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POLYACETYLENES AND OTHER CONSTITUENTS OF *LEUZEA CENTAUROIDES*

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Abstract—Roots and green parts of *Leuzea centauroides* were examined separately. Twenty-nine polyacetylenes, four tetraenic aldehydes, 16 methyl and ethyl esters, 1,8,11,14-heptadecatetraene, caffeic acid and the flavanones eriodictyol and naringenin were isolated and characterized Three trienic aldehydes and three C_{17} hydrocarbons with from one to three isolated double bonds were also isolated.

INTRODUCTION

Leuzea centauroides (L.) Holub is also known under the names *Rhapónticum centauroides* and *Cnicus centauroides* [1]. It belongs to the family Asteraceae (=Compositae) and is a rare plant only naturally occurring in the Pyrenees. *Leuzea carthamoides* has previously been investigated and four thiophene acetylenes were identified [2] To the best of our knowledge *L. centauroides* has not previously been investigated. This information is primarily based on a literature search in CAS. The UV spectra of extracts of the roots and green parts showed the presence of considerable amounts of polyunsaturated compounds, and an investigation was therefore carried out at this laboratory

It may be noticed that at the time of collecting the plant material, it had ceased the flowering. Only 60 g of material from the flower heads was collected and a complete investigation of this part was therefore not possible.

RESULTS AND DISCUSSION

The investigation revealed a large number of polyacetylenes, some of which are characteristic for the tribe Cynareae and others which are widely distributed in the Asteraceae. Especially the long chain aldehydes with non-conjugated aldehyde groups (**31–37**) present in leaves and stems are characteristic for this tribe. The compounds **2–15**, **26–30** and **38–40** are commonly found in the Asteraceae, especially in the tribes Cynareae and Heliantheae [3]. The flavanones naringenin (**17**) and eriodictyol (**16**) are not widely distributed in nature. Naringenin is found in Myrtaceae [4], Asteraceae (Cynareae) [5] and Leguminosae [6], and eriodictyol has been isolated from Hydrophyllaceae [7], Rutaceae (lemon) [8] and Leguminosae [6]. The tetraenic aldehydes (**22–25**) are commonly found in the tribe Cynareae [3].

The investigation was carried out on two separate parts, roots and leaves plus stems. The petrol extract from the leaves and stems, and the petrol, diethyl ether and methanol extracts from the roots were subjected to flash chromatography [9] and to repeated prep. TLC.

The compounds identified are shown in Tables 1–3. The *cis–trans* isomeric compounds **2** and **3** could not be separated. The ^{1}H NMR spectrum of the mixture gave

