THE GIBBERELLINS

By JOHN FREDERICK GROVE

(IMPERIAL CHEMICAL INDUSTRIES LIMITED, AKERS RESEARCH LABORATORIES, THE FRYTHE, WELWYN, HERTS.)

1. Fungal gibberellins

THE discovery of the gibberellins originated from an investigation of a soil-borne disease of rice caused by the fungus *Gibberella fujikuroi*. Infected plants eventually wilt and die, but at an early stage of the disease, called "bakanae" in Japan, the leaves and stems of some seedlings elongate more rapidly than those of healthy plants. In 1926 Kurosawa[1] showed that cell-free filtrates from cultures of the fungus produced in healthy seedlings the elongation symptons characteristic of the disease, and, eventually, in 1938 Yabuta and his collaborators[2] succeeded in isolating from such culture fluids a crystalline active material which they named gibberellin A. The chemistry and plant-growth promoting properties of this material were described in a series of papers from the University of Tokyo: these papers have been re-assembled and reviewed.[3]

More recently, in an attempt to repeat the isolation of gibberellin A from cultures of *G. fujikuroi*,[4] a group of workers at the Akers Research Laboratories of Imperial Chemical Industries Limited obtained a new active metabolite, gibberellic acid,[4] which differed from gibberellin A in its physical and chemical properties. The same compound was also isolated at the North Regional Research Laboratories, Peoria, U.S.A., where[5] it was named gibberellin X until the identity with gibberellic acid was established.[6] The American workers obtained a mixture of two compounds, gibberellic acid and gibberellin A_1, which was similar to gibberellin A. These results led to a re-examination of the crude gibberellin produced by various Tokyo University strains of *G. fujikuroi*, and gibberellic acid (gibberellin A_3), gibberellin A_1, and a third active metabolite, gibberellin A_2, were eventually obtained.[7] Gibberellin A_4, another component of the mixture, was described later,[8] and the exact composition of the gibberellin A obtained in 1938 remains obscure. Modification of the fermentation conditions used to produce gibberellic acid has led to the isolation by the

[1] Kurosawa, *Trans. Nat. Hist. Soc. Formosa*, 1926, **16**, 213.
[2] Yabuta and Sumiki, *J. Agric. Chem. Soc. Japan*, 1938, **14**, 1526; Yabuta and Hayashi, *J. Agric. Chem. Soc. Japan*, 1939, **15**, 257.
[3] Stodola, "Source Book on Gibberellin, 1828-1957," U.S. Dept. of Agriculture, 1958.
[4] Curtis and Cross, *Chem. and Ind.*, 1954, 1066.
[5] Stodola, Raper, Fennell, Conway, Sohns, Langford, and Jackson, *Arch. Biochem.*, 1955, **54**, 240; Stodola, Nelson, and Spence, *Arch. Biochem.*, 1957, **66**, 438.
[6] Cross, *J.*, 1954, 4670.
[7] Takahashi, Kitamura, Kawarada, Seta, Takai, Tamura, and Sumiki, *Bull. Agric. Chem. Soc. Japan*, 1955, **19**, 267.
[8] Takahashi, Seta, Kitamura, and Sumiki, *Bull. Agric. Chem. Soc. Japan*, 1957, **21**, 396

group at Imperial Chemical Industries Limited of gibberellin A_7[9] and gibberellin A_9.[10]

Gibberellic acid has been produced in much greater yield[11] than the other gibberellins, and a more extensive investigation of its chemistry and biological activity has been carried out than was possible with gibberellin A. The structure and some of the stereochemistry of gibberellic acid has been elucidated and the structures of the other gibberellins have been related to gibberellic acid. This contribution from the group at Imperial Chemical Industries Limited has been reviewed in a comprehensive treatise.[12]*

Despite widespread searches there is no well-authenticated example of the production of a gibberellin by a fungus other than *G. fujikuroi.*

2. Occurrence of gibberellins in higher plants

The fungal gibberellins were found to promote many normal processes of plant-growth development, and this led to the discovery that compounds similar in chemical structure and physiological properties were widely distributed in higher plants. Mitchell, Skaggs, and Anderson[13] had shown in 1951 that ether extracts of immature dwarf-bean seeds contained a substance which stimulated the elongation of seedling epicotyls to a degree much exceeding that observed after auxin application—an effect now recognised as gibberellin-like. More recently, many workers[14–16] have obtained similar extracts from the seed, roots, and shoots of a wide variety of plants.† From immature seed of the runner bean, *Phaseolus multiflorus*, MacMillan and his collaborators[17] isolated gibberellin A_1 in a yield of 2 mg./kg. fresh weight of seed together with three new compounds, gibberellins A_5,[17] A_6,[18] and A_8.[18] West and Phinney[19] have also obtained gibberellins A_1 and A_5 (bean factor II) from seed of the French bean *Phaseolus vulgaris*, and gibberellin A_1 has been isolated from young shoots of *Citrus reticulata Blanco var. unshiu.*[20] The presence of gibberellic acid

* When this treatise was written all the available evidence favoured a *trans*-fusion of rings A/B, antipodal to that normally found in diterpenes, and an α-orientation for the ring A lactone bridge: the latter is now believed to be β-oriented (see Section 6) but no ring A stereochemistry has been adopted here since no definite conclusions have been reached.

† For a more extensive bibliography see references 12 and 23.

[9] Cross, Galt, and Hanson, *Tetrahedron Letters*, 1960, No. 15, 18.

[10] Cross, Galt and Hanson, *Tetrahedron Letters*, 1960, No. 23, 22.

[11] Brit. P. 838033.

[12] Brian, Grove, and MacMillan, *Progr. Chem. Org. Nat. Prod.*, 1960, **18**, 350.

[13] Mitchell, Skaggs, and Anderson, *Science*, 1951, **114**, 159.

[14] Phinney, West, Ritzel, and Neely, *Proc. Nat. Acad. Sci. U.S.A.*, 1957, **43**, 398.

