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BIOSYNTHESIS OF GIBBERELLINS A_{12}, A_{15}, A_{24}, A_{36} AND A_{37} BY A CELL-FREE SYSTEM FROM *CUCURBITA MAXIMA*

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Key Word Index—*Cucurbita maxima*, Cucurbitaceae, biosynthesis, cell-free system, gibberellins, diterpenoids

Abstract—GA_{12}-aldehyde obtained from mevalonate *via ent*-kaurene, *ent*-kaurenol, *ent*-kaurenoic acid and *ent*-7α-hydroxykaurenoic acid in a cell-free system from immature seeds of *Cucurbita maxima* was converted to GA_{12} by the same system When Mn^{2+} was omitted from the system GA_{12}-aldehyde and GA_{12} were converted further to several products Among these GA_{15}, GA_{24}, GA_{36} and GA_{37} were conclusively identified by GC–MS With the exception of GA_{37} these GAs have not previously been found in higher plants Another biosynthetic pathway led from *ent*-7α-hydroxykaurenoic acid to very polar products *via* what was tentatively identified as *ent*-6α,7α-dihydroxykaurenoic acid An unidentified component with an MS resembling that of a dihydroxykaurenolide was also obtained from incubations with mevalonate

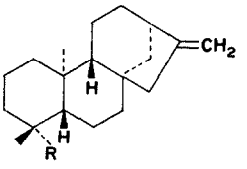
INTRODUCTION

MOST OF the information on the biosynthesis of gibberellins in higher plants has been obtained from studies of cell-free systems. The first cell-free system to biosynthesize gibberellin precursors was reported by Graebe *et al* [1] who demonstrated the incorporation of mevalonic acid (MVA) into *ent*-kaurene (**1**) and *ent*-kaurenol (**2**) in a system from immature seeds of *Echinocystis*. Using this system West and his colleagues[2] provided detailed information on the biosynthetic steps from MVA to *ent*-kaurene (**1**), *ent*-kaurenol (**2**), *ent*-kaurenal (**3**), *ent*-kaurenoic acid (**4**) and *ent*-7α-hydroxykaurenoic acid (**5**). The formation of the *ent*-kaurenoids (**1**–**4**) has also been reported in cell-free preparations from seeds of *Pisum*[3–5] and *Cucurbita*.[6,7]

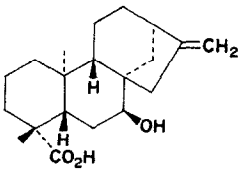
In more recent work with the *Cucurbita* system Graebe *et al.*[8] using GC–MS unambiguously identified *ent*-kaurenoic acid (**4**), *ent*-7α-hydroxykaurenoic acid (**5**) and GA_{12}-aldehyde (**6**) as conversion products from MVA. The identification of GA_{12}-aldehyde (**6**) provided the first example of the ring contraction of the *ent*-kaurenoid ring system to an *ent*-

[1] GRAEBE, J E, DENNIS, D T, UPPER, C D and WEST, C A (1965) *J Biol Chem* **240**, 1847
[2] WEST, C A (1973) Review in *Biosynthesis and its Control in Plants* (MILBORROW, B V, ed), pp 143–169, Academic Press, London
[3] ANDERSON, J D and MOORE, T C (1967) *Plant Physiol* **42**, 1527
[4] GRAEBE, J E (1968) *Phytochemistry* **7**, 2003
[5] COOLBAUGH, R C and MOORE, T C (1971) *Phytochemistry* **10**, 2401
[6] GRAEBE, J E (1969) *Planta* **85**, 171
[7] GRAEBE, J E (1972) in *Plant Growth Substances* 1970 (CARR, D J, ed), pp 151–157, Springer, Berlin
[8] GRAEBE, J E, BOWDEN, D. H and MACMILLAN, J (1972) *Planta* **102**, 261

