Phytochemistry, Vol 23, No. 9, pp 1847–1854, 1984.
Printed in Great Britain.

0031–9422/84 $3 00 + 0.00

GROWTH-PROMOTING ACTIVITY OF SOME SELECTIVELY MODIFIED GIBBERELLINS*

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(*Revised received* 19 *December* 1983)

Key Word Index—*Pisum sativum*; Leguminosae; dwarf pea; *Cucumis sativus*; Cucurbitaceae; cucumber; *Lactuca sativa*; Compositae; lettuce; bioassays; gibberellin analogues; potency scale; hormone–receptor interactions.

Abstract—Synthetic gibberellin analogues derived mainly from GA_3 were tested for their growth-promoting activity in three standard bioassay systems (dwarf pea, cucumber and lettuce). The highest potency in all the tests was displayed by 13-*O*-methyl-GA_3, while in the case of 7-homo-GA_3 a large decrease in activity was observed. No obvious correlation could be found between the partitioning of acidic analogues between ethyl acetate and water and their biological potencies. The results of the bioassays appear to be compatible with the assumption that it is the spatial correspondence between an active gibberellin (such as GA_3 or GA_7) and a specific receptor that plays the prime role in the growth response.

INTRODUCTION

In the last decade, the relationship between the structures of gibberellins and their growth-promoting activities has been the subject of several reviews [1–3] and extensive experimental studies [4–12]. At the same time, our knowledge of the metabolic fate of gibberellins in plants has been growing. This means that we are now able to discuss different inter-related aspects of the structure–activity problem in somewhat more precise terms than it was possible prior to 1974 (cf. [13–15]).

Thus, proteins capable of specific and reversible binding are found in gibberellin-responsive tissues of barley, wheat and pea, and in a few cases a correlation between the growth-promoting potency of gibberellins and their *in vitro* affinity towards these proteins has been observed [16–19]. These findings demonstrate the importance of the 'goodness of fit' between the hormone and the receptor (cf. [13]). On the other hand, experiments on the partitioning of gibberellins between lipophilic and hydrophilic phases, which imitate their partition between the lipophilic and hydrophilic elements of the plant cell, show the necessity to consider this factor as well [11, 20].

In this paper we report the results of bioassays of some specially prepared gibberellin analogues on intact seedlings of dwarf pea, cucumber and lettuce. The partition of some of these analogues between water and ethyl acetate ('lipophile') is also reported.

RESULTS AND DISCUSSION

Acidic derivatives of GA_3 and GA_7 (Table 1) were prepared according to the following procedures: for **1**, see [21]; **2**, see [22]; **3** and **4**, see [23]; **5**, see [24]; **6–8**, see [25]; **10** and **11**, see [26]; **12**, see [27]; **13**, see [28]; **14**, see [29]; **15**, see [30]; **16** and **17**, see [31]; **18**, see [32]; **19**, see [33]. 3-*O*-Methyl-GA_7 (**9**) was prepared from GA_7 in three steps as described earlier [25] for the synthesis of compound **6**. Most of the neutral analogues (Table 2) were prepared similarly (see also [34–36]).

The growth-promoting potencies were estimated with the help of a five-point scale suitable for comparing the results obtained at different times and under non-identical conditions. This scale approximates the decimal logarithmic scale; the order of potency (i.e. the potency index) of a compound in a given bioassay is deduced from the comparison of its dose–response curve (where the response is expressed in per cent of the control) with that of the statistically most active compound in the bioassay (GA_3 in the dwarf pea test, GA_7 in the cucumber test, GA_3 and GA_7 in the lettuce test) over the whole range of doses. If the curve of the tested compound is approximately parallel to that of the most potent gibberellin but displaced by some orders of the dose range, the potency index of the compound in question is assessed from the difference of the dose orders. If the shape of the curve deviates strongly from linearity, the responses at 1×10^{-2} and 1×10^{-1} μg/plant are taken as more typical while the weakly active compounds are also checked against weak gibberellins such as GA_{13}. Compounds with maximal potency in a given bioassay are rated as 4, compounds displaying potency within 10 to *ca* 99% of the maximum are rated as 3, those with potency within 1–9% of the maximum as 2, weakly active compounds with potency *ca* 0.1–0.9% of the maximum as 1, and compounds with still weaker activity are given a zero (0) potency index. The application of these principles to the assessment of potency indices is illustrated in Fig. 1.

