

## ON TERPENES. CCIX.\*

ARNICOLIDES – SESQUITERPENIC LACTONES  
FROM THE LEAVES OF *Arnica montana* L.

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From a light petroleum extract of *Arnica montana* L. leaves seven sesquiterpenic lactones and one flavone – pectolarigenin (*XIV*) were isolated. Structures were determined for four of the isolated ester-lactones, arnicolides A, B, C and D, not yet described in the literature, including absolute configurations, represented by formulae *II*, *III*, *IV*, and *V*. Other isolated lactones were identified as dihydrohelenalin (*VII*) and tetrahydrohelenalin (*VIII*).

In connection with a study of sesquiterpenic lactones from plants of the *Compositae* family we investigated the isolation of components from the leaves of *Arnica montana* L. after having determined during a preliminary screening<sup>1</sup> that the leaves of this plant contained lactonic substances.

The chemical composition of *Arnica montana* L. has often been investigated in connection with its known curative effects. A review of substances isolated from it up to the present time, as well as older literature data, may be found, for example, in the papers of Schulte and his coworkers who studied mainly the components from its roots<sup>2</sup> and flowers<sup>3</sup>. Recently an isolation and the structure of the sesquiterpenic lactone arnifolin (*I*) from the leaves of *Arnica foliosa* NUTT. and also from the leaves of *Arnica montana* L.<sup>4</sup> have also been described.

During the isolation of the components from the leaves of *A. montana* we extracted first the dry leaves collected in the vicinity of Lenora in the Šumava mountains with light petroleum and then worked up the extract as described in the experimental part of this paper. The so-called lactonic fraction was further separated chromatographically (see Experimental). We were able to isolate seven sesquiterpenic lactones, *II*–*VIII*, the main characters of which are presented in Table I, and also one flavone.

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A relatively large proportion of the light petroleum extract consisted of arnicolide A (*II*). Its IR spectrum (Table I), indicating the presence of a  $\gamma$ -lactone grouping ( $1765\text{ cm}^{-1}$ ), an acetate group ( $1730$  and  $1242\text{ cm}^{-1}$ ), and a five-membered ring keto group conjugated with a double bond ( $1712$  and  $1582\text{ cm}^{-1}$ ), is remarkably similar to the IR spectra of certain pseudoguaianolides containing similar functional groups in the molecule (as for example mexicanin C (*IX*) (ref.<sup>5</sup>), isotenulin (*X*) (ref.<sup>6</sup>), bigelovin (*XI*) (ref.<sup>6</sup>) etc.). The mass spectrum of arnicolide A (Table I) contains a molecular peak at  $m/e$  306 in addition to other fragments at  $m/e$  191, 124, 123, 122, 96, and 95 typical of pseudoguaianolides with oxygen functions at carbon atoms 4, 6, and 8 (ref.<sup>7</sup>). The fact that arnicolide A belongs among pseudoguaianolides was unequivocally confirmed by a detailed analysis of its PMR spectrum, measured by using the methods of double and triple resonance and Indor experiments. Comparing the obtained data (Table II) with published data\* on PMR spectra of pseudoguaianolides, further conclusions could be drawn, namely that arnicolide A should be identical with the acetate of dihydrohelenalin *II*.

This hypothesis was proved as true by direct chemical correlation. On one hand we prepared on hydrogenation of arnicolide A its dihydro derivative *XII*, m.p. 148 to  $149^\circ\text{C}$ , composition  $\text{C}_{17}\text{H}_{24}\text{O}_5$ , and  $[\alpha]_{\text{D}}^{20} +42.2^\circ$ , which is according to IR and PMR spectra and mixture melting point identical with acetyltetrahydrohelenalin *XII* (ref.<sup>9</sup>) prepared from helenalin (*XIII*) (ref.<sup>9</sup>) via tetrahydrohelenalin (ref.<sup>9</sup>) (*VIII*). On the other hand acetylation of dihydrohelenalin<sup>10</sup> (*VII*) gave a substance which according to IR and PMR spectra and mixture melting point was identical with arnicolide A (*II*).\*\* The absolute configuration represented in formula *II* follows from the already known stereochemical structure of dihydrohelenalin<sup>5</sup>. This is also corroborated by the ORD curve of substance *II* which fully agrees with the ORD curve of helenalin (*XIII*) (ref.<sup>11</sup>).

