Polyacetylenes of *Solidago altissima* L.

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An unknown polyacetylene was isolated from the subterranean stems of *Solidago altissima* L. in which the two polyacetylenes, dehydromatricaria ester (I) and methyl 10-[(Z)-2-methyl-2-butenoyloxy]-(2Z,8Z)-2,8-decadiene-4,6-diynoate (II) had already been found, and its structure was identified as (4Z)-2,4-decadiene-6,8-diyne-4-olide (dehydromatricaria lactone) (III). The lactone (III), as well as I, strongly inhibited the growth of the seedlings of barnyard millet (*Panicum crus-galli* L. var. *frumentaceum* TRIN.).

The seasonal variations in the polyacetylene contents of the subterranean stems were closely investigated, with the results that III occurred only in the fall of the year while I and II hardly varied throughout the year. II was a major polyacetylene component and the content was 0.9~1.0 $\mu\text{mol/g}$ fresh wt.

A number of polyacetylenes have been isolated from higher plants and higher fungi in recent years.¹⁾ However, little is known about the physiological functions of these compounds. Some polyacetylenes have been isolated as biologically active substances, and their antifungal,^{2~4)} allelopathic,⁵⁾ nematocidal⁶⁾ and plant growth inhibitory^{7~9)} properties were revealed. Dehydromatricaria ester (I) was isolated from *Solidago altissima* L. as a plant growth inhibitory substance⁷⁾ and regarded as one of the factors for the great propagating power of this plant.⁵⁾ Recently, a new polyacetylene (II) showing inhibitory effect on plant growth was isolated as the second polyacetylenic compound of *S. altissima*.⁸⁾

In the course of an investigation of the lipids of *S. altissima*, a polyacetylene containing a chromophore of conjugated double and triple bonds has been detected. The present communication deals with the structure determination of this polyacetylene (III) and its plant growth inhibitory effect. In addition, seasonal variations in the amounts of the three polyacetylenes (I, II and III) in the subterranean stems of this plant are also described. The structures of these polyacetylenes are as follows:

MATERIALS AND METHODS

UV spectra were determined in benzene, diethyl ether or ethanol with a Hitachi 124 spectrophotometer. IR spectra were measured in chloroform, carbon tetrachloride or potassium bromide with a Shimadzu infrared spectrometer IR 27C. NMR spectra were recorded in deuteriochloroform or carbon tetrachloride on a Hitachi R-24 NMR spectrometer (60 MHz).

Isolation and purification of the unknown polyacetylene (III). Lipids were extracted from the subterranean stems of *S. altissima* by the method described previously,⁸⁾ and were chromatographed on a silicic acid column (50×170 mm) with benzene as eluent. The polyacetylenes (I), (III) and (II) were separately recovered from 200~1200-ml, 800~1600-ml and 1700~2800-ml fractions, respectively. The fractions containing III were concentrated to dryness *in vacuo*, dissolved in *n*-hexane and rechromatographed on a silicic acid column (30×120 mm) with the mixtures of *n*-hexane and diethyl ether (9:1, 8:2, 7:3, 6:4, 5:5; v/v), successively. The polyacetylene (III) was eluted with *n*-hexane-diethyl ether (5:5) and separated from I which was eluted with the solvent mixture (8:2). Subsequently, III was applied on preparative plates of 0.5-mm thickness of silica gel G, and then developed

with *n*-hexane-diethyl ether (6: 4, v/v) (*R_f* values: **I** and **II**, 0.5; **III**, 0.2). A black band under ultraviolet light was scraped off and extracted several times with diethyl ether. The isolated polyacetylene (**III**) was further purified by thin-layer chromatography on preparative silica gel G plates with benzene-diethyl ether (8: 2, v/v) as the developing solvent (*R_f* values: **I** and **III**, 0.6; **II**, 0.4).

Catalytic hydrogenation of the polyacetylene (III), and reductive deoxygenation and methylation of the hydrogenation product (IV). The ethanol solution of **III** was stirred over palladium black in a stream of hydrogen at room temperature for 2 hr. After removal of the catalyst, the solvent was evaporated *in vacuo*. The product (**IV**) (10 mg) was heated under reflux for 24 hr with 4 ml of 48% hydroiodic acid and 50 mg of red phosphorus.¹⁰⁾ The reaction mixture was extracted with *n*-hexane. The extract was washed with sodium bisulfite solution, and then with water. The colorless hexane solution thus obtained was dried over anhydrous sodium sulfate and evaporated *in vacuo*. The residue was dissolved in 5 ml of methanol and refluxed for 1 hr with 1 ml of concentrated hydrochloric acid and 0.2 g of granulated zinc. Diethyl ether was added to the reaction mixture and washed with water. After the organic layer was dried and evaporated, the residue was subjected to gas-liquid chromatography.

