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5′-MONOHYDROXYPHYLLOQUINONE FROM *ANACYSTIS* AND *EUGLENA*

AH LAW*
Department of Biochemistry and Agricultural Biochemistry, University College of Wales, Aberystwyth SY23 3DD

and

GRAHAM THOMAS and DAVID R THRELFALL
Department of Botany, University of Hull, Hull HU6 7RX

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Key Word Index—*Anacystis nidulans*; *Euglena gracilis*, Algae, Cyanophyceae, Chlorophyceae, 5′-monohydroxyphylloquinone; MS, amounts

Abstract—The monohydroxy analogue of phylloquinone found in *Anacystis nidulans* and *Euglena gracilis* has been characterized as 5′-monohydroxyphylloquinone by MS analysis

INTRODUCTION

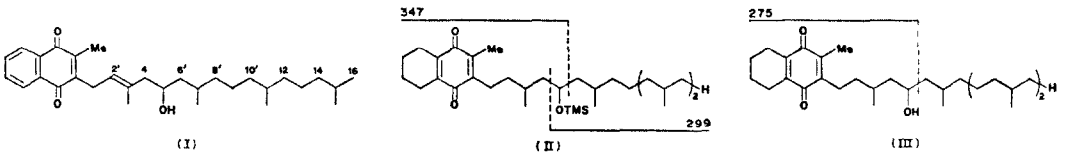
IN 1965, the blue-green alga *Anacystis nidulans* and the green alga *Chlorella pyrenoidosa* were reported to contain a polar naphthoquinone [1,2] Subsequent spectroscopic studies led to the partial characterization of the *A nidulans* quinone as a monohydroxy analogue of phylloquinone in which the hydroxyl group is in the phytyl side chain [3] Although the precise position of the hydroxyl group could not be determined with any degree of certainty, it was suggested that the NMR data were consistent with it being on either C-1′ or one of the tertiary carbon atoms C-7′, C-11′ or C-15′ [see (I) for the numbering of the 3-phytyl group] [3]

More recent studies have shown that the UV and MS (only shown in the case of the *Euglena gracilis* quinone) and TLC properties of the 'polar naphthoquinone' present in *C. pyrenoidosa* and a HOK† isolated from the green alga *E gracilis* are identical to those of the *A nidulans* quinone [4,5] These findings provided good evidence that the three algae contain the same isomer of HOK and suggested that it may be fairly widely distributed throughout the algae In the present paper the characterization by MS of the HOK found in *A nidulans* and *E. gracilis* as 5′-HOK is described

* Present address Burma Medical Research Institute, Rangoon, Burma

† *Abbreviations* HOK—monohydroxyphylloquinone, 5′-HOK—5′-monohydroxyphylloquinone

[1] HENNINGER, M D , BHAGAVAN, H. H and CRANE, F L (1965) *Arch Biochem Biophys* **110,** 69
[2] HENNINGER, M D (1965) *Biochem Biophys Res Commun* **19,** 233
[3] ALLEN, F C , FRANKE, H and HIRAYAMA, O (1967) *Biochem Biophys Res Commun* **26,** 562
[4] WHISTANCE, G R and THRELFALL, D R (1970) *Biochem J* **117,** 593
[5] GULLIS, R J (1971) B Sc Thesis, University College of Wales.



RESULTS

HOK samples (Table 1) for UV and MS studies were isolated from cells of *E gracilis* that had been grown in static culture on an acetate free medium and from cells of *A nidulans* that had been grown in the manner described by Smith, London and Stanier [6] In the course of carrying out the purification procedures no evidence was obtained for the presence of more than one isomer of HOK in the organisms