[15] Radley, *Nature*, 1956, **178**, 1070; *Ann. Bot.*, 1958, **22**, 297.

[16] Lona, *L'Alteneo Parmense*, 1957, **28**, 111.

[17] (*a*) MacMillan and Suter, *Naturwiss.*, 1958, **45**, 46; (*b*) MacMillan, Seaton, and Suter, *Proc. Chem. Soc.*, 1959, 323; (*c*) *Tetrahedron*, 1960, **11**, 60.

[18] MacMillan, Seaton, and Suter, unpublished work.

[19] West and Phinney, *J. Amer. Chem. Soc.*, 1959, **81**, 2424.

[20] Kawarada and Sumiki, *Bull. Agric. Chem. Soc. Japan*, 1959, **23**, 343.

in green malt[21] and in several higher plants[22] has also been claimed on the basis of chromatographic evidence, but the isolation of gibberellic acid from these sources has still to be achieved.

3. The significance of the gibberellins in plant physiology

The discovery of the gibberellins in plant tissues implies that they are natural hormones with a regulating function in many aspects of plant growth and development. Only very small quantities of some gibberellins are available and little biological work has been done with them; but so far as is known they all have a qualitatively similar action. In most circumstances gibberellic acid is the most active, followed by gibberellin A_1.[24] Exceptionally, members of the Cucurbitaceae respond most readily to gibberellins A_4 and A_7.[25]

In the simple case of plants whose growth is not rigorously determined by day-length or temperature, a gibberellin increases the length of the stem internodes without altering their number and only those internodes actually extending at the time of application are affected.[26] In bushy plants a gibberellin enhances apical dominance.[27] In general, genetically dwarf plants show the greatest response and treated plants are similar to the tall varieties. Increases in both cell-division[28] and cell-size[27] are involved in gibberellin-induced stem-extension.

In the more complex case of plants whose development is determined by day-length or temperature or both, the effect of a gibberellin is more intricate. Many biennials which normally require a period of cold treatment can be induced to bolt and flower by gibberellin application.[29] A gibberellin will similarly replace the long-day requirements of some plants[30] and will terminate the dormancy induced by short-days in deciduous shrubs and trees;[31] and the development of autumn foliage colours and leaf-fall can be arrested.[32]

Although the gibberellins promote cell elongation they differ from the auxins in many ways. For example, they have little or no action on root growth[33] which is inhibited by auxins; neither do they initiate roots on

[21] Lazar and Dahlstrom, personal communication.
[22] Adler, Medwick, and Johl, 138th Meeting Amer. Chem. Soc., New York, Sept. 11—16th, 1960, Abs., p. 25A.
[23] Phinney and West, *Ann. Rev. Plant Physiol.*, 1960, **11**, 411.
[24] Bukovac and Wittwer, *Nature*, 1958, **181**, 1484.
[25] Lockhart and Deal, *Naturwiss.*, 1960, **47**, 141; Brian and Hemming, *Nature*, 1961, **189**, 74
[26] Brian and Hemming, *Physiol. Plant.*, 1955, **8**, 669.
[27] Brian, Elson, Hemming, and Radley, *J. Sci. Food Agric.*, 1954, **5**, 602; Brian, Hemming, and Lowe, *Physiol. Plant.*, 1959, **12**, 15.
[28] Sachs, Bretz, and Lang. *Amer. J. Bot.*, 1959, **56**, 376.
[29] Lang, *Naturwiss.*, 1956, **43**, 257, 284.
[30] Bukovac and Wittwer, *Quart. Bull. Mich. Agric. Exptl. Stn.*, 1957, 39, 650.
[31] Lockhart and Bonner, *Plant Physiol.*, 1957, **32**, 492.
[32] Brian, Petty, and Richmond, *Nature*, 1959, **183**, 58.
[33] Brian, Hemming, and Radley, *Physiol. Plant.*, 1955, **8**, 899.

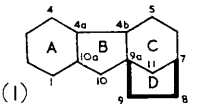
cuttings, an effect which is promoted by auxins. Exogenous auxins have a large effect on the extension of isolated plant tissues but little effect on intact plants; the gibberellins on the other hand induce large responses in intact plants but have relatively little effect on shoot or coleoptile sections unless auxins are added. This synergism between the gibberellins and auxins,[34–36] and the nature of the interaction between the gibberellins and other growth regulators, such as the kinins,[37] is not fully understood. The failure of the gibberellins to simulate the effects of cold treatment or long days in a number of cases (reviewed elsewhere[12]) may be a consequence of such hormonal interactions in which hormones other than the gibberellins are limiting.

The practical uses of the gibberellins in agriculture have been reviewed.[38] The gibberellins are used to stimulate swelling of fruits, *e.g.*, grapes and tomato; and they are more effective than auxins in inducing parthenocarpic fruit setting in tomato. They can also be used to break dormancy of seeds of, *e.g.*, lettuce, peach, and Douglas fir, and to accelerate the germination of barley.

Growth of bacteria and fungi is unaffected[27] by gibberellic acid which has negligible mammalian toxicity.[39]

4. Nomenclature

The systematic nomenclature[40] is based on the trivial name gibbane for the fully saturated tetracarbocyclic system (1), numbered as shown. The 8,9-bridge in (1) is β (absolute configuration) and the ring system derived from gibbane by inversion at positions 7 and 9a is called 7α-gibbane.



(1)

Gibberellic acid (2; R = OH) is thus 2,4a,7-trihydroxy-1-methyl-8-methylenegibb-3-ene-1,10β-dicarboxylic acid 1→4a-lactone.

