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Received for review April 23, 1973. Accepted July 23, 1973. Use of a company or product name by the department does not imply approval or recommendation of the product to the exclusion of others which may also be suitable.

Gel Chromatography of Sunflower Proteins

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The salt-extractable proteins in sunflower flour were characterized by gel chromatography, disk electrophoresis, and amino acid composition. The proteins from Commander, Majak, and Valley sunflower and a soybean control were 69–70% dispersible in 2.5% neutral salt solution. These proteins were separated into five fractions by gel chromatography on a standardized Sephadex G-200 column. The fractions I–V contained about 5, 49, 9, 24, and 12% of the extracted meal proteins, respectively, in the three sunflower varieties. After dialysis, fraction I contained a large proportion of nucleic acids, while chlorogenic acid appeared to be bound to only fraction V proteins. Molecular weight estimations indicated that, on the average, the five sunflower protein fractions were similar in molecular weight to the five soybean protein fractions. Sedimentation analyses of Valley fraction II showed that the major protein component in this fraction had a sedimentation coefficient of 12.1 *S*. The Valley proteins demonstrated fewer bands on disk electrophoresis at pH 8.9 than the soybean proteins. Amino acid analysis indicated that soybean was higher in lysine but lower in methionine than sunflower. Majak proteins were higher in lysine and methionine than Commander and Valley proteins. The major protein fraction II contained high proportions of isoleucine, phenylalanine, threonine, and nonessential amino acids. The fraction IV proteins were very rich in lysine and methionine, while the fraction V in each sunflower variety was very deficient in these essential amino acids.

In recent years, sunflower has become an important oilseed crop in many countries and ranks second in importance as a world source of vegetable oil. In addition, the defatted meals from dehulled seeds contain a higher content of protein than some other oilseed meals (Sosulski and Bakal, 1969). On the basis of their solubility in dilute salt solutions, the major portion of the sunflower proteins was shown to be globulins (Osborne and Campbell, 1897). Their low water solubility but high dispersibility in neutral salt solutions suggested that sunflower proteins may differ from soybean proteins in biochemical characteristics and functional properties (Sosulski and Bakal, 1969). While sunflower meals are presently fed to livestock, the functional properties of the protein could form the basis for utilizing sunflower flours and protein concentrates in new food products.

Few investigations have been conducted on the nature and biochemical properties of the globular proteins in sunflower meal (Gheyasuddin *et al.*, 1970; Hohlenko, 1960; Joubert, 1955). The objective of the present study was to characterize the salt-extractable proteins from sunflower flour by gel chromatography, disk electrophoresis, and amino acid analysis. The proportions of protein, nucleic acid, and chlorogenic acid were determined on the protein fractions as well as their molecular weights (*MW*). Soybean flour proteins were used as reference proteins in this study.

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EXPERIMENTAL SECTION

Seeds of Commander, Majak, and Valley sunflowers and Altona soybeans were obtained from varietal tests grown at the Research Station, Canada Department of Agriculture, Morden, Manitoba. After dehulling the seeds, the lipids were extracted from the ground sunflower kernels with *n*-hexane for 16 hr at room temperature. The solubility of the sunflower flour proteins was determined by two extractions with 0.02 *M* sodium phosphate buffer, pH 7.0, containing 2.5, 5.0, and 10% sodium chloride. Each extraction was conducted at room temperature for 45 min at a meal to solvent ratio of 1:15 (w/v). After centrifugation at 10,000 × *g* for 10 min, the yields of salt-soluble nitrogen (N) were determined by the micro-Kjeldahl procedure (AOAC, 1970).

For chromatography, the proteins were extracted from the soybean and sunflower flours by the above procedure with the 2.5% sodium chloride solution buffered to pH 7.0 and containing 0.01 *M* 2-mercaptoethanol in order to maintain the proteins and phenolic acids in a reduced state. After centrifugation, the combined supernatants were applied directly to the Sephadex gel column. In an alternate procedure, the sunflower extracts were dialyzed before column chromatography. These dialyses were conducted against distilled water containing 0.01 *M* 2-mercaptoethanol for 72 hr at 4°. The contents of the dialyses bags were freeze-dried for storage and, before chromatography, were resolubilized in the sodium chloride solution.

