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SHORT REPORTS

AMIDES FROM *ACHILLEA* SPECIES AND *LEUCOCYCLUS FORMOSUS**

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Key Word Index—*Achillea biebersteinii*; *A. crithmifolia*; *A. tomentosa*; *Leucocyclus formosus*; Compositae; Anthemideae; amides; isobutylamides; piperideine derivative; pyrrolideine derivative; acetylenic compounds.

From the large genus *Achillea* (Compositae, Anthemideae) several acetylenes and amides have been isolated [1]. We have now investigated the roots of 3 further species and those of *Leucocyclus formosus* Boiss. spp. *formosus*. The latter afforded a mixture of amides, which were only separated in part by TLC on $AgNO_3$–Si gel. The known amides **2a** and **3** [2] were isolated, the first one, however, was mixed with very small amounts of the homologous amides **2b**–**2d** (MS). The ^{1}H NMR data for the mixture were nearly identical with those of **2a**. Furthermore, two amides with isolated double bonds, the isobutylamides **8** and **9**, were present. The structures of the compounds followed from the MS and ^{1}H NMR spectra (Table 1). In the MS of **8** the ions *m/z* 166 and 167 were

Table 1. ^{1}H NMR spectral data of compounds **5**–**9** ($CDCl_3$, 400 MHz, TMS as int. standard)

	5	**6**	**7**	**8**	**9**
H-2	6.27 *d* (*br*)	5.78 *d* (*br*)	6.13 *d* (*br*)	5.77 *d* (*br*)	5.76 *d* (*br*)
H-3	7.28 *dd* (*br*)	6.83 *dt* (*br*)	6.90 *dt*	7.19 *dd*	7.19 *dd*
H-4	6.25 *dd*	2.29 *m*	2.30 *m*	6.16 *dd*	6.17 *dd*
H-5	6.17 *dt*			6.06 *dt*	6.08 *dt*
H-6	2.16 *dt*	5.77 *dt*	5.76 *dt*	2.19 *m*	2.23 *m*
H-7	1.45 *tt*	6.14 *dd* (*br*)	6.13 *dd* (*br*)		
H-8	1.30 *m*	6.53 *dd*	6.52 *dd*	5.41 *dt*	5.40 *m*
H-9		5.68 *d* (*br*)	5.66 *d* (*br*)	5.34 *dt*	
H-10	0.90 *t*	—	—	2.02 *dt*	2.78 *dd* (*br*)
H-11	—	—	—	1.3 *m*	5.40 *m*
H-12	—	5.62 *d* (*br*)	5.60 *d* (*br*)		
H-13	—	5.91 *dt*	5.88 *dt*		2.08 *dt* (*br*)
H-14	—	2.31 *dt*	2.3 *m*	0.89 *t*	0.99 *t*
H-15	—	1.46 *tq*	1.45 *tq*	—	—
H-16	—	0.95 *t*	0.92 *t*	—	—
H-1′	6.74 + 7.27 *d* (*br*)	3.16 *t*	6.61 + 7.00 *d* (*br*)	3.17 *t*	3.18 *t*
H-2′	4.97 + 5.12 *dt*	1.81 *dqq*	5.25 *m*	1.81 *dqq*	1.82 *dqq*
H-3′	2.10 *dt*	0.94 *d*	2.3 *m*	0.93 *d*	0.95 *d*
H-4′	1.87 *tt*		3.91 + 3.90 *t* (*br*)		
H-5′	3.81 + 3.68 *m*	—	—	—	—

J (Hz): compound **5**: 2,3 = 15; 3,4 = 10; 4,5 = 15; 5,6 = 6; 6,7 = 7,8 = 9,10 = 7; 1′,2′ = 8; 2′,3′ ~ 3.5; 3′,4′ = 4′,5′ ~ 6; compound **6**: 2,3 = 6,7 = 8,9 = 15; 3,4 = 5,6 = 13,14 = 14,15 = 15,16 = 2′,3′ = 2′,4′ = 7; 12,13 = 10; 1′,2′ = 1′NH = 6.5; compound **7**: 2,3 = 6,7 = 8,9 = 15: 3,4 = 5,6 = 13,14 = 15,16 = 7; 1′,2′ = 3′, 4′ ~ 7; compound **8**; 2,3 = 4,5 = 15; 3,4 = 10; 5,6 = 7,8 = 8,9 = 13,14 = 2′,3′ = 2′,4′ = 7; 1′,2′ = 1′, NH = 6.5; compound **9**: 2,3 = 4,5 = 15; 3,4 = 10; 5,6 = 8,9 = 13,14 = 2′,3′ = 2′,4′ = 7; 9,10 = 10,11 = 6; 1′,2′ = 1′, NH = 6.5.