The relationships between this scale and those used in earlier studies [14, 15] are shown in Table 3. From these relationships it is possible to estimate the growth-promoting potencies of all compounds for which either the dose–response curves or the equivalent numerical data

*Part 1 in the series "Structure–Activity Study of Gibberellins"

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Table 1. Relative growth-promoting potencies of free acid-analogues of gibberellins A_3 and A_7 as determined from comparison of the growth–response curves in the dose range 1×10^{-4}–1×10^{1} μg/plant in the dwarf pea and lettuce test and 1×10^{-3}–1×10^{1} μg/plant in the cucumber test*

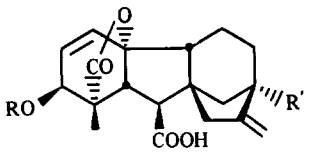
Compound	Bioassay		
	Dwarf pea	Cucumber	Lettuce
GA_3	4	2	4
iso-GA_3 (1)	2	2	2
GA_7	3	4	4
iso-GA_7 (2)	2	3	3
3,13-*O*,*O*-Diacetyl-GA_3 (3)	3	2	4
3-*O*-Acetyl-GA_3 (4)	3	2	4
13-*O*-Acetyl-GA_3 (5)	4	2	4
3,13-*O*,*O*-Dimethyl-GA_3 (6)	1	2	2
3-*O*-Methyl-GA_3 (7)	2	1	1
13-*O*-Methyl-GA_3 (8)	3	3	3
3-*O*-Methyl-GA_7 (9)	2	3	2
7-Homo-GA_3 (10)	1	0	2
7-Homo-GA_3 diacetate (11)	0	0	1
3-Dehydro-GA_3 (12)	2	0	2
3-Epi-GA_3 (13)	2	1	2
3-Dehydro-1β-OH-GA_1 (14)	1	0	1
Lactonic acid (15)	2	0	1
1(10),2-Dien-17,18-dioic acid (16)	1	0	1
3-*O*-Methyl-*iso*-GA_3 (17)	1	0	1
Phenolic acid (18)	0	0	0
1,2α-Etheno-3-dehydro-GA_1 (19)	0	—	0
GA_{13}	0	0	1

*The principle of comparison is illustrated in Fig. 1 and Table 3. Potency indices for compounds 6–14 were determined from two or more series of bioassays.

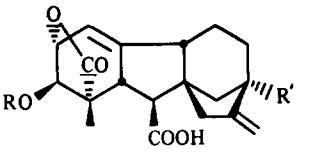
Table 2. Growth-promoting activities of the neutral gibberellin analogues at a dose level of 1 μg/plant*

Compound	Bioassay		
	Dwarf pea	Cucumber	Lettuce
Methyl esters of:			
GA_3	167.7(1)*	112 5(1)	133.3(1)
GA_7	168.8(1)	153.0(2)	149.0(1)
12	102.8(0)	109.2(0)	100.5(0)
13	108.7(0)	104.4(0)	95.1(0)
18	98.5(0)	101.1(0)	102.0(0)
1β-Ethyl-GA_1 (20)	83.0(0)	101.9(0)	110.6(0)
5,6-Seco-3-OH-epi-allogibberic acid (21)	167.1(1)	107.2(0)	139.7(1)
1,3-Di-OH-epiallogibberic acid (22)	88.0(0)	98.0(0)	95.2(0)
1,2β-Etheno-GA_1 (23)	97 3(0)	100 1(0)	100.0(0)
Dilactones:			
15α-OH-GA_3, 7 → 15 lactone (24)	175 7(1)	107.2(0)	146.2(1)
15α-OH-GA_1, 7 → 15 lactone (25)	159.2(1)	104.2(0)	129.5(0)
7-nor-5-Enes:			
from GA_3 (26)	99.4(0)	109.2(0)	98.3(0)
from GA_1 (27)	97.5(0)	102.3(0)	101.2(0)

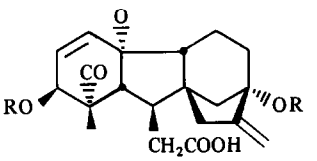
*% of water control (relative potency).



