CCLXXIII. THE AMIDE, TYROSINE AND TRYPTOPHAN CONTENTS AND THE SULPHUR DISTRIBUTIONS (CYSTINE PLUS CYSTEINE AND METHIONINE CONTENTS), OF SOME PLANT LEAF PROTEIN PREPARATIONS

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It is the purpose of this article to record partial amino-acid and amide analyses of protein preparations made here and elsewhere from time to time from the fresh leaves of various plants at different stages of growth and growing under different climatic and manurial conditions, and to compare the preparations with one another. Some work done with a few other protein preparations is included from general interest.

The preparations

The leaf protein preparations are listed in Table I, which shows in the order of the columns: the characteristic number or letter; the species of leaf material and date of extraction; the % dry wt. of the leaf material; the % total N (*t*), "albuminoid" N (*a*), coagulable N (*c*) and protein-N (*p*) in the dry leaf material; abbreviations indicating the method of extraction; the degree of extraction, usually represented by the extracted protein-N as % of the leaf-N (*t*, *a*, *c* or *p*, preferably the last, if known), but in some cases where these data are missing, by the weight of extracted dry protein as % of the leaf dry wt. (*wt*); the % N in the protein preparation; and finally, the % of ash in it. The methods of estimating most of the quantities referred to above have been described briefly in an earlier publication [Lugg, 1938, 2]. The "protein"-N in the leaf material was that left in the residue containing the coagulable N after further extraction with alcohol, dilute citric acid, alcohol and ether, as described for the purification of most of the protein preparations mentioned in the earlier article.

Of the abbreviations, "*d*" means direct maceration of the leaves [see Lugg, 1938, 2], and "*w1*" and "*w2*" mean maceration after washing the readily diffusible solutes from the leaf cells, "*1*" signifying that ether, and "*2*" that "used" ether-water, was used to plasmolyse the cells [Chibnall, 1923; Chibnall *et al.*, 1933]. Following these abbreviations, "*w*" means that water, "*ew*" that ether-water, "*es*" that ether-saturated NaCl solution, or "*pH* 7 (or 9, 10 or 11)" that a dilute buffer solution of the indicated pH value,² was the protein solvent with which the leaves were macerated; finally "*f*" and "*c*" respectively signify

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² The pH values of the juices obtained were not estimated but were certainly lower than those of the buffers employed, juices obtained with water as solvent being of about pH 6. It is considered unlikely that the proteins were irreversibly dissociated.

