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Identification and localization of gibberellins in maturing seeds of the cucurbit *Sechium edule*, and a comparison between this cucurbit and the legume *Phaseolus coccineus*

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Abstract. Twenty known gibberellins (GAs) have been identified by combined capillary gas chromatography-mass spectrometry in extracts from less than 10 g fresh weight of maturing seeds of the cucurbit *Sechium edule* Sw. The GAs are predominantly 3- and-or 13-hydroxylated. This is the first reported identification of non-conjugated 13-hydroxylated GAs in a cucurbit. Gibberellin A_8 and gibberellin A_8-catabolite are the major GAs in terms of quantity and are largely accumulated in the testa. The catabolites of 2β-hydroxylated GAs are α,β-unsaturated ketones which no longer possess a γ-lactone. They were hitherto known only in legumes. The presence of GA_8-catabolite as a major component of *Sechium* seeds indicates that the distribution of these GA-catabolites may be more widespread than previously envisaged. The localization of known GAs in maturing seeds of the legume *Phaseolus coccineus* L. was found to resemble closely that in *Sechium*. Gibberellin A_8, a putative conjugate of GA_8 and GA_8-catabolite are accumulated in the testa. The localization in the testa of end-products of the GA-biosynthetic pathway, which was first observed in maturing seeds of *Pisum sativum*, and is now described in *Phaseolus* and *Sechium*, may be a general feature of seed development.

Key words: Cucurbitaceae (gibberellins) – Gibberellin (identification, localization) – Leguminosae (gibberellins) – *Phaseolus* (gibberellins) – *Sechium* (gibberellins) – Seed maturation.

Introduction

The identification of large numbers of gibberellins (GAs) and related compounds from relatively small-scale extracts of developing seeds is now possible. This is because of recent advances in the resolution and sensitivity of combined capillary gas chromatography-mass spectrometry-computer systems (GC-MS-C) and the accumulation of mass-spectral data for the gibberellins. Recent examples in the literature report the identification of many GAs in seed or grain extracts of *Vicia faba* (broad bean) (Sponsel et al. 1979), *Hordeum vulgare* (barley) (Gaskin et al. 1984) and *Cucurbita maxima* (pumpkin) (Bleschmidt et al. 1984). Our attention is now being focussed on the distribution of GAs within constituent seed parts in an attempt to elucidate their role in seed development. We report here the identification and localization of GAs and related compounds in developing seeds of the cucurbit *Sechium edule* (chayote or cho-cho) which has been the subject of a recent study by Ceccarelli and Lorenzi (1982, 1983) and Lorenzi and Ceccarelli (1983). In addition, we describe the localization of GAs in embryos and testas of developing *Phaseolus coccineus* (runner bean) seeds, from which GAs were first isolated more than a quarter of a century ago (MacMillan and Suter 1958).

Abbreviations: GA_n = gibberellin A_n; GC-MS = combined gas chromatography-mass spectrometry

Materials and methods

Plant material. Four fruits of the tropical American cucurbit *Sechium edule* Sw. were initially obtained from Brazil. Each fruit contains one seed approx. 2.5 cm in length. The four seeds, which were at various stages of development, were separated into embryos (3.8 g fresh weight), testas (10.3 g) and endosperm. The latter was lightly homogenised and centrifuged at low speed for 15 min and the supernatant (1 ml) was retained. All material was deep-frozen before extraction.

Sechium fruits were also obtained from Italy. Seeds removed from these fruits were separated into "young" and "old" categories based on embryo size. The constituent parts of three "young" seeds (in which the embryos were < 30 mm long) were extracted, namely embryos (0.9 g), testas (4.3 g) and endosperm (0.5 g). In addition, a sample of embryos (6.5 g), testas (4.3 g) and endosperm (0.7 g) were selected from a large batch of "old" seeds which were almost devoid of endosperm. In *Se-*

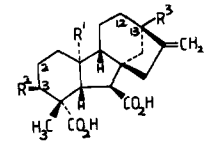
chium, the testa becomes progressively more difficult to separate from the endocarp as the seed matures.