UV Spectra

In agreement with the findings of Allen *et al* [3] and Gullis[5] the UV spectra of the HOK samples were superimposable on the UV spectrum of phylloquinone (λ_{max} 243 5, 248 5, 260, 269 and 362 nm in cyclohexane) The spectra of the 2′,3′-dihydro derivatives formed by catalytic hydrogenation were also very similar to the spectrum of phylloquinone, except that the quinone peaks had undergone the expected slight bathochromic shifts from 260 and 269 nm to 262 and 272 nm due to saturation of the 2′,3′-double bonds on further catalytic hydrogenation to form the 5,6,7,8,2′,3′-hexahydro derivatives the benzenoid absorption bands at 243 5 and 248 5 nm were lost Conversion of the quinones to their TMS derivatives had no effect on their UV spectra

The above results are consistent with each HOK being a 2-methyl-3-monohydroxyphytyl-1,4-naphthoquinone in which the hydroxyl group cannot be on C-1′, C-2′ or C-3′ The assignment of the hydroxyl group of the *A nidulans* quinone to C-1′ by Allen *et al* [3] is difficult to understand, since if it was on this carbon atom the UV spectrum of the quinone would differ markedly from that of phylloquinone

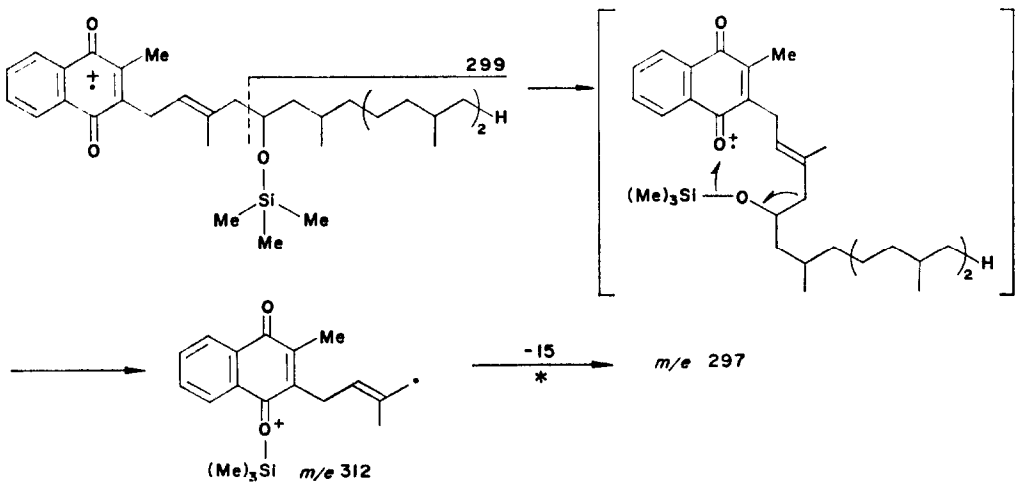
MS of the A nidulans *Quinone and its Derivatives*

In the spectrum of the *A nidulans* quinone there are no peaks that are attributable to α-cleavages about the hydroxyl group It was decided, therefore, to determine the mass spectra of the TMS and TMS-5,6,7,8,2′,3′-hexahydro derivatives of HOK, since mass spectral analysis of the TMS derivatives of hydroxy fatty acids had proved to be a useful method of establishing the positions of the in-chain hydroxyl groups in the parent acids

As expected, the spectrum of the TMS derivative of HOK showed a molecular ion of nominal mass 538 (4 1%) In addition small peaks representing ions formed by loss of a methyl group (*m/e* 523, 2 6%), trimethylsilanol (*m/e* 448, 2 6%) and a methyl group plus trimethylsilanol (*m/e* 433, 1·1%) were present The major fragmentation ions had *m/e* values of 299 2770 ($C_{18}H_{39}OSi$, 100%) and 312 1546 ($C_{19}H_{24}O_2Si$, 43%) The first ion is formed from the molecular ion by α-cleavage of the 4′,5′-bond and shows that the hydroxyl group is on C-5′ (Scheme 1) the ion formed by α-cleavage of the 5′,6′-bond gave rise to only a small peak in the spectrum (*m/e* 341, 2 1%) The even mass ion, which is a progenitor of a fragmentation ion of *m/e* 297 (6 7%), as supported by a metastable peak at 282 5, is probably formed from the molecular ion by a rearrangement process involving the migration of the TMS group to one of the keto groups and expulsion of aldehyde (Scheme 1)

[6] SMITH, A J, LONDON, J and STANIER, R Y (1967) *J Bact* **94**, 972.

