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RELATIONSHIPS BETWEEN STRUCTURE AND GROWTH-PROMOTING ACTIVITY OF THE GIBBERELLINS AND SOME ALLIED COMPOUNDS, IN FOUR TEST SYSTEMS

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Abstract—134 Compounds related to gibberellins A_1–A_9 have been examined for growth promoting activity, using the dwarf pea-stem, lettuce hypocotyl, cucumber hypocotyl and dwarf maize leaf-sheath tests. Relations between structure and growth promoting activity are discussed.

MANY workers have compared the plant-growth promoting properties of currently available gibberellins and of some related compounds, in a variety of bioassays. It is now established that, besides differing in potency in any one test system, the gibberellins show species specificity. Some derivatives[1–8] retain growth-promoting activity and a few have been reported to possess inhibitory properties.[9,10]

Seventeen gibberellins, A_1–A_{15} [A_3 = gibberellic acid], Bamboo gibberellin (I),[11] and Lupinus gibberellin I (II)[12] have so far been isolated in pure form from plants or from cultures of *Gibberella fujikuroi*. The structure of gibberellin A_{11} (VIII, compound 32)|| remains

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|| Compound numbers shown in brackets refer to numbers shown in Tables 1–6.

1 H. M. SELL, S. RAFOS, M. J. BUKOVAC and S. H. WITTWER, *J. Org. Chem.* **24**, 1822 (1959).
2 J. S. MOFFATT and MARGARET RADLEY, *J. Sci. Food Agr.* **7**, 386 (1960).
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10 F. LONA, *Giorn. Bot. Ital.* **72**, 1 (1965).
11a S. TAMURA, N. TAKAHASHI, N. MUROFUSHI, S. IRIUCHIJIMA, J. KATO, Y. WADA, E. WATENABE and T. AOYAMA, *Tetrahedron Letters* 2465 (1966).
11b N. MUROFUSHI, S. IRIUCHIJIMA, N. TAKAHASHI, S. TAMURA, J. KATO, Y. WADA, E. WATENABE and T. AOYAMA, *Agr. Biol. Chem.* (*Tokyo*) **30**, 917 (1966).
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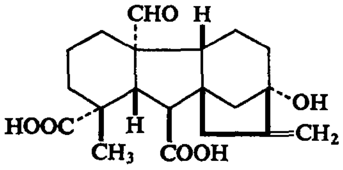
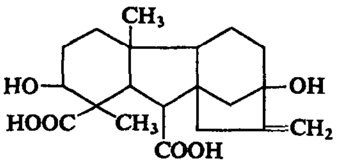
tentative;[13]* the structures of the other gibberellins are established. All are closely related modified diterpenoids containing the gibbane ring system (III), and differing in substitution pattern and degree of unsaturation, but, as far as is known, not in their absolute configuration.



(I)



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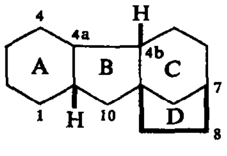

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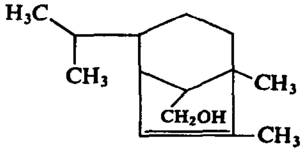


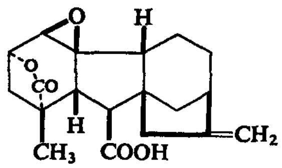
(IV)

Brian *et al.*[14] compared the potency of gibberellins A_1–A_9 in several test systems. In this paper we report results obtained during the period 1960–1964 by testing some compounds related to the gibberellins, in four of the assays used by Brian *et al.* The effect of light on biological activity in these tests[15] was not investigated. Some of the results have been discussed briefly.[16]

Compounds were tested in one or more of the following assays; dwarf pea shoot growth, lettuce and cucumber hypocotyl growth, and dwarf maize leaf-sheath growth. Only very small quantities of some compounds were available and they were not tested as extensively as gibberellins A_1–A_9; the results (Tables 1–5) are comparable with those reported by Brian *et al.*, but are less precise, and dose-response curves were not obtained. The tests were standardized by the inclusion of gibberellic acid, and the approximate growth-promoting activity was graded as shown on p. 1592.

None of the compounds listed in the tables proved inhibitory in our tests, Many were inactive in all four tests; it is, however, possible that some of these compounds would show activity if a suitable test system were used. Other compounds showed very low activity in all the assays. Apart from the possibility that such slight activity might sometimes be due to very

* A revised structure (VIIIA) has recently been proposed[36] for gibberellin A_{11} (compound 32).



(VIIIA)

J. C. BROWN, B. E. CROSS and J. R. HANSON. *Tetrahedron* In press.

[13] B. E. CROSS, Personal communication.
[14] P. W. BRIAN, H. G. HEMMING and D. LOWE, (*a*) *Nature* **193**, 946 (1962); (b) *Ann. Botany* **28**, 369 (1964).
[15] G. SEMBDNER and K. SCHREIBER, *Flora* **156**, 359 (1965).
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Table 1. Lactonic Gibbane derivatives