No cultivars of the genus *Sechium* have been identified by name (Whitaker and Davis 1962) but distinct morphological types exist. The Italian fruits possessed spines in contrast to the smooth-skinned Brazilian specimens. There were no differences in fruit shape and colour.

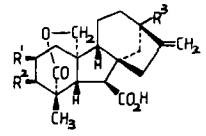
Maturing fruits of *Phaseolus coccineus* L. were harvested from a local garden during October. Seeds were separated into embryos (58 g fresh weight) and testas (32 g) and were deep-frozen. Later, plants of cv. Kelvedon Marvel were grown in an unheated greenhouse. Conditions were similar to those described previously (Sponsel and MacMillan 1977) for the growth of *Pisum sativum*. Flowers were pollinated by hand and fruits (28–40 d old) were harvested. Again seeds were separated into embryos and testas before deep-freezing.

Extraction procedure, partitioning and chromatography. Extraction and partitioning were conducted as described previously (Sponsel 1983a) to yield neutral-basic and acidic ethyl-acetate fractions and acidic butanol extracts. The latter were hydrolysed using pectolytic enzyme (The Boots Co., Nottingham, UK) (Sponsel 1983a). Ethyl-acetate fractions and hydrolysed butanol extracts from *Sechium* were not subjected to either thin-layer or high-performance liquid chromatography (HPLC) but were derivatized directly for capillary gas chromatography. Acidic ethyl-acetate fractions of the first *Phaseolus* extracts were strip-loaded onto 20·20 cm^2 plates of silica gel HF (BDH Chemicals, Poole, Dorset, UK) which had been prewashed with water-saturated ethyl acetate and were developed with ethyl acetate:chloroform:acetic acid (15:5:1, by vol.). Two strips of silica corresponding to R_f 0.2–0.5 and R_f 0.5–0.8 were scraped from the plates and material was eluted from the silica gel with water-saturated ethyl acetate. All traces of silica were removed by centrifugation prior to derivatization. Extracts of *P. coccineus* cv. Kelvedon Wonder were subjected to reverse-phase HPLC using the system described by Sponsel (1983a). A linear gradient of 30–100% methanol in 1% acetic acid was used to elute the C_{18}/5 μ Hypersil column (Shandon Southern Products, Runcorn, Cheshire, UK). Flow rate was 2.5 ml min^{-1} and four 5-min fractions were collected.

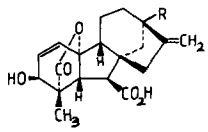
Derivatization, GC and GC-MS. All extracts were methylated using ethereal diazomethane and were trimethylsilylated using either the procedure described by Sponsel and MacMillan (1980) or N-methyl-N-trimethylsilyltrifluoroacetamide (Pierce and Warriner UK, Chester, Cheshire, UK) and dry dichloromethane. Derivatized *Sechium* samples were gas chromatographed on an OV-1 vitreous silica W.C.O.T. column fitted in a DANI-3800 gas chromatograph as described by Sponsel (1983a). The column was coupled to a VG 7050 computerised mass spectrometer (V.G. Analytical, Wythenshawe, Manchester, UK) with a source temperature of 200° C and an interface temperature of 250° C. Mass spectra, obtained at 24 eV, were recorded at 0.7 s per decade (1.3 s per cycle). A mixture of *n*-alkanes (Gaskin et al. 1971) was co-injected with each sample to obtain relative retention times. Derivatized *Phaseolus* extracts, which were analysed before the acquisition of capillary GC-MS, were gas chromatographed on 2% QF1 columns fitted in a Pye 104 gas chromatograph as described by Sponsel (1983a). The column was coupled to an A.E.I. MS30 mass spectrometer (Kratos, Manchester, UK) with a source temperature of 210° C and a separator temperature of 190° C. Mass spectra, obtained at 24 eV, were recorded at 3.0 s per decade and were processed by a VG 2035 data system. Capillary GC-MS of some later *Phaseolus* samples was performed as described above.



