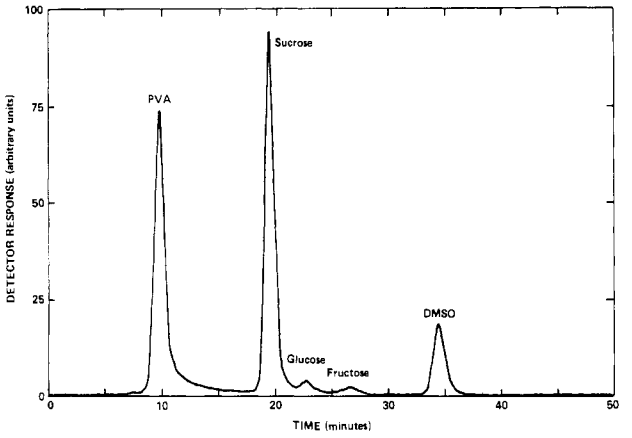

Figure 1. HPLC of polysugar prepared by the procedure described by Usmani and Salyer.

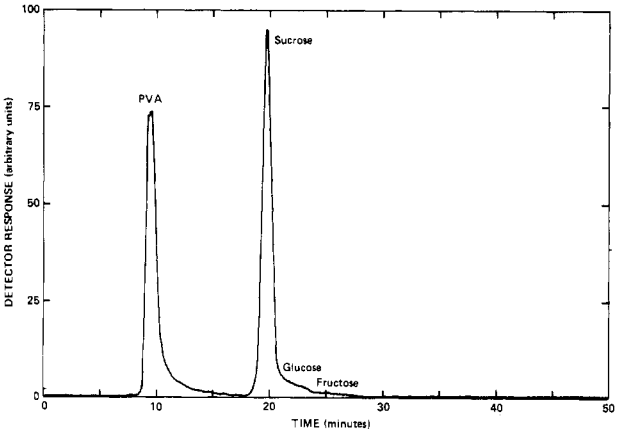

Figure 2. HPLC of a 1:1 mixture of poly(vinyl alcohol) and sucrose.

The ^1H NMR and ^{13}C NMR spectra of the isolated poly(vinyl alcohol) and starting poly(vinyl alcohol) were identical, indicating that there was no sucrose bound to the poly(vinyl alcohol).

Since we could not produce a sweet polysugar, an attempt was made to produce a bitter polysugar [polysugar sample 204257, Usmani and Salyer (1979)]. In our hands, we again produced a mixture of starting materials using Usmani and Salyer's reaction conditions of 24 h at 100 °C. The reported bitter taste of polysugar sample 204257 could have been from trans-acylation of the sucrose from the poly(vinyl acetate) giving various sucrose acetates, some of which are reported to be intensely bitter (Kononenko and Kestenbaum, 1961).

These results suggest that Usmani and Salyer did not effect an etherification of poly(vinyl alcohol) with sucrose, unless critical experimental detail omitted from their reports prevented duplication of their results. Their sweet product could have been the same as ours and sweet because of the presence of the unreacted sucrose.

EXPERIMENTAL SECTION

The poly(vinyl alcohol), 75% hydrolyzed, M_r 3000, was purchased from Scientific Polymer Products, Inc. Crystalline sucrose was purchased from Revere Sugar Co. The Me_2SO and 3A molecular sieves were purchased from Fisher Scientific Co. and Alfa Product Division of Ventron, respectively. The chemicals were used as purchased without further purification. HPLC analysis was done by using an Aminex HPX-42 300 mm × 7.8 mm carbohydrate analysis column operated at 85 °C with a refractive index detector. Integration of the detector responses was done by using a Bascom-Turner Model No. 4120 data center. ^1H NMR spectra were determined on a Bruker WM-250 spectrometer with Me_2SO-d_6 as the solvent and Me_4Si as an internal standard. ^{13}C NMR spectra were also determined on a Bruker WM-250 spectrometer with Me_2SO-d_6 as the solvent and internal reference.

