Fungal Products. Part IX.[1] Gibberellins A_{16}, A_{36}, A_{37}, A_{41}, and A_{42} from *Gibberella fujikuroi*

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Several new metabolites from the culture filtrates of *Gibberella fujikuroi*. strain TP70. are described. These include gibberellin A_{16} (4), hitherto known only as the methyl ester, an anhydride ($C_{19}H_{24}O_4$). and gibberellins A_{36} [(16) ⇌ (20)] and A_{37} (31). Structure [(16) ⇌ (20)] was deduced for gibberellin A_{36} from spectroscopic data and confirmed by chemical correlation with gibberellin A_{13} (9). The δ-lactone (31). obtained by reduction of gibberellin A_{36}. was identical with gibberellin A_{37}. previously isolated as the glucosyl ester from seed of *Phaseolus vulgaris*. The occurrence of gibberellin A_{37} in culture filtrates of the fungus was established by g.l.c.–mass spectrometry.

Gibberellin A_{13} hydrate (13) and gibberellin A_{14} hydrate (14) were also isolated. and allocated the gibberellin numbers A_{41} and A_{42}.

In connection with biosynthetic studies on the gibberellin group of plant hormones, a source of gibberellins and their possible precursors was required. After a preliminary survey by combined gas chromatography–mass spectrometry (g.l.c.–m.s.) the acidic metabolites of strain TP70 of *Gibberella fujikuroi* were selected for detailed study. The total ion current (t.i.c.) trace of the methylated-trimethylsilylated fraction, which remained after the bulk of the gibberellin A_3 (5) had been removed by crystallisation is shown in the Figure. The mass spectra, obtained by scanning the indicated peaks, established the presence of the known [2] gibberellins A_1 (1), A_3 (5), the isomer (7) of A_3, A_4 (2), A_7 (6), the isomer (8) of A_7, A_9 (3), A_{13} (9), A_{14} (10), A_{24} (11), and gibberellin A_{16} (4), previously known [3] only as its methyl ester, and of at least one new gibberellin (A_{36}).[4] G.l.c.–m.s. of the methylated crude fraction revealed the presence of two other known metabolites, gibberellin A_{25} (12) [5] and the formyl-diacid (15).[6] Large scale fractionation of the

[1] Part VIII, P. Hedden, J. MacMillan, and M. J. Grinstead, *J.C.S. Perkin I*, 1973, 2773.

[2] For leading references see J. MacMillan, ' Diterpenes. The Gibberellins,' in ' Aspects of Terpenoid Chemistry,' ed. T. W. Goodwin, Academic Press, London and New York, 1971, p. 153.

[3] R. H. B. Galt, *Tetrahedron*, 1968, **24**, 1337.

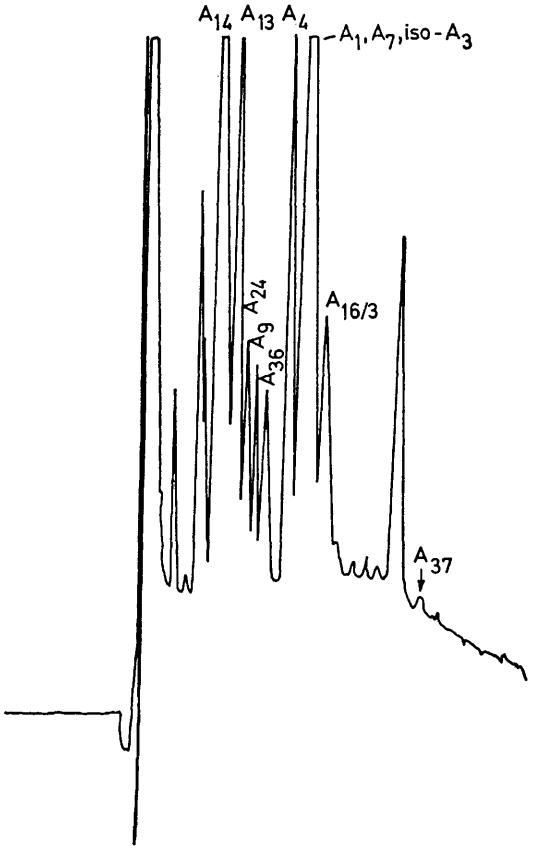
[4] J. R. Bearder and J. MacMillan, *Agric. and Biol. Chem. (Japan)*, 1972, **36**, 342.

[5] D. M. Harrison and J. MacMillan, *J. Chem. Soc.* (*C*), 1971, 631.

[6] B. E. Cross, R. H. B. Galt, J. R. Hanson, and (in part) P. J. Curtis, J. F. Grove, and A. Morrison, *J. Chem. Soc.*, 1963, 2937.

crude acid fraction by column and thin-layer chromatography allowed the separation of all of the compounds detected by g.l.c.–m.s. Three additional new compounds were isolated: the hydrates (13) and (14), and an anhydride, $C_{19}H_{24}O_4$.