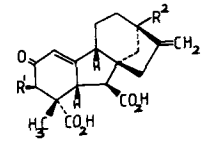
	R^1	R^2	R^3	
(1)	CH_3	H	H	GA_{12}
(2)	CH_3	H	OH	GA_{53}
(3)	CHO	H	OH	GA_{19}
(4)	CO_2H	H	H	GA_{25}
(5)	CO_2H	OH	H	GA_{13}
(6)	CO_2H	H	OH	GA_{17}



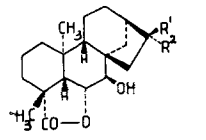
	R^1	R^2	R^3	
(9)	H	H	H	GA_{15}
(10)	H	H	OH	GA_{44}
(11)	H	OH	OH	GA_{38}
(12)	OH	OH	H	GA_{27}



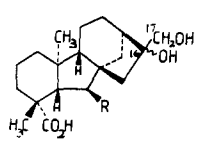
	R	
(19)	H	GA_7
(20)	OH	GA_3



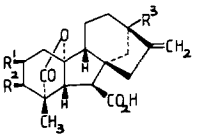
	R^1	R^2	
(23)	H	H	GA_{51}-catabolite
(24)	H	OH	GA_{29}-catabolite
(25)	OH	OH	GA_8-catabolite



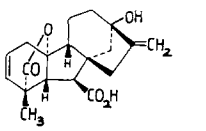
	R^1	R^2	
(28)	CH_2OH	OH	7β,16α,17-tri-OHk'ide
(29)	OH	CH_2OH	7β,16β,17-tri-OHk'ide



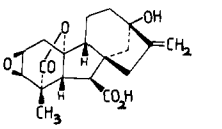
	R	
(7)	CHO	GA_{12}-aldehyde-16,17-diol
(8)	CO_2H	GA_{12}-16,17-diol



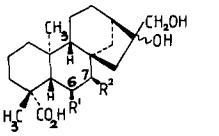
	R^1	R^2	R^3	
(13)	H	H	H	GA_9
(14)	H	OH	H	GA_4
(15)	H	H	OH	GA_{20}
(16)	H	OH	OH	GA_1
(17)	OH	H	OH	GA_{29}
(18)	OH	OH	OH	GA_8



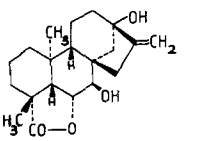
(21) GA_5



(22) GA_6



	R^1	R^2	
(26)	H	OH	7β,16,17-triOHKA
(27)	OH	OH	6β,7β,16,17-tetraOHKA



(30) 7β,13-diOHk'ide

Results and discussion

Seeds of *Sechium edule* are a rich source of gibberellins, kauranoids and other plant hormones (Table 1). Data from the Brazilian and Italian seed

Table 1. Identification and approximate quantification of gibberellins and other compounds in constituent parts of *Sechium* seeds. 16,17-diol = 16ξ, 17-dihydro, 16ξ,17-diol; KAH_2 = kauranoic acid; k'ide H_2 = kauranolide

