rected for Lorentz and polarisation effects. The structure was solved by MULTAN[8] and refined isotropically (non-hydrogen atoms only) to an $R$ value = 0.10. Further refinement is in progress. The packing of the molecule down the $a$-axis is shown in figure 2.

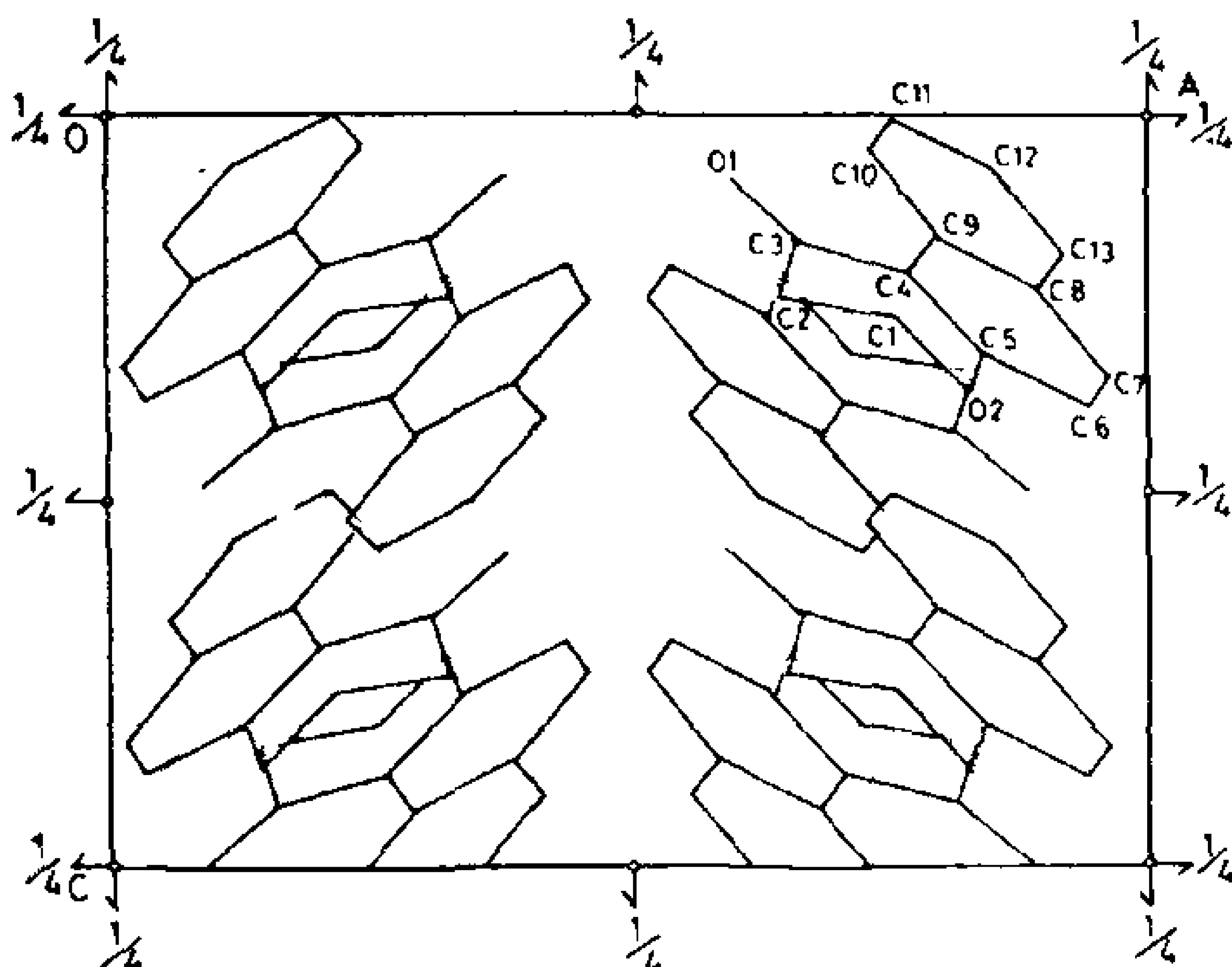


**Figure 2.** Packing of the molecules in the unit cell down $a$-axis.

The tetrahydropyran ring in the structure exists ir half-chair conformation[9]. The two benzene rings are planar. The molecule has the normal bond distances and bond angles as found in other similar structures.

