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Minor Components from Growing Buds of *Artemisia capillaris* That Act as Insect Antifeedants

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Capillin, capillarin, methyleugenol, *ar*-curcumene, and bornyl acetate were isolated as the minor component from the growing buds of *Artemisia capillaris*. These compounds showed an antifeeding activity to the larva of the cabbage butterfly. Relationships between the activity and the chemical structure of whole components are also discussed.

Isolation of 1-phenyl-2,4-pentadiyne and capillen from the growing buds of *Artemisia capillaris* and their antifeeding activity for larvae of the cabbage butterfly, *Pieris rapae crucivora*, were already reported (Yano, 1983). Relationships between the antifeeding activity for larvae and the chemical structure of phenylalkynes of the type $C_6H_5C{\equiv}CR$ were also previously studied (Yano, 1986). Miyazawa and Kameoka (1977) reported chemical components in the essential oil from aerial parts of *A. capillaris*. In this paper, isolation of the minor components in the viscous substance secreted from the growing buds of *A. capillaris* and their antifeeding activity for larvae of the cabbage butterfly are reported. Relationships between the activity and the chemical structure of whole components are also discussed.

EXPERIMENTAL SECTION

Isolation of Chemical Components. Four grams (0.23% yield) of the essential oil were obtained from the growing buds (1739 g, in June) of *A. capillaris* according to the previous paper (Yano, 1983). The oil was chromatographed on the silica gel column (100–200 mesh, 70 g, l = 63 cm, d = 1.8 cm) and divided into three fractions: terpene hydrocarbons (3% of the oil), phenylalkynes (83%), and polar components (14%).

Gas Chromatograph. A Shimadzu GC-3BT was operated to isolate the minor components under the following conditions. For γ-terpinene (**5**) and caryophyllene (**6**): 25% PEG 6000 column [3 mm × 3 m, temperature 155 °C]; carrier gas, He at 30 mL/min; range, 2 mV; filament current, 80 mA. For bornyl acetate (**7**) at 160 °C and methyleugenol at 170 °C: 10% silicone DC 560 [3 mm × 4.4 m]; carrier gas, He at 30 mL/min; range, 2 mV; filament current, 80 mA.

Biological Activity. A leaf disk (d = 2 cm) of cabbage, *Brassica oleracea* var. capitata, was punched out with a cork borer. The larvae of the cabbage butterfly were collected at cabbage field. After being held in a breeding box for 1 day, larvae in the 5th instar (weight 0.2000–0.2600 g) were used for a feeding test. In the previous paper (Yano, 1983), the sample disks were prepared by coating a liquid compound on the surface of a leaf disk. But it was difficult to coat a liquid compound equally on the surface of a disk and impossible to coat a crystalline compound in the state of solid. In these studies, sample disks were dipped into acetone solution (10^{-1} mol/L) of the compounds for 2–3 s according to the method of Hosozawa et al. (1974), and control disks were dipped into acetone. These treated disks were allowed to stand under a draft to evaporate the acetone. In the beaker (100 mL, d = 5 cm, h = 7 cm) a sample disk (left side), a control disk (right side), and larva (between both disks) were placed. The temperature was kept at 23–24 °C. After 2 h, two leaf disks were removed, the eaten area of a disk was measured, and its percentage was calculated. This leaf disk test was repeated about 10 times for every chemical components isolated from the essential oil. From the results of 10

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replicates, the average percentage of eaten area was calculated about the sample disk and control disk. Then, the relative antifeeding percentage (Yano, 1986) was calculated as

rel antifeeding % =
[1 − av % of eaten area (sample)/[av % of eaten area (sample) + av % of eaten area (control)]] × 100

When the relative antifeeding percentage is above 80%, it is judged to be active for larvae; 50% shows it to be inactive.