Compound (Structure No.)	Embryo		Endosperm		Testa	
	Free[a]	Bound[b]	Free[a]	Bound[b]	Free[a]	Bound[b]
Indole acetic acid	+	+	+ + + +	−	+ + + +	−
Indole lactic acid	−	−	−	−	−	+
Dihydrophaseic acid	+	+	−	−	+ +	+ +
C_{20}-GAs						
GA_{12} (1)	−	−	−	−	+	−
GA_{12}-16, 17-diol (8)	+	−	+	−	+	−
GA_{12}-ald-16,17-diol (7)	−	+	+	+	−	+
GA_{13} (5)	−	−	+[c]	−	+	−
GA_{15} (9)	−	−	−	−	+	−
GA_{17} (6)	+	−	+[c]	−	−	−
GA_{19} (3)	−	−	−	−	+[c]	−
GA_{25} (4)	+	−	+	−	+[c]	−
GA_{27} (12)	−	−	−	−	+	−
GA_{38} (11)	+	−	+[c]	−	−	−
GA_{44} (10)	+	+	+[c]	−	+[c]	−
GA_{53} (2)	−	−	−	−	+[c]	−
C_{19}-GAs						
GA_1 (16)	+	−	+	−	+	−
GA_3 (20)	+	−	+	−	+	−
GA_4 (14)	+	−	+	−	−	−
GA_7 (19)	+	−	+	+	+	−
GA_8 (18)	+	−	−	−	+ + +	+
GA_8-catab (25)	+	−	−	−	+ + + + +	+ +
GA_9-16,17-diol	−	+	−	−	−	−
GA_{29} (17)	+	−	−	−	+	−
Kaurenoids						
7β,16α,17-triOHKAH$_2$ (26)	−	+	+	+	+	+
7β,16α,17-triOH k'ideH_2 (28)	−	+ + +	+	+	+	+ + +
7β16β,17-triOH k'ideH_2 (29)	+	+	+	+	+	−
6β,7β,16α,17-tetra-OHKAH$_2$ (27)	+ +	+ + + +	+	+ + + +	+	+ + + +

[a] Identified in acidic or neutral-basic ethyl-acetate fractions
[b] Identified in enzyme hydrolysates of acidic butanol fractions
[c] Identified in young seed extracts only

extracts have been combined, although compounds which were identified only in "young" seed extracts have been marked in Table 1. The approximate abundance of individual compounds has been estimated from the relative ion-current intensities within each GC-MS run. Because the results were obtained from a small number of extracts from a varied supply of *Sechium* fruits, they do not allow a detailed comparison of GA contents of seeds at defined developmental stages. However some generalisations are possible. The C_{20}-GAs identified in *Sechium* appeared to be distributed between all seed components. They were present predominantly in young seed extracts, consistent with their role as biosynthetic precursors of C_{19}-GAs. They were not found to be released from butanol extracts by enzyme hydrolysis indicating that they are not conjugated or bound within the seed. The single exception, GA_{12}-aldehyde 16,17-diol, is off the main pathway to C_{19}-GAs.

The C_{19}-GAs present in *Sechium* seeds fall into two categories. The GAs which are biologically active in standard assays, namely GA_1, GA_3, GA_4 and GA_7, are present in all seed components and are not conjugated (Table 1). Gibberellin A_9, which was identified by Lorenzi and Ceccarelli (1983) in *Sechium* together with GA_1, GA_3, GA_4 and GA_7, was not identified by us even in plant material which was supplied to us by Dr. Lorenzi. Ceccarelli and Lorenzi (1983) have demonstrated the ability of cell-free systems from both endosperm and cotyledons of *Sechium* to synthesise [^{14}C]GA_9 and [^{14}C]$GA_{4/7}$ from [^{14}C]mevalonic acid. They do however note that the [^{14}C]GA_9 in the cotyledon extract was scarcely diluted by unlabelled material. The more polar biologically

