Phytochemistry, Vol. 20, No. 4, pp. 703–713, 1981.
Printed in Great Britain.

0031–9422/81/040703–11 $02.00/0

THE BIOLOGICAL ACTIVITY OF SIXTEEN GIBBERELLIN A_4 AND GIBBERELLIN A_9 DERIVATIVES USING SEVEN BIOASSAYS

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(*Received* 31 *July* 1980)

Key Word Index—Monocotyledons; dicotyledons; GA_9; GA_4; C_2- and C_3-substituted GAs; biological activity; 2,2-diMeGA_4.

Abstract—C_2- and C_3-derivatives of GA_4 and GA_9 were tested for biological activity in a range of plant assays. The activity of most of these derivatives was equal to, or less than, that of the parent GAs. However, 2β-methylGA_4 and 2,2-dimethylGA_4 had a higher activity than GA_4 in some assays and the latter derivative was shown to be the most active GA known to date in the Forward oat first leaf, Tan-ginbozu dwarf rice and d_5-maize assays. Two other derivatives, 12,16-cycloGA_9 and 19-desoxyGA_9 had less activity than GA_9.

INTRODUCTION

The metabolism of GAs involves the conversion of compounds which are biologically inactive into those which may be highly active, and then into derivatives which have low or no biological activity. The details of the GA pathways in higher plants may differ from species to species, from organ to organ in the same species, and from early to late developmental stages of a particular plant [1]. Nevertheless, progressive oxidation of the GA molecule occurs in all species of higher plants. However, the pattern of oxidation varies [2] and leads to the production of GAs which have markedly different biological activities [3–5]. The position and stereochemistry of hydroxyl groups are important. For example, 2β-hydroxylation essentially removes biological activity [5] whereas 2α-hydroxylated GAs usually retain some activity. In contrast 3β-hydroxy GAs, in the absence of a 2β-hydroxyl, are more active than 3α-hydroxy GAs [6].

To investigate further the effects of hydroxylation on biological activity, derivatives of GA_4 and GA_9 were examined in which the C_2- or C_3-positions were blocked for hydroxylation. The preparation of these compounds is described in the preceding paper. Two other derivatives of GA_9, available when this work was done, were included in the bioassays.

RESULTS

C_2-Derivatives of GA_4

The results of biossays in which 2β-MeGA_4 and 2,2-diMeGA_4 (for structures see Fig. 1) were compared with GA_4 are shown in Figs. 2–9. It can be seen that the bioactivity of the compounds varied considerably in the different bioassay systems.

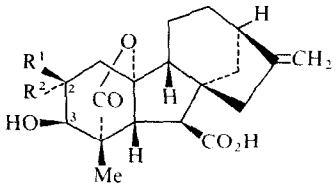
In the lettuce hypocotyl assay (Fig. 2) the GA_4 derivatives had virtually the same activity as the parent compound, whether the plants were measured after 3 days, as is the normal practice in this assay [7], or measured after 5 days.

In the cucumber hypocotyl assay (Fig. 3), measured after 3 days, 2,2-diMeGA_4 was more active than 2β-MeGA_4 and GA_4 at low concentrations. At the higher concentrations, GA_4 was the most active compound and 2,2-diMeGA_4 appeared to saturate the system. The data, obtained in a separate assay by measurement after 5 days (Fig. 3), show that each of the tested compounds saturated the system at the higher concentrations; at lower concentrations, the three compounds had similar activity.

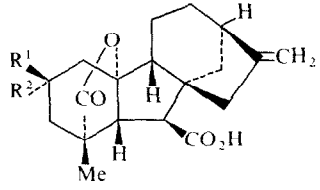
In the *Rumex* leaf senescence assay (Fig. 4) GA_4 and the two derivatives had similar activity. In the Progress No. 9 dwarf pea assay (Fig. 5) 2β-MeGA_4 was more active than 2,2-diMeGA_4 and GA_4 over the middle of the concentration range. However, at the highest concentration tested (10^2 μg/plant), 2,2-diMeGA_4 showed significantly less activity than either GA_4 or 2β-MeGA_4.

Bioassays using monocotyledonous plants showed the most striking differences between the C_2-methyl derivatives of GA_4 and the parent compound. In the oat first leaf assay (Fig. 6) there was considerably more response to the derivatives than to GA_4, with 2,2-diMeGA_4 being 100 times more active than GA_4 at the lower concentrations. The system was saturated at the higher concentrations of 2β-MeGA_4 and 2,2-diMeGA_4 even though marked further growth of the leaf sheaths occurred from 24 to 48 hr.

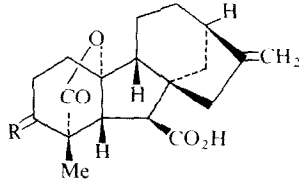
In the dwarf rice assay (Fig. 7), in which the length of the second leaf sheath was measured [8], 2β-MeGA_4 and 2,2-diMeGA_4 had the same activity as GA_4. However, when the assay was allowed to continue to day 6 and the third leaf sheath measured, 2,2-diMeGA_4 was considerably more active than 2β-MeGA_4 or GA_4.



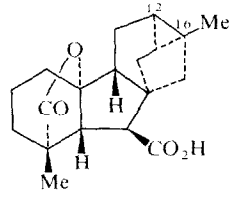
1 $R^1 = R^2 = H$
2 R^1 = Me, R^2 = H
3 $R^1 = R^2$ = Me



4 $R^1 = R^2 = H$
5 R^1 = Me, R^2 = H
6 $R^1 = R^2$ = Me
7 R^1 = F, R^2 = H
8 R^1 = H, R^2 = F
9 R^1 = OMe, R^2 = H



10 R = H, β—^{2}H
11 R = H, α—^{2}H
12 R = H, β—F
13 R = H, β—Cl
14 R = H, β—OMe
15 R = CH_2



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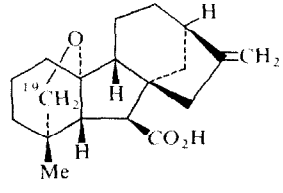

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Fig. 1. Structures of GA derivatives tested for biological activity.