On repeated column chromatography of fractions obtained during a preliminary chromatographic separation (see Experimental) we were able to isolate — as was said above — four additional arnicolides, *i.e.* arnicolides B, C, D, and E, as well as dihydrohelenalin and tetrahydrohelenalin (Table I). All mentioned arnicolides, except arnicolide E, were according to their PMR spectra closely related to helenalin. Arnicolides B, C, and D (Table I) were not identical with any native pseudoguaiano-

\* The published characteristic parameters of the PMR spectrum of dihydrohelenalin acetate measured with a 60 MHz apparatus (A-60) in deuteriochloroform did not differ appreciably from those obtained by us with a 100 MHz apparatus except for the shifts of the proton  $\text{H}_{(6)}$  signals. However, a repeated measurement of the chemical shift of proton  $\text{H}_{(6)}$  in an authentic sample of compound *II* has shown that the value 5.61 p.p.m. (ref.<sup>8</sup>) is not correct.

\*\* Both arnicolide A and the acetate prepared from native dihydrohelenalin had higher melting points ( $177$ – $178^\circ\text{C}$ ) than the acetate of dihydrohelenalin described in the literature ( $167^\circ\text{C}$ ) (ref.<sup>10</sup>). Similarly, native dihydrohelenalin melted at a higher temperature ( $233$ – $235^\circ\text{C}$ ) than was described in the literature ( $223$ – $225^\circ\text{C}$ ) (ref.<sup>10</sup>).

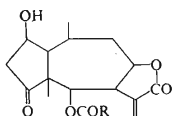
TABLE I  
Characterisation of Arnicolides

Substance	Composition (acyl residue)	M.p., °C [ $\alpha$ ] <sub>D</sub> <sup>20</sup>	IR, cm <sup>-1</sup>	Mass spectrum	R <sub>F</sub> <sup>a</sup>
Arnicolide A (II)	C <sub>17</sub> H <sub>22</sub> O <sub>5</sub> (acetyl)	177–178 –112·8°	1 765 1 730 1 712 1 585 1 242	306 264, 263, 246, 231 191, 124, 123, 122, 96, 95	0·51
Arnicolide B (III)	C <sub>20</sub> H <sub>28</sub> O <sub>5</sub> (isovaleryl)	116–117 –96·8°	1 770 1 720 1 590	264, 263, 246, 231 191, 124, 123, 122, 96, 95 85, 57	0·57
Arnicolide C (IV)	C <sub>19</sub> H <sub>26</sub> O <sub>5</sub> (isobutyryl)	137–138 –91·1°	1 765 1 720 1 590	264, 263, 246, 231 191, 124, 123, 122, 96, 95 71, 43	0·56
Arnicolide D (V)	C <sub>19</sub> H <sub>24</sub> O <sub>5</sub> ( $\alpha$ -methylacrylyl)	100–102 —	1 765 1 715 1 640 1 595	264, 263, 231 191, 124, 123, 122, 96, 95 69	0·55
Arnicolide E (VI)	C <sub>20</sub> H <sub>30</sub> O <sub>5</sub> (isovaleryl)	151–153 —	3 600 1 760 1 720 1 640	350 290, 264, 248, 230, 205 85, 57	0·41
Dihydrohelenalin (VII)	C <sub>15</sub> H <sub>20</sub> O <sub>4</sub>	230–235 —	3 600 1 765 1 705 1 590	264 246, 231 191, 124, 123, 122, 96, 95	0·37
Tetrahydrohelenalin (VIII)	C <sub>15</sub> H <sub>22</sub> O <sub>4</sub>	177–179 —	3 600 1 760 1 725	266 248, 238, 237, 233, 230, 220 198, 197, 124, 107, 97, 95	0·46

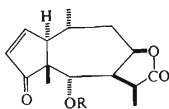
<sup>a</sup> Thin-layer chromatography on silica gel with gypsum in the system light petroleum–acetone–water saturated ether (4 : 3 : 3).

lide of the helenalin type described in the literature. However, from a detailed analysis of their PMR spectra and from their comparison with the PMR spectra of dihydrohelenalin *VII* and arnicolide A (*II*) (Table II) it followed unambiguously that these compounds, similar to arnicolide A, are also esters of dihydrohelenalin *VII* and that they differ from arnicolide A (*II*) only in the character of the ester groups bound to  $C_{(6)}$ .

