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SHORT COMMUNICATION

# INVESTIGATION OF ALGAE AND YEAST FOR α-TOCOPHEROL AND α-TOCOPHEROLQUINONE CONTENT

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**Abstract**—Five species of yeast and nine species of algae were analyzed for their content of α-tocopherol and α-tocopherolquinone. No α-tocopherol or α-tocopherolquinone was found in any of the yeasts examined. However, several of the algae contained α-tocopherol and α-tocopherolquinone. A brine water alga, *Stichococcus bacillaris*, contained the largest amount of α-tocopherol (134·2 μg/g dry weight) and a brown marine alga, *Macrocystis integrifolia*, contained the largest amount of α-tocopherolquinone (11·9 μg/g dry weight). None of these organisms would appear to be as suitable as *Euglena gracilis* for studies of the biosynthesis of α-tocopherol and α-tocopherolquinone.

## INTRODUCTION

ALTHOUGH several workers[1–5] have investigated various algae for the presence of α-tocopherolquinone, only Powls and Redfearn[6] have done so for the tocopherols. However, their search was limited to the blue–green algae. In efforts to find an organism suitable for studies of the biosynthesis of α-tocopherol and α-tocopherolquinone we surveyed a number of algae and yeasts for content of these two substances.

Yeasts analyzed were *Rhodatorula*, *Candida rugosa*, *C. pelliculosa* (SRI microbiology) and *Torula* 1 N and 3 N (Lake States Yeast, Rhinelander, Wisconsin). Algae studied were *Chlorella* (green) (Univ. of California at Berkeley), the macroscopic marine algae *Gigartina corymbifera* and *Drionitis lanceolata* (red), *Fucus distichus* and *Macrocystis integrifolia* (brown), *Cladaphora trichotoma* and *Ulva taeniata* (green) (Hopkins Marine Station, Pacific Grove, California). In addition, two green microscopic marine algae, *Stichococcus bacillaris* and *Dunaliella salina*, reportedly present in brine water (Leslie Salt Co., Hayward, California), were analyzed.

The analytical method used for α-tocopherol was that previously reported[7] while α-tocopherolquinone was analyzed by examination of the u.v. spectrogram before and after reduction with $NaBH_4$.[8]

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[1] C. BUCKE, R. LEECH, M. HALLAWAY and R. A. MORTON, *Biochim. Biophys. Acta* **112**, 19 (1966).
[2] M. D. HENNINGER, H. N. BHAGAVAN and F. L. CRANE, *Arch. Biochem. Biophys.* **110**, 69 (1965).
[3] N. G. CARR and M. HALLAWAY, *Biochem. J.* **97**, 9C (1965).
[4] N. G. CARR, G. EXELL, V. FLYNN, M. HALLAWAY and S. TALUKDAR, *Arch. Biochem. Biophys.* **120** (3), 503 (1967).
[5] K. TAKAMIYA, M. NISHIMURA and A. TAKAMIYA, *Plant Cell Physiol.* (*Tokyo*) **8** (1), 79 (1967).
[6] R. POWLS and E. R. REDFEARN, *Biochem. J.* **104** (2), 24C (1967).
[7] P. A. STURM, R. M. PARKHURST and W. A. SKINNER, *Analyt. Chem.* **38**, 1244 (1966).
[8] D. R. THRELFALL and T. W. GOODWIN, *Biochem. J.* **103**, 573 (1967).

## RESULTS AND DISCUSSION

A summary of the α-tocopherol and α-tocopherolquinone contents of the various algae and yeasts are reported in Table 1. In Table 2 are shown the chromatographic behaviour of various tocopherols and other terpenoids in several systems.