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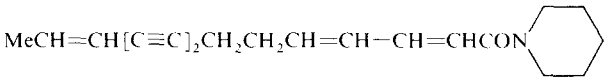
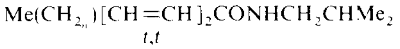
*Part 260 in the series "Polyacetylenic Compounds". For Part 259 see Bohlmann, F., Schuster, A. and Meusel, H. (1981) *Phytochemistry* **20**, 823.



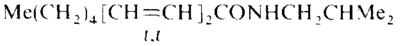
1



$$\mathrm{Me(CH_2)_n}\underset{t,t}{\mathrm{[CH{=}CH]_2}}\mathrm{CONHCH_2CHMe_2}$$

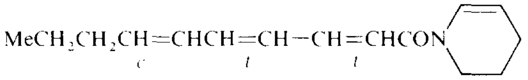
2

2a: $n = 8$, **2b**: $n = 7$, **2c**: $n = 6$, **2d**: $n = 5$



$$\mathrm{Me(CH_2)_4}\underset{t,t}{\mathrm{[CH{=}CH]_2}}\mathrm{CONHCH_2CHMe_2}$$

3



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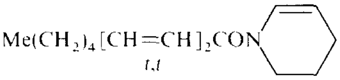
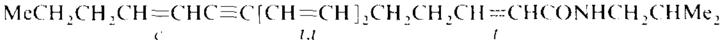
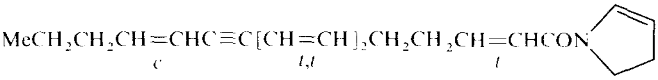


5



$$\mathrm{MeCH_2CH_2}\underset{c}{\mathrm{CH{=}CH}}\mathrm{C{\equiv}C}\underset{t,t}{\mathrm{[CH{=}CH]_2}}\mathrm{CH_2CH_2}\underset{t}{\mathrm{CH{=}CH}}\mathrm{CONHCH_2CHMe_2}$$

6



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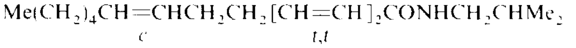
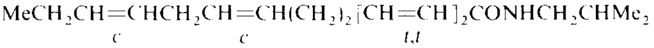


$$\mathrm{Me(CH_2)_4}\underset{c}{\mathrm{CH{=}CH}}\mathrm{CH_2CH_2}\underset{t,t}{\mathrm{[CH{=}CH]_2}}\mathrm{CONHCH_2CHMe_2}$$

8



$$\mathrm{MeCH_2}\underset{c}{\mathrm{CH{=}CH}}\mathrm{CH_2}\underset{c}{\mathrm{CH{=}CH}}\mathrm{(CH_2)_2}\underset{t,t}{\mathrm{[CH{=}CH]_2}}\mathrm{CONHCH_2CHMe_2}$$

9

obviously formed by loss of $C_5H_{11}CH{=}CHCH_2^{\cdot}$ and $C_4H_9(CH{=}CH)_2H$ (McLafferty). Furthermore, the observed loss of $^{\cdot}NHCH_2CHMe_2$ followed by the loss of CO was typical for isobutylamides. The position of the isolated double bond was supported by the ^{1}H NMR data, as the multiplet at δ 2.19 was coupled with both the signals of H-5 and H-8. The stereochemistry of the double bonds were deduced from the observed couplings. The ^{1}H NMR data of **9** (Table 1) showed that this amide contained one more double bond than **8**. The broadened triplet at δ 2.78 obviously had to be assigned to a methylene group between two double bonds. The stereochemistry of the isolated double bonds, however, could be assigned only by the missing out-of-plane vibration in the IR spectrum of **9**, as the corresponding ^{1}H NMR signal was overlapped to a multiplet similar to that of other compounds of this type.