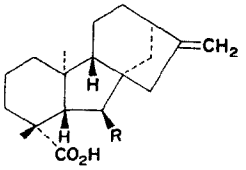
gibberellane in a cell-free system of a higher plant. We now report the further conversion of GA_{12}-aldehyde (**6**) to other GAs in the *Cucurbita* system



(**1**) R = Me
(**2**) R = CH_2OH
(**3**) R = CHO
(**4**) R = CO_2H



(**5**)



(**6**) R = CHO
(**7**) R = CO_2H

RESULTS

Incubation with MVA-[2-^{14}C] and Mn^{2+}

When the *Cucurbita* system was incubated with the standard mixture of cofactors (Mg^{2+}, Mn^{2+}, ATP, PEP, NADPH) and MVA-[2-^{14}C] up to 30% of the label was incorporated into several components, which were separated by TLC. Among the major products *ent*-7α-hydroxykaurenoic acid (**5**), GA_{12}-aldehyde (**6**) and GA_{12} (**7**) were again[8] conclusively identified by GC–MS. Likewise identified were *ent*-kaurene (**1**), *ent*-kaurenol (**2**) and *ent*-kaurenoic acid (**4**), which were obtained in variable amounts depending on the individual enzyme preparations. In addition, three well defined zones of material more polar than GA_{12}, fractions I, II and III, were detected by TLC (Fig. 1). The yields of these products and some non-diterpenoid material that was obtained as well are listed in Table 1.

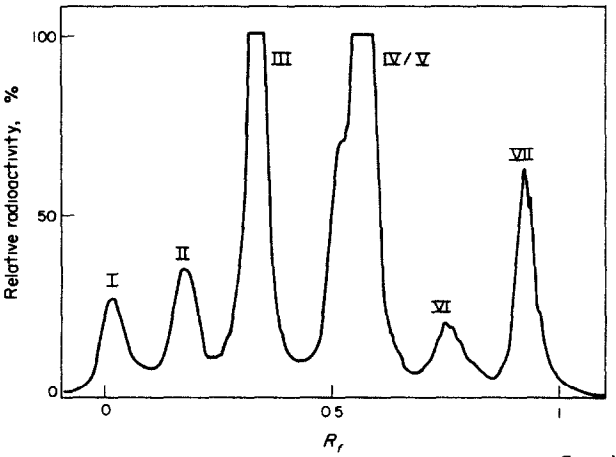


FIG 1 SEPARATION OF RADIOACTIVE PRODUCTS OBTAINED BY INCUBATION OF THE *Cucurbita* SYSTEM (50 ml) WITH D,L-MVA-[2-^{14}C] (125 μCi, 5·0 μCi/μmol) AND COFACTORS INCLUDING Mn^{2+}
Separation by TLC-system 1. 3·6 cm of a 15 cm line were scanned for radioactivity with a rate meter setting of $1·8 \times 10^5$ cpm = 100%.

Very strong evidence indicates that fraction III is identical with *ent*-6α,7α-dihydroxykaurenoic acid (**11**). Rechromatography in TLC-system 2 (see Experimental) and again in system 1 as well as investigation of a sample by GC–RC revealed a single component. This was characterized as a 6,7-dihydroxykaurenoic acid by GC–MS of the *bis*-TMS ether (**8**)

TABLE 1 YIELDS OF PRODUCTS IN THE *Cucurbita* SYSTEM*

Product	Peak†	Yield (10^6 dpm)
Unidentified	I	3·1
Unidentified	II	4 5
ent-6α,7α-Dihydroxykaurenoic acid (**11**)	III	24 0
GA_{12} (**7**)	IV/V	4·6
ent-7α-Hydroxykaurenoic acid (**5**)	IV/V	9 5
GA_{12}-aldehyde (**6**)	IV/V	22·0
ent-Kaurenol (**2**)	VI	1 9
ent-Kaurenoic acid (**4**)	VI	3 1
ent-Kaurene (**1**)	VII	1 4
Unidentified non-diterpene	VII	3·0
Squalene	VII	1 1