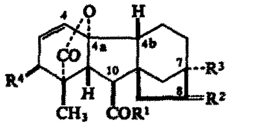
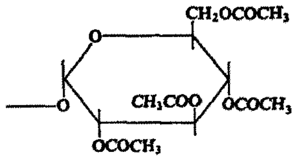
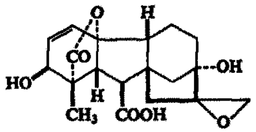
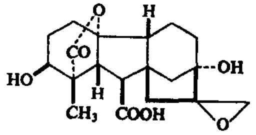
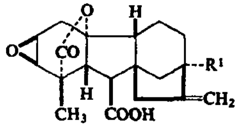
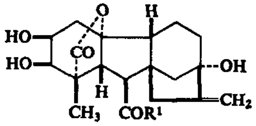
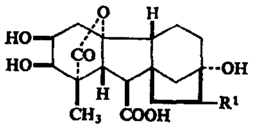
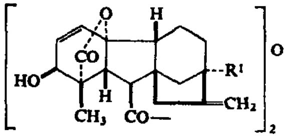
Compound		Code number	Substituents R¹	R²	R³	R⁴	Dwarf pea Shoot growth	Lettuce seedlings Hypocotyl growth	Cucumber seedlings Hypocotyl growth	Maize mutant dwarfs Leaf-sheath Mutant d-1	Leaf-sheath Mutant d-2	Extension Mutant d-3	Extension Mutant d-5
 (V)	(Gibberellic acid)	1	OH	CH_2	OH	OH	5	5	5	5	5	5	5
		2	OH	CH_2	OH	$OCOCH_3$	3	3	5	5	2	4	4
		3	OH	CH_2	OH	$OCOC_2H_5$	5	5	3	4	—	4	4
		4	OH	CH_2	OH	$OCOC_3H_7$	5	5	2	5	—	5	5
		5	OH	CH_2	$OCOCH_3$	$OCOCH_3$	4	5	5	5	5	4	5
		6	OH	CH_2	$OCOCF_3$	$OCOCF_3$	5	5	5	5	—	5	5
		7	OH	O	OH	OH	2	2	3	2	—	2	2
		8	OH	CH_2	OH	$COCH_3$	0	0	0	0	—	0	0
	(Gibberellin A_7)	9	OH	CH_2	H	OH	4	6	7	4	—	6	6
		10	OH	CH_2	H	$OCOCH_3$	5	6	7	5	—	5	5
		11	OCH_3	CH_2	OH	OH	0	0	2	1	—	1	1
		12	OC_2H_5	CH_2	OH	OH	0	2	1	1	1	—	1
		13	OC_3H_7	CH_2	OH	OH	1	3	3	2	2	—	2
		14	$OCH(CH_3)_2$	CH_2	OH	OH	1	2	1	1	1	—	2
		15	OC_4H_9	CH_2	OH	OH	0	2	0	2	—	2	2
		16	$OC(CH_3)_3$	CH_2	OH	OH	1	1	1	1	—	1	1
		17	$OCH_2C_6H_5$	CH_2	OH	OH	2	3	3	3	—	3	3
		18	$O(CH_2)_2OC_4H_9$	CH_2	OH	OH	0	1	2	1	—	0	0
		19	OC_6H_4-p-NO_2	CH_2	OH	OH	2	2	3	3	—	3	3
		20		CH_2	OH	OH	2	2	4	1	—	0	1
		21	NH_2	CH_2	OH	OH	2	0	3	1	—	1	1
		22	$NH(CH_2)_2CH_3$	CH_2	OH	OH	0	0	2	0	—	0	0
		23	$NH(CH_2)_7CH_3$	CH_2	OH	OH	0	0	2	0	—	0	0
		24	$NH\cdot C_6H_4$-p-CH_3	CH_2	OH	OH	0	—	0	—	—	—	—
		25	$NH\cdot C_6H_4$-p-$N(CH_3)_2$	CH_2	OH	OH	1	1	0	2	—	2	2
		26	$S—C_6H_5$	CH_2	OH	OH	2	2	0	1	—	1	1
		27	$S—CH_2C_6H_5$	CH_2	OH	OH	2	3	0	2	—	2	2
		28	OCH_3	CH_2	H	OH	2	2	4	0	1	—	0
 (VI)		29					0	0	0	0	—	0	0

TABLE 1.—*continued*

Compound		Code number	Substituents R[1]	R[2]	R[3]	R[4]	Dwarf pea Shoot growth	Lettuce seedlings Hypocotyl growth	Cucumber seedlings Hypocotyl growth	Maize mutant dwarfs Leaf-sheath Extension Mutant *d-1*	Mutant *d-2*	Mutant *d-3*	Mutant *d-5*
 (VII)		30					2	1	—	—	—	—	—
 (VIII)	(Gibberellin A_6)	31	OH				2	2	3	4	—	4	4
	(Gibberellin A_{11})	32*	H				0	—	3	—	—	—	—
 (IX)	(Gibberellin A_8)	33	OH				1	0	0	1	—	1	1
		34	OCH_3				1	0	0	0	0	—	0
 (X)		35	CH_3				0	0	0	—	—	—	—
		36	epi CH_3				2	0	1	1	—	1	1
 (XI)		37	OH				5	5	5	5	—	5	5
		38	H				3	3	7	4	—	4	4

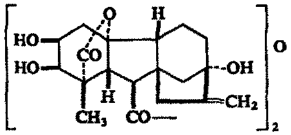
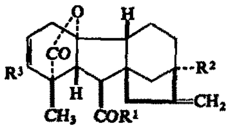
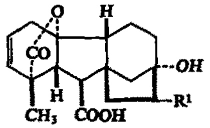
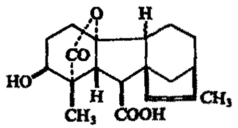
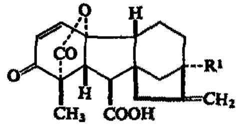
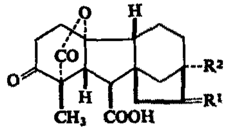
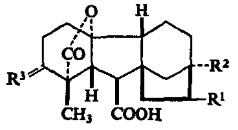
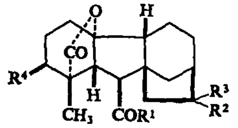
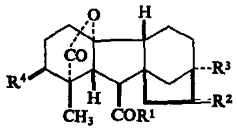
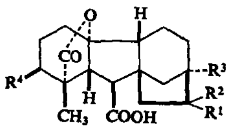
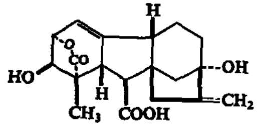
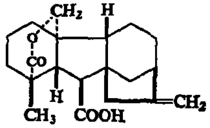
 (XII)		39				1	0	1	0	—	1	1
 (XIII)	(Gibberellin A_5)	40	OH	OH	H	3	2	3	2	—	6	6
		41	OCH_3	OH	H	1	0	3	0	1	—	0
		42	OH	OH	$COCH_3$	0	0	0	0	—	0	0
		43	OH	H	H	3	4	5	3	—	5	5
 (XIV)		44	CH_3			2	2	1	1	—	0	1
		45	epi CH_3			3	3	1	1	—	1	1
 (XV)		46				3	5	7	5	5	—	5
 (XVI)		47	OH			0	1	0	0	—	0	0
		48	H			2	2	7	4	3	—	3
 (XVII)		49	CH_2	OH		3	2	0	3	3	—	3
		50	CH_2	H		3	4	7	5	5	—	5
		51	$CH \cdot CH_3$	H		0	2	7	2	—	2	2