The roots of *Achillea biebersteinii* Afan. afforded the known amide **1** [1], while those of *A. crithmifolia* Waldst. et Kit. gave **4** [1] and the corresponding 6,7-dihydro compound **5** [^{1}H NMR data (Table 1) and MS]. The roots of *A. tomentosa* .L afforded two amides also both with an enynediene chromophore. The ^{1}H NMR data of the main compound (Table 1) showed that the isobutylamide **6** was present. The stereochemistry of the double bonds were deduced from the corresponding couplings, while the position of these bonds followed from spin decoupling, which allowed the assignment of all signals. The structure was also supported by the fragmentation pattern in the MS of **6**. The second compound obviously had the same arrangement of the double bonds and also the stereochemistry was identical with that of **6** (^{1}H NMR data, Table 1). However, the isobutylamide signals were replaced by those of a pyrrolideide amide. As in the case of piperideids [1], the presence of two conformers was deduced from the ^{1}H NMR spectrum. The structure of this amide therefore was **7**. Again the fragmentation pattern in the MS of **7** supported the proposed structure.

A preliminary survey of about 30 different *Achillea* species and provenances (Greger, unpublished results) has shown that the accumulation of amides with characteristic olefinic and acetylenic patterns represents a common chemical trend within the genus. The isolation of related amides from the monotypic *Leucocyclus* from southern Turkey supports the previous suggestion [3], that there is a close affinity between *Achillea* and *Leucocyclus*. Also in view of the amide formation *Anacyclus* fits in clearly with these genera and this is further verified by the fruit and corolla characters [4].

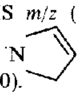
EXPERIMENTAL

Voucher specimens are deposited at the Institute of Botany, University of Vienna (WN). The roots were extracted with Et_2O–petrol (1:2) and the resulting extracts were separated first by CC (Si gel). The fractions obtained with Et_2O–petrol (1:1) and Et_2O were then further purified by repeated TLC ($AgNO_3$–Si gel, Et_2O–petrol, 4:1). *Achillea biebersteinii* (voucher A-1527) (90 g root material) afforded 5 mg **1**; *Achillea crithmifolia* (voucher A-1576) (148 g) gave 12 mg **4** and 8 mg **5**; *Achillea tomentosa* (voucher A-1602) (31 g) afforded 7 mg **6** and 1.5 mg **7**; *Leuocyclus formosus* (voucher 7-14-3) (18 g) gave 15 mg **2a**–**2d** and **3**, 8 mg **8** and 2 mg **9**.

Tetradeca-, trideca-, dodeca-undeca and deca-2t,4t-*dienoic isobutyl amide* (**2a**–**2d** *and* **3**). These were obtained as an oily mixture, IR $\nu_{max}^{CCl_4}$ cm^{-1}: 3440, 3300, 1660, 1620 (CH=CH)$_2$ CONHR); MS *m/z* (rel. int.): 279.256 (M$^+$, 12) ($C_{18}H_{23}NO$), 265.241 (M$^+$, 2) ($C_{17}H_{31}NO$), 251.225 (M$^+$, 1) ($C_{16}H_{29}NO$), 237.209 (M$^+$, 2) ($C_{15}H_{27}NO$), 223. 191 (M$^+$, 27) ($C_{14}H_{25}NO$).

Deca-2t,4t-*dienoic-2′,3′-dehydropiperideide* (**5**). Colourless gum, IR $\nu_{max}^{CCl_4}$ cm^{-1}: 1640, 1610, 1005 (CH=CH)$_2$CON$\langle$); MS *m/z* (rel. int.): 233.162 (M$^+$, 25) ($C_{15}H_{23}NO$), 151 (M – ˙N , 70), 55 (C_4H_7, 100).

Tetradeca-2t,6t,8t,-12c-*tetraen*-10-*ynoic isobutyl amide* (**6**). Colourless gum, UV $\lambda_{max}^{Et_2O}$ nm: 310, 294, 279, 211; IR $\nu_{max}^{CCl_4}$ cm^{-1}: 3460, 3320, 1690, 1640 (CH=CHCONHR), 2230 (C≡C), 990 (*trans, trans*-diene); MS *m/z* (rel. int.): 299.225 (M$^+$, 19) ($C_{20}H_{29}NO$), 284 (M – Me, 2), 270 (M – Et, 2), 270 (M – CHMe$_2$, 2), 242 (M – CH$_2$CHMe$_2$, 3), 159 (**A***, 100).