Catalytic reduction (steric control) of an 8-methylene group gives epimeric pairs of methylgibbanes in which the absolute configuration at position 8 is not known with certainty. Only one epimer is obtained by chemical reduction (thermodynamic control) of gibberellic acid and is arbitrarily called an 8-methylgibbane: the epimer is called an 8-epimethylgibbane. The 8-methyl compounds may be expected to have the less

[34] Brian and Hemming, *Nature*, 1957, **179**, 417; *Ann. Bot.*, 1958, **22**, 1.
[35] Purves and Hillman, *Physiol. Plant.*, 1958, **11**, 29.
[36] Galston and Warburg, *Plant Physiol.*, 1959, **34**, 16.
[37] Wickson and Thimann, *Physiol. Plant.*, 1958, **11**, 62.
[38] Wittwer and Bukovac, *Econ. Bot.*, 1958, **12**, 213.
[39] Peck, McKinney, Tytell, and Byham, *Science*, 1957, **126**, 1064.
[40] Grove and Mulholland, *J.*, 1960, 3007.

hindered configuration and in this event will be 8α-methyl compounds. The term gibberellic acid nor-ketone is used to describe the 8-ketone obtained by oxidative removal of the 8-methylene substituent.

Trivial names, *e.g.*, allogibberic acid (18) and gibberic acid (8), are retained for degradation products in which ring A is aromatic. Within this class the prefix epi is reserved for those compounds in which the 4b-hydrogen atom is β-oriented.

Degradation products in which ring D of gibbane has been opened are named as derivatives of fluorene, *e.g.*, 9β-carboxy-4bα,5,6,7,8,8a-hexahydro-1-methyl-7-oxofluorenyl-8aβ-acetic acid (24; R = H).

The gibberellins are defined as a group of naturally occurring plant hormones containing the tetracyclic system (1). As new gibberellins are isolated they are allotted trivial names in the series gibberellin, $A_1 \ldots . A_n$. This procedure is adopted because the names gibberellin B and gibberellin C were given by the Japanese workers to allogibberic acid (18) and the 7α-gibbane (34; X = H, OH; R = H), respectively, of which the former has no plant-growth promoting properties.[41]

5. The gibberellins and their structural relationships

The structures and physical properties of the gibberellins are listed in the Table. All are gibbane-10β-carboxylic acids with a 1→4a lactone bridge. Gibberellic acid and gibberellin A_7 have an 8-methylene substituent

Compound	Structure	Formula	M.p.[a]	$[\alpha]_D^b$	Source[c]
Gibberellic acid	2; R=OH	$C_{19}H_{22}O_6$	233—235°	+92	F
Gibberellin A_1	4; R=R′=OH, R″=H	$C_{19}H_{24}O_6$	285 255—258	+36	F, P
Gibberellin A_2	5	$C_{19}H_{26}O_6$	256 235—237	+12	F
Gibberellin A_4	4; R=R″=H, R′=OH	$C_{19}H_{24}O_5$	255 214—215	−3	F
Gibberellin A_5	3	$C_{19}H_{22}O_5$	260—261	−77	P
Gibberellin A_6[d]	6	$C_{19}H_{24}O_6$	222—225 206—209	−28	P
Gibberellin A_7	2; R=H	$C_{19}H_{22}O_5$	202	+20	F
Gibberellin A_8	4; R=R′=R″=OH	$C_{19}H_{24}O_7$	210—215	+30	P
Gibberellin A_9	4; R=R′=R″=H	$C_{19}H_{24}O_4$	208—211	−12	F

[a]Decomposition point. The alternative values given are for polymorphic forms.
[b]In ethanol or methanol. [c]Isolated from fungus (F) or higher plant (P).
[d]Later work shows this to be a 2,3-epoxide, $C_{19}H_{22}O_6$.

and Δ^3 double bond: in gibberellin A_5 the latter is replaced by a Δ^2 double bond. Gibberellins A_1, A_4, A_6, and A_9 have the 8-methylene substituent as the only unsaturated centre: in gibberellin A_2 this has been saturated by the addition of the elements of water. With the exception of

[41] Brian, Grove, Hemming, Mulholland, and Radley, *Plant Physiol.*, 1958, **33**, 329.

gibberellins A_5, A_6, and A_9 all have a 2(*ax*)-hydroxyl group while gibberellic acid and gibberellins A_1, A_5, A_6, and A_8 have a 7(*eq*)-hydroxyl substituent. Gibberellin A_8 is unique in having a hydroxyl group at position 3. The position of one hydroxyl group in gibberellin A_6 has still to be located (see, however, the Table).

The relations between the gibberellins were elucidated as follows (for practical convenience the methyl esters were frequently used).

Reduction of the 3,4- double bond in gibberellic acid[42] and gibberellin A_7[9] gave gibberellin A_1 and, with simultaneous reduction of the 8-methylene substituent, dihydrogibberellin A_4 respectively. Reduction with zinc and acetic anhydride[43] of the 7-hydroxy-8-ketone obtained from gibberellin A_1 by ozonolysis gave gibberellin A_4 nor-ketone (as its acetyl derivative). The relation between gibberellins A_1 and A_4 has been confirmed[10] by an alternative route. Treatment of gibberellin A_4 with dilute mineral acid[44] gave gibberellin A_2; and the action of collidine on the 2-toluene-*p*-sulphonyl derivative of gibberellin A_1 gave gibberellin A_5.[17c]

The 7-methyl-8-oxo-7α-gibb-2-ene derived from gibberellin A_5 by the acid-induced rearrangement of rings C/D (see p. 65) proved to be a key compound in relating gibberellin A_5 to gibberellin A_6 and to A_8.[18] Hydroxylation with osmium tetroxide of the 2,3- double bond gave the corresponding 7-methyl-8-ketone obtained from gibberellin A_8; and catalytic reduction of the 2,3- double bond gave a 7-methyl-8-oxo-7α-gibbane obtained from gibberellin A_6 by a reaction sequence in which, after rearrangement of rings C/D, the remaining hydroxyl group was replaced by halogen and the halogen removed by treatment with Raney nickel.

Collidine treatment of the toluene-*p*-sulphonyl derivative of gibberellin A_4 nor-ketone methyl ester followed by catalytic reduction gave gibberellin A_9 nor-ketone methyl ester.[10]

6. The chemistry of gibberellic acid

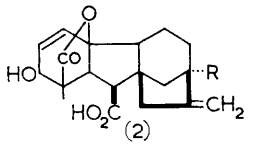
The evidence for the structure and stereochemistry of gibberellic acid is discussed first in paragraphs (a)—(d) and some of the reactions commonly encountered with the gibberellins are mentioned in paragraph (e).

