therefore of interest in relation to these other nitrogenous substances.

RESULTS AND DISCUSSION

The seeds of 38 species of 29 genera in 21 tribes were examined (Table 1). Stizolamine was detected in 19 species of the subfamily Lotoideae. It could not be detected in *Delonix regia* Raf., *Cassia occidentalis* L., and *Cercis chinensis* Bunge. belonging to Caesalpinioideae or in *Leucaena glauca* Benth. of the Mimosoideae, but its absence from these two subfamilies cannot be assumed until a proper sample has been examined. Its occurrence in Lotoideae was wide (82% in tribes, 56% both in genera and species tested) but very sporadic. The content was fairly varied (0.249–9540 nmol/g of seeds), and always less than that originally found in *Stizolobium hassjoo*. The maximum content was obtained in *Lespedeza cuneata* G. Don., and the minimum in *Indigofera decora* Lindl.

Turner and Harborne [5] have reported that canavanine is found only in the subfamily Lotoideae and stizolamine, also a guanidino compound, seems to have a similar distribution.

EXPERIMENTAL

Plant materials. The seeds of *Cercis chinensis* Bunge., *Indigofera pseudotinctoria* Matsum., *Glycine soja* Sieb. et Zucc. and *Vicia unijuga* Al. Br. were collected at Saruhashi in Yamanashi Prefecture, *Rhynchosia acuminatifolia* Makino, *Pueraria thunbergiana* Benth., *Lespedeza pilosa* Sieb. et Zucc., *Kummerowia striata* Schindl., *Dumasia truncata* Sieb. et. Zucc. and *Desmodium racemosum* DC. at Kakio in Kanagawa Prefecture and *Erythrina* sp. at Izu-kogen in Shizuoka Prefecture. The seeds of *Sophora japonica* L., *Cytisus scoparius* Link., *Indigofera decora* Lindl., *Amphicarpa edgeworthii* Benth. var *japonica* Oliver and *Lespedeza cuneata* G. Don. were gathered at Fukazawa, Setagaya-ku in Tokyo, *Wistaria floribunda* DC. in Akasaka, Minato-ku in Tokyo and *Cassia occidentalis* L., *Sophora fomentosa* L., *Canavalia lineata* DC. and *Leucaena glauca* Benth. at Ogasawara in Tokyo. Further, the seeds of *Delonix regia* Raf., *Kobinia pseudo-Acacia* L., and *Vicia sativa* L. were supplied by Dr. S. Kobayashi, Makino Herbarium, Tokyo Metropolitan University. The other plant materials were all commercial sources.

Isolation and determination of stizolamine. Five g (or 3 g) of seeds were crushed in 30 ml hot H_2O and extracted with 4 × 100 ml hot 50% MeOH containing 1 ml of HOAc for each 1 hr. The combined extracts were concentrated *in vacuo*, the remaining residue dissolved in 100 ml of hot H_2O and insoluble materials filtered off. The filtrate was passed through the Amberlite IRA 410 (OH^- form) column, the effluent was concentrated and dissolved in 100 ml 0·01 N NCl. The solution was concentrated and dissolved in 5 ml of H_2O (or 3 ml of H_2O). 50 μl of this crude extract was spotted on 2 cm wide papers and developed with *n*-BuOH–HOAc–H_2O (6:1:2) and 6% HOAc, separately. Stizolamine was detected on chromatograms by its blue fluorescence and colour reactions with alkaline nitroprusside–ferricyanide and Dragendorff's reagents at R_f value 0.3 (the former solvent) and at 0·7 (the latter solvent) [the limitation value of detectable concentration by fluorescence; 3.33 pmol per 2 cm width]. An aliquot (1.5 ml) of the crude extract was spotted on paper (0.5 ml/20 ml of width) and developed with the former solvent. The blue fluorescent substance at R_f 0.3 was extracted with 50% MeOH, concentrated and dissolved in 20 ml H_2O. The soln was applied on CM-cellulose column (1.6 × 20 cm) and column was washed with 50 ml H_2O. The amine adsorbed on the column was then eluted with 1 N HOAc. The eluate was concentrated *in vacuo*, dissolved in 10 ml of H_2O, passed through the Amberlite IRA 410 (OH^- form) column. The effluent was concentrated, dissolved in 1.5 ml of H_2O, applied on Sephadex G-10 column (2 × 49 cm) saturated with 0.1 M NH_4Ac and chromatographed with the same solvent (flow rate; 6 ml/hr, fraction volume; 2 ml). In this procedure, the most abundant amount of stizolamine was detected at 34th fraction. Stizolamine was measured fluorometrically (Excited at 350 nm, Analysed at 390 nm).

