Phytochemistry, Vol. 20, No. 9, pp. 2071–2075, 1981.
Printed in Great Britain.

0031–9422/81/092071-05 $02.00/0

THE SEED GLOBULINS OF *LUPINUS ALBUS*

MARCELLO DURANTI, PATRIZIA RESTANI, MALGORZATA PONIATOWSKA* and PAOLO CERLETTI

Department of General Biochemistry, University of Milan, Milano, Italy

(*Revised received* 8 *January* 1981)

Key Word Index—*Lupinus albus*; Leguminosae; white lupine; seed proteins; glycoproteins; seed amino acids.

Abstract—The seed globulins of *Lupinus albus* were extracted and 12 different proteins were separated: four of them correspond to vicilins and two to legumins. The two groups together represent 77.4% of total seed protein. Two other globulins, 9b and 1, are respectively 12.5 and 6.0% of total. The rest are very minor components. All proteins except 9b have high MW. The legumins undergo association–dissociation equilibria. The amino acid composition is similar for proteins of the same group. It corresponds to a poor nutritional value for the vicilins, which contain only very little or no sulphur amino acids; the legumins are significantly better. Protein 1 has a very good composition and 9b contains more than 40% glutamate/glutamine. All proteins contain carbohydrate, mostly neutral sugars. The amount and composition are typical for proteins of the same group.

INTRODUCTION

Lupine is one of the legumes with the highest protein content in the seeds; the actual amount depends on the species and ranges from 31% of the whole seed in *Lupinus angustifolius* to 44% in *L. luteus* compared to 35% in the soybean [1]. One interesting aspect as a protein source is that it grows under soil and climate conditions that soybean does not tolerate.

Lupine seed proteins were investigated by many approaches, e.g. molecular types and distribution in related varieties [2–7], amino acid composition [8–10] and, for one subunit of one globulin, amino acid sequence [11], influence of nutrients on the protein and amino acid composition of the seed [9, 12, 13]; nevertheless, knowledge of lupine proteins is not as advanced as it is for soybean and other legumes.

As in other legumes, most of the lupine seed proteins are globulins [5]. When analysed by ultracentrifugation two or three components were shown according to the species [2], and electrophoresis separated three bands in all species examined [3]. In particular, *L. albus* yielded three globulin components by both methods [2, 3]. Three globulin fractions which were separated by electrophoresis were also isolated from *L. angustifolius* by conventional preparative protein chemistry [4]. The data reported, however, indicate that these techniques did not satisfactorily resolve all species present.

In a preliminary attempt to resolve the globulins of *L. albus* by other procedures we found more components than those previously described [5]. These studies were continued to characterize each individual globulin, paying special attention to information able to provide a biochemical basis for breeding and selection, or which might be significant for food uses. The results are reported in the present paper.

*On leave of absence from the Institute of Plant Genetics, Polish Academy of Sciences, Poznan, Poland.

RESULTS

Separation and molecular properties of globulins

The globulin extract was desalted on a column of Sephadex G-25 equilibrated with 0.05 M phosphate buffer, pH 7.5, and then fractionated on Whatman DE 52 DEAE-cellulose with increasing NaCl concentration. A typical separation is shown in Fig. 1. Arrows indicate where a new concentration was applied.

Fractions 1 and 2 were not retained on the column. Nine more peaks were separated by increasing the salt concentration stepwise, a procedure that in this case gave better results than continuous gradients. A few very small peaks were also visible, but they varied in different preparations and sometimes did not appear at all. Total recovery was 92.1% of the applied extract.

Gel filtration on Ultrogel AcA 54 (fractionation MW 5000–70 000) resolved peak 9 into two components, globulins 9a and 9b. Globulin 9b was separated as an individual component also by gel filtrating the total globulin extract on Ultrogel AcA 54. As shown in Table 1, isolated 9b was still eluted by 0.25 M NaCl from the DEAE-cellulose ion exchanger but it had a larger elution volume than when chromatographed together with purified 9a or 8 or in the unfractionated globulin extract containing both these proteins. This behaviour suggests interactions occurring between 9b and the other two proteins. Elution volumes for the isolated globulins given in Table 1 indicate that their acidic character increases from 8 to 9a and 9b. This finding is confirmed by isoelectric focusing [14].

