31}NO_3$, requires 321.2304), 236 $[M-Me_2CHCH_2CO]^+$ (2), 220 $[M-Me_2CHCH_2COO]^+$ (6), 141 (100), 85 $[Me_2CHCH_2CO]^+$ (37).

10-Hydroxyspilanthol-(3-methylacrylate) (**5**). MS *m/z* (rel. int.): 319.2142 $[M]^+$ (7) ($C_{19}H_{29}NO_3$, requires 319.2147), 236 $[M-Me_2C{=}CHCO]^+$ (16), 141 (80), 83 $[Me_2C{=}CHCO]^+$ (100).

Hydrospilanthol (= *2,3-Dihydrospilanthol*) (**6**). MS *m/z* (rel. int.): 223.1976 $[M]^+$ (57) ($C_{14}H_{25}NO$, requires: 223.1937), 128 (38), 115.1017 $[C_6H_{13}NO]^+$ (100) requires: 115.0997), 81 (28), 72 $[NHR_1]^+$ (26).

Acknowledgements—We thank Professor Dr. Jansen, Department of Botany and Plant Pathology, Michigan State University, East Lansing, for identifying the plant species. We are grateful to Mr. Rubik, Institut für Biochemie (Director Prof. Dr. Hecker), DKFZ, for running the mass spectra and Dr. Kramer and Mrs. Jost, Pharm. Chem. Inst. (Director Prof. Dr. Neidlein), Universität Heidelberg, for running the ^{1}H NMR spectra.

REFERENCES

1. Jacobson, M. (1957) *Chem. Ind.* **50**.
2. Gokhale, V. G. and Bhide, B. V. (1945) *J. Indian Chem. Soc.* **22**, 250.
3. Bohlmann, F., Ziesche, J., Robinson, H. and King, R. M. (1980) *Phytochemistry* **19**, 1535.
4. Martin, R. and Becker, H., *Fresenius Z. Anal. Chem.* (in press).
5. Bohlmann, F. and Hoffmann, H. (1983) *Phytochemistry* **22**, 1173.
6. Pretsch, E., Clerc, T., Seibl, J. and Simon, W. (1981) *Strukturaufklärung organischer Verbindungen*, 2nd edn. Springer, Berlin.