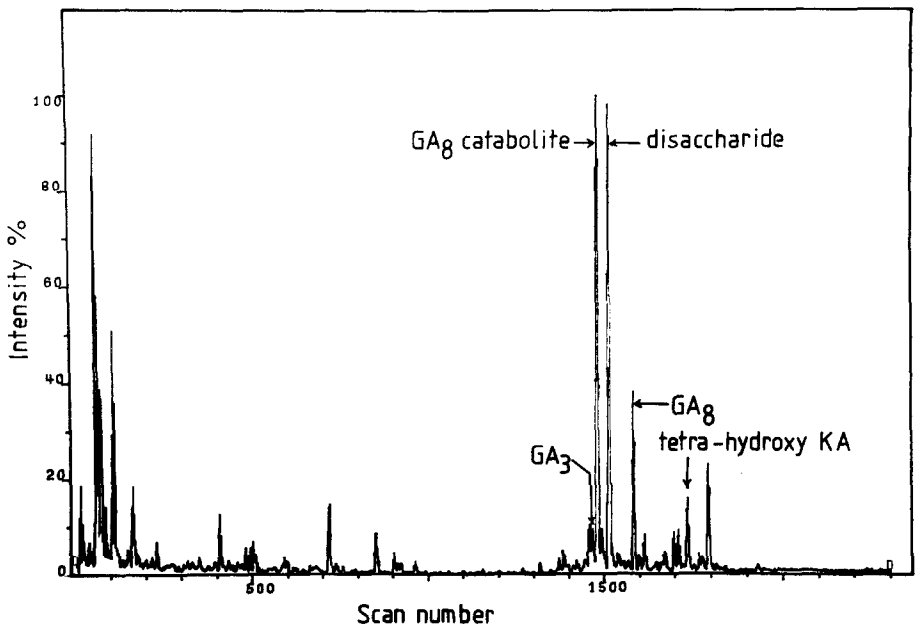


Fig. 1. Mass chromatogram (m/z 250–650) of derivatized ethyl-acetate extract of *Sechium* testa

inactive GAs, notably GA_8 and GA_8-catabolite (Table 1) are not present in the endosperm but instead are predominantly localized in the testa. Gibberellin A_8 and its catabolite are presumably end-products of the GA biosynthetic pathway in *Sechium* and they accumulate in the testa in large amounts (Fig. 1). The polyhydroxylated kauranoids also accumulate. They are present in all seed components and are largely conjugated.

In all, twenty known GAs have been identified in *Sechium* extracts. These are predominantly 3- and/or 13-hydroxylated GAs together with their 2β-hydroxylated derivatives. Surprisingly 13-hydroxylated GAs, although of widespread occurrence in angiosperms (see Sponsel 1983b) have not previously been identified in cucurbits despite extensive investigation of several genera, e.g. *Cucumis* (Hemphill et al. 1972), *Marah* (formerly *Echinocystis*; Beeley et al. 1975), *Cucurbita* (Fukui et al. 1977a, b; Blechschmidt et al. 1984), and *Lagenaria* (Fukui et al. 1978). There has been a single report of the identification of GA_1- and GA_3-*n*-propyl esters in mature seeds of *Cucumis* (Hemphill et al. 1973). The predominant GAs in these genera are 3-hydroxylated, in conjunction with 11-hydroxylation (*Lagenaria*) and 12-hydroxylation (*Cucurbita*), together with their 2β-hydroxylated derivatives. There is a taxonomic distinction between these genera which may account for the dissimilarity in GA contents. *Cucumis, Cucurbita* and *Lagenaria* are in the tribe Cucurbiteae whilst *Sechium* is in the Sicyoideae (Hutchinson 1967). A re-examination of seeds of *Marah*, which is in the Sicyoideae too, would be most interesting. Elson et al. (1964) tentatively identified GA_1 and GA_3 in mature seed extracts by thin-layer chromatography and bioassay although later examination of immature endosperm extracts by GC-MS failed to confirm these identifications (Beeley et al. 1975). The presence

Table 2. Identification and approximate quantification of gibberellins and other compounds in constituent parts of *Phaseolus coccineus* seeds. KAH_2-kauranoic acid; k'ide = kaurenolide