Assay for plant growth. The inhibitory effects of the polyacetylenes (**I**) and (**III**) on the growth of the seedlings of barnyard millet (*Panicum crus-galli* L. var. *frumentaceum* TRIN.) were measured under the same conditions as described previously,⁸⁾ except that the incubation period was 3 days.

Seasonal variations in polyacetylene contents. The subterranean stems of *S. altissima* were harvested monthly and the lipids were extracted by the method described in the previous paper.⁸⁾ The three polyacetylenes in the lipids were separated each other by the column-chromatographic procedure similar to that described above. A mixture of **I** and **III** was separated from **II** by the first silicic acid column chromatography with benzene, and then rechromatographed on a silicic acid column with the solvent system of *n*-hexane-diethyl ether. Each polyacetylene thus isolated was determined by UV spectrometry with the ϵ values described in the references on **I**,¹¹⁾ **II**⁸⁾ and **III**.¹²⁾

RESULTS AND DISCUSSION

The structure of the unknown polyacetylene (III)

As shown in Fig. 1, the IR spectrum of **III**, which was obtained as yellow crystals by the above chromatographic procedure, exhibited

FIG. 1. IR Spectrum of Polyacetylene **III** (in chloroform).

FIG. 2. UV Spectrum of Polyacetylene **III** (in ethanol).

a sharp absorption band at 2220 cm^{-1} , interpreted as a disubstituted $\text{C}\equiv\text{C}$ stretching frequency. Two very strong absorption bands at 1790 and 1750 cm^{-1} indicated the presence of an unsaturated-lactone ring. The UV spectrum of **III** (Fig. 2) was identical with that of dehydromatricaria lactone isolated from *Anthemis brachycentros* F. Gay.¹²⁾ This indicated the presence of a diyne-diene-one chromophore $[(\text{C}\equiv\text{C})_2(\text{C}=\text{C})_2\text{C}=\text{O}]$ in the structure. The catalytic hydrogenation of **III** required 5.9 moles/mole of hydrogen and the gas-liquid chromatographic retention time of the hydrogenation product (**IV**) agreed with that of authentic decan-4-olide. The mass spectrum of **IV** clearly showed that it was a γ -lactone (Fig. 3). The IR and NMR spectra of **IV** also

FIG. 3. Mass Spectrum of the Saturated Product (IV) Prepared by Hydrogenation of III.

supported the idea that it was decan-4-olide. As was expected, the hydrolysis of IV with 1 N potassium hydroxide in ethanol gave a hydroxy-acid (IR: 3200 cm^{-1} , OH; 1540 cm^{-1} , COO). The saturated fatty acid methyl ester prepared by reductive deoxygenation and methylation of IV was identified as methyl decanoate (V) by gas-liquid chromatography.

TABLE I. NMR SPECTRAL DATA OF POLYACETYLENE III

$ \begin{array}{c} \text{a} \quad \text{b} \quad \text{c} \quad \text{d} \\ \text{CH}_3(\text{C}\equiv\text{C})_2\text{CH}=\text{C} \begin{array}{c} \text{CH}=\text{CH} \\ \diagup \quad \diagdown \\ \text{O} \quad \text{C=O} \end{array} \end{array} $		
H	δ (ppm)	J (Hz)
a	2.00 (s)	—
b	5.24 (m)	—
c	7.33 (d)	4.7
d	6.20 (d)	4.7

On the basis of these data, the structure of this polyacetylenic lactone (III) was established as dehydromatricaria lactone, 2,4-decadiene-6,8-diyn-4-olide. A sharp absorption band at 940 cm^{-1} in the IR spectrum of III (Fig. 1)

showed that the double bond at C-4 position was (Z)-configuration [(E)-dehydromatricaria lactone lacks this absorption band.¹²⁾]. This structure was well confirmed by the NMR data (Table I) and the results of the elemental analysis (Found: C, 76.07; H, 3.97. Calcd. for $\text{C}_{10}\text{H}_6\text{O}_2$: C, 75.94; H, 3.82%).