Globulins 8 and 9a when gel filtered on Ultrogel AcA 34 (fractionation MW 20 000–360 000) resolved in part into two components with apparent MWs close to a 2:1 ratio (Fig. 2); however, each protein displayed on sodium dodecyl sulphate-polyacrylamide gel electrophoresis (SDS-PAGE) its distinctive subunit pattern over all the peaks (unpublished results). This behaviour suggested that they were liable to association–dissociation

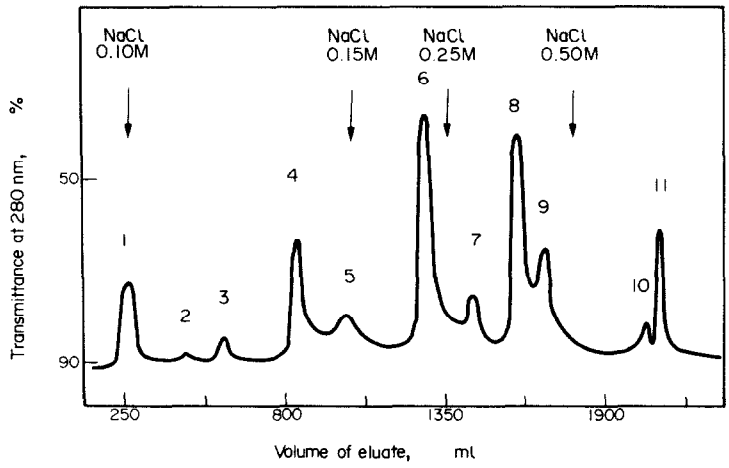

Fig. 1. Separation of globulins of white lupine seeds on Whatman DE 52 cellulose. The desalted extract (600 mg proteins) was applied on the column (24 × 5 cm, V_0 = 250 ml) equilibrated with 0.05 M phosphate buffer, pH 7.5. Elution was at 20°, using the buffer with stepwise additions of NaCl. Flow rate 6 ml/cm²/hr. Fractions of 7 ml were collected.

Table 1. Elution volumes on DEAE-cellulose of globulins eluted by 0.25 N NaCl

Proteins applied	Tube No. at peak maximum	Compound(s) eluted
8	59	8
9a	71	9a
9b	85	9b
8 + 9a	59	8
	75	9a
8 + 9b	57	8
	79	9b
9a + 9b	75	9a + 9b
Peak 9	75	9a + 9b

Peak 9 from the total globulin extract (46 mg), or the isolated globulins after gel filtration (8 mg each), was concentrated and then applied separately or in a mixture on a Whatman DE 52 cellulose column (24 × 2 cm) in 0.05 M phosphate buffer, pH 7.5, containing 0.05 N NaCl. The column was percolated with the buffer containing 0.15 and then 0.25 N NaCl, which eluted the proteins. Proteins in the eluate were identified by gel filtration and by SDS-PAGE.

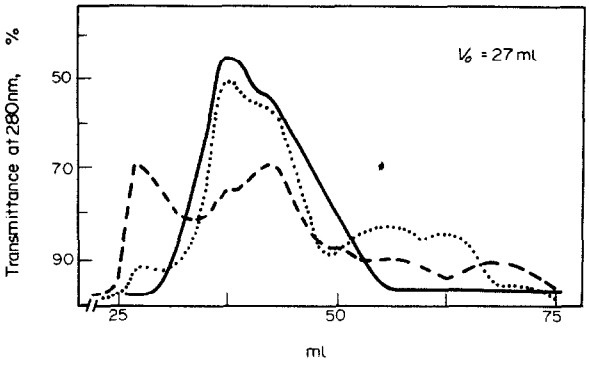

Fig. 2. Elution curves from an AcA 34 column of globulin 8. —— untreated, ··· after 24 hr in saturated sodium chloride solution at 4°, – – – after incubation at 4°, 1 hr each, at pH 5.8, 4.0, 3.0, 5.0, 8.0, 9.0 and overnight at pH 8.2.

phenomena: this was confirmed when they were submitted to modified conditions of pH and ionic strength. As shown in Fig. 2, treated preparations of globulin 8 still displayed the typical peak, with unmodified SDS-PAGE pattern, but it was reduced in size and the ratio of maxima was changed. Other peaks formed, corresponding to higher or smaller MWs, and here the protomer pattern of the original protein was modified. This indicates that the association of subunits was altered under these conditions. Globulin 9a behaved in a similar way.