Table I

Prep.	Source and date	% dry wt.	% N in dry leaf (t, a, c, p)		Method of extr.	Extent of extr.	% N prep.	% ash prep.
A	(Edestin)	—	—	—	—	—	18.5	0.2
B	(Casein)	—	—	—	—	—	15.05	2.0
C	(<i>Brassica campestris</i>) (Swede turnip)	—	—	—	—	—	13.0	16.4
1	<i>Dactylis glomerata</i> (cocksfoot grass) 3. vi. 35	17.9	2.98 (t),	2.80 (a)	d, ew, f	25.5 (a)	13.1	3.4
2	" 7. xi. 35	19.0	3.20 (t),	2.82 (a)	d, ew, f	27.7 (a)	13.0	1.0
3	" 7. xi. 35	19.0	3.20 (t),	2.82 (a)	d, es, f	19.9 (a)	13.1	1.0
4	" 29. ix. 36	12.5	6.81 (t),	4.50 (p)	w2, w, f	39.4 (p)	14.1	1.1
5	" 27. v. 37	12.4	4.15 (t),	3.41 (p)	d, w, f	31.1 (p)	11.8	4.8
6	" 27. v. 37	12.4	4.15 (t),	3.41 (p)	d, pH 7, f	37.6 (p)	12.05	3.6
7	" 27. v. 37	12.4	4.15 (t),	3.41 (p)	d, pH 9, f	37.3 (p)	12.7	2.7
8	" 27. v. 37	12.4	4.15 (t),	3.41 (p)	d, pH 11, f	41.2 (p)	12.7	2.2
9	" 20. ix. 37	16.6	5.57 (t),	4.33 (p)	d, w, c	30.3 (p)	13.1	1.0
10	" 20. ix. 37	16.6	5.57 (t),	4.33 (p)	, pH 10, c	5.7 (p)	13.2	4.8
11	" 20. ix. 37	16.6	5.57 (t),	4.33 (p)	d, pH 10, c	36.7 (p)	13.1	1.2
12	<i>Phalaris tuberosa</i> (Too- woomba canary-grass) 20. viii. 35	20.4	3.08 (t),	2.72 (a)	d, ew, f	15.2 (a)	15.4	0.7
13	" 20. viii. 35	20.4	3.08 (t),	2.72 (a)	d, es, f	9.2 (a)	15.35	0.8
14	<i>Lolium perenne</i> (peren- nial rye-grass) 6. xi. 33	18.1	4.6 (t),	3.8 (c)	w2, w, f	21.8 (c)	12.85	1.1
15	<i>Lolium italicum</i> (Italian rye-grass) 12. vi. 33	16.0	—	—	w2, w, f	4.8 (wt)	14.2	1.1
16	" 26. vi. 33	15.0	5.9 (t),	5.2 (c)	w2, w, f	20.5 (c)	13.5	1.1
17	<i>Poa trivialis</i> (rough- stalked meadow-grass) 12. vi. 33	22.0	—	—	w2, w, f	2.3 (wt)	12.7	1.5
18	" 27. vi. 33	14.0	6.2 (t),	5.3 (c)	w2, w, f	13.7 (c)	14.0	0.8
19	<i>Festuca rubra</i> v. <i>fallax</i> (Chewing's fescue-grass) 26. ix. 32	—	—	—	w2, w, f	17.8 (c)	14.25	0.8
20	" 9. vi. 33	26.0	—	—	w2, w, f	1.3 (wt)	14.4	2.0
21	" 29. vi. 33	15.0	—	—	w2, w, f	0.9 (wt)	14.2	1.7
22	<i>Cynosurus cristatus</i> (crested dog's tail grass) 12. vi. 33	24.0	—	—	w2, w, f	6.6 (wt)	14.1	1.3
23	<i>Medicago sativa</i> (lucerne) 18. vii. 35	20.1	5.1 (t),	4.4 (a)	d, ew, f	31.6 (a)	13.85	1.4
24	" 18. vii. 35	20.1	5.1 (t),	4.4 (a)	, es, f	1.1 (a)	13.85	1.7
25	" 29. vi. 33	21.0	3.25 (t),	2.8 (c)	w2, w, f	12.6 (c)	14.2	1.2
26	<i>Trifolium repens</i> (wild white clover) 18. x. 33	13.6	5.09 (t),	4.3 (c)	w2, w, f	28.0 (c)	13.1	1.5
27	<i>Trifolium pratense</i> (red clover) 23. x. 33	23.7	2.71 (t),	2.4 (c)	w2, w, f	24.3 (c)	12.75	1.6
28	<i>Phaseolus vulgaris</i> (runner bean) 7. x. 36	—	—	—	w2, w, f	—	13.3	3.2
29	<i>Atriplex nummularium</i> (old-man salt-bush) 6. vi. 35	15.6	3.48 (t),	2.54 (a)	d, ew, f	17.9 (a)	12.1	4.5
30	<i>Spinacia oleracea</i> (spinach) 1923	—	—	—	1, , f	ca. 2 (p)	15.5	1.3
31	" "	—	—	—	w1, w	—	11.7	14.8
32	" "	—	—	—	w1, w, f	ca. 20 (p)	15.1	2.1

that the leaf juice, after squeezing through cloth, was either filtered through filter paper, or centrifuged for 30 min. in a field of $500\times$ gravity, before flocculating the protein. Whereas most of the chloroplast and nuclear material was removed with the cell debris by filtration, much of the chloroplast material was not removed by centrifuging. In one instance, 30, protein was flocculated from the solution carrying the readily diffusible cell constituents; in another, 31, protein was flocculated from juice which had not been centrifuged or filtered through filter paper; in two other instances, 10 and 24, residues of leaf material from the extraction of 9 and 23 respectively, were extracted with other solvents but without further maceration.