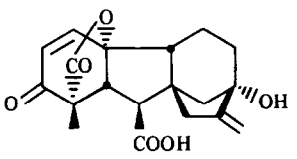
GA_3 R = H, R' = OH
GA_7 R = R' = H
3 R = Ac, R' = OAc
4 R = Ac, R' = OH
5 R = H, R' = OAc
6 R = Me, R' = OMe
7 R = Me, R' = OH
8 R = H, R' = OMe
9 R = Me, R' = H



1 R = H, R' = OH
2 R = R' = H
17 R = Me, R = OH



10 R = H
11 R = Ac



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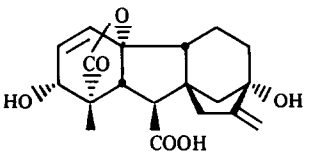

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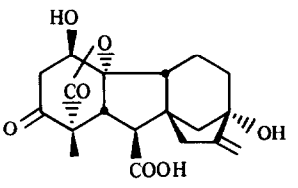

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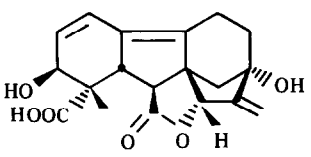

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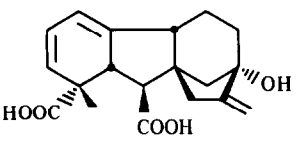

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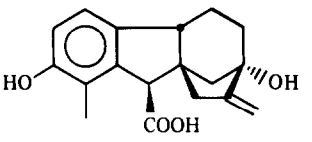

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are available. The reliability of this approach was proved by the results obtained for three *O*-methylated analogues of GA_3 (**6–8**) and two oxo-compounds (**12** and **14**) in several series of bioassays on dwarf pea (1974, 1976, 1977), cucumber (1974, 1977) and lettuce (1974, 1977, 1978). In spite of the large difference in the absolute values of the growth response, the relative position of the dose–response curves for **6**, **7**, **8**, **12** and **14** with respect to those of GA_3, GA_7 and GA_{13} did not change. This can be illustrated by the data in Tables 4 and 5 or by the corresponding curves. For a more reliable assessment of the potencies, overlapping series of bioassays including at least one more reference compound common to a pair of series in addition to GA_3, GA_7 or GA_{13} were systematically compared. The data thus obtained (available on request from the first author) are given in Table 1.

The activities of the neutral analogues were measured in comparison with that of GA_3 methyl ester whose potency indices in all three bioassays were assessed as unity, being three orders of dose weaker than GA_3 in the dwarf pea and lettuce test or GA_7 in the cucumber test. The generalized results of the bioassays for the neutral analogues ae given in Table 2.

The highest activities in all the bioassays were displayed by the compounds closely similar to GA_3 and GA_7 in terms of the nature, number and position of functional groups. In this respect the results of the bioassays are compatible with the idea that the spatial correspondence between the hormone and the receptor is very important for the development of the physiological response. The polarity of the gibberellin analogues or their lipophilicity (as approximated by the partition between the aqueous buffer phase and ethyl acetate) seems to be of secondary importance. Comparing the partition coefficients, *P*, for the compounds listed in Table 6 with the growth-promoting potencies of these compounds (Table 1), one can see that the acids with similar *P* values can differ considerably in their potency indices, and, vice versa, compounds with different *P* values can belong to the same activity group.

