occur for greater durations, due to complicating or secondary reactions.

A series of experiments was carried out in which 10 g. of Preparation A were extracted with 100 cc. of solution, filtered through paper, and 10 cc. of the filtrates tested, each with 28.1 mg. urea, 5 cc. of added water, for 18 hours.

Extracting sol.:	Water, 1 *M* NaCl, 0.05 *M* $MgSO_4$, 0.1 *M* Na_2HPO_4, 0.1 *M* KH_2PO_4.
% Urea hydrolyzed:	10 6 8 7 1

Water extracted more urease apparently than the disodium hydrogen phosphate solution, although the solid preparation tested directly with the solutions showed greater activity with the latter than with the former. From the previous results it was to be expected that the monopotassium phosphate extract would show little activity, and that the other salt extracts less activity than the aqueous extract.

Conclusions.

Castor bean preparations hydrolyzed much less urea under comparable conditions than did similar soy bean preparations. This indicates that the urease of castor beans is less active than that of soy beans, or that less urease is present in castor beans than in soy beans.

The action of acids, bases, and salts on the hydrolysis of urea by castor bean urease was studied. Relations, similar to those observed by others with soy bean urease, were found.

[CONTRIBUTION FROM THE NEVADA AGRICULTURAL EXPERIMENT STATION.]

ENZYMES PRESENT IN ALFALFA. ALFALFA INVESTIGATION, V.

BY C. A. JACOBSON AND AUGUST HOLMES.

Received August 14, 1914.

The present work is a continuation of that begun by one of us in Prof. Hedin's laboratory in Uppsala, on the enzymes present in alfalfa seeds.[1]

The same general methods of work there used have been followed in the present investigation, which embraces the enzymes of the green as well as of the dried stems and leaves and of the fresh roots. In addition to the enzymes tested for and determined in the seeds, we have included three carbohydrases not infrequently encountered in juices and extracts of plants, namely, maltase, lactase, and pectinase. All standard solutions employed in the investigation were derived titrimetrically from normal hydrochloric acid, which had been standardized, gravimetrically, against silver.

All experiments were run in duplicate, of which only the mean will be recorded.

[1] THIS JOURNAL, **34**, 1730 (1912).

Enzymes in the Dried Stems and Leaves.

A water extract of air dried and finely ground alfalfa hay of the same quality as that used in Alfalfa Investigation I[1] and II[2] was prepared by adding 400 g. water to 100 g. of the ground alfalfa, and then heating the well stirred mixture for two hours at 37°, pressing out the extract in a small hand press and filtering through a soft filter paper. The reddish brown filtrate was clear and remained so for several hours, but if allowed to stand over night it became cloudy and a light precipitation was found at the bottom of the containing vessel. In every experiment the clear fresh extract was used.

Lipase.—No positive evidence of the existence of a lipase was found in alfalfa seeds and it was hardly to be expected that this enzyme should exist in the other parts of the plant, in measurable quantity. This supposition was also sustained by the following experiments:

Experiment 1.—(*a*) 5 cc. ethyl benzoate + 25 cc. extract + 50 cc. water + a few drops toluene, the mixture heated at 37°, after which 25 cc. of the liquid were withdrawn and titrated with 0.5 *N* NaOH, giving 3.04 cc. for neutralization.

(*b*) 5 cc. ethyl benzoate + 75 cc. water, treated the same as in (*a*). 25 cc. of this mixture were then withdrawn and titrated with 0.5 *N* NaOH giving 0.10 cc. for neutralization.

(*c*) 25 cc. extract + 55 cc. H_2O digested and titrated like (*b*), gave 2.38 cc. 0.5 *N* NaOH.

The sum of the titration values of (*b*) and (*c*) is a little less than (*a*), therefore a slight saponification of the ester had taken place.

Experiment 2.—(*a*) 5 cc. olive oil + 25 cc. extract + 50 cc. water were heated together as in Expt. 1 (*a*). 25 cc. of the resulting liquid were withdrawn and titrated with 0.5 *N* NaOH, giving 3.86 cc. for neutralization.

(*b*) 5 cc. olive oil + 75 cc. H_2O, digested and titrated as in (*a*), giving 0.12 cc. for neutralization.

(*b*) + (*c*) of Expt. 1 gave a lower value than (*a*), therefore, a slight saponification of olive oil. From these results we conclude that there is a suggestion of a lipase present in the alfalfa extract to about the same extent as was found in the seeds.

Amylase.—It was found that the alfalfa extract itself reduced an alkaline copper solution, so that this enzyme could only be determined by obtaining the difference of the reducing power with and without starch. No attempt was made to extract and isolate the ferment.

To determine whether or not amylase was present in the extract the following experiment was carried out:

Experiment 1.—(*a*) 5 cc. extract + 50 cc. H_2O + 10 cc. of a 1% starch solution, and the mixture heated at 60° for one hour, after which it was heated to boiling and filtered.