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## DEVELOPMENTAL PATTERNS OF α-AMYLASE AND LEUCINE-AMINO-PEPTIDASE ACTIVITY DURING SHOOT DIFFERENTIATION IN SUGARCANE (*SACCHARUM OFFICINARUM* Cv Co-740) CALLUS.

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DIFFERENTIATION of organised structures from an explant involves a shift in metabolism which leads to changes in content and spectrum of both structural and enzymic proteins. As amylolysis and proteolysis represent the major hydrolytic enzyme systems supplying assimilable material to the developing tissues, the developmental patterns of two enzymes, one from each class, namely α-amylase and leucine aminopeptidase (LAP), respectively, were investigated and compared in shoot forming and non-shoot forming callus cultures of sugarcane.

Callus cultures from young leaves of sugarcane (*Saccharum officinarum* Cv Co-740) were initiated and maintained in the dark at 26 ± 2°C on a basal Murashige and Skoog mineral salt medium (MS)[1] containing organic constituents as mentioned below for Medium A. Callus grown on this medium was transferred to 100 ml Erlenmayer flasks containing 20 ml of the two experimental media listed below (1000 mg inoculum/flask).

| | |
|---|---|
| *Medium A* (Non shoot forming) | : MS + 1.0 mg/l thiamine hydrochloride + 120 mg/l myo-inositol + 10% coconut milk (v/v) + 2% Sucrose + 3 mg/l 2,4-dichlorophenoxyacetic acid (2,4-D). |
| *Medium B* (Shoot forming) | : MS + 1.0 mg/l thiamine hydrochloride + 120 mg/l myo-inositol + 10% coconut milk (v/v) + 2% sucrose. |

The cultures were incubated at 26 ± 2°C in light (ca 3000 lux) for a period of ten days.

Before homogenisation callus was sponged dry under folds of filter paper. A known amount of callus was homogenized with 0.1 M sodium phosphate buffer, pH 7.0 (2 ml buffer/g tissue) at 4°C. The contents were centrifuged at 10,000 X g for 20 minutes and the supernatant which served as the crude enzyme preparation was collected.

Protein contents were determined according to the method of Lowry *et al*[2].

α-amylase activity was measured by using 3,5 dinitrosalicylic acid reagent as reported[3].

Leucine aminopeptidase activity was assayed by using L-leucyl β-naphthyl amide hydrochloride as substrate[4].

These experiments were repeated three times and the enzyme activities found to be reproducible.

Sugarcane callus remained undifferentiated on Medium A during the culture period of 10 days. Medium B, however, induced visible differentiation of shoots on day 8 of culture. Figure 1 shows the developmental patterns of α-amylase activity in shoot forming (SF) and non-shoot forming (NSF) sugarcane callus. In both SF and NSF tissues, the activity initially decreased sharply till day 2 of the culture period. After this, the activity in NSF tissue increased sharply till day 3, followed by a sharp decline till day 4. In contrast to this, there was no significant change in activity in SF callus during the corresponding period. From day 4–6, the activity level in both SF and NSF tissues remained unchanged, though the SF tissue exhibited a slightly higher level of activity. From day 6 onwards till the end of the culture period, the activity in NSF tissue increased continuously. In contrast the activity in SF tissue after a small increase till day 7 reverted back to its original level till the end of the culture period. Summarising, the main differences in the developmental pattern of this enzyme between the two tissues (SF and NSF) were clearly evident between days 2 to 4 and day 7–10. Thus, in NSF tissue the activity increased sharply between day 2 and 4 showing a peak on day 3 while activity was at its minimum in SF tissue during the corresponding period. Further, an increasing trend in activity was observed in NSF tissue after day 7. The activity in SF tissue, in contrast, showed a decreasing trend during the corresponding period. The data suggest better mobilization of starch in NSF than in SF callus, during the whole culture period. A comparatively lower amylase activity in SF tissue, (and hence an accumulation of starch) suggest its role in differentiation, as reported earlier[5-7].