The highly potent acetates **3–5** might undergo enzymic deacetylation back to GA_3 in the course of the bioassay due to the action of esterases present in the plant. On the other hand, since enzymes capable of splitting the aliphatic ether linkage are not likely to occur in plants, *in vivo* regeneration of GA_3 or GA_7 from their *O*-methylated derivatives (**6–9**) is unlikely under the bioassay conditions.

In the dwarf pea bioassay, the highest activity among the irreversibly modified gibberellins was found for 13-*O*-methyl-GA_3 (**8**). Its isomer (**7**) and 3-*O*-methyl-GA_7 (**9**)

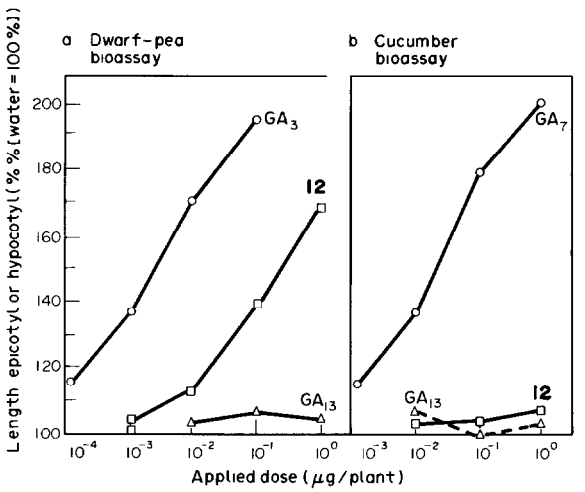

Fig. 1. Assessment of the growth-promoting potency of 3-dehydro-GA_3 (**12**) in the dwarf pea test (a) and the cucumber test (b) from the growth–response curves. In (a) the curve of **12** is approximately parallel to that of GA_3 but displaced by about two orders of the dose range, meaning that the potency index of **12** is two points lower than that of GA_3. In (b) the curve of **12** nearly coincides with that of GA_{13} and hence the potency of **12** in this test is zero. Reference compounds: GA_3 (maximal activity in the dwarf pea test), GA_7 (maximal activity in the cucumber test) and GA_{13} (zero activity in both tests). The mean length of the control plants (in mm) is taken to be 100%, the mean lengths of treated plants (in mm) are expressed in per cent to the control for the convenience of potency assessment.

were about one order of magnitude less potent while 3,13-*O*,*O*-dimethyl-GA_3 (**6**) showed only weak activity. Hence, in this bioassay the presence of the free 3β-hydroxyl group is more important than that of the 13-hydroxyl group.

In the cucumber hypocotyl bioassay, the high activity observed for 13-*O*-methyl-GA_3 (**8**) is compatible with the assumption that in the cucumber hypocotyl test, a hydrophobic interaction between the region around C-13 in the gibberellin molecule and the complementary locus in the putative receptor is of great importance. The lipophilicity constant of the CH_3O group ($\pi = -0.02$) is very close to that of H ($\pi = 0.00$) and much higher than that of the hydroxyl group ($\pi = -0.67$) [37]. This may be the reason why the 13-*O*-methyl derivative (**8**) is more active than GA_3 in spite of the fact that the inductive constants, σ_I, are nearly the same for CH_3O and hydroxyl groups (see [38]). This hydrophobic interaction does not appear to be very sensitive to the steric factor: although the volume of the CH_3O group is greater than that of the hydroxyl group, compound **8** is more active than GA_3, and compound **6** more active than **7**.

In the lettuce hypocotyl bioassay, the dose–response curves for GA_3 and GA_7 were practically identical. However, from the comparison of pairs **1/2**, GA_3 methyl ester/GA_7 methyl ester, GA_3/**8** and **7/9**, one may conclude that in this bioassay an increase in the lipophilicity of substituents at C-13 brings about some increase in the potency.