[1] THIS JOURNAL, **33**, 2048 (1911)

[2] *Ibid.*, **34**, 300 (1912).

(*b*) 5 cc. extract + 65 cc. H_2O, heated as in (*a*).

(*c*) 10 cc. starch solution + 55 cc. H_2O, also heated as in (*a*).

The reducing sugar in the filtrate was determined according to the method of Kendall,[1] the copper being titrated with 0.1 *N* sodium thiosulfate, which resulted as follows: (*a*) 26.27 cc.; (*b*) 23.86 cc.; and (*c*) none, showing that amylase is present in small amount.

The following experiment was carried out to determine the diastatic power[2] of the extract:

Experiment 2.—(*a*) 100 cc. of a 1% starch solution + 1 cc. extract.

(*b*) 100 cc. of a 1% starch solution + 2 cc. extract.

(*c*) 100 cc. of a 1% starch solution + 3 cc. extract.

(*d*) 100 cc. of a 1% starch solution + 5 cc. extract.

The above solutions were heated simultaneously, one hour at 61° and then the reducing sugar determined as above, giving for (*a*), 20.84 cc. sodium thiosulfate solution of such a strength that 1 cc. is equivalent to 8.13 milligrams of copper; (*b*), 48.54 cc.; (*c*), complete reduction; and (*d*), 19.43 cc.

Having obtained the value of the reduction by the pure extract in (*d*), we are able to find by difference the reduction due to the amylase. This gives (*a*) 16.95 cc., and (*b*) 40.76 cc. of thiosulfate solution, or 137.8 mg. of copper in (*a*) and 331.4 mg. copper in (*b*). These values of copper are equivalent to 120.4 mg. and 292.3 mg. of maltose, respectively, making the diastatic power 24 and 33 in the two cases, or a mean diastatic power of 28.5.

Coagulase.—Having found this enzyme in the alfalfa seed extract, it was of interest to learn if it also occurred in the stem and leaves of the dried plant. Fresh milk was taken and treated with one-tenth its volume of a 20% solution of calcium chloride and then treated with the extract as given in the following experiment:

Experiment 1.—(*a*) 10 cc. prepared milk + 1 cc. extract.

(*b*) 10 cc. prepared milk + 1 cc. water.

(*c*) 10 cc. prepared milk + 0.5 cc. 0.1 *N* acetic acid.

(*d*) 10 cc. prepared milk + 0.5 cc. 0.1 *N* *n*-butyric acid.

The two latter representing an excess of the amount of acidity developed in 1 cc. of extract during 24 hours' digestion. The above mixtures were then heated in the incubator at 37° for 24 hours and the process of change watched from time to time during that period. No marked change took place and at the end of 24 hours the milk could quite easily be poured, showing that there is no coagulase, on the order of rennin, in the alfalfa extract.

Emulsin.—This enzyme is determined by its power of splitting up the glucoside amygdaline into glucose, benzaldehyde and hydrocyanic acid

[1] THIS JOURNAL, 24, 317 (1904).

[2] If 1 cc. of amylase solution develops maltose equivalent to 500 mg. of copper when digested with 100 cc. of a 1% starch solution it is said to have a diastatic power of 100.

and the estimation of any one of the decomposition products. Whether or not the enzyme is a typical amygdalase containing hydroxynitrilase and yielding mandelonitrile glucoside as an intermediate product, we did not consider of sufficient importance for the present investigation. We at first attempted to determine the emulsin by titrating with iodine, the hydrocyanic acid generated, but were obliged to abandon this procedure for the reason that the extract itself absorbs considerable quantities of iodine. The determination of the glucose was therefore resorted to, and the results given in the following experiments:

Experiment 1.—(*a*) 25 cc. extract + 0.25 g. amygdaline + 25 cc. H_2O.

(*b*) 25 cc. extract + 25 cc. H_2O.

(*c*) 0.25 g. amygdaline + 25 cc. H_2O.

(*d*) 0.05 g. Merck's emulsin + 0.25 g. amygdaline + 25 cc. H_2O.

All four portions digested for 24 hours at 37° and the reducing power of 5 cc. of the supernatant liquid used for the determinations, which resulted as follows:

(*a*) 16.20 cc. sodium thiosulfate solution were required to titrate the reduced copper. (*b*), 10.60 cc.; and (*d*), 3.22 cc. In (*c*) there was no reduction.

These results are not quantitative in character, but suffice it to say, that emulsin is present in considerable quantity in the dried leaves and stems of alfalfa.

Invertase.—To determine the presence of invertase the following experiments were carried out:

Experiment 1.—(*a*) 2 cc. of 10% cane sugar solution + 5 cc. extract + 50 cc. H_2O.

(*b*) 5 cc. extract + 52 cc. H_2O.

(*c*) 2 cc. —10% cane sugar solution + 5 cc. 0.2 *N* HCl + 50 cc. H_2O.

(*d*) 2 cc. 10% cane sugar solution + 0.83 cc. 0.2 *N* HCl + 50 cc. H_2O.