Gibberellic acid (2; R = OH) formed mono- and di-acetyl derivatives each of which yielded a monomethyl ester as did the acid itself.[6] One of the hydroxyl groups is secondary, since, in the reduction products of gibberellic acid it was oxidised to a ketone:[45] the other was considered to be tertiary from the difficulty of acylation. When gibberellic acid was kept with excess of alkali at room temperature a second equivalent was consumed: this, together with the fact that the infrared spectra of the acid and

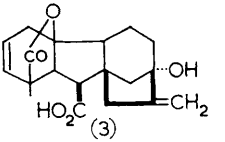
[42] Grove, Jeffs, and Mulholland, *J.*, 1958, 1236; Takahashi, Seta, Kitamura, and Sumiki, *Bull. Agric. Chem. Soc. Japan*, 1957, **21**, 327.
[43] Kitamura, Takahashi, Seta, Kawarada, and Sumiki, *Bull. Agric. Chem. Soc. Japan*, 1959, **23**, 344.
[44] Grove, unpublished work.
[45] Cross, *J.*, 1960, 3022.

its derivatives showed a strong band near 1780 cm.$^{-1}$ indicated the presence of a γ-lactone ring. Microhydrogenation revealed the presence of two ethylenic double bonds.

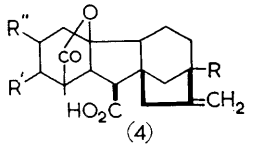
This evidence[6] showed that gibberellic acid was a tetracarbocyclic dihydroxylactonic carboxylic acid. Further information about the ring



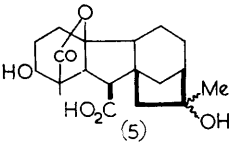
(2)



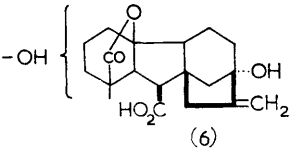
(3)



(4)



(5)



(6)

(See, however, the Table, p. 60.)

system was derived largely from the study of two products of acid hydrolysis, allogibberic acid (18) and gibberic acid (8).

(a) *Structure of gibberene.* Selenium dehydrogenation of both allogibberic and gibberic acid has earlier given[46] a hydrocarbon, gibberene, which from its ultraviolet spectrum was regarded, correctly, as a substituted fluorene but was incorrectly given the formula $C_{16}H_{16}$, and 4-ethyl-5-methylfluorene was suggested as a possible structure. By degradation to the known fluorene-1,7-dicarboxylic acid Mulholland and Ward[47] showed that gibberene was 1,7-dimethylfluorene (11; R = Me) $C_{15}H_{14}$ and this was confirmed by unambiguous synthesis[47] from 2-amino-5-methylbenzoic acid and *o*-tolylmagnesium bromide, *via* the intermediate benzophenone (17).

(b) *Structure of gibberic acid.* Treatment of gibberellic acid or allogibberic acid with boiling dilute mineral acid gave[6] gibberic acid (8), $C_{18}H_{20}O_3$, m.p. 153—154° or 174—175°, $[\alpha]_D$ −7°, together with an isomer, epigibberic acid (23), m.p. 227—230° or 252—255°, $[\alpha]_D$ + 131°. The formation of an ester, an oxime, and an oxime ester showed that gibberic acid was a keto-acid, and a band at 1741 cm.$^{-1}$ in the infrared spectrum indicated that the ketone group was present in a five-membered ring. Microhydrogenation showed the absence of ethylenic double bonds but the ultraviolet spectrum (λ_{max}265, 274 mμ; log ϵ 2·56, 2·47) revealed the presence of a benzenoid ring. The presence of the hexahydrofluorene nucleus in the tetracarbocyclic system (8) was established by stepwise degradation,[6,48] *via* the α-diketone (9) and the tricarboxylic acid (12), to 1,7-dimethylfluorene, and by oxidation of gibberic acid to benzene-1,2,3-tricarboxylic acid.[48] The substitution pattern of the five-membered ring

[46] Yabuta, Sumiki, Aso, Tamura, Igarashi, and Tamari, *J. Agric. Chem. Soc. Japan*, 1941, **17**, 975.
[47] Mulholland and Ward, *J.*, 1954, 4676.
[48] Cross, Grove, MacMillan, and Mulholland, *J.*, 1958, 2520.

containing the ketone group was deduced from ultraviolet absorption studies on the α-diketone (9) which was found to have no enolisable hydrogen atom. The position of the carboxyl group was established[48] by dehydrogenation of the methyl ester of (9) to methyl 1,7-dimethylfluorene-9-carboxylate, identical with a synthetic specimen prepared by carboxylation of the 9-lithium derivative of 1,7-dimethylfluorene followed by methylation.

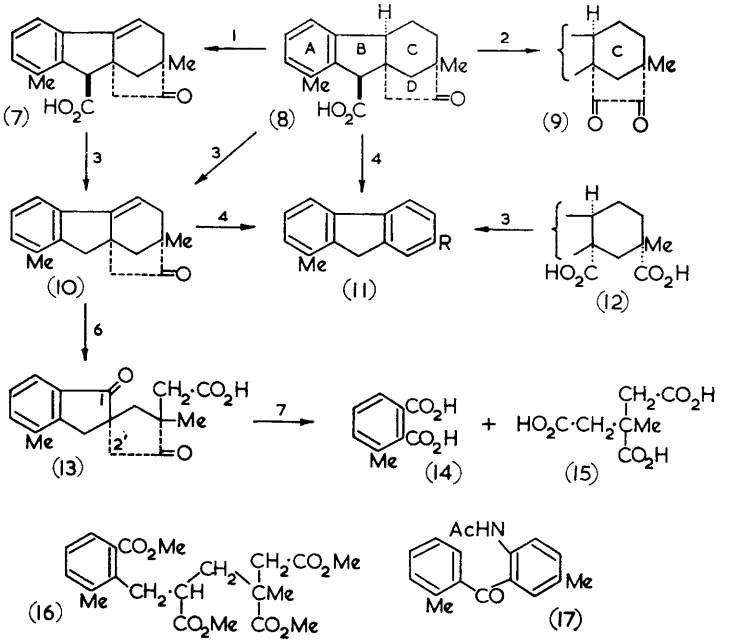


CHART 1

Reagents: 1, $KMnO_4$. 2, SeO_2. 3, Pd–C. 4, Se. 5, H_2O_2–NaOH. 6, CrO_3. 7, $KMnO_4$–$Mg(NO_3)_2$.