* Incubated with D,L-MVA-[2-^{14}C] (355 μCi, 5 μCi/μmol) and the standard mixture of cofactors (total volume 142 ml)

† See Fig 1 and Experimental Section

and of the Me *n*-butylboronate (**9**).[9,10] The MS of the Me *bis*-TMS ether (**8**) (Table 2) showed a very weak M^+ at *m/e* 492 and strong M^+-15 and M^+-90 ions. A vicinal *bis* -TMS ether was indicated by the presence of an ion at *m/e* 147[11] and by a base peak at *m/e* 269 which is assigned structure (**10**) and which could arise by cleavage *a* as shown in structure (**8**). An ion at *m/e* 209 was present corresponding to the loss of 60 *amu* (HCO_2Me) from the base peak, *m/e* 269. The MS of the Me *bis*-TMS of fraction III was different from the MS of the corresponding derivatives of the known *ent*-6β,7β- and -6β,7α-dihydroxykaurenoic acids. Although an authentic sample of the known *ent*-6α,7α-dihydroxykaurenoic acid (**11**) was not available for direct comparison this structure (**11**) is assigned to fraction III* from the striking similarity between the MS of its methyl ester TMS ether and of the methyl ester TMS ether of the norketone (**12**) (Table 3).[12,13] These MS showed analogous fragmentations including a base peak at *m/e* 269 (**10**) and ions at *m/e* 209 and 147.

TABLE 2. MS OF FRACTION III-[^{14}C] METHYL ESTER TMS ETHER

492 (0·7), 481 (5), 480 (8), 479 (27), 478 (21), 477 (59), 404 (7), 403 (6), 402 (22), 271 (22), 270 (21), 269 (100), 253 (5), 211 (9), 209 (19), 151 (5), 147 (13), 75 (9), 73 (24)

Structure (**11**) for fraction III received further support from the ready formation of an *n*-butyl boronate (**9**). The MS (Table 4) of this derivative gave a M^+ at *m/e* 414, showing the correct ^{11}B:^{10}B isotopic ratio 81:19. The base peak at *m/e* 285 (M^+-129) contained boron and is assigned structure (**13**); an ion at M^+-84 corresponds to the loss of $OB(CH_2)_3Me$[10] and a strong ion at *m/e* 137 is assigned the structure (**15**). The methyl *n*-butyl boronate of the norketone (**12**) had a very similar MS (Table 5)[13] which showed M^+ 416, a base peak at M^+-129 (*m/e* 287) (**14**) and an ion, *m/e* 137 (**15**).

* *Note added in proof* Comparison of fraction III with *ent*-6α,7α-dihydroxykaurenoic acid (11) (kindly donated by Profs C A West and P R Jefferies) by GC–MS, conclusively proved their identity

[9] BROOKS, C J W and HARVEY, D J (1971) *J Chromatogr.* **54,** 193
[10] BROOKS, C. J W and MACLEAN, I (1971) *J Chromatogr. Sci* **9,** 18
[11] BINKS, R., MACMILLAN, J and PRYCE, R J. (1969) *Phytochemistry* **8,** 271
[12] HANSON, J R and HAWKER, J (1972) *Tetrahedron* **28,** 2521
[13] BEARDLR, J R (1973) Ph D Thesis, Bristol University

TABLE 3 MS OF METHYL *ent*-6α 7α-DIHYDROXY-16-OXO-17-NORKAURAN-19-OATE *bis*-TMS[13]

494 (0·6), 481 (10), 480 (25), 479 (75) 405 (5) 404 (15), 361 (10), 330 (5), 329 (16), 271 (7), 270 (20), 269 (100), 255 (11), 210 (7) 209 (37), 151 (9) 146 (5), 145 (35), 137 (6), 133 (5), 103 (7), 75 (12) 73 (57)

