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MASS SPECTROMETRIC IDENTIFICATION OF ADENOSINE 3′:5′-CYCLIC MONOPHOSPHATE ISOLATED FROM A HIGHER PLANT TISSUE

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Abstract—Mass spectrometric evidence is presented confirming the identification of the adenosine nucleotide previously isolated from tissues of *Phaseolus vulgaris* as adenosine 3′:5′-cyclic monophosphate.

INTRODUCTION

Early reports of the occurrence of adenosine 3′:5′-cyclic monophosphate (cyclic AMP) in the tissues of higher plants were criticized as being based either upon indirect physiological evidence or upon equivocal chromatographic identification data. The occurrence of cyclic AMP in plant tissues was, however, subsequently demonstrated by Brown and Newton [1] using a rigorous multistage chromatographic and electrophoretic sequence employing ion-exchange, paper and thin-layer chromatography, and high voltage electrophoresis. This procedure separated all known adenosine derivatives, including the 2′:3′-isomer, from cyclic AMP. The amount of cyclic AMP isolated was determined spectrophotometrically. Later determination of the cyclic AMP content of the same tissue using a specific cyclic AMP binding-protein assay with appropriate controls, gave a closely similar result [2].

Despite these findings and the demonstrated presence in plant tissues of a specific cyclic AMP phosphodiesterase [3–6], a specific cyclic AMP binding-protein [2], together with adenylate cyclase [2], some authors (e.g. refs. [7–9]) continue to express doubt that the compound isolated was cyclic AMP as distinct from a cyclic AMP-like adenosine nucleotide of unknown structure. The aim of the work reported here was therefore to re-isolate a sample of the compound using the previously described method [1] and to subject it to mass spectrometric analysis.

RESULTS AND DISCUSSION

The mass spectrum of trimethylsilylated adenosine 3′:5′-cyclic monophosphate (**1**) has been recorded by Lawson *et al.* [10] who have described the structural correlations and the principal fragmentation pathways. Their spectrum shows a molecular ion (*m/z* 545) which corresponds to the addition of three trimethylsilyl groups. The recorded specific ion products clearly indicate the cyclic nature of the molecule and enable 3′:5′-cyclic AMP to be easily distinguished from the adenosine non-cyclic phosphates. These characteristic ions also clearly distinguish 3′:5′-cyclic AMP from the 2′:3′-cyclic monophosphate isomer as well as from the deoxyribose analogues.

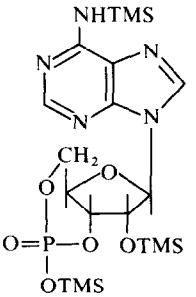

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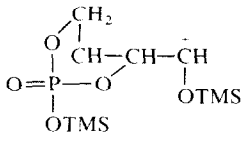
Table 1 shows the major ion products in (a) the published spectrum of cyclic AMP-$(TMS)_3$ [10], (b) the spectrum of the TMS derivative prepared in the present work from an authentic sample of cyclic AMP and (c) the mass spectrum of the TMS derivative of the adenosine nucleotide isolated from *Phaseolus vulgaris*. Comparison of the three sets of data in Table 1 shows that the major ion products observed by Lawson *et al.* [10] in the spectrum of cyclic AMP-$(TMS)_3$ are also present in (b) and (c). Additional ion products which appear in both (b) and (c) are *m/z* 473, the molecular ion of cyclic AMP-$(TMS)_2$, and *m/z* 251 and 250 which are fragment ions from cyclic AMP-$(TMS)_2$. The presence of these three ion products in the spectrum of the TMS derivative of the authentic sample of cyclic AMP and also in that of the derivatized sample from the plant source, indicate that their presence is due to incomplete silylation of some of the molecules.

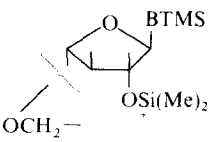
The ion products at *m/z* 310 and 378 are of crucial significance in that they correspond to structures (**2**) and (**3**), respectively. These are unique to the 3′:5′-cyclic structure [10] and cannot derive from cyclic deoxyAMP because their structures have been shown to include the

m/z 310



2

m/z 378



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trimethylsilylated 2′-hydroxyl group which would be absent in cyclic deoxyAMP. The mass spectral data presented here therefore confirm the identification of the adenosine nucleotide isolated from leaves of *Phaseolus vulgaris* as adenosine 3′:5′-cyclic monophosphate.

EXPERIMENTAL

Extraction. Seeds of *Phaseolus vulgaris* cv The Prince were surface-sterilized by immersion for 1 hr in a 5% (w/v) soln of $Ca(OCl)_2$ containing 0.2% (v/v) Stergene as a wetting agent. After thorough washing, seeds were germinated and grown for 9 days at 25° in a light cycle of 18 hr light (5.5 klx) and 6 hr dark. Batches of leaves were collected, rapidly immersed in liquid N_2, then freeze-dried. The freeze-dried material (70 g sample) was homogenized in an ice-cold monophasic mixture of MeOH–$CHCl_3$–HCO_2H (12:5:3). Care was taken to avoid a rise in temp. during homogenization. The cyclic nucleotide was extracted from the homogenate and purified using the procedure previously described [1]. In order to obtain a maximum efficiency during the subsequent derivatization, it was found advisable to pass the nucleotide sample, from the final step of the described purification procedure, through the Dowex-50 column for a second time. The UV-absorption characteristics of the final sample were recorded and confirmed to be those of a spectrophotometrically-pure adenosine derivative.

Derivatization and mass spectrometry. The trimethylsilyl (TMS) derivative of an authentic sample of cyclic AMP (Boehringer, Mannheim, Germany) and the TMS derivative of the compound isolated from the leaves were separately prepared by heating each

Table 1. Major ions in mass spectra of TMS-derivatized samples of cyclic AMP

Ion product (*m/z*)	Cyclic AMP-$(TMS)_3$ published data [10]	Cyclic AMP-$(TMS)_3$ prepared from authentic sample	TMS derivative of plant nucleotide
545	+	+	+
530	+	+	+
473	–	+	+
458	+	+	+
384	+	+	+
383	+	+	+
378	+	+	+
338	+	+	+
337	+	+	+
323	+	+	+
322	+	+	+
314	+	+	+
311	+	+	+
310	+	+	+
306	+	+	+
299	+	+	+
279	+	+	+
266	+	+	+
265	+	+	+
258	+	+	+
251	–	+	+
250	–	+	+
243	+	+	+
236	+	+	+
230	+	+	+
227	+	+	+
225	+	+	+
220	+	+	+
211	+	+	+
208	+	+	+
207	+	+	+
192	+	+	+
179	+	+	+
169	+	+	+
155	+	+	+
153	+	+	+
147	+	+	+

sample (50 μg) with 10 μl of acetonitrile and 90 μl of *N,O*-bis(trimethylsilyl)trifluoroacetamide (Pierce Chemical Co., Rockford, Illinois, U.S.A.) in a sealed vial at 100° for 4 hr. In order to remove the volatile reagents, the solns were evapd to dryness over P_2O_5 in a vacuum desiccator. Mass spectra of the TMS derivatives were recorded on a VG-Micromass ZAB-2F mass spectrometer with the source in the E1 mode; accelerating voltage 6 kV; ionizing voltage 70 eV; trap current 100 μA; source pressure $1–3 \times 10^{-7}$ Torr; source temperature 180°; probe temp. 140°.

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