The new gibberellin (16), briefly described in a preliminary communication [4] and named A_{36}, was present in concentrations of *ca.* 0·7 mg l^{-1} of culture fluid. The molecular formula, $C_{20}H_{26}O_6$, was determined by combustion analysis and by the mass spectra of the dimethyl



Total ion current (t.i.c.) trace of methylated, trimethylsilylated gibberellin A_3 mother liquors on 2% QF-1 (temp. programmed 200—260° at 2° min^{-1})

ester (17) and the dimethyl ester trimethylsilyl ether (18). Since the structural arguments for gibberellin A_{36} itself are complicated by the aldehyde–lactol equilibrium [(16) ⇌ (20)], they are presented for the dimethyl ester (17). The latter compound gave an n.m.r. spectrum (Table) which showed the presence of a tertiary methyl group, a tertiary aldehyde group, a secondary hydroxy-group, two methoxycarbonyl groups, and an exocyclic methylene function; an AM double doublet was present, characteristic of the 5- and 6-protons of a gibberellin. I.r. absorption of a solution in carbon tetrachloride confirmed the presence of hydroxy- (3635 cm^{-1}), aldehyde (2720 and 2760), methoxycarbonyl (1735), and exocyclic methylene (3070, 1658, and 883) functions. The base peak at *m/e* 129 in the mass spectrum of the trimethylsilyl ether (18) and the chemical shift of the 5-proton, deshielded (Table) by 0·56 p.p.m. in comparison with the methyl ester of gibberellin A_{24} (11), are both characteristic [7,8] of a 3β-hydroxygibberellin. These data are also compatible with a 1β-hydroxy-group which, however, was excluded by the chemical shift of the 18-protons. These protons showed the characteristic behaviour [5,8,9] of 3β-hydroxygibberellins, being deshielded in the methyl ester (17) by 1·1 p.p.m. in comparison with those in the methyl ester of the unhydroxylated analogue (11). Their signal was restored by acetylation to the higher field position for the 18-protons in the methyl ester of (11) (Table).

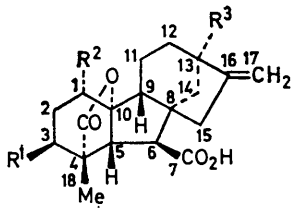
The tertiary nature of the aldehyde function and the formation of a lactol diacetate (21) indicated the presence of a 4- or 10-aldehyde. The 10-position was indicated by the chemical shift of the 18-protons. Comparison of the aldehydes (22) and (24) with their respective methoxycarbonyl analogues (23) and (25) shows that the 18-protons in the 4-aldehydes are deshielded by 0·17 [10] and 0·13 [11] p.p.m. in the respective pairs. Since the 18-protons in gibberellin A_{36} dimethyl ester (17) have chemical shifts (Table) almost identical with those in the trimethyl ester of gibberellin A_{13} (9), a 4-aldehyde function in A_{36} is excluded.

Structure (17) for gibberellin A_{36} dimethyl ester was unambiguously established by a chemical correlation with gibberellin A_{13} (9) *via* the acetate monocarboxylic acid (26). The latter was prepared from gibberellin A_{13} (9) by way of the known [9] acetate anhydride (28) and the corresponding methyl ester (29), which was methanolysed at 150° in a sealed tube; methanolysis of such anhydrides has been shown [12] to occur in the direction indicated in (26). The monocarboxylic acid (26) was also obtained in poor yield by oxidation of acetylgibberellin A_{36} dimethyl ester (19); it was identified by g.l.c.–m.s. in a complex mixture of oxidation products as the trimethylsilyl ester (27). Attempts to oxidise gibberellin A_{36} dimethyl ester (17) directly to the corresponding monoacid or to a 20-nor-derivative by irradiation [13] in a stream of oxygen gave starting material only.

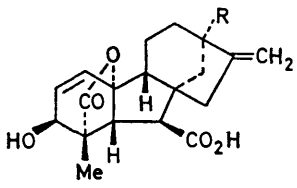
Gibberellin A_{36}, like gibberellin A_{24} (11) [5] and gibberellin A_{23},[14] exists mainly as the lactol (20). At 28° the n.m.r. spectrum (Table) of a solution in [$^2H_5$]pyridine showed no aldehyde proton, and the 6-proton signal occurred at higher field than expected for a free 10-aldehyde. At −80° the lactol methine proton signal occurred at τ 4·38 as a singlet, whereas in the diacetate (21) the lactol methine proton signal occurred at τ 3·56.

Reduction of gibberellin A_{36} [(16) ⟵ (20)] with

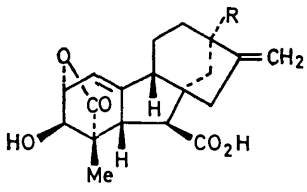
[7] R. Binks, J. MacMillan, and R. J. Pryce, *Phytochemistry*, 1969, **8**, 271.
[8] J. R. Hanson, *J. Chem. Soc.*, 1965, 5036.
[9] R. H. B. Galt, *J. Chem. Soc.*, 1965, 3143.
[10] J. R. Hanson and A. F. White, *Tetrahedron*, 1969, **25**, 2743.
[11] H. J. Bakker, P. R. Jefferies, and J. R. Knox, *Tetrahedron Letters*, 1972, 2723.
[12] B. E. Cross and J. C. Stewart, *J. Chem. Soc.* (*C*), 1971, 245.
[13] M. E. Wolff and S.-Y. Chang, *Tetrahedron Letters*, 1966, **23**, 2509.
[14] H. Fukui, H. Ishii, K. Koshimizu, M. Katsumi, Y. Ogawa, and T. Mitsui, *Agric. and Biol. Chem.* (*Japan*), 1972, **36**, 1003.



