# Auxin in a Seaweed Extract: Identification and Quantitation of Indole-3-acetic acid by Gas Chromatography-Mass Spectrometry

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## Summary

Indole-3-acetic acid (IAA) has been unequivocally identified by gas chromatography-mass spectrometry in the commercial seaweed extract, Maxicrop, which is derived from the brown alga *Ascophyllum nodosum* Le Jol. Using a [1-$^{14}$C]IAA internal standard to enable correction for losses during the procedure, we have estimated that one gram of the dried Maxicrop powder tested contains 6.63 ± 0.29 μg IAA.

*Key words: Algal extract, Ascophyllum nodosum Le Jol, auxin, gas chromatography-mass spectrometry, indole-3-acetic acid, Maxicrop, seaweed, seaweed extract, seaweed concentrate.*

## Introduction

The distributors of liquid seaweed extracts such as Maxicrop (Bell-Booth Group, N.Z.), Sea Magic and S.M.3 (Arthur Yates Ltd., N.Z.) claim that their products contain plant growth «stimulants», in particular cytokinins, auxins and gibberellins. The presence of these compounds is consistent with such claimed responses as increased fruit set (auxins and gibberellins), reduced fruit drop (auxins) and improved fruit quality (auxins, cytokinins and gibberellins). However, van Staden and co-workers consider the beneficial effects of seaweed products to be a consequence of improved root growth and they suggest that the cytokinins present in Kelpak 66 could be responsible (Finnie and van Staden, 1985).

Featonby-Smith and van Staden (1983) have reported the presence of cytokinin-like compounds in Kelpak 66 which is prepared from *Ecklonia maxima* (Osbeck) Papenf., while we have shown extensive cytokinin-like activity in the tobacco callus bioassay and have tentatively identified several cytokinins in the liquid seaweed extract Maxicrop obtained from *Ascophyllum nodosum* (Sanderson and Jameson, 1986). Further, Tay et al. (1985) have identified cytokinins by GC-MS-multiple ion detection in

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*Abbreviations:* BHT = butylated hydroxytoluene, BSTFA = N,O-*bis*(trimethylsilyl)trifluoroacetamide, DW = dry weight, FW = fresh weight, GC-MS = gas chromatography-mass spectrometry, IAA = indole-3-acetic acid, MS = mass spectrometry, SIM = selected ion monitoring, TMCS = trimethylchlorosilane, TMSi = trimethylsilyl.

Seasol, a liquid seaweed extract derived from the brown seaweed *Durvillea potatorum* (Labillardière) Areschoug.

The reports on the presence of auxin in liquid seaweed extracts are, by contrast, contradictory. Mowat (1965) detected auxin-like compounds in Maxicrop using a bioassay, as well as with Salkowski's and Ehrlich's reagents. Kingman and Moore (1982), using GLC, reported the presence of indole-3-acetic acid (IAA) in extracts of «dehydrated *A. nodosum* concentrate» (Maxicrop), whereas Williams et al. (1981), using bioassay techniques, did not detect any auxin-like activity in the commercial seaweed extracts Maxicrop, Marinure or S.M.3.

The presence of IAA in seaweeds has been confirmed. Recently, Jacobs et al. (1985) reported the unequivocal identification by GC-MS of IAA in the green seaweed *Caulerpa paspaloides* (Bory) Grev. and earlier Abe et al. (1972) identified IAA by GC-MS in the brown seaweed *Undaria pinnatifida* Suringar.

Accordingly, we have reinvestigated the claim that auxin is present in Maxicrop, by using GC-MS and incorporating a radioactive internal standard to account for losses during purification.

## Materials and Methods

*Purification of [1-$^{14}$C]IAA*

[1-$^{14}$C]IAA (58 mCi mmol$^{-1}$, Radiochemical Centre, Amersham) was repurified by anion exchange on DEAE-Sephadex A25 (Sigma Chemical Co.) in the acetate form, using the methods of Bialek et al. (1983) and made up in propan-2-ol : water : acetic acid (50 : 49 : 1 v/v).

*Liquid seaweed extract*

Maxicrop is processed in England (Maxicrop International Ltd., Cambridge) from *Ascophyllum nodosum* Le Jol. by alkaline hydrolysis of the seaweed. The subsequent extract is then dried by film evaporation and distributed in a soluble powder form.

*Extraction of IAA*

Two 1 g replicates of dried Maxicrop powder were each dissolved in 5 ml of water at 25 °C. Absolute methanol containing 1.2 mg · ml$^{-1}$ butylated hydroxytoluene (BHT) as antioxidant (Iino et al., 1980) was added to the aqueous solution to give a final concentration of 80 % methanol/BHT to water (v/v). 0.0511 $\mu$Ci of [1-$^{14}$C]IAA (in 1.5 ml of solution) was added to the methanol solution as an internal standard and the solution was stirred for 24 h at room temperature. The mixture was vacuum-filtered through Whatman No. 3 paper. The residue was resuspended in 5 ml of water and again made up to 80 % (v/v) methanol/BHT : water. After four hours of re-extraction the suspension was again filtered. The combined filtrates were quantitatively transferred to a flask and reduced to the aqueous phase by rotary film evaporation at 30 °C.

A partitioning sequence was adapted from Bandurski and Schulze (1974) and Badenoch-Jones et al. (1982, 1983) to extract IAA from the aqueous solution. This involved acidification with 2 N HCl (to pH 3) and centrifugation at 2500 g for 20 min then extracting the supernatant three times with equal volumes of ethyl acetate. The combined organic phase was then re-extracted with three equal volumes of 5 % (w/v) sodium hydrogen carbonate at pH 8 and the organic phase discarded. The alkaline phase was partitioned four times against equal volumes of dichloromethane and the organic phase discarded. The pH of the aqueous phase was adjusted to 3 using 12 N sulphuric acid and this was partitioned four times against equal volumes of dichloro-

methane with the organic phase being retained. This dichloromethane phase was evaporated to dryness, the extract redissolved in absolute methanol, transferred to a suitable microvial and dried over phosphorus pentoxide.