The gel filtration behaviour of globulin 1 on the gels mentioned and on AcA 22 (fractionation MW 60 000–1 000 000) indicated the presence of several components, with apparent MWs 92 000, 150 000 and 300 000. They all produced the same pattern on SDS-PAGE, suggesting an association–dissociation equilibrium. Indeed, as detailed in a subsequent paper, the values mentioned correspond to multiples of the subunits found in the protein [14]. Globulins 4, 5, 6 and 7 gave one symmetrical peak on all gel filtration media. As described elsewhere [14], all globulins mentioned have their distinctive pattern on SDS-PAGE.

The MW of the globulins and their relative amount are given in Table 2. Except globulin 9b, all the others are large molecules. Globulins 6, 8, 9a and 9b which include the major components of the extract were about constant in amount. Proteins 1, 4, 5 and 7 showed some variability; the other fractions are a minor part of total globulins and varied remarkably in different preparations.

Cellulose acetate electrophoresis of the total globulin extract at pH 7 yielded four bands γ, β, α and δ in order of increasing anodic mobility. Band γ corresponds to globulin 1, globulin 4, 5, 6 and 7 move as band β, 8 and 9a as band α, and 9b yields band δ. The composite bands

Table 2. Molecular weight and distribution of protein and neutral sugar in the globulins of *Lupinus albus*

Globulin	Electrophoretic Component	MW ($\times 10^{-3}$)	Protein		Neutral sugar			
			% of total globulins	v.c.*	% of total	v.c.	mg/100 mg peptide	v.c.
1	γ	92–150–300†	6.0	0.13	11.7	0.37	4.3	0.07
2	—	n.d.‡	0.5	0.40	8.4	0.50	36.9	0.04
3	—	123	0.7	0.57	4.9	0.39	15.5	0.50
					25.0	0.02		
4	β	187	10.0	0.15	10.9	0.09	2.4	0.04
5	β	225	2.1	0.19	3.5	0.38	3.7	0.03
					14.4	0.10		
6	β	260	30.2	0.03	28.9	0.06	2.1	0.09
7	β	143	1.9	0.21	2.9	0.06	3.4	0.09
8	α	330	21.2	0.06	14.5	0.12	1.5	0.07
9a	α	430	12.0	—	4.4	0.11	0.8	0.15
9b	δ	44	12.5	0.04	4.0	0.05	0.7	0.14
10	—	226	2.6	0.46	1.8	0.12	1.5	0.53
11	—	n.d.	0.4	0.25	3.9	0.47	21.5	0.49

*v.c. = variability coefficient.
†See text.
‡n.d. = not determined.

could not be resolved by changing the pH from 7 to 8.6. Fractions 2, 3 and 10 and 11 were too scarce to be evidenced by protein staining.

Amino acid composition

The amino acid compositions of the globulins are given in Table 3. Dicarboxylic amino acids and their amides were measured together: the acidic character of proteins from 4 to 9b on electrophoresis and also on isoelectric focusing [14] is in line with their relatively low lysine and also indicates the presence of unsubstituted carboxyls. Globulin 9b contains unusually much glutamate: its entire composition is peculiar, with extremely high cysteine and/or cystine and low lysine, valine, threonine and aromatic amino acids. Also fraction 11 is rather uncommon with very pronounced lysine, leucine, isoleucine and methionine but no cysteine. All essential amino acids are high in globulin 1, but especially valine and threonine: indeed its MEAA index is close to that of the FAO provisional pattern. On the other hand, globulins 4, 5 and 6 lack sulphur amino acids and tryptophan and have a very poor MEAA index, but 8 and 9a, which include 33.2% of total seed protein, have a better pattern than the total globulins.

Table 3 also includes the index for potential bitter taste of the protein: its value depends on the content in hydrophobic amino acids and in all samples it remains below the level for bitterness.

Carbohydrate component of globulins

Carbohydrate assay in the total extract and in the separated globulins gave the same results before and after precipitation with 10% cold trichloroacetic acid and after gel sieving chromatography on Sephadex G 50. Sugar, however, can be hydrolysed from the proteins with 3 N hydrochloric acid at 160° for 4 hr. This behaviour and the fact that upon dissociation of the oligomeric proteins sugars are found together with peptide and are present only on some subunits [14] indicates that carbohydrates are covalently bound to the peptide.