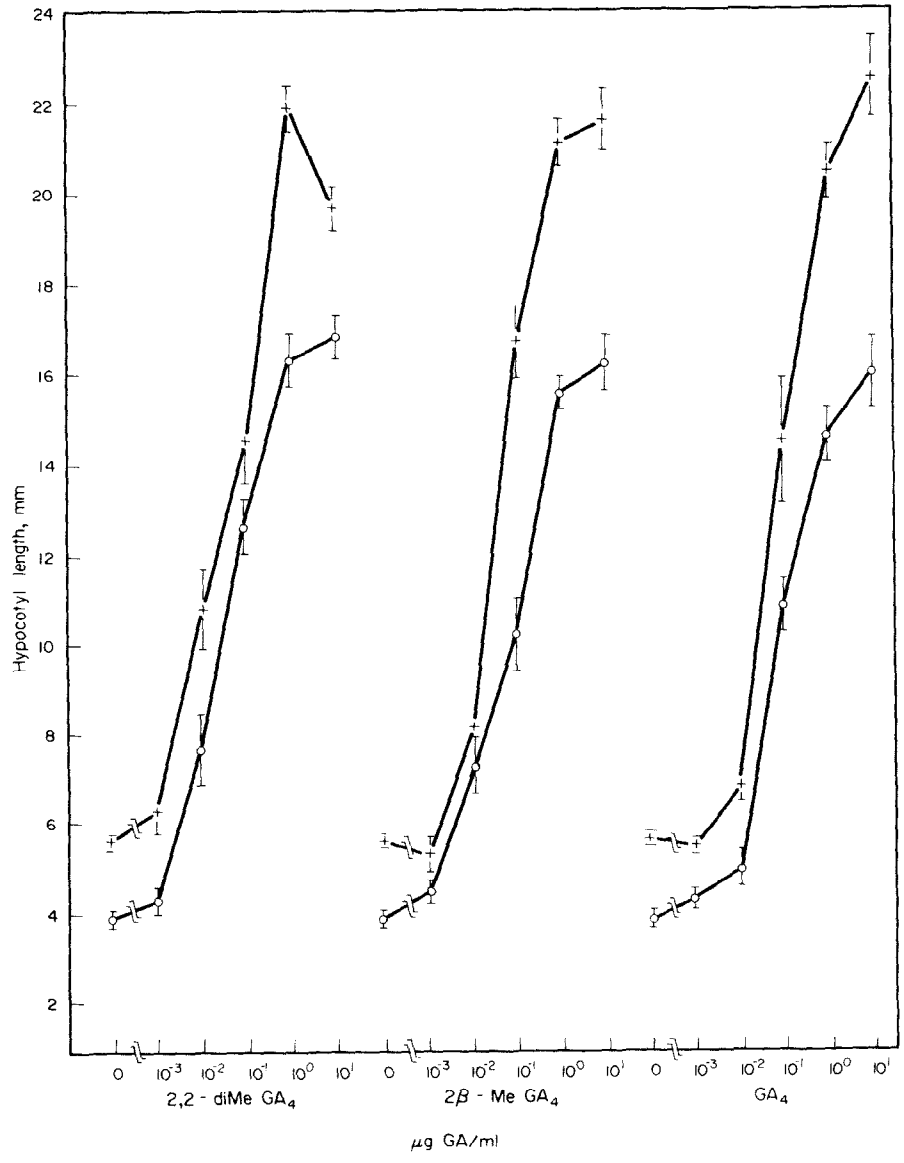

Fig. 2. Activities of 2,2-diMeGA$_4$, 2β-MeGA$_4$ and GA$_4$ in the Arctic King lettuce assay. Growth response of the hypocotyl is shown after 3 days (○–○) and 5 days (+–+).

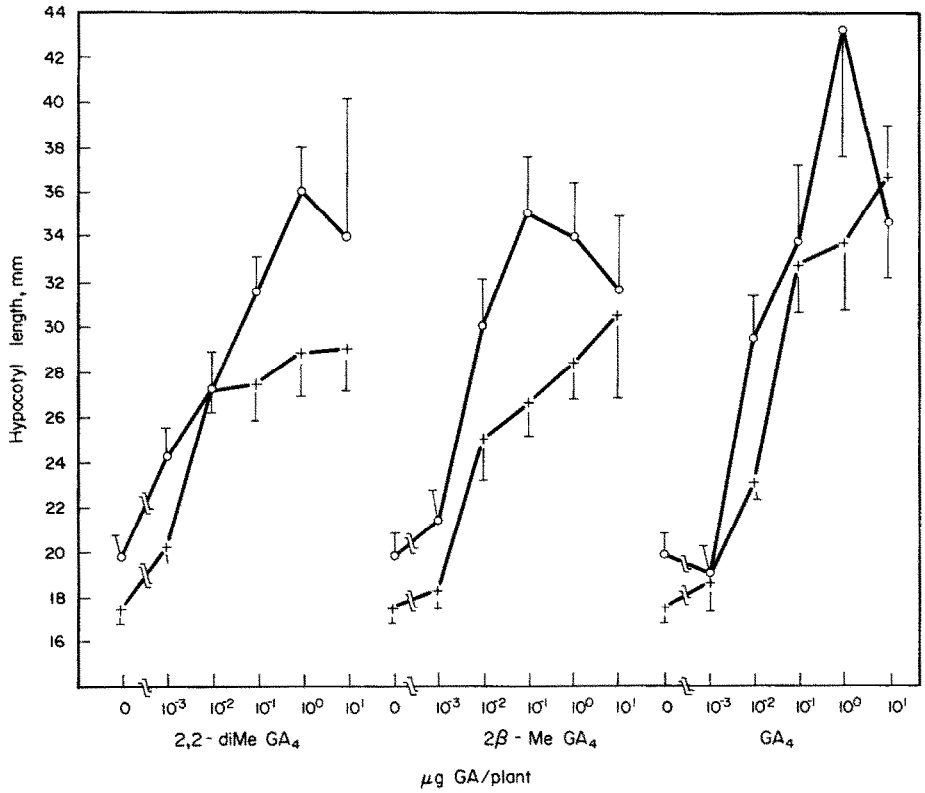

Fig. 3. Activities of 2,2-diMeGA$_4$, 2β-MeGA$_4$ and GA$_4$ in the Perfection Ridge cucumber assay. Growth response of the hypocotyl is shown after 3 days (+–+) and 5 days (○–○).

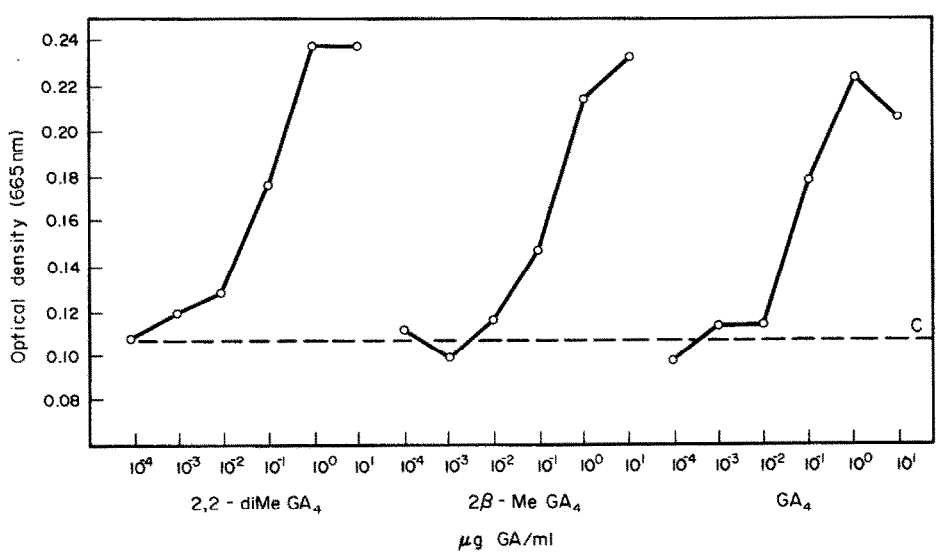

Fig. 4. Activities of 2,2-diMeGA$_4$, 2β-MeGA$_4$ and GA$_4$ in the dock leaf senescence assay. The control value is indicated (——C).