In the spectrum of the TMS-hexahydro derivative all of the ions discussed above, with the exception of the *m/e* 299 ion, were displaced by 6 m.u. The base peak was still formed by the ion produced by α-cleavage of the 4',5'-bond; however, the ion produced by α-cleavage of the 5',6'-bond now made a substantial contribution to the spectrum (*m/e* 347; 65%) (II) and was itself a progenitor of a fragmentation ion of *m/e* 229, as shown by a metastable peak at 151 1.



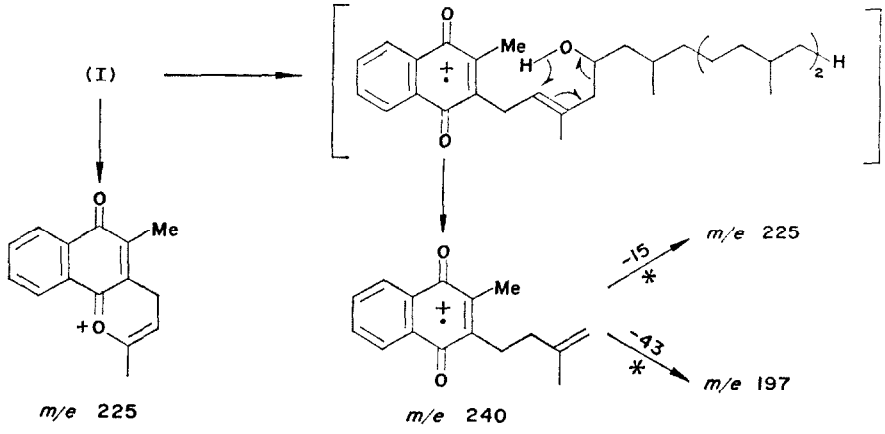
SCHEME 1 FRAGMENTATION OF 5'-*O*-TMSPHYLLOQUINONE

With the knowledge that the hydroxyl group was on C-5' the MS of HOK was re-examined. In agreement with the studies of Allen *et al* [3] the spectrum was found to show a molecular ion of nominal mass 466 ($C_{31}H_{86}O_3$; 3%) (in some spectra an M + 2 ion, formed as a result of a dismutation reaction in the spectrometer, was present). Small peaks at *m/e* 448 (2·5%) and 443 (0·5%) indicated the formation of ions by the facile loss of H_2O and by the loss of H_2O plus the 3'-methyl group respectively The base peak was at *m/e* 240·1182 and in addition strong peaks were present at *m/e* 225 (85%) (in some spectra this was the base peak) and 197·0602 (25%) The even mass ion ($C_{15}H_{13}O_2$), whose genesis may be ascribed to a McClafferty rearrangement of the type shown in Scheme 2, is a progenitor of fragment ions of *m/e* 225 and 197, as shown by the presence of metastable peaks at 211·1 and 161·9 However, since a major peak at *m/e* 225 is observed in all phylloquinone and menaquinone spectra,[7] it follows that the one in the HOK spectrum is also contributed to by the ion formed from the molecular ion by α-cleavage of the 3',4'-bond (Scheme 2). It is of note that the even mass ion is isomeric with the molecular ion of menaquinone 1, which also gives rise to *m/e* 225 and 197 ions.[7]