Neutral sugar in the total globulin extract was 2.3 $\pm$ 0.2 mg per 100 mg protein; the content in individual globulins is given in Table 2. No sugar was found in the eluate between protein peaks.

The proteins differ considerably in carbohydrate content. The very small fractions 2, 3 and 11 represent a special group with very high sugar content. The other proteins contain much less carbohydrate: among them globulin 1 has highest sugar per mg peptide followed by 5, 7, 4 and 6; protein 8 is intermediate and 9a and 9b are the poorest. Globulins 4, 6 and 8 are quantitatively constant components of the extract both for sugar and for protein. Globulins 1, 2 and 5 preserve their specific quantitative carbohydrate composition although they considerably oscillate in amount in the seed. In 3, 10 and 11 protein and sugar vary independently.

As for the type of sugar (Table 4), mannose is most abundant and frequent, followed by galactose; other sugars are also present and the distribution in each protein has distinctive traits.

Amino sugars are scarce: 0.19 mg per 100 mg protein in the total globulin extract. Only *N*-acetylglucosamine was found. It is highest in 8 and in 9a.

DISCUSSION

Globulins are by far the major proteins of seed also in *L. albus*. This study has documented the unsuspected complexity of their pattern. Interestingly, all these molecules to a different extent contain carbohydrate which appears protein-bound.

Table 3. Amino acid composition of seed globulins in *Lupinus albus*, %

	1	3	4	5	6	7	8	9a	9b	11
His	5.53	3.40c	1.21b	1.79	1.35	1.28	1.86	2.06	1.84	2.57
Lys	6.34	5.90	4.10	3.78	3.51	3.08	3.98	3.60	1.02	7.85
Arg	4.04	12.41b	12.80	12.35	13.77b	13.35	11.86	9.98	11.14	9.91
Asp	11.81	9.25b	13.43	12.17	11.74	12.86	10.12	10.39	9.81	7.08b
Thr	7.43c	4.68	3.04b	2.85b	2.63	2.71	3.85	4.78	1.36c	3.60
Ser	8.79b	7.13	5.65	6.61	5.53	4.91	4.91b	4.11	5.07	6.95
Glu	10.10	21.15	23.34	25.27	24.66	27.31	24.76	23.56	41.06	12.10
Pro	5.98	5.07	4.04	4.64	4.08	3.70b	4.38c	4.34	2.00c	5.12
Gly	4.05b	4.85	3.31b	4.09	3.90	3.19	3.91b	4.25	2.00b	6.95b
Ala	4.21	3.23	2.88	2.73c	2.66	2.75	2.63	2.84b	0.83	3.86b
Cys	2.62	0.83	tr.	0.03	0	0	1.26c	1.14c	6.55	0
Val	6.30	3.96	3.09b	2.39	2.95b	3.13	3.81	3.88b	1.73	2.96
Met	0.75	0.28	tr.	tr.	0.00	0.00	0.18	0.54	0.50c	3.99
Ile	4.91	3.40	4.89	3.39	4.09	4.46	5.81	5.40	3.33b	5.66
Leu	9.35	5.96	7.31	6.49	7.00	6.84	7.54	8.61b	8.76	11.71
Tyr	3.02	3.63	4.49	5.27b	6.69	6.32	5.34	4.80	1.33	4.89
Phe	4.82	3.57	4.91	4.41b	4.36	3.14	3.96	3.78	1.10	5.79
Trp	1.11	1.67	0.04b	0.00	0.00	0.00	0.96	1.19c	0.03c	n.d.
Index for potential bitter taste	1202	1088	1132	1090	1159	1135	1197	1183	856	1322
MEAA	99.46	80.77	33.23	25.12	25.91	26.39	82.82	83.97	35.12	91.50

Each value is the average of at least three separate determinations. Variability coefficients are below 0.05 except where indicated, b (0.05 ÷ 0.10) and c (>0.10). Index for potential bitter taste was calculated according to Ney [25]; values below 1300 indicate no bitterness. MEAA (Modified Essential Amino Acid index) was calculated according to Mitchell [26].