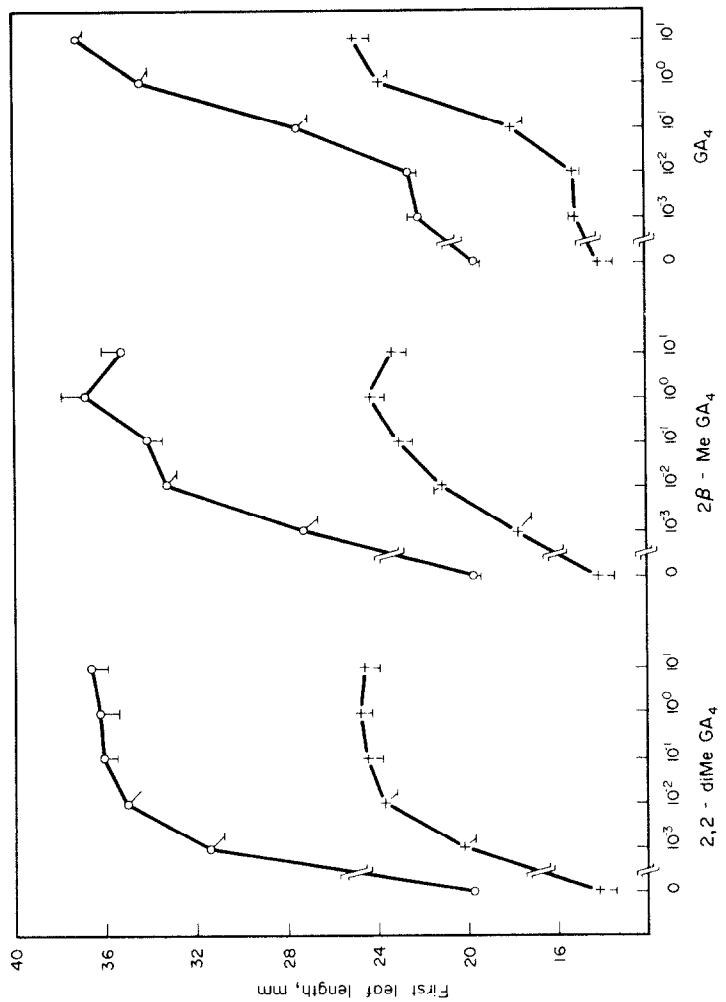

Fig. 6. Activities of 2,2-diMeGA_4, 2β-MeGA_4 and GA_4 in the Forward oat assay. Growth response of the first leaf is shown after 2 days (+ – +) and 4 days (○–○).

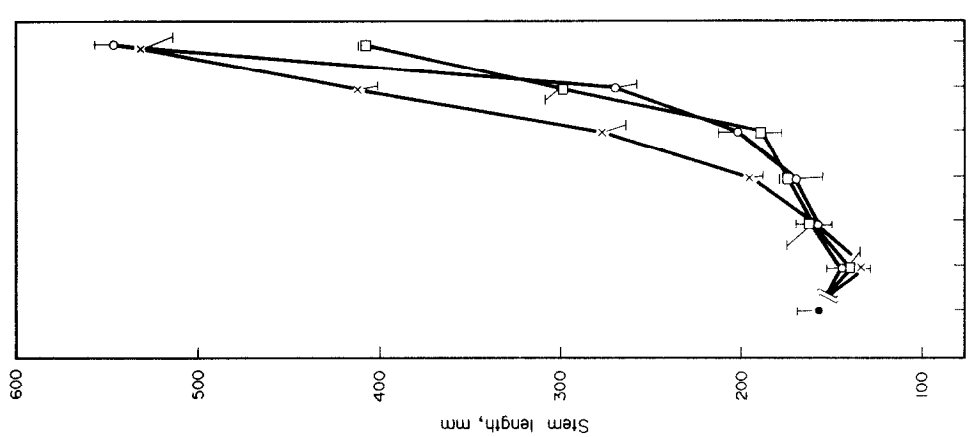

Fig. 5. Activities of 2,2-diMeGA_4 (□–□), 2β-MeGA_4 (× – ×) and GA_4 (○–○) in the Progress No. 9 pea stem assay.