It is well established [14] that in all gibberellin bioassays the presence of a free carboxyl group at C-6 is a necessary condition for high activity. Alkali-metal and ammonium salts of gibberellins are as active as free acids [39, 40], which implies the importance of the ionized form, at least, for the uptake stage. In this connection, it is noteworthy that 7-homo-GA_3 (**10**), which has nearly the same degree of lipophilicity as GA_3 (see Table 6), displays but weak activity. The low potency of 7-homo-derivatives, **10** and **11**, indicates that the carboxyl group may be necessary not only to provide the ionized form but also to play a part in the recognition of the hormone by the receptor. Our results agree well with the recent observation [12] that **10** and 6-epi-GA_3 are considerably weaker than GA_3 in the dwarf pea and four other bioassays. In the case of 7-homo-GA_3 (**10**), its binding to the putative receptor can be accompanied with a limited rotation around the C-6/C-7 bond necessary to insert the carboxyl into the complementary locus of the receptor.

In this connection, it is noteworthy to compare the zero potencies of 7-nor-5-enes (**26**) and **27** with that of 7-nor-GA_3, which in the dwarf pea test is quite low [12]. Even though the carboxyl group at C-6 may not be absolutely

Table 3. Relationship of scales for the estimation of growth-promoting potency

Criteria		
Potency with respect to gibberellic acid (= 100) [14]	Lowest response-producing dose μg/plant) [15]	Potency with respect to the most active compound in the test (this paper)
⩾1000 7		
> 100 6	< 10^{-2} ++++	Maximal 4*
ca 100 5	*ca* 10^{-2} +++	0.1 of max 3
> 10 4	⩽ 10^{-1} ++	
ca 10 3		
> 1 2	⩽ 10^{0} +	0.01 of max 2
ca 1 1		
None 0	No activity at 10^{0} μg 0	0.001 of max 1 0.001 of max 0

*Dwarf pea test: GA_3; cucumber test: GA_7; lettuce test: GA_3 and GA_7 were of equal potency.

