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Steroid Degradation Compound Associated with Sex Expression in Nutmeg (*Myristica fragrans* L.)*

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Thin-layer chromatography of leaf extracts of male and female lines of nutmeg indicated the presence of steroid degradation compound specific for male lines. β-sitosterol also showed similar degradation. Structural elucidation of this compound was accomplished by IR and MS and was tentatively identified as 1-dimethyl 2-ethyl butyl methyl ketone with molecular weight as 150. The results of the present study suggest the possible use for identification of sexual characteristics of nutmeg in seedling stage itself.

Key Words: Steroid degradation, β-sitosterol, Nutmeg, *Myristica fragrans* L.

Introduction

Characterization of sex in nutmeg *Myristica fragrans* L. has been investigated without much success so far. Currently, sex determination is possible only after flowering commences—which is five to six years after transplantation. Identification of sex in nutmeg was attempted by morphological, chemical and physiological methods (Choudhary et al. 1957, Flach 1966, Phadnis et al. 1971).

In the present work, we studied the role of compounds, obtained on degradation of steroids, in the determination of sex (Genus 1978).

Material and Methods

Leaf samples from 32 males and female lines of eight-year-old nutmeg plants were collected and the enzymes were inactivated (Loomis et al. 1937). Dried leaf samples were finely powdered and 5 g of the samples were extracted with chloroform for 24 hr at ambient temperature. The extracts were centrifuged at 19,980 g for 5 min. Clear supernatant fraction was flash-evaporated to near dryness and redissolved in 2 ml of chloroform. The leaf extract was chromatographed on thin-layer plates coated with silica gel G (0.25 mM) and activated at 60 C (1 hr), using benzene and ethyl acetate (4:3 v/v). Two chromogenic sprays viz. 20% sulphuric acid and 5% alcoholic phospho-molybdic acid, were tried respectively for consistency in identifying steroids and their degradation products. The plates were heated at 110°C for 2 min (Touchstone et al. 1978). β-sitosterol and stigmasterol were co-chromatographed and treated as above.

Spectra in Perkin Elmer 3400 IR spectrophotometer (from 4000 to 600 $\times$ cm^{-1}) and in Hewlett Packard mass spectrometer operating at 70 ev and an ion source temperature of 170°C were recorded every 6 sec and processed by V G Digispec display data system which produced standard bar graphs for direct comparison.

Results and Discussion

The chloroform extracts were spotted on TLC plates —a typical chromatogram, comprising of male and female chloroform extracts, β-sitosterol and stigmasterol in chloroform is presented in figure 1. Under the conditions of TLC development, stigmasterol and sitosterol have similar Rf values (0.8). Sitosterol undergoes degradative changes on storage. These degradative changes resulted in a compound having Rf value 0.5.

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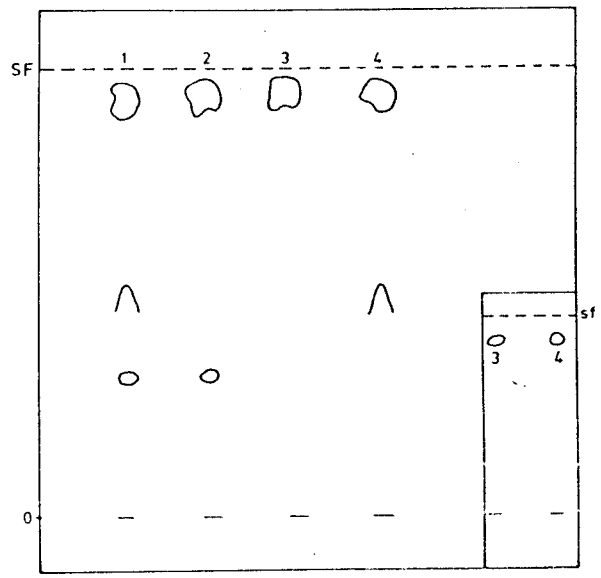

Figure 1 TLC trace diagram of chloroform extracts of nutmeg. **SF**, solvent front; **0**, origin; **1**, male; **2**, female; **3**, stigmasterol; **4**, β-sitosterol; Inset: purified compounds of sito- and stigma-sterols

Chloroform extracts of the male line also exhibit a similar pattern. Oogoniol, a sterol having a similar structure as sitosterol, undergoes degradative change—the resultant compound is reported by Popplestone and Unrau (1973, 1974) as precursors of steroid hormone. The structural details of sitosterol and probable cleavage site are indicated in figure 2. Even though sitosterol is cited as an example of Barbier-Wieland (quoted by Finar 1975) degradative change, this compound can result from similar steroids like cholesterol, campesterol, etc. Such type of degradation is found in the male lines of nutmeg. By changing the solvent proportions, attempts were made to differentiate the sito- and

