optimum at 9·0. Experiment on thermal stability of the enzyme shows that it can withstand a temperature of 50°C for 10 minutes with little change in enzyme activity (about 20% activity is lost). At 60°C only 37% of the activity is retained. The effect of metal ions on the enzyme activity shows that magnesium is absolutely required for the enzyme activity. No other metal ions can replace magnesium appreciably. Only Co^{2+} and Mn^{2+} have a little activating effects (7% and 12% respectively with respect to Mg^{2+} ion). In presence of 10 mM magnesium, other metal ions at 10 mM concentration exert inhibitory effects on the enzyme activity. The order of inhibition is $Ca^{2+} > Cd^{2+}, Zn^{2+} > Pb^{2+} > Hg^{2+} > Mn^{2+} > Ba^{2+} > Co^{2+} > Sr^{2+} > Cu^{2+}$. Fluoride severely inhibits the enzyme activity. Chromate, cyanide and azide have no inhibitory effects but tungstate and molybdate are somewhat inhibitory. Iodoacetate (10 mM) causes 40% inhibition whereas *p*-chloromercuribenzoate (10 mM) has no inhibitory effect.

For fixed concentration of tetrasodium pyrophosphate (1 mM) the enzyme activity was measured at variable concentration of magnesium chloride. The comparison of variation in the concentrations of free Mg^{2+}, free PP_i^{4-}, $MgPP_i^{2-}$ and Mg_2PP_i (determination of concentration of each ion species has been described in 'Method' portion) with the activity as a function of total $MgCl_2$ (Fig. 1) reveals that $MgPP_i^{2-}$, and not free PP_i^{4-}, is the true substrate of the enzyme in presence of free Mg^{2+} ion. Mg_2PP_i may also possibly act as a substrate as evident from Fig. 1. Effect of pH on Mg^{2+} requirement shows that the optimum pH shifts towards lower pH values with larger excess of Mg^{2+} as shown in Fig. 2.

From the findings of the present study it appears that *Cynodon dactylon* leaves give rise to a rich source of alkaline inorganic pyrophosphatase and this enzyme is very much similar to maize leaf inorganic pyrophosphatase[5].

