2. Both fractions show graded ease with which they can be rendered soluble in aqueous solvents, considerable overlapping existing around the end-point of chlorination cellulose preparation.

3. Tentative distinction of two hemicellulose types seems to remain of value as a working hypo-

thesis for further botanical and constitutional investigations.

4. Factors affecting the water-solubility of hemicellulose fractions are discussed; there is only partial parallelism between hemicellulose solubility and degree of delignification.

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Large-scale Extraction of Protein Samples Reasonably Representative of the Whole Proteins in the Leaves of some Plants: the Amide, Tyrosine, Tryptophan, Cystine (plus Cysteine) and Methionine Contents of the Preparations

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Limited experimental evidence supports the statement (Lugg, 1943) that the whole proteins of the somatic, photosynthesizing, gametophyte tissues of bryophytes, and sporophyte tissues of pteridophytes and spermatophytes, appear to be of similar composition. The evidence, though meagre, was held to justify the view that for the purpose of animal nutrition studies, comprehensive analyses of pasture proteins might well be confined in the first instance to those of only a few species belonging to the most important families of pasture plants.

Considerable data already exist concerning the amide, tyrosine, tryptophan, cyst(e)ine (cystine plus cysteine) and methionine contents of the whole-leaf proteins of several spermatophytes, and of numerous protein preparations which, in most instances, may not have been properly representative of the whole protein in the leaves from which they were obtained (Lugg, 1938 *b, c*, 1939, 1943; Chibnall, 1939). The arginine, histidine and lysine contents of various preparations of uncertain representativeness have been estimated (Tristram, 1939), and in some instances the amounts of aspartic and glutamic acid have also been determined (Chibnall, 1939). The proline content of one such preparation has been estimated (Miller, 1936), and the glycine,

alanine, valine, leucine (plus isoleucine and nor-leucine) and phenylalanine contents of another preparation have been estimated only very approximately and uncertainly by Lugg (quoted by Chibnall, 1939, and by Lugg, 1941).

The presence of cellulose, polyuronides and similar contaminants in protein preparations introduces no apparent systematic errors in the estimation of amide, tyrosine, tryptophan, cyst(e)ine and methionine contents when suitable, tested methods are employed. The methods available for the estimation of other amino-acids, however, are known to be subject to systematic errors or are of uncertain reliability, under these conditions. For the most satisfactory estimation of these other amino-acids, it is therefore necessary to work with preparations which are freed as far as possible from such contaminants, and if the estimations are intended to refer to the whole proteins of the leaves, it is necessary also to ensure that the preparations are properly representative of these.

The principles of methods designed to provide representative preparations of reasonable purity have been discussed elsewhere (Lugg, 1939). In the present article there will be described the extraction of large samples of such protein from the leaves of

four pasture species of great importance in Australian agriculture. Partial amino-acid compositions were determined and were used in testing representativeness.

EXPERIMENTAL

The plant materials

The plant species selected were: *Phalaris tuberosa* L. (*Phalaris*, Toowoomba canary grass), *Hordeum murinum* L.* (barley grass, wall-barley grass, mouse-barley grass), *Medicago sativa* L. (lucerne, alfalfa) and *Medicago denticulata* Willd. (burr medic†). The first two were chosen to represent the family Gramineae, and the second two the family Leguminosae. The first and third are perennials and are normally cultivated in Australian agricultural practice, whereas the second and fourth are annuals and are not usually cultivated but grow 'wild'. None of the plants is native to Australia. They are believed to have had their origins in Europe or Asia.

The plant materials were harvested from plants growing in soil which had been kept well watered and had been lightly manured with ammonium sulphate and 'super' (essentially a mixture of calcium sulphate and primary phosphate). The leaves of *M. denticulata*, with attached petioles and some short lengths of stalk, were harvested from plants which had never been cut back. Two-thirds of the material came from fairly young plants growing in seed boxes, and the rest from older plants growing in a garden. A few of the young plants had begun to flower, whilst among the older plants there were some flowers and a few burrs. Flower and burr materials were rigorously excluded. Leaves of the other species were harvested from plants which had been cut back at about the time the manures were applied and then allowed to grow for several weeks. The petioles and some short lengths of stalk were left attached to the *M. sativa* leaves. The average lengths of the *P. tuberosa* and *H. murinum* leaves were about 15 cm. in each case.