The salt-soluble proteins were fractionated at 4° on a 2.5 × 83 cm column packed with Sephadex G-200. The proteins were eluted from the column by upward flow of the above sodium chloride solution containing 0.02% sodium azide (a bacteriostatic agent). The flow rate was

Table I. Effect of Sodium Chloride Concentrations on the Extractability of Soybean and Sunflower Flour N

	Percentage of flour N in the extracts									
		Commander			Valley			Majak		
Extraction	Soybean, 2.5%	2.5%	5%	10%	2.5%	5%	10%	2.5%	5%	10%
1st	61.3	54.6	60.0	62.2	63.0	68.3	69.1	58.3	62.4	63.5
2nd	16.5	14.8	15.4	13.1	15.2	10.6	10.1	13.0	11.4	10.0
Total	77.8	69.4	75.4	75.3	78.2	78.9	79.2	71.3	73.8	73.5

Table II. Percent N, Uv Absorption Characteristics and Molecular Weight (*MW*) of Salt-Extractable Soybean Protein Fractions Separated on a Sephadex G-200 Column and Water-Extractable Protein Fractions Reported in the Literature

Salt-extractable proteins in the present study					Water-extractable proteins (Obara and Kimura, 1967)		Water-extractable proteins (Wolf, 1970)		
Sephadex G-200 fractions	% N in fractions	λ_{max}, nm	280:260 nm ratio	*MW*	Sephadex G-200 fractions	λ_{max}, nm	Ultracentrifugation fractions	% of N in fractions	*MW*
A	19	~260	0.9	~600,000	A	~260	15S	11	~600,000
B	57	280	1.3	400,000	B	278	11S	31	350,000
C	5	275	1.0	120,000	C	270–278	7S	37	61,000–210,000
D	11	275	1.1	30,000	D	270–278	2S	22	8000–21,000
E	1	265	0.8		E	~260			

maintained at 10 ml/hr. Using Blue Dextran-2000 (*MW* = 2,000,000), the void volume (*Vo*) of the column was found to be 164 ml. For the estimation of *MW*, the column was calibrated by determining the elution volumes (*Ve*) of five known proteins. The standard proteins were ribonuclease A (*MW* 13,700), chymotrypsinogen A (*MW* 25,000), ovalbumin (*MW* 45,000), aldolase (*MW* 158,000), and apoferritin (*MW* 480,000). A regression equation was derived between the reduced elution volume (*Ve*/*Vo*) and the logarithm of the *MW* of the standard proteins (Determann and Mitchel, 1966). The regression between *Ve*/*Vo* and the logarithm of the *MW* was highly significant (P = 0.001). The regression equation

$$Ve/Vo = 5.312 - 0.749 \log MW$$

was used to draw a selectivity curve for the determination of *MW* in the soybean and sunflower protein fractions.

The five protein peaks obtained in the gel chromatography of both the undialyzed and dialyzed salt extracts were designated as fractions A, B, C, D, and E for soybean and as fractions I, II, III, IV, and V for the sunflower varieties in order of their elution from the column. The five fractions were scanned between 220 and 350 nm for the spectrophotometric determination of the absorption maxima (λ_{max}), the total composition of protein (Groves *et al.*, 1968), nucleic acid content using yeast ribonucleic acid (RNA) as a standard (Webb, 1958), and the amount of chlorogenic acid (Moores *et al.*, 1948) in the samples. The protein fractions were then purified and concentrated by dialysis in the presence of the reducing agent and freeze-dried.

Analytical ultracentrifugation was performed using a Beckman Spinco Model E analytical ultracentrifuge operated at 20° and 60,000 rpm.

The disk electrophoresis of the protein fractions from soybean sample and the sunflower variety, Valley, was conducted according to the procedure of Davis (1964). Protein bands were detected by staining with Coomassie Brilliant Blue (Crambach *et al.*, 1967). The relative electrophoretic mobilities (*Rm*) of stained protein bands were obtained with respect to a marker dye, Brom Phenol Blue.

Amino acid analyses of the soybean and sunflower flours and the major sunflower protein fractions II, IV, and V were conducted on a Beckman model 120C analyzer according to the two-column procedure of Spackman *et al.* (1958). Before analysis, the proteins were hydrolyzed with 6 *N* HCl at 110° for 24 hr under vacuum in sealed tubes.