The preparations were made from leaves ranging in age from very young to quite old, and in general, as regards a single species, a high % dry wt. connotes old age and relative toughness and a high % total N (ages being similar) connotes good manuring. The proteins were extracted at room temperature (10–25°). With materials of much the same degree of toughness the macerating machinery (a mincing machine or a corn mill) permitted much the same degree of maceration. Even with the tenderest leaves, however, many of the cells were not ruptured. Preps. 14–22 and 30–32 were drawn from supplies made by Prof. A. C. Chibnall and co-workers in the past. Preps. 1, 2, 3, 4, 12, 13, 23, 24, 25 and 29 have been described in an earlier article [Lugg, 1938, 2] wherein their characteristic numbers were 1–10 in succession.

Prep. A was the sample of edestin used in earlier work [Lugg, 1938, 1, 2]; B was a sample of casein used by Vickery & White [1933] and was kindly supplied by Dr H. B. Vickery; and C, a protein obtained from the juice of macerated turnips (see Williams [1917]) and believed to have a high S content, was kindly supplied by Mr F. W. Foreman.

Methods of analysis

Amide. The general scheme behind the procedure for estimating this quantity (acid hydrolysis of amide linkages and estimation of the liberated NH_3) is to insure virtually complete amide hydrolysis with minimum deamination of peptides and amino acids (notably arginine). The problem has been studied with special care by Shore *et al.* [1936], and whilst the most appropriate estimate appears to require many estimations with increasing periods of hydrolysis and an approximate elimination (by extrapolation) of the contribution of NH_3 by the deamination reactions, reasonably representative values can be obtained by a standard period hydrolysis. The procedure adopted was to heat 0.1 g. of the preparation with 5 ml. of 2*N* HCl at 100° for 3 hr. and to distill the NH_3 off at pH 10.0 (borate buffer), by boiling under reduced pressure at 45°, into standard acid, the excess of which was back-titrated with alkali.

The procedure was checked with edestin and edestin plus 20 % by weight of arabinose, the carbohydrate being entirely without effect and therefore, presumably, introducing no new systematic errors. In comparison with values obtained by hydrolysing the leaf protein preparations for the standard (3 hr.) period, those obtained in a 2 hr. hydrolysis period were generally a few % lower, and in a 5 hr. period a few % higher; and by hydrolysing with 5*N* HCl at the boiling point for 20 hr. they were generally increased by some 20 %.

Tyrosine and tryptophan. 0.2 g. of the preparation was hydrolysed with alkali stannite reagent as described by Lugg [1938, 1] and tyrosine and tryptophan were estimated in the hydrolysate by Lugg's [1937] methods. Diiodotyrosine could not be detected in a few preparations by comparing with values obtained by plain alkali hydrolysis, and was assumed to be absent in all cases. Most of the leaf protein preparations yielded small amounts of extraneous phenol during hydrolysis and the legume preparations 23, 24, 25, 26 and 28 yielded some extraneous indole as well. These substances presumably had their origin in the impurities which appear almost always to contaminate such preparations, and the legume preparations therefore must have contained some nitrogenous impurity. This non-protein-N (presumably very small in amount) has been ignored in all calculations. Whereas the phenol was readily removed by the ether extraction advocated, the indole was not adequately removed either by the ether or the toluene extraction. It slightly affected both the tint and the fading rate in the tryptophan colour reaction and so, to an unknown but

apparently small degree, vitiated the tryptophan estimations in these cases. Correction had to be made for adventitious coloration, which was more marked in the tryptophan than in the tyrosine estimations. The tyrosine and tryptophan contents of A have already been reported [Lugg, 1938, 1].

S-distribution (cystine plus cysteine and methionine). The S-distributions of A, 1, 2, 3, 4, 12, 13, 23, 24, 25 and 29 and the methods employed have been discussed in an earlier article [Lugg, 1938, 2]. Estimations were made with the other preparations by the HI digestion (titration) and differential oxidation procedures, there described.

RESULTS AND DISCUSSION

The results are recorded in Table II. The amide and amino-acid contents are all reported on a "nitrogen basis", namely, the N contained in the chemical species as % of the N in the protein preparation. This scheme facilitates comparison of the protein moieties of impure preparations if the nitrogenous impurities are negligibly small, and they are believed to have been so here. The