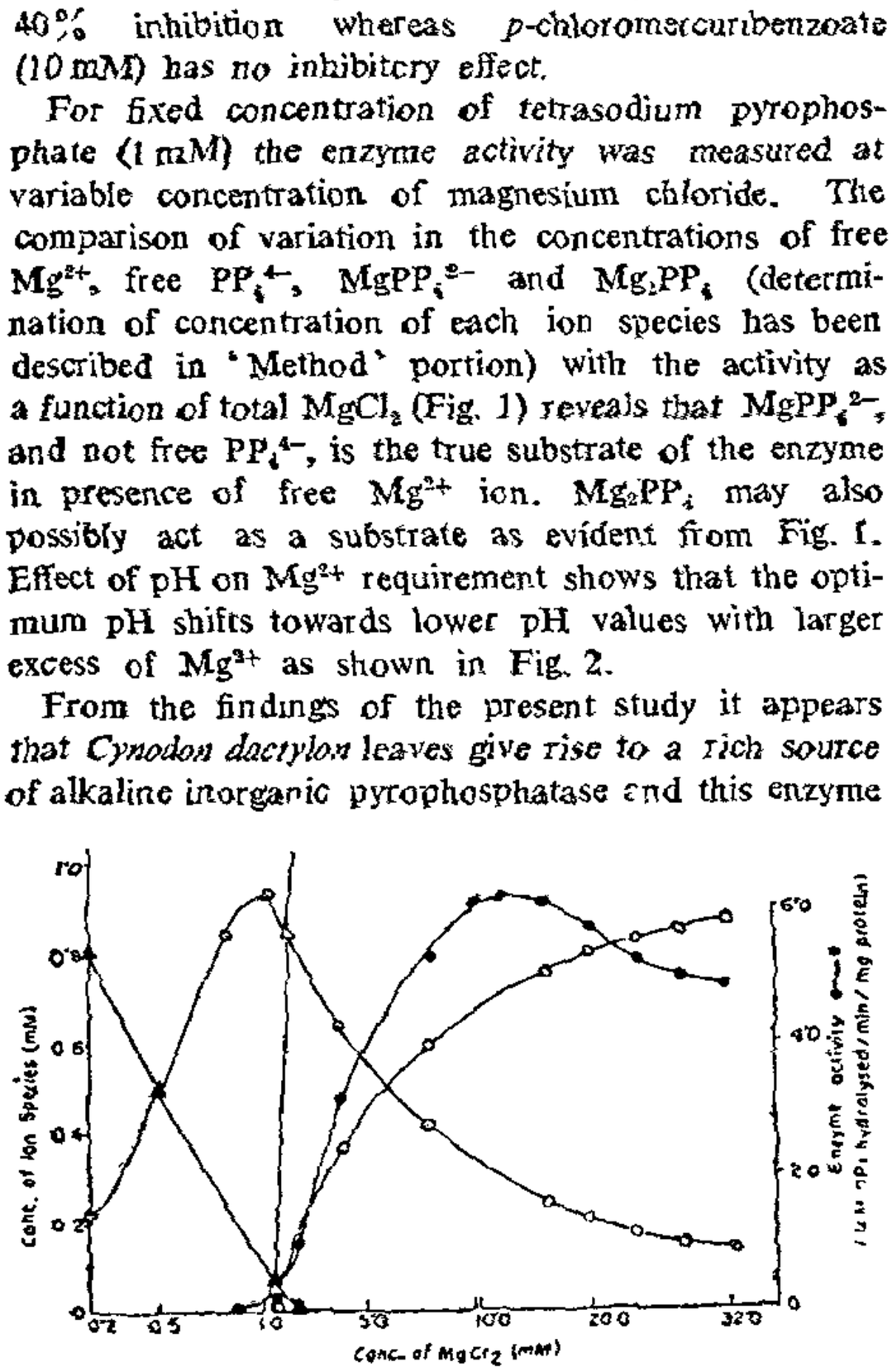

FIG. 1. Comparison of the variation in the concentration of free PP_i^{4-} (▲——▲), free Mg^{2+} (△——△), $MgPP_i^{2-}$ (⊙– –⊙), Mg_2PP_i (○——○) in *Cynodon dactylon* inorganic pyrophosphatase catalysed reaction mixture with the activity (●——●) as a function of total $MgCl_2$.

