*Phytochemistry*, Vol. 26, No. 8, pp. 2267–2269, 1987.
Printed in Great Britain.

0031-9422/87 $3.00 + 0.00


# CARBOHYDRATE COMPOSITION AND CHARACTERIZATION OF TWO UNUSUAL SUGARS FROM THE BLUE GREEN ALGA, *SPIRULINA PLATENSIS*

K. MADHAVI SHEKHARAM, L. V. VENKATARAMAN and P. V. SALIMATH

Central Food Technological Research Institute, Mysore 570 013, India

(*Revised received* 2 *December* 1986)

**Key Word Index**—*Spirulina platensis*; 2-*O*-methyl-rhamnose; 3-*O*-methyl rhamnose.

**Abstract**—*Spirulina platensis* contains 13.6% carbohydrate, the sugar composition of which is comprised principally of glucose along with rhamnose, mannose, xylose, galactose and two unusual sugars. The latter were identified by a combination of GC-MS, NMR and de-*O*-methylation as 2-*O*-methyl-L-rhamnose and 3-*O*-methyl-L-rhamnose. Water-soluble polysaccharides were complex and heterogeneous, while the acid-soluble polysaccharide was a homogeneous glucan.

## INTRODUCTION

Algae are used traditionally as human food, since they are a rich source of protein [1]. Even though a considerable amount of work has been done on the characterization of the proteins [2] and the toxicological evaluation [3] of *Spirulina*, no systematic work has been done on the carbohydrates of this alga.

## RESULTS AND DISCUSSION

*Spirulina* contains 13.6% carbohydrate and 55% protein (for sugar composition see Table 1). The acidic sugar in the hydrolysates of the alga and its water-soluble polysaccharides was identified as mannuronic acid (PC in solvent system (e); glucuronic, galacturonic and mannuronic acids as standards). This is in contrast to the glucuronic and galacturonic acids reported in *Anabaena cylindrica* [4] and *Nostoc commune* [5] respectively. No starch was detected in *Spirulina*.

The free sugars, isolated in 0.8% yield, consisted of glucose (40%), mannose (30%) and xylose (26%) with a small amount of an oligosaccharide (4%, $R_{Glc}$ 0.48). The latter yielded only glucose upon isolation in pure form and hydrolysis.

The residue left after extraction of the free-sugars contained 18.2% carbohydrate, the constituent sugars of which are shown in Table 1. Cold water, hot water and acid-soluble polysaccharides were isolated sequentially in yields of 5.1, 0.6 and 0.5% respectively from the alcohol-insoluble residue.

The cold water-soluble extract was highly coloured due to the presence of phycocyanins, the bulk of which were removed by isoelectic pH precipitation (pH 3.0). The resultant cold water-soluble polysaccharide (CWSP) was rich in carbohydrates (60%) and had a protein content of 22.8%. The sugar composition of the CWSP was very complex, c.f. complex water-soluble polysaccharides reported for *Nostoc muscorum* [6]; *Anabaena cylindrica* [4] and *Nostoc Commune* [5]. It contained 9.5% uronic acid and 2.3% sulphate and attempts to fractionate it on DEAE-cellulose gave six fractions which differed from each other both qualitatively and quantitatively. The water-eluted neutral fraction (2.2% recovered material) had glucose (55.6%) and sugar $X_1$ (28.6%) as the major sugars with small amounts of galactose, rhamnose and mannose. Two minor fractions (6.7%) were eluted with 0.05 and 0.1 M borax. The fraction eluting with 0.05 M borax had glucose (95.6%) as the major sugar with small amount of sugar $X_1$ (4.4%). The other fraction eluting with 0.1 M borax had glucose and sugar $X_1$ in approximately equal concentrations. The cause of variation in these two fractions is not clear as uronic acid and sulphate estimations were not done. The three major fractions (90.8%) eluting with NaOH had differences in uronic acid and sulphate contents. The fraction eluting with 0.05 M NaOH had glucose as the major sugar (40.3%), followed by mannose (23.4%), rhamnose (18.5%), xylose (11.1%) with small amounts of sugars $X_1$ and $X_2$. The fraction eluting with 0.1 M NaOH had rhamnose (49.1%) and mannose (20.8%) as the major sugars. The last fraction (0.4 M NaOH) contained mannose and rhamnose in an approximate ratio of 1:2 with small amounts of other sugars. Even though the composition of all these fractions varied qualitatively and quantitatively, none of them were pure as assessed by microzone electrophoresis [7] and gel permeation chromatography [8].