RESULTS AND DISCUSSION

The water- and salt-soluble soybean proteins were highly dispersible in the two extractions with 2.5% neutral salt solution containing 0.01 *M* 2-mercaptoethanol (Table I). Nash and Wolf (1967) reported that the proteins in soybean isolates were 66–78% dispersible in salt solutions containing 0.01 *M* reducing agent. The Valley sunflower proteins were similar in solubility to soybean in the 2.5% salt solution but higher concentrations were only slightly beneficial in improving the extractability of the flour proteins. While slightly less soluble than Valley, the other two sunflower varieties were also more soluble in the 5 and 10% neutral salt solutions.

The first extraction removed a variable proportion of sunflower flour nitrogen (55–69%) (Table I). The second extraction of each sample recovered an additional 10–15% of the flour nitrogen, the higher levels occurring when the first extraction was less efficient. In total, the differences in extractability among the three salt concentrations were not large. Two extractions with the 2.5% neutral salt solution were adopted for the present investigation on protein fractions in sunflower and soybean.

The salt-soluble proteins from soybean were fractionated on the Sephadex G-200 column into five peaks whose ultraviolet (uv) absorption characteristics were similar to those reported by Obara and Kimura (1967) (Table II). With very dilute protein solutions and a long Sephadex G-200 column (3 × 210 cm), Hasegawa *et al.* (1963) fractionated the water-extractable soybean proteins into 11 peaks but higher concentrations eliminated the smaller peaks.

The peak absorption of fraction B corresponded to that of a pure protein solution (280 nm), while the other fractions showed varying degrees of contamination with nucleic acids. Pure nucleic acids absorb at 260–265 nm and fractions A and E with their low 280:260 nm ratios appeared to be primarily nucleic acids, as were the A and E fractions described by Obara and Kimura (1967).

The *MW* of the four peaks in the present study (A, B, C, and D) corresponded to the values reported by many investigators for the ultracentrifugation fractions of the water-soluble soybean proteins, 15 *S*, 11 *S*, 7 *S*, and 2 *S* (Table II). However, the proportions of salt-soluble proteins in the Sephadex G-200 fractions appeared to be widely different than the distribution of proteins in the ultracentrifugation study. Roberts and Briggs (1965) demonstrated that the 7 *S* component of soybean protein would

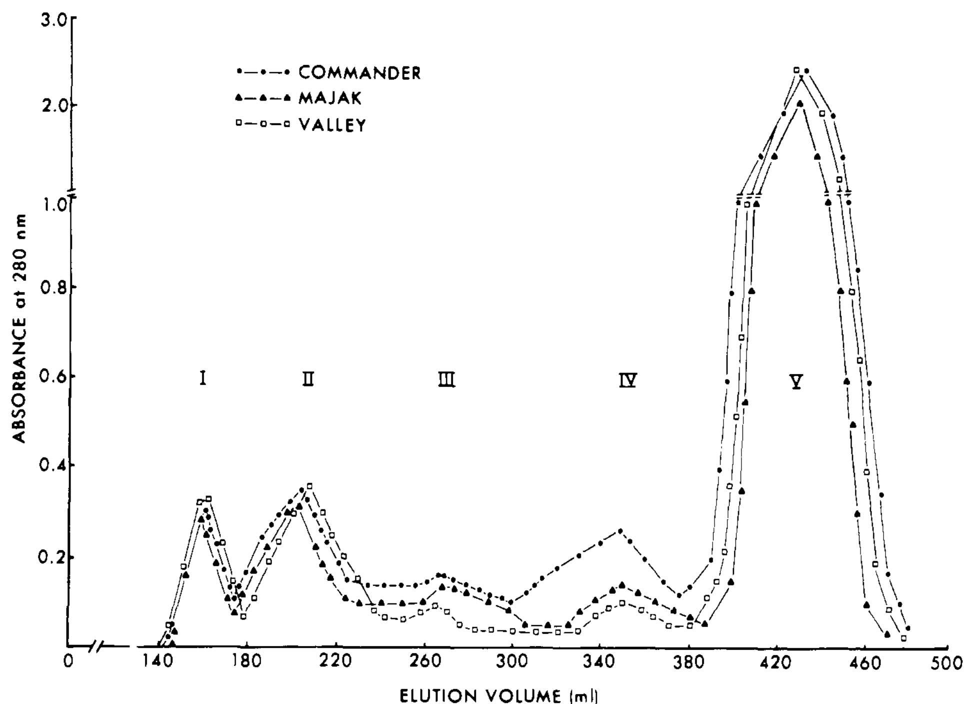

Figure 1. Fractionation of the salt-extractable sunflower proteins on a Sephadex G-200 column with neutral salt solution.