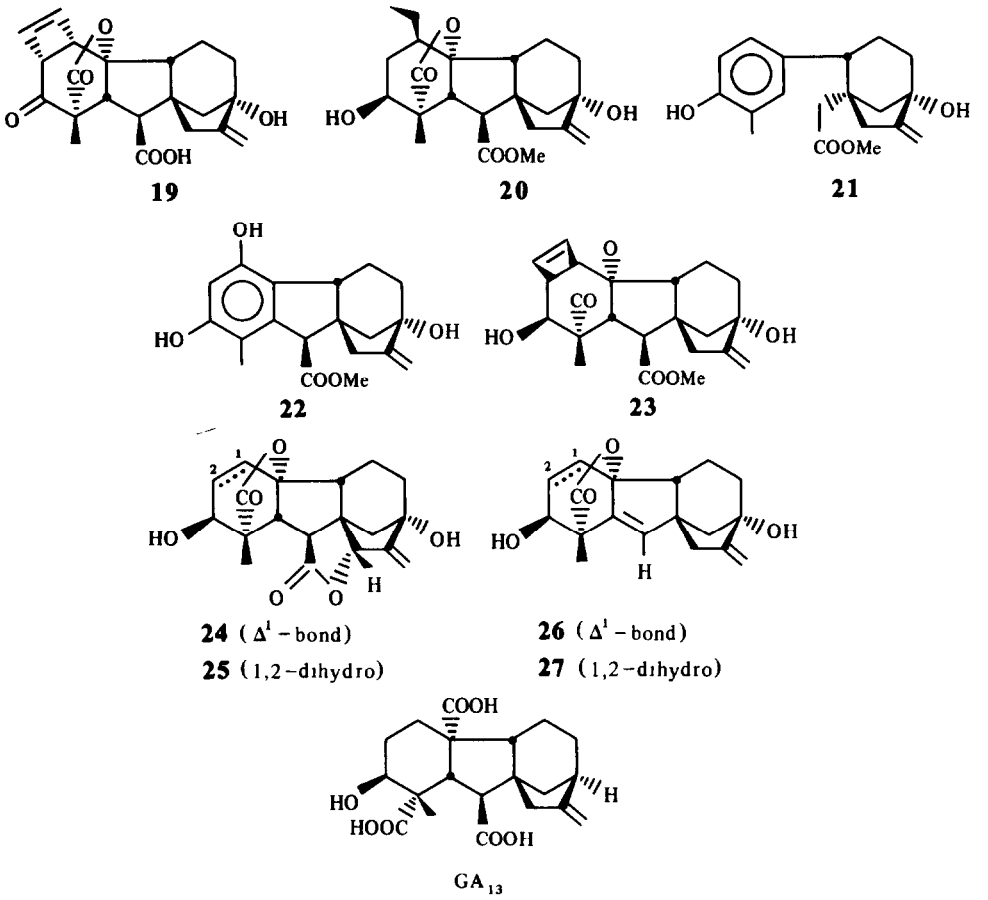

Table 4. The growth-promoting activities of three *O*-methylated analogues of GA_3 in the dwarf pea bioassay

Compounds	Dose (μg/plant) 1×10^{-4}	1×10^{-3}	1×10^{-2}	1×10^{-1}	1×10^{0}	1×10^{1}	Relative potency
First series of bioassays (Summer 1974)							
Control (0.05% EtOH in water)			18.0 ± 0.8 (100)*				
GA_3	—	20.8 (116)	26.4 (146)	31.2 (173)	—	—	4
6	—	—	18.0 (100)	18.6 (105)	25.1 (139)	—	1
7	—	—	19.3 (107)	22.8 (126)	28.0 (155)	—	2
8	—	—	21.8 (121)	25.8 (143)	32.2 (178)	—	3
GA_{13}	—	—	18.0 (100)	17.8 (98)	19.2 (106)	—	0
Second series of bioassays (Spring 1977)							
Control (0.05% EtOH in water)			16.8 ± 0.9 (100)				
GA_3	17.8 (106)	24.8 (147)	30.2 (180)	35.8 (214)	—	—	4
6	16.8 (100)	18.2 (108)	19.0 (113)	25.1 (149)	28.8 (171)	30.4 (181)	1
7	17.5 (104)	19.3 (115)	22.7 (135)	25.2 (150)	30.3 (181)	34.6 (206)	2
8	16.2 (96)	18.8 (112)	26.9 (160)	29.4 (175)	34.4 (205)	40.5 (241)	3
GA_{13}	—	—	17.0 (102)	16.7 (100)	17.7 (105)	18.4 (110)	0

*Length of epicotyls in mm (% control).
Mean values obtained and statistically evaluated on the basis of *t*-distribution.

necessary for the display of activity in all bioassays (cf. [12]), it is of prime importance in the dwarf pea test as in many others (cf. [41]).

From what is known about the metabolism of C_{20}-gibberellins in Leguminosae and Cucurbitaceae [3] and their activity in the dwarf pea and cucumber bioassays [2, 6, 8], it may be concluded that the potency of C_{20}-gibberellins depends on their ability either to be metab-

Table 5. The activities of the GA_3 analogues in the lettuce seedling bioassay

Compounds	Dose (µg/plant) 1×10^{-3}	1×10^{-2}	1×10^{-1}	1×10^{0}	Relative potency
May, 1974					
Control (0.05% EtOH in water) 2.66 ± 0.15 (100)*					
GA_3	3.28 (126)	4.10 (157)	—	—	4
12	2 67 (100)	2.90 (112)	3.64 (137)	4.40 (169)	2
14	2.65 (99)	2.68 (103)	2.90 (112)	3.15 (122)	1
GA_{13}	—	2.66 (100)	2.80 (108)	3.64 (140)	1
June, 1977					
Control (0.05% EtOH in water) 2.60 ± 0.20 (100)					
GA_3	3.8 (146)	9.2 (352)	—	—	4
10	2.6 (100)	3.6 (139)	5.9 (225)	8.8 (338)	2
11	2.6 (100)	2.6 (100)	2.6 (100)	3.3 (128)	0
14	2.6 (100)	2.6 (100)	3.3 (123)	3.7 (143)	1