In an attempt to show α-cleavage about the 5'-hydroxyl group the 5,6,7,8,2',3'-hexahydro derivative of the *A nidulans* quinone was subjected to MS analysis. The molecular ion, which was also the most abundant ion, had a nominal mass of 472 Substantial peaks were also found at *m/e* 456 (M + 2-H_2O; 83%), 454 (M-H_2O; 57%) and 191 (M + 2-$C_{19}H_{39}O$; 83%). The presence of an *m/e* 275 (25%) ion showed that α-cleavage of the 5',6'-bond had taken place (III). An even mass ion was present at *m/e* 246 (53%) and since saturation of the

[7] DIMARI, S. J., SUPPLE, J. H. and RAPOPORT, H. (1966) *J. Am Chem. Soc.* **88**, 1226.

2',3'-double bond precluded a rearrangement of the type responsible for the *m/e* 240 ion in 5'-HOK it could only be formed by the hydroxyl hydrogen atom taking part in a rearrangement process analogous to that undergone by the TMS group (see above)



SCHEME 2 FRAGMENTATION OF 5'-HOK

MS of the E gracilis *Quinone and its Derivatives*

The MS of the *E. gracilis* HOK and its various derivatives were identical to those obtained for the *A. nidulans* quinone and its derivatives This shows that *E gracilis* also contains the 5'-monohydroxy isomer of HOK

TABLE 1 LEVELS OF 5'-HOK, PHYLLOQUINONE (K), PLASTOQUINONE-9 (PQ-9) AND UBIQUINONE-9 (Q-9) IN *E gracilis* STRAIN *Z*, *E gracilis* Y_1 ZSmL, *A nidulans* AND *C pyrenoidosa*

Organism	Conditions of growth	Age (days)	Vol of medium (l)	Dry wt (g)	Quinone levels (μmol/g dry wt) 5'-HOK	K	PQ-9	Q-9
E gracilis strain z	−Ac, static	2	4	1 2	0 075	0	0 98	0 189
	−Ac, static	5	20	12 0	0 136*	0	1 29	0 212
	−Ac, static	10	2	2 1	0 238*	0	2 50	0 276
	−Ac, shake	5	10	3 9	0 135	0	1 25	0 180
	+Ac, static	4	10	12 0	0 032	0	0 69	0 374
	+Ac, shake	4	10	21 7	0 031	0	0 47	0 540
E gracilis Y_1ZSmL	+Ac, shake	5	10	13 2	0	0	0	0 326
A nidulans	See Exptl	4	20	6 0	0 175*	0 080	0 400	0
		8	4	1 9	0 257*	0 257	0 585	0
C pyrenoidosa†	See Ref †	5	10	6 0	0 013	0 005	0 311	0

* Samples used for mass spectral analysis

† Taken from Whistance and Threlfall [4] It should be noted that in this paper all the wts of algal cells were incorrectly referred to as wet wts they should have been dry wts

Synthesis of 5'-HOK by E gracilis, A nidulans *and* C. pyrenoidosa

In the course of these and related investigations 5'-HOK was isolated from algal cells grown under a variety of conditions The amounts present, together with the amounts of phylloquinone (a possible precursor), ubiquinone-9 (an extrachloroplastidic quinone) and plastoquinone-9 (an intrachloroplastidic quinone) are given in Table 1.

DISCUSSION

Allen *et al*,[3] by the application of UV, NMR, IR and MS spectroscopy, deduced that the polar naphthoquinone found in *A nidulans* is a monohydroxy analogue of phylloquinone Gullis[5] demonstrated that the same quinone is present in *E gracilis*, and Whistance and Threlfall[4] provided good evidence for its presence in *C pyrenoidosa* We have now extended these observations further by presenting MS evidence to show that it is the 5'-monohydroxy isomer of HOK which is contained in *A nidulans* and *E gracilis* To our knowledge this is the first time that the hydroxyl group in the side-chain of an isoprenoid quinone has been located by MS analysis An obvious further application of this procedure is in the location of the hydroxyl groups in the nonaprenyl groups of the plastoquinones -C and -Z and the plastochromanols-C