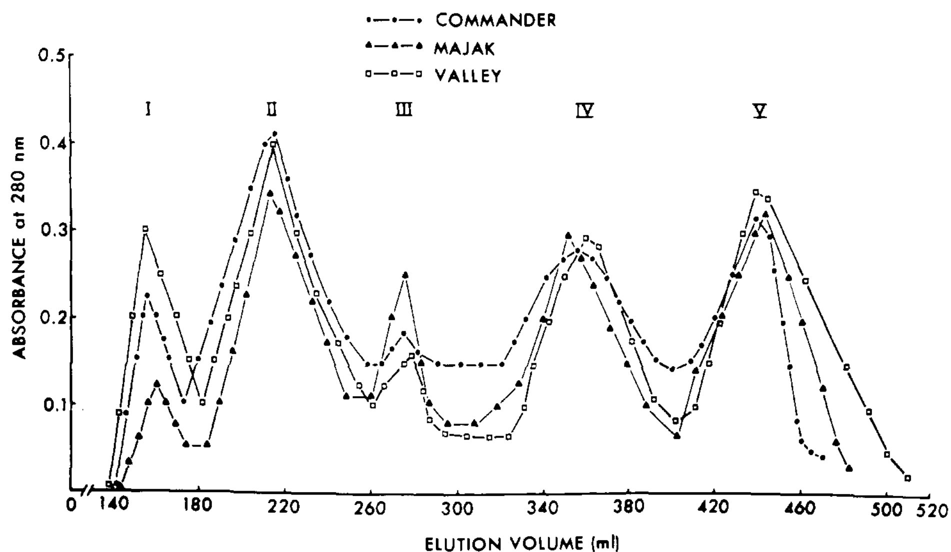

Figure 2. Fractionation of the dialyzed salt-extractable sunflower proteins with neutral salt solution.

exhibit an apparent *MW* of 330,000 at low protein concentrations (below 0.1%), as occurred during the present column fractionation. It appeared that most of the 7 *S* fraction was eluted with the B component and the total yield of B + C components corresponded to the proportions of 11 *S* + 7 *S* in the literature (Wolf, 1970). Possibly the main reason for the poor separation of the 7 *S* and 11 *S* fractions is the length of the Sephedex column. Apart from this discrepancy the gel chromatography technique gave a satisfactory estimate of the uv absorption and *MW* characteristics of soybean proteins.

The sunflower proteins also fractionated into five peaks, with varietal differences being relatively small (Figure 1). Fraction V showed far greater absorption at 280 nm than the higher *MW* fractions. However, after dialysis to remove the low *MW* compounds (below *MW* 12,000), this peak became proportional in size to the other fractions (Figure 2). Dialysis of the salt extracts also permitted the application of greater protein loads on the Sephadex G-200 column with superior resolution of the peaks than was possible with the nondialyzed samples (Figure 1).

Fraction 1 from the salt-extractable proteins in sunflower was eluted in the void volume of the Sephadex G-200 (Figure 2) and would have a molecular weight of over 600,000, the exclusion limit of the gel (Table III). However, this fraction constituted only 5-7% of the sample protein from each variety and much of the absorbance at 280 nm originated from a high peak at 260-265 nm due to nucleic acids. After dialysis, the absorbance maxima for fraction I remained near 260 nm and the absorbance ratios were still less than 1.0, suggesting that some of the nucleic acids may be bound to the proteins. The limited contamination of fractions II, III, and IV with nucleic acids was largely eliminated by dialysis and the absorption maxima for these dialyzed fractions approached 280 nm in the three sunflower varieties.