Registry No. Sucrose, 57-50-1; poly(vinyl alcohol), 9002-89-5.

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Inhibitory Effect of Poly(2-methyl-5-vinylpyridine *N*-oxide) on Tobacco Mosaic Virus Multiplication in Tobacco Leaf Protoplasts

Tobacco mosaic virus (TMV) multiplication was markedly inhibited when tobacco mesophyll protoplasts infected with TMV in vitro were incubated in poly(2-methyl-5-vinylpyridine *N*-oxide) solution. No remarkable decrease in the infectivity of TMV occurred by mixing with the polymer.

In animal cells, it was suggested that an interaction occurred between monosilicic acid and poly(2-vinylpyridine *N*-oxide) and that certain of these oxides inhibited fibrogenesis normally associated with the presence of quartz in the lungs and the cytotoxic actions of silica in cultures of phagocytic cells (Holt et al., 1967; Holt, 1971). When administered to animals by injection, the polymer prevented the pathological effects normally associated with inhaled silica dust. In the rice seedling grown with nutrient solution containing the polymer, the particular epidermal cells of leaves took on the ability of accumulating great amounts of silica (Parry, 1975).

We have examined whether or not poly(vinylpyridine *N*-oxide) and related polymers possess additional biological

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activities, in particular antiviral activity against plant virus infection. For evaluation of antiviral activity, the tobacco mosaic virus (TMV)/ tobacco mesophyll protoplast system was chosen since use of protoplasts for TMV multiplication has been well established and inhibitory action can be studied in more detail in relation to the virus multiplication process (Takebe, 1977). The results of the reported research demonstrate that poly(2-methyl-5-vinylpyridine *N*-oxide) (PVNO) is a potent inhibitor against TMV multiplication in tobacco leaf protoplasts.

EXPERIMENTAL SECTION

Preparation of PVNO. The polymerization of 2-methyl-5-vinylpyridine was carried out in the presence of benzoyl peroxide at 15 °C for 12 days. The resulting product was converted into the *N*-oxide form by adding hydrogen peroxide. The average molecular weight was estimated to be 30 000, based on viscosity measurement.

Inoculation of Protoplasts. Protoplasts of palisade parenchyma cells were enzymatically isolated from the leaves of *Nicotiana tabacum* L. cv. Xanthi by the method reported previously (Sugimura and Ushiyama, 1975). Isolated protoplasts were inoculated by incubation with 1 μg/mL TMV and 1 μg/mL poly(L-ornithine) ($M_r \simeq 130\,000$). They were transferred aseptically into the protoplast incubation medium (Sugimura and Ushiyama, 1975) and cultured at 25 °C under continuous illumination of 2800 lx. The protoplasts were examined in a light microscope at the beginning and end of each experiment to check their appearance.

Fluorescent Antibody Staining. Antibody to TMV was prepared and conjugated with fluorescein isothiocyanate (FITC) following the method described previously (Sugimura and Ushiyama, 1975). The viral antigen produced in infected protoplasts reacted specifically with obtained antibody and could be detected as visible materials under a fluorescence microscope. Thus, the infected protoplasts were distinguished from noninfected ones by the presence of fluorescent materials in protoplasts. About 200 protoplasts were observed 24 h after inoculation to determine the infection percentage in each sample.

Infectivity Assay. To test if PVNO directly inactivate TMV, purified TMV (1 μg/mL) was mixed with an equal volume of PVNO solution at different concentrations. After incubation at room temperature, the mixture was inoculated on 10 half-leaves of *N. tabacum* L. cv. Xanthi-nc. The opposite control halves were inoculated with TMV treated similarly with water. Infectivity of protoplast extract was assayed on tobacco leaves (*N. tabacum* L. cv. Xanthi-nc) as described previously (Otsuki et al., 1972).

Each experiment was conducted 3 times at least. The typical results were represented in Tables I–III.

