

TABLE I

STO-3G energy differences for various conformations in methanediol and methanedithiol (in Kcal mol<sup>-1</sup>) using standard bond lengths and bond angles

| Conformation | Methanediol <sup>a</sup> | Methanedithiol |
|--------------|--------------------------|----------------|
| (sc, sc)     | 0 <sup>b</sup>           | 0 <sup>c</sup> |
| (sc ap)      | 2.24                     | 0.18           |
| (ap, ap)     | 6.60                     | 0.97           |

<sup>a</sup> 4-31G and 6-31G\* calculations on methanediol are reported in reference 9 and reference 10 respectively. STO-3G calculations on methanediol were done in this study, to carry out the comparison of methanediol and methanedithiol at the same level of approximation.

<sup>b</sup> Total energy for this conformation is -187.38249 a.u.

<sup>c</sup> Total energy is -834.223984 a.u.

mation which is never observed and is known to be least favoured due to exoanomeric effect in the oxygen systems.

The results can also be applied to the preferences of ring conformations. Though the ring conformation depends on the environment<sup>12,13</sup> and the nature of hydrogen bonds involved<sup>14</sup>, some features are apparent from the present study. In  $\beta$ -D-ribose, the <sup>4</sup>C<sub>1</sub> conformation has only one axially oriented OH group (at C3) and <sup>1</sup>C<sub>4</sub> conformation has three axially oriented hydroxyl groups (two of the hydroxyl groups are involved in 1-3 diaxial interactions). Hence non-bonded interaction energies favour <sup>4</sup>C<sub>1</sub> conformation for  $\beta$ -D-pyranoside<sup>15</sup>. However, because of predominance of anomeric effect ( $\Delta E_1$ ), <sup>1</sup>C<sub>4</sub> conformation becomes more favoured for  $\beta$ -D-ribose. This is in agreement with the observed conformation of  $\beta$ -D-ribose both in solution<sup>2</sup> and solid state<sup>3</sup>. The present calculation suggests that the anomeric energy ( $\Delta E_1$ ) is reduced considerably in S-C-S system and hence the resulting ring conformation in  $\beta$ -1,5 dithio-D-ribose may be decided mainly by non-bonded interactions. This explains the existence of this molecule in <sup>4</sup>C<sub>1</sub>(D) conformation in solid state and its predominance in solution.

In the case of  $\alpha$ -D-ribose, <sup>1</sup>C<sub>4</sub> conformation is slightly more favoured from non-bonded energy calculations. Since the anomeric effect ( $\Delta E_1$ ) is small for S-C-S system, theory predicts that  $\alpha$ -1,5 (d) dithioribopyranoside exist in <sup>4</sup>C<sub>1</sub>  $\rightleftharpoons$  <sup>1</sup>C<sub>4</sub> equilibrium in solution and either of them in solid state depending on the lattice energy. This is in agreement with the experimental studies in solution<sup>2</sup> and solid state<sup>3</sup>.