The materials were thus neither very immature nor senescent, and manuring had been light. Previous work has shown that the composition of the whole leaf proteins of a species probably varies but little under these conditions.

* Referred to as '*Hordeum leporinum* Link' in *Council for Scientific and Industrial Research Bulletin*, no. 156 (1942).

† Referred to as 'toothed medic' by Black (1922-9).

Procedure for extraction of protein

The freshly harvested material was macerated at room temperature (about 20°) with about 2.5 times its weight of a pH 9.2 borate buffer containing 1.1 g. B/l. It was chopped and torn (Lugg, 1939) rather than crushed during its single passage through a power-driven mincing machine. After breaking the surface froth with a few drops of octanol, the mash was poured on to a sieve (holes 1 mm. sq.) with continual agitation, to remove most of the fibrous material and some cell debris. The maceration was not fine, and microscopic examination of the fibrous residues revealed many unruptured cells.

The resulting green, opaque juice was adjusted carefully with NaOH to pH 9.2, and a cold (0-5°) mixture of 0.8 vol. ethanol and 0.2 vol. ether was added slowly with vigorous stirring. After about 15 min. the liquid was poured through muslin to remove most of the remaining cell debris. The bulk of the 'disintegrated granule residues' (Lugg, 1939) and some other impurities were then removed by passing the liquid through a centrifugal cream separator, running continuously except for stoppages to clean the residues from the centrifuge bowl. The liquid was submitted for about 1 min. to a field intensity of approx. 5000 $\times g$. The emergent liquid was pale green and somewhat opalescent, whereas centrifugates obtained after 30 min. at 25,000 $\times g$, and filtrates passing through paper-pulp, were found by Lugg (1939) to be almost transparent. However, large quantities of material could be treated rapidly by the cream separator.

By addition of acetic acid the liquid was brought to a pH corresponding with that of a pH 4.5 aqueous acetate buffer to which had been added 0.8 vol. ethanol, and it was then heated to about 70°. The coagulum was filtered or centrifuged from the liquid, washed, and then extracted with boiling ethanol, dilute citric acid, ethanol again and finally warm ether (Lugg, 1938b), to give the 'extracted' protein preparation. 'Whole' and 'residual' protein preparations were made as described by Lugg (1939); the former from a sample of the dried leaf material and the latter from a sample of the leaf residues (to which had been added the residues from the muslin filter and the centrifuge).

Data concerning the extractions are shown in Table 1. In the order of the columns are: the label of the preparation (experiment number followed by W, E or R, signifying respectively 'whole', 'extracted' or 'residual'); plant species and date;

Table 1. *Extraction of protein from the plant leaves*

Preparation	Source and date	% dry wt.	% N in dry leaf			% extracted N			% N in preparation	% ash in preparation	Dry wt. of E (g.)
			Total	Coagulable	Protein	Total	Coagulable	Protein			
1W	<i>Phalaris tuberosa</i> 9. x. 41	13.8	4.71	3.94	3.75				6.05	5.6	
1E						46.1	44.4	45.5	14.90	1.8	209
1R									2.88	6.1	
2W	<i>Hordeum murinum</i> 14. ix. 43	14.3	4.90	3.94	3.78				6.27	4.6	
2E						57.1	53.5	54.9	14.10	2.8	216
2R									3.05	6.1	
3W	<i>Medicago sativa</i> 17. ii. 41	13.7	5.07	4.00	3.88				6.78	2.0	
3E						56.2	50.5	51.1	14.15	2.3	222
3R									4.01	3.4	
4W	<i>Medicago denticulata</i> 24. ix. 41	13.1	4.91	3.84	3.75				6.87	2.7	
4E						38.0	32.3	31.4	14.55	1.4	133
4R									4.45	3.5	