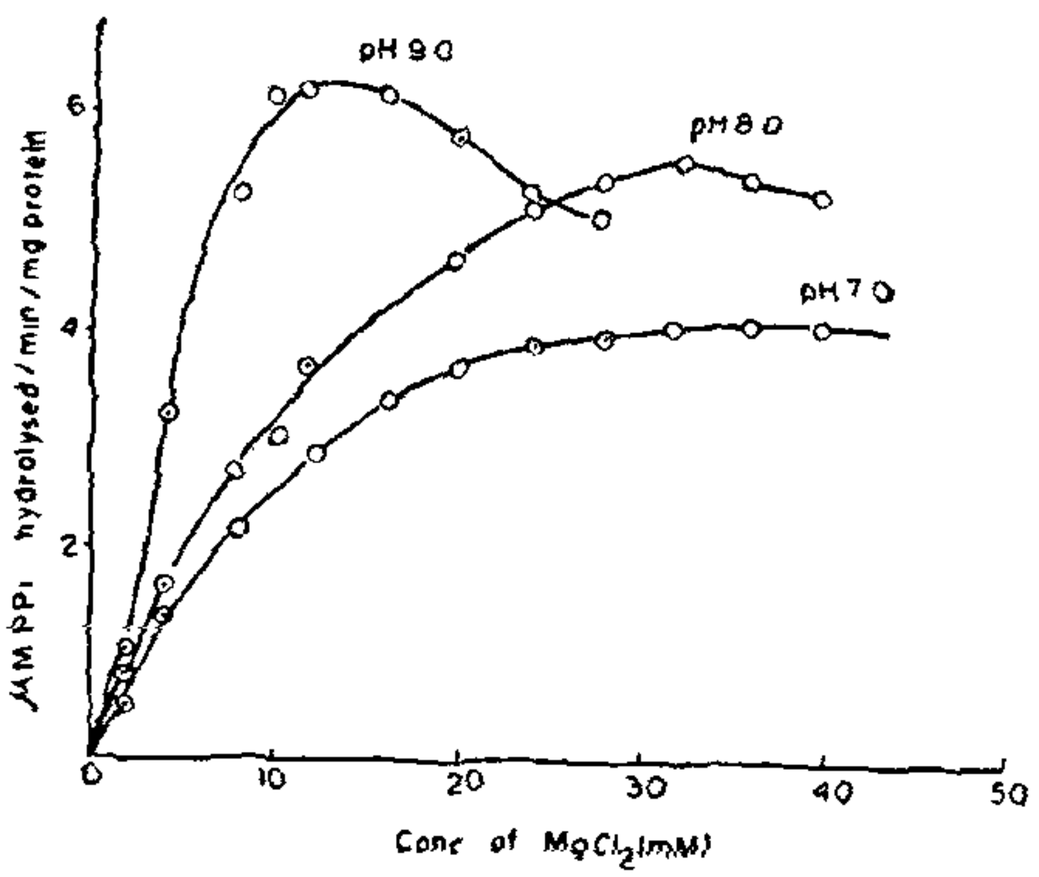

FIG. 2. Effect of the concentration of magnesium chloride on the activity of *Cynodon dactylon* inorganic pyrophosphatase at different levels of pH.

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t-BUTANOLYSIS OF LECANORIC ACID

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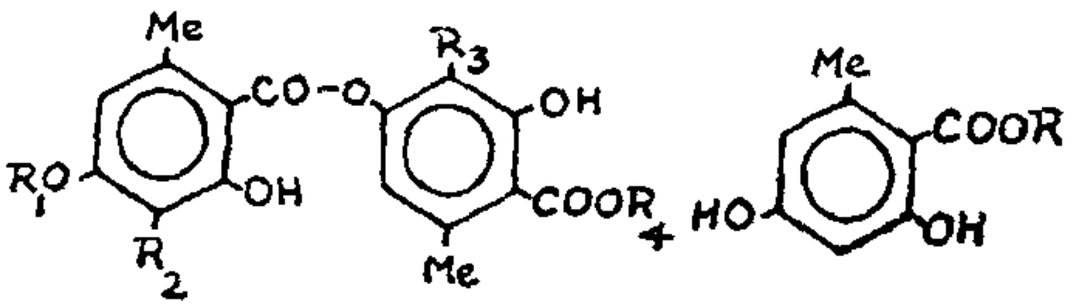
DEPSIDES, a class of naturally occurring compounds, almost unique to lichens, are dimeric esters of variously substituted orsellinic acids and their analogues. The cleavage of the depside link is frequently effected by alcoholysis with methanol in the presence of sodium hydroxide[1], whereby the left side acid half

of the depside is obtained as the methyl ester. This method leads to some ambiguity when applied to methyl esters of depsides which are also of common occurrence in lichens.

Recently Bachelor *et al.*[2] reported the alcoholysis of depsides using *t*-butanol which seems to circumvent the disadvantages in other methods and accomplished the cleavage of two *β*-orcinol depsides, atranorin (I) and barbatic acid (II). They have concluded that the *t*-butanolysis brings about the cleavage of those depsides possessing only the *β*-orcinol skeleton.

Since there is no report of similar study on any simpler depsides, we have studied the *t*-butanolysis of lecanoric acid (III), a typical orcinol depside and the results are recorded here.