Table 1 Compounds isolated from the roots of *L centauroides*

1	$\mathrm{MeCH_2(CH\overset{c}{=}CHCH_2)_3(CH_2)_4CH{=}CH_2}$
2	$\mathrm{MeCH\overset{c}{=}CH(C{\equiv}C)_4CH{=}CH_2}$
3	$\mathrm{MeCH\overset{t}{=}CH(C{\equiv}C)_4CH{=}CH_2}$
4	$\mathrm{Me(C{\equiv}C)_4(CH{=}CH)_2H}$
5	$\mathrm{Me(C{\equiv}C)_3(CH{=}CH)_3H}$
6	$\mathrm{MeCH{=}CH(C{\equiv}C)_2(CH{=}CH)_2(CH_2)_4CH{=}CH_2}$
7	$\mathrm{Me(C{\equiv}C)_5CH{=}CH_2}$
8	$\mathrm{MeCH{=}CH(C{\equiv}C)_2(CH{=}CH)_2(CH_2)_3OAc}$
9	$\mathrm{MeCH{=}CH(C{\equiv}C)_2(CH{=}CH)_2\underset{\underset{OAc}{\vert}}{CH}CH_2\underset{\underset{OAc}{\vert}}{CH_2}}$
10	$\mathrm{Me(C{\equiv}C)_3(CH{=}CH)_2\underset{\underset{OAc}{\vert}}{CH}CH_2\underset{\underset{OAc}{\vert}}{CH_2}}$
11 **12**	$\mathrm{MeCH{=}CH(C{\equiv}C)_2(CH{=}CH)_2(CH_2)_{\mathit{n}}OH}$, $n=3,5$
13	$\mathrm{Me(C{\equiv}C)_3(CH{=}CH)_2(CH_2)_3OH}$
14	$\mathrm{MeCH{=}CH(C{\equiv}C)_2(CH{=}CH)_2\underset{\underset{OH}{\vert}}{CH}CH_2\underset{\underset{OH}{\vert}}{CH_2}}$
15	$\mathrm{Me(C{\equiv}C)_3(CH{=}CH)_2\underset{\underset{OH}{\vert}}{CH}CH_2\underset{\underset{OH}{\vert}}{CH_2}}$
16	Eriodictyol
17	Naringenin
18	Caffeic acid
19	$\mathrm{Me(CH_2)_{14}CH{=}CH_2}$
20	$\mathrm{Me(CH_2)_7CH\overset{c}{=}CHCH_2(CH_2)_4CH{=}CH_2}$
21	$\mathrm{Me(CH_2)_4(CH\overset{c}{=}CHCH_2)_2(CH_2)_4CH{=}CH_2}$

(c = *cis*-double bond, t = *trans*-double bond)

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*Author to whom correspondance should be addressed the following signals δ1 93 (*dd*, $J = 7$0, 1 7 Hz, Me), 5 47–5 89 (*m*, $\mathrm{CH{=}CH_2}$ and $\mathrm{MeCH\overset{c}{=}CH}$), 6 29 (*dq*, $J = 10$ 9, 7 0 Hz, $\mathrm{MeCH\overset{c}{=}CH}$) for **2** and δ 1 83 (*dd*, $J = 7.0$, 1 8 Hz, Me), 5 47–5 89 (*m*, $\mathrm{CH{=}CH_2}$ and $\mathrm{MeCH\overset{t}{=}CH}$), 6.45 (*dq*, $J = 15$ 9, 7 0 Hz, $\mathrm{MeCH\overset{t}{=}CH}$) for **3** From the ^{1}H NMR spectrum of **2** and **3** it appears that they have slightly different chemical shifts for the methyl group and the double bond proton next to the methyl group It was therefore possible to find the ratio between compounds **2** and **3**, and this was determined to be 1 5

From the fraction containing **1** three other hydrocarbons (**19–21**) were isolated These compounds could only be separated by the use of GC and were therefore not fully characterized The GC/MS data yielded three molecular ion peaks at *m/z* 238 for **19**, 236 for **20** and 234 for **21**, suggesting the presence of C_{17} hydrocarbons with one, two and three double bonds, respectively Compounds **1** and **19–21** have been isolated from the Asteraceae [10–13], and may have been formed from linolenic, stearic, oleic, and linoleic acids, respectively, by β-oxidation From another and more polar fraction linoleic, stearic and palmitic acid were isolated The proposed structures of **19–21** are shown in Table 1

From the methanol extract free caffeic acid (**18**) was isolated. Compound **18** probably occurs in plants mainly as caffeoyl esters [14] It is therefore possible that a caffeoyl ester has been hydrolysed to caffeic acid during the isolation. However, a great amount (250 mg/kg) of caffeic acid was isolated from the root material, pointing to the presence of free caffeic acid in the plant, at least for a part of it