Compound (Structure No.)	Embryo		Testa	
	Free[a]	Bound[b]	Free[a]	Bound[b]
Abscisic acid	+	−	−	−
Phaseic acid	+	−	−	−
Dihydrophaseic acid	+	−	−	−
C_{20} GAs				
GA_{17} (6)	+	+	+	−
GA_{19} (3)	+	−	+	−
GA_{38} (11)	+	−	+	−
GA_{44} (10)	+	−	+	−
C_{19} GAs				
GA_1 (16)	+	+	+	+
GA_5 (21)	+	−	+	−
GA_6 (22)	+	−	++	−
GA_8 (18)	−	+	+++++	+++
GA_8-catab (25)	−	−	++	−
GA_{20} (15)	+	−	+	−
GA_{29} (17)	−	−	+	−
Kaurenoids				
7β,13-diOH k'ide (30)	+	−	−	+
6β,7β,16α,17-tetra-OHKAH$_2$ (27)	−	++	−	−

[a] Identified in acidic or neutral-basic ehtyl-acetate fractions
[b] Identified in enzyme hydrolysates of acidic butanol fractions

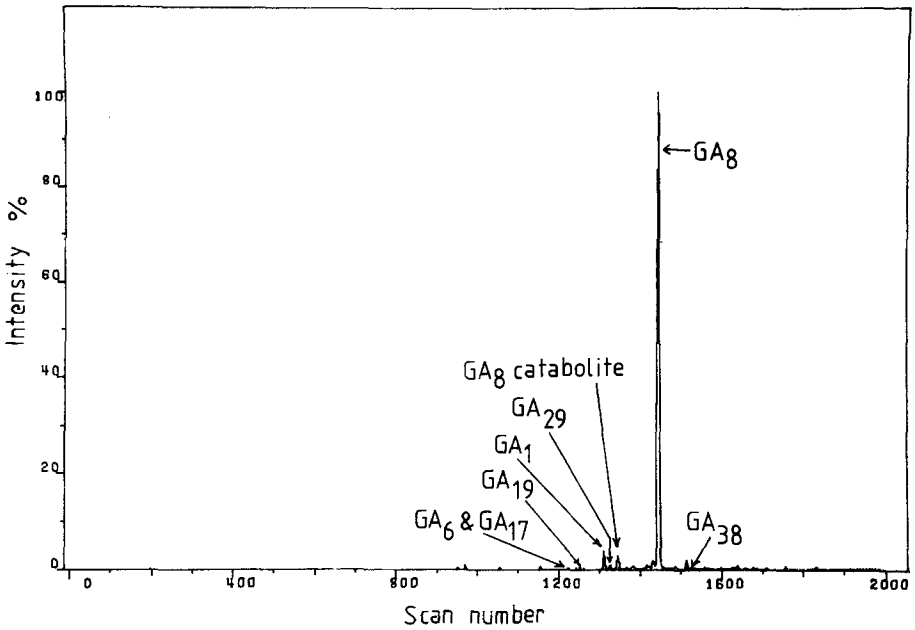


Fig. 2. Mass chromatogram (m/z 250–650) of derivatized ethyl-acetate extract of *Phaseolus* testa (R_f zone 0.2–0.5)

of 3,13-dihydroxylated GAs in cotyledons and testas of *Marah* may be anticipated.

The distribution of GAs between embryos and testa of *Phaseolus coccineus* seeds is shown in Table 2. Most, though not all, of the GAs known to be present in *Phaseolus coccineus* (Durley et al. 1971; Sponsel et al. 1979) were identified in this series of extracts. The distribution of GAs in *Phaseolus* seeds bore a marked similarity to that of GAs in *Sechium*. For instance, the C_{20}-GAs and less polar biological active C_{19}-GAs, such as GA_1, GA_5, GA_6 and GA_{20}, were present in both embryos and testa. In addition, there was a massive accumulation in the testa of the more polar biologically inactive GA, GA_8 (Fig. 2). Hydrolysed butanol extracts of testas contained large quantities of GA_8 too (Table 2) indicating the presence of a GA_8-conjugate. Results were the same for both the unnamed cultivar extracted first and for cv. Kelvedon Marvel.