TABLE 1. ANALYSES OF YEAST AND ALGAE FOR α-TOCOPHEROL AND α-TOCOPHEROLQUINONE*

| No. | Organism | Dry wt (g) | Extract (g) | α-Tocopherol[a] | α-Tocopherolquinone[a] |
|---|---|---|---|---|---|
| | | | | (μg/g dry wt) | |
| | | Yeast | | | |
| 1 | *Torula* 1 N | 50·0 | 4·45 | ND (2·0) | ND (0·4) |
| 2 | *Torula* 3 N | 50·0 | 4·69 | ND (2·0) | ND (0·4) |
| 3 | *Torula* 1 N[b] | 50·0 | 5·87 | ND (2·0) | ND (0·4) |
| 4 | *Rhodatorula* | 21·3 | 1·57 | ND (4·75) | ND (0·9) |
| 5 | *Candida rugosa* | 8·0 | 1·10 | ND (12·5) | ND (2·5) |
| 6 | *C. pelliculosa* | 39·8 | 2·36 | ND (2·5) | ND (0·5) |
| | | Algae | | | |
| 7 | *Chlorella*[c] (green) | 100·0 | 12·7 | 7·58 (1·0) | 0·74 |
| 8 | Brine water algae A[d] (green) | 44·5 | 3·0 | 63·8 (1·74) | ND (0·3) |
| 9 | Brine water algae B[d] (green) | 11·7 | 2·02 | 134·2 (8·75) | ND (1·7) |
| 10 | *Gigartina corymbifera* (red) | 100·0 | 2·37 | ND (1·0) | 0·92 |
| 11 | *Drionitis lanceolata* (red) | 117·5 | 2·24 | ND (0·85) | 2·42 |
| 12 | *Ulva taeniata*[e] (green) | 48·5 | ·75 | ND (2·1) | 3·43 |
| 13 | *Cladaphora trichotoma*[e] (green) | 154·5 | 1·79 | 0·74 (0·65) | 3·00 |
| 14 | *Macrocystis integrifolia* (brown) | 73·7 | 1·67 | 12·2 (1·36) | 11·9 |
| 15 | *Fucus distichus* (brown) | 105·3 | 1·26 | 11·1 (0·95) | 9·78 |

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* Extracts from analyses 1, 2, 3, 5, and 6 were saponified and the unsaponifiable lipids examined by thin-layer chromatography for the presence of α-tocopherol and α-tocopherolquinone. In remaining analyses, the extracts were chromatographed on columns of silicic acid and the appropriate fractions examined by thin-layer chromatography. ND = none detected.

[a] Figures in parentheses show the lowest possible chromatographic limits of detection.

[b] Sample was homogenized in a Brownwell homogenizer before extraction.

[c] An unknown substance with four sharp bands at 244, 249, 255, 261 mμ was isolated in conjunction with the α-tocopherol area.

[d] Brine water A, salinity 74 g/kg, contained both *Stichococcus bacillaris* and *Dunaliella salina*; brine water B, salinity 36 g/kg, contained predominantly *S. bacillaris*. The 20 per cent E-PE fractions of each, upon TLC, yielded a substance with u.v. maximum at 243 mμ (A) and 245 mμ (B).

[e] A substance with u.v. maxima at 242, 247, 253, and 259 mμ was isolated in conjunction with the α-tocopherol area.

TABLE 2. CHROMATOGRAPHIC BEHAVIOR OF TOCOPHEROLS AND OTHER TERPENOIDS

| Compound | $R_f$ value in system*: SiGF–$CHCl_3$ | $R_f$ value in system*: KiG–90% $Me_2CO$ in $H_2O$ |
|---|---|---|
| Ubichromenol-9 | | 0·056 |
| Ubichromanol-3 | 0·53 | 0·61 |
| α-Tocopherol | 0·64 | 0·42 |
| γ-Tocopherol | | 0·695 |
| δ-Tocopherol | | 0·81 |
| Ubiquinone-10 | 0·76 | 0·87 |
| α-Tocopherolquinone | 0·32 | |

* SIGF, Silica gel-GF; KiG, Nujol-impregnated Kieselguhr.

Brine water algae "B", which contained primarily *Stichococcus bacillaris* because of the lower salinity, had the highest concentration of α-tocopherol, 134·2 μg/g, while *Macrocystis integrifolia*, a brown marine algae, contained the largest amount of α-tocopherolquinone, 11·9 μg/g. No detectable amounts of either α-tocopherol or α-tocopherolquinone could be found in any of the yeasts investigated. Our limit of detection of α-tocopherol for *Torula* yeasts 1 N and 3 N was 2 μg/g. Diplock *et al.*[9] reported 2·5–4·2 μg/g of α-tocopherol in *Torula* obtained from the same source. We did find an ubichromenol in this yeast with an $R_f$ value on silica gel GF in benzene of 0·75 compared with 0·67 for α-tocopherol. Stevenson *et al.*[10] reported ubichromenol-6 to be present in *Torula*.