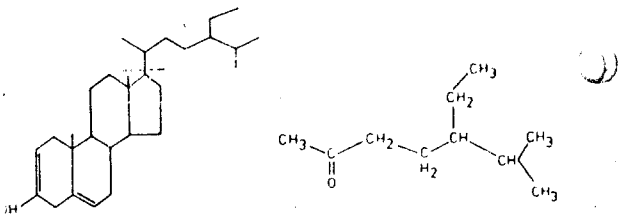

Figure 2 β-sitosterol with the possible degradation compound (-----cleavage site)

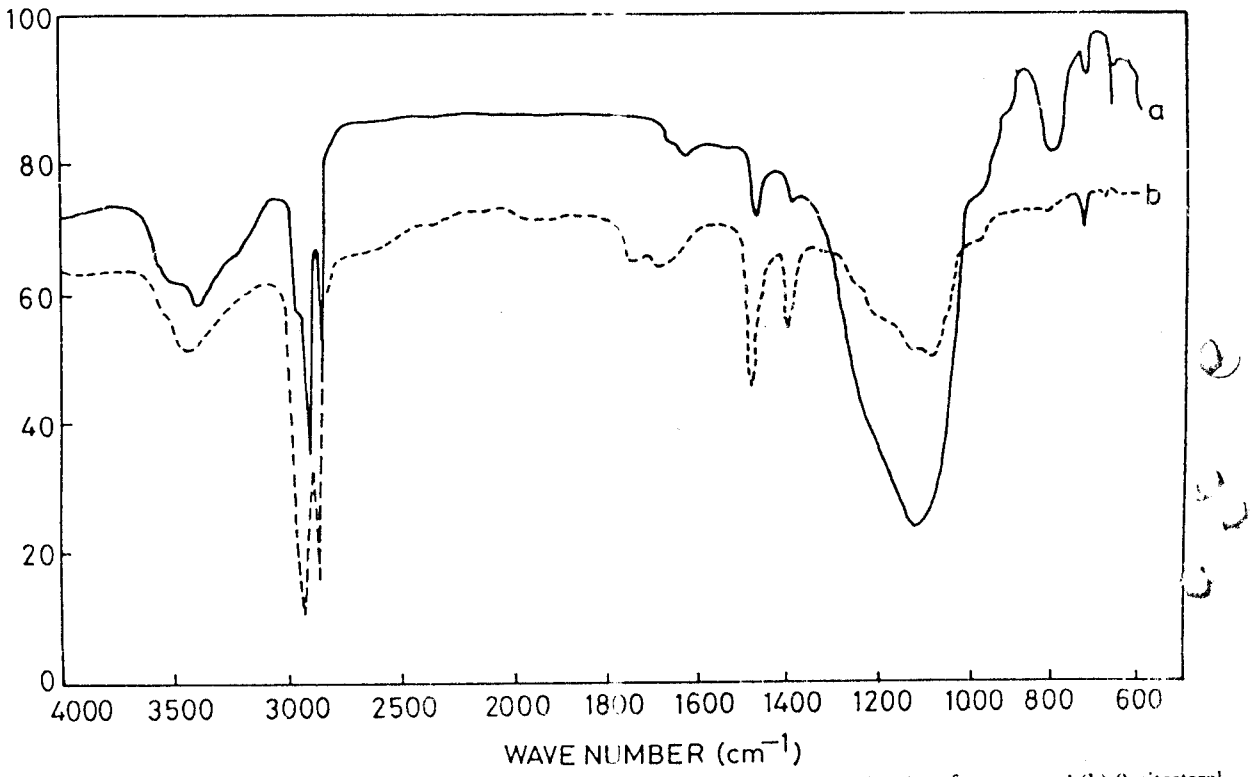

Figure 3 IR spectra of the degradation products of (purified chloroform extracts) (**a**) male line of nutmeg and (**b**) β-sitosterol