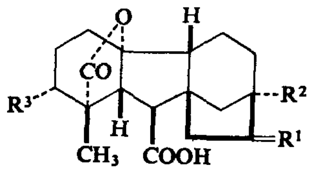
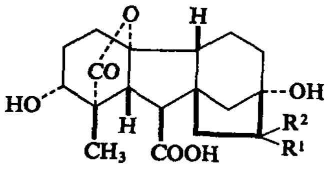
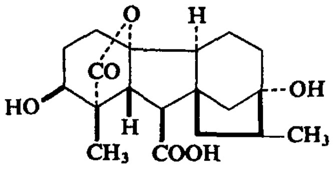
TABLE 1.—*continued*

Compound		Code number	Substituents R¹	Substituents R²	Substituents R³	Substituents R⁴	Dwarf pea Shoot growth	Lettuce seedlings Hypocotyl growth	Cucumber seedlings Hypocotyl growth	Maize mutant dwarfs Leaf-sheath Extension Mutant d-1	Maize mutant dwarfs Leaf-sheath Extension Mutant d-2	Maize mutant dwarfs Leaf-sheath Extension Mutant d-3	Maize mutant dwarfs Leaf-sheath Extension Mutant d-5
 (XVIII)		52	CH_3	OH	O		2	1	1	1	1	—	1
		53	epi CH_3	OH	O		3	2	5	0	—	0	0
		54	epi CH_3	OH	N OH		0	1	0	—	—	—	—
		55	CH_3 (mixed epimers)	H	O		3	4	7	3	0	—	1
		56	CH_3 (mixed epimers)	H	N·OH		1	2	7	2	2	—	2
 (XIX)	(Gibberellin A_2)	57	OH	CH_3	OH	OH	2	2	6	2	—	2	2
		58	OCH_3	CH_3	OH	OH	0	0	0	0	0	—	0
		59	OH	OH	CH_3	OH	3	3	7	1	1	—	1
	(Gibberellin A_{10})	60	OH	CH_3	OH	H	0	3	5	1	—	4	4
 (XX)	(Gibberellin A_1)	61	OH	CH_2	OH	OH	4	2	6	4	—	4	4
		62	OCH_3	CH_2	OH	OH	0	1	3	1	0	—	0
		63	OH	O	OH	OH	2	2	3	2	—	2	2
	(Gibberellin A_4)	64	OH	CH_2	H	OH	2	4	7	4	—	5	4
		65	OH	CH_2	H	OSO_2CH_3	2	2	7	1	—	2	1
		66	OCH_3	CH_2	H	OH	0	2	5	3	2	—	2
	(Gibberellin A_9)	67	OH	CH_2	H	H	1	4	7	1	—	6	4
		68	OCH_3	CH_2	H	H	0	1	5	0	2	0	1
		69	OH	O	H	OH	2	3	7	2	—	2	2
		70	OH	O	H	H	0	2	5	0	—	2	2
 (XXI)		71	CH_3	H	OH	OH	4	4	3	3	—	3	3
		72	H	epi CH_3	OH	OH	4	4	5	2	—	2	2
		73	CH_3	H	OH	H	2	2	1	0	—	1	1
		74	H	epi CH_3	OH	H	2	3	1	1	1	1	1
		75	CH_3 and H (mixed 8-epimers)	H and CH_3	H	OH	4	4	7	5	5	—	4
		76	CH_3 and H (mixed 8-epimers)	H and CH_3	H	H	2	3	7	1	—	2	2
		77	OH or H (mixed 8-epimers)	H or OH	H	H	0	1	3	0	—	2	2
		78	H	H	H	H	2	2	7	4	—	5	3

 (XXII)		79					3	2	
 (XXIII)	(Gibberellin A_{15})	80	1	1	5	1	—	5	5

* See footnote on page 1476

Table 2. Lactonic gibbane derivatives with altered configuration at 2, 4b, 7, 9a

Compound	Code number	Substituents			Dwarf pea Shoot growth	Lettuce seedlings	Cucumber seedlings	Maize mutant dwarfs Leaf-sheath Extension			
		R^1	R^2	R^3		Hypocotyl growth		Mutant *d*-1	Mutant *d*-1	Mutant *d*-1	Mutant *d*-1
 (XXIV)	81	CH_2	OH	OH	1	0	0	2	2	—	3
	82	CH_2	H	OH	1	2	7	3	—	3	3
	83	$CH.CH_3$	H	OH	1	2	7	2	—	3	3
 (XXV)	84	CH_3	H		0	0	0	—	—	—	—
	85	H	epi CH_3		2	1	2	0	—	1	1
 (XXVI)	86				—	1	—	3	—	3	3