Fractions II, III, and IV appeared within the fractionation range of the gel and, after dialysis, their *MW* were estimated to be 320,000-340,000, 90,000, and 20,000, respectively (Table III). Fraction II was the major sunflower protein, constituting about 50% of the salt-soluble proteins in each variety. This fraction was similar in molecular

Table III. Protein Content, Uv Absorption Characteristics, RNA Distribution and the *MW* in Nondialyzed and Dialyzed Salt-Extractable Sunflower Protein Fractions Separated on a Sephadex G-200 Column

	Nondialyzed					Dialyzed			
Variety and protein fractions	% of protein in fractions[a]	λ_{max}, nm	280:260 nm ratio	Distribution of RNA mg/100 mg of protein	*MW*	% of protein in fractions[b]	λ_{max}, nm	280:260 nm ratio	*MW*
Commander									
I	5	260	0.7	1.02	⩾600,000	4	260	0.9	⩾600,000
II	42	276	1.3	1.91	370,000	48	280	1.3	340,000
III	12	276	1.1	0.70	100,000	8	280	1.2	90,000
IV	20	276	1.0	1.55	20,000	27	280	1.1	20,000
V	17	328		3.11		11	328		
Majak									
I	7	265	0.9	1.04	⩾600,000	4	265	0.9	⩾600,000
II	50	278	1.4	2.34	370,000	50	278	1.5	330,000
III	9	275	1.1	0.84	100,000	7	280	1.2	90,000
IV	20	275	1.2	1.85	20,000	27	280	1.4	20,000
V	12	328		3.04		9	328		
Valley									
I	6	260	0.7	1.56	⩾600,000	4	260	0.8	⩾600,000
II	48	278	1.1	2.10	360,000	51	280	1.2	320,000
III	9	275	1.1	0.69	100,000	9	280	1.2	90,000
IV	23	275	1.1	1.71	20,000	24	280	1.2	20,000
V	10	328		3.30		9	328		

[a] Protein content was determined by the spectrophotometric procedure of Groves *et al.* (1968). [b] Protein content by the micro-Kjeldahl procedure (AOAC, 1970).

weight and quantity to the B fraction in soybean (Table II). Fraction III appeared to parallel C in soybean but the low molecular weight Fraction IV occurred in much higher concentration than the D fraction of soybean. However, the limitations of the Sephadex G-200 fractionation technique would make this a tentative conclusion.

The major component of the Valley fraction II proteins had a sedimentation coefficient ($s^\circ_{20,\ 0.15\ M\ NaCl}$) of 12.1 *S* (Figure 3). However, the fraction also contained components whose sedimentation coefficients were 9 *S* and 4 *S*. These possibly represent contamination with fractions III and IV but could also be due to dissociation of the 12.1 *S* protein. Because of the impurity of this fraction, the ultracentrifugation study was not continued on the other fractions but a more detailed investigation of sunflower proteins has been initiated with more homogeneous proteins obtained by ion-exchange chromatography.

Fraction V constituted 10–17% of the salt-soluble proteins in the three sunflower varieties and, after dialysis, the fraction was reduced to about 10% of the extracted protein (Table III). The *MW* of fraction V could not be estimated because the peaks were eluted under the lower fractionation range of the column (*MW* 5000) (Figure 2). The large peaks obtained for fraction V before dialysis reflected the composition of low *MW* nonprotein constituents such as phenolic and nucleic acids (Figure 1). High proportions of the nucleic acids were concentrated in fraction V, as indicated by the RNA contents of 3 mg/100 mg of protein in each variety (Table III). The RNA determination was based on the ribose content of the extracts and interference may result from pentoses associated with proteins or presented in the free form in fraction V from undialyzed protein extracts. The 280:260 nm ratio could not be used to estimate the nucleic acid contamination because of the large absorption peak at 328 nm. Chlorogenic acid, which absorbs at 328 nm, has been identified by numerous investigators as the principal phenolic acid in sunflower meal (Sosulski *et al.*, 1972). Dialysis removed a high proportion of the chlorogenic acid from the fraction V proteins but a significant proportion was still detected in this fraction. Apparently this phenolic acid was bound to the fraction V proteins. Phenolic acids are also capable of strong hydrogen bonding with the Sephadex dextran gels