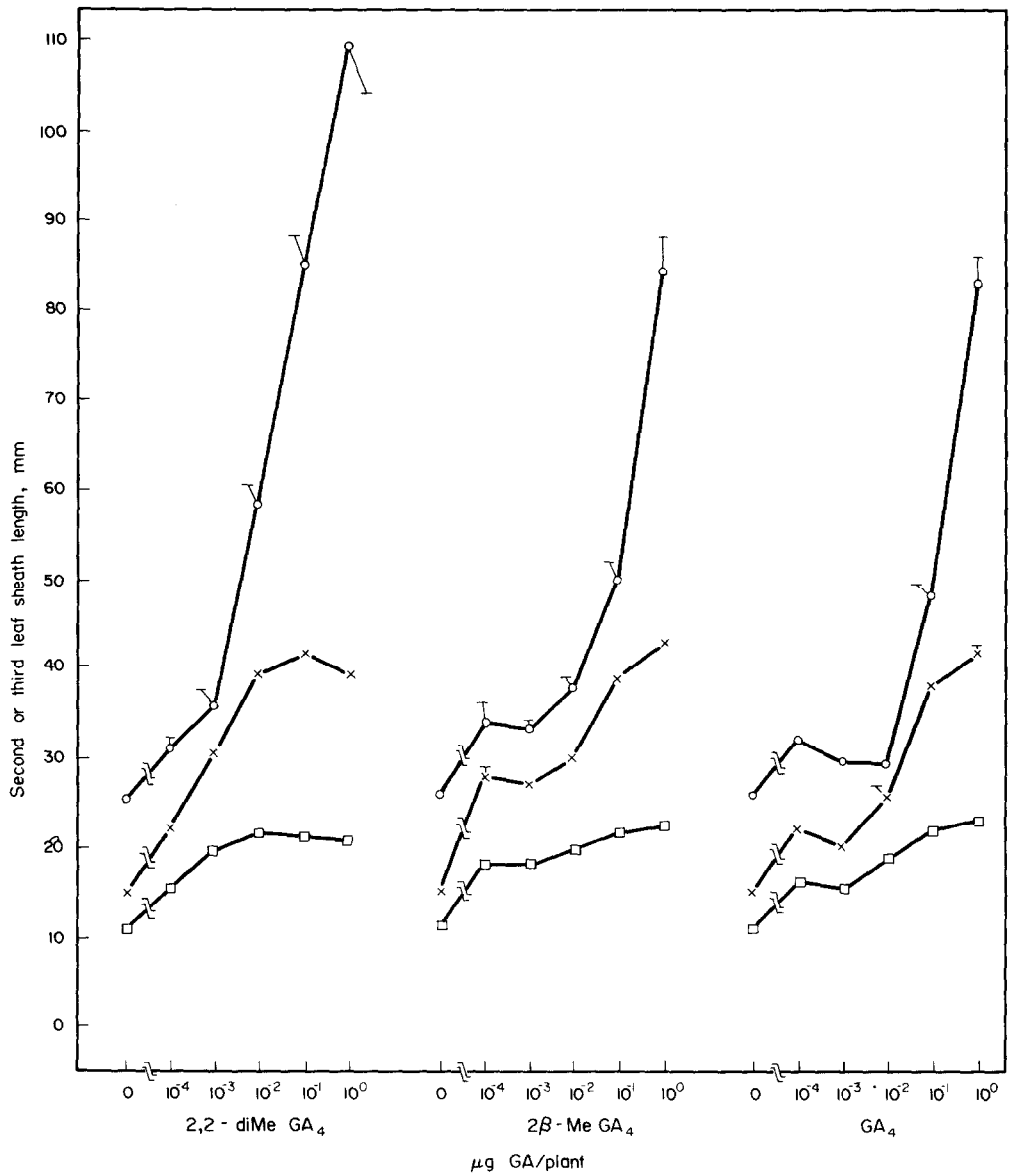

Fig. 7. Activities of 2,2-diMeGA$_4$, 2β-MeGA$_4$ and GA$_4$ in the Tan-ginbozu dwarf rice assay. Growth response of the second leaf sheath is shown after 1 day (□–□), 3 days (× – ×) and the third leaf sheath after 6 days (○–○).

In the d_5-maize assay, only leaf sheaths 1 and 2 are usually measured [9]. However, more information can be obtained by measuring successive leaf sheaths over a longer period of time. A selection of the data obtained with GA$_4$, 2β-MeGA$_4$ and 2,2-diMeGA$_4$ over 11 weeks is shown in Fig. 8. The effect of the compounds on the growth of leaf sheath 1 was minimal, but increased progressively as successive leaf sheaths were measured. Not only did the response to each compound increase over the concentration range but also the two derivatives had considerably more activity than GA$_4$. In particular, 2,2-diMeGA$_4$ increased leaf sheath lengths of the d_5-dwarf above those of the normal maize controls. A similar picture emerged (Fig. 9) on measurement of the lengths of the stem (shoots) and tassel (male inflorescence). The 2,2-diMeGA$_4$ derivative increased the length of both stem and tassel of the d_5-maize, to the extent that they were considerably longer than the normal maize controls.

C_2-*Derivatives of* GA_9

The results of the bioassays in which the activity of the C_2-derivatives of GA$_9$ was compared with GA$_9$ are shown in Tables 1–4. The orders of activities in the different assays were:

Lettuce hypocotyl assay (*Table* 1)

2β-MeOGA$_9$ < 2,2-diMeGA$_9$ < 2β-fluoroGA$_9$ < 2α-fluoroGA$_9$ < 2β-MeGA$_9$ = GA$_9$.

Cucumber hypocotyl assay (*Table* 2)

2β-MeOGA$_9$ < 2,2-diMeGA$_9$ < 2β-MeGA$_9$ < 2β-fluoroGA$_9$ < 2α-fluoroGA$_9$ = GA$_9$.

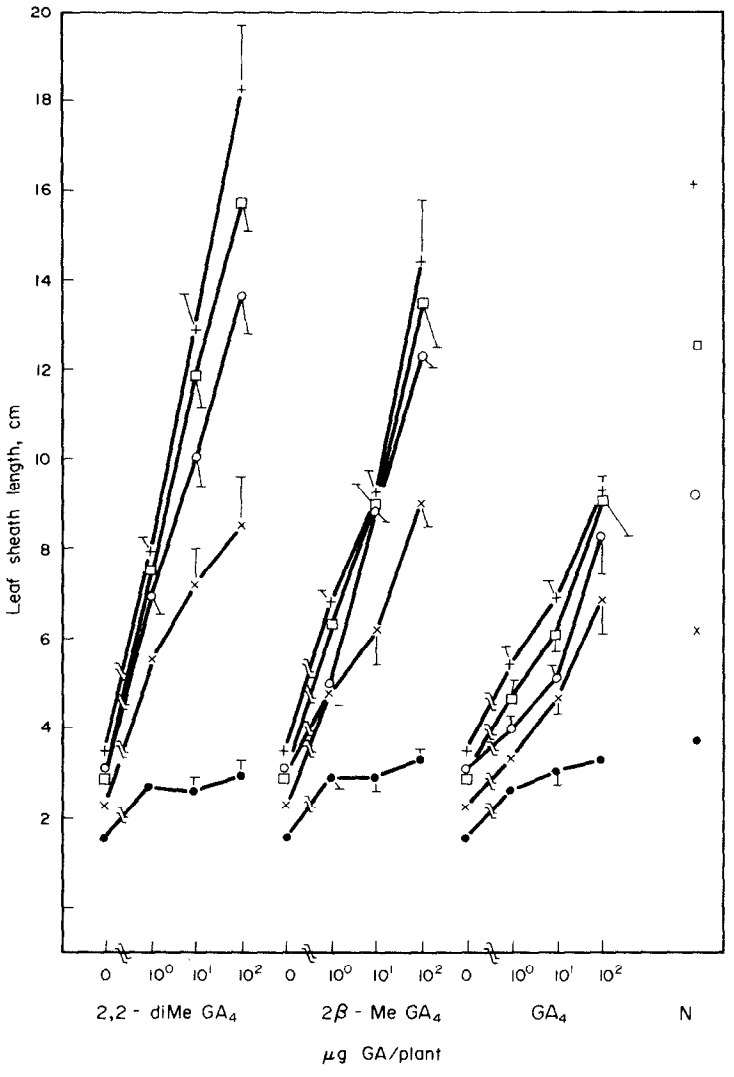

Fig. 8. Activities of 2,2-diMeGA$_4$, 2β-MeGA$_4$ and GA$_4$ in the d_5-maize assay. The growth responses of leaf sheaths 1(●–●), 2(×–×), 3(○–○), 4(□–□), and 5(+–+) is shown after 11 weeks. Values for the leaf sheaths of untreated normal (N) maize plants are also given.

Dwarf rice leaf sheath assay (*Table* 3a, b)

Measured after 3 days: 2β-MeOGA$_9$ < 2β-MeGA$_9$ = 2,2-diMeGA$_9$ < 2β-fluoroGA$_9$ < 2α-fluoroGA$_9$ = GA$_9$.