Table II

Prep.	% N	% amide N	% tyr.-N	% try.-N	% cyst.-N (d.o.)	% meth.-N (d.o.)	% cyst. + meth.-N (d.o.)	SO ₄ -S × 100/N (d.o.)	% cyst.-N (HI t.)	% meth.-N (HI t.)
A	18.5	9.63	1.80	1.09	0.94	1.24	2.18	0.07	0.90	1.18
B	15.05	—	3.14	1.17	—	—	—	—	0.18	1.77
C	13.0	—	—	—	1.36	1.65	3.01	0.35	1.24	1.62
1	13.1	5.05	2.37	1.83	1.51	1.44	2.95	0.33	1.38	1.21
2	13.0	5.11	2.32	1.82	1.47	1.35	2.82	0.27	1.28	1.20
3	13.1	5.06	2.36	1.86	1.53	1.47	3.00	0.19	1.28	1.19
4	14.1	5.26	2.34	1.82	1.68	1.49	3.17	0.40	1.50	1.30
5	11.8	5.05	2.39	1.81	—	—	—	—	—	—
6	12.05	5.35	2.40	1.86	—	—	—	—	—	—
7	12.7	5.16	2.38	1.87	—	—	—	—	—	—
8	12.7	5.38	2.38	1.86	—	—	—	—	—	—
9	13.1	4.94	2.38	1.89	—	—	—	—	1.52	1.24
10	13.2	4.48	2.09	1.65	—	—	—	—	—	—
11	13.1	5.09	2.29	1.81	—	—	—	—	1.43	1.31
12	15.4	4.85	2.48	1.86	1.63	1.48	3.11	0.52	1.50	1.45
13	15.35	5.13	2.34	1.58	1.58	1.44	3.02	0.89	1.40	1.36
14	12.85	5.02	2.26	1.74	1.65	1.60	3.25	0.37	1.26	1.16
15	14.2	4.72	2.34	1.80	1.58	1.57	3.15	0.33	1.31	1.43
16	13.5	4.70	2.33	1.74	1.70	1.69	3.39	0.58	1.42	1.50
17	12.7	5.28	2.33	1.74	1.65	1.63	3.28	0.36	1.34	1.33
18	14.0	5.13	2.31	1.80	1.52	1.66	3.18	0.33	1.36	1.50
19	14.25	4.64	2.34	1.87	—	—	—	—	1.41	1.36
20	14.4	5.12	2.36	1.88	1.54	1.58	3.12	0.79	1.31	1.41
21	14.2	4.95	2.38	1.93	1.66	1.62	3.28	0.25	1.37	1.46
22	14.1	4.73	2.36	1.96	1.65	1.55	3.20	0.26	1.50	1.41
23	13.85	5.29	2.70	<1.98	1.19	1.23	2.42	0.29	1.11	1.22
24	13.85	5.55	2.72	<1.88	1.31	1.23	2.54	0.34	—	—
25	14.2	5.16	2.74	<2.00	1.29	1.35	2.64	0.41	1.25	1.31
26	13.1	5.68	2.49	<1.88	1.23	1.33	2.56	0.30	0.99	1.23
27	12.75	5.40	2.50	1.70	1.25	1.29	2.54	0.27	1.19	1.22
28	13.3	5.08	2.36	<1.55	1.30	1.26	2.56	1.22	1.05	1.01
29	12.1	5.98	2.55	1.74	1.44	1.53	2.97	1.92	1.30	1.55
30	15.5	5.76	2.57	1.43	—	—	—	—	1.43	1.09
31	11.7	5.36	2.63	1.72	—	—	—	—	1.22	1.27
32	15.1	5.64	2.71	1.69	—	—	—	—	1.37	1.30

results have been corrected for the losses undergone by the pure, free amino-acids in the procedures concerned. The random errors of estimation are believed to lie within the limits indicated in the following description of the table's contents. In the order of the columns are shown: the characteristic number or letter;

the % N in the preparation (error 0.5 %, affecting all the others); the amide content (error 1 % for A, 1.5 % for the others); the tyrosine content (error 1 % for A and B, 2 % for the others); the tryptophan content (error 1.5 % for A and B, 3 % for the others, excepting what were almost certainly over-estimations at least several % in the case of 23, 24, 25, 26 and 28); the cystine plus cysteine content by differential oxidation (error 2 % for A, 4 % for the others); the methionine content by differential oxidation (error 3 % for A, 5 % for the others); the sum of these last two quantities (error 2 %); the $\text{SO}_4\text{-S}$ as % of the weight of N in the preparation (error from 2 % for the larger to 20 % for the smaller values); the cystine plus cysteine content by titration of HI digests (error 1.5 % for A, 3 % for the others); and finally the methionine content by titration of HI digests (error 2 % for A and B, 4 % for the others).

