tone-dried tissue and becomes dialyzable only after hydrolysis.

Since tartronate facilitates oxalosuccinic decarboxylation, and since it is a constituent of animal tissues, it may reasonably be considered a significant factor in the citric acid cycle. A tissue deficiency of tartronate (a plant, but not an animal product, *5*), which might be caused by an insufficient intake because of dietary habits, loss in the preparation of food (volatility with steam, *6*, and also *7*, discarding of water extracts), faulty assimilation or retention, and so forth, could bring about a disturbance of the cycle. The resultant accumulation of pyruvate and acetate would lead to an abnormal amount of fat formation (*6*) and thus an excessive requirement for insulin (*8*). If this state were sufficiently prolonged, disorders of the endocrine control of carbohydrate metabolism might be induced. Hindrance in the formation of succinyl coenzyme-A, which makes possible the degradation of the fatty acids (*9*) and entrance into the cycle of acetoacetyl coenzyme-A (*10*) from both fatty acid and carbohydrate catabolism might also be caused by tartronate deficiency.

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Occurrence of Diffusible Auxin in Psilotum

Auxin first became known as a growth hormone of flowering plants, of the oat coleoptile in particular (*1*). Since its discovery in the Pteropsida, Seidl (*2*) has reported auxin from the Lycopsida, Wetmore and Morel (*3*) from the Sphenopsida, von Witsch (*4*) from Bryophyta, Nielsen (*5*) from Fungi, and van der Weij (*6*) from Algae. Thus auxin has been reported to occur in at least some member of every major group of plants, with the sole exception of Psilopsida. From the standpoint of comparative biochemistry and because the Psilopsida is an extremely primitive, rootless, leafless, and mostly extinct group of vascular plants, it is of interest to see whether living members of this group also produce auxin.

Stem tips 5 millimeters long were cut from aerial stems of *Psilotum nudum*, placed basal cut surface down on 1.5-percent agar blocks, and allowed to stand for 3 hours in normal diffuse room light. During the diffusion the agar blocks were placed on glass slides in a petri dish containing wet filter paper, to prevent desiccation of the agar. The standard *Avena* bioassay for auxin was used (*7*).

When thick, fast-growing stems were used for diffusion tests, substantial curvatures were obtained in the *Avena* bioassay—for example, two thick tips diffused onto 12 blocklets gave mean curvature per blocklet of 12.5°. When slower-growing stem tips were tested, no detectable auxin was found.

The absence of roots in Psilopsida is of particular interest to a student of auxin physiology, because a stimulating effect of added auxin on the number of roots or rhizoids developed has been observed in many plant groups, particularly in the Angiospermae. Although this rhizogenic activity has not been confirmed from as many major plant groups as has the occurrence of auxin, yet pure auxin has been shown to have a rhizogenic stimulation per se in Pteropsida by Thimann and Koepfli (*8*), in Lycopsida by Williams (*9*), in Bryophyta by Fitting (*10*), and in Algae by Jacobs (*11*). Accordingly, cuttings from both aerial and underground stems were treated with various concentrations of synthetic auxins (indole-acetic acid, naphthalene acetic acid, indole butyric acid), alone and in combination, with a medium containing substances known to limit the growth of excised angiospermous roots—that is, thiamine, nicotinic acid, sucrose, and mineral salts. Cuttings were checked macroscopically, under a binocular dissecting microscope, and finally under a compound microscope, after they had been paraffin imbedded, serially sectioned, and double stained. In no case were roots or root primordia detected.

Both the normal presence of auxin in *Psilotum* stems and the absence of root initiation in the auxin-treated cuttings support the interpretation that auxin is not the limiting factor for root initiation in *Psilotum*. However, since the reports for other plant groups show that auxin stimulates root formation only in groups where roots are normally formed, while it stimulates rhizoids in the groups which normally form rhizoids, it may well be that auxin does have a rhizogenic effect in *Psilotum*, but acts on the initiation of rhizoids rather than on the initiation of roots.

Attempts to induce rooting in *Psilotum* are continuing.

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Isomeric Substituted-Vinyl Phosphates as Systemic Insecticides

Substituted-vinyl phosphates have been frequently noted for their high insecticidal activity (*1, 2*). Their pharmacological action on mammals has also been investigated (*3*). One of these materials, designated as compound 2046 or 0,0-dimethyl 2-carbomethoxy-1-methylvinyl phosphate, is a very efficient short-residual systemic insecticide (*4*). This carbomethoxy material was studied along with its carbethoxy analog, 0,0-diethyl 2-carbethoxy-1-methylvinyl phosphate and its chloro analog, 0,0-diethyl 2-chlorovinyl phosphate (*3*).

Different preparations of the carbethoxy analog were found to vary greatly in systemic insecticidal activity, even though all were colorless liquids with identical sharp boiling points. Fractionation of several samples of the three analogs by partition chromatography on silica gel columns yielded two fractions from the carbomethoxy and carbethoxy materials and three components from the chlorovinyl phosphate. The first material eluted with organic solvents (α) was 5 to 100 times more toxic to insects than the more water-soluble fractions next eluted (β and γ).

Two geometric isomerides are possible with substituted-vinyl phosphates. *Trans* isomers are known to be generally more stable than *cis* isomers, because of the greater strain at the double bond in the *cis* materials. With the carbomethoxy, carbethoxy, and chlorovinyl phosphates, the α fractions were always the most active antiesterases, the least stable to

oxidative attack and hydrolysis of the phosphoric anhydride bonds, and the least water-soluble materials. An infrared absorption peak appeared between 11.00 and 11.10 μ with the α fractions but not with the β and γ fractions. Starting with either the α or β fraction from the carbethoxy analog, irradiation with ultraviolet light yielded a mixture of approximately 30-percent α and 70-percent β material as shown by infrared analysis. If the α and β fractions are isomeric, treatment of either isomer with ultraviolet light should produce a mixture of isomers with the more stable *trans* configuration predominating. All the experimental evidence is consistent with the hypothesis that the active α fraction is the *cis* isomer for each of the substituted-vinyl phosphates.