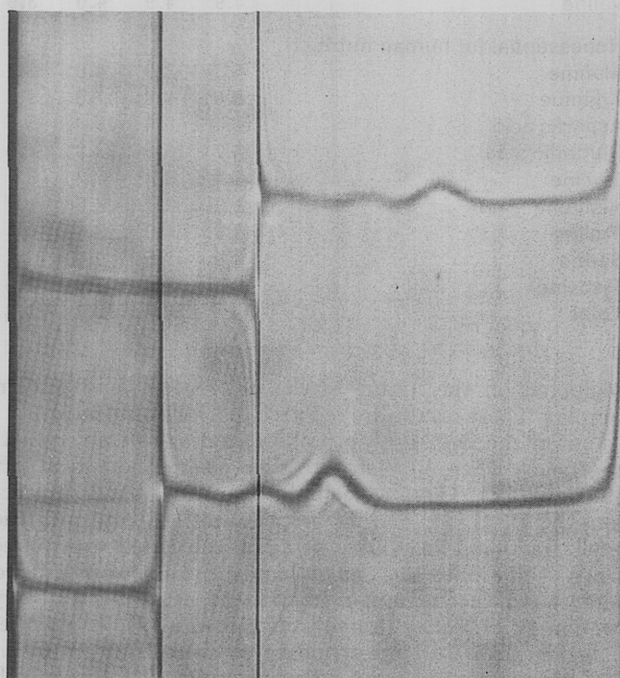

Figure 3. Sedimentation pattern of Valley fraction II proteins in 0.15 *M* NaCl. Lower pattern 0.750%, upper pattern 0.375%. Analyzer angle 70°. The photograph was taken after 24 min at 60,000 rpm.

and a chlorogenic acid–protein complex would be retarded in elution during the protein fractionation (Brook and Munday, 1970). This would explain why proteins which failed to be dialyzed from the fraction (over *MW* 12,000) were eluted under the lower fractionation range of the column (below *MW* 5,000).

Under alkaline conditions, fraction V proteins exhibited the characteristic green color of chlorogenic acid, while the other four fractions remained white. Because the color problem has been the principal factor in limiting the use of sunflower proteins in foods, further investigations were

Table IV. Relative Electrophoretic Mobilities (pH 8.9) of Salt-Extractable Soybean and Sunflower Proteins in the Fractions Separated on a Standardized Sephadex G-200 Column[a]

Fractions from soybean	*Rm* values for soybean proteins	Fractions from sunflower	*Rm* values for sunflower proteins, Valley
A	0.02, 0.18, 0.25, 0.37, 0.42, 0.55, 0.65, 0.70, 0.83, 0.95	I	0.22, 0.45, 0.65
B	0.05, 0.20, 0.25, 0.38, 0.69, 0.80, 0.89, 0.95	II	0.08, 0.20, 0.35, 0.65, 0.75, 0.83
C	0.02, 0.15, 0.33, 0.55, 0.65, 0.78, 0.90	III	0.22, 0.55, 0.65, 0.75, 0.95
D	0.25, 0.33, 0.38, 0.63, 0.70, 0.75, 0.80, 0.90	IV	0.05, 0.12, 0.22, 0.32, 0.45, 0.60, 0.70, 0.82, 0.88
E	Not stained	V	0.20, 0.60, 0.66

[a] Major bands are underlined.

Table V. Amino Acid Compositions of Soybean and Sunflower Flours and Protein Fractions (g of Amino Acid/16 g of N)

