sition of 4α-methyl sterol fractions from five legume seed oils is given in Table V. Obtusifoliol (9.5–39.5%), gramisterol (14.7–58.7%), and citrostadienol (8.1–47.6%) were the prominent components of this fraction. Our results for *A. hypogea* are quite similar with those given by Itoh et al. (1974 a) since these authors have found in peanut oil 25% obtusifoliol, 28% cycloeucalenol plus gramisterol, and 24% citrostadienol.

Table VI shows approximate composition of triterpene alcohol (and 4,4-dimethyl sterol) fractions from the five legume seed oils. Tentative identification was based on the comparison of their RRT by GLC. Among these compounds, cycloartanol (3.0–26.4%), butyrospermol (1.4–59.1%), cycloartenol (1.4–22.4%), 24-methylenecycloartanol (0–42%), and cyclobranol (trace–14.4%) were the main components of this fraction. α-Amyrin (38.3%) was found as the prominent component in *P. lunatus*. Butyrospermol (59.1%) was the highest 4,4-dimethyl sterol of *V. sinensis*. In some cases it was difficult to determine precisely the peak area of individual GLC peaks, particularly for the RRT range of 1.0–1.1, as in the case of *L. esculentus* (Table VI). β-Amyrin was found in all legume seed oils (1.5–9.3%) except in *P. lunatus*. The results observed for *A. hypogea* are quite similar to those given by Itoh et al. (1974b) since these searchers found 33% cycloartenol, 46% 24-methylenecycloartanol, 2% cycloartanol, and 8% cyclobranol for peanut oil. Jeong et al. (1975) showed, in a study of the 4,4-dimethyl sterol fraction from 20 vegetable oils, that cycloartanol, 24-methylenecycloartanol, β-amyrin, and cycloartenol are common in most of the oils. Butyrospermol, α-amyrin, lupeol, and cyclobarnol occur in some of these oils.

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Registry No. Cholesterol, 57-88-5; campesterol, 474-62-4; stigmasterol, 83-48-7; β-sitosterol, 83-46-5; Δ^5-avenasterol, 18472-36-1; Δ^7-stigmasterol, 481-19-6; Δ^7-avenasterol, 23290-26-8; lophenol, 481-25-4; obtusifoliol, 16910-32-0; 31-norcycloartenol, 60485-38-3; cycloeucalenol, 469-39-6; gramisterol, 1176-52-9; 24-ethyllophenol, 36735-29-2; citrostadienol, 474-40-8; cycloartanol, 4657-58-3; lanosterol, 79-63-0; β-amyrin, 559-70-6; butyrospermol, 472-28-6; α-amyrin, 638-95-9; cycloartenol, 469-38-5; lupeol, 545-47-1; 24-methylenecycloartanol, 1449-09-8; cyclobranol, 25692-13-1.

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Free Amino Acids from Different Cultivars of *Vicia faba*

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The free amino acid composition of the seeds of some *Vicia faba* L. and *Vicia faba minor* cultivars was determined by using gas chromatography. Very high levels of L-Dopa and methionine are found, whereas the alanine, threonine, lysine, and serine contents are relatively high in certain samples. The observed variability in the L-Dopa and methionine contents of the variants is briefly discussed in relation to the potential for selecting *V. faba* genotypes with improved seed amino acid quality.

Leguminous seeds constitute an important sources of food protein and energy for a large sector of the world population. The increasing interest in the food legumes has been demonstrated by several international symposia on this topic in the last decade (Milner, 1973; Wall, 1973; Jaffé, 1977).

The *Vicia faba* seeds, widely cultivated in Europe and in remarkable development in Africa and Asia in these last few years, contain a high percent of proteins (up to 25% dry weight) so they are very advantageous as human and animal food. Therefore, the economic interest in the cultivation could be very great, in spite of the presence of

