Table 5. Substrate specificity of ADH

Alcohol	Alcohol concn. (M)	Relative Rate of Oxidation
EtOH	0.01	100
n-PrOH	0.01	48
2-Propen-1-ol	0.01	155
n-BuOH	0.01	40
2-Butane-1-ol	0.01	34
Isoamyl alcohol	0.01	12
4-Pentene-1-ol	0.01	6
n-Hexanol	satd	11
Cinamyl alcohol	satd	12
2-Phenylethanol	satd	7

to 50% saturation. This fraction was desalted on Sephadex G-25. The desalted sample containing roughly 100 mg protein was then purified by means of chromatography on DEAE-cellulose. A column, 2.4 × 45 cm in size was eluted with Tris–acetate buffer pH 6.4 with a linear gradient from 0.1 to 0.6M, using a vol of 1000 ml. Fractions of 12 ml were taken. All steps in enzyme purification were carried out in an ice box. The active fractions obtained were concentrated by means of lyophylisation; activity of the lyophilised preparations was maintained for several weeks.

Enzyme activity measurement. We determined the activity of the alcohol dehydrogenase by a procedure similar to that of Racker's method [11]. We determined proteins by the Lowry method [12]. The MW was determined by means of gel filtration on a column of Sephadex G-200, 1.6 × 18 cm in size. Elution was done with 0.01M Tris–acetate buffer pH 6.4 at a flow-rate of 3 ml/20 min.

REFERENCES

1. Leblová, S., Vaníčková, V. and Sinecká, E. (1974) *Biol. Plant.* **16,** 406.
2. Arslanian, M. J., Pascoe, E. and Reihold, J. G. (1971) *Biochem. J.* **125,** 1039.
3. Hayes, J. E. and Velick, S. F. (1954) *J. Biol. Chem.* **207,** 225.
4. Shore, J. D., and Theorell, H. (1967) *European J. Biochem.* **2,** 32.
5. Reynolds, C. H., Morris, D. L. and McKinley-McKee, J. S. (1970) *European J. Biochem.* **14,** 14.
6. Theorell, H. and McKinley-McKee, J. S. (1961) *Acta Chem. Scand.* **15,** 1811.
7. Reynolds, C. H. and McKinley-McKee, J. S. (1975) *Arch. Biochem. Biophys.* **168,** 145.
8. Nachman, M., Lester, D. and LeMagnen, J. (1970) *Science* **168,** 1244.
9. Lester, D. and Benson, G. D. (1970) *Science* **169,** 282.
10. Theorell, H. and McKinley-McKee, J. S. (1961) *Acta Chem. Scand.* **15,** 1834.
11. Racker, E. (1950) *J. Biol. Chem.* **184,** 313.
12. Lowry, H. O., Rosebrough, N. J., Farr, A. L. and Randall, R. J. (1951) *J. Biol. Chem.* **193,** 265.

Phytochemistry, 1976, Vol. 15, pp. 815–816 Pergamon Press. Printed in England.

THE *N*-TERMINAL AMINO ACID SEQUENCE OF CYTOCHROME *f* FROM *SPIRULINA PLATENSIS*

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Key Word Index—*Spirulina platensis*; amino acid sequence; cytochrome *f*; blue–green algae.

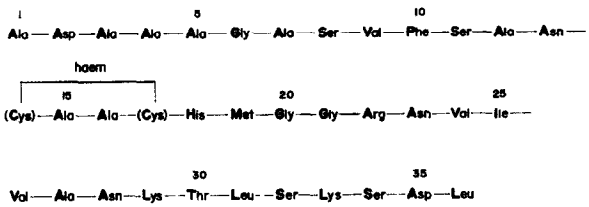
Ambler *et al.* [1] have recently reported the amino acid sequence of cytochrome *f* from the blue–green alga, *Spirulina maxima*, comparing it with other known cytochrome *f* sequences. We had at that time begun a study of this protein from a related species, *S. platensis*, from which we obtained a partial sequence.

Our data suggest a moderate degree of divergence between the proteins from the two species, since they differ at two residues out of the first 36 of the *N*-terminal region, namely at residue positions 1 and 3 (Fig. 1), where Gly and Val in *S. maxima* are replaced by alanine.