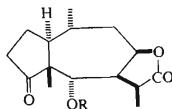
For correlation of the PMR spectra those of authentic samples of helenalin (*XIII*), dihydrohelenalin *VII*, tetrahydrohelenalin *VIII*, and of their acetates were analysed at 100 MHz. Characteristic parameters of the PMR spectra of these substances are also presented in Table II unless they were identical with some of the arnicolides. An interesting feature of substances of this type, *i.e.* with a double bond between  $C_{(2)}$  and  $C_{(3)}$ , is the long-range coupling of protons  $H_{(2)}$  and  $H_{(6)}$  over five bonds. This coupling was always proved by decoupling experiments and in view



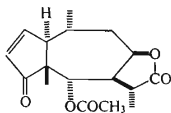
*I*, R =  $C(CH_3)=CH.CH_3$   
(tiglic acid)



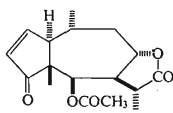
*II*, R =  $CO.CH_3$   
*III*, R =  $CO.CH_2.CH(CH_3)_2$   
*IV*, R =  $CO.CH(CH_3)_2$   
*V*, R =  $CO.C(CH_3)=CH_2$   
*VII*, R = H



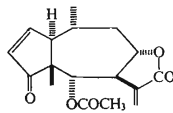
*VIII*, R = H  
*XII*, R =  $CO.CH_3$



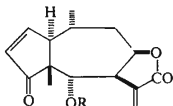
*IX*



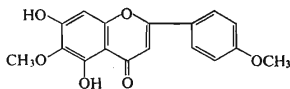
*X*



*XI*



*XIII*, R = H  
*XV*, R =  $CO.CH_3$



*XIV*

of the known stereospecificity of the mechanism of such interaction it can be expected that this coupling might serve as a useful indication of the fusion of a five-membered ring with a seven-membered one, or also of the configuration at  $C_{(6)}$  in pseudoguaianolides of helenaline type. This problem is presently under further study.

Arnicolide B (*III*) contains according to its mass spectrum an unsaturated ester grouping with five carbon atoms, as can be deduced from the fragments of  $m/e$  85 and 57 (Table I). In the PMR spectrum of the mentioned substance (Table II) a doublet is present of a relative intensity corresponding to 6 protons at 0.89 p.p.m. ( $J = 6.5$  Hz), which indicates the presence of an isopropyl group and hence also of the isovaleric acid residue in the molecule of arnicolide B. The fragment of  $m/e$  71 and 45 in the mass spectrum of arnicolide C (*IV*; Table I) indicates that its molecule contains a residue of a saturated acid containing 4 carbon atoms. The PMR spectrum of arnicolide C (Table II) also contains two doublets of secondary methyl groups at 1.08 p.p.m. ( $J = 7.1$  Hz) and at 1.04 p.p.m. ( $J = 7.1$  Hz) which indicate the presence of an isopropyl group and hence also the isobutyrate residue in the molecule of arnicolide C. The mass spectrum of arnicolide D (*V*), *i.e.* the fragment of  $m/e$  69 (Table I) in it, shows that an unsaturated acid residue is present in its molecule. The residue is composed of 4 carbon atoms and a double bond. In its PMR spectrum (Table II) signals of protons are present at 5.50 and 5.89 p.p.m., displaying mutual coupling as well as a coupling with the protons of the methyl groups bound to the double bond. The latter form a complex signal at 1.83 p.p.m.. From the mentioned results it follows that the residue of  $\alpha$ -methylacrylic acid is present in the molecule of arnicolide D. ORD curves of arnicolides B, C, and D had the same form as the ORD curves of arnicolide A (*II*) and helenalin (*XIII*) (ref.<sup>11</sup>). In view of the identity of relative configurations of arnicolides A, B, C, and D, deduced from their PMR spectra (Table II) the absolute structure *III* can be assigned to arnicolide B, structure *IV* to arnicolide C, and formula *V* to arnicolide D.