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Table I. Free Amino Acids in *V. faba* Seeds[a]

amino acid	*V. faba* L.		*V. faba minor*		
	Muchamiel	Paceco	L. Monti 26	Scuro T. L.	Manfredini
alanine	10.51 (±0.07)	11.26 (±0.04)	11.59 (±0.04)	8.75 (±0.02)	8.46 (±0.03)
glycine	0.72 (±0.04)	1.63 (±0.06)	0.48 (±0.03)	0.66 (±0.03)	0.79 (±0.03)
valine	3.94 (±0.03)	4.48 (±0.04)	4.52 (±0.04)	4.86 (±0.03)	3.43 (±0.04)
leucine	1.00 (±0.04)	1.31 (±0.03)	1.23 (±0.03)	tr	1.31 (±0.03)
isoleucine	1.36 (±0.04)	1.80 (±0.03)	1.17 (±0.03)	tr	2.39 (±0.03)
proline			tr	3.35 (±0.04)	tr
threonine	8.49 (±0.03)	11.65 (±0.07)	9.13 (±0.05)	9.51 (±0.03)	14.55 (±0.05)
serine	6.49 (±0.05)		3.72 (±0.03)		
methionine	34.25 (±0.05)	23.79 (±0.04)	18.77 (±0.04)	35.26 (±0.04)	32.48 (±0.04)
cysteine	0.86 (±0.06)				
glutamic acid	7.96 (±0.06)				
Dopa	7.87 (±0.06)	37.88 (±0.06)	49.38 (±0.04)	37.60 (±0.04)	28.21 (±0.04)
histidine	1.60 (±0.07)				
tyrosine	3.44 (±0.04)				
ornithine	1.01 (±0.05)				
lysine	6.99 (±0.08)	7.20 (±0.03)			4.61 (±0.02)
tryptophan	3.08 (±0.04)				3.75 (±0.03)

[a] Values in μg/100 μg. Results are the mean of five replicates and the parenthetical values are 95% confidence limits (Student's *t* test). Value for glutamic acid includes glutamine. tr = traces.

some metabolites (vicine, convicine, L-Dopa β-glucoside) that show hemolytic properties in people sensitive to the so-called "favism" illness (Sisini et al., 1981).

Little information is available on the chemical composition in the different lines and cultivars of *V. faba* over the world (Jamalian, 1978). Some results seem remarkable, i.e., the evidence of the very great variability of the content of L-dihydroxyphenylalanine (L-Dopa) (Sisini et al., 1981), a very useful drug in the treatment of Parkinson's disease, first isolated from *V. faba* in 1913 (Torquati, 1913; Guggenheim, 1913).

This led us to investigate the free amino acid fraction of some *V. faba* seeds because the quantitative determination of the levels of L-Dopa, methionine, an amino acid generally limiting in the plants but not in the cotyledons of *faba* (Dini and Pizza, 1977), and other free amino acids can be useful in the selection of lines and cultivars to be used as human food.

Total free amino acids were extracted with boiling aqueous ethanol, removed by ion-exchange chromatography, and, then, lyophilized. After derivation, the identification of each amino acid was on a gas chromatographic analysis and coinjection with standards.

The presence of free methionine in the test samples was also confirmed on two-dimensional paper chromatograms by a specific reaction based on the color which forms after treatment with a solution of α-naphthylamine diazonium chloride in HCl (Blazsek, 1957).

EXPERIMENTAL SECTION

Samples. The seeds of *V. faba minor* varieties (Scuro Torre Lama, Manfredini, Linea Monti 26) were kindly supplied by Dr. F. Basso, Instituto di Agronomia e Coltivazioni Erbacee della Facoltà di Agraria, University of Naples, whereas the *V. faba* L. cv. (Muchamiel, Paceco) was purchased from Zorzi Sementi, Padova, Italy.

Reagents. The following reagents were used: AG 50W-X4 (H^+) resin, 400–800 mesh (Bio-Rad Laboratories, Richmond, CA); ammonium hydroxide, analytical reagent grade (Merck, Darmstadt, West Germany); nitrogen or dry air; acetic acid, and acetic anhydride, 99% (C. Erba, Milano, Italy); 1-butanol (C. Erba); triethylamine (C. Erba); sodium hydroxide (C. Erba); dichloromethane (C. Erba); boron trifluoride–propanol, 14% (Alltech Associates, Arlington Heights, IL 60004); amino acids (Supelco); norleucine (Supelco) for use as an internal standard; *n*-propyl, *N*-acetyl amino acids (Alltech).