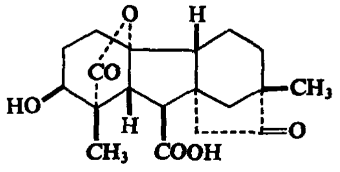
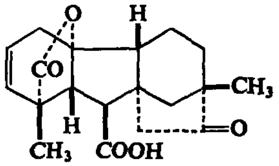
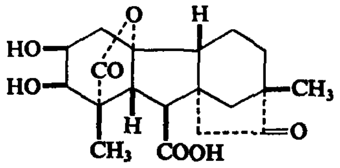
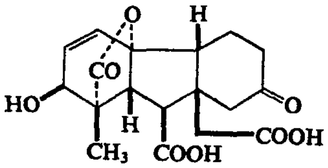
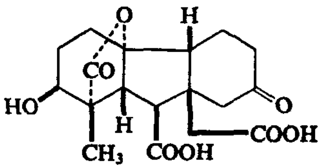
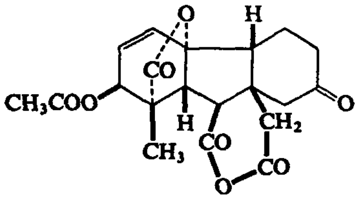
 (XXVII)	87	1	1	2	2	2	0	2
 (XXVIII)	88	1	1	0	0	—	0	0
 (XXIX)	89	1	1	0	0	—	0	0

TABLE 3. LACTONIC SECO DERIVATIVES LACKING RINGS A OR D

Compound	Code number	Dwarf pea Shoot growth	Lettuce seedlings Hypocotyl growth	Cucumber seedlings Hypocotyl growth	Maize mutant dwarfs Leaf-sheath Extension Mutant *d*-1	Mutant *d*-2	Mutant *d*-3	Mutant *d*-5
 (XXX)	90	0	0	0	0	0	—	0
 (XXXI)	91	0	0	0	0	0	—	0
 (XXXII)	92	0	0	—	—	—	—	—

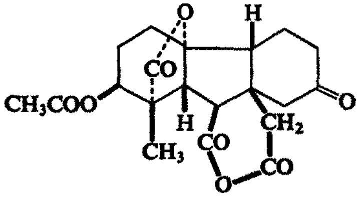
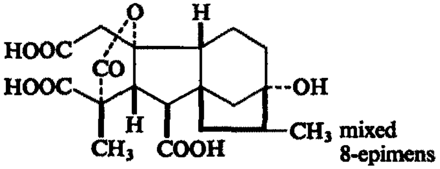
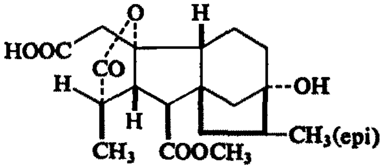
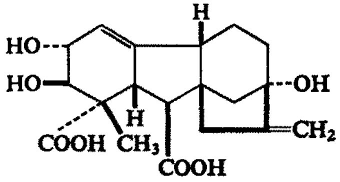
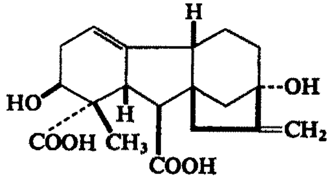
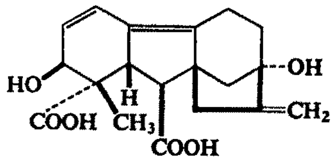
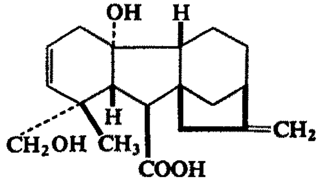
 (XXXIII)	93	0	—	—	—	—	—	—
 (XXXIV)	94	0	1	1	0	—	0	0
 (XXXV)	95	0	1	0	—	—	—	—

TABLE 4. NON-LACTONIC GIBBANE DERIVATIVES

Compound	Code number	Substituents R^1	Substituents R^2	Substituents R^3	Dwarf pea Shoot growth	Lettuce seedlings Hypocotyl growth	Cucumber seedlings Hypocotyl growth	Maize mutant dwarfs Leaf-sheath Extension Mutant d-1	Mutant d-2	Mutant d-3	Mutant d-5
 (XXXVI)	96				2	2	3	2	3	—	3
 (XXXVII)	97				0	0	0	0	—	0	0
 (XXXVIII)	98				0	0	0	—	—	—	—
 (XXXIX)	99				1	0	0	0	—	0	0

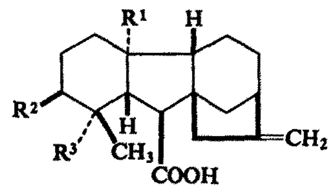
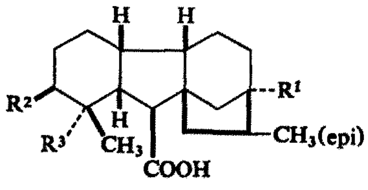
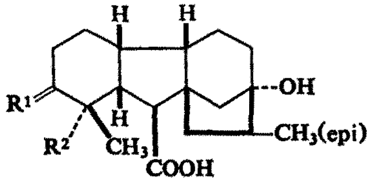
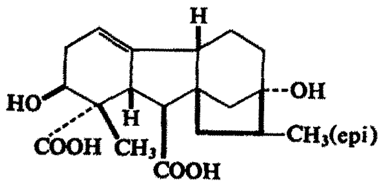
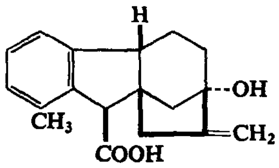
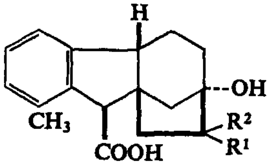
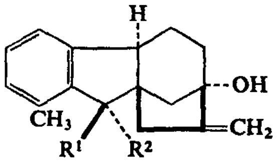
 (XL)	(Gibberellin A_{13}) (Gibberellin A_{14}) (Gibberellin A_{12})	100 101 102 103	OH COOH CH_3 CH_3	H OH OH H	CH_2OH COOH COOH COOH	0 0 3 —	0 0 1 —	2 2 1 —	0 2 1 0	— — — —	0 0 3 3	0 0 1 3
 (XLI)		104 105 106	OH $OCOCH_3$ OH	OH $OCOCH_3$ H	COOH COOH H	0 0 0	0 0 0	0 0 0	0 0 0	0 0 0	— — —	0 0 0
 (XLII)		107 108 109	O O $S—CH_2$ | $S—CH_2$	COOH H H		0 0 0	0 0 0	0 0 0	0 0 —	0 0 —	— — —	0 0 0
 (XLIII)		110				0	1	0	0	0	—	0