Tetradeca-2t,6t,8t,12c-*tetraen*-10-*ynoic 2′,3′-dehydro pyrrolideide* (**7**). Colourless gum, UV $\lambda_{max}^{Et_2O}$ nm: 311, 294, 281; IR $\nu_{max}^{CCl_4}$ cm^{-1}: 1660, 1608 (CH=CHCON), 990 (*trans, trans*-diene); MS *m/z* (rel. int.): 295.194 (M$^+$, 8) ($C_{20}H_{25}NO$), 227 (M – ˙N , 4), 199 (227 – CO, 8), 159 (A,* 30), 69 (C_4H_7N, 100).

Tetradeca-2t,4t,8c-*trienoic isobutyl amide* (**8**). Colourless gum, IR $\nu_{max}^{CCl_4}$ cm^{-1}: 3440, 3290, 1660, 1620, 1000 [(CH=CH)$_2$ CONHR], 1630 (CH=CH); MS *m/z* (rel. int.): 277.241 (M$^+$, 14) ($C_{18}H_{31}NO$), 262 (M – Me, 4), 248 (M – Et, 2), 205 (M – NHCH$_2$CHMe$_2$, 38), 167 (M – $C_4H_9{=}_2H$, 50, McLafferty), 166 (M – $C_8H_{15}^{\cdot}$, 27), 57 ($C_4H_9^+$, 100).

Tetradeca-2t,4t,8c,11c-*tetraenoic isobutyl amide* (**9**). Colourless gum, IR $\nu_{max}^{CCl_4}$ cm^{-1}: 3450, 3300, 1675, 1620, 1000 [(CH=CH)$_2$ CONHR], 1605 (CH=CH); MS *m/z* (rel. int.): 275.225 (M$^+$, 5) ($C_{18}H_{29}NO$), 260 (M – Me, 3), 246 (M – Et, 2), 203 (M – NHCH$_2$CHMe$_2$, 6), 175 (203 – CO, 11), 167 (M – $Et{=}_3H$, 15, McLafferty), 166 (M – Et=CH$_2$=CH$_2$, 11), 67 ($C_5H_7^+$, 100).

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***A** = $C_3H_7{=}{\equiv}{=}_2CH_2^+$.

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TWO NOVEL HEXASACCHARIDES FROM THE ROOTS OF *ASPARAGUS OFFICINALIS*

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Key Word Index—*Asparagus officinalis*; Liliaceae; novel hexasaccharides; fructo-oligosaccharides; 1^F-β-fructofuranosyl-6^G(1-β-fructofuranosyl)$_3$sucrose; 1^F(1-β-fructofuranosyl)$_2$-6^G(1-β-fructofuranosyl)$_2$sucrose.

Abstract—Two non-reducing hexasaccharides isolated from the roots of *Asparagus officinalis* were identified as 1^F-β-fructofuranosyl-6^G(1-β-fructofuranosyl)$_3$sucrose and 1^F(1-β-fructofuranosyl)$_2$-6^G(1-β-fructofuranosyl)$_2$sucrose by examination of the constituent saccharides, GLC analysis of methyl derivatives, and investigation of partial acid hydrolysates and β-fructofuranosidase-catalysed hydrolysis products.

In a series of studies on fructo-oligosaccharides of Liliaceae, my colleagues and I have shown the presence of neokestose in onion bulbs [1], and neokestose and its five related higher oligosaccharides in the roots of asparagus [2, 3]. The present paper deals with the isolation and structural elucidation of hexasaccharides concerned with neokestose from the roots of asparagus.

Two fructo-oligosaccharides, saccharides A($[\alpha]_D^{20}$ –14.9°) and B($[\alpha]_D^{20}$ –14.4°), were isolated from an extract of asparagus roots by repeated carbon–Celite column chromatography and preparative paper chromatography. The saccharides were shown to be homogeneous by PC (solvent I) and TLC (solvent III). They were both non-reducing and on hydrolysis with 0.1 M hydrochloric acid or β-fructofuranosidase gave glucose and fructose (PC). The degrees of polymerization were established by measurements of the [M + Na]$^+$ ions (*m/z*, 1013) on FDMS and of the molar ratios (A, 5.86; B, 5.75) of reducing sugar to glucose in acid hydrolysates of the isolated saccharides. These findings showed that saccharides A and B were non-reducing hexasaccharides made up of 1 mol of D-glucopyranose and 5 mol of β-D-fructofuranose.

To clarify the bond structures of the component sugars,