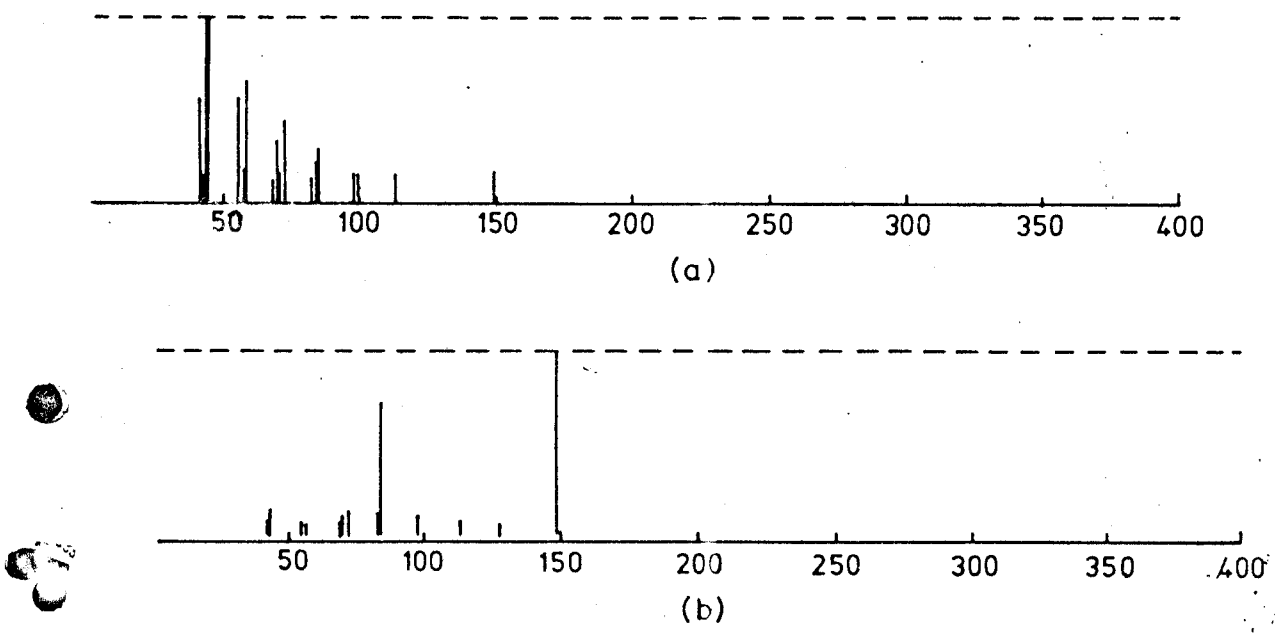

Figure 4. Mass spectra of degradation products (purified chloroform extractive) of (a) male line of nutmeg and (b) β-sitosterol

stigma-sterols. When the zones corresponding to stigma- and sito-sterols were re-chromatographed after a preliminary clean up, Rf values remain as in the pure compound. This indicates that degradation is partial and complete degradation does not occur. Phadnis and Choudhary (1972) reported differences in chlorophyll pigments, *p*H, N and K contents as well as in the carbohydrates between the male and female lines but could not arrive at a tangible conclusion with any degree of certainty.

IR Spectra of purified fraction of plant origin (a) and sitosterol (b) at Rf 0.5 are presented in figure 3. A peak at 1680 cm^{-1} and 1750 cm^{-1} represent C=O and CH_3CO groupings respectively. The fingerprint region of spectra indicates the long chain hydrocarbon. Superimposition of (a) over (b) indicates the similarity in the nature of the compound.

Pure compounds obtained after TLC clean up are subjected to Mass Spectroscopy (MS) and scanned through 40 to 600 a.m.u. at a fixed voltage. The MS of the purified fraction of plant origin (a) and sitosterol (b) are presented in figure 4. Hundred per cent abundance of a compound having a molecular weight of 150 units is found in both the above fractions. A tentative identification of the compound is made as 1-dimethyl 2-ethyl butyl methyl ketone on the basis of IR, MS and known reactions of sitosterol and its analogues.

Under ambient conditions of oxidation sitosterols and its analogues undergo degradative change resulting in steroid hormone and ketone (as quoted by Finar 1975).

In nutmeg plants, the consistent association of the degradation of steroids in the male and the absence of such degradation in the female seems to be indicating a role for steroids in the determination of sex in this plant species. The authors in a pilot study noted similar differences in one-year-old nutmeg seedlings also. Out of 20 seedlings so evaluated, steroid degradation was noted in eight seedlings only. It may be possible that the process of degradation of steroids sets in during the early development stages itself and this may be influencing the differentiation of sex in nutmeg. Efforts are now under way to screen the seedlings based on the above difference in order to correlate the data with the adult plant sex later when the plants come to bearing.

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