The hot water-soluble polysaccharide (HWSP) had a carbohydrate content of 38.3% with a uronic acid content of 9.1% and a sulphate content of 0.4%. Glucose (38.7%) and rhamnose (24.4%) were the major sugars (Table 1).

The acid-soluble polysaccharide (ASP) isolated as a creamy solid was rich in carbohydrates ($\simeq$ 80%). This had mainly glucose (97.4%) and hence was a glucan-type polysaccharide. ASP was negative to the starch-$I_2$ test and had a sulphate content of 4.9%. The coloured acid-insoluble residue (yield 43.1%) contained only glucose.

### *Characterization of unusual sugars*

Two unusual sugars ($X_1$, $T_{Rha}$ 0.79; $X_2$, $T_{Rha}$ 0.9) were observed on GC analysis of the alga and its alcohol-insoluble residue and water-soluble (cold and hot) polysaccharide fractions. They moved as a single spot ahead of

Table 1. Chemical composition (%) of *S. platensis* and its fractions

| | Alga | Alcohol insoluble residue | CWSP | HWSP | ASP | Acid insoluble residue |
|---|---|---|---|---|---|---|
| Yield | (100) | 74.4 | 5.1 | 0.6 | 0.5 | 43.1 |
| Moisture | 8.1 | 10.5 | 5.4 | ND | ND | ND |
| Protein | 55.0 | 60.1 | 22.8 | 38.0 | 1.1 | 16.1 |
| Sulphate | ND | ND | 2.3 | 0.4 | 4.9 | ND |
| Uronic acid | ND | ND | 9.5 | 9.1 | — | ND |
| Total sugar | 13.6 | 18.2 | 60.0 | 38.3 | 79.9 | 19.9 |
| *Sugars detected* | | | | | | |
| Sugar $X_1$ | 1.1 | 1.2 | 2.1 | 6.1 | — | — |
| Sugar $X_2$ | 3.2 | 2.3 | 6.0 | 14.5 | — | — |
| Rhamnose | 22.3 | 18.3 | 50.6 | 24.4 | — | — |
| Xylose | 7.0 | 0.8 | 2.3 | 2.8 | 0.6 | — |
| Mannose | 9.3 | 1.2 | 17.1 | 9.9 | 1.1 | — |
| Galactose | 2.6 | 1.1 | 1.0 | 3.6 | 0.9 | — |
| Glucose | 54.4 | 75.1 | 20.9 | 38.7 | 97.4 | 100.0 |

CWSP, Cold water-soluble polysaccharide; HWSP, hot water-soluble polysaccharide; ASP, acid-soluble polysaccharide; ND, not determined.

rhamnose on PC and TLC ($R_{Rha}$ 1.44 in solvent system 'b' on PC) in at least eight different solvent systems and could be detected with aniline phthalate spray reagent but not with $AgNO_3$/NaOH or urea/HCl reagents.

$^1$H NMR analysis of a mixture of the two sugars isolated in pure form from HWSP showed the presence of signals at $\delta$1.6 and 1.7 indicative of a C–Me group, while others at $\delta$3.8 suggested the presence of a O–Me group. The increase in peak intensity due to protons for Me and O–Me gave further support for the presence of these groups.

Further characterization of these sugars was carried out by GC-MS analysis of their alditol ($NaBH_4$) acetate derivatives. The first sugar eluted ($T_{Rha}$ 0.79, sugar $X_1$) gave primary fragments *m/z* 275 (12%) and 118 (100%). The latter is derived by cleavage between C-2 and C-3, when C-1 is labelled. Fragment *m/z* 275 is indicative of a deoxy group probably at C-6. This indicated that sugar $X_1$ may be 2-*O*-methyl-6-deoxy hexose. The second sugar eluted (sugar $X_2$) gave primary fragments *m/z* 203 (33%) and 190 (48%) due to cleavage between C-2 and C-3, and C-3 and C-4 respectively. These fragments on further elimination of acetic acid gave two secondary fragments, *m/z* 143 and 130. Thus sugar $X_2$ was identified as 3-*O*-methyl-6-deoxy hexose.