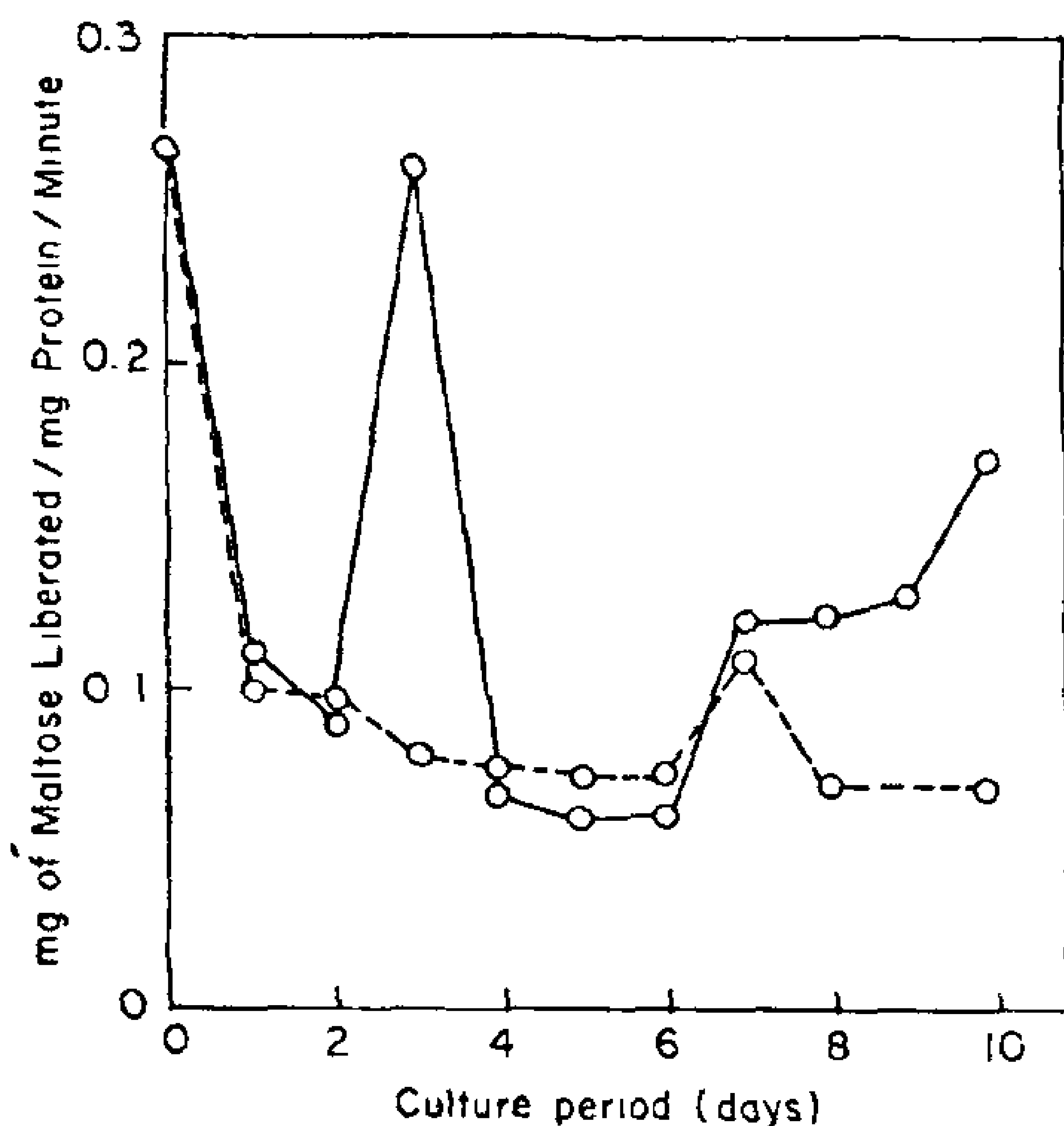


**Figure 1.** Developmental patterns of α-amylase activity under shoot forming (o----o) and non-shoot forming (o—o) conditions. Activity is expressed as mg of maltose liberated per min per mg protein.

Figure 2 shows the developmental patterns of leucine aminopeptidase (LAP) in SF and NSF sugarcane callus. In both SF and NSF callus the activity initially increased sharply till day 2 followed by a rapid decrease till day 3. From day 4–6, the activity in SF tissue remained constant. In contrast to this, activity in NSF tissue increased almost two folds during the corresponding period. In both SF and NSF tissue the activity decreased between day 6–7 and 6–8, respectively. From day 8–10 the NSF tissue showed more or less a constant level of LAP activity while the activity in SF tissue increased sharply during the corresponding period. Thus, initially both types of tissue showed identical patterns (day 0–3). From day 4 onwards distinct differences in the pattern of this enzyme in SF and NSF tissue were observed (days 4–6 and 8–10). From days 4–6 NSF tissue showed increased level of activity which was more or less constant in SF tissue. The reverse was observed between day 8–10 where SF tissue showed an increased level of activity unlike NSF tissue. The data suggest high proteolytic activity during initial period of growth in culture (day 0–2) in both SF and NSF tissues. From day 3 the proteolytic events are at a moderate rate in SF tissue while this is high in NSF tissue during the corresponding period.