Catabolites of 2-hydroxy GAs were first shown to be native to maturing seeds of *Pisum sativum* (Sponsel and MacMillan 1980). Catabolites of GA_8, GA_{29} and GA_{51} are now known to be native to several legumes (Sponsel 1980) and their structures have been confirmed by synthesis (Gaskin et al. 1981). They are α,β-unsaturated ketones which no longer possess a γ-lactone. Metabolic work (Sponsel and MacMillan 1978, 1980; Durley et al. 1979) has indicated that 2β-hydroxylation followed by catabolism is an alternative to conjugation for the disposal of biologically active GAs in legume seeds. The identification of GA_8-catabolite as a major component of maturing *Sechium* seeds indicates that the occurrence of GA-catabolites may be far more widespread than hitherto envisaged.

Of interest too is the localization of GAs in different seed components. In *Pisum sativum* seeds, GA_{20} and GA_{29} are present in the cotyledons whilst GA_{29}-catabolite is located predominantly in the testa (Sponsel 1983a). Results of in-vivo and in-vitro feeds indicated that the 2β-hydroxylated GA, GA_{29}, moves from the cotyledons to the testa where it is rapidly and quantitatively converted to GA_{29}-catabolite. The presence of GA_8 and GA_8-catabolite in testa extracts of *Sechium* indicates that a similar mechanism may also operate in some cucurbits. In *Phaseolus*, GA_8 and its putative conjugate together with lesser amounts of its catabolite also accumulate in the testa, indicating that the testa is the primary site for the formation of GA_8-glucoside. The interesting possibility that GA-glucosyl esters are formed in the embryo from biologically active GAs located there, and that GA glucosyl ethers are formed in the testa from biologically inactive GAs localised there is currently being examined.

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References

Beeley, L.J., Gaskin, P., MacMillan, J. (1975) Gibberellin A_{43}, and other terpenes in endosperm of *Echinocystis macrocarpa*. Phytochemistry **14**, 779–783

Blechschmidt, S., Castel, U., Gaskin, P., Hedden, P., Graebe, J.E., MacMillan, J. (1984) GC/MS analysis of the plant hormones in seeds of *Cucurbita maxima*. Phytochemistry **23**, 553–558

Ceccarelli, N., Lorenzi, R. (1982) Biosynthesis of GA_9 in a cell-free system of *Sechium edule* S. Z. Pflanzenphysiol. **108**, 477–479

Ceccarelli, N., Lorenzi, R. (1983) Gibberellin biosynthesis in endosperm and cotyledons of *Sechium edule* seeds. Phytochemistry **22**, 2203–2206

Durley, R.C., MacMillan, J., Pryce, R.J. (1971) Investigation of gibberellins and other growth substances in the seed of *Phaseolus multiflorus* and of *Phaseolus vulgaris* by gas chromatography and by gas chromatography-mass spectrometry. Phytochemistry **10**, 1891–1908

Durley, R.C., Sassa, T., Pharis, R.P. (1979) Metabolism of tritiated gibberellin A_{20} in immature seeds of dwarf pea cv. Meteor. Plant Physiol. **64**, 214–219

Elson, G.W., Jones, D.F., MacMillan, J., Suter, P.J. (1964) Plant hormones. IV. Identification of the gibberellins of *Echinocystis macrocarpa* Greene by thin layer chromatography. Phytochemistry **3**, 93–101

Fukui, H., Koshimizu, K., Nemori, R. (1978) Two new gibberellins A_{50} and A_{52} in seeds of *Lagenaria leucantha*. Agric. Biol. Chem. **42**, 1571–1576

Fukui, H., Koshimizu, K., Usuda, S., Yamazuki, Y. (1977a) Isolation of plant growth regulators from seeds of *Cucurbita pepo* L. Agric. Biol. Chem. **41**, 175–180

