LETTER TO THE EDITOR

Preliminary Observation of the Chemical Composition of Callus Derived from Immature Seeds of *Vernonia galamensis*, Var. Ethiopica, Gilbert

Sir,

Seeds of *Vernonia galamensis* are an important source of an epoxytriglyceride oil that shows promise in industry. The oil contains about 80% epoxytriglyceride that currently is produced synthetically and used in the manufacture of epoxy resins, chemical coatings, and plasticizer/stabilizers used in plastic formulations (1–3). This is a report of preliminary work to obtain and cultivate *Vernonia* immature seed callus *in vitro* and to ascertain its oil-producing potential.

Callus was initiated from immature seeds of *Vernonia galamensis*, var. Ethiopica, Gilbert. The flower heads were removed from 9-week-old plants, sterilized by immersing them in a 10% Chlorox solution for 3 minutes followed by three rinses of sterile distilled water, immersed in 70% ethanol and again rinsed in three changes of sterile distilled water. The seeds were teased out of the flower heads and placed on a semi-solid medium (4) supplemented with .05 mg/l 2,4-D. Cultures were maintained in tubes 25 mm × 150 mm in a chamber at 24–26°C and under a 16/8-hour light and dark cycle.

After 6 weeks in culture, slow-growing brown, succulent callus tissues were produced in the explants. Callus tissues from the fourth subculture on the initiation medium and approximately 22-weeks old were selected for oil extraction and subsequent chemical analysis.

The extraction of 350 mg of tissues was carried out at room temperature using all glass homogenizer, with pentane as a solvent. The extract was dried over anhydrous sodium sulfate and the solvent was removed under nitrogen gas.

The extract, methylated with an ethereal solution of diazomethane, was analyzed for free fatty acids using a Finnigan 4500 computerized gas chromatograph-mass spectrometer equipped with a Supelco fused silica SPB—1 column. Transesterification of the ethereal solution of the methylated extract was carried out with ca. 3.3 M sodium methoxide solution (5). Gas chromatographic/mass spectrometric analyses of the methylated pentane extract indicated the presence of stearic and palmitic acids. Transesterification of the extract similarly showed only palmitic and stearic acids.

Consequently, we concluded that no vernolic acid formation has taken place at this stage in the callus. In order to promote the formation of this epoxy acid, it may be necessary to modify the composition of the medium as well as the physical conditions. Further work is in progress to establish the optimum conditions that will promote the formation of oil in the callus of *Vernonia galamensis*.

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