The amounts of 5'-HOK are markedly affected by the period of growth and the nature of the growth medium (Table 1) The findings that, as is the case with plastoquinone-9, the quinone is present in reduced amounts in cells of *E gracilis* grown under the more extreme heterotrophic conditions and is entirely absent from streptomycin-bleached cells of *E gracilis* provides good evidence that it is a chloroplast component It is of interest that phylloquinone, a possible precursor of 5'-HOK, is rarely detected in *E gracilis* but is present in substantial amounts in *A nidulans* and *C pyrenoidosa*

EXPERIMENTAL

Biological material E gracilis strain *Z*, obtained from the Culture Collection of Algae and Protozoa, Cambridge, U K and *E gracilis* Y_1ZSmL, prepared by treatment of *E gracilis* strain *Z* with streptomycin, were grown for 4 days at 28° with constant illumination and, if required, agitation [180 rpm, Psychrotherm Incubator Shaker (New Brunswick)] in a medium containing either 0 5% NaOAc, 0 5% proteose peptone (Oxoid) and 0 2% yeast extract (Difco) in tap water (+Ac) or 0 5% proteose peptone and 0 2% yeast extract in tap H_2O (–Ac) The medium (2, 4, 10 or 20 l) was dispensed in 1 l vol in 2-l conical flasks Growth was started by the addition of a 10% inoculum of a liquid sub-culture

A nidulans (M B Allen), a gift from Dr A J Smith, was grown in 10 l batches in the manner described by Smith *et al*[6]

Isolation of 5'-HOK, phylloquinone, plastoquinone-9 and ubiquinone-9 These were isolated from the wet cell masses and purified by routine procedures [4,8] The 5'-HOK samples for MS analysis were further purified by chromatography on thin-layers of Ag^+-impregnated silica gel G developed with methyl ethyl ketone-*iso*-octane (2 3) to remove small traces of contaminating pigments

Catalytic hydrogenation of 5'-HOK Catalytic reduction (PtO_2–H_2) of 5'-HOK and the subsequent reoxidation of the hydrogenated product was carried out under standard conditions [9] It is of interest that the product produced at Aberystwyth after a 1 hr hydrogenation period over old catalyst was 2',3'-dihydro-5'-HOK, whilst that produced at Hull after a 10 min period over fresh catalyst was 5,6,7,8,2',3'-hexahydro-5'-HOK

Preparation of 5'-O-TMSphylloquinone and 5,6,7,8,2',3'-hexahydro-5'-O-TMSphylloquinone The TMS ethers of 5'-HOK and its hexahydro derivative were prepared in 0 5–1 μmol amounts using a standard silylation method [10] The ethers (formed in 90–100% yield) were purified by TLC on Rhodamine-6G impregnated silica gel G developed with C_6H_6–light petrol (b p 40–60°) (2 3) (5'-*O*-TMS phylloquinone, R_f 0 35, 5,6,7,8,2',3'-hexahydro-5'-*O*-TMSphylloquinone, R_f 0 45)

[8] WHISTANCE, G R , THRELFALL, D R and GOODWIN, T W (1967) *Biochem. J* **105,** 145
[9] WHISTANCE, G R and THRELFALL, D R (1970) *Phytochemistry* **9,** 213
[10] KLEBE, J F , FINKBEINER, H and WHITE, D M (1966) *J Am Chem Soc* **88,** 3390

UV spectra were determined in a Unicam SP800 spectrophotometer Phylloquinone, and 5'-HOK and its TMS derivative were assayed in cyclohexane by using a molar extinction coefficient of 19 000 (λ_{max} 248 5 nm) Ubiquinone-9 and plastoquinone-9 were assayed by reduction with $NaBH_4$ [9]

Mass spectra were determined in either an MS-12 (A E I Ltd) mass spectrometer or an MS-902 (A E I Ltd) mass spectrometer

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