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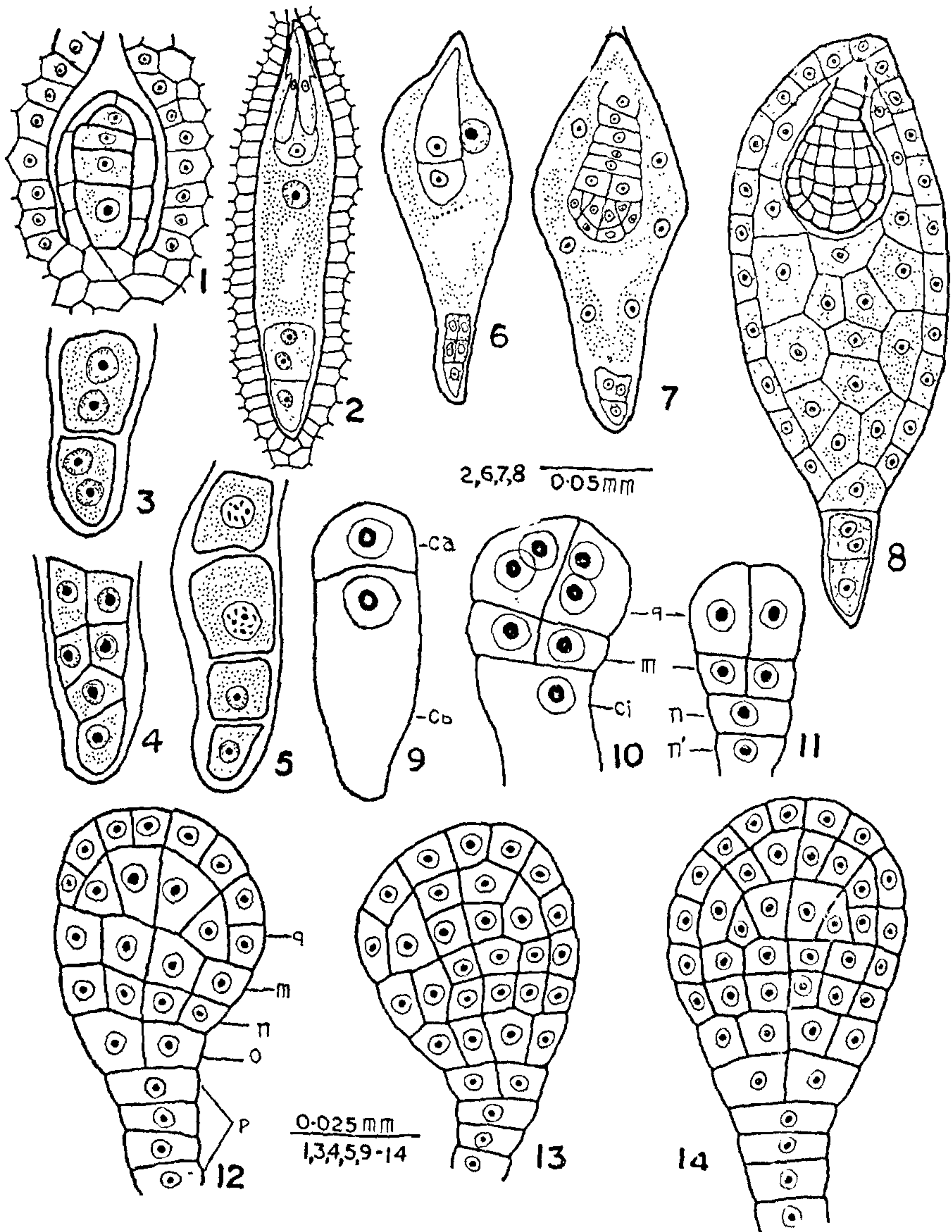
## EMBRYOLOGY OF ACANTHOSPERMUM HISPIDUM DC

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ALTHOUGH considerable literature is available on the embryology of the tribe Heliantheae of the family Compositae (see Pullaiah<sup>1</sup>), information on the genus *Acanthospermum* is inadequate. Vaidya<sup>2</sup> studied a few aspects of embryology of *Acanthospermum hispidum*, but no detailed information is available regarding fertilisation, endosperm and embryo development. The present investigation on *Acanthospermum hispidum* DC was undertaken to bridge the gap in our present knowledge of the embryology of the genus.

Pollen grains in *Acanthospermum hispidum* at the time of shedding are three-celled with thick spinous



FIGS, 1-14. Fig. 1. Megaspore tetrad. Fig. 2. Organised embryo sac. Figs. 3-5. Antipodal cells. Fig. 6. Embryo sac showing two-celled embryo and undivided endosperm nucleus. Fig. 7. Embryo sac showing embryo and few free endosperm nuclei. Fig. 8. Embryo sac showing globular embryo and cellular endosperm, Figs. 9-14. Various stages in the development of the embryo,

exine, a report which is contrary to the earlier one<sup>2</sup>. Brewbaker<sup>5</sup> studied a number of Compositae and concluded that three-celled pollen grain (at the anthesis) is a characteristic feature of the family Compositae.