percentage dry weight of the leaf material; the percentage of total N (*t*), coagulable N (*c*) and protein N (*p*) in the dry leaf; the percentage of the leaf N (*t*, *c* and *p*) appearing in the centrifuged liquid from the cream separator; the percentage of N in the dry protein preparation; and the percentage of ash in it after ignition in air at about 600°. The terms 'coagulable nitrogen' and 'protein nitrogen' have been explained fully in earlier articles (e.g. Lugg, 1939). Briefly 'protein N' means N not removed by successive extractions with boiling ethanol, dilute citric acid, ethanol and ether, from any material containing 'coagulable N'; and 'coagulable N' (in the present work) means N not removed from a material containing 'total N' by extraction at about 70° with a mixture of dilute acetic acid and 0.8 vol. of ethanol at a pH corresponding with that of an aqueous acetate buffer at pH 4.5 to which 0.8 vol. of ethanol had been added.

Analytical work

Nitrogen estimations were made by the Kjeldahl method, with selenium as catalyst and digestion at the boiling-point in the presence of considerable NaHSO₄ for at least 8 hr. (see Chibnall, Rees & Williams, 1943) after the digest had cleared.

The amide, tyrosine, tryptophan, cyst(e)ine and methionine contents of the protein preparations were estimated by the methods described by Lugg (1937, 1938 *a*, *b*, *c*). Some of the total S determinations, required in one of these methods, were made as described by Lugg & Weller (1944) (peroxide fusion, after alkali fusion under reducing conditions).

lated values: N (0.5, affecting all the other errors), amide (1.5), tyrosine (2), tryptophan (3), cyst(e)ine, by differential oxidation (4), methionine, by differential oxidation (5), the sum of the last two values (2), SO₄²⁻-S (5 for the higher, to 100 for the lower values), cyst(e)ine, by titration of HI digests (3), and methionine, by titration of HI digests (4).

DISCUSSION

Only the values for amide, tyrosine, tryptophan, cyst(e)ine (differential oxidation method, d.o.), methionine (d.o.), and the sum of the last two (which has a smaller random percentage error than either), may be used for demonstrating differences or similarities in the protein moieties of the preparations. The cyst(e)ine and methionine values by HI titration, being subject to systematic errors in these instances, cannot be considered entirely reliable. In a general way, however, they tend to confirm the rather larger values obtained by the less specific differential oxidation method. The sulphate-S values are to be regarded as measures of some impurity.

It is considered that the differences in composition between the W, E and R protein preparations from any one material were so small, and so uncertain in view of the magnitudes of the random errors, as to support the belief (based upon the method of preparation) that the E preparations were reasonably representative of the whole proteins in the leaf

Table 2. *Partial compositions of the 'whole', 'extracted' and 'residual' protein preparations*

Preparation	N (%)	Amide-N (%)	Tyrosine-N (%)	Tryptophan-N (%)	By differential oxidation				By HI titration	
					Cyst(e)ine-N (%)	Methionine-N (%)	Cyst(e)ine + methionine-N (%)	100(SO ₄ ²⁻ -S) N	Cyst(e)ine-N (%)	Methionine-N (%)
1W	6.05	4.76	2.43	2.04	1.35	1.46	2.81	0.21		
1E	14.90	4.74	2.34	2.08	1.39	1.57	2.96	0.79	1.15	1.38
1R	2.88	4.75	2.52	2.07	1.49	1.53	3.02	0.52		
2W	6.27	5.17	2.33	2.09	1.35	1.38	2.73	0.21		
2E	14.10	5.08	2.31	2.02	1.34	1.42	2.76	0.63	1.14	1.25
2R	3.05	5.04	2.38	1.93	1.48	1.45	2.93	0.56		
3W	6.78	5.34	2.43	1.93	1.14	1.24	2.38	0.05		
3E	14.15	5.12	2.30	1.94	1.17	1.36	2.53	0.35	1.01	1.19
3R	4.01	5.18	2.45	1.78	1.23	1.30	2.53	0.05		
4W	6.87	5.11	2.45	1.86	1.19	1.23	2.42	0.06		
4E	14.55	5.18	2.42	1.87	1.27	1.32	2.59	0.38	1.03	1.13
4R	4.45	5.10	2.45	1.79	1.19	1.32	2.51	0.42		