Table 2 Compounds isolated from the leaves and stems of *L centauroides*

22–25	$\mathrm{MeCH_2(CH{=}CH)_4(CH_2)_nCHO}$, $n = 4$–7
26–27	$\mathrm{MeCH{=}CH(C{\equiv}C)_2(CH{=}CH)_2(CH_2)_nOAc}$, $n = 2,4$
28–30	$\mathrm{Me(C{\equiv}C)_3(CH{=}CH)_2(CH_2)_nOAc}$, $n = 2$–4
31–34	$\mathrm{MeCH{=}CH(C{\equiv}C)_2(CH{=}CH)_2(CH_2)_nCHO}$, $n = 3$–6
35–37	$\mathrm{Me(C{\equiv}C)_3(CH{=}CH)_2(CH_2)_nCHO}$, $n = 3$–5
38, 39	$\mathrm{Me(C{\equiv}C)_3(CH{=}CH)_2(CH_2)_nOH}$, $n = 4,5$
40	$\mathrm{MeCH{=}CH(C{\equiv}C)_2(CH{=}CH)_2(CH_2)_4OH}$
41–43	$\mathrm{MeCH_2(CH{=}CH)_3(CH_2)_nCHO}$, $n = 5$–7

Compounds **8**, **11–13** and the compounds shown in Table 2 were identified from leaves and stems The different chain lengths being determined by MS. from the fraction containing compounds **22–25** three other aldehydes (**41–43**), with a triene chromophore were isolated ($\lambda_{\max}^{Et_2O}$ 252, 266, 282 nm) These compounds were not fully characterized, because they could only be separated from **22–25** by GC The GC/MS data exhibited molecular ion peaks at *m/z* 206 for **41**, 220 for **42** and 234 for **43**, indicating the presence of C_{13}, C_{14} and C_{15} trienic aldehydes, respectively Both the tetraenic and the trienic aldehydes may be by-products in the oxidative degradation of C_{18}-or C_{17} compounds The proposed structures of **41–43** are shown in Table 2 To our knowledge compounds **41–43** have not previously been reported in the literature

In addition we isolated and characterized 16 different saturated and unsaturated normal chain methyl and ethyl esters (**44–59**), from roots, and leaves plus stems (Table 3) The GC/MS spectra of the saturated methyl and ethyl esters (**44–54**) yielded the following diagnostic peaks at *m/z* 74 $[\mathrm{CH_2{=}C(OH)OMe}]^+$, 87 $[\mathrm{(CH_2)_2COOMe}]^+$. 143 $[\mathrm{(CH_2)_6COOMe}]^+$ and *m/z* 88 $[\mathrm{CH_2{=}C(OH)OEt}]^+$, 101 $[\mathrm{(CH_2)_2COOEt}]^+$, 157 $[\mathrm{(CH_2)_6COOEt}]^+$, respectively [15, 16], and, together with the overall fragmentation pattern, it was possible to make a complete characterization of **44–54.** The unsaturated methyl and ethyl esters (**55–59**) were characterized by comparing the GC/MS spectra obtained from these compounds, with literature values [17]. The positions of the double bonds in **56–59** are suggested to be in accordance with those of linoleic and linolenic acids, respectively

It should be noted that the compounds **1–15**, **17–40** are found in Centaureinae Dumort [3, 5]. In this subtribe

Table 3 Methyl and ethyl esters (**44–59**) found in roots, and leaves plus stems

		Roots	Leaves and stems
44	$\mathrm{Me(CH_2)_{12}COOMe}$		+
45	$\mathrm{Me(CH_2)_{12}COOEt}$	–	+
46	$\mathrm{Me(CH_2)_{13}COOMe}$	–	+
47	$\mathrm{Me(CH_2)_{13}COOEt}$	–	+
48	$\mathrm{Me(CH_2)_{14}COOMe}$	+	+
49	$\mathrm{Me(CH_2)_{14}COOEt}$	–	+
50	$\mathrm{Me(CH_2)_{15}COOMe}$	–	+
51	$\mathrm{Me(CH_2)_{16}COOMe}$	–	+
52	$\mathrm{Me(CH_2)_{18}COOMe}$	–	+
53	$\mathrm{Me(CH_2)_{20}COOMe}$	–	+
54	$\mathrm{Me(CH_2)_{20}COOEt}$	–	+
55	$\mathrm{Me(CH_2)_7CH{=}CH(CH_2)_7COOMe}$	+	+
56	$\mathrm{Me(CH_2)_4(CH{=}CHCH_2)_2(CH_2)_6COOMe}$	+	+
57	$\mathrm{Me(CH_2)_4(CH{=}CHCH_2)_2(CH_2)_6COOEt}$	–	+
58	$\mathrm{MeCH_2(CH{=}CHCH_2)_3(CH_2)_6COOMe}$	–	+
59	$\mathrm{MeCH_2(CH{=}CHCH_2)_3(CH_2)_6COOEt}$	–	+