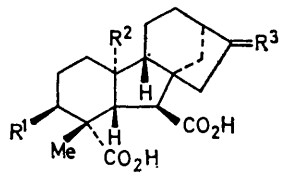
(1) $R^1 = R^3 = OH$, $R^2 = H$
(2) $R^1 = OH$, $R^2 = R^3 = H$
(3) $R^1 = R^2 = R^3 = H$
(4) $R^1 = R^2 = OH$, $R^3 = H$



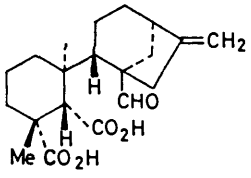
(5) R = OH
(6) R = H



(7) R = OH
(8) R = H



	R^1	R^2	R^3
(9)	OH	CO_2H	CH_2
(10)	OH	Me	CH_2
(11)	H	CHO	CH_2
(12)	H	CO_2H	CH_2
(13)	OH	CO_2H	OH, Me
(14)	OH	Me	OH, Me



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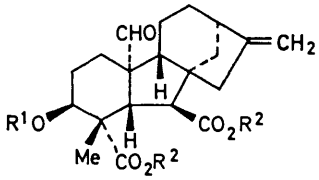
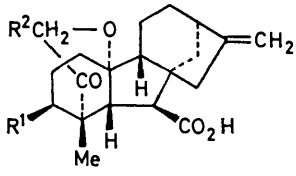
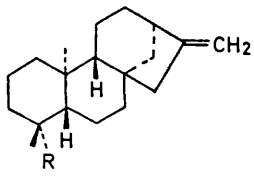


(16) $R^1 = R^2 = H$
(17) $R^1 = H$, $R^2 = Me$
(18) $R^1 = SiMe_3$, $R^2 = Me$
(19) $R^1 = Ac$, $R^2 = Me$



(20) $R^1 = R^2 = OH$
(21) $R^1 = R^2 = OAc$

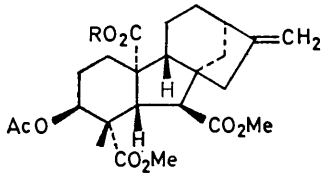


(22) R = CHO
(23) R = CO_2Me
(24) R = CHO, 2-ene
(25) R = CO_2Me, 2-ene

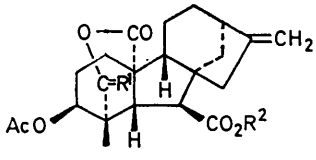
Chemical shifts (τ) [a] of protons in some gibberellins

Gibberellin	Solvent	3-H [b]	5-H [c]	6-H [c]	17-H [d]	20-H	18-H	Other signals
A_{36} [(16) ⇌ (20)]	*e*	5·65	6·59 [g]	6·49 [g]	5·07, 5·20	*h*	8·14	
A_{36} diMe ester (17)	*e*	5·52	5·71	6·66	5·06, 5·14	0·04	8·41	6·29 and 6·36 (2 × OMe)
	f	5·89	7·25	6·09	5·08, 5·16	0·32	8·78	6·28 and 6·36 (2 × OMe)
A_{24} (11) diMe ester [5]	*f*		7·93	6·22	5·20, 5·27	0·38	8·89	6·34 and 6·43 (2 × OMe)
A_{13} (9) triMe ester	*f*	6·11	7·49	6·21	5·20, 5·27		8·79	6·36, 6·41 and 6·48 (3 × OMe)
A_{36} diacetate (21)	*f*	4·98	7·29	7·09	5·05, 5·19	3·56	8·81	7·86 (2 × OAc)
A_{37} (31)	*e*	5·87	6·72	6·72	5·08, 5·22	5·39, [j] 5·87 [j]	8·16	
A_{37} Me ester	*f*	6·26	7·26	7·26	5·10, 5·22	5·60, [i] 5·93 [j]	8·81	6·35 (OMe)

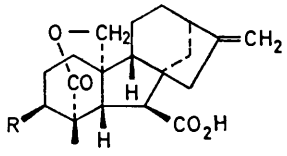
[a] Measured at 100 MHz with tetramethylsilane as internal standard. [b] Triplet (*J ca.* 3 Hz). [c] Doublet (*J* 13 Hz) except for A_{37} and A_{37} Me ester. [d] Broad. [e] In [$^2H_5$]pyridine. [f] In $CDCl_3$. [g] Or *vice versa*. [h] τ 4·38 at −80°. [i] Double doublet (*J ca.* 1 and 12 Hz). [j] Doublet (*J* 12 Hz).



