dimethyl uracil (DMU) exhibited the same reversible type of photoreaction as uridylic acid, and its physical and chemical properties made it particularly suitable for the isolation of the product. We therefore irradiated 0.2*M* aqueous DMU with 254 mμ light (G.E. 15-w germicidal lamps), fractionated the product by partition between chloroform and water, and purified it by chromatography on alumina and by repeated recrystallization from mixtures of chloroform and *n*-hexane (*4*).

The isolated material is a white, crystalline solid, (mp 102°C) readily soluble in water and in ethanol, slightly soluble in chloroform and insoluble in *n*-hexane. Its aqueous solutions show only end-absorption in the ultraviolet (Fig. 1). On treatment either with acid (*p*H 1) or alkali (*p*H 10) it is rapidly and quantitatively reconverted to DMU, identifiable both by the detailed agreement of its ultraviolet absorption curve with that of known DMU (Fig. 1) and by its characteristic distribution coefficient of 3.47 between chloroform and water at 20°C.

Analysis of the isolated product of irradiation gave the results shown in Table 1. The mean value found for its molecular weight by the cryoscopic method was 158.3. This may be compared with an estimated equivalent weight of 157 with respect to reversion to DMU (mol. wt. 140).

The molecular weight, which differs by 18 from that of DMU, and the analytic data of Table 1 point to the conclusion that the product of irradiation arises by the addition of a molecule of water to DMU. The disappearance during irradiation of DMU of the characteristic reaction with bromine water (*5*), which is known to involve addition at the 5 : 6 double bond, together with the loss of the absorption band at 265 mμ, which

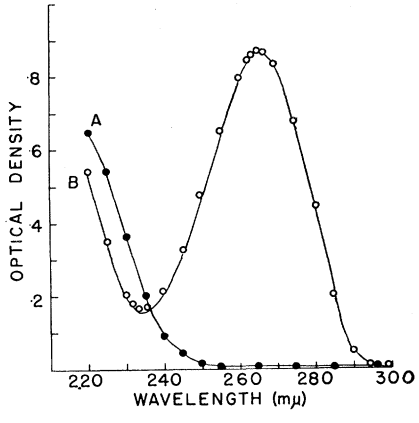

Fig. 1. Absorption curves. *A*, product of irradiation, $10^{-4}M$ in water. Points on *B*, product of irradiation, $10^{-4}M$, after heating at 100°C for 5 min at *p*H 1.0. Solid curve *B*, $10^{-4}M$ DMU in water.

Table 1. Analysis of the product of irradiation of DMU.

	Calculated for DMU $C_6H_8O_2N_2$ (%)	Calculated for $C_6H_{10}O_3N_2$* (%)	Observed (mean values)
Carbon	51.4	45.58	45.55
Hydrogen	5.71	6.33	6.38
Nitrogen	20.00	17.72	17.69

* That is, DMU plus a molecule of water.

also involves this double bond as part of the chromophore (*6*), suggests that the product is either the 6- or 5-hydroxy-1,3-dimethyl hydrouracil. The presence of an —OH group is further indicated by the observation of a strong band at 2.98 μ in the infrared absorption spectrum of the isolated product in alcohol-free chloroform, a band that is absent from similar solutions of DMU. This is also in keeping with the increased polarity of the product over DMU, as is evidenced by its much lower distribution coefficient (0.03) in a chloroform-water system.

Attempts to synthesize 6-hydroxy-1,3-dimethyl hydyrouracil have so far been unsuccessful. Reduction of 6-hydroxy-5, 5-dibromo-1,3-dimethyl hydrouracil catalytically with platinum and hydrogen, with continuous neutralization of the hydrobromic acid liberated, has yielded mainly DMU. However, it was found that the platinum catalyst itself also catalyzed the recovery of the isolated irradiation product to DMU, even in neutral solution, so that this result is in keeping with the postulated structure, although it does not prove it. Reduction methods involving the use of strong acid or alkali are ruled out because of the lability of the desired compound at extremes of *p*H.

That uracil and uridylic acid react similarly to DMU on irradiation is indicated by a number of parallels in their behavior. (i) The product of irradiation in each case can regenerate the original compound on treatment with either acid or alkali. [It should be noted that with respect to the alkaline conditions our finding differs from that of Sinsheimer (*3*).] (ii) Simultaneously with the loss in ultraviolet absorption during irradiation, all these compounds lose their ability to react rapidly with bromine water, indicating loss of the 5 : 6 double bond. (iii) The product of irradiation of uracil is more polar than uracil itself, as is indicated by its lower R_f on a paper chromatogram employing *n*-butanol saturated with water as mobile phase. The product in this case is easily located by the ultraviolet photographic method of Markham and Smith (7) after the developed chromatogram has been exposed to HCl vapor to regenerate the uracil.

