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# SHORT REPORTS

## N-TERMINAL AMINO ACID SEQUENCE OF β-SUBUNITS OF LEGUMIN FROM *PISUM SATIVUM*

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**Key Word Index**—*Pisum sativum*; Leguminosae; pea; seed storage proteins; legumin β-subunits; N-terminal amino acid sequence.

### INTRODUCTION

Legumin, one of the major storage proteins of pea seeds, is composed of equimolar amounts of α- (MW *ca* 40 × $10^3$) and β- (MW *ca* 20 × $10^3$) subunits [1–4]. Each class of subunit displays microheterogeneity, both on SDS gels [1, 3, 5] and on two-dimensional (isofocusing/SDS) gels [5, 6]. Sequence studies on the β-subunits of legumin from *Vicia faba* and of the corresponding protein (glycinin) from *Glycine max* [7] have shown that the β-subunits from each protein are homologous and that there is sequence microheterogeneity in at least five positions within the first 29 N-terminal residues. This report describes the N-terminal amino acid sequence of the legumin β-subunits from a variety of *Pisum sativum* (cv Dark Skinned Perfection).

### RESULTS AND DISCUSSION

The purified legumin β-subunits showed the same three-banded pattern, characteristic of cv Dark Skinned Perfection, irrespective of whether the subunits were carboxymethylated, pyridethylated or unalkylated. The MWs of the subunits were estimated as *ca* 22 000, 21 000 and 19 000, similar to values reported elsewhere for another genotype [6]. Dansylation of the mixture of β-subunits showed a single N-terminal glycine residue [1, 8].

The sequence of the first 32 amino acid residues of the β-subunits is shown in Table 1, where it is compared with the published N-terminal sequences of the corresponding proteins from *V. faba* and *G. max* [7]. It is clear that the β-subunits of *P. sativum* legumin are homologous to those of *V. faba* legumin and *G. max* glycinin and that the N-terminal sequences of the legumin β-subunits are more similar to each other than each is to that of the β-subunits of glycinin, an observation which is consistent with the relative taxonomic positions of *Pisum*, *Vicia* and *Glycine*.

The β-subunits of *P. sativum* exhibit sequence microheterogeneity in three of the same positions as the *Vicia* subunits. This heterogeneity suggests the existence of multiple, similar β-subunits; the fact that there seems to be a 2:1 ratio of one amino acid to another at the sites of heterogeneity implies the possible presence of three β-subunits. It is likely that β-subunit gene duplication and diversification has produced this microheterogeneity, although there may be other explanations. At two of the three positions where sequence heterogeneity exists, the alternative residues are consistent with single point mutations; for instance, at position 16 glycine is coded by GGC/G/A/U whilst the codon for alanine is GCC/G/A/U. The exception is at position 13, where apparently leucine (codons CUC/G/A/U or UUA/G) and glutamic acid (codons GAA/G) are the alternatives. It is possible that position 13 is glutamine (codons CAA/G) which may have been deamidated during handling. It has been demonstrated [9] that the α- and β-legumin subunits are initially synthesized as a precursor molecule of MW 60 × $10^3$, which raises the possibility that α- and β-subunit loci may be adjacent in the *Pisum* genome; alternatively, the loci may be physically separated, but their transcripts joined in some way to produce a messenger RNA which codes for the MW 60 × $10^3$ precursor. Any model for the arrangement of the α- and β-loci must take into account the β-sequence (and thus, presumably, β-gene) multiplicity described here.

### EXPERIMENTAL

Legumin from *P. sativum* cv Dark Skinned Perfection was purified as before [5, 10] and the purity was ascertained by analytical ultracentrifugation, SDS-gel elctrophoresis and dansylation [1]. Legumin β-subunits were isolated by ion-exchange chromatography on Dowex [11] but using 1 mM dithiothreitol and 8 M, instead of 6 M, urea. After removal of salts, urea and excess of thiol by dialysis against $H_2O$, the protein was freeze-dried, reduced and either *S*-carboxymethylated [1, 11] or *S*-pyridethylated [12]. The alkylated β-subunits were dialysed against 0.2 M HOAc and recovered by freeze-drying.

The alkylated subunits (4–10 mg) were subjected to sequence analysis as described elsewhere [13]. Two sequence determinations were made using carboxymethylated, and two using pyridethylated, β-subunits. The data presented in Table 1 are a combination of the four independent analyses.