The position of the $-CH_2{\cdot}CO-$ bridge followed from a second stepwise degradation[48] in which the key compound was a ketone, gibberone, $C_{17}H_{18}O$ (10) obtained directly from gibberic acid by dehydrogenation over palladium–charcoal or indirectly by decarboxylation of dehydrogibberic acid (7) ($\lambda_{max.}$ 260, 269 mμ; log ε 4·14, 4·09), a permanganate oxidation product of gibberic acid. Oxidation of gibberone with chromic oxide gave the 1-oxoindanespirocyclopentanone (13) which was stable to hydrolysis and was not therefore a 1,2′-diketone. Further oxidation of the indanone (13) gave 3-methylphthalic acid (14) and β-methyltricarballylic acid (15); and opening of both five-membered rings by second-order Beckmann rearrangement of the 2′-oximino-compound gave, after hydrolysis

and methylation, two diastereoisomeric tetramethyl esters (16), m.p. 83—84°, $(\alpha)_D$ −6°, and m.p. 47—48°, $(\alpha)_D$ +12°. The structure (16) of the esters was confirmed by the unambiguous synthesis of their racemates.[49] This synthesis completed the elucidation of the structure of the indanone (13) and consequently of gibberone (10). It followed that the methylene carbonyl bridge in gibberic acid must be attached as in (8). Racemic gibberone has recently been synthesised.[50]

(c) *Structure and stereochemistry of allogibberic acid.* With cold dilute mineral acid both gibberellic acid[6,41] and the intermediate, gibberellenic acid (19)[51] gave allogibberic acid (18), $C_{18}H_{20}O_3$, m.p. 201—203°, $(\alpha)_D$ −84°, and 1 mol. of carbon dioxide, With hydrazine hydrate, gibberellenic acid gave both allogibberic acid and an isomer epiallogibberic acid (20),[40] m.p. 244°, $(\alpha)_D$ +87°. Allogibberic acid ($\lambda_{max.}$ 266, 274 mμ; log ε 2·50, 2·35) contained a benzenoid ring and an ethylenic double bond, present in an exocyclic methylene grouping, since ozonolysis[52] gave formaldehyde and a nor-ketone $C_{17}H_{18}O_4$ (21). The carboxyl group was attached in the same position as in gibberic acid since the methyl ester was isomerised with acid to methyl gibberate. The third oxygen atom was present as a hydroxyl group ($\nu_{max.}$ 3460 cm.$^{-1}$), considered to be tertiary because of the difficulty of acylation and the failure to oxidise dihydroallogibberic acid to a ketone. These facts together with the following evidence established allogibberic acid as a tetracarbocyclic hydroxy-acid (18): the presence of an α-ketol system in a five-membered ring in the nor-ketone (21) was shown by the infrared spectrum ($\nu_{max.}$ 1742 cm.$^{-1}$) and by oxidation with sodium bismuthate[52] to a tricyclic dibasic keto-acid $C_{17}H_{18}O_5$ (24; R = H), in which the carbonyl group was contained in a saturated six-membered ring. The position of this carbonyl group and hence of one point of attachment of the five-membered ring in allogibberic acid was ascertained by selenium dehydrogenation[52] to 8-methylfluoren-2-ol (11; R = OH), whose structure was proved by synthesis.[53]

With aqueous alkali the keto-ester (24; R = Me) gave the acid (24; R = H) together with a new dibasic keto-acid, $C_{17}H_{18}O_5$, epimeric at position 9. With acetic anhydride both dibasic acids gave the same *cis*-anhydride, but hydrolysis of the anhydride regenerated only the acid (24; R = H) derived directly from allogibberic acid. The 10-carboxylic acid substituent and 8,9-two-carbon bridge are therefore *cis* in allogibberic acid.[40]

Since catalytic reduction of dehydrodihydroallogibberic acid (26), a 4b(5)-ene related to dehydrogibberic acid (7), will take place from the less-hindered side of the molecule opposite to the 10-carboxyl substituent

[49] Morrison and Mulholland, *J.*, 1958, 2536.
[50] Loewenthal, *Proc. Chem. Soc.*, 1960, 355.
[51] Moffatt, *J.*, 1960, 3045.
[52] Mulholland, *J.*, 1958, 2693.
[53] Morrison and Mulholland, *J.*, 1958, 2702.

and 8,9-bridge, and since this process regenerated the original stereochemistry at position 4b,[40] it followed that rings B/C were *trans*-fused in allogibberic acid. The absolute configuration (18) followed from measurements of optical rotatory dispersion on the keto-ester (24; R = Me).[40,54]