Arnicolide E (*VI*) which we isolated from the combined chromatographic fractions 301–500 (Experimental; Table III) in a negligible yield was characterised by its melting point, 151–153°C, composition  $C_{20}H_{30}O_5$ , IR spectrum indicating the presence of a  $\gamma$ -lactone group ( $1760\text{ cm}^{-1}$ ), an ester group ( $1720\text{ cm}^{-1}$ ), a double bond ( $1640\text{ cm}^{-1}$ ), and hydroxy groups ( $3600\text{ cm}^{-1}$ ), and mass spectrum (Table I). In view of the negligible amount of the isolated substance *VI* we were unable to study it further. As regards the native substance with a m.p. 230–235°C and of the composition  $C_{15}H_{20}O_4$  we proved by comparison of its melting point, IR, PMR, and mass spectra, as well as by mixture melting point with a standard sample that it is identical with dihydrohelenalin *VII* (ref.<sup>10</sup>). In the same manner we have shown that the native compound of m.p. 177–179°C is identical with tetrahydrohelenalin *VIII* (ref.<sup>9</sup>).

From chromatographic fractions 44–60 (Table III) we isolated a substance,  $C_{17}H_{14}O_6$  and m.p. 218–220°C, of which we have shown, by comparison of its

TABLE II

Characteristic Parameters of the PMR (first-order values) Spectra

The spectra were measured in deuteriochloroform on a Varian HA-100 spectrometers using tetramethylsilane as standard.

A) Chemical shifts ( $\delta$ -scale)

Compound	H <sub>1</sub>	H <sub>2</sub>	H <sub>3</sub>	H <sub>6</sub>	H <sub>7</sub>	H <sub>8</sub>	H <sub>9</sub>	H <sub>9</sub> '	H <sub>10</sub> <sup>a</sup>	H <sub>11</sub> <sup>a</sup>	H <sub>13</sub>	H <sub>14</sub>	H <sub>15</sub>
II <sup>b</sup>	3.03 <sup>a</sup>	7.65	6.04	5.42	2.83	4.74	2.45	1.64	2.15	3.05	1.47	1.20	0.99
III <sup>c</sup>	3.04 <sup>a</sup>	7.66	6.05	5.44	2.83	4.74	2.47	1.63	2.17	3.07	1.51	1.22	1.02
IV <sup>d</sup>	—	7.67	6.06	5.41	2.81	4.75	—	1.63	—	—	1.51	1.24	1.03
V <sup>e</sup>	3.07 <sup>a</sup>	7.68	6.06	5.48	2.90	4.75	2.48	—	2.20	3.07	1.54	1.24	1.06
VII	3.14 <sup>a</sup>	7.71	6.06	4.37	2.95	4.78	2.41	1.72	2.10	3.04	1.35	1.21	0.98
VIII	—	—	—	4.30	2.89	4.75	2.25 <sup>a</sup>	—	2.01	3.04	1.34	1.03	0.80
XII <sup>f</sup>	—	—	—	5.37	2.78	4.70	2.32	1.54	2.14	3.02	1.47	1.07	0.83
XIII	3.03	7.65	6.06	4.45	3.57	4.97	2.25 <sup>a</sup>	1.80 <sup>a</sup>	2.05	—	<sup>h</sup>	1.26	0.98
XV <sup>g</sup>	3.01	7.65	6.06	5.38	3.52	4.88	2.37	1.71	2.15	—	<sup>i</sup>	1.27	0.99