Measured after 6 days: 2β-MeOGA$_9$ < 2,2-diMeGA$_9$ ≪ 2β-MeGA$_9$ < 2β-fluoroGA$_9$ < 2α-fluoroGA$_9$ = GA$_9$.

d_5-*Maize stem assay* (*Table* 4)

Measured after 11 weeks: 2β-MeOGA$_9$ = GA$_9$. Measured after 16 weeks: 2β-fluoroGA$_9$ < 2,2-diMeGA$_9$ < 2β-MeGA$_9$ < 2α-fluoroGA$_9$ = GA$_9$.

In the Progress No. 9 dwarf pea assay, GA$_9$ and 2,2-diMeGA$_9$ were inactive and 2β-MeGA$_9$ had very low activity; no data are therefore presented.

C$_3$-*Derivatives of* GA$_9$, 12,16-*cyclo*GA$_9$ *and* 19-*desoxy*GA$_9$

The responses in the different assays of the C$_3$-derivatives of GA$_9$ are shown in Tables 5–8. For convenience the data for 12,16-cycloGA$_9$ and 19-desoxyGA$_9$ are included in these tables. In the lettuce hypocotyl assay (Table 5). the cucumber hypocotyl assay (Table 6) and the dwarf rice assay (Table 7), 12,16-cycloGA$_9$ shows the lowest activity followed by 3-exomethylene GA$_9$. The other compounds ranged from equal activity to one-tenth the activity of GA$_9$. In the d_5-maize stem assay, GA$_9$ showed little activity (Table 8) and there was no indication that any of the derivatives tested had substantially higher activities than the parent compound, GA$_9$.

DISCUSSION

The C$_2$-derivatives of GA$_4$ and GA$_9$ were prepared and bioassayed to test the thesis that 2β-hydroxylation is an important factor in controlling the effective concentration of bioactive, native GAs. If so, it might be expected that 2β-substituted derivatives in which potential 2β-hydroxylation is blocked would show either higher, or

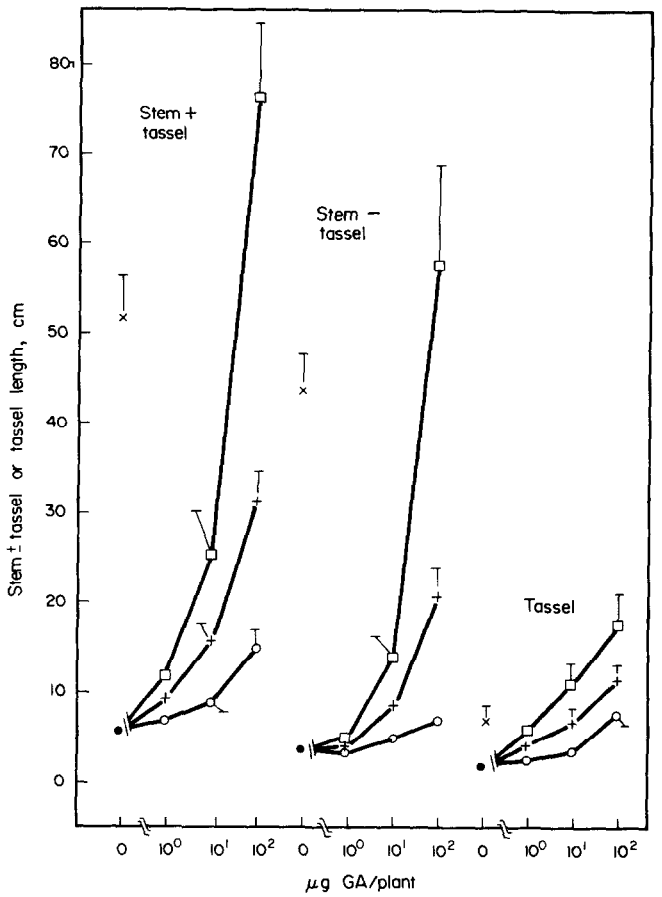

Fig. 9. Activities of 2,2-diMeGA$_4$ (□–□), 2β-MeGA$_4$ (+–+) and GA$_4$ (○–○) in the d_5-maize assay. The growth responses of stem + tassel, stem − tassel and tassel are shown after 11 weeks. The growth of control normal maize (×) is also indicated.

Table 1. Activities of C_2-GA$_9$ derivatives in the lettuce hypocotyl assay (length in mm)

Compound	μg/ml				
	10^{-3}	10^{-2}	10^{-1}	10^{0}	10^{1}
2β-MeGA$_9$	3.9 ± 0.2	5.3 ± 0.4	9.0 ± 0.5	13.7 ± 0.5	14.3 ± 0.7
2,2-DiMeGA$_9$	3.5 ± 0.2	3.3 ± 0.1	5.1 ± 0.4	7.2 ± 0.4	10.8 ± 0.5
2β-FluoroGA$_9$	3.0 ± 0.1	2.9 ± 0.1	4.1 ± 0.3	8.1 ± 0.8	14.8 ± 0.7
2α-FluoroGA$_9$	2.5 ± 0.1	3.1 ± 0.1	6.3 ± 0.5	13.1 ± 0.7	16.5 ± 0.3
2β-MeOGA$_9$	2.5 ± 0.1	2.8 ± 0.1	2.9 ± 0.2	2.6 ± 0.1	4.2 ± 0.2
GA$_9$	3.4 ± 0.2	5.0 ± 0.3	11.1 ± 0.6	16.5 ± 0.7	19.1 ± 0.5
Control	2.85 ± 0.1				

Table 2. Activities of C_2-GA_9 derivatives in the cucumber hypocotyl assay (length in mm)

Compound	μg/plant				
	10^{-3}	10^{-2}	10^{-1}	10^{0}	10^{1}
2β-$MeGA_9$	20.0 ± 0.4	20.1 ± 1.3	23.1 ± 1.8	33.0 ± 0.9	33.4 ± 1.4
2,2-$DiMeGA_9$	19.3 ± 1.0	19.4 ± 0.5	22.6 ± 1.1	26.3 ± 1.6	31.3 ± 2.7
2β-$FluoroGA_9$	21.3 ± 0.9	21.0 ± 1.2	25.1 ± 1.2	32.5 ± 3.7	44.3 ± 3.5
2α-$FluoroGA_9$	20.1 ± 0.8	23.6 ± 2.0	30.4 ± 2.4	34.8 ± 2.2	38.1 ± 2.0
2β-$MeOGA_9$	19.5 ± 0.8	21.4 ± 1.3	22.0 ± 1.0	23.9 ± 1.0	27.3 ± 1.4
GA_9	21.3 ± 1.0	21.9 ± 1.1	31.8 ± 2.5	40.9 ± 3.1	45.1 ± 3.4
Control			19.6 ± 0.5		

more persistent, activity than the parent GAs. However, in considering the results, it must be borne in mine [10,11] that the plants used in the bioassays may differ qualitatively and quantitatively in their capacity to 2β-hydroxylate the applied GAs. There is no evidence, for example, that lettuce will C_2-hydroxylate applied GAs [12,13]. Thus a 2β-hydroxylating enzyme may not be present in lettuce, accounting for the fact that C-2 derivatives of GA_9 and GA_4 were no more active than the parent compounds.