Acknowledgements—The authors are grateful to Prof. S. Yoshida, Department of Biology, Faculty of Science, Tokyo Metropolitan University, for his advice on this study and to Dr. S. Kobayashi, Makino Herbarium, Tokyo Metropolitan University, for help on the classification of plant materials.

REFERENCES

1. Yoshida, T. (1977) *Phytochemistry* **16** (in press).
2. Kitagawa, M. and Tomiyama, T. J. (1926) *J. Biochem.* (Tokyo) **11**, 269.
3. Lin, Y. and Ling, K. (1962) *Taiwan Ta Hsueh Huitsa Chih.* **61**, 484; *CA* **65**, 4143.
4. Bell, E. A. and Foster, R. G. (1962) *Nature* **194**, 91.
5. Turner, B. L. and Harborne, J. B. (1967) *Phytochemistry* **6**, 863.

Phytochemistry, 1977, Vol. 16, pp 132–133. Pergamon Press. Printed in England

FUCOIDAN IN *PADINA SANCTAE-CRUCIS* SPORES

KYRSIS R. RODRÍGUEZ* and LARRY B. LIDDLE

Department of Biology, University of Puerto Rico, Río Piedras, PR 00931, U.S.A.

(*Revised received* 10 *May* 1976)

Key Words Index—*Padina sanctae-crucis*; Dictyotaceae; fucoidan; sulphated polysaccharide, spore.

Abstract—The presence and distribution of fucoidan in the vegetative fronds and spores of the brown alga *Padina sanctae-crucis* Børg. was studied. Autoradiography using ^{35}S showed that fucoidan is localized in the walls of the

* Part of this work was presented in partial fulfilment of the requirements for the degree of Master in Science.

meristematic cells of the rhizoid of the developing spore. It is suggested that fucoidan is structurally involved in the formation of the new cell walls.

INTRODUCTION

Morphological and biochemical studies of polar axis formation in the zygotes of brown algae have shown that RNA [1], certain organelles [2], and a sulphated polysaccharide [2–5] accumulate in the rhizoidal protuberance. Quatrano [2] demonstrated that the sulphated polysaccharide is fucoidan.

Fucoidan, first isolated and described by Kylin [6, 7], is a sulphated polysaccharide polymer of fucan [8]. Fucan is composed of 65% L-fucose and 10% sulphate and has a MW of 7.8×10^4 [9]. A calcium ion attaches two fucan molecules together in the polymerization process [10]. Whistler and Smart [11] found fucoidan to be insoluble in alcohols but soluble in cold dilute acids in which it precipitates as a fine white powder.

The sulphation and polymerization of L-fucose in the fertilized egg of *Fucus* occurs only at the time and site of rhizoid formation [3, 12]. Quatrano and Crayton [13] suggested that fucoidan might be playing a major role in the establishment of the polar axis, but they later proved [14] that this was not so. They also hypothesized that the net negative charge introduced in fucoidan by sulphation could be involved in localizing this substance into the rhizoidal part of the cell by a self-electrophoresis mechanism in which the negative part of the molecule makes it migrate to the more positive side.

Although fucoidan has been isolated from many algal phyla and several Orders of the Phylum Phaeophyta [8], it has not been demonstrated in tropical brown algae. *Padina sanctae-crucis* grows abundantly in Puerto Rico and other Caribbean islands on rocks, old corals and shells in shallow water of moderately protected habitats [15]. *Padina* spores develop like *Fucus* zygotes. The rhizoidal protuberance appears about 14 hr after liberation and the first cell dvision is completed two hr later.

RESULTS AND DISCUSSION

Purified fucoidan from fronds of *Padina sanctae-crucis* was identified by the peak A at 620 nm on complexing with toluidine blue to form the *O*-fucoidan complex [13].

Formation of fucoidan requires sulphation [9, 13, 14]. Autoradiographic studies showed that normal germinating spores incorporate labeled sulphate ions. Radioactivity in granules was observed in the rhizoidal part of the spore. The grains were also present in the vicinity of the walls of the new cells of the rhizoid. The optimum exposure time for incorporation of labelled sulphate ions was 30 days. These results corroborate those of *Fucus* zygotes in which fucoidan migrates and accumulates in the rhizoidal protuberance where cell wall formation is occurring [14]. This polysaccharide may not be exclusively synthesized and used by reproductive cells (spores and zygotes). Any cell undergoing division may use fucoidan to build up a new wall.