Table 4. Monosaccharide composition of carbohydrate in globulins, %

Sugar	Total extract	Globulins						
		1	4	5	6	8	9a	9b
Xylose	tr	3.9	—	—	—	tr	tr	3.3
Arabinose	tr	20.8	—	—	—	tr	tr	7.5
Galactose	9.2	61.2	—	tr	tr	2.9	20.1	31.2
Mannose	83.2	10.7	96.3	72.0	94.2	79.9	68.9	41.5
Glucose	tr	2.8	—	22.9	—	6.3	1.3	13.1
N-Acetyl-glucosamine	7.6	0.6	3.7	5.1	5.8	10.9	9.7	3.4

tr = trace.

The scatter of data giving the globulin composition of different seed samples indicates how far each protein is a specific species in the dormant seed or if it may undergo fluctuations. The pattern of distribution, however, refers to a given set of field conditions: it was shown that deficit of inorganic sulphur in the soil drastically changed in *L. angustifolius* the ratio of the two major groups of seed globulins, namely vicilins and legumins [9].

Globulins 4, 5, 6, 7 and respectively 8 and 9a are similar in amino acid composition, electrophoretic behaviour and in the type of bound sugars. From its properties the first group can be ascribed to the vicilins and the second one to the legumins by analogy with the vicilins and legumins in other legumes [16]. The vicilins correspond to conglutin β (2, 3) and to the 7.4–7.8 S fractions, MW 173 000–181 000, isolated from *L. luteus* and *L. angustifolius* by Gerritsen [8]; the legumins can be assimilated to conglutin α [2, 3] and to the 11.6 S fraction of Joubert, MW 336 000 [6]. Dissociation of glycinin, the soybean legumin, at the ionic strength that we used in the gel filtration of globulins 8 and 9a has been reported [17]. Vicilins from soybean do not dissociate under these conditions, and this was the same in our case. The presence of carbohydrate on the three conglutins is reported by Eaton-Mordas and Moore [18] with a similar ratio between conglutins α and β as found between legumin and vicilin in our studies. The nutritional difference is striking: legumins have a reasonable content

in tryptophan and sulphur amino acids and their MEAA indexes are higher than for total globulins, whereas vicilins are defective for both aspects.

A third interrelated group of proteins appears if attention is given to carbohydrate distribution, namely proteins 1, 2, 3: each varies remarkably as per cent of total sugar, but taken together they are a constant share of the total. A possible reason for this is that carbohydrate is deposited alternatively on these molecules. Proteins 1 and 3 differ in amino acid composition, but both are high in serine, threonine and asparagine which are likely binding sites for carbohydrate chains.

Globulin 1 matches conglutin γ isolated from several lupine species for amino acid composition and electrophoretic mobility and also for existing in a number of oligomeric species [2, 3]. All MWs determined are close to multiples of that of the subunit evidenced in the protein [14]. Globulin 9b has similar amino acid composition to the 2.0 S component, MW 27000, identified in *L. luteus* [8] and in other lupine species [4, 6]. Due to its low content in aromatic amino acids and tryptophan, it is underestimated in assays based on UV absorbancy.

Globulin 1, although it represents only 6% of total globulins, with its equilibrated amino acid composition contributes in raising the overall nutritional value of the globulins and the seed. Its amount varies to a lesser extent between species and between cultivars [3]. In *L. angustifolius* its concentration in the seed as well as that of legumins is considerably decreased by the increased nitrogen to sulphur ratio in the nutrient [9], which gives indications for improving cultural practice.

EXPERIMENTAL

Materials and methods. All chemicals were reagent grade. Ultrogels were from LKB, and Sephadex from Pharmacia. Standard proteins for MW determinations were obtained from Sigma, namely bovine tyroglobulin, jack bean urease, bovine fibrinogen, bovine liver catalase, rabbit muscle aldolase, yeast alcohol dehydrogenase, human transferrin, BSA, egg albumin, chymotrypsinogen, horse heart myoglobin and cytochrome *c*.

L. albus seeds were of commercial origin. The characteristics quoted in the present paper remained the same in various lots and throughout storage.

Proteins were assayed by a biuret and a micro-biuret method [19, 20]. Total N was estimated by the AOAC micro-Kjeldahl procedure [21]; the 6.25 conversion factor was used. Protein extraction was according to ref. [5].

Cellulose acetate electrophoresis was performed as described in ref. [22] in phosphate or Tris barbiturate buffer, $I = 0.1$, with a current of 0.75 mA/cm for 75 min at 25°. Proteins were stained with Amido Black.