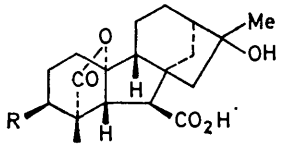
(26) R = OH
(27) R = $SiMe_3$



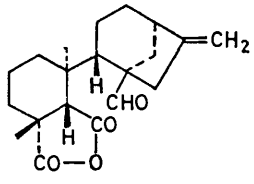
(28) $R^1 = O$, $R^2 = H$
(29) $R^1 = O$, $R^2 = Me$
(30) $R^1 = H_2$, $R^2 = H$



(31) R = OH
(32) R = H



(33) R = OH
(34) R = H



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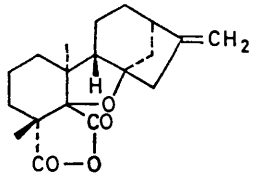

(36)

sodium borohydride gave the δ-lactone (31), the identity of which with gibberellin A_{37} confirmed the structure of this new gibberellin, isolated as the glucosyl ester from mature seed of *Phaseolus vulgaris* by Hiraga *et al.*[15] The spectroscopic properties of this δ-lactone (31) are unexceptional. The n.m.r. spectrum (Table) is similar to that of the isomeric lactone (30)[12] except that the signal due to the 18-proton occurs at lower field. The n.m.r. spectrum of the methyl ester of gibberellin A_{37} (31) is similar to that of gibberellin A_{15} (32) apart from the deshielding of the 3α-, 5β-, and 18-protons by the 3β-hydroxy-group; the deshielding of the 5-proton results in the accidental equivalence of the 5- and 6-protons. This accidental equivalence appears to be a feature[14,16] of 3β-hydroxygibberellins with a 19,20-lactone. The mass spectrum of gibberellin A_{37} methyl ester shows fragment ions typical[7] of a gibberellin methyl ester. The main feature of the mass spectrum of the trimethylsilyl ether of the methyl ester is the base peak at *m/e* 129, characteristic of the 3-trimethylsilyloxy-group.

The co-occurrence of gibberellins A_{36} and A_{37} in *G. fujikuroi* seemed likely; a retrospective g.l.c.–m.s. analysis of the crude acid fraction from strain TP70 did in fact reveal (Figure) the presence of gibberellin A_{37}. Thus gibberellin A_{37} joins the relatively few gibberellins which have been found both in the fungus and in higher plants. Structure (30) for gibberellin A_{37}, and hence structure [(16) ⇌ (20)] for gibberellin A_{36}, has been verified recently[17] by a synthesis from gibberellin A_{13} (9).

Gibberellin A_{16} (4), previously known[3] only as the methyl ester, was obtained as a crystalline solid with the expected n.m.r. properties. It was identified by direct comparison of the mass spectra of the methyl ester and the trimethylsilyl ether of the methyl ester with reference spectra.[7]

The hydrates (13) and (14) were characterised by their spectroscopic properties. The n.m.r. spectrum of the methyl ester of gibberellin A_{14} hydrate (14) and of gibberellin A_{14} (10) were similar except for the absence of the vinylic proton signals, and the presence of a third methyl singlet at τ 8·65 in the spectrum of the hydrate. The mass spectra of both methyl esters were also similar; that of the trimethylsilyl ether of the methyl ester of gibberellin A_{14} hydrate (14) showed strong peaks at $M^+ - 130$ and at *m/e* 130, both ions being characteristic[7] of ring D fragmentation in the corresponding derivatives of gibberellins A_2 (33) and A_{10} (34), and it was identical to the mass spectrum of the trimethylsilyl ether of the methyl ester of the major product obtained[18] from the reaction of gibberellin A_{14} with 1·0N-hydrochloric acid at 100° for 3 h. Similar mass spectral evidence determined the structure of gibberellin A_{13} hydrate (13), including the identity of the methyl ester trimethylsilyl ether with that of the product from gibberellin A_{13} and 1·0N-hydrochloric acid. The hydrate (13) of gibberellin A_{13} has also been isolated from *G. fujikuroi* by Takahashi and his co-workers (personal communication). The status of the hydrates (13) and (14) as enzyme-derived metabolites is the same as that of gibberellins A_2 (33)[19] and A_{10} (34).[20] The gibberellin numbers A_{41} and A_{42} are accordingly allocated[21] to gibberellin A_{13} hydrate (13) and gibberellin A_{14} hydrate (14), respectively.

Gibberellins A_9 (3) and A_{24} (11), fujenal (35), the diacid (15), and a new anhydride were shown to be present by g.l.c.–m.s. in the last fraction, eluted by acetone from the charcoal–Celite column of the crude acids from strain TP70. Further fractionation on a silica gel column gave the gibberellins A_9 (3) and A_{24} (11), the new anhydride, and fujenal (35) but none of the corresponding diacid (15). From the amount of fujenal (35) isolated, it appeared that the diacid (15) had been converted into the anhydride (35) during chromatography; this conversion was shown to occur by adsorption of the unfractionated mixture from a solution in acetone on silica gel, which was then eluted to give only the anhydride (35). Other investigators (*e.g.* ref. 6) have also isolated the anhydride (35) from the acidic fraction of the metabolites of *G. fujikuroi*. A reversible interconversion of the anhydride (35) and the diacid (15) is compatible with these results.