*Analysis*

Trimethylsilyl (TMSi) derivatives were prepared by treating the sample with a mixture of 25 µl of pyridine and 75 µl of BSTFA containing 1% TMCS (Alltech Assoc.) in a sealed Reacti-vial (Lab Supply Pierce) and heating at 30 °C overnight. A series of IAA (Sigma Chemical Co.) standards were also prepared in duplicate and derivatised concurrently with the samples. GC-MS analysis was performed on a Hewlett-Packard 5840A GC interfaced to a 5985 MS system. A fused-silica SE-54 capillary column (10 m × 0.30 mm id) was operated isothermally at 150 °C using helium as the carrier gas with a flow rate of 2 ml · min$^{-1}$. The injector was at 250 °C and 1 µl aliquots were injected on to the column via a Hewlett-Packard «duckbill» injector unit. The GC-MS interface was an SGE open-split design incorporating a 0.23 mm id fused-silica capillary held at 275 °C which allowed 1.0 – 1.5 ml into the MS. The MS was operated in the electron ionisation mode at 70 eV, with the ion source at 200 °C.

Duplicate aliquots of 20 µl were taken for radioactive determinations from the solutions in the Reacti-vials; correction was made for quenching caused by the derivatisation reagents. All extracts were counted to give a minimum of 10,000 cpm, i.e. ± 2% counting error.

## Results

The background-subtracted mass spectrum recorded of the trimethylsilylated IAA standard gave fragment ions characteristic of *bis*-TMSi-IAA. Ions of m/z 319(M+), 304, 202, 75 and 73 are considered by McDougall and Hillman (1979, 1980) as diagnostic of *bis*-TMSi-IAA. These ions along with m/z 276 and 186 were used in the selected-ion analysis of the total ion trace of the samples. The percent relative abundance of the diagnostic ions detected in the standard and sample A is compared in Table 1. Since the discrepancies in relative abundance are within acceptable experimental error we conclude that IAA is present in the sample.

As an unidentified compound with a fragment ion of m/z 319 almost co-eluted with *bis*-TMSi-IAA ($R_t$ = 9.18 min cf $R_t$IAA = 9.07 min) the peak areas of m/z 202 were used for quantitation. To ensure that the peak quantified was that of *bis*-TMSi-IAA, the peak area ratio of m/z 304 : 202 was compared to that found in the standard spectrum and this was found to be consistent for the samples. From the calibration curve drawn using the m/z 202 peak areas of the standards, the quantities of IAA present in the two samples were determined as 18.2 ng · µl$^{-1}$ (Sample A) and

Table 1: Relative abundance of ions diagnostic of *bis*-TMSi-IAA in the background-subtracted mass spectra obtained following injection of i) 10 ng of trimethylsilylated IAA standard and ii) a 1 µl aliquot of sample A extracted from 1 g of Maxicrop powder.

| | m/z 319 (M+) | 304 | 276 | 202 | 186 | 75 | 73 |
|---|---|---|---|---|---|---|---|
| Rel. abundance (Standard) | 14.3 | 4.0 | 2.5 | 100 | 2.5 | 3.2 | 32.9 |
| Rel. abundance (Sample A) | 16.3 | 4.7 | 1.9 | 100 | 2.8 | 5.3 | 26.8 |

14.2 ng · $\mu l^{-1}$ (Sample B). Correction for scintillation counter efficiency (89.2 %), radiolabel recovery (25.3 % and 22.3 % respectively), and the amount of internal standard added (113538 dpm or 0.154 $\mu$g per sample) gave the total IAA in Sample A as 7.04 $\mu$g and in Sample B as 6.22 $\mu$g. Hence, the average amount of IAA in the Maxicrop powder tested was $6.63 \pm 0.29$ $\mu g \cdot g^{-1}$ DW.

## Discussion

The above data establish unequivocally the presence of IAA in Maxicrop (soluble powder) which is in contrast to the report by Williams et al. (1981). The quantity of IAA present ($6.63 \pm 0.29$ $\mu g \cdot g^{-1}$ DW) is similar to that found by Jacobs et al. (1985) in *Caulerpa paspaloides* of 6.25 $\mu g \cdot g^{-1}$ DW, which is equivalent to approximately 1 mg · $kg^{-1}$ FW and is similar to the concentration of IAA found in angiosperms ($1-10^3$ $\mu g \cdot kg^{-1}$ FW: see Davis et al., 1985). By contrast, Kingman and Moore's (1982) report of 50 mg IAA per gram dried *A. nodosum* concentrate (presumably Maxicrop) is several orders of magnitude higher. Since Maxicrop is manufactured by a high pressure alkaline hydrolysis procedure it is possible that any bound IAA could be released as free IAA (Cohen et al., 1986) and hence increase the auxin content of the liquid seaweed extract. However, Buggeln and Craigie (1971) subjected the residue of methanol-extracted *Ascophyllum nodosum* to alkaline hydrolysis but did not detect any freed IAA using Ehrlich's reagent.

Auxins other than IAA were reported by Abe et al. (1974) in the brown alga *Undaria pinnatifida*. Phenylacetic acid and *p*-hydroxyphenylacetic acid were identified by IR, UV, and NMR spectra. The presence of these auxins in Maxicrop is currently being investigated.

The physiological significance of the presence of IAA and cytokinins in Maxicrop remains to be determined, however, by foliar uptake, translocation and metabolic studies.

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