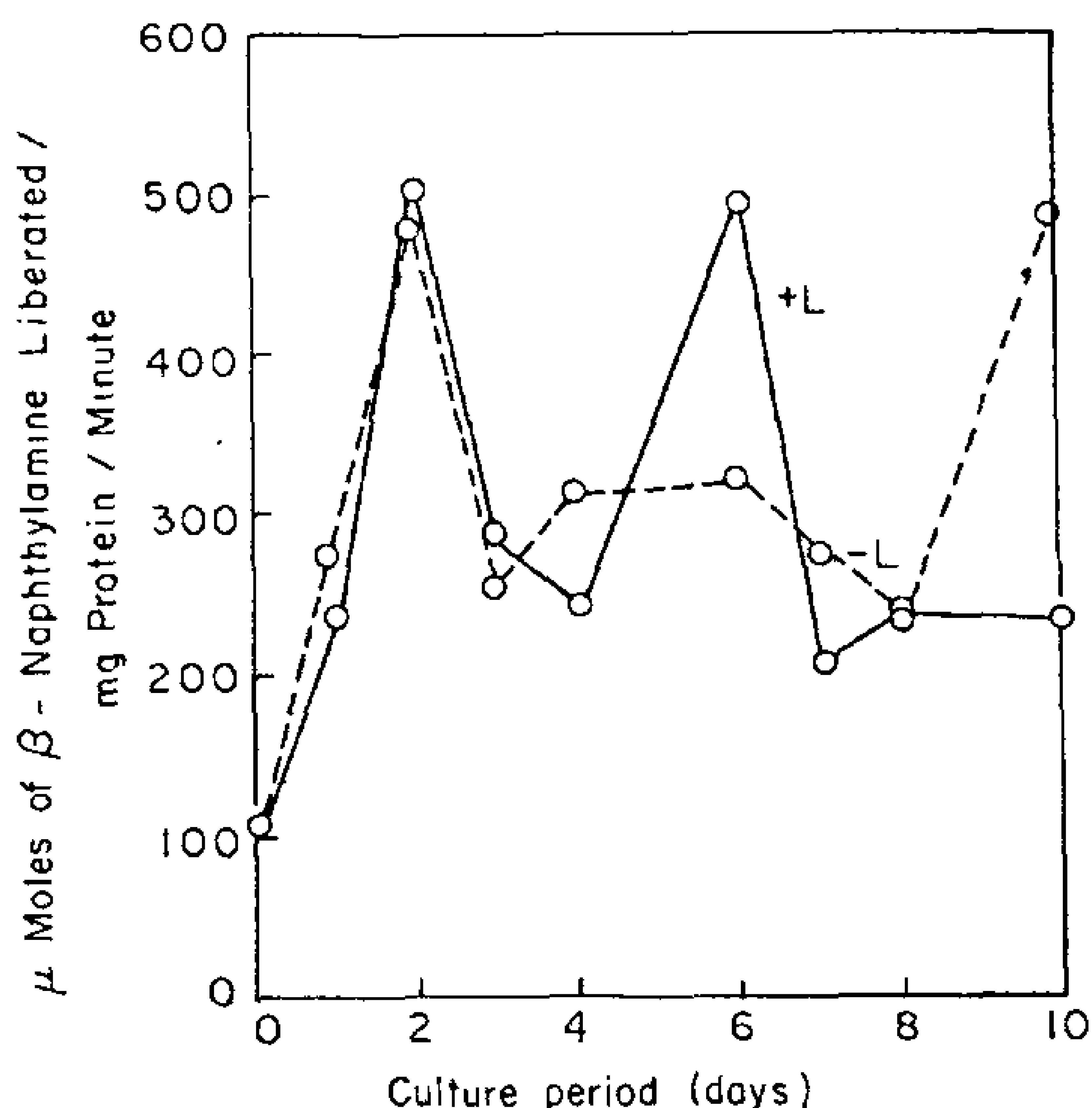


**Figure 2.** Changes in leucine aminopeptidase activity during growth of sugarcane callus under shoot forming (o----o) and non-shoot forming (o—o) conditions. Activity is expressed as $\mu$ moles of $\beta$-naphthylamine liberated per min per mg protein.