Seed of *Pisum sativum* cv Progress No. 9 contains a 2β-hydroxylase. Thus the endogenous GA_9 and GA_{20} are converted into GA_{51} and GA_{29} respectively [14,15]. In the pea stem assay (Fig. 5) there was some indication that 2β-$MeGA_4$ was more active than GA_4 at intermediate concentrations. However, if 2β-hydroxylation were a limiting factor in the growth response to GA_4, stem elongation might have been expected to be more marked for 2β-$MeGA_4$ and 2,2-$diMeGA_4$ than was the case in this assay. Also, if the inactivity of GA_9 in this bioassay were due to 2β-hydroxylation, then 2β-$MeGA_9$ and 2,2-$diMeGA_9$ would have been expected to show considerably greater activity than GA_9, which they do not.

Results from the cucumber hypocotyl bioassay (Fig. 2, Table 2), show no enhanced activity of the C_2-derivatives of GA_4 and GA_9 over that of the parent compounds and do not provide evidence for the proposition that 2β-hydroxylation is a regulatory process in this plant.

In Tan-ginbozu rice seedlings, the metabolism of GA_4 to the 2β-hydroxylated derivative, GA_{34}, was reported by Durley and Pharis [16]. Thus, in this plant, a 2β-substituent might be expected to enhance activity. However, none of the C_2-derivatives of GA_9 and GA_4, except for 2,2-$diMeGA_4$, had higher activities than the parent GAs (Fig. 7; Tables 3a,b). Indeed 2α-$fluoroGA_9$

Table 3. Activities of C_2-GA_9 derivatives in the Tan-ginbozu dwarf rice assay: (a) lengths (mm) of second leaf sheaths measured after 3 days, and (b) lengths (mm) of third leaf sheaths after 6 days

	Compound	μg/plant				
		10^{-3}	10^{-2}	10^{-1}	10^{0}	10^{1}
(a)	2β-$MeGA_9$	19.3 ± 0.6	23.8 ± 0.8	32.3 ± 1.4	38.7 ± 0.6	36.9 ± 0.9
	2,2-$DiMeGA_9$	18.3 ± 0.7	18.7 ± 0.4	21.6 ± 0.4	31.3 ± 0.8	39.8 ± 0.9
	2β-$FluoroGA_9$	18.4 ± 0.3	25.2 ± 0.6	37.2 ± 0.5	41.3 ± 0.8	39.9 ± 0.7
	2α-$FluoroGA_9$	18.9 ± 0.5	24.9 ± 0.5	37.7 ± 0.6	46.0 ± 0.8	41.2 ± 0.8
	2β-$MeOGA_9$	20.4 ± 0.6	21.6 ± 0.5	20.8 ± 0.3	25.1 ± 0.4	33.4 ± 1.0
	GA_9	19.7 ± 0.4	28.3 ± 0.5	40.6 ± 1.0	45.4 ± 0.9	43.4 ± 1.2
	Control			15.2 ± 0.3		
(b)	2β-$MeGA_9$	22.5 ± 0.8	26.3 ± 0.9	37.7 ± 1.9	70.8 ± 2.9	115.0 ± 3.5
	2,2-$DiMeGA_9$	23.2 ± 0.9	22.3 ± 0.9	24.6 ± 1.0	38.0 ± 1.6	56.2 ± 2.0
	2β-$FluoroGA_9$	23.8 ± 1.1	26.9 ± 0.8	43.1 ± 2.0	85.6 ± 6.8	138.9 ± 3.1
	2α-$FluoroGA_9$	23.6 ± 1.0	30.8 ± 1.3	55.5 ± 1.4	104.0 ± 2.9	136.3 ± 4.4
	2β-$MeOGA_9$	23.9 ± 1.3	25.2 ± 2.0	27.4 ± 0.7	32.2 ± 1.4	53.4 ± 0.9
	GA_9	24.7 ± 0.9	33.2 ± 0.8	53.6 ± 4.1	99.6 ± 7.3	154.3 ± 4.0
	Control			23.5 ± 0.8		

Table 4. Activities of C_2-GA_9 derivatives in the d_5-maize assay. Lengths (mm) of stems measured after (a) 11 weeks, and (b) 16 weeks

		μg/plant		
		10^0	10^1	10^2
(a)	2β-MeO-GA_9	105 ± 18.1	67 ± 7.8	105 ± 9.2
	GA_9	67 ± 6.5	93 ± 11.6	147 ± 9.6
	d_5-Controls		57 ± 8.7	
	'Normal' controls		516 ± 4.7	
(b)	2β-MeGA_9	244 ± 12.1	280 ± 16.3	354 ± 24.3
	2,2-DiMeGA_9	234 ± 17.4	240 ± 15.2	300 ± 15.2
	2β-FluoroGA_9	184 ± 11.7	260 ± 12.3	256 ± 20.6
	2α-FluoroGA_9	248 ± 11.9	276 ± 18.2	360 ± 21.8
	GA_9	254 ± 18.9	290 ± 12.6	318 ± 8.5
	d_5-Controls		240 ± 26.9	
	'Normal' controls		1949 ± 121	

was as active as 2β-fluoroGA_9. It is of interest that the high activity of 2,2-di$MeGA_4$ was only observed for the third leaf sheath (see later).

In the d_5-maize assay, leaf sheath elongation, and stem extension, were measured over 11 and 16 weeks. This was done to examine, in more detail, the finding that enhanced activity of 2,2-di$MeGA_4$ over GA_4 in the dwarf rice assay was only observed for the third, and not for the second, leaf sheath. None of the C_2-derivatives of GA_9 showed enhanced activity (Table 4) and 2α-fluoroGA_9 was as active as 2β-fluoroGA_9. However, 2β-MeGA_4 and 2,2-di$MeGA_4$ were more active than GA_4. The activities of these derivatives in promoting stem and tassel elongation are remarkable (Fig. 8), particularly that of 2,2-di$MeGA_4$ which was 100 times that of GA_4 and 50 times that of GA_3 observed in the same experiment. The log dose–response curves for the first to fifth leaf sheaths treated with GA_4, 2β-MeGA_4 and 2,2-di$MeGA_4$ are not parallel, perhaps indicating that 2,2-di$MeGA_4$ is inactivated slower than 2β-MeGA_4 which, in turn, is inactivated slower than GA_4. A similar conclusion may be drawn from the oat first leaf assay which confirms the very high activity of 2,2-di$MeGA_4$ in monocotyledonous plants (Fig. 6).