Fukui, H., Nemori, R., Koshimizu, K., Yamazuki, Y. (1977b) Structures of gibberellins A_{39}, A_{48}, A_{49} and a new kaurenolide in *Cucurbita pepo* L. Agric. Biol. Chem. **41**, 181–187

Gaskin, P., Gilmour, S.J., Lenton, J.R., MacMillan, J., Sponsel, V.M. (1984) Endogenous gibberellins and kauranoids identified from developing and germinating barley grain. J. Plant Growth Regul. **2**, 229–242

Gaskin, P., Kirkwood, P.S., MacMillan, J. (1981) Partial synthesis of *ent*-13-hydroxy-2-oxo-20-norgibberella-1(10), 16-diene-7,19-doic acid, a catabolite of GA_{29}, and of related compounds. J. Chem. Soc. Perkin Trans. **1**, 1083–1091

Gaskin, P., MacMillan, J., Firn, R.D., Pryce, R.J. (1971) 'Parafilm' a convenient source of *n*-alkane standards for the determination of gas chromatographic retention indices. Phytochemistry **10**, 1155–1157

Hemphill, D.D., Baker, L.R., Sell, H.M. (1972) Isolation and identification of the gibberellins of *Cucumis sativus* and *Cucumis melo*. Planta **103**, 241–248

Hemphill, D.D., Baker, L.R., Sell, H.M. (1973) Isolation of novel conjugated gibberellins from *Cucumis sativus* seed. Can. J. Biochem. **51**, 1647–1653

Hutchinson, J. (1967) The genera of flowering plants. Clarendon Press, Oxford

Lorenzi, R., Ceccarelli, N. (1983) Endogenous gibberellins in endosperms and cotyledons of *Sechium edule* during seed growth and maturation. Phytochemistry **22**, 2189–2192

MacMillan, J., Suter, P.J. (1958) The occurrence of gibberellin A_1 in higher plants: isolation from the seed of runner bean (*Phaseolus multiflorus*). Naturwissenschaften **45**, 46

Sponsel, V.M. (1980) Gibberellin metabolism in legume seeds. In: Gibberellins – chemistry, physiology and use. British Plant Growth Regulator Group, Monograph No. 5, pp. 49–62, Lenton, J.R., ed. British Plant Growth Regulator Group, Wantage

Sponsel, V.M. (1983a) The localization, metabolism and biological activity of gibberellins in maturing and germinating seeds of *Pisum sativum* cv. Progress No. 9. Planta **159**, 454–468

Sponsel, V.M. (1983b) In vivo gibberellin metabolism in higher plants. In: The biochemistry of gibberellins, vol. 1, pp. 151–250, Crozier, A., ed. Praeger, New York

Sponsel, V.M., Gaskin, P., MacMillan, J. (1979) The identification of gibberellins in immature seeds of *Vicia faba*, and some chemotaxonomic considerations. Planta **146**, 101–105

Sponsel (née Frydman) V.M., MacMillan, J. (1977) Further studies on the metabolism of gibberellins (GAs) A_9, A_{20} and A_{29} in immature seeds of *Pisum sativum* cv. Progress No. 9. Planta **135**, 129–136

Sponsel, V.M., MacMillan, J. (1978) Metabolism of gibberellin A_{29} in seeds of *Pisum sativum* cv. Progress No. 9; use of [^{2}H] and [^{3}H]GAs, and the identification of a new GA catabolite. Planta **144**, 69–78

Sponsel, V.M., MacMillan, J. (1980) Metabolism of [$^{13}C_1$]gibberellin A_{29} to [$^{13}C_1$]gibberellin catabolite in maturing seeds of *Pisum sativum* cv. Progress No. 9. Planta **150**, 46–52

Whitaker, T.W., Davis, G.N. (1962) Cucurbits botany, cultivation and utilization. Interscience, New York

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