(*e*) 5 cc. extract + 5 cc. 0.2 *N* HCl + 50 cc. H_2O.

The above solutions were heated for 24 hours in the thermostat at 37° and then brought to boiling and filtered. The reducing sugar in the filtrate was determined by Kendall's method. By a previous experiment it was found that 5 cc. of the extract heated under the same conditions developed an acidity equivalent to 0.83 cc. 0.2 *N* HCl and hence the reason for the (*d*) part of the experiment. In terms of cubic centimeters of standard thiosulfate solution, the reducing sugar obtained from the above solutions was found to be

(*a*)—57.51 cc.; (*b*)—25.14 cc.; (*c*)—35.21 cc.; (*d*)—23.00 cc.; (*e*)—24.18 cc.

It is seen that the value of (*a*) is larger than the sum of (*b*) and (*d*) which indicates the presence of invertase. From part (*c*) we see that even 5 cc. 0.2 *N* HCl does not produce as large an inversion of cane sugar as the same volume of alfalfa extract.

It was then decided to try the action of the enzyme in neutral solution and the following experiment carried out:

Experiment 2.—(*a*) 2 cc. 10% cane sugar solution + 5 cc. extract + 50 cc. H_2O + excess of powdered $CaCO_3$.

(*b*) 5 cc. extract + 52 cc. H_2O + excess of powdered $CaCO_3$.

The titration of the reduced copper in presence of an excess of potassium iodide resulted as follows: (*a*) 42.50 cc., and (*b*) 22.30 cc. sodium thiosulfate solution, showing that an active invertase is present in neutral solution of the alfalfa extract.

Peroxidase.—The tests for oxidases and peroxidases were carried out as follows:

Experiment 1.—(*a*) 1 g. pyrogallol + 1 cc. extract + 10 cc. of a 1% H_2O_2 solution diluted to 50 cc. with water.

(*b*) Same as (*a*), except no H_2O_2, also diluted to 50 cc. There was no change in (*a*) or (*b*) even after standing over night.

Experiment 2.—(*a*) A guaiacum emulsion, obtained by dissolving a small amount of gum guaiacum in alcohol and adding water until a cloudiness appeared, + 1 cc. extract + 3 cc. of a 3% solution of H_2O_2 and the mixture diluted to 10 cc. with water.

(*b*) Same as (*a*), without the H_2O_2, also diluted to 10 cc. A bluish precipitate developed in (*a*) after standing for a few minutes, which became heavier upon longer standing. There was no change in (*b*) until next morning when a very light, bluish precipitate had settled out.

These experiments go to show that no oxidase, but an extremely small amount of a peroxidase is present in the alfalfa extract. No test could be made with cresol for lack of this reagent.

Maltase.—To investigate whether maltase was present or not the following method was employed: The optical activity of a maltose solution was observed before and after digesting with a portion of the extract.

Experiment 1.—(*a*) Rotation of a 2% maltose solution, + 2.54°.

(*b*) Rotation of a mixture of 50 cc. of 2% maltose solution + 10 cc. extract before digestion, + 2.05°.

(*c*) Rotation of same mixture as (*b*), which had been digested 24 hours at 37°, + 1.84°.

The change in rotation is so slight that we would scarcely be justified in saying that maltase is present in the extract.

Lactase.—Experiment 1.—(*a*) The rotation of a 2% lactose solution was found to be + 1.03°.

(*b*) Rotation of a mixture of 50 cc. 2% lactose solution + 10 cc. extract before digestion, +1.01°.

(*c*) Rotation of the same mixture as (*b*) after digesting for 24 hours at 37°, +0.89°.

The small change in rotation would scarcely warrant the statement that a lactase is present.

Pectinase.—A good grade of pectin was prepared from dried pears by the following method: About 4 pounds of dried pears were passed through a meat chopper and soaked in enough water to cover the same, for about 14 hours at room temperature, and the resulting infusion separated by decantation and pressing and finally filtration. The clear amber colored filtrate was freed from calcium and albuminates by precipitating with oxalic acid and tannin and the solution again filtered. It was then concentrated on the water bath to about one-third of its original volume

and after cooling to the room temperature the pectin was precipitated by adding gradually, with constant stirring, three to four volumes of 95% alcohol. The thick, gelatinous precipitate was separated by filtering through an ordinary filter paper. As this was a very slow process, the filtration was arranged to proceed over night and the following day. The small, brown and horny looking material left in the filter papers, was then dissolved in a small quantity of water and the precipitation with 95% alcohol repeated. The process was repeated four times after which the gelatinous precipitate resulting was almost a pure white, but when dry it was dark, and its properties suggested dried glue, although the pectin was not quite so elastic.

An equal volume of extract was added to a 2% solution of this pear pectin. The mixture gelatinized almost instantaneously, showing the presence of a pectinase in considerable quantity in the water extract of dry alfalfa hay.