<sup>a</sup> Approximate values found by decoupling and Indor experiments (frequency swept); <sup>b</sup> CH<sub>3</sub>CO : 1.90; <sup>c</sup> —COCH<sub>2</sub>CH(CH<sub>3</sub>)<sub>2</sub> : CH 2.41, CH<sub>3</sub> 0.89 (d, 6 H, J = 6.5); <sup>d</sup> —COCH(CH<sub>3</sub>)<sub>2</sub> : CH<sub>3</sub> 1.08 (d, 3 H, J = 7.1) a 1.04 (td, 3 H, J = 7.1); <sup>e</sup> —COC(CH<sub>3</sub>)<sub>2</sub>=CH<sub>2</sub>(b, c): H<sub>a</sub> 1.83, H<sub>b</sub> 5.50 (J<sub>a, b</sub> = 1.4), H<sub>c</sub> 5.89 (J<sub>b, c</sub> = 1.5, J<sub>a, c</sub> = 1); <sup>f</sup> CH<sub>3</sub>CO : 1.95; <sup>g</sup> CH<sub>3</sub>CO : 1.98; <sup>h</sup> H<sub>13</sub> 6.36 (J<sub>7, 13</sub> = 3.0), H<sub>13</sub> 5.79 (J<sub>7, 13</sub> = 2.8); <sup>i</sup> H<sub>13</sub> 6.43 (J<sub>7, 13</sub> = 2.8), H<sub>13</sub> 6.12 (J<sub>7, 13</sub> = 2.5), <sup>2</sup>J = 0.

TABLE II  
(Continued)  
B) Coupling constants (Hz)

Compound	$J_{1,2}$	$J_{1,3}$	$J_{1,10}^a$	$J_{2,3}$	$J_{2,6}$	$J_{6,7}$	$J_{7,8}^b$	$J_{7,11}^c$	$J_{8,9}^b$	$J_{8,9}^b$	$J_{9,9}$	$J_{9,10}^e$	$J_{9,10}^e$	$J_{10,14}^d$	$J_{11,13}^d$
II	1.5	3.0	9.5	6.0	±0	±0	6.0	10	6.0	2.0	14.5	<3	11	6.0	7.0
III	1.9	3.0	10.5	6.0	±0	—	6.5	10	6.5	1.5	15	1-2	11	6.5	7.0
IV	1.8	3.0	—	6.0	±0	—	6.5	10	6.0	1.7	15	—	11	6.4	7.1
V	1.6	3.0	—	6.0	—	±0	6.0	10	6.0	1.8	15	—	—	6.6	6.9
VII	2	3.0	10.5	6.0	±0	—	6.0	—	6.0	2.5	15	—	10	6.6	7.0
VIII	—	—	—	—	—	±0	6.5	10	6.5	—	—	—	—	6.0	7.0
XII	—	—	—	—	—	±0	6.0	11	6.0	—	15	—	11	6.0	7.1
XIII	1.8	2.9	11.1	6.0	±0	1.8	7.9	—	7.9	2.6	14.5	—	5.5	6.5	—
XV	1.9	3.0	11.1	6.0	0.5 <sup>f</sup>	1.3	7.5	—	7.5	2.2	15	3	8	6.5	—

<sup>a</sup> Read from double- and triple-resonance spectra (frequency-swept)  $H_1 - \{H_2, H_3\}$ ; <sup>b</sup> from the multiplet  $H_8$ ; <sup>c</sup> from the multiplet  $H_7 - \{H_6\}$ ; <sup>d</sup> the value of doublet splitting of methyl groups signals; <sup>e</sup> from the multiplet  $H_9$  and  $H_9$ ; <sup>f</sup> from the multiplet  $H_2$ .

IR spectrum and mixture melting point with standard, that it is identical with pectolin-arigenin (XIV) (ref.<sup>12</sup>).

The presence of substances of pseudoguaianolide type in the species of the *Arnica* genus is remarkable from the chemotaxonomical point of view because substances of the mentioned type have not yet been found in plants of the *Senecioneae* tribe, in which the *Arnica* genus is still classified. Chemotaxonomical conclusions following from this paper, completed by morphological evaluation of characters, represent the basis of a study on the systematic position of the *Arnica* genus, which will be published elsewhere.