The new anhydride, $C_{19}H_{24}O_4$, was shown by high resolution mass spectrometry to contain two tertiary methyl groups (τ 9·02 and 8·64), an exocyclic methylene group (τ 5·05 and 5·12; $\nu_{max.}$ 3075, 1664, and 878 cm^{-1}), and an anhydride system (1788 cm^{-1}). The presence of the anhydride function was confirmed by methanolysis to a monomethyl ester, characterised by g.l.c.–m.s. as the monotrimethylsilyl ester monomethyl ester and the dimethyl ester. Reduction of the latter with lithium aluminium hydride gave a diol which formed a bistrimethylsilyl ether, the mass spectrum of which contained a base peak at $M^+ - 103$ and an intense peak at *m/e* 103, assigned the structure $CH_2{=}\overset{+}{O}{-}SiMe_3$. This fragmentation suggests that at least one of the carboxy-groups in the original anhydride is directly attached to a tertiary centre.

The presence of an anhydride group in the new metabolite leaves one unassigned oxygen function, which is presumed to be an ether since hydroxy-absorption is absent in the i.r. spectrum and since a trimethylsilyl ether was not obtained. The absence of a carbonyl group is also indicated by the i.r. spectrum and by the formation of a diol and no triol on hydride reduction. Attempts to open the ether function with 10% sulphuric acid in acetone or with acetyl tosylate in acetonitrile failed. In the latter case attack at the exocyclic double bond occurred to give a *C*-acetyl derivative

[15] K. Hitaga, T. Yokota, M. Murofushi, and N. Takahashi, *Agric. and Biol. Chem.* (*Japan*), 1972, **36**, 345.
[16] T. Yokota, N. Murofushi, N. Takahashi, T. Yokota, and S. Tamura, *Agric. and Biol. Chem.* (*Japan*), 1971, **35**, 573.
[17] D. H. Bowen, D. M. Harrison, and J. MacMillan, *J.C.S. Chem. Comm.*, 1972, 808.
[18] P. Gaskin and J. MacMillan, unpublished results.
[19] J. F. Grove, *J. Chem. Soc.*, 1961, 3545.
[20] J. R. Hanson, *Tetrahedron*, 1966, **22**, 701.
[21] J. MacMillan and N. Takahashi, *Nature*, 1968, **217**, 170.

(=CH·COMe). Insufficient of the anhydride was available for its structure to be defined, although structure (36) is an attractive possibility which accommodates the available evidence and has a biogenetically feasible origin from a precursor such as the anhydride (35). However, as pointed out by a referee, the anhydride (36) could be an artefact formed by auto-oxidation of the anhydride (35) to the corresponding 7-nor-5,7-diol (*cf.* ref. 22).

EXPERIMENTAL

For general experimental details see Part V.[23] Analytical t.l.c. plates were viewed in visible or u.v. light after spraying with 5% sulphuric acid in ethanol and heating at 120°. G.l.c.–m.s. was performed either with a Varian MAT CH7 instrument as described in Part VI [24] or with a G.E.C.–A.E.I. MS30 instrument as described in Part VII.[25]

Isolation of Gibberellins.—A concentrate of the gibberellin A_3 mother liquors from an acidic butyl acetate of a commercial fermentation of *G. fujikuroi* strain TP70 was donated by I.C.I. Ltd. A portion of the acids (750 g) was adsorbed on silica gel from a solution in acetone and placed on a column of charcoal (2·6 kg) and Celite (1·3 kg). The column was eluted with water–acetone mixtures in fractions (10 l) which were evaporated to the aqueous phase *in vacuo*, acidified to pH 2·5, and extracted with ethyl acetate.

(a) *Fraction* 1 (25% acetone in water) contained no organic material and was discarded.

(b) *Fraction* 2 (40% acetone in water) gave a gum (2 g) which contained 80% (g.l.c.) of gibberellin A_{13} hydrate (13) identical (g.l.c.–m.s. of trimethyl ester and bistrimethylsilyl ether trimethyl ester) with the product obtained [18] by treatment of gibberellin A_{13} with 1N-hydrochloric acid at 100° for 3 h; *m/e* (trimethyl ester) 438 (M^+, 0·5%), 420 (1·5), 406 (1), 388 (13), 360 (11), 328 (100), 310 (21), 300 (36), 283 (31), 269 (25), 241 (15), 223 (24), and 164 (19); *m/e* (bistrimethylsilyl ether trimethyl ester) (g.l.c.–m.s.) 582 (M^+, 2%), 567 (11), 550 (6), 523 (10), 460 (6), 283 (12), 231 (10), 209 (17), 160 (25), and 129 (100).