Protease.—In the paper[1] already referred to; the discovery of a peptolytic enzyme, on the order of vegetable erepsin, in alfalfa seed, is recorded; but this protease is not a peptonizing one like pepsin. It was, therefore, of considerable interest whether or not the presence of a similar enzyme could be confirmed in the extract of the dried plant.

Digestion experiments were set up with extracts on egg albumin, serum albumin, and fibrin, similar to those carried out on the seeds, the results in every case turning out negative.

The following experiments were then arranged to see if casein and Witte peptone could be digested with the extract. Solutions of these modified proteins were prepared exactly as specified in the paper just mentioned, with the exception that the casein solution required only 9.5 cc. *N* NaOH for neutrality to litmus instead of 10 cc.

Experiment 1.—(*a*) 25 cc. extract + 50 cc. casein solution + 1 cc. toluene.
(*b*) 25 cc. extract + 50 cc. water + 1 cc. toluene.
(*c*) 50 cc. casein solution + 25 cc. water + 1 cc. toluene.
(*d*) 25 cc. (boiled) extract + 50 cc. casein solution + 1 cc. toluene.
(*e*) 25 cc. (boiled) extract + 50 water + 1 cc. toluene.

The above mixtures were digested 24 hours at 37°, after which 25 cc. of the supernatant liquid of each were withdrawn, 50 cc. water and 10 cc. neutral formol phenolphthalein solution[2] added to each and the mixtures titrated with 0.2 *N* NaOH solution, resulting as follows: (*a*) 6.47 cc., (*b*) 2.72 cc., (*c*) 1.22 cc., (*d*) 3.30 cc., and (*e*) 2.35 cc.

Experiment 2.—(*a*) 25 cc. extract + 50 cc. 2% peptone solution + 1 cc. toluene.
(*b*) 25 cc. extract + 50 cc. water + 1 cc. toluene.
(*c*) 25 cc. water + 50 cc. peptone solution + 1 cc. toluene.

[1] THIS JOURNAL, **34**, 1734 (1912).

[2] Made by adding 1 cc. of a 1% alcoholic phenolphthalein solution to 50 cc. of a 40% formaldehyde solution and titrated to a faint pink with 0.5 *N* NaOH.

(*d*) 25 cc. (boiled) extract + 50 cc. peptone solution + 1 cc. toluene.
(*e*) 25 cc. (boiled) extract + 50 cc. water + 1 cc. toluene.

The mixtures digested and titrated as in Expt. 1, with the following results: (*a*) 5.82 cc., (*b*) 2.70 cc., (*c*) 1.80 cc., (*d*) 4.31 cc., and (*e*) 2.33 cc. These two experiments go to show that the extract of the dried alfalfa as well as that of the seeds, contains a protease capable of splitting up both casein and peptone.

Reaction Influence.—Experiments were arranged to determine whether or not the action of the protease would be influenced by an acid or alkaline solution, which was found to be the case with the seed protease.

Experiment 1.—(*a*) 25 cc. extract + 50 cc. H_2O + (toluene).
(*b*) 25 cc. extract + 50 cc. casein solution + (toluene).
(*c*) 25 cc. extract + 50 cc. casein solution + 0.5 g. sodium carbonate.
(*d*) 25 cc. extract + 50 cc. casein solution + Na_2CO_3 to make a 0.5% solution.
(*e*) 25 cc. extract + 50 cc. casein solution + Na_2CO_3 to make a 1.0% solution.
(*f*) 25 cc. extract + 50 cc. casein solution + Na_2CO_3 to make a 2% solution.
(*g*) 25 cc. water + 50 cc. extract.

The mixtures were digested for 24 hours at 37°, after which the calculated amount of *N* HCl was added to neutralize the Na_2CO_3 in each portion, and the liquids titrated as before, making due allowance for the volume change upon neutralization of the carbonate. (*a*) gave 1.56 cc. of 0.2 *N* NaOH, (*b*) 3.42 cc., (*c*) 4.97 cc., (*d*) 4.82 cc., (*e*) 5.29 cc., (*f*) 7.51 cc., and (*g*) 1.39 cc., showing that an alkaline medium favors the digestion of casein.

Experiment 2.—(*a*) 25 cc. extract + 50 cc. casein solution + HCl to 0.05% solution.
(*b*) 25 cc. extract + 50 cc. casein solution + HCl to make a 0.1% solution.
(*c*) 25 cc. extract + 50 cc. casein solution + HCl to make a 0.2% solution.
(*d*) 25 cc. extract + 50 cc. casein solution + HCl to make a 0.3% solution.
(*e*) 25 cc. extract + 50 cc. casein solution + HCl to make a 0.4% solution.

The digestions were carried out under the same conditions as those above and then the added HCl exactly neutralized with *N* NaOH and the formol titration carried out in the usual manner, giving for (*a*) 2.36 cc. 0.2 *N* NaOH, (*b*) 2.16 cc., (*c*) 2.11 cc., (*d*) 1.84 cc., and (*e*) 1.67 cc., showing that an acid solution acts unfavorably upon the digestion of casein, which was likewise the case with the seed protease.