The amide and amino-acid contents are expressed in Table 2 as percentages of the protein N appearing in these forms. The values, taken mostly from replicate estimations, have been corrected for such losses as the pure, free amino-acids undergo in the analytical procedures. The random errors with W and R preparations are believed to have been not more than 1.5 times those with the E preparations, which in turn are believed not to have exceeded the following (parenthesized) percentages of the tabu-

materials. E preparations of tested representativeness and of such degrees of purity (the uncontaminated proteins would probably contain about 17% N: see Chibnall (1939)), have not been made previously on a scale involving the preparation of 100–200 g. of material. These seem to be the first E preparations to have been made from the leaves of *H. murinum* and *M. denticulata*. Two earlier, small-scale preparations from *P. tuberosa* (Lugg, 1938*b*) contained 15.4 and 15.35% N, but could not

be regarded as representative of the whole-leaf proteins. Of the earlier preparations from *M. sativa* leaves, one by Osborne, Wakeman & Leavenworth (1921) contained 16.26 % N and one by Chibnall & Nolan (1924-5) contained 15.73 % N, but they were largely deficient in proteins associated with the chloroplasts and other granular protoplasmic bodies, and probably they both, more especially the former, contained degraded protein.

The degrees of extraction of the proteins were very good, in view of the coarse maceration technique used. The leaves of *M. denticulata*, being thin and pliant, are believed to have passed through the machine with less injury than the others, thus yielding less of their protein.

The partial compositions of the protein preparations are seen to be similar to those found for the W and representative E preparations from the leaves of other members of the Gramineae and Leguminosae. The impression gained earlier (Lugg, 1938 *b, c*) from work with preparations of uncertain representativeness, and supported by subsequent work (Lugg, 1939, 1943), that the whole-leaf proteins of the Leguminosae are probably, in general, of slightly smaller cyst(e)ine and methionine content than those of the Gramineae, has been further substantiated.

SUMMARY

1. The extraction of large samples of protein of reasonable purity from the leaves of four important

species of pasture plants has been described. The procedure, which was designed to secure reasonably representative extraction, involved the liberation (presumed) of protein from the chloroplasts and possibly other granular protoplasmic bodies, with the aid of a mildly alkaline (pH 9.2) buffer in the presence of lipid solvents (ethanol and ether). The differences in partial composition between the extracted proteins, and those in the original leaves and in the leaf residues, were so small and uncertain that the extracted proteins were considered to be reasonably representative of the whole proteins in the leaves.

2. The plant species were *Phalaris tuberosa*, *Hordeum murinum*, *Medicago sativa* and *Medicago denticulata*. The extracted protein preparations contained 14.90, 14.10, 14.15 and 14.55 % N, respectively. Of the N in these preparations there occurred respectively, 4.74, 5.04, 5.12 and 5.18 % as amide-N; 2.34, 2.31, 2.30 and 2.42 % as tyrosine-N; 2.08, 2.02, 1.94 and 1.87 % as tryptophan-N; 1.39, 1.34, 1.17 and 1.27 % as cyst(e)ine-N; and 1.57, 1.42, 1.36 and 1.32 % as methionine-N.

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The Analysis of Hair Keratin

4. A NOTE ON THE OCCURRENCE OF SERINE IN HUMAN HAIR

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Vickery & Schmidt (1931) in their review make the statement that 'serine is one of the most difficult of all the amino-acids to isolate from proteins and is even yet an exceedingly rare substance'. A reagent

which has been used for precipitating *l*-serine from hydrolyzed silk fibroin, viz. *p*-hydroxy-azobenzene-*p*-sulphonic acid (Stein, Moore & Bergmann, 1941; Stein, Moore, Stamm, Chou & Bergmann, 1942),