In addition to racemisation at position 9, the ester (24; R = Me), on

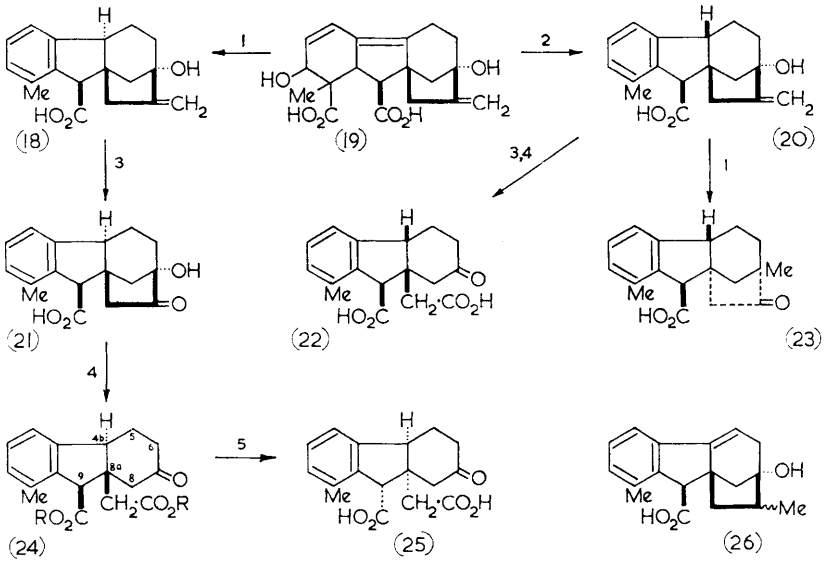


CHART 2

Reagents: 1, H^+. 2, N_2H_4,H_2O. 3, O_3. 4, $NaBiO_3$. 5, OH^-.

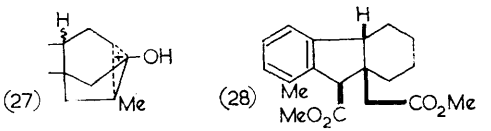
treatment with base, underwent[40] an intramolecular Claisen-type condensation at position 6: this was followed by fission of the 6,7-bond and hydrolysis, liberating the 4bα,8aα,9α-isomer (25) of the acid (24; R = H). Epiallogibberic acid, which gave epigibberic acid (23) on acid treatment, was chemically similar to allogibberic acid, and yielded the 4bβ,8aβ,9β-enantiomer (22) of (25) on ozonolysis followed by fission of the resulting α-ketol. It followed that epiallogibberic acid differed from allogibberic acid only in configuration at position 4b.[40]

The acid-catalysed rearrangement of these acids takes place by a Wagner–Meerwein mechanism[52,55] *via* the cation (27), and in the resulting gibberic acids the 8,9-bridge has the opposite configuration (α) to that which it occupied in the allogibberic acids.

The racemate of the ester (28), a reduction product of the ketone (22), has been synthesised.[56]

[54] Stork and Newman, *J. Amer. Chem. Soc.*, 1959, **81**, 3168.
[55] Grove, MacMillan, Mulholland, and Turner, *J.*, 1960, 3049.
[56] House, Paragamian, and Wluka, *J. Amer. Chem. Soc.*, in the press.

(d) *Structure of gibberellic acid.* The position of the carboxyl substituent in gibberellic acid was the same as in allogibberic acid since the methyl ester with acid gave methyl gibberate. An early suggestion[57] that the two acids had the same B/C/D structure was confirmed[45] by the ozonolysis of methyl gibberellate which took the same course as the ozonolysis of methyl allogibberate giving formaldehyde and, ultimately, a keto-acid



(32; R = H). The methyl ester (32; R = Me) of the latter gave the ester (24; R = Me) with acid, and the formation of allogibberic acid from gibberellic acid therefore involves only the aromatisation of ring A.

Ring A of gibberellic acid must accommodate the methyl group, which appeared at position 1 in the fluorene degradation products, the saturated γ-lactone ring, and the secondary hydroxyl group which was shown to be allylic by oxidation with manganese dioxide[45] of methyl gibberellate to the Δ^3-2-one (29) ($\lambda_{max.}$ 228mμ; log ϵ 3·99). Catalytic hydrogenation of the ketone (29) afforded the 2-ketones (31), epimeric at position 8, also obtained

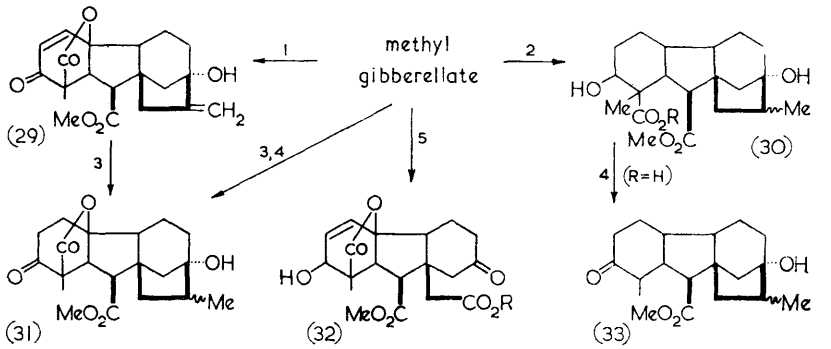


CHART 3

Reagents: 1, MnO_2. 2, H_2/Pt. 3, H_2/Pd. 4, CrO_3. 5, O_3.

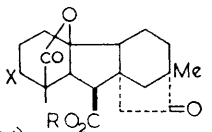
by oxidation with chromic oxide of methyl tetrahydrogibberellate and its 8-epimer. The position of the allylic hydroxyl group was established[45,58] by selenium dehydrogenation of the ketones (35; R = H, X = H_2, Y = O) and (34; R = Me, X = O), both derived from gibberellin A_1, to 1-methylfluoren-2-ol and 1,7-dimethylfluoren-2-ol[59] respectively. The position of

[57] Cross, Grove, MacMillan, and Mulholland, *Chem. and Ind.*, 1956, 954.

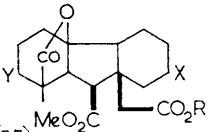
[58] (*a*) Seta, Takahashi, Kitamura, Takai, Tamura, and Sumiki, *Bull. Agric. Chem. Soc. Japan*, 1958, **22**, 61; (*b*) Seta, Takahashi, Kitamura, and Sumiki, *Bull. Agric. Chem. Soc. Japan*, 1958, **22**, 429.

[59] Cross and Melvin, *J.*, 1960, 3038.