I–III IV, V

I, $R_1 = H$; $R_2 = CHO$; $R_3 = R_4 = Me$
II, $R_1 = R_2 = R_3 = Me$; $R_4 = H$
III, $R_1 = R_2 = R_3 = R_4 = H$
IV, $R = CMe_3$
V, $R = Me$

Lecanoric acid (III), isolated from the lichen, *Parmelia tinctorum*[3], was heated under reflux for 48 hr with *t*-butanol. The solvent was removed *in vacuo*, the residue was dissolved in ether and extracted with sodium bicarbonate solution. The crude solid, obtained on evaporation of the ether layer, when purified by passing through a silica gel column built in benzene, afforded a colourless crystalline compound, m.p. 155–56° (EtOH). It gave a violet colour with alcoholic ferric chloride and when refluxed with 10% methanolic NaOH followed by acidification, it yielded orsellinic acid (2,4-dihydroxy-6-methylbenzoic acid). It exhibited the following spectral characteristics:

UV : λ_{max}^{EtOH} 218, 266, 305 nm.

IR : ν_{max}^{Nujol} 3300, 2840, 1640, 1610, 1570, 1518, 1440, 1380, 1280, 1210, 990, 830, 750 cm^{-1}

PMR signals (DMSO d_6, 90 MHz, δ values, ppm): 1·65 (*s*, 9H, -COOC(C<u>H</u>$_3$)$_3$; 2·50 (*s*, 3H; Ar-C<u>H</u>$_3$); 6·25 (*s*, 2H, Ar-<u>H</u>); 9·50 (*s*, 1H, -O<u>H</u>) and 11·90 (*s*, 1H, chelated -O<u>H</u>).

On acetylation (Ac_2O + Py, room temp., 24 hr), the compound yielded a crystalline acetate, m.p. 92–93° (EtOH), exhibiting the following PMR signals ($CDCl_3$, 90 MHz, δ values, ppm): 1·60 [*s*, 9H, -COOC(C<u>H</u>$_3$)$_3$]; 2·30 (*s*, 6H, 2 -OCOC<u>H</u>$_3$); 2·45 (*s*, 3H, Ar-C<u>H</u>$_3$) and 6·85 (*s*, 2H, Ar-<u>H</u>). Based on the above data, the compound has been characterized as *t*-butyl orsellinate (*t*-butyl 2,4-dihydroxy-6-methylbenzoate) (IV). From the bicarbonate solution mentioned above, orsellinic acid could be isolated by acidification with ice-cold HCl which was identified by mmp and co-TLC with an authentic sample.

From the present work it can be concluded that orcinol depsides also, like *β*-orcinol depsides, undergo cleavage with *t*-butanol. It is interesting to note that the PMR spectrum of *t*-butyl orsellinate exhibits only a singlet due to the two aromatic protons which are *meta* to each other instead of the expected *m*-coupled doublets. However, it is observed that in the PMR spectrum of methyl orsellinate (V) also, only a singlet is observed for the two aromatic protons instead of the *m*-coupled doublets. [($CDCl_3$, 90 MHz, δ values, ppm) : 2·50 (*s*, 3H, Ar-C<u>H</u>$_3$); 3·90 (*s*, 3H, -COOC<u>H</u>$_3$), 6·30 (*s*, 2H, Ar-<u>H</u>), 9·30 (*s*, 1H, -O<u>H</u>) and 11·70 (*s*, 1H, chelated -O<u>H</u>)]. The precise reason for this does not appear to be clear and work is in progress to examine this aspect in detail.

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TWO NEW SPECIES OF *PSEUDOCERCOSPORA* FROM INDIA

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In January, 1978 two leaf spotting Hyphomycetes were collected on *Stephania discolar* Spr. and *Murraya koenigii* Spreng. respectively from North Gorakhpur Forest Division (U.P.). The present communication describes these collections as *Pseudocercospora menispermacearum* Kumar *et* Kamal sp. nov. and *P. murrayicola* Kumar *et* Kamal sp. nov. respectively.

Pseudocercospora menispermacearum Kumar *et* Kamal sp. nov.

Leaf spots indefinite, olivaceous brown, irregular, hypophyllous; colonies hypophyllous, effuse; myce-