with the base peak at $m/z$ 43 The $^1$H NMR spectrum was identical with the spectrum of *neo*-inositol hexaacetate (**2a**) (Calc for $C_{18}H_{24}O_{12}$ C, 50 00, H, 5 55, Found C, 50 04, H, 5 48%)

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# CHEMODIFFERENTIATION OF DIOSGENIN IN *DIOSCOREA COMPOSITA*

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**Key Word Index**—*Dioscorea composita*, Dioscoreaceae, chemodifferentiation, diosgenin, tissue culture

**Abstract**—Diosgenin was isolated from different parts of a three-year-old plant of *Dioscorea composita* The amounts (% on a dry wt basis) present were tubers, 3 6, vine internodes and nodes with their leaves from first 20 nodes from the tubers, 1 6, similarly from intermediate 20 nodes, 0 039 and from upper 20 nodes, 0 03 The amounts (% on a dry wt basis) from tissue culture of nodal explants were 30-day-old callus, 0 89, 90-day-old callus, 1 61, emergent shoots, 2 5, regenerated roots, 0 08

## INTRODUCTION

Diosgenin is used as a starting material for the manufacture of steroid drugs, including corticosteroids and oral contraceptives It is present in different amounts in the same plant growing in different localities [1, 2], but little is known regarding its levels in different parts of the plant and its synthesis in relation to organogenesis in culture

## RESULTS AND DISCUSSION

In three-year-old plants harvested prior to flowering the upper young 20 nodes with leaves contained 0 030% diosgenin on a dry weight basis, whereas the nodes (20) adjacent to the tubers contained 1 6%, the intermediate 20 nodes contained 0 039% and the tubers contained 3 6% diosgenin. The side shoots arising at the nodes were not included in these analyses Young callus (30-day-old) obtained from $MS_1$ medium contained 0 89% and the highest content, 1 61%, was observed in 90-day-old culture The content was further enhanced to 2 52% in the emergent shoots derived from callus cultures However, regenerated roots contained less diosgenin (0 08%)

Studies with tissue cultures of *D composita* have shown that supplementation of the growth medium with 0 5 mg benzyladenine stimulates diosgenin biosynthesis [3, 4], and it has been reported that diosgenin synthesis is greater in unorganised tissue culture than in organised root culture of *D deltoidea* [5, 6] In the present study, however, it was found that diosgenin synthesis is greatest in organised shoot cultures of *D composite*, a finding which is reflected in the *in situ* production pattern of the plant Perhaps, chemodifferentiation of diosgenin is influenced by organogenesis A similar response has been reported in the case of cardenolide biosynthesis in *Calotropis gigantea* [7]

## EXPERIMENTAL

Plant materials (aerial part, tubers) were collected from the University garden Three-year-old plants were divided into four parts viz the internodes and leaves of the upper 20 nodes, similarly of the 20 nodes adjacent to the tuber and likewise the 20 intermediate nodes, and tubers Nodal explants of healthy growing plants were cultured in RT (revised tobacco medium), supplemented with 2,4-D (2 mg/l) and Kn (0 5 mg/l) for callus initiation and organogenesis Diosgenin was also determined from the initiated callus (1-month-old), old callus (90-days-old), the emergent shoots derived from callus, proliferated roots in culture and regenerated plants in culture

*Extraction procedure of diosgenin* Dried finely powdered plant material (0 5 kg in the case of tubers and aerial parts, 10 g in the

case of callus tissues and regenerated shoots and roots for each phase of growth) was hydrolysed with 2 5 l of HCl (5%) under reflux for 6 hr, the volume of the mixture was maintained constant by adding $H_2O$ from time to time After cooling, the mixture was filtered and the residue washed thoroughly with $H_2O$ (to free from acid) and then dried at 60° The dried mass was then extracted in a Soxhlet apparatus with *n*-hexane for 10 hr, after which the extract was distilled (to remove solvent) on a waterbath and then chromatographed over aluminium oxide (neutral grade I, 20 g of $Al_2O_3$ per g extract) The fractions were monitored on TLC for diosgenin A fraction of *n*-hexane–benzene (1 1) eluates showed the presence of only diosgenin (compared by co-TLC with reference diosgenin) The residue from these eluates were combined and crystallized from $Me_2CO$ Quantitation of diosgenin from callus and regenerated shoots and roots in culture was performed by IR and HPLC analysis [8]

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# STEROLS FROM *CANDIDA LIPOLYTICA* YEAST GROWN ON *n*-ALKANES

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**Key Word Index**—*Candida lipolytica*, Ascomycetes, fungi, sterols

**Abstract**—The sterols of *Candida lipolytica* grown on *n*-alkanes were isolated by reverse phase HPLC and found to be mainly ergosterol, with small quantities of ergost-7-en-3$\beta$-ol, ergosta-7,22-dien-3$\beta$-ol, ergosta-7,24(28)-dien-3$\beta$-ol and ergosta-5,7,9(11),22-tetraen-3$\beta$-ol

Recently much attention has been given to the yeasts *Candida tropicalis* and *Candida lipolytica*, which, being capable of utilizing aliphatic hydrocarbons as the sole carbon source, attracted commercial interest for production of microbial proteins which may be utilized as components in animal feeds [1] In continuing our work on sterols from fungi [2, 3], we examined the sterol composition of *C lipolytica* grown on *n*-alkanes

The residue from the chloroform–methanol extract of *C lipolytica* upon saponification followed by column chromatography, gave the 4-demethylsterol mixture, which was acetylated The preliminary GC of the steryl acetates showed four small peaks beside the major peak identical with that of standard ergosteryl acetate The sterol acetate mixture, subjected to reverse phase HPLC, yielded ergosta-5,7,9(11),22-tetraen-3$\beta$-yl acetate, ergosteryl acetate, ergosta-7,24(28)-dien-3$\beta$-yl acetate, ergosta-7,22-dien-3$\beta$-yl acetate and ergost-7-en-3$\beta$-yl acetate The sterols were identified on the basis of their mass, UV and $^1$H NMR spectra The percent composition of the sterols in *C lipolytica* and the chromatographic mobility data are summarized in Table 1 A previous investigation of the sterol mixture of *C lipolytica* grown on *n*-alkanes revealed the presence of ergosterol [4]

## EXPERIMENTAL

*C lipolytica* was grown on *n*-alkanes by the industrial process of Italproteine HPLC was on a Waters instrument equipped with a differential refractometer and Whatman Partisil 5/25 ODS-3 column (4 6 mm × 25 cm), $^1$H NMR, 270 MHz, $CDCl_3$, TMS as internal standard, UV, MeOH, GC, DB-1 fused silica capillary column (30 m × 0 25 mm) at 265°, MS, 70 eV

*Extraction and separation of sterols C lipolytica* (58 g) was extracted × 3 at room temp with $CHCl_3$–MeOH (1 1) The solvents were evapd to give a viscous oil (8 4 g), which was