Gel filtration columns with Ultrogels (81 × 1.1 cm for MW determinations) were equilibrated and eluted with 0.1 M NaCl in 0.05 M phosphate buffer, pH 7.5. For those of Sephadex (25 × 5 cm) only the buffer was used. Elution was monitored at 280 nm.

Solns were concd with CX Immersibles from Millipore, having a cut-off limit of 12000 daltons. MW determinations were performed as indicated in ref. [23]. Amino acid analyses were performed as described in ref. [10]. Tryptophan was assayed according to ref. [24].

Neutral sugars were determined according to ref. [27]; assayed samples were all made 0.25 M with respect to NaCl. Individual carbohydrates were separated and determined by GC [28].

Acknowledgements—The contribution of Dr. R. Ghidoni and Dr. S. Sonnino in performing gas-chromatographic techniques is gratefully acknowledged.

REFERENCES

1. Hudson, B. J. F., Fleetwood, J. G. and Zand Moghaddam, A. (1976) *Plant Food for Man* **2,** 81.
2. Joubert, F. J. (1956) *Biochim. Biophys. Acta* **19,** 172.
3. Gillespie, J. M. and Blagrove, R. J. (1975) *Aust. J. Plant Physiol.* **2,** 29.
4. Blagrove, R. J. and Gillespie, J. M., (1975) *Aust. J. Plant Physiol.* **2,** 13.
5. Cerletti, P., Fumagalli, A. and Venturin, D. (1978) *J. Food Sci.* **43,** 1409.
6. Joubert, F. J. (1957) *J.S. Afr. Chem. Inst.* **10,** 21.
7. Blagrove, R. J., Gillespie, J. M. (1978) *Aust. J. Plant Physiol.* **5,** 651.
8. Gerritsen, T. (1956) *Biochim. Biophys. Acta* **22,** 269.
9. Blagrove, R. J., Gillespie, J. M. and Randall, P. J. (1976) *Aust. J. Plant Physiol.* **3,** 173.
10. Duranti, M. and Cerletti, P. (1979) *J. Agric. Food Chem.* **27,** 977.
11. Ellman, T. C. (1977) *Aust. J. Biol. Sci.* **30,** 33.
12. Gillespie, J. M., Blagrove, R. J. and Randall, P. J. (1978) *Aust. J. Plant Physiol.* **5,** 641.
13. Herbert, S. J. and Hill, G. D. (1976) *Proc. Agric. Soc. N. Z.* **6,** 65.
14. Restani, P., Duranti, M., Cerletti, P. and Simonetti, P. (1981) *Phytochemistry* **20,** MS. 560.
15. (1973) *FAO Rep. Joint FAO/WHO Committee*, 63.
16. Derbyshire, E., Wright, D. J. and Boulter, D. (1976) *Phytochemistry* **15,** 3.
17. Badley, R. A., Atkinson, D., Hauser, H., Oldani, D., Green, J. P. and Stubbs, J. M. (1975) *Biochim. Biophys. Acta* **412,** 214.
18. Eaton-Mordas, C. and Moore, K. G. (1979) *Phytochemistry* **18,** 1775.
19. Gornall, A. G., Bardawill, C. J. and David, M. M. (1949) *J. Biol. Chem.* **177,** 751.
20. Itzaki, R. F. and Gill, D. M. (1964) *Analyt. Biochem.* **9,** 401.
21. (1970) *Official Methods of Analysis of the A.O.A.C.*, 11th edn 42.014, p. 858.
22. Chin, H. P. (1970) *Cellulose Acetate Electrophoresis. Techniques and Applications.* Ann Arbor Press, Ann Arbor, Michigan.
23. Andrews, P. (1965) *Biochem. J.* **96,** 595.
24. Hugli, T. E. and Moore, S. (1972) *J. Biol. Chem.* **9,** 2828.
25. Ney, K. H. (1974) in *The Contribution of Chemistry to Food Supplies* (Morton, I. and Rhodes, D. N., eds.) p. 411, London.
26. Mitchell, H. M. (1954) in *Symposium on Methods for Evaluation of Nutritional Adequacy and Status* (Spector, H. *et al.*, eds.) p. 13. National Research Council, Washington, D.C.
27. McKelvy, J. F. and Lee, Y. C. (1969) *Arch. Biochem. Biophys.* **132,** 99.
28. Zanetta, J. P., Breckenridge, W. C. and Vincendos, G. (1972) *J. Chromatogr.* **69,** 291.