EXPERIMENTAL

Specimens of *Padina sanctae-crucis* with high spore density were collected from the intertidal region in the Condado area in San Juan, Puerto Rico. The plants were held from 10 to 12 h in aerated seawater containers before use. Methods previously described in ref. [13] were used to isolate, purify and identify the fucoidan. Aliquots of 0.05–0.30 ml of the isolated and purified fucoidan were pipetted onto Whatman filter paper disks (3MM) (25 mm diam) under a stream of warm air. Dry filter paper was stained for 15 min in 0.1% toluidine blue 0 in 1% HOAc. The stain was then removed by washing the disks 3–4 times in 7% HOAc solns. The metachromatic color of the toluidine blue *O*-fucoidan complex was removed by shaking the disks in 25 ml of 90% EtOH containing 1% NH_4OH. The colored soln was centrifuged at 5000 rpm and read at 10 nm intervals in a range of 350–700 nm. Autoradiographic studies followed the methods of ref [16–18]. Spores of *Padina* were liberated on slides in Petri dishes containing 40 ml of Millipore-filtered sea water. Spores were exposed to 0.56 mCi of Na_2SO_4 (^{35}S) (sp.act.677 mCi/mmol) for 1, 10 and 16 hr after the initiation of germination. Three Petri dishes containing a soln of 2.06×10^{-5} M in Na_2SO_4 were used for each exposure time. After different time intervals, spores were fixed for 1 h in 4% HcHO in sea water and subsequently transferred for 30 mins to fresh 4% HcHO. The slides were then put in 10% TCA for 10 min, rinsed in cold H_2O, transferred to 5% TCA for 5 min and then washed in running H_2O for 5 min to remove any unbound isotope. After air drying, the slides were placed in black slide boxes (plastic) and sealed with opaque tape. They were stored in the refrigerator until development. Slides were developed in Dektol 11 photographic developer after 4, 7, 10, 14, 18, 20 and 30 days of exposure. The developed slides were stained using the hematoxylin-eosin method [19]. The number of developing spores per slide, as well as the number of spores showing radioactivity granules per slide were counted. The percentage of spores that incorporated the isotope was used to establish the optimum exposure time.

REFERENCES

1. Kochler, L. D. and Linskens, H. F. (1967) *Protoplasma* **64**, 209.
2. Quatrano, R. S. (1972) *Exp. Cell Res.* **70**, 1.
3. McCully, M. E. (1969) *XIth. Intl. Botanical Congress* (Abstract) Seattle, Washington.
4. Nakazawa, S. and Takamura, K. (1966) *Cytologia* **32**, 408.
5. Quatrano, R. S. (1970) *J. Cell Biol.* (Abst. no. 436) **47**, 164a.
6. Kylin, H. (1913) *Z. Physiol. Chem.* **83**, 171.
7. Kylin, H. (1915) *Z. Physiol. Chem.* **94**, 337.
8. Percival, E. G. V. and McDowell, R. H. (1967) *Chemistry and Enzymology of Marine Algal Polysaccharides*. Chapt. 7, p. 157. Academic Press, New York.
9. Bernardi, G. and Springer, G. F. (1962) *J. Biol. Chem.* **237**, 75.
10. Percival, E. G. V. and Ross, A. G. (1950) *J. Chem. Soc.* No. 145, 717.
11. Whistler, R. L. and Smart, C. L. (1953) *Polysaccharide Chemistry*. Chapt. XII, p. 355. Academic Press, New York.
12. McCully, M. E. (1970) *Ann. N.Y. Acad. Sci.* **175**, 702.
13. Quatrano, R. S. and Crayton, M. A. (1973) *Dev. Biol.* **30**, 29.
14. Crayton, M. A., Wilson, E. and Quatrano, R. S. (1974) *Dev. Biol.* **39**, 164.
15. Taylor, W. R. (1960) *Marine Algae of the Eastern Tropical and Subtropical Coasts of America*. p. 237. The University of Michigan Press, Ann Arbor.
16. Caro, L. G. (1966) *Methods in Cell Physiology*. Volume I. (Prescott, D. M. ed.), Chapt. 16. p. 327. Academic Press, New York.
17. Prescott, D. M. (1966) *Methods in Cell Physiology*. Volume I. (Prescott, D. M. ed.) Chapt. 19, p. 370. Academic Press, New York.
18. Stevens, A. R. (1966) *Methods in Cell Physiology*. Volume II. (Prescott, D. M. ed.), Chapt. 10, p. 255. Academic Press, New York.
19. Cowdry, E. V. (1943) *Laboratory Technique in Biology and Medicine*. 2nd. Edition-1948. p. 118. Williams & Wilkins, Baltimore, Md U.S.A.