RESULTS AND DISCUSSION

In this paper, seven minor components were isolated from the growing buds of *A. capillaris*. γ-Terpinene (**5**; 1% of the oil) was isolated by gas chromatography from the hydrocarbon fraction: $\nu_{C=C}$ (liquid film) 1660 cm^{-1}, δ_{CH} 1385, 1365 cm^{-1}. The IR spectrum was identical with that of **5** reported by Mitzner et al. (1965). Caryophyllene (**6**; trace) was isolated by the same method with **5**: $\nu_{=CH_2}$ (liquid film) 3070 cm^{-1}, $\nu_{C=C}$ 1675, 1635 cm^{-1}, δ_{CH} 1390, 1375 cm^{-1}, $\delta_{=CH_2}$ 895 cm^{-1}. The IR spectrum was identical with **6** in Wenninger et al. (1967). *ar*-Curcumene (**4**; trace) was isolated as the last eluate of hydrocarbon fraction: $\nu_{ArC=C}$ (liquid film) 1520 cm^{-1}, δ_{CH} 825 cm^{-1}. The IR spectrum was identical with **4** in Wenninger et al. (1967). Methyleugenol (**3**; 1%) was isolated by gas chromatography from the last eluate of the polar fraction: $\nu_{CH_3(ArO)}$ (liquid film) 2840 cm^{-1}, $\nu_{C=C}$ 1640 cm^{-1}, $\nu_{ArC=C}$ 1595 cm^{-1}, $\nu_{=COC}$ 1245 cm^{-1}. The IR spectrum was identical with **3** in Nagasawa (1961). Bornyl acetate (**7**; 8%) was isolated by gas chromatography from the first eluate of the polar fraction: $[\alpha]^{20}_D$ +41°; $\nu_{C=O}$ (liquid film) 1740 cm^{-1}, δ_{CH} 1385, 1370 cm^{-1}, ν_{CO} 1250 cm^{-1}. The IR spectrum was identical with **7** of Yukawa and Itō (1973). Capillin (**1**; trace) was isolated as the crystalline mass from the middle eluate of the polar fraction: mp 79.5–80.5 °C (recrystallized from acetone); ^1H NMR ($CDCl_3$, Me_4Si internal standard) δ 2.02 (3 H, s, C≡CC*H*$_3$), 7.51 (3 H, m, Ar*H*), 8.12 (2 H, dd, J = 8.2, 1.0 Hz, Ar*H*); IR $\nu_{C\equiv C}$ (KBr disk) 2240, 2140 cm^{-1}, $\nu_{C=O}$ 1637 cm^{-1}, $\nu_{ArC=C}$ 1600, 1580 cm^{-1}. The IR spectrum was identical with **1** in Imai (1956). Capillarin (**2**; trace) was isolated as the crystalline mass from the last eluate of the polar fraction: mp 121–122 °C (recrystallized from ethyl acetate); ^1H NMR δ 1.86, (3 H, tm, J = 3 Hz, C≡CC*H*$_3$), 3.44 (2 H, sm, C*H*$_2$C≡C), 6.64 (1 H, s, C=C*H*), 7.60 (3 H, m, Ar*H*), 8.26 (1 H, d, J = 8 Hz, Ar*H*); IR $\nu_{C=O}$ (KBr disk) 1730 cm^{-1}, $\nu_{ArC=C}$ 1600, 1570, 1485 cm^{-1}. The IR spectrum was identical with **2** of Harada et al., (1960).

A sample disk treated with acetone solution (10^{-1} mol/L) of capillarin (**2**) and a control disk were fed to a larva (5th instar) at 23–24 °C. After 2 h, average percentages of eaten area of both disks were calculated respectively as 1% (sample disk) and 38% (control disk) from 10 replicates of this test. Then, the relative antifeeding percentage of **2** was calculated as 97%. It shows that **2** has a strong antifeeding activity for larvae. Next, the leaf disk tests of the other chemical components were examined in the same manner. The results of the leaf disk tests of these seven chemical components, 1-phenyl-2,4-pentadiyne (**8**), and capillen (**9**) are listed in Table I, to discuss relationships between the relative antifeeding percentage and the chemical structure.