TABLE 5. DERIVATIVES WITH AN AROMATIC RING A

Compound		Code number	Substituents R[1]	Substituents R[2]	Dwarf pea Shoot growth	Lettuce seedlings Hypocotyl growth	Cucumber seedlings Hypocotyl growth	Maize mutant dwarfs Leaf-sheath Extension Mutant *d-1*	Mutant *d-2*	Mutant *d-3*	Mutant *d-5*
 (XLIV)	(Epiallogibberic acid)	111			0	0	0	0	0	0	0
 (XLV)		112	CH_3	H	—	—	—	0	0	—	0
		113	H	epi CH_3	0	0	0	0	0	—	0
 (XLVI)	(Allogibberic acid)	114	COOH	H	0	0	0	3	3	3	3
		115	H	COOH	0	0	2	0	—	0	0

94

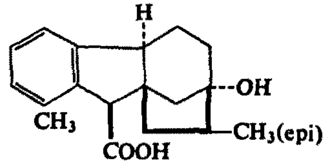
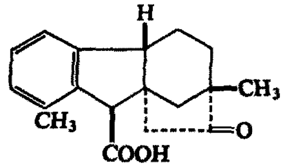
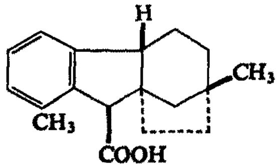
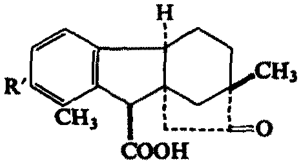
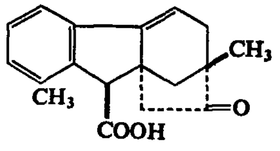
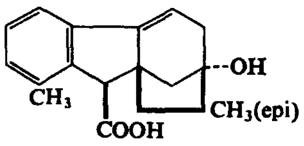
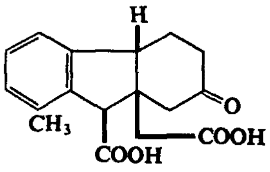
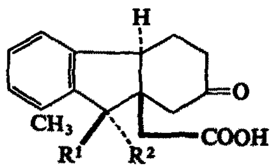
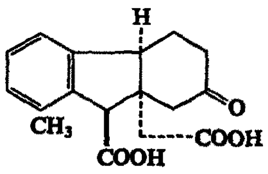
 (XLVII)		*116*		0	0	0	0	0	0	0
 (XLVIII)	(Epigibberic acid)	117		0	0	0	0	0	—	0
 (XLIX)		118		0	0	0	0	0	—	0
 (L)	(Gibberic acid)	119	H	0	0	0	0	0	—	0
		120	NH_2	0	0	0	0	0	—	0
		121	OH	0	0	0	0	0	—	0
		122	NO_2	0	0	0	0	0	—	0
		123	Cl	0	0	0	0	0	—	0

Table 5.—*continued*

Compound	Code number	Substituents R[1]	Substituents R[2]	Dwarf pea Shoot growth	Lettuce seedlings Hypocotyl growth	Cucumber seedlings Hypocotyl growth	Maize mutant dwarfs Leaf-sheath Extension Mutant *d*-1	Mutant *d*-2	Mutant *d*-3	Mutant *d*-5
 (LI)	124			0	0	0	0	0	—	0
 (LII)	125			1	0	0	0	0	—	0
 (LIII)	126			0	0	—	0	0	—	0

 (LIV)	127	COOH	H	0	0	—	0	0	—	0
	128	H	COOH	0	0	—	—	—	—	—
 (LV)	129			0	0	—	—	—	—	—

slow metabolic conversion into active analogues, it is uncertain if the activity was always "real" or was due to traces of an active impurity. An example is the 7α-gibbane (XXVII, 87, Table 2) which has been reported[3] to show substantial activity on rice seedlings. Initially our material showed slight activity which, on further purification of the material, progressively diminished without quite disappearing. This residual activity may not be real activity.

Grade	*Potency (Gibberellic acid = 100)*
0	Inactive
1	<1
2	1–9
3	*ca.* 10
4	>10, <100
5	*ca.* 100
6	>100, <1000
7	1000 and higher

RESULTS

(i) *General Conclusions*

As judged by our four assays, no one particular feature of the active compounds could be regarded as essential for activity, but there appeared to be requirements for the higher grades of activity.

(a) *The ring system.* The highly active compounds contained an intact gibbane ring system. Fission of rings A (94, 95) or D (90, 93) gave inactive products (Table 3). Compounds (94) and (95) lacked the exocyclic methylene group at position 8, but loss of unsaturation at this position does not in itself destroy activity (e.g. the tetrahydrogibberellic acids, 71 and 72, Table 1). Three synthetic lactonic analogues (130–132) of ring A were inactive, as was a lactone (133) formally related to ring B (Table 6).

No compound with an aromatic ring A (Table 5), or with the normal bi-, tri-, or tetracyclic diterpenoid ring system (Table 6) showed high activity in our assays. With one exception, aromatization of ring A destroyed the activity. Highly purified allogibberic acid (114) showed some activity on maize mutants whereas its 10-epimer (115) and 4b-epimer (epiallogibberic acid, 111) showed little or no activity in any assay. Allogibberic acid has been reported to be active on maize mutants[8] and also in other test systems,[4,7,17] but rigorous purification of this compound is difficult.[18]

A lactone ring is absent from gibberellins A_{12}–A_{14} (101–103, Table 4) and is no longer regarded as essential for activity, but the most active compounds contained it, The possibility that the activity of gibberellins A_{12}–A_{14} is due to metabolic conversion to lactones is not excluded. A similar argument may be applied to the non-lactonic compound (96) (which, in agreement with Sembdner *et al.*,[8] we found to possess slight activity, Table 4), but cannot easily be applied to allogibberic acid. Although gibb-4-ene- and gibb-4a(4b)-ene-1α-carboxylic acids are readily relactonized, compounds (97) and (98) were inactive (Table 4). Sembdner *et al.*[8] found compound (98) to have moderate activity against maize.