On the whole the results do not support the hypothesis that 2β-hydroxylation regulates the biological activity of applied GAs even in plants in which 2β-hydroxylation has been shown to occur (cf. refs. [10,11]). However, they do reveal that C_2-methylated derivatives of GA_4, in particular 2,2-di$MeGA_4$, are highly active compounds in monocotyledonous plants. The high activity of these derivatives may be due to their slower metabolic deactivation, but it is difficult to explain, on this basis, why the corresponding derivatives of GA_9 do not show enhanced activity. An alternative explanation, at least in

Table 5. Activities of C_3-derivatives of GA_9, 12,16-cyclo- and 19-desoxyGA_9 in the lettuce hypocotyl assay (length in mm)

	μg/ml				
Compound	10^{-3}	10^{-2}	10^{-1}	10^0	10^1
3β-MeOGA_9	—	2.4 ± 0.2	2.7 ± 0.1	7.0 ± 0.5	14.7 ± 0.4
3β-FluoroGA_9	—	2.2 ± 0.1	2.9 ± 0.1	10.7 ± 0.5	15.5 ± 0.5
3β-ChloroGA_9	—	2.5 ± 0.1	3.1 ± 0.1	8.1 ± 0.4	15.0 ± 0.5
3β-^{2}H-GA_9	2.6 ± 0.1	3.7 ± 0.3	7.9 ± 0.3	12.7 ± 0.4	16.0 ± 0.4
3α-^{2}H-GA_9	2.7 ± 0.2	3.7 ± 0.2	8.4 ± 0.4	14.8 ± 0.5	14.8 ± 0.6
3-ExomethyleneGA_9	—	2.1 ± 0.1	2.2 ± 0.1	4.2 ± 0.2	12.6 ± 0.5
12,16-CycloGA_9	—	2.4 ± 0.1	3.9 ± 0.2	6.7 ± 0.3	8.0 ± 0.2
19-DesoxyGA_9	—	2.9 ± 0.1	5.2 ± 0.3	11.8 ± 0.3	14.8 ± 0.5
GA_9	2.6 ± 0.1	3.4 ± 0.2	8.3 ± 0.3	14.3 ± 0.3	—
Control			2.1 ± 0.1		

Table 6. Activities of C_3-derivatives of GA_9, 12,16-cyclo- and 19-desoxyGA_9 in the cucumber hypocotyl assay (length in mm)

	μg/plant				
Compound	10^{-3}	10^{-2}	10^{-1}	10^{0}	10^{1}
3β-MeOGA_9		19.4 ± 1.1	20.3 ± 1.8	29.7 ± 1.5	26.7 ± 0.8
3β-FluoroGA_9		16.2 ± 0.8	18.6 ± 0.7	27.9 ± 0.8	27.3 ± 3.1
3β-ChloroGA_9		14.4 ± 1.1	20.3 ± 1.1	23.9 ± 1.5	28.1 ± 2.3
3β-^{2}H-GA_9		17.3 ± 0.8	24.1 ± 1.0	31.4 ± 2.1	33.7 ± 2.2
3α-^{2}H-GA_9		18.1 ± 1.3	20.9 ± 1.2	28.4 ± 2.4	25.7 ± 1.8
3-MethyleneGA_9		16.1 ± 0.4	16.6 ± 1.0	17.7 ± 1.1	27.3 ± 1.4
12,16-CycloGA_9		13.3 ± 0.3	17.3 ± 0.9	20.1 ± 0.5	22.1 ± 2.0
19-DesoxyGA_9		15.1 ± 0.6	17.9 ± 2.0	21.0 ± 1.7	26.1 ± 1.4
GA_9	17.3 ± 1.2	20.6 ± 0.8	27.4 ± 1.6	33.3 ± 2.6	
Control			14.1 ± 0.8		

Table 7. Activities of C_3-derivatives of GA_9, 12,16-cyclo- and 19-desoxyGA_9 in the Tan-ginbozu rice assay. Length (mm) of second leaf sheath

	μg/plant				
Compound	10^{-3}	10^{-2}	10^{-1}	10^{0}	10^{1}
3β-MeOGA_9		26.3 ± 0.9	36.0 ± 2.0	50.8 ± 1.1	47.2 ± 1.9
3β-FluoroGA_9		26.3 ± 0.8	28.1 ± 2.2	41.5 ± 1.9	47.9 ± 1.1
3β-ChloroGA_9		20.1 ± 0.6	27.5 ± 1.0	39.1 ± 1.5	44.9 ± 1.1
3β-^{2}H-GA_9		22.4 ± 0.9	35.7 ± 1.2	41.0 ± 1.1	47.7 ± 1.1
3α-^{2}H-GA_9		25.3 ± 1.2	36.8 ± 1.2	41.7 ± 1.6	43.0 ± 1.8
3-MethyleneGA_9		20.4 ± 0.4	23.9 ± 1.4	29.8 ± 1.1	41.2 ± 1.4
12,16-CycloGA_9		21.3 ± 0.8	23.2 ± 0.6	26.4 ± 0.6	29.2 ± 0.8
19-DesoxyGA_9		19.3 ± 0.5	25.3 ± 0.9	36.3 ± 1.4	47.2 ± 1.4
GA_9	19.0 ± 0.8	26.9 ± 1.7	38.8 ± 1.5	45.4 ± 1.5	47.2 ± 1.1
Control			14.4 ± 0.4		

Table 8. Activities of C_3-derivatives of GA_9, 12,16-cyclo- and 19-desoxyGA_9 in the d_5-dwarf maize assay (length in mm)

	μg/plant							
	0		10^{0}		10^{1}		10^{2}	
Compound	Stem	Stem − tassel	Stem	Stem − tassel	Stem	Stem − tassel	Stem	Stem − tassel
3β-MeOGA_9			223 ± 17.2	110 ± 16.7	228 ± 34.1	114 ± 14.5	228 ± 32.1	144 ± 14.5
3β-FluoroGA_9			257 ± 19.0	134 ± 7.6	220 ± 19.6	110 ± 8.1	250 ± 26.0	123 ± 13.6
3β-ChloroGA_9			212 ± 42.0	106 ± 22.6	282 ± 16.4	156 ± 8.2	318 ± 24.0	185 ± 15.3
3β-^{2}H-GA_9			240 ± 12.0	108 ± 8.3	236 ± 15.0	105 ± 5.6	312 ± 25.0	190 ± 14.5
3-ExomethyleneGA_9			267 ± 16.0	95 ± 6.8	251 ± 15.3	116 ± 8.3	239 ± 23.0	125 ± 13.8
19-DesoxyGA_9			190 ± 39.9	92 ± 20.7	219 ± 8.4	120 ± 7.0	290 ± 15.2	170 ± 14.6
GA_9			193 ± 17.2	91 ± 7.8	282 ± 24.9	148 ± 13.1	332 ± 8.5	190 ± 11.3
d_5-Controls	240 ± 26.9	131 ± 10.0						
Normal controls	1949 ± 121	1746 ± 100						