Studies on ferredoxin sequences [2] and DNA base compositions [3] also support the suggestion that morphological divergence has not kept pace with DNA and protein evolution in the blue–green algae. As a consequence, a relatively large number of sequences will need to be determined before the phylogenetic relationships between this and other groups of organisms can be established.

EXPERIMENTAL

A gift of partially purified cytochrome *f*, separated by an ammonium sulphate precipitation (55–80%



Residues 1–13, 15 and 16, and 19–36 were obtained by the sequencer method; residue 19 (His) has not been unambiguously identified. Residues 1–4 were confirmed by the manual method [5].

Fig. 1. *N*-terminal amino acid sequence. Sample 17 contained haem, thus allowing the positioning of Cys 14 and 17.

saturation) and chromatography on DE 23 Cellulose, was received from Professor D. O. Hall (King's College, London). It was further purified by gel filtration and rechromatographed on DEAE cellulose. The purified protein $E550$ red/$E250$ red = 1·1, $E410$ oxid/$E280$ red = 5·2 was then sequentially degraded on a Beckman 890C sequenator [4]. The identity of the variant residues was also verified by manual Dansyl–Edman analysis [5].

REFERENCES

1. Ambler, R. P. and Bartsch, R. G. (1975) *Nature* **253**, 285.
2. Glazer, A. N. and Kang, S. (1973) *J. Biol. Chem.* **248**, 659.
3. Stanis, R. Y., Kunisawa, R., Mandel, M. and Cohen-Bazire, G. (1971) *Bact. Rev.* **35**, 171.
4. Scawen, M. D. and Boulter, D. (1974) *Biochem. J.* **143**, 257.
5. Thompson, E. W., Laycock, M. V., Ramshaw, J. A. M. and Boulter, D. (1970) *Biochem. J.* **117**, 183.

Phytochemistry, 1976, Vol. 15, p. 816. Pergamon Press Printed in England.

MAJOR ALKALOIDS OF *LEBECKIA PLUKENETIANA**

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Key Word Index—*Lebeckia plukenetiana*; Leguminosae; lupin alkaloids; sparteine; lupanine; nuttalline.

Plant. Lebeckia plukenetiana E. and Z. (Voucher specimen no. van Breda 796 deposited in the Botanical Research Institute, Pretoria). *Source.* Western Cape, South Africa. *Plant parts examined.* Leaves and twigs.

Extraction. Dry, powdered plant material was continuously extracted with hot MeOH and the residue obtained after evaporation was dissolved in 2M HCl. After filtration, the filtrate was basified with NH_4OH and extracted with $CHCl_3$ to give a brown gum. TLC—silica gel; cyclohexane Et_2NH (4:1)— showed the presence of five bases with R_f values 0.89, 0.53, 0.39, 0.25, 0.18.

Separation. Column chromatography—alumina; Et_2O–MeOH (99:1)—gave pure samples of the three bases with higher R_f values: attempts to identify the minor bases (R_f 0.25, 0.18) by comparison with known lupin alkaloids using GLC and filter-disc chromatography were inconclusive. *Sparteine* (R_f 0.89) was identified by comparison of IR and MS with an authentic specimen. Mercurichloride identified by mp and mmp. *Lupanine* (R_f 0.53) was identified by comparison of IR and MS with those of an authentic specimen. Methiodide identified by mp, mmp, and IR.

Nuttalline (R_f 0.39). Detailed analysis of the MS, used in conjunction with the data of Spiteller *et al.*, [1] confirmed the structure as a 4-hydroxylupanine. The IR and MS were identical with those obtained by Goldberg and Balthis [2] for nuttalline. However, the mp (64–65°) does not agree with that reported (108°) [2]. Many members of the plant family Leguminosae have been investigated and found to contain various lupin alkaloids, but to our knowledge this is the first reported investigation of a member of the genus *Lebeckia*. Furthermore the alkaloid nuttalline is not of common occurrence having been only once before reported (from *Lupinus nuttallii*).

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REFERENCES

1. Schumann, D., Neuner-Jehle, N. and Spiteller, G. (1968) *Monatsh.* **99**, 390.
2. Goldberg, S. I. and Balthis, V. M. (1969) *Chem. Commun.* 660.

*Details may be found in the Ph.D. Thesis of M. J. Nattrass, University of the Witwatersrand, Johannesburg.