TABLE 4 MS OF FRACTION III-[^{14}C] METHYL ESTER *n*-BUTYLBORONATE

416 (6·4), 415 (7·8), 414 (21), 413 (5·7), 399 (12), 398 (16), 397 (12), 396 (38), 395 (9), 357 (8), 356 (9), 355 (14), 354 (21), 353 (6), 341 (22), 240 (18), 339 (51), 338 (15), 332 (38), 331 (26), 330 (100), 314 (13), 313 (16), 312 (18), 300 (27), 299 (27), 298 (60), 297 (15), 287 (26), 286 (28), 285 (99), 284 (28), 272 (13), 271 (12), 270 (21) 257 (13), 255 (23), 254 (15), 253 (30), 246 (23), 245 (21), 206 (36), 205 (19), 203 (20), 147 (22), 145 (19), 139 (23), 137 (71), 121 (28), 119 (26) 108 (33) 106 (29), 104 (42), 101 (26) 95 (30), 94 (31), 93 (38), 91 (26), 81 (29) 79 (26) 55 (26), 41 (23), 40 (38)

Note strong [^{14}C]-isotopic peaks, e g *m e* 416 = M$^+$ + 2 These are also apparent for the stronger peaks in Table 2

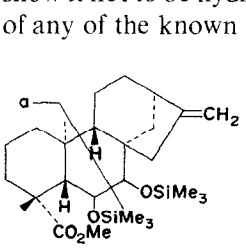
TABLE 5 MS OF METHYL *ent*-6α,7α-DIHYDROXY-16-OXO-17-NORKAURAN-19-OATE *n*-BUTYLBORONATE[13]

416 (3), 401 (1), 356 (6), 342 (5) 341 (21) 340 (5), 302 (6), 288 (18), 287 (100) 286 (23) 137 (10), 109 (5) 101 (7), 95 (8), 93 (5), 81 (5), 79 (5), 55 (5), 44 (14)

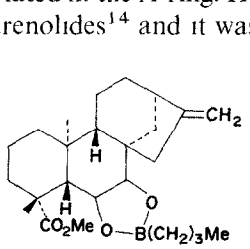
TABLE 6 MS OF FRACTION II 3-[^{14}C] METHYL ESTER TMS ETHER

476 (2), 461 (2), 433 (4), 387 (4), 386 (13), 343 (4), 298 (5), 297 (24), 296 (100), 281 (8), 270 (5), 269 (4), 268 (7), 221 (6), 208 (5), 195 (5), 193 (5), 182 (5), 181 (11), 173 (5), 172 (18), 169 (5), 160 (16), 159 (6) 157 (7), 156 (12), 149 (5), 147 (19), 145 (8), 143 (8), 137 (27), 131 (11) 130 (16), 129 (12), 125 (10) 124 (8), 119 (6), 118 (9), 117 (6), 110 (7) 109 (83), 107 (6) 105 (9), 104 (5), 103 (19), 95 (6) 93 (7), 92 (6) 91 (5), 81 (7), 75 (30), 74 (7), 73 (74)

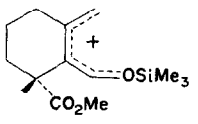
Fraction II was further separated by TLC-system 3 into three subfractions, II.1 (R_f 0·33, 3·0 × 10^5 dpm), II.2 (R_f 0·43, 3·1 × 10^5 dpm) and II 3 (R_f 0·55, 2·3 × 10^6 dpm) The MS of the methyl ester TMS derivative of II 3 (Table 6) showed the correct M$^+$ and the typical fragmentation of a dihydroxykaurenolide The presence of strong ions at *m/e* 109 and 137 show it not to be hydroxylated in the A-ring. However, the MS did not correspond to that of any of the known kaurenolides[14] and it was not possible to assign an exact structure



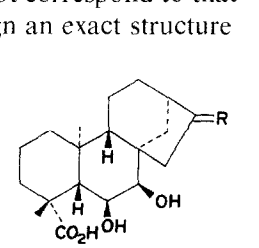
(8)



(9)



(10)



(11) R = CH_2
(12) R = O

Fractions II.1, II.2 and the very polar material in fraction I could not be identified from their MS