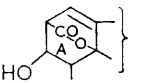
the lactone bridge was deduced from the high yield of acidic hydrogenolysis products on catalytic reduction of methyl gibberellate, which suggested an allylic lactone system, and from the formation of a heteroannular conjugated diene, gibberellenic acid[51] (19) ($\lambda_{max.}$ 253 mμ; log ϵ 4·35) from gibberellic acid in aqueous solution. It was concluded[60] that



(34)



(35)



(36)

gibberellic acid has structure (2; R = OH), and this conclusion was supported by nuclear magnetic resonance studies.[61] An alternative ring A structure (36) has been suggested[58b] but is inconsistent with much of the foregoing evidence.

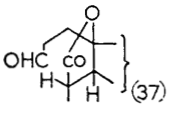
The quasi-axial nature of the 2-hydroxyl substituent in gibberellic acid was deduced from the base-catalysed isomerisation of gibberellin A_1 to the more stable 2(*eq*)-hydroxy-epimer.[62] The rotary dispersion curves for the keto-esters (35; R = Me, X = O, Y = H,OH) and (24; R = Me) were almost identical[63] and significantly different from that for the methyl ester of the keto-acid (22) obtained from epiallogibberic acid. Gibberellic acid therefore probably has the same *trans*-B/C ring fusion as allogibberic acid but more evidence on this point would be desirable. Oxidation and decarboxylation of the 8-epimethyl acid (30; R = H), obtained by hydrogenolysis of methyl gibberellate, gave the keto-ester (33), the rotatory dispersion curve of which showed a negative Cotton effect,[63] but this fact is in itself insufficient to determine unequivocally the nature of the A/B fusion in the acid (30; R = H). The assignment,[63] from nuclear magnetic resonance studies, of a *trans*-orientation to the hydrogen atoms at positions 10 and 10a now appears to be unwarranted.[61]

Some controversy arose over the interpretation of the physical evidence for the orientation of the 1→4a lactone bridge.[64] The chemistry of the ester (30; R = Me)[65] indicated that the 2-hydroxyl substituent was equatorial and that, consequently, hydrogenolysis of methyl gibberellate involved inversion of configuration at position 4a. This chemical evidence is decisively in favour of a β-orientation for the lactone bridge, but a rigorous proof of the configuration at position 10a is still required.

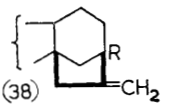
[60] Cross, Grove, MacMillan, Moffatt, Mulholland, Seaton, and Sheppard, *Proc. Chem. Soc.*, 1959, 302.
[61] Sheppard, *J.*, 1960, 3040.
[62] Cross, Grove, and Morrison, *J*, 1961, in the press.
[63] Cross, Grove, McCloskey, Mulholland, and Klyne, *Chem. and Ind.*, 1959, 1345.
[64] (*a*) Stork and Newman, *J. Amer. Chem. Soc.*, 1959, **81**, 5518; (*b*) Edwards, Nicolson, Apsimon, and Whalley, *Chem. and Ind.*, 1960, 624.
[65] Grove and McCloskey, unpublished work.

(e) *General chemical reactions of the gibberellins.* Some of the more important reactions encountered with the gibberellins are indicated below.

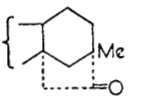
(i) 2(*ax*)-Hydroxygibbane 1→4a-lactones are epimerised in dilute alkali to the more stable 2(*eq*)-hydroxy-compounds, and a retroaldol mechanism *via* the intermediate (37) has been suggested.[62] Under the same conditions[62] 2(*ax*)-hydroxygibb-3-ene 1→4a-lactones undergo an allylic type of rearrangement to gibb-4-ene 1→3-lactones without concomitant epimerisation of the hydroxyl substituent.



(37)



(38)



(39)

(ii) In the presence of a 1→4a-lactone bridge, catalytic reduction of a 3-ene precedes that of an 8-methylene substituent; but the 8-methylene group is reduced before a 4-ene group in gibb-4-ene 1→3-lactones. The latter are difficult to reduce, and hydrogenolysis of the lactone predominates.[62]

(iii) 2-Hydroxyl groups are smoothly oxidised to 2-ketones only in gibbanes in which an 8-methylene substituent has been reduced or eliminated.[45] The 2-ketones are reduced by alkali-metal hydrides to the 2(*eq*)-hydroxy-compounds.[62]

(iv) In gibbanes 2(*ax*)-hydroxy-substituents are readily eliminated,[17c] either directly under the influence of nucleophilic reagents or *via* the toluene-*p*-sulphonate, giving gibb-2-enes.

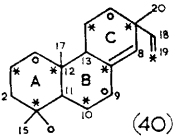
(v) Compounds containing the C/D partial structure (38; R = OH) undergo Wagner–Meerwein rearrangement with acid to give ketones of partial structure (39).[55] Under the same conditions the elements of water are added to the 8-methylene group in compounds (38; R = H).

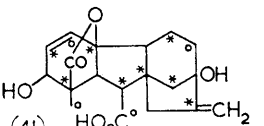
(vi) Among degradation products in which ring A is aromatic, compounds having a 10-methoxycarbonyl substituent in the less stable configuration undergo base-catalysed racemisation at this centre. Only compounds in which the 4b-hydrogen atom is *trans* to the 10-carboxylic acid substituent are oxidised by permanganate to 4b(5)-enes: in some cases neighbouring groups cause steric inhibition of this reaction.[40,55] The configuration at position 10 also determines the steric course of the catalytic reduction of 4b(5)-enes, hydrogenation occurring *trans* to a 10-carboxyl or 10-methoxycarbonyl substituent.