Amino acid	Soy flour	Commander Flour	Commander II	Commander IV	Commander V	Majak Flour	Majak II	Majak IV	Majak V	Valley Flour	Valley II	Valley IV	Valley V
Essential for human nutrition													
Isoleucine	4.8	3.9	4.4	3.1	3.9	3.9	4.9	3.6	3.7	3.7	4.8	3.4	3.4
Leucine	7.8	5.9	5.7	5.3	5.2	6.0	5.9	5.5	4.9	6.0	5.5	5.5	4.7
Lysine	6.2	3.0	2.2	3.0	1.8	3.6	3.1	4.1	1.7	3.2	3.6	4.3	2.5
Methionine	1.5	1.7	2.4	3.5	2.0	2.0	3.0	6.8	2.0	1.7	4.0	5.8	2.0
Phenylalanine	4.8	4.5	5.0	2.8	4.7	4.1	4.9	1.8	4.2	4.2	4.6	2.4	4.3
Threonine	3.8	2.8	3.0	1.8	2.2	2.9	3.2	2.0	2.3	3.1	2.8	2.1	2.3
Valine	4.9	4.9	4.0	3.9	4.7	4.7	4.0	3.8	4.5	4.8	4.9	4.0	4.7
Nonessential for human nutrition													
Alanine	4.3	3.9	4.0	2.2	3.5	4.0	4.5	2.6	3.5	3.7	4.2	2.6	3.6
Arginine	8.0	8.8	7.8	9.4	6.7	9.0	8.5	8.8	8.5	9.4	9.6	10.5	7.9
Aspartic acid	11.1	9.0	10.3	5.6	9.2	8.6	9.3	6.8	8.9	8.7	9.2	7.3	8.6
Glutamic acid	17.7	23.5	23.8	34.0	24.2	21.6	22.6	33.0	25.1	22.6	20.4	28.1	23.2
Glycine	4.4	5.2	6.2	3.5	4.0	6.0	7.3	3.6	3.9	5.1	7.8	3.7	4.5
Histidine	1.8	2.1	2.5	1.1	2.1	2.2	2.8	1.4	2.0	2.2	2.7	1.3	2.1
Proline	4.8	4.0	3.6	4.4	4.0	4.3	2.9	4.7	3.1	3.9	3.0	4.4	3.6
Serine	3.9	3.5	4.0	2.6	3.5	3.3	3.9	2.7	3.3	4.0	4.0	2.8	4.2
Tyrosine	3.6	2.8	1.8	2.6	1.9	2.3	1.8	3.2	1.7	2.2	1.6	2.9	1.7
Total	93.4	89.5	90.7	88.8	83.6	88.5	92.6	94.4	83.3	88.5	92.7	91.1	83.3

conducted on the nature of the chlorogenic acid–protein complex. These results are reported in a following paper.

Four of the five soybean protein fractions were very heterogeneous when subjected to disk electrophoresis at pH 8.9 (Table IV). There were 10, 8, 7, 8, and 0 bands in fractions A, B, C, D, and E, respectively. In contrast, the small fraction I in Valley proteins contained only three bands, while only five major bands and one minor band were found in the major fraction II. Numerically, only fraction IV of Valley proteins contained a greater number of bands than the corresponding soybean fraction. However, three proteins were detected in fraction V, while E in soybean did not react with the protein stain. It appeared that the sunflower had a simpler protein system than soybean. As the similarities in *Rm* values would suggest, each protein fraction was contaminated to some degree with proteins from other fractions.

The amino acid compositions of the soybean and sunflower flours and protein fractions II, IV, and V are presented in Table V. The soybean flour proteins were characterized by high levels of essential amino acids but lower proportions of glutamic acid than sunflower proteins. Specifically, soybean contained over 6% lysine, while the mean for the sunflower flours was only slightly above 3%. On the other hand, sunflower flours were generally higher in methionine than Altona soy flour.

Differences between the sunflower varieties were relatively small. Majak flour was higher in lysine and methionine than Commander and Valley (Table V). Previously, another Russian variety, Peredovik, was found to be higher than Commander in these two essential amino acids (Sosulski and Sarwar, 1973). In both studies, Commander flour was also higher than other sunflower varieties in the nonessential glutamic acid.

Differences in amino acid composition of sunflower flours were also reflected in variations among the protein fractions but differences between fractions were even greater. The major protein fraction II contained high levels of isoleucine, phenylalanine, threonine, alanine, and several nonessential amino acids. Fraction IV, which constituted about one-quarter of the salt-soluble proteins, was very rich in lysine and methionine, but deficient in phenylalanine. Fraction IV was also unusually high in arginine, glutamic acid, proline, and tyrosine. The amino acid composition of fraction V was characterized by the low levels of lysine and methionine and the rather poor recovery of total amino acids. The differences between the total amino acids recovered on the analyzer and the Kjeldahl nitrogen determinations represented degradation products of serine, tryptophan, amide nitrogen from asparagine, and glutamine and nonprotein nitrogen from the samples.

ACKNOWLEDGMENT

This investigation was supported by a scholarship from the Canadian International Development Agency and a research grant from the National Research Council of Canada.

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Received for review May 3, 1973. Accepted July 30, 1973.

