II.—ENZYMES FROM THE SEEDS OF *CAESALPINIA BONDUCELLA.*

By Vinayak Narayan Patwardhan.

The seeds of *Cæsalpinia bonducella* commonly known in the vernacular as *Gajga* have a very thick and hard seed-coat which is not easily softened. The decorticated resting seed, containing 15 to 20 per cent. of oil and 2 to 3 per cent. sugar, has been found to contain the following enzymes :—protease, urease, amylase, peroxidase, catalase and oxidases in very small quantity ; it does not contain invertase.

An unexpected result in this examination was that lipase could not be detected. Experiments with seeds containing fat and also with fat-free samples all failed to reveal this enzyme. After germination, which took nearly five weeks, the seed was again tested for lipolytic activity with the same result ; this is difficult to explain in view of the high percentage of oil stored in the cotyledons.

When searching for enzymes in resting seeds the seed-powder was previously extracted with ether for 40 hours, but as lipase sometimes becomes inactivated during extraction the untreated material was also used. In many experiments extracts were made with 20 per cent. glycerol, but this also may act as an inhibiting factor. In special experiments for lipase, therefore, water alone was used and the experiments conducted with shaking, without shaking,with finely emulsified oil and oil temporarily emulsified by shaking. Acetic acid having an accelerating influence on lipase was tried, but without success, and similarly esterase could not be detected, as was shown in experiments with ethyl butyrate. Conclusive proofs of the absence of lipase were obtained by examining the extract, the residue after centrifuging and the top layer of the centrifugate, which according to Hoyer contains most active lipase. Experiments to obtain lipase by extracting the centrifugate with petrol failed to give any results.

The activity of the other enzymes increases during germination.

EXPERIMENTAL.

Extraction of the Enzyme.—Dry fatty or fat-free seed-powder, as the case required, was ground in a mortar with sufficient 20 per cent. glycerol or water to make a loose paste. The mixture after half an hour was centrifuged at 4000–5000 r. p. m. for 10–15 minutes. The supernatant liquid was made up to a known volume, and with addition of a little toluene could be preserved at zero.

Methods used.—The substrates used for protease, urease, amylase and invertase were casein, urea, soluble starch and cane-sugar respectively, the first three giving positive results while inversion was not found in the case of sucrose. All the experiments were conducted at ordinary room temperature except in one or two tests for lipase when the reaction mixtures were incubated for some time at 37°. Tests for peroxidase were made with solutions of α-napthol, *p*-phenylenediamine hydrochloride and hydrogen peroxide solutions (indophenol reaction). Catalase was traced by its action on neutral hydrogen peroxide.

A typical example of experiments for investigating the lipase-content is as follows:—The fat-free seed-powder (10 gms.) was extracted with 20 per cent. glycerol, centrifuged and the centrifugate made up to 100 c.c. Reaction mixtures prepared from 5 gms. of oil, 10 c.c. of N/10 acetic acid, 25 c.c. of water and 10 c.c. of (*a*) extract and (*b*) boiled extract, each with a few drops of toluene, were shaken continually in a machine. Portions of 10 c.c. were withdrawn at intervals, 5 c.c. of alcoholic potash added and the mixture titrated with standard sulphuric acid, which showed no change at reaction periods of 19 hrs. 45 mins. and 43 hrs. 15 mins.

The residue was tested for lipase by extracting 10 gms. fat-free seed-powder with water and preparing reaction mixtures from 33 c.c. of oil, 10 c.c. of N/10 acetic acid with 20 c.c. of (*a*) residue, (*b*) the top-layer of centrifugate and (*c*) the remainder of the centrifugate. A few drops of toluene were added to each and the bottles shaken by hand at frequent intervals; 5 c.c. portions were titrated as above and showed no change after 24 hours.

Experiments with fatty seeds extracted with water or with water containing 10–15 c.c. of N/10 acetic acid and conducted on lines similar to above, failed to prove the presence of lipase. In some of the latter experiments the oil was emulsified with gum arabic.

Department of Bio-Chemistry,
Indian Institute of Science,
Bangalore.

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