[14] CROSS B E., GALT, R H B and HANSON, J R (1963) *J Chem Soc* 2944., CROSS, B E GALT, R H B and HANSON, J R (1963) *J Chem Soc* 3783., SEREBRYAKOV, E P SIMOLIN, A V., KUCHEROV V F and ROSYNOV, B V (1970) *Tetrahedron* **26**, 5215., HANSON, J R and WHITE, A F (1968) *Tetrahedron* **24**, 6291., BATESON, J H and CROSS, B E (1972) *J C S Perkin* I, 1117., HEDDEN, P., MACMILLAN, J and GRUNSTED M J (1973) *J C S Perkin* I, 2773

Interconversion of intermediates in the presence of Mn^{2+}

The radioactive fractions and compounds that had been obtained were re-incubated with the system to establish the sequence of their formation. Incorporation of *ent*-kaurene (**1**) into *ent*-kaurenol (**2**), *ent*-kaurenal (**3**) (tentative identification), *ent*-kaurenoic acid (**4**) and unidentified acids was reported earlier.[7] After the acids had been identified,[8] another incubation (10 ml) with *ent*-kaurene-[^{14}C] ($6{\cdot}5 \times 10^6$ dpm) yielded *ent*-kaurenol (**2**) and *ent*-kaurenoic acid (**4**) (together $1{\cdot}3 \times 10^5$ dpm), *ent*-7α-hydroxykaurenoic acid (**5**) and GA_{12}-aldehyde (**6**) (together $9{\cdot}1 \times 10^5$ dpm), GA_{12} (**7**) ($4{\cdot}7 \times 10^5$ dpm), fraction III ($2{\cdot}9 \times 10^5$ dpm), fraction II ($2\,8 \times 10$ dpm), fraction I ($1\,4 \times 10^5$ dpm) and unconverted *ent*-kaurene (**1**) ($1\,4 \times 10^5$ dpm) In a separate experiment *ent*-kaurenol-[^{14}C] yielded the same products except *ent*-kaurene (**1**). Incubation of *ent*-kaurenoic acid (**4**), *ent*-7α-hydroxykaurenoic acid (**5**), GA_{12}-aldehyde (**6**) and fraction III gave the results shown in Table 7 which represents one of twenty incubations of identical composition but with different enzyme preparations. The amounts of products obtained with the individual preparations varied markedly but the products always gave a similar TLC pattern. *ent*-7α-Hydroxykaurenoic acid (**5**) was converted to GA_{12}-aldehyde (**6**), GA_{12} (**7**), fraction III and fraction I but GA_{12}-aldehyde (**6**) was only converted to GA_{12} (**7**) and fraction III was only converted to fraction I. Resolution of the *ent*-7α-hydroxykaurenoic acid/GA_{12}-aldehyde pair by TLC-system 2 or 5 showed ratios varying between 4:1 and 1:4 when *ent*-kaurenoic acid (**4**) was used as a precursor. When *ent*-7α-hydroxykaurenoic acid (**5**) was used as a precursor it was often completely consumed. GA_{12}-aldehyde (**6**) was not converted back to *ent*-7α-hydroxykaurenoic acid (**5**) to a detectable extent. Fraction I, fraction II and GA_{12} (**7**) were not converted further in the standard system. These results establish the following sequence of formation: *ent*-kaurene (**1**) → *ent*-kaurenol (**2**) → *ent*-kaurenoic acid (**4**) → *ent*-7α-hydroxykaurenoic acid (**5**) → [fraction III → fraction I]/[GA_{12}-aldehyde (**6**) → GA_{12} (**7**)]. Each of these steps was shown to be enzymatic by control incubations in which the enzyme preparations had been denatured by boiling. In no case was conversion obtained with the boiled preparations. The exact origin of fraction II is unknown at the moment.