Influence of Albumin in the Digestion of Casein and Peptone.

It was found that the presence of small amounts of egg albumin or serum in the digestion liquid of seed protease inhibited the action of the enzyme upon both casein and peptone, and therefore experiments were carried out to learn if the action of the plant protease is similarly inhibited.

Experiment 1.—(*a*) 25 cc. extract + 10 cc. H_2O + 50 cc. casein solution and the mixture titrated at once.

(*b*) 25 cc. extract + 10 cc. H_2O + 50 cc. casein solution digested at 37° for 24 hours.

(*c*) 25 cc. extract + 50 cc. casein solution + 10 cc. albumin solution (made by dissolving the white of one hen's egg in 1 liter of water) and the mixture titrated at once.

(*d*) 25 cc. extract + 50 cc. casein solution + 10 cc. albumin solution digested for 24 hours at 37°.

(*e*) 25 cc. extract + 10 cc. albumin solution mixed and left in contact for one-half hour at room temperature, then 50 cc. casein sol. added, and the mixture titrated at once.

(*f*) Same as (*e*) except after adding the casein the mixture was digested 24 hours at 37°.

(*g*) 25 cc. extract + 10 cc. albumin solution allowed to act together for 2 hours at room temperature, then 50 cc. casein solution added, and the mixture titrated at once.

(*h*) 25 cc. extract + 10 cc. albumin solution left 2 hours at room temperature, then 50 cc. casein solution added and mixture digested at 37° for 24 hours and titrated.

(*i*) 25 cc. extract + 10 cc. albumin solution left in contact at room temperature 21 hours, then 50 cc. casein solution added and the mixture titrated at once.

(*j*) Same as (*i*), except after adding casein the mixture was digested for 24 hours at 37° and then titrated.

Results of Titrations.—(*a*) 2.93 cc. 0.2 *N* NaOH, (*b*) 3.36 cc., (*c*) 2.93 cc., (*d*) 3.20 cc., (*e*) 2.94 cc., (*f*) 3.19 cc., (*g*) 2.91 cc., (*h*) 3.14 cc., (*i*) 2.97 cc., (*j*) 3.05 cc., showing a small but definite inhibitory action of the albumin upon the casein digestion, which is proportional to the time of action.

Experiment 2.—(*a*) 25 cc. extract + 10 cc. H_2O + 50 cc. 2% peptone solution titrated at once.

(*b*) 25 cc. extract + 10 cc. H_2O + 50 cc. peptone solution heated 24 hours at 37° and then titrated.

(*c*) 25 cc. extract + 10 cc. albumin solution + 50 cc. peptone, titrated at once.

(*d*) 25 cc. extract + 10 cc. albumin solution + 50 cc. peptone, heated 24 hours at 37° and then titrated.

(*e*) 25 cc. extract + 10 cc. albumin solution left at room temperature for one-half hour and then 50 cc. peptone added, and the mixture titrated at once.

(*f*) 25 cc. extract + 10 cc. albumin, left at room temperature for one-half hour, then 50 cc. peptone added, digested at 37° for 24 hours and titrated.

(*g*) Same as (*e*) except extract and albumin left for 2 hours at room temperature.

(*h*) Same as (*f*) except extract and albumin left for 2 hours at room temperature.

(*i*) Same as (*e*) except extract and albumin left together for 24 hours at room temperature before adding the peptone.

(*j*) Same as (*f*) except extract and albumin left together for 24 hours at room temperature.

The formal titrations resulted as follows: (*a*) 3.10 cc. 0.2 *N* NaOH, (*b*) 4.24 cc., (*c*) 3.09 cc., (*d*) 4.22 cc., (*e*) 3.12 cc., (*f*) 4.21 cc., (*g*) 3.05 cc., (*h*) 4.22 cc., (*i*) 3.20 cc., (*j*) 4.24 cc., showing that egg albumin does not perceptibly influence the digestion of peptone by alfalfa protease.

A series of experiments were then run to determine whether the time or the temperature plays the greater influence in the inhibition of plant protease on casein by egg albumin, but it would seem unnecessary to reproduce this mass of detail. The results all pointed to the conclusion that it is the time of action and not the temperature that determines the extent of inhibition.

Enzymes in the Fresh Alfalfa Plant (Stem and Leaves).

Young growing plants were collected and ground to a fine pulp in a

meat chopper, and after stirring it up with an equal volume of water, the mixture was allowed to stand for 2 hours at a temperature of 37°, after which the extract was pressed out and filtered. The filtrate was clear and of an amber brown color. Methods identical with the foregoing were employed for the determinations, except in the determination of the reducing sugars, in which cases the cuprous oxide was titrated by means of the ferric sulfate and permanganate method. This method, however, is not to be recommended for such work. The iodometric copper method of Kendall is to be preferred.