The differences between the cystine plus cysteine and methionine contents estimated by titration of HI digests and by differential oxidation, have already been discussed in relation to some of the preparations [Lugg, 1938, 2]. The disparities are again apparent with the rest of the preparations and the same uncertainty as to their significance remains. Again it may be pointed out that while the estimations by titration of HI digests are much the more specific they are likely to be low, and that the values obtained by differential oxidation must be used when comparing compositions. $\text{SO}_4\text{-S}$ values are regarded here simply as measures of some impurity.

Whereas significant differences in composition, even in respect of only one chemical species, prove the non-identity of the protein moieties of two preparations, their absence leaves only an indeterminate degree of probability (increasing with the number of chemical species considered) that they are identical.

Of the preparations from cocksfoot grass, 5, 6, 7 and 8 show that slightly increased extraction may result when the pH of the extracting liquid is increased beyond that of the mixed cell contents. 5 is of slightly lower amide content. Incidentally, when leaves of the Gramineae are macerated alone or with water the resulting juice is usually found to be of about pH 6. Prep. 10, made by extracting the leaf residue from 9 with a pH 10 buffer, is of materially lower amide, tyrosine and tryptophan contents than any of the other cocksfoot preparations which, except for small differences, are remarkably uniform in composition. In fact, except for 10, all the preparations from leaves of the Gramineae (1-22) show relatively small variations in composition, the greatest being in the S-distributions and the amide contents.

In comparison with these, the preparations from leaves of the Leguminosae (23-28) are rather less uniform in composition, but in general, their tyrosine contents are a little higher and their cystine plus cysteine and methionine contents are lower; and the preparations from leaves of the Chenopodiaceae (29-32) are generally of higher amide and tyrosine contents.

No definite variations in composition of extracted protein can be associated with the age of the leaves or with the climatic and manurial conditions during growth. It is to be noted too, that preps. 9, 11 and 30, which presumably contained more representative amounts of the proteins associated with the chloroplasts, do not differ markedly in composition from the others.

Taken as a group for comparison with preparations like A and B, the leaf protein preparations from monocotyledons (1-22) and dicotyledons (23-32) appear from these analyses to be of fairly uniform composition, and judged by the usual standards of animal nutritional requirements are by no means deficient in their contents of tyrosine, tryptophan, cystine plus cysteine and methionine.

The analyses of A and B are in reasonable agreement with the more recently published work of others on edestin and casein preparations. There appears to be nothing remarkable about the S distribution of C (from Swede turnips), but then, contrary to expectation, its S content was not abnormally high.

SUMMARY

Estimated amide, tyrosine, tryptophan, cystine (and/or cysteine) and methionine contents of protein preparations made from the fresh leaves of various mono- and di-cotyledonous plants, are recorded, together with those of a few other protein preparations.

The leaf protein preparations were made from *Dactylis glomerata*, *Phalaris tuberosa*, *Lolium perenne*, *L. italicum*, *Poa trivialis*, *Festuca rubra* var. *fallax* (Hack) and *Cynosurus cristatus*, of the Gramineae; *Medicago sativa*, *Trifolium repens*, *T. pratense* and *Phaseolus vulgaris*, of the Leguminosae; and *Atriplex nummularium* and *Spinacia oleracea*, of the Chenopodiaceae. For the most part they represented some 10–40% of the leaf protein, but were probably rather deficient in the proteins associated with the plastids and nuclei of the leaf cells.

In comparison with preparations from the first plant order, those from the second and third were generally a little higher in tyrosine contents, those from the second a little lower in cystine (and/or cysteine) and methionine contents, and those from the third a little higher in amide contents. The ranges over all the leaf protein preparations were: 4.70–5.98% amide-N, 2.09–2.74% tyrosine-N, 1.43–1.98% tryptophan-N, 0.99–1.70% cystine (plus cysteine)-N, and 1.01–1.69% methionine-N, the lower limits in the last two cases probably being underestimations, and the upper limits being possibly too high owing to lack of specificity in estimation in these same cases.

In regard to their contents of the amino-acids here estimated, the leaf protein preparations compare favourably with many other proteins which enter into the dietary of animals.

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