If our conclusion regarding DMU is correct, the analogous product for uracil should be either 6- or 5-hydroxy hydrouracil. The 5-hydroxy compound is known (*8*) and does not have the required properties. The 6-hydroxy compound, on the other hand, does not appear to have been described. We believe that this omission is a result of the extreme facility with which it loses water to form uracil.

A. M. Moore
C. H. Thomson
Biology Division, Atomic Energy of Canada, Ltd., Chalk River, Ontario

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25 April 1955

Tartronate as a Possible Coenzyme of Oxalosuccinic Carboxylase

Oxalosuccinic carboxylase catalyzes the oxalosuccinate⟶α-ketoglutarate decarboxylation in the citric acid cycle and is known to be activated by Mn^{++} in acid solution (*1*). In the work reported here, tartronate (hydroxymalonate) was found to act as coenzyme of oxalosuccinic carboxylase at physiological *p*H. This was indicated by two methods: (i) titration of the carbonate formed by the decarboxylation and (ii) measurement of the optical activity of the reaction mixture.

Method A, titration of the carbonate. Veal heart was ground, extracted with acetone, and dried in a vacuum (*1*). Water (2 ml) added to the sample (0.5 g) was hard-frozen and melted; the sample was pressed and washed (4 × 1 ml water) with a 10-minute interval between washings. This extract was dialyzed 18 hours against cold water as a thin (2- to 3-mm) layer in a closed cellulose sack from which most of the air was expelled before it was tied off, and which was clamped between two sheets of 10-mesh,

silver-plated copper gauze. The volume of liquid in the sack remained quite constant. The volume was made 5 ml, the *p*H was made 7.2, and the CO_2 was expelled by applying low pressure (Hyvac, 16- by 150-mm Pyrex test tube) a number of times (the tube was vented as the foam rose). After each cycle, the *p*H was readjusted to 7.2, and low pressure was again applied, until a constant *p*H of 7.2 was obtained. The volume was maintained constant by the addition of water.

The substrate was prepared by shaking oxalosuccinic triethyl ester (*2*) (0.8 g) with HCl (2 ml, 37 percent) for 1 hour, after which it was allowed to stand at room temperature overnight. A portion of this solution (0.2 ml, 60 mg oxalosuccinic acid) was neutralized (ice bath) with 3*N* NaOH, tartronic acid (*3*) (1 or 5 mg, 1 ml, *p*H 7.2) or water (1 ml) was added, and the CO_2 was expelled. Extract (1 ml, equivalent to 0.1 g of the acetone-dried tissue) and, finally, Mn^{++} (1 drop, 0.20 mg $MnCl_2 \cdot 4\,H_2O$, 0.06 mg Mn^{++}) were added (total volume, 3 ml), and the time was noted. The CO_2 from the decarboxylation was expelled at frequent intervals for 15 minutes. The standardized HCl (a convenient concentration is 1.85 ml concentrated HCl in 100 ml) required during this interval measured the $NaHCO_3$ formed by the decarboxylation (Table 1). This titration method was tested, using the same procedure on a solution similarly prepared, except that concentrated HCl (0.2 ml) was substituted for the oxalosuccinic-HCl; $NaHCO_3$ (5 mg) was added to the solution that had been stabilized at *p*H 7.2, and the CO_2 was expelled as described in the preceding paragraph: found, 4.7 mg $NaHCO_3$.

Method B, measurement of optical activity. The rotation appearing in the reaction mixture because of the enzymatic decarboxylation of a portion of one optically active enantiomorph of oxalosuccinic acid to the inactive α-ketoglutaric acid was measured to compare the relative activity of the carboxylase with and without the additives. The moistened (with 4 ml water) acetone-extracted tissue (1 g) was hard-frozen, washed (4 × 2 ml), and dialyzed as it was for method A. The substrate was prepared by cooling (ice-salt) the oxalosuccinic-HCl solution (2 ml, 0.6 g substrate) in a 15-ml centrifuge tube, adding NaOH (0.7 g in 1 ml water) with stirring, centrifuging, and then pouring off the supernatant from the precipitated NaCl for further neutralization with 3*N* NaOH. Tartronic acid (5 or 50 mg) was added in small portions, with stirring, to the neutralized substrate, and the *p*H of the solution was kept at 8.0 to 8.5 to avoid a temporary drop to below 7.0. The total dialyzed extract was added, the *p*H was adjusted to 7.2, and finally Mn^{++} (10 drops, 0.6 mg Mn^{++}) was added. The reaction mixture was maintained at *p*H 7.2 to 7.3 without removal of the CO_2, and, at the end of 15 minutes at 25°C, powdered NaCl (2 g) and 3*N* NaOH (1 ml, producing a *p*H of 10.8) were added to slow further decarboxylation. The rotation was measured after centrifuging and filtering through asbestos (two Gooch crucibles for greater speed). The measurements (Table 2) were made with a Landolt precision polarimeter, an Osram lamp, and a 200-mm tube (capacity, 17 ml).