Seasonal variations in polyacetylene contents and inhibition of dehydromatricaria lactone (III) on plant growth

Table II shows that dehydromatricaria lactone (III) inhibits the growth of the seedlings of barnyard millet as effectively as dehydromatricaria ester (I) does. However, the season when III appeared in the subterranean stems of *S. altissima* was confined to the fall of the year and it was scarcely detected in the other seasons, while the amounts of I varied with the months in the range of 0.27 to $0.46\text{ }\mu\text{mol/g}$ fresh wt. (Fig. 4). As III can be metabolized into some thioether compounds,¹³⁾ the decrease of this polyacetylene in the winter may indicate its conversion into other polyacetylenes. At-

TABLE II. INHIBITION OF POLYACETYLENES I AND III ON GROWTH OF THE SEEDLINGS OF BARNYARD MILLET

Polyacetylene	Coleoptile length (mm)			
	Concentration (ppm)			
	0	1	10	100
I	32.7	25.3	11.0	6.0
III	32.7	26.7	11.4	4.0

FIG. 4. Seasonal Variations in Polyacetylene Contents of the Subterranean Stems of *S. altissima*.

▲—▲, I; ●—●, II; ○—○, III.

tempts to find these possible metabolites were nevertheless unsuccessful. Therefore, **III** will be metabolized into a non-polyacetylenic compound or excreted to the soil in the winter. It was worth noting that the variations in the amounts of **III** did not affect apparently those for **I** regarded as a precursor of **III**.¹³⁾ The amounts of both **I** and **III** showed maxima in October (Fig. 4). Once **I** is synthesized as a precursor of **III**, it seems to be scarcely accumulated in the cells. Kobayashi *et al.*⁵⁾ have found that *S. altissima* contains **I** in February (maximum in wt.) twice as much as in June (minimum in wt.). Their results did not so much differ from the variation curve of **I** in Fig. 4. The amounts of **II** hardly varied in the subterranean stems throughout the year.

It was particularly interesting that the major polyacetylene component was not **I** which was one of the most widespread polyacetylenes, but **II**. Since **I** has been detected in the soil where the plants grow (5 to 6 ppm per dry wt.),⁵⁾ it is probable that **II** also accumulates there. If so, **II** may be a more important polyacetylene for allelopathy than the other two. But it is a problem to be solved whether **II** can actually inhibit the growth of wild plants or not. A difference in growth inhibition of **II** on rice⁹⁾ and barnyard millet⁸⁾ seedlings may show species-specific inhibition of this compound.

The polyacetylenes (**II**) and (**III**) may also inhibit seed germination of *S. altissima* itself as strongly as **I** did.⁵⁾ Although this inhibition appears inexpedient for breeding of the plant, it is not necessarily so. When its seed germinates and roots at a place where no polyacetylene is present, it rapidly spreads in all directions with the subterranean stems. Therefore, if these polyacetylenes do not inhibit the growth of the subterranean stems, this plant can propagate without being affected by the polyacetylenes excreted to the soil on which it grows. It may be possible that the growth of

the subterranean stems is not inhibited with the polyacetylenes, because this organ itself contains a relatively large amount of these compounds.

Solidago virgaurea L., which is nearly related to *S. altissima*, also contains several polyacetylenes.¹⁴⁾ The major polyacetylenes of the former are matricaria ester* and matricaria lactone** which are the dihydro-derivatives of **I** and **III**, respectively. In spite of structural resemblances (between **I** and matricaria ester and between **III** and matricaria lactone) and a near relationship between both the plant species, the seasonal variation curves of **I** and **III** in *S. altissima* differed from those of matricaria ester and matricaria lactone in *S. virgaurea*,¹⁴⁾ respectively. Both the species, however, contained the polyacetylenes only in their subterranean parts. It can therefore be presumed that these parts of the genus *Solidago* are the main sites for polyacetylene synthesis and metabolism.

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* $\text{CH}_3\text{CH}=\text{CH}(\text{C}\equiv\text{C})_2\text{CH}=\text{CHCOOCH}_3$

** $\text{CH}_3\text{CH}=\text{CHC}\equiv\text{CCH}=\text{C}\begin{array}{c} \text{CH}=\text{CH} \\ \diagup \quad \diagdown \\ \text{O} \quad \text{CO} \end{array}$

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