[17] F. LONA, *L'Ateneo Parmense* **33**, *Suppl.* **6**, 1 (1962).
[18] P. W. BRIAN, JOHN FREDERICK GROVE, H. G. HEMMING, T. P. C. MULHOLLAND and MARGARET RADLEY, *Plant Physiol.* **5**, 329 (1958).

TABLE 6. MISCELLANEOUS COMPOUNDS

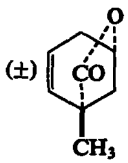
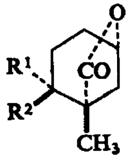
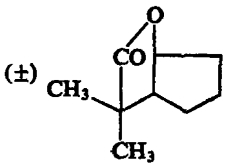
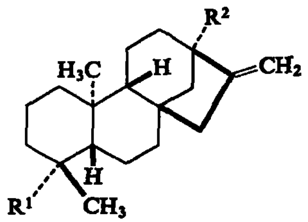
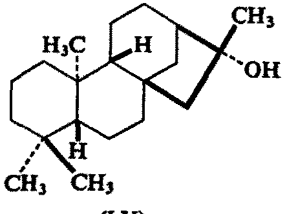
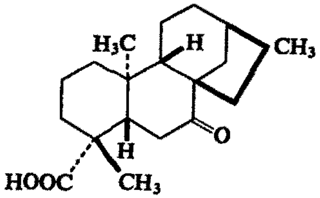
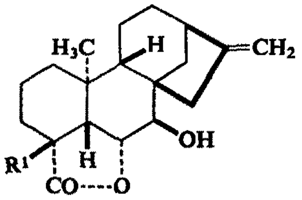
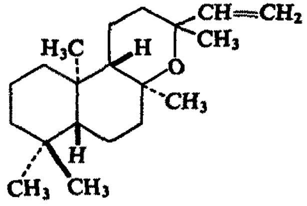
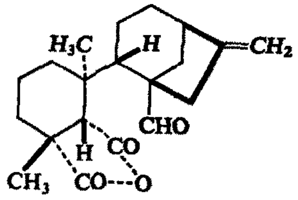
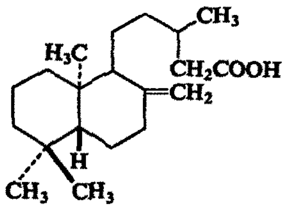
Compound		Code number	Substituents R¹	Substituents R²	Dwarf pea Shoot growth	Lettuce seedlings Hypocotyl growth	Cucumber seedlings Hypocotyl growth	Maize mutant dwarfs Leaf-sheath Extension Mutant d-1	Mutant d-2	Mutant d-3	Mutant d-5
 (LVI)		130			0	0	0	0	—	0	0
 (LVII)		131	OH	H	0	0	0	0	—	0	0
		132	H	OH	0	—	0	0	—	0	0
 (LVIII)		133			0	0	0	0	—	0	0
 (LIX)	[(−)-Kaurene]	134	CH_3	H	0	0	0	0	—	0	0
	(Steviol)	135	COOH	OH	0	0	0	0	—	0	1
		136	COCH	H	0	0	0	0	—	0	1

TABLE 6.—*continued*

Compound	Code number	Substituents R¹	Substituents R²	Dwarf pea Shoot growth	Lettuce seedlings Hypocotyl growth	Cucumber seedlings Hypocotyl growth	Maize mutant dwarfs Leaf-sheath Extension Mutant *d*-1	Mutant *d*-2	Mutant *d*-3	Mutant *d*-5
 (LX)	137			0	1	0	0	—	0	0
 (LXI)	138			0	0	0	0	0	—	0
 (LXII)	139	CH_3		0	0	0	0	0	—	0
	140	CH_2OH		—	—	—	0	0	—	0

 (LXIII)		141	—	—	—	0	0	—	0
 (LXIV)		142	—	—	—	0	0	—	0
 (LXV)	(Eperuic acid)	143	0	0	0	0	0	—	0

In two examples (99, 100) reductive fission of the lactone ring almost destroyed the activity. Some activity was retained by the 1→3-lactones (79) and (32) and the 6-ring lactone gibberellin A_{15} (80). The inactivity of compounds (99) and (100) possibly reflects inability of the semi-synthetic non-lactonic gibbanes, as opposed to the non-lactonic gibberellins, to serve as precursors of more active products.

It was confirmed (cf. refs.[19, 20]) that steviol (135) is slightly active on maize mutant *d*-5. Steviol is known[21] to be converted into a gibberellin-like compound by *G. fujikuroi*, and it is conceivable that a similar process might occur in plants. On the other hand (−)-kaurene (134), a known precursor of gibberellic acid[22] was inactive (Table 6), as were the 7-oxokauran-19-oic acid (138) and the kauranolides (139–140). (−)-Kaur-16-en-19-oic acid (136) showed activity similar to that of steviol, in agreement with Katsumi *et al.*,[23] who also found activity in (−)-kaur-16-en-19-ol. (−)-Kauran-16-ol (137) appeared to show a trace of activity in the lettuce hypocotyl assay at the highest concentration (10 ppm) tested.

(b) *Configuration.* The steric complexity of the gibberellins suggests that alteration of the configuration might be expected to have an important effect, particularly as the shape of the molecule is altered by epimerization at ring junctions. Some epimers are listed in Table 2. Inversion of ring D as in the 7α-gibbanes (87–89) almost destroyed the activity. Only one 4b-epimer was available for testing; this (86) retained some activity (cf. compound 72). The available 4aβ-gibbanes (Table 4) were inactive, but lacked the lactone bridge.