(c) *Fraction* 3 (45% acetone in water) gave a semi-crystalline brown oil (19 g) which was crystallised from ethyl acetate–light petroleum to give gibberellin A_{13} (9) (3·1 g). The recovered material (1·5 g) from the mother liquors was chromatographed on a column of silica gel (500 g), which was eluted with light petroleum containing ethyl acetate in 500 ml fractions. The fraction eluted with ethyl acetate–light petroleum (4 : 1) gave crude gibberellin A_{16} (1·7 g). A portion (417 mg) was purified by t.l.c. on silica gel G in ethyl acetate–light petroleum–acetic acid (50 : 40 : 1; 3 elutions). The band at R_F 0·40 was extracted with ethyl acetate to give *gibberellin* A_{16} (4) (93 mg), m.p. 137—140° (from ethyl acetate–light petroleum) (Found: M^+, 348·157. $C_{19}H_{24}O_6$ requires M, 348·156); $\nu_{max.}$ 3520, 3440, 1752, 1708, 1655, and 897 cm^{-1}; τ 8·33 (s, 20-H_3), 6·72 (d, J 12 Hz, 5-H), 6·04 (d, J 12 Hz, 6-H), 5·77 (q, J 1 and 4 Hz, 3-H), 5·39 (q, J 6 and 10 Hz, 1-H), 5·12br (17-H), and 5·01br (17-H); *m/e* 348 (M^+, 12%), 330 (24), 312 (14), 302 (26), 286 (65), 284 (24), 274 (100), 268 (67), and 228 (31).

A further quantity of pure gibberellin A_{16} (116 mg) was obtained by chromatography of the crude gibberellin A_{16} (485 mg) on a column (100 × 2·5 cm) of Sephadex G-25 (100 g; fine) with the solvent system benzene–ethyl acetate–acetic acid–water (55 : 25 : 30 : 50) as described by Pitel *et al.*[26]

The fraction eluted from the silica gel column of the gibberellin A_{13} mother liquors with ethyl acetate–light petroleum (9 : 1) gave hydrated gibberellin A_{14} (14), which was further purified by rechromatography on silica gel as before, then by t.l.c. on silica gel G in ethyl acetate–light petroleum–acetic (70 : 30 : 1; 3 elutions; R_F 0·30). Precipitated from ethyl acetate by light petroleum, it had m.p. 174—182°; $\nu_{max.}$ 2300—3650, 3400br, and 1700br cm^{-1}; *m/e* 366 (M^+, absent), 348 (23%), 330 (100), 315 (40), 302 (17), 287 (23), 284 (25), 271 (28), and 269 (34); the bistrimethylsilyl ether dimethyl ester was identical (g.l.c.–m.s.) with that of the product obtained [18] by heating gibberellin A_{14} with 1N-hydrochloric acid at 100° for 3 h and had *m/e* 538 (M^+, 3%), 523 (24), 481 (11), 448 (11), 416 (19), 376 (100), 299 (18), 287 (88), 269 (54), 231 (80), 130 (40), and 129 (75); the methyl ester had τ 9·33 (s, 20-H_3), 8·83 (s, 18-H_3), 8·65 (s, 17-H_3), 7·74 (d, J 12 Hz, 5-H), 6·70 (d, J 12 Hz, 6-H), 6·33 and 6·30 (both s, 2 × CO_2Me), and 5·88br (3-H).

(d) *Fraction* 4 (50% acetone in water) gave a yellow oil (254 g) of which a portion (40 g) was chromatographed on a silica gel column (500 g) and eluted with ethyl acetate–light petroleum in 100 ml fractions. Elution with ethyl acetate–light petroleum (3 : 2) gave a fraction (229 mg) rich in gibberellin A_{36}, which was subjected to t.l.c. on silica gel HF with ethyl acetate–light petroleum–acetic acid (50 : 50 : 1; 2 elutions; R_F 0·4) to give crude gibberellin A_{36} (*ca.* 85% by g.l.c. of methyl ester). Repeat t.l.c. under the same conditions (3 elutions) gave *gibberellin* A_{36} [(16) ⇌ (20)] (39 mg), m.p. 205—208° (from ethyl acetate–light petroleum) (Found: C, 66·0; H, 7·5. $C_{20}H_{26}O_6$ requires C, 66·3; H, 7·2%); $\nu_{max.}$ 3460, 3350br, 3070w, 1720, 1710, 1698, 1659, and 878 cm^{-1}; for τ values see Table; *m/e* 362 (M^+, 4%), 344 (100), 326 (42), 318 (33), 316 (38), 298 (49), 270 (62), and 91 (79). Gibberellin A_{36} was also purified by chromatography on a column (30 × 5·5 cm) of Sephadex G-25 (fine) by the method of Pitel *et al.*,[26] with benzene–ethyl acetate–acetic acid–water (55 : 25 : 30 : 60) as eluant. It was obtained in fractions 18—32 (20 ml fractions).