Gas Chromatography. A Perkin-Elmer Model F33 and a Packard Model 427 gas chromatographs fitted with heated FID were used together with a Model 90 Spectra Physic SP 9100 (Data System) recorder. Two different glass columns were employed; the first was a 2 m × 3 mm i.d. column packed with GP 2% SP 2510 DA on Supelcoport, 100–200 mesh; the second column (2 m × 3 mm i.d.) was packed with 0.31% Carbowax 20M, 0.28; Silar 5CP, and 0.06% Lexan stationary phase on Chromosorb W AW, 100–200 mesh. Nitrogen as the carrier gas was used (40 mL/min); detector and injection temperatures were 280 and 300 °C, respectively. Temperature for both columns was programmed at 120 °C for the first 2 min after injection and then from 120 to 270 °C at 10 °C/min, holding at the upper limit. Attenuation was set at 64 × 10. Two microliters of the derivatized material in CH_2Cl_2 are taken for the GC analysis, after addition of norleucine derivative as the internal standard.

Extraction and Separation. In a typical experiment, the powdered seeds (200 g) of Muchamiel bean were extracted for 1 h with hot 80% (v/v) aqueous ethanol (4 × 1500 mL). The combined extracts were concentrated under reduced pressure at less than 40 °C (Bell et al., 1977). The aqueous suspension was extracted repeatedly with Et_2O to remove lipids and pigments and then lyophilized.

A 10-mL solution of 50 mg of sample (in 0.01 N HCl) was passed through a column of AG 50W-X4 resin in the acid form, previously treated as suggested by Lazarus (1973). The resin was washed with 20 mL of 0.01 N HCl followed by 30 mL of distilled water. The amino acids were eluted with 30 mL of 2 N ammonium hydroxide and lyophilized again.

After conversion into the respective *n*-propyl, *N*-acetyl derivatives according to Adams (1974), the quantitative evaluation of individual amino acids was carried out by the triangulation of peaks from the GLC traces. Detection of methionine was carried out also on paper chromatography.

The amino acid mixture was separated on Whatman 3MM paper by two-dimensional chromatography in a direct comparison with a parallel paper chromatogram of a pure reference methionine run in the same solvent systems; BAW (1-butanol–acetic acid–water, 4:1:1 v/v) and phenol–water (3:1 by weight) were used respectively for the first and the second development (Harborne, 1980).

The resulting chromatograms were dried and sprayed with a mixture composed of equal volumes of 0.1% α-

naphthylamine in 10% hydrochloric acid and 0.5% $NaNO_2$ in water. The chromatograms were dried again for 5 min at 80 °C, after which the methionine spots appeared as an orange-yellow hues exhibiting a dark red fluorescence under ultraviolet light.

RESULTS AND DISCUSSION

The free amino acid composition from seed extracts is listed in Table I. All amino acid values are expressed as $\mu g/100$ μg of total recovered amino acids; glutamine is converted to its respective acid during propylation step, and this adds to the glutamic acid value reported.

The most significant finding are the very high concentration of Dopa and methionine which constitute on the average about 60% of the available amino acid pool.

All species contain low concentrations of glycine, leucine, and isoleucine and show a general prevalence of alanine, valine, and threonine.

Small amounts of aromatic amino acids, tyrosine and tryptophan, are also evident, but no phenylalanine is found in any samples. Aspartic acid and arginine were not detected in the cultivars examined, whereas proline is present in appreciable level only in one of the samples (Scuro Torre Lama).

Although the species were basically similar, an interesting difference in the content of L-Dopa between the two cultivars of *V. faba* L. was found. The low L-Dopa in Muchamiel was balanced mainly by a higher methionine percentage. The Muchamiel variety is also unique among the cultivars investigated by its content of cysteine, glutamic acid, histidine, ornithine, and its considerable concentration of serine.

The high L-Dopa amount is in good agreement with the previous data reported by Longo et al. (1974), who found in some *Vicia* seeds also moderate amounts of glutamic and aspartic acids: sulfur-containing amino acids were almost entirely absent in the free form. In spite of this, our data indicate a very high free methionine content in all samples.

The nature of this result led us to confirm the presence of methionine also by a second analytical method in addition to the gas chromatographic one.

The so-observed presence of substantial level of methionine in these cultivars could be of interest from a food technological point of view. It has been considered that an increase in this amino acid would be an important objective in the breeding of proteins with increased nutritional quality (Smartt, 1975).

The increase in methionine accompained by a relative decrease in L-Dopa seems an interesting result. More detailed investigations are necessary, however, to get a clearer picture of this finding; further studies are needed on the composition of the amino acids of the protein hydrolysate before any chemotaxonomic conclusions.

Registry No. L-Dopa, 59-92-7; L-methionine, 63-68-3.

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