TABLE 7 INTERCONVERSION OF SUBSTANCES OBTAINED IN THE *Cucurbita* SYSTEM

	Incorporation into products (dpm)					
Precursor	Fraction I	Fraction II	Fraction III	GA_{12} (7)	*ent*-7α-OHKA (5) GA_{12}-ald (6)	*ent*-Kaurenoic acid (4)
ent-Kaurenoic acid (**4**)	4760	1340	4320	5740	7060	5540
ent-7α-OHKA (**5**)	6670	0	4950	9010	12400	0
GA_{12}-aldehyde (**6**)	0	0	0	9570	9550	0
Fraction III	6750	0	6290	0	0	0

Standard incubation mixtures (0 2 ml) incubated for 2 hr at 30° Products were extracted, separated by TLC, located by scanning and scraped into liquid scintillation vials

Incubation of GA_{12}*-aldehyde* (**6**) *in absence of* Mn^{2+}

Further conversion of GA_{12}-aldehyde (**6**) and GA_{12} (**7**) to more highly oxidized GAs could not be achieved using the standard system. However, incubation of GA_{12}-aldehyde-[^{14}C] in the standard system (10 ml) but without Mn^{2+} resulted in almost complete conversion of this compound to 5 new fractions, A–E, which were separated by TLC (Fig.

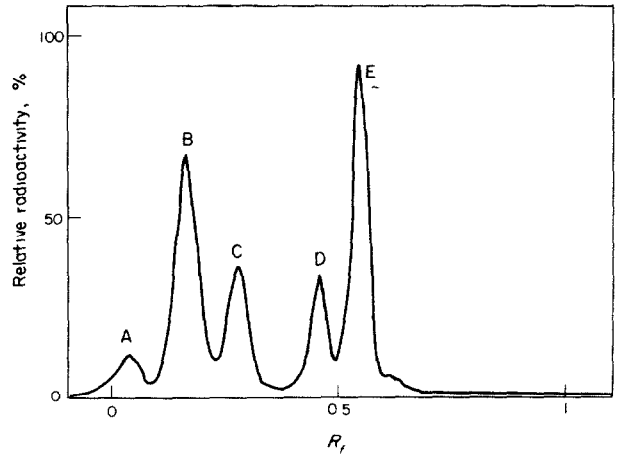
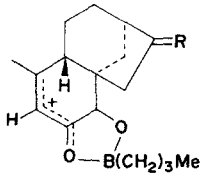
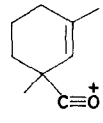


FIG. 2. SEPARATION OF RADIOACTIVE PRODUCTS OBTAINED BY INCUBATION OF THE *Cucurbita* SYSTEM (10 ml) WITH GA_{12}-ALDEHYDE-[^{14}C] (20 μCi/μmol) AND COFACTORS EXCLUDING Mn^{2+}. SEPARATION IN TLC-SYSTEM 1.
Fractions A–C are not identical with fractions I–III (Fig. 1) although their R_f values are similar. 3·6 cm of a 10 cm line were scanned at a rate meter range of 6×10^4 cpm = 100%.

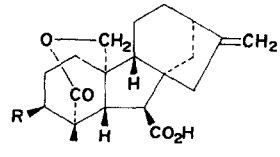
Fraction E ($21{\cdot}6 \times 10^5$ dpm after elution) was rechromatographed in TLC-system 2 and again in system 1, which separated a main component ($13{\cdot}4 \times 10^5$ dpm) and a minor one ($1{\cdot}9 \times 10^5$ dpm). The main component was methylated and shown by GC–RC to contain only two radioactive compounds which were identified as GA_{12} (**7**) and GA_{15} (**16**) by GC–MS. GA_{15} (**16**) was the major component of the fraction, accounting for 95% of the radioactivity.



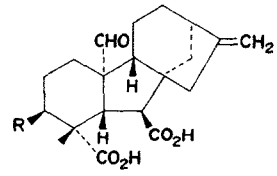
(**13**) R = CH_2
(**14**) R = O



(**15**)



(**16**) R = H
(**17**) R = OH



(**18**) R = H
(**19**) R = OH

Fraction D ($5{\cdot}75 \times 10^5$ dpm) was shown to be homogeneous by chromatography in system 2 followed by system 1, after which $4{\cdot}1 \times 10^5$ dpm were recovered. Methylation and examination by GC–RC confirmed that the fraction contained a single radioactive compound which was identified as GA_{24} (**18**) by GC–MS.