RESULTS AND DISCUSSION

There are two types of antiviral substances to protect plants from viral disease (Hirai, 1977). (1) The first is inhibitors against virus infection. They inactivate virus infectivity in vitro or inhibit the establishment of virus infection when mixed in inoculum. The inhibitory activity can be estimated from the number of local lesions in treated plants. (2) The second is inhibitors against virus multiplication. When they are satisfactorily spreaded into cells, the concentration of virus produced in cells decreases due to the blockage of virus multiplication. With regard to PVNO, experiments were designated to examine the type of inhibitor.

Effect of PVNO on TMV Infectivity. When the polymer was mixed in TMV inoculum in vitro for at least 6 h, no significant decrease in the number of local lesions was observed at 250–500 μg/mL compared with those produced by control inoculum (Table I). Although slight inhibition in local lesion formation occurred at the concentration of 1000 μg/mL, there was no correlation with incubation time. From these results, the polymer has no or little activity on TMV inactivation under present conditions.

Table I. Infectivity of TMV Inoculum Containing Different Concentrations of PVNO

PVNO concn, μg/mL	local lesion no., % of control, for incubation time				
	0.5 h	1 h	6 h	8 h	20 h
250	99	108	107	102	95
500	116	105	93	*a*	*a*
1000	78	79	*a*	65	70

[a] Not tested.

Table II. Inhibition of TMV Production in Protoplasts Which Were Treated by Adding PVNO to the Medium 30 min Postinoculation and Harvested 24 h Postinoculation

concn, μg/mL	infected protoplasts, % of protoplasts scored	infectivity, % of control
0	73	100
250	3	7
400	1	2

Table III. Inhibition of TMV Production in Protoplasts Which Were Incubated in the Medium without PVNO for 10 h Postinoculation and Then Treated with the Polymer for 18 h

concn, μg/mL	infectivity, % of control
100	72
500	53
1000	19

Inhibition of TMV Multiplication in Protoplasts. Isolated leaf protoplasts are a useful tool for detecting antiviral activity at cellular level, because protoplasts obviously provide better conditions than leaf tissue conventionally used, and complexities arising from distribution of chemicals applied in leaf tissue can be avoided (Takebe, 1977).

TMV growth in tobacco mesophyll protoplasts was examined by infectivity assay. The virus multiplication was evident 6 h postinfection and proceeded exponentially until 12 h postinfection. The rate of virus multiplication then decreased gradually but continued until 42 h postinfection. This sigmoid growth was essentially consistent with that of Takebe and Otsuki (1969). PVNO was added to protoplast incubation medium 30 min and 10 h after inoculation in vitro in order to investigate the effects in the initial stage and rapid growth stage of virus multiplication.

From 30 min postinoculation when the polymer at the concentrations of 250–400 μg/mL was added to the medium and for the remaining 23 h 30 min of the 24 h postinoculation period, during which the protoplasts were treated continuously, the protoplasts which were stained with FITC-labeled TMV antibody could be found with very low frequency (Table II). They had small size of fluorescent materials, whereas large crystal were observed in the infected protoplasts which were cultured without the polymer for 24 h. This reflects that the synthesis of viral protein was restricted by such treatment. The infectivity in treated protoplasts reduced by over 90% of that of nontreated protoplasts (Table II), indicating that virus production was inhibited completely at these dosages.

On the basis of the above-mentioned virus growth, the extensive production of progeny virus took place after a 10 h infection. Addition of the polymer 10 h postinoculation followed by incubation continuously for 18 h showed that a decrease in infectivity occurred substantially at relatively high concentrations (Table III). Therefore, TMV multiplication was directly inhibited during treatment in proportion to the different concentrations tested.

The light microscopic observation showed that the morphology in treated protoplasts was similar to that in nontreated ones at the end of the experiment, regardless of the concentrations applied and timing of treatment.

