DOLICHOL IN CANOLA

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INTRODUCTION

Polyisoprenoid alcohols are widely distributed in nature, having been found in bacteria, plants and animals (Rothfield and Romeo 1971; Hemming 1974; Elbein 1979; Rip et al. 1985). Dolichol consists of a series of homologous isoprenoid alcohols containing 14-24 isoprene units, with the α-isoprene residue being saturated (Fig.1). They occur as free alcohols, fatty acid esters or various phosphorylated forms. Dolichyl phosphate is an obligatory intermediate in the biosynthesis of N-linked glycoproteins (Staneloni and Leloir 1982; Rip et al. 1985), which include many biologically important proteins. Dolichol may also influence membrane fluidity (Murgolo et al. 1989).

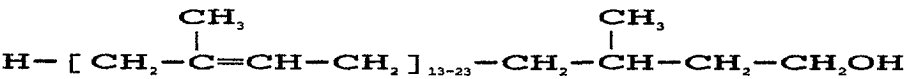

Fig. 1 Structure of dolichol

As part of our ongoing studies on polyisoprenoid alcohols, we were interested in obtaining quantities of dolichol as starting material for the preparation of analogues and derivatives. It appeared that seeds or seed oils might be a good source, since earlier experiments in our laboratory showed that dolichol was present in seeds of a number of commercially important crops (Ravi et al. 1984). Among the seeds examined, rapeseed was found to contain the highest concentration of dolichol (160 μg/g). Since canola is now the major type of rapeseed grown in Canada, further experiments were done to investigate canola and canola oil as possible sources of dolichol. The results are presented in this communication.

MATERIALS AND METHODS

Canola seed and various intermediate fractions produced during the extraction of canola oil (Fig.2) were kindly provided by Petros Loutas, formerly with Central Soya of Canada, Ltd., Hamilton, ON. Docosaprenol (prenol 22) from Sigma Chemical Co. (St. Louis, MO) served as a recovery standard. All solvents and chemicals used were of at least reagent grade.

Isolation and Quantitation of Dolichol

Samples (1 g) of finely ground seed, flaked seed and meal were saponified and extracted as described previously by Rip and Carroll (1987) to obtain the non-saponifiable fraction containing the dolichol. A modification of the method of Itoh et al. (1973) was used to obtain the non-saponifiable fraction

from oils and gums. Briefly, 1 g of oil or gum was saponified in 10 ml of 1.0N ethanolic KOH for 1 hour. The mixture was then diluted with 20 ml of water and extracted with 3 x 10 ml of diethyl ether. The ether fractions were pooled and washed with 3 x 10 ml of water. The solvent was evaporated under nitrogen and the residue dissolved in chloroform/methanol (2:1, v/v) for analysis by HPLC.

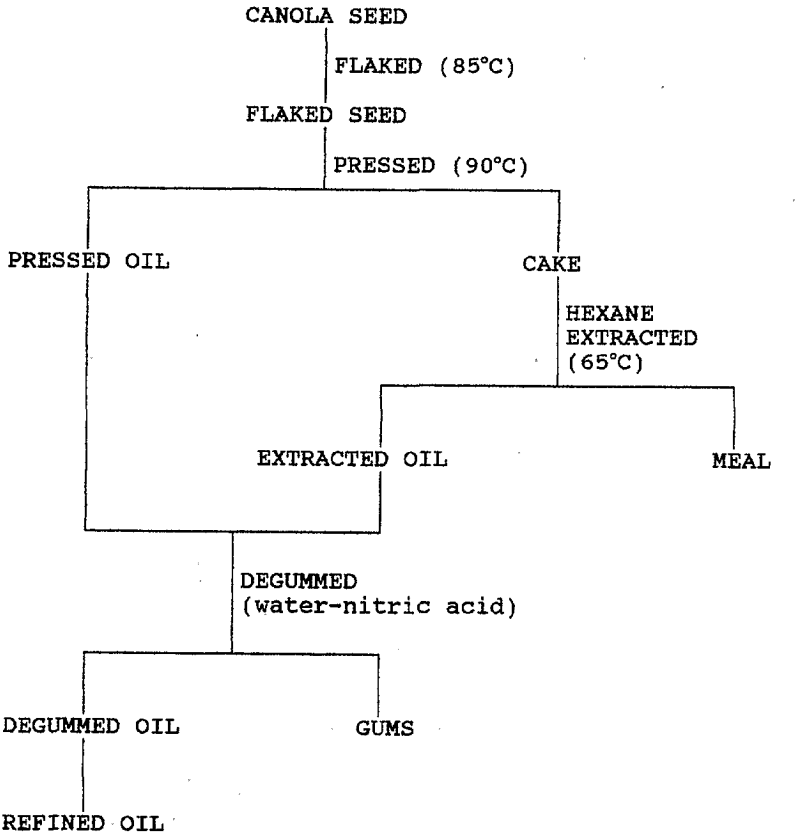

Fig. 2 Extraction and Refining of Canola Oil (Information provided by Petros Loutas)

The dolichol was separated on a reverse-phase C18 column (Hewlett Packard #799150d17) in a Hewlett Packard 1084-B HPLC and absorbance was monitored at 210 nm with a reference wavelength of 430 nm (Chaudhary et al. 1982). The mobile phase, delivered at 2 ml/min., was a methanol-isopropanol gradient in which the proportion of isopropanol increased from 10% at time 0 to 35% at 5 minutes and to 80% at 20 minutes. Quantitation was achieved by summing the areas of homologs 14 to 18 (Fig.3) correcting for recovery, and converting the areas to mass units using a conversion factor determined with known amounts of dolichol.