A linear tetrad of megaspores is formed consequent upon the two meiotic divisions in the megaspore mother cell (Fig. 1). Embryo sac is of the polygonum type (Fig. 2). Antipodal cells in the newly formed embryo sac are either two or three in number. In some cases the antipodal cells increase in number (Figs. 4, 5) while in others nuclear divisions and fusions result in multinucleate and polyploid cells (Fig. 3). This disproves the opinion so generally held by Harling<sup>4</sup> and Eliasson<sup>5</sup> that the tribe Heliantheae is characterised by two or three secondarily multinucleate, but not dividing antipodal cells.

Fertilisation is porogamous. Endosperm development is of the nuclear type. The primary endosperm nucleus undergoes a few divisions resulting in free nuclei which migrate to the periphery of the embryo sac where they divide further (Fig. 7). Eventually the endosperm enters the cellular phase and fills the entire embryo sac with cellular tissue. By the time a globular embryo is formed the endosperm cells undergo divisions such that a narrow layer of small peripheral cells is formed (Fig. 8). These peripheral endosperm cells resemble the endothelial cells in their appearance and in their glandular nature, but differ from them in being longitudinally elongated. The entire endosperm excepting this peripheral layer is consumed by the growing embryo. Similar condition of formation of peripheral layer in Heliantheae is reported earlier by Kapil and Sethi<sup>6</sup> in *Tridax trilobata*, Padmanabhan<sup>7</sup> in *Tridax procumbens* and Pullaiah<sup>1</sup> in *Tithonia rotundifolia*. The primary endosperm nucleus in a few cases even after formation of two-celled embryo, remains undivided (Fig. 6)

The zygote undergoes transverse division resulting in a terminal cell, *ca* and a basal cell, *cb* (Fig. 9). The former undergoes a vertical division and the latter divides transversely resulting in a 'T' shaped four-celled proembryo. The derivatives of the basal cell *cb* are termed as *m* and *ci* (Fig. 10). The cell *ci* divides by a transverse wall and produces two cells *n* and *n'* (Fig. 11). The cell *n'* after undergoing one more transverse division produces two cells *O* and *p* (Fig. 12). The cell *p* undergoes two or three transverse divisions and produces an uniseriate suspensor (Figs. 13, 14).

The four-celled proembryo is 'T' shaped and both the cells *ca* and *cb* contribute to the development of the embryo proper (Figs. 9-14). Further development of the embryo follows the Senecio variation of Asterad type.

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#### A NOVEL IMMUNOGENIC STRAIN : *MYCOBACTERIUM HABANA* AGAINST *MYCOBACTERIUM ULCERANS* (BURULI ULCER) INFECTION IN MICE

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'BURULI ULCER', is a local ulcerative disease of humans caused by *Mycobacterium ulcerans*. Its response to known antitubercular regimens is very poor<sup>1,2</sup>, and at present no prophylaxis is available. Bacillus Calmette and Guerin (BCG), the only vaccine strain of mycobacteria has shown little protection against this ailment<sup>3</sup>. Search for immunogenic strain(s) amongst mycobacteria and/or other eubacteria, is of great interest. In a general screening programme for search of immunogenic strains against experimental tuberculosis of mice, a strain of atypical mycobacteria designated *M. habana* (TMC 5135) was found to afford high degree of protection against *M. tuberculosis* H<sub>37</sub>Rv challenge in mice<sup>4</sup> and guinea pigs<sup>5</sup>. This strain of *M. habana* was followed in further studies for its immunogenic spectrum against various models. One such model available with us was *M. ulcerans*-mouse model. When *M. ulcerans* is given to the mouse through intravenous route, it produces fulminating type of disease with high mortality rate and in chronic conditions ulceration of the tail is the most common symptom. *M. habana* was tested in this model in two successive experiments.

*Expt. No. 1* : Five groups of mice comprising of 20 animals each were taken. In the first and second groups *M. habana* alone was administered subcutaneously in 4 and 1 mg/mouse doses respectively. In the third and fourth groups, the same two doses were