*Elongation of hypocotyl in mm between the third and fifth day after germination (% control).
Mean values verified by *t*-distribution

olized to the more active lactonic acids of the C_{19}-series or to mimic them. The nature of the contact being species-dependent, the requirements for the optimal 'response-triggering' contact between gibberellins and the hypothetical specific receptors might be tentatively deduced from the results of bioassays of C_{19}-gibberellins and their close analogues. For the dwarf pea test and the cucumber test, such requirements are schematically presented in Fig. 2(a, b). These idealized pictures do not take into account the possibility of metabolic activation or deactivation of C_{19}-compounds in the course of bioassay (see, for example, [11, 42]) which whilst ever-present are difficult to evaluate at this stage of our knowledge.

Although this approach to the structure–activity problem remains largely speculative, it provides a working hypothesis for the synthesis of gibberellin analogues with predictable growth-promoting activity.

EXPERIMENTAL

3-O-Methyl-GA_7 (**9**) was prepared from GA_7 by *O*-methylation of its *p*-methoxyphenacyl ester and subsequent splitting of the ester group upon photolysis in EtOH (see [25]) in 19% yield overall Mp 127–129° (from EtOAc–hexane); IR ν_{max}^{KBr} cm^{-1}: 3080, 3045, 1760, 1715, 1665, 1190, 895, MS: $[M]^+$ 344.

Partition coefficients of acidic analogues. The analogues (10.0 mg) were dissolved in 10 ml EtOAc (distilled and pre-equilibrated with H_2O), the solns were shaken for 15 min with 10.0 ml 1 M NaPi buffer, pH 6.4 or 3.5, pre-equilibrated with EtOAc and then left for 24 hr at room temp. The organic layer was separated and evapd under vacuum, the residues being azeotropically dried with C_6H_6 to a constant wt. From that wt, the average wt of the inorganic contaminant (0.9 mg at pH 3.5 or 0.4 mg at pH 6 4) was subtracted and the partition coefficients ($P = c_{H_2O}/c_{EtOAc}$) were calcd from the ratio $(10-m)/m$, where m

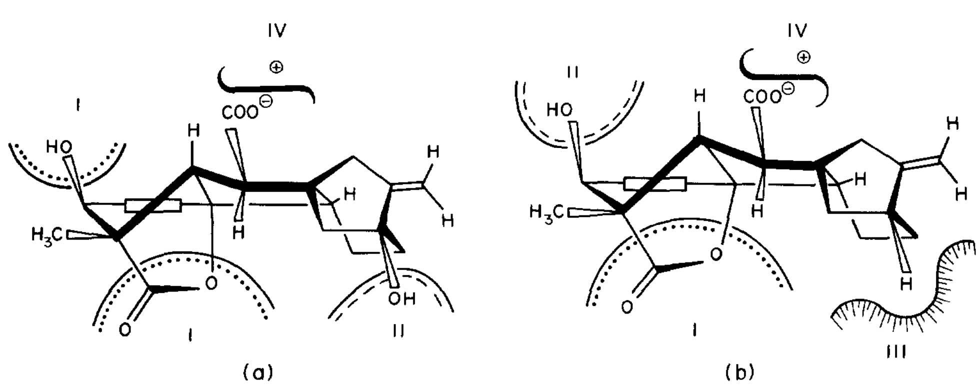

Fig. 2. Hypothetical sites of binding between a C_{19}-gibberellin and a specific receptor in the case of optimal spatial correspondence: (a) GA_3 and the 'dwarf pea receptor'; (b) GA_7 and the 'cucumber receptor'. The following sites may be conceived on the basis of bioassays data: (I) Site of obligatory binding—a good fit here is necessary for high activity; (II) sites of ancillary binding—a good fit here increases the activity by some degree; (III) specific site of obligatory hydrophobic interaction; (IV) site of electrostatic interaction between the ionized carboxyl group of the hormone and a positively charged group on the receptor surface. The binding at the alcoholic hydroxyl groups and at the lactone bridge may be assured either by hydrogen bonds or by transient formation of an ester linkage.