TABLE III

Chromatographic Separation of the Lactonic Fraction of the Light Elution Petroleum Extract with Benzene-Ether (4 : 1)

Fraction	Volume, ml	Weight, g	Main compound
1— 20	300	2.00	mixture
21— 31	165	2.91	arnicolide B
32— 43	180	1.94	mixture
44— 60	255	0.84	pectolin-arigenin
			arnicolide C
61— 80	300	0.30	arnicolide D
81—130	750	1.13	arnicolide A
131—300	2 550	0.74	mixture
301—500 <sup>a</sup>	3 000	0.66	tetrahydrohelenalin
			arnicolide E
			dihydrohelenalin

<sup>a</sup> Eluent: benzene-ether 1 : 1.

## EXPERIMENTAL

Melting points were determined on a Kofler block and they were not corrected. Silica gel for chromatography was prepared according to Pitra and Štěrba<sup>13</sup>, particle size 30—60  $\mu$ . IR spectra were measured in chloroform on a Unicam SP-200 or Zeiss UR-10 spectrometer. The UV spectrum was measured in ethanol on a CF 4 (Optica Milano) apparatus. Optical rotations and ORD curves were determined on a Jasco (ORD/UV-5) spectropolarimeter in methanol, and mass spectra on a MCH 1303 (USSR) spectrometer.

### Chromatography of the Light Petroleum Extract

Dry, powdered leaves of *Arnica montana* L., *Compositae*, (16 kg), were extracted exhaustively with light petroleum. The solvent was evaporated and the residue (130 g) was mixed with 1.3 l of light petroleum. The undissolved fraction was filtered off and the solution was extracted with 60% aqueous ethanol. The combined aqueous-ethanolic fractions were evaporated under reduced pressure until only water remained in the distillation flask, and the aqueous residue was extracted



with ether. The combined ethereal extracts were washed with water, dried over anhydrous sodium sulfate, and filtered, and the filtrate was evaporated. The residue (11 g) was dissolved in benzene and chromatographed on a silica gel column (330 g). The course of the chromatography is represented in Table III.

#### Arnicolide A (II)

From the combined fractions 81–130 (Table III) arnicolide A crystallised out, m.p. 177–178°C, (ether),  $[\alpha]_D^{20} - 112.8^\circ$  (c 0.13). For  $C_{17}H_{22}O_5$  (306.3) calculated: 66.66% C, 7.24% H; found: 66.63% C, 7.00% H. ORD:  $[\phi]_{400} - 1263^\circ$ ,  $[\phi]_{352} - 3693^\circ$ ,  $[\phi]_{301} + 2332^\circ$ ,  $[\phi]_{255} - 2235^\circ$ .

#### Acetyltetrahydrohelenalin X

A) Arnicolide A (II; 100 mg) was dissolved in 6 ml of ethanol, mixed with 20 mg of a 5% Pd/SrCO<sub>3</sub> catalyst, and saturated with hydrogen at 25°C for 2 hours (consumption 9 ml H<sub>2</sub>). The mixture was diluted with methanol, filtered, and the filtrate evaporated under reduced pressure. From the residue dihydroarnicolide A (X) was obtained, m.p. 148–149°C (ethyl acetate–light petroleum) and  $[\alpha]_D^{20} + 42.4^\circ$  (c 0.21). For  $C_{17}H_{24}O_5$  (308.4) calculated: 66.20% C, 7.84% H; found: 66.26% C, 7.84% H.

B) Tetrahydrohelenalin<sup>9</sup> VIII (50 mg) was acetylated in the conventional manner with acetic anhydride in pyridine, and the reaction mixture was worked up as usually to give the acetate X, m.p. 148–149°C (ethyl acetate–light petroleum) and  $[\alpha]_D^{20} + 47.1$  (c 0.20). For  $C_{17}H_{24}O_5$  (308.4) calculated: 66.20% C, 7.84% H; found: 66.24% C, 7.89% H. Mixture melting point with the substance obtained under A was undepressed.