+ Detected
– Not detected

differently unsaturated aldehydes with chain lengths from C_{14} to C_{17} typically occur in leaves and stems, while the roots always contain ene-tetrayene-enes. Also characteristic are bifunctional ene-triyen-enes and the ene-diyen-dienes In addition we find a remarkable chemical resemblance between *Centaurea scabiosa* belonging to Centaureinae and *L. centauroides*, when we consider roots, and leaves plus stems [10]. Flower heads from *C. scabiosa* contain some chlorohydrins, characteristic for the tribe Cynareae [3, 10]. As mentioned before, we did not have enough material to make a complete investigation of the flower heads of *L. centauroides.* Only compound **4** was isolated from this part of the plant. Therefore it can not be excluded that the flower heads of *L. centauroides* also contain chlorohydrins. It is now clear that *L. centauroides* belongs to Centaureinae which is a subtribe in the tribe Cardueae Cass. [18].

EXPERIMENTAL

General. GC/MS spectra were recorded on a VG Trio-2 mass spectrometer, using an OV-101 column. Prep. TlC: silica gel 60 (ART No 7748) Flash CC· silica gel 60, 230–400 mesh (ART No 9385) For complete identification UV, IR, NMR, MS and TLC were used

Plant material. Roots (3.2 kg) (washed and air-dried) and 1.4 kg of leaves and stems from *Leuzea centauroides* were obtained from the Botanical Garden of Aarhus (15th October 1987). Voucher specimen remain in the herbarium at the University of Aarhus (Botanical Institute).

Extraction and isolation. Roots, leaves and stems were ground and extracted Roots were extracted first with petrol, then with Et_2O, and lastly with MeOH Leaves and stems were extracted with petrol The petrol and Et_2O extracts were subjected to flash chromatography using petrol and petrol containing increasing proportions of Et_2O as eluents In the case of the MeOH extract, only Et_2O was used as an eluent For further separation repeated prep TLC (silica gel) was used. Silica gel containing 5 to 10% of caffeine was used to separate unsaturated low-polar compounds with a slight difference in the number of π-electrons [19, 20]

Compounds isolated from roots 250 mg of **1**, 4 mg of **2**, 20 mg of **3**, 1 mg of **4**, 0 5 mg of **5**, 2 mg of **6**, 1 mg of **7**, 3 mg of **8**, 15 mg of **9**, 0 8 mg of **10**, 49 mg of **11**, 2 mg of **12**, 2 mg of **13**, 9 mg of **14**, 0 1 mg of **15**, 700 mg of **16**, 140 mg of **17**, 800 mg of **18**, and 25 mg of **19–21** (not separated).

Compounds isolated from leaves and stems 15 mg of **22–25** (not separated), 2 mg of **8**, **26**, **27** (not separated), 2 mg of **28–30** (not separated), less than 0 1 mg of **31**, 7 mg of **32**, 7 mg of **33**, 2 mg of **34**, less than 0.3 mg of **35**, 3 mg of **36**, less than 0 3 mg of **37**, 0.4 mg of **13**, **38**, **39** (not separated), 1 mg of **11**, **12**, **40** (not separated) and less than 1 mg of **41–43** (not separated)

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