Poly(vinylpyridine *N*-oxide) has been demonstrated to counteract the pathogenic effects of mineral dust for animal cells, and it was suggested that the protective action may be attributed to stabilization of cell membrane (Gabor et al., 1975; Schulz and Beck, 1976; Hahon, 1976). From observation of the mode of action in animal cell systems, the polymer used in this experiment may have some effect on TMV incorporation by protoplasts, which is associated with endocytosis of plasma membrane (Takebe, 1977). The early process of infection including endocytotic uptake of virus particles would be affected by addition of the polymer to the medium 30 min postinoculation, resulting in the unsuccessful infection established in most of the protoplasts (Table II). In addition to inhibition of virus infection in this early stage, the polymer blocked virus multiplication during the rapid growth stage (Table III). Preliminary experiments showed that, in protoplasts treated with PVNO at 1000 μg/mL for 24 h, the uptake of [^{14}C]uridine and [^{14}C]leucine into RNA and protein was 48% and 43% less than that in nontreated protoplasts. TMV multiplication, therefore, is possibly inhibited due to the disturbance of RNA and protein synthesis in host protoplasts by the polymer.

In conclusion, it was demonstrated that PVNO reduced TMV multiplication in tobacco protoplasts without inducing visible phytotoxicity under a light microscope.

Registry No. PVNO, 27555-41-5.

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Insect Antifeeding Phenylacetylenes from Growing Buds of *Artemisia capillaris*

Two phenylacetylenes, 1-phenyl-2,4-pentadiyne and capillen, were isolated from the growing buds of *Artemisia capillaris*. These acetylenes revealed an antifeeding activity to the larva of the cabbage butterfly.

Many phenylacetylenes (Figure 1), capillon [**3** (Harada, 1956)], capillin [**4** (Imai, 1956)], capillen [**2** (Harada, 1957)], capillarin [**5** (Harada et al., 1960)], *o*-methoxycapillen [**6** (Miyazawa and Kameoka, 1975)], capillanol [**7** (Miyazawa and Kameoka, 1975)], norcapillen [**8** (Miyazawa and Kameoka, 1976)], and neocapillen [**9** (Miyazawa and Kameoka, 1976)], have been isolated from the essential oil of *Artemisia capillaris*. However, the viscous substance of the growing buds of the plant in June has not been investigated. It is supposed that the substance is effective in protecting the plant from dryness, because it has many long lateral roots to suck up water and the fine thread leaves to prevent an evaporation of water. But in Japan, June is a rainy season and it is the time of most growing for the plant. From this point, it is deduced that the antifeeding compounds to the insect may be contained in this viscous substance. In this report, 1-phenyl-2,4-pentadiyne (**1**) and capillen (**2**) given in Figure 1 were isolated as the main component from the growing buds. On phenylacetylenes, the strong antifungal activity of **4** was reported by Imai (1956), but very little has been known about the biological activity of the natural acetylenes. The activities of **1** and **2** to the insect were examined, and consequently two compounds revealed an antifeeding activity to the larva of cabbage butterfly.

Isolation of Phenylacetylenes. The growing buds 634 g of *A. capillaris* were collected in June on the bank of the Onga River in Fukuoka Prefecture. By the steam distillation and the extraction of the distillate with ether, the essential oil was obtained (1.48 g, 0.23% yield). The essential oil was chromatographed on a silica gel column (100–200 mesh, 55 g l = 55 cm, d = 1.8 cm). Hexane eluted terpene hydrocarbons (3% of the essential oil), hexane–ethyl acetate (4:1) eluted phenylacetylenes (83%), and hexane–ethyl acetate (1:1) eluted polar components (14%). 1-Phenyl-2,4-pentadiyne (**1**, 9% of the essential oil) was isolated from the first eluate of the phenylacetylene fraction: ^{1}H NMR ($CDCl_3$, Me_4Si internal standard) δ 2.00 (1 H, t, J = 1 Hz, C≡C–H), 3.69, (2 H, d, J = 1 Hz, Ph–CH_2–C≡C), 7.34 (5 H, s, ArH); MS (rel intensity) m/z

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