The effect of epimerizing a 2-hydroxyl group varied (see below). In an aqueous medium 2α-hydroxygibbane 1→4a lactones are in equilibrium with a small amount of the 2β-epimers and the activity shown by 2α-hydroxy compounds could arise in this way.

Epimerization at position 8 usually had little effect on the activity (*e.g.* gibberellin A_2, 57 and its 8-epimer, 59; and the 8-methyl compounds (44, 45) and 71, 72) (Table 1).

(c) *Substituents.* In our assays the presence of a free 10-carboxyl group or of the corresponding acid anhydride (e.g. 37–38) which readily yields the free 10-carboxylic acid in aqueous media, was essential for high activity in all the assays. Although gibberellins A_1–A_9 vary in acid strength,[24] it seems unlikely that the relatively small variation has a major effect on specificity. Neutral derivatives (e.g. 11–28, Table 1) were of low activity compared with the parent gibberellin.[2]

One example (46) of an 8-methyl-8,9-gibbene had similar activity to the isomeric gibberellin A_4 (64) with an 8-methylene group.

General rules could not be established for the relation between substituents at positions 2, 7, and 8, and biological activity, since some specificity of action in the various tests seemed to be associated with these substituents (see below).

Although hydroxyl groups were important in this respect, acylation of these groupings caused little change in activity. It seems likely, therefore, that the acyl groups undergo hydrolytic fission at an early stage in the movement of these compounds to the site of action.

(ii) *Individual Assays*

(a) *Pea stem growth.* Gibberellic acid (1) and some *O*-acyl derivatives (2–6) remain the most active compounds tested in this assay. Modification of the substitution pattern at

[19] M. RUDDATT, A. LANG and E. MOSETTIG, *Naturwiss.* **50**, 23 (1963).
[20] J. P. NITSCH and C. NITSCH, *Ann. Physiol. Végetale* **7**, 259 (1966).
[21] M. RUDDATT, E. HEFTMANN and A. LANG, *Arch. Biochem. Biophys.* **111**, 187 (1965).
[22] B. E. CROSS, R. H. B. GALT and J. R. HANSON, *J. Chem. Soc.* 2944 (1963).
[23] M. KATSUMI, B. O. PHINNEY, P. R. JEFFERIES and C. A. HENRICK, *Science* **144**, 849 (1964).
[24] B. K. TIDD, *J. Chem. Soc.* 1521 (1964).

positions 2, 3, 7, and 8, or of the degree of unsaturation, led to diminished activity. High activity was retained by gibberellin A_7 (9), lacking the 7-hydroxyl group, by gibberellin A_1 (61), and the 8-epimeric tetrahydro-derivatives (71, 72) of gibberellic acid which lacked respectively one and both olefinic bonds.

Other modifications of the gibberellic acid structure reduced the activity further. Some resulted in almost complete inactivity (e.g. the 8-spiro epoxides, 29–30); in other cases the residual activity appeared to be roughly the result of a balance of favourable and unfavourable factors involving the four positions mentioned above.

Retention of moderate activity (grade 3) was compatible with the presence of a saturated ring A, a 2, 3-olefinic bond (40, 43) or a 2, 3-epoxide ring (31). A 2-acetyl derivative (8), the 2-oximes (54, 56), compounds with a 2α-hydroxyl group (81, 84, 85), or with additional hydroxyl group at position 3 (33) showed little or no activity (Table 1). 8-Epitetrahydrogibberellin A_5 (74), unsubstituted at position 2, was active, but most derivatives lacking the 2β-hydroxyl group, and particularly those (67, 76, 78) lacking both hydroxyl groups, showed little activity in this assay. Some 2-oxo-derivatives (48–50, 53) were active, though the 2-oxo-derivative (47) of gibberellic acid itself was virtually inactive.

At a moderate level of activity (grade 3) the presence of a 7-hydroxyl group was often beneficial to activity (cf. 57, 60), but was not always so (47–48).

At position 8, reduction as in the tetrahydro-derivatives (71–72) of gibberellic acid and other compounds (e.g. 45, 55, 75), or hydration (57, 60) of the methylene group, was compatible with activity. Two 8-epoxides (29, 30), the 8-nor-ketones (7, 63) and an 8-ethylidene analogue (51) showed very little activity.

(b) *Lettuce hypocotyl growth.* Brian *et al.*[14] have shown that gibberellins A_1–A_9 show a different order of activity in this test as compared with that in the growth of pea stems. Only gibberellin A_7 (9) and its *O*-acetate (10) were more active than gibberellic acid. Comparison of several pairs of compounds with, and without, a 7-hydroxyl group shows that the presence of this group is generally, but not always, disadvantageous. The effect was much less marked than in the cucumber hypocotyl assay (below).

A 2-hydroxyl group was present in most of the more active compounds; its removal from some less active analogues had little effect on the activity.

The substitution pattern at position 8 seemed to be somewhat less critical than in the pea-stem assay, but no striking differences between activities in the two assays were noted.

(c) *Cucumber hypocotyl growth.* We have confirmed that in this assay high activity is found in, and so far is limited to, the 7-deoxylactonic gibberellins (A_4, A_7, A_9) and their derivatives. A few such derivatives (e.g. 43, 77) were no more active than gibberellic acid, but the majority were highly active.

It was noticeable that in the 7-deoxy series, modification of the substitution pattern usually had less effect on the activity than in the other assays. Thus, the 2α-epimer (82) of gibberellin A_4, dihydrogibberellin A_4 (75), an 8-nor-ketone (69), a 2-oxime (56), and 8-desmethylene gibberellin A_9 (78) retained activity greater than that of gibberellic acid (Table 1). Several 2-oxo-derivatives appeared to be particularly active.

