

Table 1 Production of cellulases from *C. lunata*

Substrate	Biomass g/l	FPase ^a IU/ml	CMcase ^b IU/ml	Cellobiase IU/ml	Protein mg/ml	Specific activity ^c	Level of substrate ^d
Cellulose	0.65	0.02	0.06	0.04	0.06	0.33	64
Lactose	3.25	0.21	1.27	0.17	0.18	1.17	800
Glucose	4.71	0.22	1.23	0.18	0.31	0.71	816

^a, filter paper activity; ^b, carboxymethyl cellulase activity; ^c, filter paper units/mg of protein, units/mg; ^d, estimated in terms of glucose, $\mu\text{g/ml}$.

Production of three different cellulase components from the eight-day-old cultures grown in three different substrates is shown in table 1. Among the three cellulases, carboxymethyl cellulase was higher than the other two enzyme systems. The cellulase system was poor in cultures grown on cellulose. The specific activity in terms of filter paper units produced per mg of protein was greater in lactose than in glucose, though glucose supported more biomass. It is interesting to note that cellulases are induced even in the presence of substrate (glucose). Lactose and glucose have been established as good inducers of cellulase by many workers⁶ and lactose as the good productive substrate in specific enzyme (protein) productivity⁷. Further improvements in cellulase production are in progress.

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OCCURRENCE OF URSOLIC ACID AND RELATED COMPOUNDS IN *EUCALYPTUS* HYBRID LEAVES

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EUCALYPTUS hybrid (Mysore gum, mainly *E. tereticornis*) is extensively grown in India under the social forestry programme due to its high biomass yield in a short span of time. The wood is mainly used for paper and pulp while the leaves give an essential oil consisting of cineole, pinenes and other monoterpenes¹. Since there is no report on the other chemical constituents of the leaves, the present investigation was taken up.

The leaves (400 g) were extracted with petroleum ether, acetone and alcohol respectively. Column chromatography of the acetone extract over silica gel gave three pure compounds A, B and C.

Compound A (800 mg) crystallized from chloroform-methanol as colourless needles m.p. 255–56°, acetate m.p. 260–62°. It was identified as ursolic acid lactone² by NMR, MS and direct comparison with an authentic sample (m.m.p., Co-TLC and superimposable IR spectra).

Compound B (4.800 g) crystallized from chloroform methanol as white needles, m.p. 291–92°, acetate m.p. 285–86°, $[\alpha]_D + 58.5^\circ$ (chloroform), methyl ester acetate m.p. 232–33° and gave + ve L.B. test (pink \rightarrow blue). It was characterized as ursolic acid³ by NMR, MS and by direct comparison with an authentic sample (m.m.p., Co-TLC and superimposable IR spectra).

Compound C (400 mg) crystallized from methanol as white needles, m.p. 243–44°. It formed diacetate, m.p. 196–97°, methyl ester m.p. 208–209°, $[\alpha]_D + 48^\circ$ (chloroform) and methyl ester diacetate m.p. 144–45°. Its identity was confirmed as 2- α -hydroxy ursolic acid⁴ by NMR studies of the methylester diacetate and direct comparison with an

authentic sample (Co-TLC, m.m.p. and superimposable IR spectra).

This is the first report of the occurrence of the above three compounds in *Eucalyptus* hybrid leaves. 2- α -Hydroxy ursolic acid is reported for the first time in the genus *Eucalyptus* and for the third time in the family Myrtaceae. It also occurs in the leaves of *Psidium guajava* Linn⁵, and *Callistemon lanceolatus* DC⁴.

Ursolic acid is of medicinal value. It is a well-known anti-inflammatory, antibiotic, antiarthritic, antiulcer and hypolipidemic agent⁶. About 4.28 lakh hectares of *Eucalyptus* hybrid were planted⁷ in India up to 1977. Plantation programmes of various states include plantation of this species in large areas and by now the area under this species will be much more. About 1.2% of ursolic acid occur in the leaves of *E. hybrid* as reported above. Thus, the large quantities of the leaves available in the country may be utilized for the isolation of ursolic acid.

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NEW RECORD OF A BACULOVIRUS DISEASE IN *LEUCINODES ORBONALIS* GUEN

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LEUCINODES ORBONALIS Guen is a serious pest of eggplant throughout India often resulting up to 80% infestation¹. It has also been reported on potato² and other wild plants³. The information on natural biotic mortality factors of the pest is limited and has been reviewed by Tewari and Krishna Moorthy⁴.

During September–October, 1985, the survey studies conducted on the natural mortality factors of *L. orbonalis* revealed the presence of sluggish larvae with symptoms of loss of appetite on fruits of eggplant. With passage of time they became fragile with flaccid body and died inside the fruit. The disease infection varied from 1.06 to 6.38% (table 1).

As the larvae were suspected to be virus-infected, further studies were carried out on isolation, purification and identification of virus in the laboratory. The infected larvae were macerated in 0.05 M phosphate buffer (pH 7.2) at 1 ml of buffer per g of infected larvae in a pestle mortar for 5 min and filtered through double muslin cloth. The filtrate was centrifuged at 10,000 g for 15 min and the supernatant was mixed with 6% polyethylene glycol (6000 MW) and 0.1 M NaCl, shaken for 30 min and then centrifuged at 30,000 g for 1 hr. The supernatant was discarded and the pellet was dissolved in 0.05 M phosphate buffer (pH 7.2). The virus was further purified by differential ultracentrifugation at 1,50,000 g for 2 hr. The pellet was collected and dispersed in 0.05 M phosphate buffer (pH 7.2) and again centrifuged at 7,000 g for 10 min. The supernatant was collected and this constituted the purified virus preparation. The purified virus suspension was sprayed on formvar coated copper grids stained with

Table 1 Occurrence of baculovirus disease in *L. orbonalis*

Date	No. of larvae observed	No. of larvae diseased	Infection (%)
4-9-1985	188	2	1.06
10-9-1985	242	9	3.72
23-9-1985	164	8	4.88
3-10-1985	47	3	6.38