In prevous studies (Yano, 1986), it was reported that the C≡C triple bonds in a side chain of **8** and **9** caused antifeeding activity since those compounds lacking the C≡C triple bonds lacked activity. In terms of chemical structure (Figure 1), two compounds have the same structure with the exception of the terminal group in a side chain. **9** has

Table I. Antifeeding Leaf Disk Test[a] for Larva[b] of the Cabbage Butterfly

compound	uneaten disk no.	av % eaten area	rel antifeeding %
capillin (**1**)			
sample disk[c]	10	0	100
control	0	36	
capillarin (**2**)			
sample disk	8	1	97
control	0	38	
methyleugenol (**3**)			
sample disk	10	0	100
control	0	32	
ar-curcumene (**4**)			
sample disk	5	3	85
control	0	17	
γ-terpinene (**5**)			
sample disk	2	9	61
control	1	14	
caryophyllene (**6**)			
sample disk	3	6	73
control	1	16	
bornyl acetate (**7**)			
sample disk	10	0	100
control	0	24	
1-phenyl-2,4-pentadiyne (**8**)			
sample disk	6	7	83
control	0	35	
capillen (**9**)			
sample disk	9	3	91
control	0	31	

[a] Test was repeated 10 times; temperature was kept 23–24 °C; and feeding time was 2 h. [b] 5th instar was used (weight 0.2000–0.2600 g). [c] Cabbage leaf disk was dipped into acetone solution (10^{-1} mol/L) of compound and allowed to stand under a draft to evaporate the acetone.

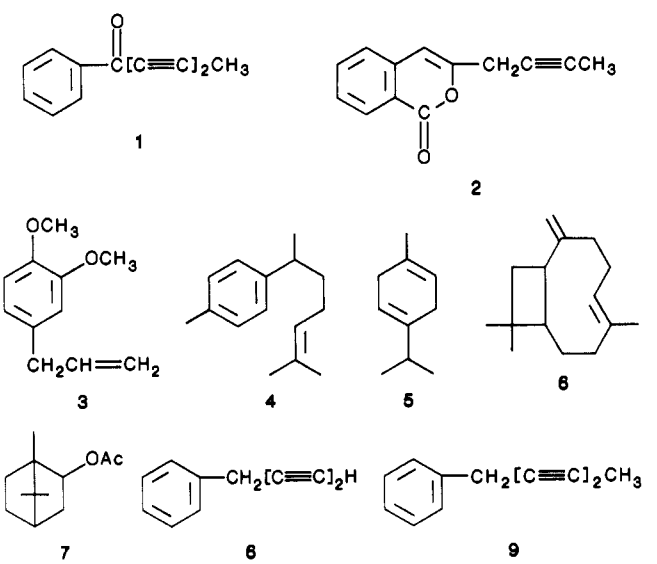

Figure 1. Whole components from growing buds of *A. capillaris*: **1**, capillin; **2**, capillarin; **3**, methyleugenol; **4**, *ar*-curcumene; **5**, γ-terpinene; **6**, caryophyllene; **7**, bornyl acetate; **8**, 1-phenyl-2,4-pentadiyne; **9**, capillen.

a methyl group as a terminal group, and its relative antifeeding percentage (91%) is active against larvae. On the other hand, **8** has a hydrogen atom instead of a methyl group, and its relative antifeeding percentage (83%) is less than that of chemicals possessing a methyl group in this position. Also, the number of the uneaten sample disk of **8** is 6 of 10 replicates, and that of the uneaten control disk is 0. Concerning **9**, the uneaten sample disk is 9 and uneaten control disk 0. These results agree with a tendency of relative antifeeding percentage. This phenomenon (Yano, 1986) was also observed about phenylethyne C_6-

$H_5C{\equiv}CH$ (relative antifeeding percentage 64%) and 1-phenylpropyne $C_6H_5C{\equiv}CCH_3$ (96%).

The relative antifeeding activities of capillin (1; 100%) and capillarin (2; 97%) are strongly active against larva. Also, the numbers of uneaten sample disks of 1 and 2 are 10 and 8, respectively, and show the same tendency with percentage. In terms of chemical structure, 1 has a C=O carbonyl group instead of a CH_2 methylene group in a side chain of 9. 2 has a δ-lactone ring formed by cyclization of a side chain of 9 and a C≡C triple bond in a side chain. It is therefore suggested that a C=O carbonyl group and a C≡C triple bond in a side chain and a lactone ring (Rodriquez et al., 1976) are of the many factors that contribute to a compound being antifeeding active.