7. Biogenesis of the gibberellins

Inspection of structure (2; R = OH) showed that it could have arisen by a variant of the processes leading to the tricyclic diterpene skeleton (40) in which (i) the 17-carbon atom had been lost, (ii) contraction of ring B to

a five-membered ring had occurred with extrusion of a carboxyl group, and (iii) formation of the phyllocladene-type of bridged-ring structure had occurred from ring C and its substituents according to the scheme suggested by Wenkert.[66] The correctness of these speculations was established[67] by the degradation of gibberellic acid obtained from *G. fujikuori* grown on


(40)


(41)

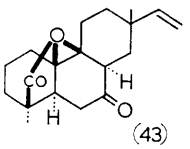

(42)

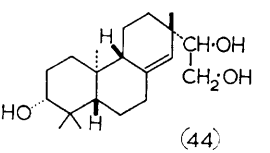
[*carboxy*-^{14}C]acetic acid [*indicates labelled atom in (40)] and [2-^{14}C] mevalonic lactone (42); the results of the degradation were consistent with the labelling pattern (41) expected from the usual mode of incorporation (40) of these precursors in a tricyclic diterpene.

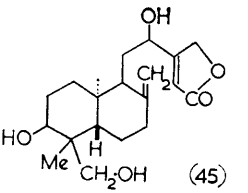
The stereochemical implications of this important work have been the subject of much speculation.[67,68] Two points are clear however: first, oxidation at position 7 in the gibberellins is subsidiary to the main biogenetic process and this may also be true of the hydroxylation of ring A. Secondly, since the 17-carbon atom is lost and contraction of ring B is likely to involve an intermediate 10-oxo-derivative, it is not necessary to postulate that gibberellic acid is derived from other than the normal (11α) type of *trans-anti-trans*-hydrophenanthrene precursor (40).

8. The gibberellins in diterpene chemistry

Many terpenoids[69] and, more commonly, compounds with isoprenoid side chains[70] are known among fungal metabolic products, but few diterpenoids have been isolated. Of these, only the groups related to


(43)


(44)


(45)

rosenonolactone[71] (43) and gibberellic acid have been extensively investigated.

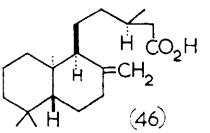
Few diterpenes have the 2-hydroxy-substituent which occurs frequently

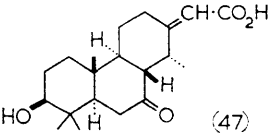
[66] Wenkert, *Chem. and Ind.*, 1955, 282.
[67] Birch, Rickards, and Smith, *Proc. Chem. Soc.*, 1959, 192; Birch, Rickards, Smith, Harris, and Whalley, *Tetrahedron*, 1959, **7**, 241.
[68] Djerassi, Cais, and Mitcher, *J. Amer. Chem. Soc.*, 1959, **81**, 2386.
[69] (*a*) Haagen-Smit, *Progr. Chem. Org. Nat. Prod.*, 1955, **12**, 1; (*b*) Jones and Halsall, *ibid.*, p. 44.
[70] Birch, English, Massy-Westropp, and Smith, *J.*, 1958, 369.
[71] Harris, Robertson, and Whalley, *J.*, 1958, 1799, 1807; Freeman, Morrison, and Michael, *Biochem. J.*, 1949, **45**, 191.

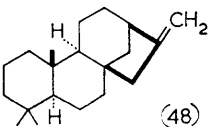
in the gibberellins. Among those possessing this feature, several, *e.g.*, darutigenol[72] (44) and andrographolide[73] (45), also have the abnormal antipodal *trans*-A/B stereochemistry, which however is not invariably associated with the presence of a 2-hydroxyl group, *e.g.*, eperuic acid[74] (46) and cassaic acid[75] (47). The axial configuration of the 2-hydroxyl substituent in the gibberellins is also unusual, although similarly situated axial hydroxyl groups have been recorded in the triterpenoids, *e.g.*, in the mould product, polyporenic acid A.[69b]

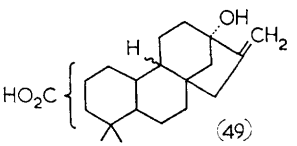
The 8,9-two-carbon bridge in ring C, carrying an 8-methylene substituent, is found in the tetracarbocyclic diterpenes related to phyllocladene (48).[76] The structure of steviol has[77] been amended to (49) and the steviol → isosteviol rearrangement is now recognised as analogous to the acid-induced conversion of allogibberic into gibberic acid.

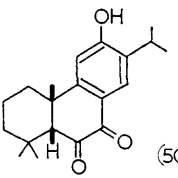
Contraction of ring B in a diterpene skeleton, such as (40), may be assumed to involve a 9,10-dioxygenated intermediate and xanthoperol (51)[78] provides an example of a diterpenoid 9,10-diketone. Benzilic acid rearrangement of the enantiomer of methyl-9,10-dioxopodocarpa-5,7,13(14)-trien-16-oate has been shown to give the hydrofluorene-carboxylic acid (51).[79]

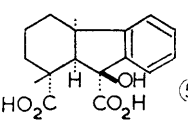

(46)


(47)


(48)


(49)


(50)


(51)

The gibberellins differ from all other tri- and tetra-cyclic diterpenoids in that selenium dehydrogenation gives substituted fluorenes instead of substituted phenanthrenes. Apart from this fact, the more characteristic reactions (see Section 6e) are associated with the 2-hydroxygibb-3-ene 1→4a-lactone and 2-hydroxygibbane 1→4a-lactone systems, for which there are no exact analogies among natural products.

[72] Diara, Asselineau, and Lederer, *Bull. Soc. chim. France*, 1959, 693.
[73] Cava and Weinstein, *Chem. and Ind.*, 1959, 851; Chan, Haynes, and Johnson, *Chem. and Ind.*, 1960, 22.
[74] Barltrop and Bigley, *Chem. and Ind.*, 1959, 1447.
[75] Turner, Herzog, Morin, and Riebel, *Tetrahedron Letters*, 1959, No. 2, 7.
[76] Briggs, Cain, Davis, and Wilmshurst, *Tetrahedron Letters*, 1959, No. 8, 13.
[77] Dolder, Lichti, Mosettig, and Quilt, *J. Amer. Chem. Soc.*, 1960, **82**, 246.
[78] Bredenberg, *Acta Chem. Scand.*, 1960, **14**, 385.
[79] Grove and Riley, *J.*, 1961, 1105.