Table 1. Relative activity, as indicated by method A, of oxalosuccinic carboxylase, with and without tartronate and Mn^{++}, at a temperature of about 25°C and *p*H 7.2 to 7.3

Expt.	Extract added to oxalosuccinic acid (60 mg)	$NaHCO_3$	Percentage of theoretical
1	Boiled, dialyzed extract, 5 mg tartronic acid, 0.06 mg Mn^{++}	0.80 mg	
2	Undialyzed extract	2.06*	17
3	Dialyzed extract	0.05*	0.4
4	Dialyzed extract, 5 mg tartronic acid	0.48*	4
5	Dialyzed extract, 0.06 mg Mn^{++}	0.96*	8
6	Dialyzed extract, 1 mg tartronic acid, 0.06 mg Mn^{++}	1.60*	13
7	Dialyzed extract, 5 mg tartronic acid, 0.06 mg Mn^{++}	2.72*	22
8	Same as for No. 7, but without oxalosuccinic acid	0	
9	Theoretical value for complete reaction†	12.46*	

* Corrected for spontaneous decarboxylation, and so forth, as represented by the value of No. 1.
† Assuming the formation of 1 molecule of $NaHCO_3$ per molecule of one enantiomorph.

Table 2. Relative activity, as indicated by method B, of oxalosuccinic carboxylase, with and without tartronate and Mn^{++}, at 25°C and *p*H 7.2 to 7.3

Expt.	Extract	Rotation*
1	Undialyzed extract without oxalosuccinic acid†	−0.10 ± 0.02°
2	Dialyzed extract without oxalosuccinic acid†	−0.06 ± 0.02°
3	Undialyzed extract with 0.60 g oxalosuccinic acid	−0.19 ± 0.02°‡
4	Dialyzed extract with 0.60 g oxalosuccinic acid	−0.07 ± 0.01°‡
5	No. 4 with 50 mg tartronic acid	−0.05 ± 0.02°‡
6	No. 4 with 0.6 mg Mn^{++}	−0.06 ± 0.02°‡
7	No. 4 with 5 mg tartronic acid and 0.6 mg Mn^{++}	−0.14 ± 0.02°‡
8	No. 4 with 50 mg tartronic acid and 0.6 mg Mn^{++}	−0.17 ± 0.03°‡
9	No. 7 with 200 mg K_2HPO_4	−0.18 ± 0.03°‡
10	No. 8, except that the dialyzed extract was boiled	0 ± 0.01°‡

* The rotation corresponding to a known amount of one enantiomorph of this extremely unstable acid is not known, and hence percentage values cannot be given. For comparison: the rotation given by 0.21 g L-malic acid, equimolar with one enantiomorph of the oxalosuccinic acid used, alkalinized by NaOH to *p*H 10.8, was measured and found to be −0.32°.
† HCl (2 ml, 37 percent) was substituted for the oxalosuccinic-HCl solution (2 ml); otherwise the procedure was the same as for No. 8. This rotation did not diminish with time, as did that produced by enzymatic decarboxylation (for example, a No. 3 sample lost 0.06° in 3 hours at 25°C). Approximately ½ hour was required for filtration and measurement.
‡ Corrected for No. 1 or 2, respectively.

Table 3. Relative concentration of the components used in methods A and B

Component	Method A	Method B
Extract	33%	60%
Oxalosuccinic acid	2	3.5
Tartronic acid	0.033 or 0.165	0.03 or 0.3
Mn^{++}	0.002	0.0035

The desirability of increasing the angle of rotation required an increase in the concentration of the components in method B from that in method A (Table 3). This necessitated a partial removal of the NaCl, which is enzyme-inhibitory in too great concentration, since the limited volume of the polarimeter tube did not allow dilution, as was done in method A. In method A a similar increase was not advisable, because the time required for the removal of the CO_2 would have been unnecessarily prolonged.

Thus, although the results obtained by methods A and B do not entirely agree concerning the relative rates of decarboxylation, it may be seen that in both methods a marked increase was produced by tartronate at physiological *p*H when tartronate was used in conjunction with the activator Mn^{++}.

The amount of tartronic acid dialyzed in 18 hours from acetone-extracted veal heart was about 10 mg/g (*4*). Apparently, the tartronate is present in combined, acetone-insoluble form in the ace-

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.

LAURENCE G. WESSON*
Research Department, Forsyth Dental Infirmary for Children, Boston, Massachusetts

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* Present address: Massachusetts Institute of Technology, Cambridge, Mass.

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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.

WM. P. JACOBS
Biology Department, Princeton University, Princeton, New Jersey

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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