(d) *Dwarf maize leaf-sheath growth.* In this assay the presence of a 7-hydroxyl group did not have a major effect on the activity of active gibbanes, but the substitution pattern at position 8 was important. High activity on at least one mutant was usually associated with unsaturation at this position, either endocyclic (46) or exocyclic; though the 8-unsubstituted compound (78) was also active. Most compounds with 8-methyl groups (e.g. dihydrogibberellin A_9, 76) showed low activity on any mutant (Table 1).

Three gibberellins, A_5 (40), A_9 (67)[14, 25–27] and Bamboo gibberellin (I)[11] have been reported to show markedly less growth-promoting activity on mutant *d*-1 than on other mutants. It has been suggested[27] that this type of specificity may be due to a metabolic block in mutant *d*-1, resulting in inability to utilize 2-unsubstituted gibberellins.

In our series the effect of altering the substitution pattern at position 2, though clearly important, was not clear-cut. Like dihydrogibberellin A_9 (76), the 8-epimeric dihydrogibberellins A_5 (44–45) were almost inactive on all the mutants, presumably owing to lack of unsaturation at position 8 (Table 1). Some other 2-unsubstituted gibbanes (43, 60, 80, Table 1; 103, Table 4) were least active on mutant *d*-1; the last (gibberellin A_{12}) has been shown[28] to be capable of serving as a biogenetic source of gibberellic acid, at least in *G. fujikuroi*. One 2-αhydroxygibbane (83, Table 2) also seemed to be least active on mutant *d*-1, whereas a 2-oxogibbane (55, Table 1) and a 2β-hydroxygibbane (101, gibberellin A_{13}, Table 4) were more active on mutant *d*-1 than on other mutants.

DISCUSSION

The mechanism of gibberellin activity at a molecular level is incompletely understood, but results are consistent with the theory that the gibberellins may bind to specific receptor sites. Intrinsic biological activity is then controlled by chemical and steric factors, and, since the compound must first reach the site of action, is influenced by physical factors such as lipoid solubility. The high water solubility of the gibberellins is ascribed to the 10-carboxylate anion, a feature common to all. The oil-water partition ratios of the anions must nevertheless be influenced by the substituents, particularly hydroxyl or potential hydroxyl substituents, and will be at a minimum in the almost inactive trihydroxygibbanes. Some portion of the plant specificity effects may be attributed to this factor.

Additional factors likely to affect the activity and specificity of the exogeneous gibbanes are their varying stability *in vivo*, and the possibility that they may act wholly, or partly, by releasing active endogenous gibberellin bound to non-specific receptors. This, and related effects, will be at a maximum if the exogenous gibbane is identical with an endogenous gibberellin of the test plant.

Derivatives of helminthosporol (IV) show some gibberellin-like activity[20, 29, 30] and this has led Briggs[30] to suggest that in gibberellins rings C and D may form an "effector" part and rings A and B an "affinity" part of the molecule. This seems too simplified a view since it appears that the shape and size of the molecule, and the nature of the substituents, considered as a whole, control activity.

The only chemically reactive groupings which have yet been shown to be essential for high activity are the 10-carboxyl group which may bind to a receptor, and possibly a lactone ring. The biological activity of some non-lactonic gibbanes may well be a result of preliminary conversion to lactones *in vivo*. though it is difficult to explain the activity of allogibberic acid on this basis. The final assessment of the latter compound on plant growth may have to await the availability, in quantity, of synthetic material.

The varying activity of the gibbane derivatives differently substituted at positions other

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than 10 may be due mainly to steric factors. The most clear-cut example remains the specificity resulting from changes at position 7; in this connection it would be of interest to test gibbanes with substituents other than hydrogen or hydroxyl at this position.

On the other hand the effects of alterations to the ring A substitution pattern are not wholly due to steric reasons. The effect of epimerizing a 2-hydroxyl group may be due to the change in molecular shape, but the failure of maize mutant *d*-1 to metabolize effectively several 2-unsubstituted gibbanes is most easily explained in terms of failure to convert these compounds into active products.

EXPERIMENTAL

Compounds tested were purified to analytical standard and have been described in the literature, with the exception of compounds (32),[31] (88–89),[32] and (120–123).[33] Compound (46) contained[34] a small amount of dihydrogibberellin A_4.

Many acidic compounds were further purified, when appropriate, by chromatography on Celite buffered at pH 6·2. An example is described below. The method separates gibbane derivatives containing one or no alcoholic hydroxyl groups from those (e.g. gibberellic acid) containing two. The latter are not eluted with light petroleum or chloroform.

Allogibberic acid (by D. C. Aldridge) The acid (3·0 g) was deposited in acetone on a small amount of "Hyflo Super Cel" Celite and the dried Celite was placed on a column of Celite (300 g) previously mixed with a 2 M phosphate buffer[35] of pH 6·2 (300 ml), and made up in chloroform-light petroleum (b.p. 60–80°) (2:1). Elution of the column with chloroform slowly removed allogibberic acid. When part (1·5 g) had been recovered it was crystallized from ethyl acetate-light petroleum and from benzene-methanol, giving plates (1·0 g) m.p. 199–201°. No gibberellic acid or gibberellin A_1 was detected in this material by TLC (Kieselguhr, benzene-acetic acid-water [8:3:5]).

Activity grades for gibberellins A_1–A_9 are adapted from published values.[14b]

Bioassays (gibberellic acid as control) were carried out as described by Brian *et al.*,[14] using ten-fold dilutions. (i) Dwarf Pea (Dwarf Cultivar Meteor); shoot growth (10·0, 1·0, 0·1 μg/plant), applied to the first true leaf. (ii) Lettuce hypocotyl growth (Cultivar Arctic King); five concentrations, (10·0→0·001 μg/ml). (iii) Cucumber hypocotyl growth (Cultivar Perfection Ridge); 10·0, 1·0, 0·1 μg/plant. (iv) Dwarf maize, leaf-sheath growth (mutants *d*-1, *d*-2, *d*-3, *d*-5); 10·0, 1·0, 0·1 μg/plant.

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