Lipase.—Experiment 1.—(*a*) 5 cc. ethyl benzoate + 25 cc. extract + 50 cc. H_2O.
(*b*) 25 cc. extract + 50 cc. H_2O.
(*c*) 5 cc. ethyl benzoate + 75 cc. H_2O.
(*d*) 5 cc. olive oil + 25 cc. extract + 50 cc. H_2O.
(*e*) 5 cc. olive oil + 75 cc. H_2O.

The titration values are as follows: (*a*) 3.29 cc., (*b*) 2.93 cc., (*c*) 0.11 cc., (*d*) 3.15 cc., (*e*) 0.05 cc., showing that there is a small, but appreciable, amount of lipase present.

Amylase.—Eperiment 1.—(*a*) 5 cc. extract + 10 cc. starch solution + 50 cc. H_2O.
(*b*) 5 cc. extract + 60 cc. H_2O.
(*c*) 10 cc. starch solution + 55 cc. water.

The number of cubic centimeters of permanganate required for the precipitated cuprous oxide were found to be: (*a*) 17.41 cc., (*b*) 15.09 cc., and (*c*) none. Therefore, a small amount of amylase is present.

Coagulase.—Experiment 1.—(*a*) 10 cc. prepared milk + 1 cc. extract.
(*b*) 10 cc. milk + 1 cc. water.
(*c*) 10 cc. milk + 0.37 cc. 0.1 *N* acetic acid.
(*d*) 10 cc. milk + 0.37 cc. 0.1 *N* butyric acid.

Results: (*a*), coagulated to a firm mass in seven hours, whereas the others remained practically unchanged, showing that a coagulase on the order of rennin is present.

Emulsin.—Experiment 1.—(*a*) 5 cc. extract + 5 cc. of a 5% amygdaline solution + 50 cc. H_2O.
(*b*) 5 cc. extract + 55 cc. H_2O.
(*c*) 5 cc. amygdaline solution + 55 cc. H_2O.
(*d*) 5 cc. amygdaline solution + 5 cc. of a 0.5% emulsin solution + 50 cc. H_2O.

The number of cubic centimeters of permanganate required for the cuprous oxide is as follows: (*a*) 28.53 cc., (*b*) 15.30 cc., (*c*) 1.10 cc., and (*d*) 39.34 cc. The results indicate that emulsin is present in the fresh plant to a marked degree.

Invertase.—Experiment 1.—(*a*) 2 cc. 10% cane sugar solution + 5 cc. extract + 50 cc. H_2O.
(*b*) 5 cc. extract + 52 cc. H_2O.
(*c*) 2 cc. sugar solution + 55 cc. H_2O.
(*d*) 2 cc. sugar + 2 cc. 0.2 *N* HCl + 50 cc. H_2O.
(*e*) 2 cc. sugar + 5 cc. extract + 50 cc. H_2O + (powdered) $CaCO_3$.
(*f*) 5 cc. extract + 52 cc. H_2O + (powdered) $CaCO_3$.

Results: (*b*), developed an acidity of 1.91 cc. 0.2 *N* NaOH. The titrations with permanganate were (*a*) 51.41 cc., (*b*) 15.10 cc., (*c*) no reduction, (*d*) 21.96 cc., (*e*) 21.45 cc., and (*f*) 13.18 cc., which indicate the presence of invertase in small amount.

Peroxidase.—Experiment 1.—(*a*) 1 g. pyrogallol + 10 cc. of a 1% solution of H_2O_2 + 1 cc. extract, diluted to 50 cc. with water.

(*b*) Same as (*a*) but without hydrogen peroxide.

(*c*) Guaiacum emulsion + 1 cc. extract + 3 cc. of a 3% H_2O_2 solution + water to 10 cc.

(*d*) Same as (*c*), but without the H_2O_2.

(*e*) α-Naphthol + 1 cc. extract + 1 cc. 3% H_2O_2 + H_2O to 10 cc.

(*f*) Same as (*e*) but without the H_2O_2.

Results: (*a*), immediately assumed a yellowish brown color, and in a few minutes a heavy brown precipitate appeared. In (*b*) no change, (*c*) developed the characteristic bluish precipitate at once, (*d*) no change for a long time, (*e*) a violet colored precipitate settled out in a few minutes, (*f*) remained unchanged. All these facts point to the presence of a peroxidase but not to an oxidase.

Pectinase.—The test for this enzyme with pectin from pears gave a more positive result than with the extract of the dry alfalfa.

Protease (Casein Digestion).—Experiment 1.—(*a*) 25 cc. extract + 50 cc. casein solution.

(*b*) 25 cc. water + 50 cc. casein solution.

(*c*) 25 cc. extract + 50 cc. water.

(*d*) 25 cc. extract (boiled) + 50 cc. casein solution.