The increase in activity of LAP after day 8 (the day of visual manifestation of shoots) in SF tissue suggest that LAP has some major role in later stages of differentiation. Higher levels of LAP activity during initial stages have been reported[8] in bush bean cotyledon suspension cultures grown in presence of 2,4-D and kinetin as compared to the corresponding cultures grown without growth regulators. They have also reported higher LAP activity, in absence of growth regulator, only during later stages of growth. A sharp peak in activity in NSF tissue on days 2, and 6 and in SF tissue on day 10 may be attributed to the appearance of some new isoenzyme forms during growth, as suggested for LAP activity profile in bush bean cotyledon suspension cultures[8]. Efforts in this direction are in progress.

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## SYNTHESIS OF 4-HYDROXYCOUMARINS

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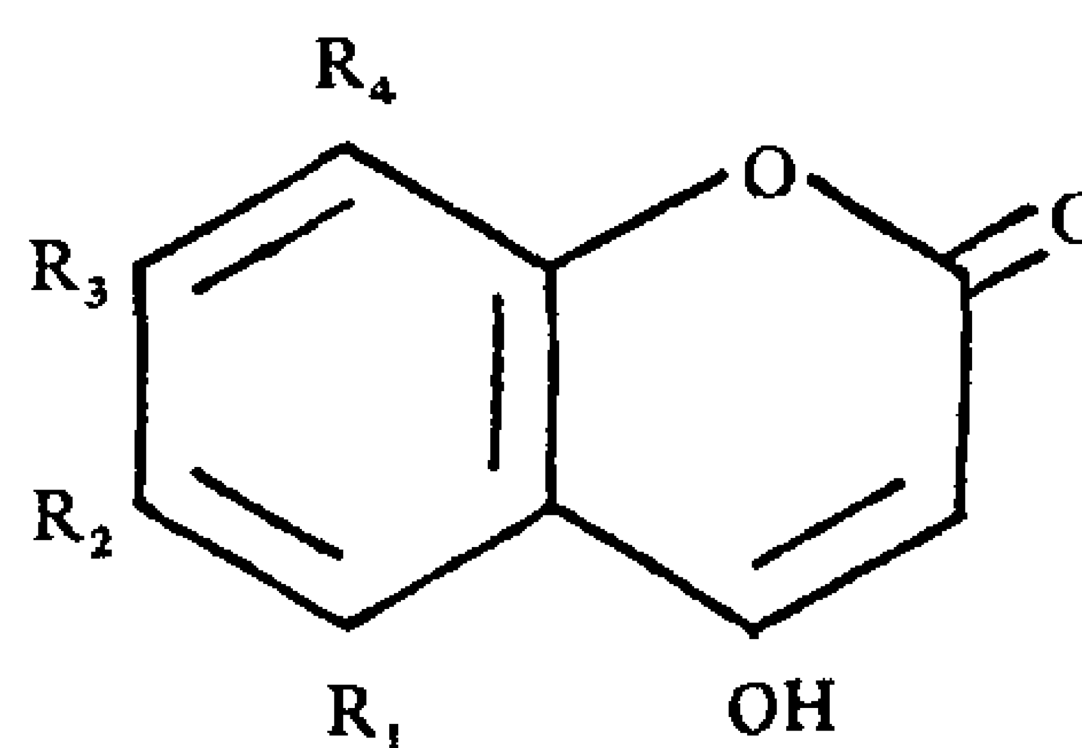
SOME 4-hydroxycoumarins have been prepared by the method of Shah *et al*[1]. The coumarins have been characterised by preparing the corresponding, bromo, nitro, sulphonic acid and phenylazo derivatives.




I $R_1 = R_3 = CH_3$, $R_2 = R_4 = H$
II $R_1 = R_2 = H$, $R_3 = R_4 = CH_3$
III $R_1 = R_4 = H$, $R_2 = Cl$, $R_3 = CH_3$
IV $R_1 = R_2 = R_3 = H$, $R_4 = Ph$

Various phenols were condensed with malonic acid in presence of phosphoryl chloride and fused zinc chloride at 70°C for 10–12 hr when the corresponding 4-hydroxycoumarins were obtained in 60–70% yield. The period of reaction had to be varied according to the nature of the phenol.

Thus, 3,5-xylenol gave 4-hydroxy-5,7-dimethylcoumarin [I, m.p. 210–11°, UV $\lambda_{max}^{95\%, EtOH}$ (log $\varepsilon$) 239