The relative antifeeding percentage of methyleugenol (3; 100%) is strongly active against larvae. Also, the number of the uneaten sample disk is 10 and shows the same tendency with percentage. 3 has *o*-dimethoxybenzene as a partial structure. And the contribution to antifeeding activity of this group is continuously studied in this laboratory.

ar-Curcumene (4) is weakly active (relative antifeeding percentage 85%, uneaten sample disk 5). On the other hand, the relative antifeeding percentages of γ-terpinene (5) and caryophyllene (6) are 61% and 73%, respectively. Also, the numbers of the uneaten sample disk of 5 and 6 are 2 and 3, respectively. These data show inactivity. But, bornyl acetate (7) is strongly active (relative antifeeding percentage 100%, uneaten sample disk 10).

In the present and previous studies, it was observed that 1–4 and 7–9 among whole components, which were isolated from the growing buds of *A. capillaris*, showed antifeeding activity for larvae of the cabbage butterfly. It is a very interesting point in the food chain cycle of the natural world that these components prevent insects from eating the growing buds of the plant.

Registry No. Capillin, 495-74-9; capillarin, 3570-28-3; methyleugenol, 93-15-2; *ar*-curcumene, 644-30-4; bornyl acetate, 76-49-3; caryophyllene, 87-44-5; γ-terpinene, 99-85-4.

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Quantitation of Hymenoxon and Related Sesquiterpene Lactones

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A rapid and reproducible method for the quantitation of hymenoxon and related sesquiterpene lactones has been developed using reversed-phase HPLC. The methanol extract of a dried and ground plant sample was analyzed on an RP-8 column eluted with methanol–water (1:1, v/v) and monitored at 235 nm. The hymenoxon content of the above ground whole plant of *Hymenoxys odorata* ranged from 0.21 to 1.27% according to the site, season, and year of collection. Helenalin and mexicanin E were concentrated in the flowering heads (8.95 and 4.66%, respectively) of *Helenium microcephalum* while tenulin was concentrated in the leaves (6.14%) of *Helenium amarum*. The detection limit was 25 ng for helenalin and mexicanin E and 75 ng for hymenoxon and tenulin. The recovery rate of hymenoxon was 102.5 ± 4.5%.

Hymenoxon and related sesquiterpene lactones (Figure 1) are widely distributed among well-known livestock poisoning plants such as *Helenium*, *Hymenoxys*, and *Baileya spp.* The reported lethal dose of smallhead sneezeweed (*Helenium microcephalum*) in sheep and cattle is approximately 2.5 g/kg when the animals are force-fed the freshly ground flowering plants as a single dose whereas the LD_{50} for bitter sneezeweed (*Helenium amarum*) in sheep is 2.0 g/kg per day for 2 consecutive days (Dollahite et al., 1964, 1972). The oral LD_{50} values of dried and ground bitterweed (*Hymenoxys odorata*) in sheep range from 2.9 to 8.5 g/kg according to the site, season, and/or year of collection (Rowe et al., 1973). Sesquiterpene lactones helenalin and mexicanin E, among others, have been isolated from *H. microcephalum* (Clark, 1939; Kim, 1980); tenulin and aromaticin have been isolated from *H. amarum* (Clark, 1939; Lucas et al., 1964); hymenoxon has been isolated from *H. odorata* (Kim et al., 1975; Ivie et al., 1975a). Both hymenoxon and bitterweed (*H. odorata*) elicit common toxic effects in sheep (Terry et al., 1981); thus, the toxicity of a bitterweed sample appears to be dependent on the hymenoxon content.

When *H. amarum* is eaten by dairy cattle, tenulin is excreted in the milk, making it unpalatable due to the bitter taste (Ivie et al., 1975b). Helenalin causes electrocardiographic aberrations consistent with myocardial damage, respiratory paralysis, and progressive hypotension

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