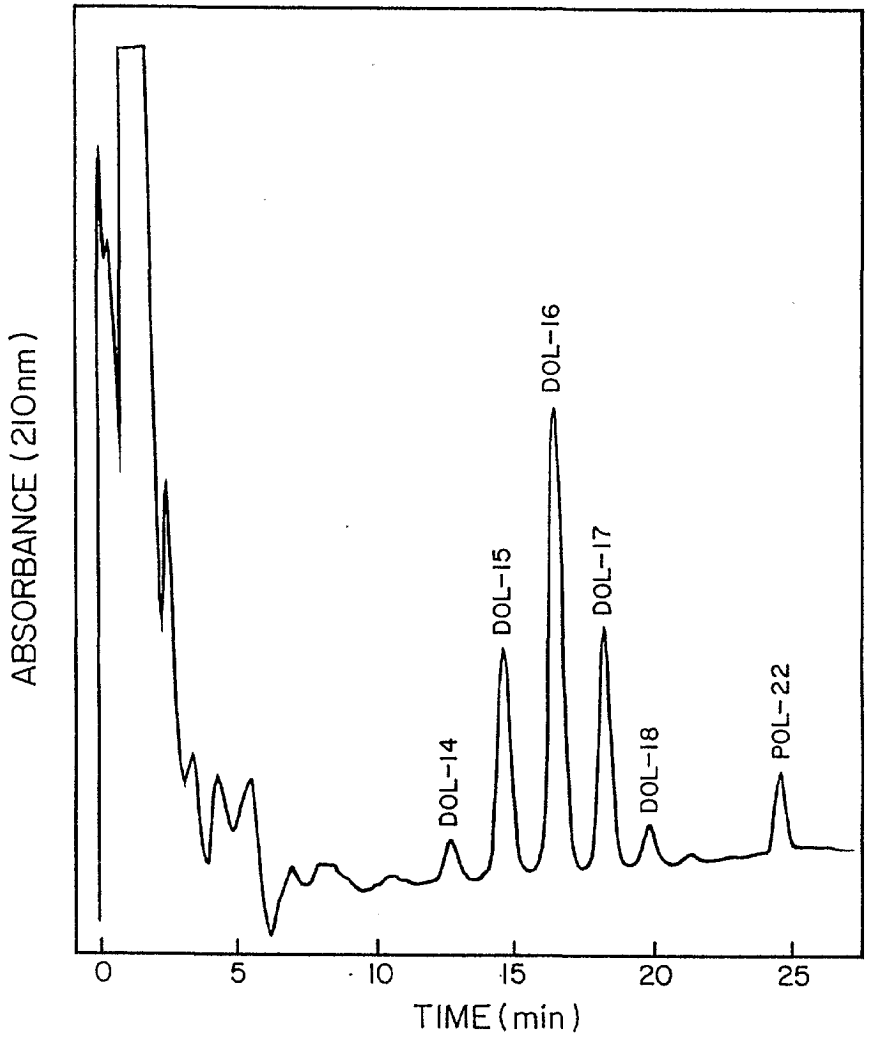

Fig. 3. Reverse phase HPLC of dolichol from canola oil

RESULTS AND DISCUSSION

The concentrations of dolichol in canola seed, canola oil and intermediate fractions produced in the process of extraction and refining the oil are given in Table 1. The concentration in canola seed was similar to that observed previously for rapeseed (Ravi et al. 1984). The concentration in refined oil was substantially lower and since the oil makes up only about 40% of the weight of the seed, the amount in refined oil represents only about 15% of that present in the seed.

Analysis of the intermediate fractions indicated that most of the loss occurred during the flaking process since the flaked seed contained less than 25% of the dolichol in the whole seed. Small amounts were also found in the extracted

meal and in the gums (Table 1). It seemed possible that the loss during the flaking process was due to alteration or destruction of dolichol through exposure to heat and air. However, a sample of oil provided by Central Soya that was prepared by cold pressing of canola seed had much the same concentration of dolichol as oil extracted by conventional means (Table 1). More work is required to determine why a much higher yield of dolichol can be obtained from seed extracted in the laboratory.

It appears from these experiments that canola oil provides a good source of dolichol, particularly if ways can be found to minimize losses during extraction of oil from the seed. One of the advantages of canola as a source of dolichol is that the end product is relatively pure compared to that extracted from tissues that are rich in dolichol (Rupar and Carroll 1978). The non-saponifiable fraction of canola can be applied directly to an HPLC column for isolation of dolichol whereas further preliminary purification is required in the case of tissue extracts.

Table 1 Dolichol Content of Canola Seed, Canola Oil and Intermediate Fractions

Source	Concentration of Dolichol (μg/g ± S.E.M.)
Canola seed	184±9
Refined oil	71±3
Flaked seed	42±0.2
Meal	5±0.05
Pressed oil	79±2
Extracted oil	94±5
Degummed oil	96±4
Gums	32±4
Cold pressed oil	75±4

ACKNOWLEDGEMENTS

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