The properties of the *cis* and *trans* carbethoxy analog are shown in Table 1. Comparison of the vinyl phosphates as anticholinesterase agents showed that the isomeric dimethyl phosphates were combining in a different manner with the enzyme than the diethyl phosphates (the comparison was based on the slopes of the inhibition curves). The difference in anticholinesterase activity of the isomers increased as the size of the group hindered in rotation by the rigidity of the double bond increased. A consideration of isomeric yields from different synthesis methods explained the difference in biological activity of various preparations.

The biological distribution and fate of the *cis* isomer of compound 2046 was studied by means of radiotagged material (P^{32}). It was found that in contrast to other systemic insecticides currently available (*5*), compound 2046 does not require a preliminary "metabolic activation" within the plant (this finding is based on parallel radioactivity, bioassay, and anticholinesterase analyses) or within the insect or mammal (these findings are based on activation experiments with rat liver slices and cockroach intestines, *6*) to produce the effective toxicant. Accordingly, the anticholinesterase method was standardized to give a residue analysis procedure sensitive to 0.05 ppm. Field residual studies on a technical sample of compound 2046 (67 percent *cis*, 33 percent *trans*, Shell Development Co.) on 13 vegetable crops showed less than 0.1 ppm of residue 4 days after treatment of the soil or foliage with ¼ lb per acre of the insecticide. The substituted-vinyl phosphates had the shortest residual period of 20 organophosphates studied on carrots, potatoes, and cabbage. Immediately following treatment, the major loss of compound 2046 from plants was the result of volatilization, but about 12 hours later the decomposition within the plant became the significant factor. This enzymatic detoxification occurred throughout the plant and yielded a half-life in young greenhouse pea plants of about 20 hours for *cis* 2046 and about 48 hours for the *trans* isomer.

The distribution, detoxification, and esterase specificity of *cis* 2046 was investigated for the white rat and American cockroach. Detoxification appeared to take place in the plasma of the rat and in the gastric caeca and nerve cord of the roach. Compound 2046 did not appear to be selectively localized in the rat but accumulated in the mid- and hindgut of the roach. Esterase specificity tests *in vivo* and *in vitro* showed this *cis* carbomethoxy phosphate to be one of the most selective inhibitors of acetylcholinesterase that we have found in our studies to date.

Biological specificity of geometric isomerides has been frequently observed. To my knowledge, this is the first demonstration of *cis-trans* specificity with organophosphate antiesterases. A description of the experimental details of this study on vinyl-substituted phosphates is in preparation.

Table 1. Properties of *cis* and *trans* 0,0-diethyl 2-carbethoxy-1-methylvinyl phosphate

Item	*cis*	*trans*	Biological activity ratio *cis/trans**
Partition coefficients†			
CCl_4/H_2O	58	14	
n-Hexane/H_2O	5.5	1.1	
Chemical reactivity			
Hydrolytic half-life (—P≠O—C=C) (hr)‡	3.4	9.0	
Oxidative half-life (MnO_4^-) (min)§	8.9	15.4	
Biological activity			
Blood cholinesterase, *in vitro* pI_{50}‖	7.75	5.93	66
Rat LD_{50}, intraperitoneal (mg/kg)#	0.35	35	100
Fly LD_{50}, topical (mg/kg)**	2.0	59	30
Aphid LD_{50}, systemic (ppm)††	10.5	770	73
Method of synthesis			
Schrader (*1*) (% yield)	5–9	91–95	
Stiles (*7*) (% yield)	67	33	

* A ratio of the reciprocal of the amount of the *cis* isomer divided by the reciprocal of the amount of the *trans* isomer required to produce the same end-point.
† 2.0 mg organophosphate partitioned between 2.0 ml organic solvent and 2.0 ml distilled water at 28°C.
‡ Half-life in hours of vinyl phosphate bond at 28°C and *p*H 11.6 (0.1*M* Na_2CO_3) based on loss of partitioning properties into chloroform as diethyl phosphoric acid is formed on hydrolysis.
§ Half-life in minutes of 0.0004*M* $KMnO_4$ in acetone solution in the presence of 0.015*M* vinyl phosphate at 28°C, determined colorimetrically at 530 mμ.
‖ Negative logarithm of molar concentration effecting 50-percent inhibition of esterase activity of whole human blood on acetylcholine with 1-hour preincubation of phosphate and enzyme prior to addition of substrate.
Based on mortality of 250-g white rats 24 hours after intraperitoneal injection of organophosphate in isotonic saline.
** Based on mortality 24 hours after application of organophosphate in 1.3 μlit of acetone to the pronotum of 3-day-old adult male houseflies.
†† Insecticide needed for 50-percent mortality of pea aphids after 8 hours of feeding on pea plants pretreated for 24 hours by immersing the roots in the indicated insecticide solution.

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Atomic Charges in Monosubstituted Benzenes

Newly available information on charge distribution in molecules (*1, 2*), even though it is somewhat speculative, should certainly be of interest in the field of aromatic substitution where no completely satisfactory interpretive theory has yet been developed (*3*). This paper presents such data for a number of monosubstituted benzenes.

Table 1 lists the substituents in order of decreasing electron release to, or increasing withdrawal from, the ring. The partial charge on the atom of the substituent group that is directly attached to