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Table 1. N-Terminal amino acid sequence of the β-subunits from *Pisum sativum* legumin, *Vicia faba* legumin and *Glycine max* glycinin

| | 1 | | | | | | | | | 5 | | | | | | | | | | 10 | | | | | | | | | | 15 | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| *Vicia faba* | G | – | L | – | E | – | E | – | T | | $V^2/I^1$ | – | C | – | T | – | $V^2/A^1$ | – | K | – | L | – | R | – | L/E | – | N | – | I | – | $A^2/G^1$ | – | | | | | | | |
| *Pisum sativum* | G | – | L | | E | – | E | – | T | | $V^{2*}/I^1$ | – | C | – | T | – | A | – | K | – | L | – | R | – | $L^2/E^1$ | – | N | – | I | – | $A^2/G^1$ | – | | | | | | | |
| *Glycine max* | G | – | $V^1/I^2$ | – | D | – | E | – | N/T | – | I | – | C | – | T | – | M | – | K | – | L | – | R | – | E | – | N | – | I | – | $G^2/A^2$ | – | | | | | | | |
| | | | | | | | 20 | | | | | | | | | | 25 | | | | | | | | | | 30 | | | | | | | | | | | | |
| *Vicia faba* | (Q) | | P | – | (A) | – | R | – | $P^2/A^1$ | – | D | | L | – | Y | – | N | – | P | – | (Q) | – | A | | G | | | | | | | | | | | | | | |
| *Pisum sativum* | P | – | (S)† | | (S) | – | (A) | – | P | – | D | – | L | – | Y | – | N | – | P | – | E | – | A | – | G | – | X | – | (L) | – | K | | | | | | | | |
| *Glycine max* | Q | – | P | | (S) | – | R | | $P^2/A^1$ | – | D | – | L | – | Y | – | N | | P | – | (Q) | – | A | – | G | | | | | | | | | | | | | | |

The *Glycine max* and *Vicia faba* sequences are from ref. [7].

* Where two residues were identified in the same position the approximate ratio of the yields is indicated by the superscripts.

† Data in brackets indicate uncertainty.

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# PHELLANDRENE ENDOPEROXIDES FROM THE ESSENTIAL OIL OF *CHENOPODIUM MULTIFIDUM*

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**Key Word Index**—*Chenopodium multifidum*; Chenopodiaceae; essential oil; *p*-menthanic monoterpenes; phellandrene endoperoxides.

**Abstract**—The essential oil of *Chenopodium multifidum* does not contain ascaridole, but does contain two isomeric endoperoxides related to α-phellandrene, besides other structurally and biogenetically related *p*-menthanic monoterpenes.

## INTRODUCTION

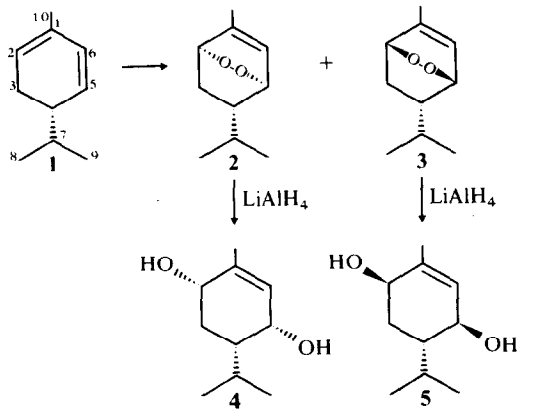
Ascaridole is the most characteristic component of the essential oils from Chenopodiaceae and is responsible for the anthelmintic properties of these oils [1].

*Chenopodium multifidum* L. (*Rouvieva multifida*, Moq.)* has been little studied with reports only on the isolation of α-phellandrene and anethole from Californian plants [2]; ascaridole, *p*-cymene, limonene and camphene from Brazilian plants [3]; and ascaridole, limonene, *cis*- and *trans*-carveol ('paicol') from Argentinian plants [4], with a vague allusion to the likeness of the *C. ambrosioides* essential oil.

We have re-examined the essential oil from *C. multifidum* collected at the end of October, near Babilafuente (Salamanca) in western Spain.

## RESULTS AND DISCUSSION

The essential oil contained monoterpenes, but it did not contain any ascaridole. The main fraction consisted of two stereoisomeric endoperoxides (**2**, **3**) related to α-phellandrene (**1**) and so far not reported as natural products, although they were obtained by Schenck *et al.* [5, 6], by photo-oxidation of α-phellandrene (**1**).




* The material for this work was identified by Prof. B. Casaseca Mena, Department of Botany, Salamanca University, where a specimen is held (Herbarium No. 19587).