After digestion the formol titration resulted in: (*a*) 6.80 cc. 0.2 *N* NaOH, (*b*) 1.31 cc., (*c*) 2.95 cc., (*d*) 4.18 cc., which brings out the fact that a stronger casein digesting ferment is present in the fresh than in the dried plants.

(*Peptone Digestion.*)—*Experiment 2.*—(*a*) 25 cc. extract + 50 cc. peptone solution.

(*b*) 25 cc. extract + 50 cc. H_2O.

(*c*) 25 cc. water + 50 cc. peptone solution.

(*d*) 25 cc. (boiled) extract + 50 cc. peptone solution.

Titrations were as follows: (*a*) 7.07 cc. 0.2 *N* NaOH, (*b*) 3.29 cc., (*c*) 1.78 cc., (*d*) 4.61 cc., showing that peptone is easily digested by the enzyme.

(*Egg Albumin Digestion.*)—*Experiment 3.*—(*a*) 25 cc. extract + 50 cc. albumin solution.

(*b*) 25 cc. extract + 50 cc. H_2O.

(*c*) 25 cc. H_2O + 50 cc. albumin solution.

The titrations resulted as follows: (*a*) 3.15 cc., (*b*) 3.17 cc., (*c*) 0.12 cc. These results show conclusively that the protease in question is unable to digest albumin, and therefore not a peptonizing enzyme.

Enzymes in the Fresh Alfalfa Root.

Fresh alfalfa roots were dug and ground up in a meat chopper, treated with water, digested for a short time at 37°, and the extract pressed out and filtered. The clear filtrate was used for the following experiments:

Lipase.—Experiment 1.—(*a*) 25 cc. extract + 2 cc. ethyl benzoate + 50 cc. H_2O.
(*b*) 25 cc. extract + 52 cc. H_2O.
(*c*) 2 cc. ethyl benzoate + 75 cc. H_2O.
(*d*) 25 cc. extract + 2 cc. olive oil + 50 cc. H_2O.
(*e*) 2 cc. olive oil + 75 cc. H_2O.

The titration with 0.2 *N* NaOH resulted in the following values: (*a*) 2.48 cc., (*b*) 2.24 cc., (*c*) 0.07 cc., (*d*) 2.26 cc., (*e*) 0.08 cc., indicating that lipases are absent.

Amylase.—Experiment 1.—(*a*) 5 cc. extract + 10 cc. starch solution + 50 cc. H_2O.
(*b*) 5 cc. extract + 60 cc. H_2O.
(*c*) 10 cc. starch + 55 cc. H_2O.

In these experiments the reduced copper was determined iodometrically and the titrations with sodium thiosulfate resulted as follows: (*a*) 7.20 cc., (*b*) 5.85 cc., (*c*) no reduction, showing that amylase is present although to a less extent than in the stems and leaves.

Coagulase.—Identical experiments with those under coagulase in the foregoing section were set up and after digesting the mixtures for 5 hours at 37°, a thickening of the milk in tube (*a*) was observed, whereas the milk in the other tubes remained unchanged. This points to the presence of a coagulase in the root extract also.

Emulsin.—Experiments were run on the root extract corresponding to (*a*), (*b*) and (*c*) in the foregoing section, and after heating, the thiosulfate titration resulted as follows: (*a*) 7.13 cc., (*b*) 5.80 cc., (*c*) no reduction. Emulsin is present in very small amount in the roots.

Invertase.—Experiment 1.—(*a*) 2 cc. cane sugar solution + 5 cc. extract + 50 cc. H_2O.
(*b*) 2 cc. sugar solution + 55 cc. H_2O.
(*c*) 2 cc. sugar solution + 2 cc. 0.2 *N* HCl + 50 cc. H_2O.
(*d*) 5 cc. extract + 52 cc. H_2O.
(*e*) 2 cc. sugar solution + 1 cc. 0.2 *N* HCl + 50 cc. H_2O.

The thiosulfate titrations resulted as follows: (*a*) 10.01 cc., (*b*) no reduction, (*c*) 22.76 cc., (*d*) 5.80 cc., (*e*) 12.40 cc. Invertase is thus present to a limited extent and nearly equivalent to 1 cc. of 0.2 *N* HCl.

Peroxidase.—The same experiments as (*a*), (*b*), (*c*) and (*d*), of the foregoing section were carried out using root extract. The results may be given as follows: (*a*), formed a heavy brown precipitate immediately; (*b*), remained unchanged; (*c*), developed a deep blue precipitate immediately; while (*d*), remained unchanged for several hours. These results indicate that peroxidases are present in the roots to a larger degree than in the stems and leaves, but no oxidases are present.

Pectinase.—The results of the pectinase tests on the root extract resulted positive.

Protease (Casein Digestion).—Experiment 1.—(*a*) 25 cc. extract + 50 cc. casein solution.
(*b*) 25 cc. H_2O + 50 cc. casein solution.

(*c*) 25 cc. extract + 50 cc. H_2O.
(*d*) 25 cc. (boiled) extract + 50 cc. casein solution.