Further elution of the silica gel column of fraction 4 with ethyl acetate–light petroleum (1 : 1) gave a gum, a portion (200 mg) of which was chromatographed on Sephadex G-25 (fine) with the biphasic solvent system benzene–light petroleum–acetic acid–water (6 : 2 : 5 : 3).[26] Fractions (20 ml) were collected, giving gibberellin A_{14} (71 mg) (fractions 26—31), gibberellin A_4 (24 mg) (fractions 40—50), gibberellin A_7 (13 mg) (fractions 60—71), and the 19,2-lactone (8) (48 mg) (fractions 85—100). These compounds were identified as their methyl esters by mass spectroscopy and by g.l.c. retention times.

(e) *Fraction* 5 (40% acetone in water) and *fraction* 6 (60% acetone in water) had compositions similar to fraction 4 by g.l.c. after methylation.

(f) *Fraction* 7, eluted with acetone, gave a gum (50 g), a portion (3 g) of which was chromatographed on a silica gel

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column (200 g). Elution with ethyl acetate–light petroleum (1 : 9) gave gummy crystals (42 mg) which were purified by t.l.c. on silica gel G with ethyl acetate–light petroleum (1 : 4). Recovery from the zone at R_F 0·40 in ethyl acetate gave an anhydride (23 mg), m.p. 112—114° (from ethyl acetate–light petroleum) (Found: M^+, 316·167. Calc. for $C_{19}H_{24}O_4$: M, 316·167); $\nu_{max.}$ 3070, 1855w,br, 1788br, 1656, and 880 cm^{-1}; τ 9·02 (3H, s), 8·64 (3H, s), and 5·12br and 5·05br (both 1H); *m/e* 316 (M^+, 53%), 301 (6), 273·113 (3; calc. for $C_{16}H_{17}O$: 273·113; calc. for $C_{17}H_{21}O_3$: 273·149, with rel. intensity 3 : 1), 245·117 (35; calc. for $C_{15}H_{17}O_3$: 245·118), 244·182 (43; calc. for $C_{17}H_{24}O$: 244·183), and 229·158 (100; calc. for $C_{16}H_{21}O$: 229·159).

Fractions eluted from the silica gel column with ethyl acetate–light petroleum (1 : 5) and (1 : 4) gave *ent*-7-oxo-6,7-secokaur-16-ene-6,19-dioic anhydride (35) (221 mg), m.p. 168—170°. Fractions eluted with ethyl acetate–light petroleum (35 : 65 and 40 : 60) gave gibberellin A_9 (43 mg). Fractions eluted with ethyl acetate–light petroleum (1 : 1 and 55 : 45) gave impure gibberellin A_{24}, which was purified by t.l.c. on silica gel (2 elutions; R_F 0·45) with ethyl acetate–light petroleum–acetic acid (40 : 60 : 1) to give pure gibberellin A_2 (11) (15 mg).

Derivatives of Gibberellin A_{36}.—(a) The *dimethyl ester* (17) had m.p. 105—108° (from ethyl acetate–light petroleum) (Found: M^+, 392·204. $C_{22}H_{30}O_6$ requires M, 390·204); $\nu_{max.}$ (CCl_4) 3635, 3540br, 3070, 2760w, 2720w, 1735br, 1658, and 883 cm^{-1}; for τ values see Table; *m/e* 390 (M^+, 1·5%), 372 (1·5), 358 (37), 340 (32), 330 (67), 326 (94), 312 (35), 302 (26), 298 (34), 284 (100), 225 (67), and 172 (70); the trimethylsilyl ether dimethyl ester had *m/e* (g.l.c.–m.s.) 462 (M^+, 10%), 447 (11), 444 (5), 430 (48), 402 (19), 374 (14), 340 (19), 312 (52), 284 (98), 231 (9), 225 (53), 211 (76), 171 (32), and 129 (100).

(b) 3-O-*Acetylgibberellin* A_{36} *dimethyl ester* (19), prepared from gibberellin A_{36} dimethyl ester with acetic anhydride in pyridine, was obtained as an oil, homogeneous by g.l.c. and t.l.c., $\nu_{max.}$ (CCl_4) 1761, 1658, and 885 cm^{-1}; τ 8·89 (s, 18-H_3), 7·90 (s, OAc), 6·28 and 6·35 (each s, 2 × OMe), 7·32 (d, J 13 Hz, 5-H), 6·05 (d, J 13 Hz, 6-H), 5·15br and 5·07br (17-H_2), 4·65 (t, J *ca.* 3 Hz, 3-H), and 0·32 (s, 20-H); *m/e* 432 (M^+, absent), 400 (4%), 372 (16), 340 (21), 328 (18), 312 (70), 284 (100), 231 (86), 225 (76), 223 (42), 216 (37), and 171 (81).

(c) *Gibberellin* A_{36} *diacetate* (21), prepared from gibberellin A_{36} with acetic anhydride in pyridine, had m.p. 233—237° (from ethyl acetate–light petroleum) (Found: C, 64·4; H, 7·0. $C_{24}H_{30}O_8$ requires C, 64·6; H, 6·8%); $\nu_{max.}$ 3270, 1770, 1754, 1735, 1700, 1660, and 877 cm^{-1}; for τ values see Table; *m/e* 446 (M^+, absent), 395 (99%), 349 (14), 293 (10), 220 (10), 162 (21), and 43 (100). The methyl ester had *m/e* 460 (M^+, absent), 429 (6%), 418 (3), 400 (6), 386 (3), 382 (5), 371 (7), 368 (13), 358 (34), 340 (40), 326 (36), 284 (100), and 225 (68).