16-week assay.

part, may be based on the relative lipid and water solubilities of these methylated GAs. From their structures, it would be predicted that C_2-methylated GA_4 and GA_9 are more lipophilic than the parent GAs and that GA_4 and its C_2-methyl derivatives are more water-soluble than GA_9 and its C_2-methyl derivatives. Thus it may be that high lipid solubility at the site of action is necessary for high biological activity but that sufficient water solubility is required for transport to the site of action; in other words, the partition coefficient between lipid and aqueous phases may be *one* of the important factors in determining the observed growth response of a plant to applied GAs. It can then be argued that 2,2-diMeGA_4 is more lipophilic than 2β-MeGA_4 and GA_4 but has sufficient water solubility, by virtue of the 3β-hydroxyl group, to reach the site of action. On the other hand, the highly lipophilic 2,2-diMeGA_9 may be a very active compound, *per se*, but is not sufficiently water-soluble to reach the site of action. These speculations could also explain why 2,2-diMeGA_4 is more active on monocotyledons than dicotyledons since transport of the applied GAs to the intercalary meristem of monocotyledons may be less of a barrier than transport to the apical meristem of dicotyledons.

The C_3-derivatives of GA_9 were prepared and bioassayed to examine the possibility that GA_9 was not active *per se*, but required 3β-hydroxylation to GA_4. The results indicate that 3β-hydroxylation of GA_9 is not an absolute requirement for biological activity.

Four further points of interest emerge from the results. The first is that more information can be obtained by measuring the growth response in the d_5-maize, Tan-ginbozu rice and Avena assays over longer periods than those normally used. Secondly, the 2β-methoxyGA_9 had low biological activity in all assays indicating that inactivation may not be due to the presence of a 2β-hydroxyl group, *per se*, but to a 2β-oxygen function. Thirdly, the activity of 19-desoxyGA_9 shows that the carbonyl group of the lactone bridge in GA_9 is not necessary for biological activity. This indicates that the primary function of the lactone bridge is to confer steric rigidity on the molecule. Lastly, the retention of biological activity in 12,16-cycloGA_9, albeit at a low level, indicates that the bicyclo[3,2,1]octane of rings C/D is not an absolute requirement for GA-activity.

EXPERIMENTAL

The compounds (Fig. 1) were: 2β-methylGA_4 (structure **2**); 2,2-dimethylGA_4 (**3**); 2β-methylGA_9 (**5**); 2,2-dimethylGA_9 (**6**); 2β-fluoroGA_9 (**7**); 2α-fluoroGA_9 (**8**); 2β-methoxyGA_9 (**9**); 3β-deuterioGA_9 (**10**); 3α-deuterioGA_9 (**11**); 3β-fluoroGA_9 (**12**); 3β-chloroGA_9 (**13**); 3β-methoxyGA_9 (**14**); 3-exomethyleneGA_9 (**15**); 12,16-cycloGA_9 (**16**); and 19-desoxyGA_9 (**17**). They were homogeneous by TLC, GLC, and GC/MS except for 2β-fluoroGA_9 (**7**) which contained *ca* 10% of the 15-double bond isomer.

Their biological activities were compared with the parent compounds, GA_4 (**1**) and GA_9 (**4**), in the following assays: lettuce hypocotyl (*Lactuca sativa* cv Arctic King); intact dwarf pea stem (*Pisum sativum* cv Progress No. 9); cucumber hypocotyl (*Cucumis sativus* cv Perfection Ridge); oat first leaf (*Avena sativa* cv Forward); dwarf rice (*Oryza sativa* cv Tan-ginbozu); dwarf maize (*Zea mays*, d_5-mutant); and dock leaf (*Rumex obtusifolius*). Details of the bioassay methods are given in previous publications [17–19]. Where the bioassay methods have been modified, further details are given in the Results.

In the graphic presentation of the data, the mean values + and − one standard error are given wherever possible; but, in some cases, only mean values + or − one standard error are shown for clarity of presentation.

Acknowledgements—Financial assistance from N.A.T.O., A.R.C., N.S.F., and S.R.C. is gratefully acknowledged. We also thank Miss P. J. Hughes-Games for valuable technical assistance.

REFERENCES

1. Bearder, J. R. and Sponsel, V. M. (1977) *Biochem. Soc. Trans.* **5**, 569.
2. Hedden, P., MacMillan, J. and Phinney, B. O. (1978) *Annu. Rev. Plant Physiol.* **29**, 149.
3. Reeve, D. R. and Crozier, A. (1975) in *Gibberellins and Plant Growth* (Krishnamoorthy, H. N., ed.) pp. 35–64. Wiley, Eastern Press, New Delhi.
4. Agnistikova, V. N., Kobrina, N. S., Kucherov, V. F. and Serebryakov, E. P. (1974) in *Biochemistry and Chemistry of Plant Growth Regulators, Proc. Int. Symp. Cottbus* (Schreiber, K., Schutte, H. R. and Sembdner, G., eds.) pp. 63–77. Inst. Plant Biochemistry, Acad. Sci. GDR, DDR-Halle.
5. Sponsel, V. M., Hoad, G. V. and Beeley, L. J. (1977) *Planta (Berlin)* **135**, 143.
6. Brian, P. W., Grove, J. F. and Mulholland, T. P. C. (1967) *Phytochemistry* **6**, 1475.
7. Frankland, B. and Wareing, P. F. (1960) *Nature (London)* **185**, 255.
8. Murakami, Y. (1968) *Bot. Mag. (Tokyo)* **81**, 33.
9. Phinney, B. O. (1961) in *Plant Growth Regulation* (Klein, R. M., ed.) pp. 489–501. Ames, Iowa State University Press.
10. Rappaport, L., Davies, L., Lavee, S., Nadeau, R., Patterson, R. and Stolp, C. F. (1974) in *Plant Growth Substances* 1973, pp. 314–331. Hirokawa, Tokyo.
11. Railton, I. D. (1980) *Z. Pflanzenphysiol.* **96**, 103.
12. Nash, L. J., Jones, R. L. and Stoddart, J. L. (1978) *Planta (Berlin)* **140**, 143.
13. Durley, R. C., Bewley, J. D., Railton, I. D. and Pharis, R. P. (1976) *Plant Physiol.* **57**, 699.
14. Frydman, V. M. and MacMillan, J. (1975) *Planta (Berlin)* **125**, 181.
15. Sponsel, V. M. and MacMillan, J. (1977) *Planta (Berlin)* **135**, 129.
16. Durley, R. C. and Pharis, R. P. (1973) *Planta (Berlin)* **109**, 357.
17. Hoad, G. V. (1969) *Planta (Berlin)* **87**, 164.
18. Hoad, G. V. and Kuo, C. C. (1970) *Can. J. Botany* **48**, 1323.
19. Crozier, A., Kuo, C. C., Durley, R. C. and Pharis, R. P. (1970) *Can. J. Botany* **48**, 867.