Formol titrations: (*a*) 4.52 cc. 0.2 *N* NaOH, (*b*) 1.28 cc., (*c*) 2.23 cc., (*d*) 4.22 cc. These results indicate that no protease is present in the root extract.

(*Peptone Digestion.*)—*Experiment 2.*—(*a*) 25 cc. extract + 50 cc. peptone solution.
(*b*) 25 cc. H_2O + 50 cc. peptone solution.
(*c*) 25 cc. extract + 50 cc. H_2O.
(*d*) 25 cc. extract (boiled) + 50 cc. peptone solution.

The solutions were heated in the usual way and then titrated, giving the following values: (*a*) 5.33 cc., (*b*) 2.18 cc., (*c*) 2.23 cc., (*d*) 5.36 cc. The values for (*a*) and (*d*) are seen to be almost identical in this case also, and therefore no digestion of peptone.

(*Albumin Digestion.*)—*Experiment 3.*—(*a*) 25 cc. extract + 50 cc. albumin solution.
(*b*) 25 cc. H_2O + 50 cc. albumin solution.
(*c*) 25 cc. extract + 50 cc. H_2O.

After heating, the titrations resulted as follows: (*a*) 2.38 cc., (*b*) 0.10 cc., (*c*) 2.23 cc., showing that no digestion of albumin has taken place.

Conclusion.

The present investigation was designed to cover the more common enzymes encountered in vegetable juices and extracts, but no attempt has been made to isolate the different ones from their media, nor to study them in minute detail. Their presence in the water extracts of the dried and fresh alfalfa stems and leaves, as well as in the alfalfa roots has been determined qualitatively and in some instances with reference to the approximate amount. The following table sets forth the results obtained in the present investigation, together with those obtained on alfalfa seeds:

Enzyme.	Dried plants.	Fresh plants.	Fresh roots.	Seeds.
Lipase	—	+(*s*)	—	—
Amylase	+(*s*)	+(*s*)	+(*s*)	+
Coagulase	—	+	+	+
Emulsin	+(*l*)	+(*l*)	+(*s*)	+
Invertase	+	+(*s*)	+	—
Peroxidase	+(*s*)	+	+(*l*)	+
Maltase	—			
Lactase	—			
Pectinase	+(*l*)	+(*l*)	+	
Protease (peptonizing)	—	—	—	—
Protease (peptolytic)	+	+	—	+

The presence of the enzyme is denoted by + and the absence by —, the (*s*) after the sign denotes, in small amount; and the (*l*) in considerable, or large amount.

The diastatic power of the water extract of the dried plants was determined and found to be approximately 20.

A slight alkalinity favors the action of the protease on casein, whereas

an acid solution above that spontaneously produced in the extract retards or inhibits this action.

No digestion of egg albumin could be detected by the proteases in any part of the plant, but this substance invariably retards the action of the enzyme on casein. It was also found that the inhibiting influence of egg albumin on the casein digestion was proportional to the time of action and not to the temperature.

It is hoped that a more detailed investigation of individual members of the alfalfa enzymes can be undertaken in the near future.

RENO, NEVADA.

THE VOLATILE OIL OF CALYCANTHUS FLORIDUS.

BY EMERSON R. MILLER, G. W. TAYLOR AND M. H. ESKEW.

Received August 20, 1914.

The plant family Calycanthaceae comprises two genera and six or seven species, natives of North America and eastern Asia. The family is represented in the United States by four or five species of the genus *Calycanthus* L. (*Butneria Duhamel*), all but one of which are found in the south eastern states. The remaining one is native to California. All of these are aromatic shrubs from two to ten feet high, growing on hillsides and along streams. The flowers of the eastern species appear early in the spring, are brownish to brownish purple and exhale a delightful fragrance, compared by some to that of strawberry. Owing to their aromatic properties these plants are known commonly as Sweet Scented Shrub, Strawberry Shrub, Carolina Allspice, Florida Allspice, etc. The California species is known also by the names Spice Bush, Spice Wood, Wine Flower, etc. Plants of this latter species are a little larger than the others and have somewhat larger flowers of a livid red color.

Though this genus may not be rightly considered as being of much economic importance, two of its species, namely, *Calycanthus floridus* and *C. fertilis* (*glaucus*) are cultivated as ornamental shrubs both in this country and in Europe. Of further interest may be mentioned its reputed medicinal and poisonous properties. Thus, according to the National Standard Dispensatory, the root, leaves and bark of *C. fertilis* are much used as an antiperiodic. This undoubtedly means as a so-called domestic remedy. Kings' American Dispensatory states that the same species has been suggested as a stimulant, antiperiodic and an aromatic, while the root is said to be emetic. There are also reports of its having been poisonous to cattle. Other writers on medicinal plants include also the *C. floridus*. With the exception of the single phytochemical group of alkaloids practically nothing is known of the chemistry of this genus.

In 1888 the alkaloid calycanthine was discovered in the seeds of *Caly-*