Identification of the nature of the deoxy hexose was done by de-*O*-methylating the native sugars with boron trichloride [9] to give rhamnose (PC and GC). The optical rotation $[\alpha]_D$ of the mixture of sugars $X_1$ and $X_2$ in water of +50° indicates that the sugars belong to the L-series. The reported $[\alpha]_D$ for L-acofriose is +39.1° [10] and that of D-acofriose −27° [11]. The $[\alpha]_D$ of 2-*O*-methyl D-rhamnose is −22° [11].

Thus the two unusual sugars present in *S. platensis* were identified as 2-*O*-methyl-L-rhamnose (Sugar $X_1$) and 3-*O*-methyl-L-rhamnose (sugar $X_2$) also called L-acofriose. The latter has been identified as a constituent of the lipopolysaccharides of *Rhodopseudomonas capsulata* [12], *Anabaena variabilis* [13] and *Batrachospermum* [14].

Different types of *O*-methyl sugars are reported in algae. 3-*O*-Methyl-xylose, and 3- and 4-*O*-methyl-galactose are found in *Porphyridium aerugineum* [15], 3-*O*-methyl-xylose in *Rhodella maculata* [16], 3-*O*-methyl-D-galactose in *Batrachospermum* [14] and 3- and 4-*O*-methyl mannose in *Anacystis nidulans* [17].

## EXPERIMENTAL

*Cultivation of the algae. Spirulina platensis* was cultivated in fresh $H_2O$ at alkaline pH (8–10) under defined conditions with $NaHCO_3$ as the carbon source (8 g/l) and $NaNO_3$ (1.5 g/l) as the $N_2$ source. The other nutrients in the medium were $K_2HPO_4$ (0.5 g/l), NaCl (1 g/l), $MgSO_4 \cdot 2H_2O$ (0.2 g/l), $K_2SO_4$ (1 g/l), $CaCl_2 \cdot 2H_2O$ (0.04 g/l) and $FeSO_4 \cdot 7H_2O$ (0.01 g/l). The alga was harvested by gravity filtration and freeze dried.

*Extraction of carbohydrates.* Freeze dried *Spirulina* was defatted with $CHCl_3$–MeOH (2:1) and the free sugars extracted with hot 70% EtOH (4 hr × 3). The combined extract was deionized, concentrated and dried. Cold and hot water-soluble polysaccharides were extracted (8 hr × 3) from the alcohol-insoluble residue and purified by isoelectric pH precipitation (pH 3.0). The purified extracts after dialysis were precipitated with 3 vol. of EtOH and lyophilized to give creamy cold and hot water-soluble polysaccharides. The water-insoluble residue was extracted with HCl (pH 2.0) at 70° (8 hr × 3) [18] and concentrated. This was dialysed and lyophilized to get acid-soluble polysaccharide.

*General methods.* Most of the general methods employed for hydrolysis, PC, TLC, GC and GC-MS have been described [19]. PC was done on Whatman No. 1 or No. 3 papers with the following solvent systems: (a) 1-PrOH–EtOH–$H_2O$ (7:1:2), (b) 1-BuOH–EtOH–$H_2O$ (10:1:2), (c) 1-BuOH–$C_6H_5N$–$H_2O$ (6:4:3) (d) EtOAC–$C_6H_5N$–HOAc–$H_2O$ (5:5:1:3), (e) EtOAc–HCOOH–HOAc–$H_2O$ (18:1:3:4), and TLC using either precoated Silica gel G plates or cellulose strips in the following solvent systems: (f) $C_6H_6$–EtOH (5:1), (g) *n*-BuOH–$C_6H_5N$–$H_2O$ (6:4:3), (h) $C_6H_6$–BuOH–$C_6H_5N$–$H_2O$ (1:5:3:3), (i) Butanone saturated with water. The spray reagents used were modified $AgNO_3$/NaOH [20], aniline hydrogen phthalate [21], and urea hydrochloride [22]. Sulphate was

estimated by a turbidometric method [23]. De-*O*-methylation was affected with $BCl_3$ [9].