Fraction C ($11{\cdot}7 \times 10^5$ dpm) was separated into a major component ($4{\cdot}1 \times 10^5$ dpm) and a minor one ($0{\cdot}22 \times 10^5$ dpm) by TLC systems 2 and 1. The major component was identified as GA_{37} (**17**) by GC–MS of the methyl ester TMS derivative. No other radioactive component was present in the purified material.

Fraction A ($9{\cdot}0 \times 10^5$ dpm) and fraction B ($22{\cdot}1 \times 10^5$ dpm) were further fractionated by TLC systems 2 and 1 to provide several radioactive components which, however, yielded no useful MS because of the presence of non-radioactive substances.

Incubation with GA_{12} (7) in the absence of Mn^{2+}

Incubation of GA_{12}-[^{14}C] in the standard system (10 ml) without Mn^{2+} gave the same fractions A–E as described from the incubation with GA_{12}-aldehyde (**6**)

Fraction E was methylated and separated by TLC-system 4 into two components ($2{\cdot}5 \times 10^5$ and $3{\cdot}6 \times 10$ dpm) which were identified by GC–MS as the methyl esters of GA_{12} and GA_{15} respectively. In addition GC–RC showed that each methyl ester was accompanied by a minor radioactive peak of slightly shorter retention time. Only the peak accompanying GA_{15} was present in sufficient amount for GC–MS. It had the same molecular weight as the methyl ester of GA_{15} but has not yet been identified.

The other fractions were examined without further TLC purification. As in the incubation with GA_{12}-aldehyde (**6**), fraction D ($0{\cdot}69 \times 10^5$ dpm) contained only GA_{24} (**18**) and fraction C ($3{\cdot}5 \times 10^5$ dpm) only GA_{37} (**17**) by GC–MS and GC–RS.

Fraction B ($2{\cdot}75 \times 10^5$ dpm) was examined as its methyl ester TMS derivative and separated into four radioactive peaks containing 10, 38, 33 and 19% of the radioactivity by GC–RC. The last three components were further examined by GC–MS and the one accounting for 38% of the radioactivity was identified as GA_{36} (**19**). The other two have not yet been identified.

In each incubation all the compounds identified had the same specific radioactivity as judged by GC–MS.[15] This indicates a direct incorporation of the precursors as well as a direct relationship between the intermediates.

DISCUSSION

Our previous paper[8] demonstrated the formation of GA_{12}-aldehyde (**6**) from MVA in a cell-free system from a higher plant. The present results show that GA_{12}-aldehyde (**6**) is converted further to GA_{12} (**7**), GA_{15} (**16**), GA_{24} (**18**), GA_{36} (**19**) and GA_{37} (**17**) by the same system.

GA_{12} (**7**) was identified in our earlier work[8] although its origin as an enzymatic product from MVA was interpreted with caution since we had shown that GA_{12}-aldehyde (**6**) was readily converted non-enzymatically to GA_{12} (**7**). Our results now definitely establish that not only is GA_{12} (**7**) an enzymatic product of GA_{12}-aldehyde (**6**) but is also converted further to the other identified GAs. In the meantime West[2] has referred to unpublished results of Nakata which provide chromatographic evidence that GA_{12} (**7**) is also a metabolite of GA_{12}-aldehyde (**6**) in the *Echinocystis* cell-free system.

GA_{37} (**17**) has recently been isolated from mature seeds of *Phaseolus vulgaris*.[16] With this exception the GAs obtained in the cell-free system have not been found previously in higher plants.

