

TRANSFORMED CELL SUSPENSION CULTURE OF *GALPHIMIA GLAUCA* PRODUCING SEDATIVE NOR-FRIEDELANES.



The Mexican species Galphimia glauca (Cav.) Kuntze (Malphigiaceae) synthesises a family of sedative and anxiolytic nor-secofriedelanes, designated as galphimines. These active principles accumulate at low concentration in the aerial parts of plants from wild populations. Transformed calluses and cell suspension cultures of this species were established in order to induce a greater production of nor-friedelanes. The cell suspension line GgBa was selected and grown over a period of two years of continuous subculturing in MS nutrient medium in the absence of growth regulators. PCR and Southern blot analyses were employed in order to confirm that the rol A gene had been integrated into the plant genome. Batch cultures of the GgBa cell line were grown over a 32-day period and first-order growth kinetics was observed, reaching a specific growth rate ( $\mu$ ) of 0.13 d<sup>-1</sup>. The production of glaucacetalin A (10), a triterpenoid related to the known galphimines, was quantified in the nutrient medium by HPLC. The transformed cell suspension culture GgBa also synthesised a novel nor-friedelane, given the name glaucacetalin D (13). High-resolution spectroscopic and spectrometric techniques were employed to elucidate the structure of 13. This triterpene has never been observed in wild plant tissues or in other in vitro cultures. Maslinic acid (14) was identified in cell biomasses. The triterpene production of the cell line GgBa was as follows: glaucacetalin A, 2.7 mg/L; glaucacetalin D, 2.9 mg/L and maslinic acid, 2.4 mg/g dry weight. The sedative activity of compounds 10 and 13 was demonstrated in ICR mice by using the sodium pentobarbital-induced hypnosis model. No cytotoxicity of 10 and 13 was exhibited against KB, MCF-7 and HF6 human cancer cell lines.

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