#### Acetylation of Dihydrohelenalin II

Dihydrohelenalin (VII) (30 mg) was acetylated with acetic anhydride in pyridine to give acetyldihydrohelenalin, m.p. 178–179°C, which according to its IR and PMR spectrum and mixture melting point with arnicolide A was identical with the latter.

#### Arnicolide B (III)

Combined fractions 21–31 (Table III) were dissolved in benzene and rechromatographed on a column of silica gel (300 g) with light petroleum–acetone–water saturated ether (5 : 1 : 1). From the first fractions arnicolide B (III), was isolated, m.p. 116–117°C (light petroleum) and  $[\alpha]_D^{20} - 96.8^\circ$  (c 0.23). For  $C_{20}H_{28}O_5$  (348.4) calculated: 68.94% C, 8.10% H; found: 66.63% C, 7.96% H. ORD:  $[\phi]_{400} - 1316^\circ$ ,  $[\phi]_{352} - 4230^\circ$ ,  $[\phi]_{301} + 2538^\circ$ ,  $[\phi]_{250} - 1645^\circ$ .

#### Arnicolide C (IV)

Combined fractions 44–60 (Table III) were allowed to crystallise and the crystals were separated. The mother liquors were rechromatographed on 80 g of silica gel with light petroleum–acetone–water saturated ether mixture (5 : 1 : 1) as solvent. From the central fractions arnicolide C (IV) was isolated, m.p. 137–138°C (acetone–light petroleum),  $[\alpha]_D^{20} - 91.1^\circ$  (c 0.25). For  $C_{19}H_{26}O_5$  (334.4) calculated: 68.24% C, 7.84% H; found: 68.36% C, 7.72% H. ORD:  $[\phi]_{400} - 1261^\circ$ ,  $[\phi]_{352} - 3613^\circ$ ,  $[\phi]_{298} + 2607^\circ$ ,  $[\phi]_{255} - 757^\circ$ .

#### Arnicolide D (V)

Combined fractions 61–80 (Table III) were rechromatographed on a silica gel column (30 g) with the same eluent as in the preceding experiments. From the last fractions arnicolide D (V) was isolated, m.p. 100–102°C (light petroleum). For  $C_{19}H_{24}O_5$  (332.4) calculated: 68.65% C, 7.28% H; found: 68.06% C, 7.22% H. ORD:  $[\Phi]_{400} -1223^\circ$ ,  $[\Phi]_{351} -3233^\circ$ ,  $[\Phi]_{300} +1094^\circ$ ,  $[\Phi]_{254} -4370^\circ$ .

#### Arnicolide E (VI)

Combined fractions 301–500 (Table II) were rechromatographed on a silica gel column (60 g) with the same solvent mixture as in the preceding experiment. From the medium fractions arnicolide E (VI) was obtained, m.p. 151 to 153°C (acetone–light petroleum). For  $C_{20}H_{30}O_5$  (350.4) calculated: 68.55% C, 8.61% H; found: 68.82% C, 8.84% H.

#### Isolation of Dihydrohelenalin VII

During the chromatography described above substance VII was isolated from the latter fractions, m.p. 230–235°C (acetone–ether). According to its IR and PMR spectrum this substance was identical with dihydrohelenalin (VII) (ref.<sup>10</sup>), as was proved by the mixture melting point with authentic dihydrohelenalin.

#### Isolation of Tetrahydrohelenalin VIII

The same chromatography as above afforded fractions (initial fractions) which contained a compound of m.p. 177–179°C, which according to its IR spectrum and mixture melting point with an authentic sample was identical with tetrahydrohelenalin VIII.

#### Pectolinarigenin XIV

From the combined fractions 44–60 (Table III) pectolinarigenin<sup>12</sup> (XIV) crystallised out, m.p. 218–220°C (ethanol). For  $C_{17}H_{14}O_6$  (314.3) calculated: 64.96% C, 4.49% H, 0.64% H act.; found: 64.71% C, 4.38% H, 0.58% H act. The IR and UV spectrum of it was identical with the spectra of an authentic sample of pectolinarigenin<sup>12</sup>. Mixture melting point was also undepressed.

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