Our results indicate a branch point in the pathway from *ent*-kaurene (**1**) in the *Cucurbita* system. It occurs at *ent*-7α-hydroxykaurenoic acid (**5**) which is converted either to what is tentatively identified as *ent*-6α,7α-dihydroxykaurenoic acid (**11**) and hence to fraction I or to GA_{12}-aldehyde (**6**) and the gibberellins. This accords with findings in the fungus *Gibberella fujikuroi*.[2]

The steps from *ent*-kaurene (**1**) to all of the identified products represent oxidative reactions. However, the conversion of GA_{12} (**7**) to the other GAs is inhibited by Mn^{2+} whereas its formation is not. Thus one or more of the enzymes in the steps from GA_{12} (**7**) have properties distinct from those catalysing its formation.

[15] Bowen, D H, Macmillan, J and Graebe, J E (1972) *Phytochemistry* **11**, 2253
[16] Hiraga, K, Yokota, T, Murofushi, N and Takahashi, N (1972) *Agric Biol. Chem* **36**, 345

EXPERIMENTAL

The large fruit commercial variety of *Cucurbita maxima* L was the same as used in earlier publications[7, 8] where it was incorrectly identified as *C pepo*

Cell-free extracts consisted of endosperm removed from immature seeds as described,[7] centrifuged at 20000 *g* dialysed against phosphate buffer (0 05 M, pH 8 0) with $MgCl_2$ (2 5 mM) and kept frozen in liquid N_2 Standard incubation mixtures contained $MgCl_2$ (10 mM), $MnCl_2$ (1 mM) ATP (5 mM), phosphoenol pyruvate (10 mM) and NADPH (0 5 mM) in addition to the phosphate and $MgCl_2$ present in the dialysed endosperm preparation which constituted 75% of the total vol

Preparation of ^{14}C-labeled compounds for identification and for use as substrates was performed by a large scale incubation containing endosperm preparation (106 ml), D,L-MVA-[2-^{14}C] (355 μCi 5 μCi/μmol) and the standard mixture of cofactors (total vol 142 ml) After incubation for 3 hr at 30 the products were extracted and separated on 3 plates (20 × 20 cm) by TLC-system 1 (Fig 1 shows one of these plates) Each fraction was rechromatographed in the same system to afford complete separation from the others Fraction IV V (Fig 1) was further separated into GA_{12} (R_f 0), GA_{12}-aldehyde (R_f 0 43) and *ent*-7α-hydroxykaurenoic acid (R_f 0 61) by TLC-system 5 Fraction VII was chromatographed in system 6 which separated a subfraction of unknown composition but without diterpenoids (R_f 0 0), squalene (R_f 0 5, identified by co-chromatography) and *ent*-kaurene (R_f 1) The yields of fractions and compounds at this point were as listed in Table 1 Fraction III for identification was obtained from a separate incubation of the same composition but with MVA-[2-^{14}C] of specific activity 6 7 μCi/μmol

TLC-systems used on silica gel G were 1 $CHCl_3$–EtOAc–HOAc (70 30 1), 2 $CHCl_3$ EtOAc–HOAc (70 30 1) with 5% $AgNO_3$ in the layer, 3 $CHCl_3$ EtOAc–HOAc (30 70 1) 4 Petrol (40–60°) EtOAc (1 1), 5 Petrol (40–60°)–acetone (70 30), 6 Petrol (40–60°)

GC-radiocounting (GC-RC) and GC-MS were performed on glass columns 3 mm × 1 5 m packed with 2% SE-31 for GC–RC and 1 5 mm × 1 5 m packed with 2% SE-33 for GC-MS In GC RC the A CO_2 gas flow was 100 ml/min and the temp was programmed from 190° at 4°/min In GC MS the He gas flow was 30 ml/min and the temp was programmed from 190° at 3°/min The data from GC–MS was processed by an on-line Linc 8 computer to give normalized spectra from which the peaks due to column bleed had been substracted

The n-*butylboronates* were prepared by the method of Brooks and Harvey[9] The authentic nor-ketone (**12**) was a gift from Dr J R Hanson

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