$^1H$ NMR *analysis* was performed at 60 MHz. The sample after purification from PC was desalted on a Biogel P 2 column (1.5 × 50 cms), exchanged with $D_2O$ and finally taken up in 99.98% $D_2O$ for analysis.

*DEAE-Cellulose CC.* The CWSP (2.2 g) was loaded on a DEAE-Cellulose (borate form) column (5 × 35 cm) [24]. The column was eluted with water, 0.05–0.5 M borax and 0.05–0.4 M NaOH. The flow rate was adjusted to 60 ml/hr and 15 ml fractions were collected.

*Acknowledgements*—We thank Dr H. Mayer, MPI, Freiburg, F.R.G. for GC-MS analysis and Dr R. N. Tharanathan (this Institute) for useful suggestions. One of the authors, (M.M.S.) wishes to thank the Department of Science and Technology for a fellowship during the course of this study.

## REFERENCES

1. Becker, E. W. and Venkataraman, L. V. (1980) in *Algae Biomass* (Shelef, G. and Soeder, C. J., eds) p. 35. Elsevier, Amsterdam.
2. Venkataraman, L. V., Becker, E. W., Rajasekaran, T. and Mathew, K. R. (1980) *Food. Cosmet. Toxicol.* **18**, 271.
3. Anusuya Devi, M. and Venkataraman, L. V. (1983) *J. Food. Sci.* **49**, 24.
4. Bishop, C. T., Adams, G. A. and Hughes, E. O. (1954) *Can. J. Chem.* **32**, 999.
5. Hough, L., Jones, J. K. N. and Wadman, W. H. (1952) *J. Chem. Soc.* 3393.
6. Biswas, B. B. (1957) *Sci. Cult.* **22**, 696.
7. Anderson, D. M. W., Hendrie, A., Miller, J. K. A. and Munro, A. C. (1971) *Analyst* **96**, 870.
8. Determann, H. (1968) *Gel Chromatography*. Springer, New York.
9. Allen, S., Bonner, T. G., Bourne, E. J. and Saville, M. N. (1958) *Chem. Ind.* 630.
10. Muhr, H., Hunger, A. and Reichstein. T. (1954) *Helv. Chim. Acta.* **37**, 403.
11. Morrison, I. M., Young, R., Perry, M. B. and Adams, G. A. (1967) *Can. J. Chem.* **45**, 1987.
12. Weckesser, J., Mayer, H. and Drews, G. (1970) *Eur. J. Biochem.* **16**, 158.
13. Weckesser, J., Katz, A., Drews, G., Mayer, H. and Fromme, I. (1974) *J. Bacteriol.* **120**, 672.
14. Turvey, J. R. and Griffiths, L. M. (1973) *Phytochemistry* **12**, 2901.
15. Percival, E. and Foyle, R. A. J. (1979) *Carbohydr. Res.* **72**, 165.
16. Fareed, V. S. and Percival, E. (1977) *Carbohydr. Res.* **53**, 276.
17. Weise, G., Drews, G., Jann, B. and Jann, K. (1970) *Arch. Mikrobiol.* **71**, 89.
18. Carlberg, G. E. and Percival, E. (1977) *Carbohydr. Res.* **57**, 223.
19. Sajjan, S. U. and Salimath, P. V. (1986) *Carbohydr. Res.* **145**, 348.
20. Trevelyan, W. E., Procter, D. P. and Harrison, J. S. (1950) *Nature* **166**, 444.
21. Partridge, S. M. (1949) *Nature* **164**, 443.
22. Dedonder, R. (1952) *Bull. Soc. Chem. Biol.* (Paris) **34**, 144.
23. Sperber, I. (1948) *J. Biol. Chem.* **172**, 441.
24. Siddiqui, I. R. and Wood, P. J. (1974) *Carbohydr. Res.* **36**, 35.