Synthesis and Laboratory Evaluation of 1-(2,6-Disubstituted benzoyl)-3-phenylureas, a New Class of Insecticides. II. Influence of the Acyl Moiety on Insecticidal Activity

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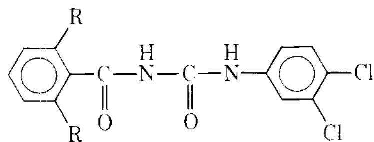
The insecticidal properties of a number of 1-acyl-3-phenylureas were evaluated with larval stages of *Aëdes aegypti* L., *Pieris brassicae* L., and *Leptinotarsa decemlineata* Say. Only mono- and di-ortho-substituted benzoyl derivatives showed interesting larvicidal activities. For compounds with the common formula



the order of effectiveness was R = H, OH, OCH_3, C_2H_5 < CH_3 < Br ≪ Cl < F. The most powerful toxicant proved to be 1-(2,6-difluorobenzoyl)-3-(4-trifluoromethylphenyl)urea. Together with 1-(4-chlorophenyl)-3-(2,6-dichlorobenzoyl)urea (PH 60-38), a second chemical has been chosen for further development, namely 1-(4-chlorophenyl)-3-(2,6-difluorobenzoyl)urea (PH 60-40). A short survey is given of the properties of both PH 60-38 and PH 60-40.

In part I of this series of articles (Wellinga *et al.*, 1973) we concluded that 1-(4-chlorophenyl)-3-(2,6-dichlorobenzoyl)urea (PH 60-38) was the most promising larvicide out of a series of 157 1-(2,6-dichlorobenzoyl)-3-phenylureas. In the present article we report the synthesis and laboratory evaluation of another series of 1-acyl-3-phenylureas, where acyl in most cases represents substituted benzoyl, with the 2,6-dichlorobenzoyl moiety excluded.

CHEMICAL METHODS

Microanalyses were carried out in the Analytical Department of the Institute for Organic Chemistry TNO, Utrecht, the Netherlands, under the supervision of W. J. Buis. Nuclear magnetic resonance spectra were recorded in deutero-dimethyl sulfoxide solution on a Varian HA 100 spectrometer. Chemical shifts are reported in parts per million (δ) downfield from the internal reference tetramethylsilane. All melting points have been determined on a Kofler hot stage apparatus because of severe decomposition at higher temperatures.

All compounds referred to in this paper were prepared according to the methods already described (Wellinga *et al.*, 1973). Method B (Figure 1) especially proved to be of great value because of the simplicity of the procedure and the high purity of the products obtained.

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Most of the amides RC(=O)NH_2 or the corresponding acids and nitriles are described in the literature (*e.g.*, Koopman and Daams, 1965). In the following examples only some particular cases are recorded.

Preparation of 2,6-Difluorobenzamide. To a solution of 172.0 g (1.0 mol) of 2,6-dichlorobenzonitrile in 300 ml of dry sulfolane was added 290.0 g (5.0 mol) of finely powdered potassium fluoride. While being stirred vigorously, the mixture was heated at 230–235° for 8 hr. After cooling to 80°, the reaction mixture was poured into 2 l. of water. The resulting suspension was extracted three times with dichloromethane. In order to remove the sulfolane the combined dichloromethane extracts were washed thoroughly with water. After drying, the solvent was removed and the residual oily liquid distilled to give 109.2 g of 2,6-difluorobenzonitrile (78.5%), bp 81–83° (12 mm), mp 30–31°. 2,6-Difluorobenzonitrile was easily hydrolyzed to the corresponding amide in 90% sulfuric acid at 70°, mp 143–145°.

Anal. Calcd for $C_7H_5F_2NO$ (157.12): C, 53.51; H, 3.21; F, 24.18; N, 8.91. Found: C, 53.5; H, 3.4; F, 23.9; N, 9.1.

Preparation of 2-Dimethylamino-6-fluorobenzamide. 2,6-Difluorobenzonitrile (27.8 g, 0.20 mol) was dissolved in 100 ml of acetonitrile. While stirring and cooling to keep the temperature below 30°, dry dimethylamine gas was passed in. After 2 hr the solvent was distilled off at reduced pressure. To the oily residue was added 1 l. of water, after which the pH was adjusted to 9 with a 10% solution of sodium hydroxide. The desired nitrile was iso-