Various strains of *Euglena gracilis* have been investigated for content of α-tocopherol and α-tocopherolquinone and concentrations of α-tocopherol up to 846 μg/g and of α-tocopherolquinone up to 555 μg/g have been reported.[8]

From Table 2 it can be seen that α-tocopherol and α-tocopherolquinone can easily be separated from one another using silica gel-GF–$CHCl_3$ TLC. Ubichromanol-3 does not interfere with α-tocopherol separation in this system. By the use of Nujol-impregnated kieselguhr plates (KiG) one can separate ubichromenol-9, ubichromenol-3, α-tocopherol, δ-tocopherol, and γ-tocopherol from one another, but ubiquinone-10 interferes with δ-tocopherol.

It appears that none of the algae investigated are as rich a source of α-tocopherol or α-tocopherolquinone as is *E. gracilis*. However, variation of growth conditions was not attempted.

## EXPERIMENTAL

The six macroscopic marine algae (10–15, Table 1; Hopkins Marine Station) were ground in a Hobart grinder with a No. 10 dye, lyophilized and extracted for a 24 hr period at room temperature with excess 25 per cent methanol in light petroleum (30–60°). *Torula* and *Chlorella* were obtained in dry form, while the remaining samples required lyophilization before similar extraction.

The algae content of brine water was removed by the use of a Sharples centrifuge. Organisms centrifuged from brine water with salinity of 74 per cent (A) are *Stichococcus* and *Dunaliella* while *Stichococcus* alone predominates in brine with salinity of 36 per cent (B).[11] Salinity is expressed as total salts in g per kg of solution.

The concentrated extracts either underwent saponification[7] or column chromatography followed by TLC, examination by u.v. and subsequent reduction by $NaBH_4$ (α-tocopherolquinone) and analysis by the Tsen-modified[12] Emmerie-Engel method[13] for α-tocopherol. The column chromatography was carried out by placing extracts dissolved in light petroleum (30–60°) on silicic acid (usually 50 g/g of extract; Gallard-Schlesinger, 90–200 mesh) columns packed in light petroleum and eluting with 0, 2, 4, 6, 10, 20, 30, 100 per cent diethyl ether in light petroleum; each fraction being 10 ml/g silicic acid. α-Tocopherol was found to predominate in the 4 per cent ether–light petroleum fraction with some in the 6 per cent fraction, while α-tocopherolquinone predominated in the 20 and 30 per cent fraction.

Thin-layer adsorptive and reversed-phase partition chromatography were used to further characterize those fractions of interest. Benzene (1 ml) was added to each fraction after evaporation to dryness and aliquots (10–20 μl) were spotted along with standard α-tocopherol and α-tocopherolquinone on silica gel GF thin-layer plates. Development was with $CHCl_3$ and spots were detected with 10 per cent phosphomolybdic acid in ethanol. Tocopherols and other strongly reducing substances were blue. Heating the plate brought out other less easily oxidizable materials.

Tocopherol fractions were chromatographed on silica gel GF (1 mm) plates and the appropriate area removed and extracted. The extracts were further separated on Nujol-impregnated Kieselguhr thin-layer plates and eluted with 90 per cent acetone in water. This system is known to separate tocopherols from

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[9] A. T. Diplock, J. Green, E. E. Edwin and J. Bunyan, *Nature* **189**, 749 (1961).

[10] J. Stevenson, P. J. Hayward, F. W. Hemming and R. A. Morton, *Nature* **196**, 1291 (1962).

[11] L. H. Carpelan, *Hydrobiology of the Alviso Salt Ponds* (doctorate thesis), Stanford University (1953).

[12] C. C. Tsen, *Analyt. Chem.* **33** (7), 849 (1961).

[13] A. Emmerie and C. Engel, *Nature* **142**, 873 (1938).

tocotrienols.[14] U.v. absorption spectra in ethanol were obtained and quantitative measurement was as previously described.[7]

Preparative silica gel GF using 1:4 diethyl ether/$CHCl_3$ or $CHCl_3$ was sufficient to separate α-tocopherolquinone in pure form. Using the phosphomolybdic acid spray on a silica gel GF plate, the limits of detection for α-tocopherol were 0·5 μg before heating. For α-tocopherolquinone, after heating, 0·1 μg was detectable.

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[14] K. J. WHITTLE, P. J. DUNPHY and J. F. PENNOCK, *Biochem. J.* **100**, 138 (1966).