Table 6. Partition coefficients (P) of some close semi-synthetic analogues of GA_3 for the water–EtOAc system

	$P = c_{H_2O}/c_{EtOAc}$									
pH	GA_3	GA_7	1	6	7	8	10	12	13	18
6.4	9.64	0.52	9.85	0.19	0.76	0.85	9.90	9.20	9.90	∞
3.5	0.18	0 02	0.20	0.00	0.09	0.10	0.25	0.30	0.22	0.16

= the corrected wt of the dry residue from the organic phase. No chemical changes occurred during the partitioning of gibberellin analogues between EtOAc and buffer solution (TLC data). The results are given in Table 6.

The growth-promoting potency was studied on whole seedlings of dwarf pea (*Pisum sativum* L., cv Pioneer), cucumber (*Cucumis sativus* L., cv Podmoskovnye) and lettuce (*Lactuca sativa* L., cv Berlinsky). The seeds of dwarf pea were obtained from the collection of the Timiriazev Institute of Plant Physiology, Moscow; the others were commercially available from the all-Union 'Soyuzsortsemovozsch' Firm. Free acids were tested over a wide range of dose ($1 \times 10^{-4} - 10^0$ μg/plant and sometimes at 10^1 μg/plant) while neutral compounds were tested mainly at the 10^0 μg/plant level. All bioassays were carried out according to ref. [1].

The seeds of dwarf pea were left to germinate in darkness in Petri dishes on wet filter paper (48 hr, 24°). Germinated seeds of equal size were selected and cut in half across the cotyledons. The halves with rootlets and shoots were left for 18 hr in a fridge (+2°) and then put in Petri dishes with 0.75% aq. agar (10 such halves in a dish, 2 dishes for each variant). One drop (5 μl) of the test soln was pipetted onto each hypocotyl (control—0.05% EtOH in H_2O). The doses were adjusted by appropriate dilutions of alcoholic solns of the tested compounds with H_2O. The dishes were covered and kept in darkness for 48 hr at 24°. Then the stems were cut off close to the seed and their lengths to the upper internode were measured. With 20 replicates, the 10% difference between two variants was within the 95% reliability range.

The seeds of cucumber were left to germinate in darkness in large Petri dishes (*ca* 200 seeds per dish) on wet filter paper for 72 hr at 24°. Germinated seeds of equal size with hypocotyl lengths of 4–7 mm and easily removable husks were then planted in 0.75% agar (3–5 mm thick) in Petri dishes (10 seeds per dish). One drop (5 μl) of the test soln was applied to each hypocotyl (control—0.05% EtOH in H_2O), the dishes were covered by crystallization vessels, and the seedlings grown at 20–22° for 48 hr under continuous illumination provided by a luminescent daylight lamp Then they were removed from the agar and the lengths of their hypocotyls measured With 20 replicates, the 10% difference between two variants was within the 95% reliability range

The seeds of lettuce were left for 72 hr to germinate on wet filter paper in Petri dishes (first 24 hr at 20–21° with continuous illumination, then 48 hr at 24° in the dark). Seedlings of the same size with hypocotyl lengths of 5–6 mm were stripped of their husks and transferred into Petri dishes with 0.75% agar (3–5 mm thick, 10 seeds per dish) with rootlets oriented from the periphery to the centre of the dish and covered by wet filter paper. One drop (5 μl) of the test soln was applied to each hypocotyl, the dishes were covered and the seedlings left to grow under continuous illumination, provided by a daylight lamp, for 48 hr at 23–24°. Then the seeds were removed from the agar, the lengths of their hypocotyls measured, and the elongation of the hypocotyls between the third and the fifth day calculated. With 20 replicates, the 10% difference between two variants was within the 95% reliability range.

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