3-O-*Acetylgibberellin* A_{13} *Anhydride Monomethyl Ester* (29).—Prepared by methylation of acetyl gibberellin A_{13} anhydride (29) [9] with diazomethane, this was obtained as a homogeneous *oil* (t.l.c. and g.l.c.) (Found: M^+, 416·184. $C_{23}H_{28}O_7$ requires M, 416·183); $\nu_{max.}$ (CCl_4) 1805, 1768, 1752, 1738, 1665, and 886 cm^{-1}; τ 8·84 (s, 18-H_3), 7·88 (s, OAc), 7·54 (d, J 12 Hz, 5-H), 7·24 (d, J 12 Hz, 6-H), 6·28 (s, OMe), 5·06br (3-H and 17-H), and 4·95br (17-H).

3-O-*Acetylgibberellin* A_{13} *Dimethyl Ester* (26).—The monomethyl ester (29) (113 mg) in methanol (2 ml) was heated at 150° for 15 h in a sealed tube. Evaporation of the methanol and t.l.c. of the product on silica gel G in ethyl acetate–light petroleum–acetic acid (30 : 60 : 1; R_F 0·5) gave *acetyl gibberellin* A_{13} *dimethyl ester* (26) (90 mg), m.p. 190—193° (from ethyl acetate–light petroleum) (Found: M^+, 448·210. $C_{24}H_{32}O_8$ requires M, 448·210); $\nu_{max.}$ 3255, 3070, 1734, 1713, and 1657 cm^{-1}; τ 8·98 (s, 18-H_3), 7·91 (s, OAc), 6·29 and 6·35 (each s, 2 × OMe), 7·44 (d, J 13 Hz, 5-H), 6·23 (d, J 13 Hz, 6-H), 5·17br and 5·09br (17-H_2), and 4·75 (t, J *ca.* 3 Hz); *m/e* 448 (M^+, 9%), 416 (24), 370 (39), 356 (85), 328 (88), and 296 (100). The dimethyl monotrimethylsilyl ester had *m/e* (g.l.c.–m.s.) 520 (M^+, 2%), 505 (18), 488 (34), 460 (21), 428 (50), 400 (22), 370 (48), 368 (93), 340 (23), 310 (33), 283 (100), 282 (53), 268 (20), 251 (30), and 223 (71).

Oxidation of Acetylgibberellin A_{36} *Dimethyl Ester* (19).—The acetate (2 mg) in acetone (5 ml) was treated with Jones reagent in slight excess at 0° for 30 min. Work-up in the usual way gave a complex mixture (t.l.c., g.l.c.). Trimethylsilylation and g.l.c.–m.s. of the product showed the presence of, *inter alia*, acetylgibberellin A_{13} dimethyl ester monotrimethylsilyl ester (27).

A similar result was obtained with chromium trioxide. With silver nitrate–potassium hydroxide the acetate (19) was recovered unchanged.

Reduction of Gibberellin A_{36} *to Gibberellin* A_{37} (31).—Gibberellin A_{36} (55 mg) in ethanol (10 ml) was treated with sodium borohydride (250 mg) at 20° for 6 h. Work-up in the usual way gave an oil which was purified by t.l.c. on silica gel G in ethyl acetate–light petroleum–acetic acid (60 : 40 : 1). The band at R_F 0·45 was extracted with ethyl acetate to give *gibberellin* A_{37} (21 mg), m.p. 228—232° (from ethyl acetate–light petroleum) (Found: M^+, 346·177. $C_{20}H_{26}O_5$ requires M, 346·178); $\nu_{max.}$ 3495, 3080w, 1715, 1687, 1660, and 885 cm^{-1}; for τ values see Table; *m/e* 346 (M^+, 87%), 328 (36), 318 (50), 310 (22), 300 (57), 282 (100), 270 (85), 237 (57), and 43 (73). The *methyl ester* was obtained with diazomethane as an oil, homogeneous by t.l.c. and g.l.c. (Found: M^+, 360·194. $C_{21}H_{28}O_5$ requires M, 360·194); $\nu_{max.}$ (CCl_4) 3630, 3460br, 3075, 1740, 1720, 1660, and 887 cm^{-1}; for τ value see Table; *m/e* 360 (M^+, 32%), 342 (19), 332 (42), 329 (23), 328 (25), 314 (12), 310 (32), 300 (56), 296 (23), 284 (100), 283 (50), 282 (89), and 237 (71); the methyl ester trimethylsilyl ether had *m/e* (g.l.c.–m.s.) 432 (M^+, 8%), 342 (14), 327 (8), 316 (11), 310 (26), 284 (22), 283 (8), 282 (13), 258 (11), 237 (8), and 129 (100).

Reactions of the Unknown Anhydride.—(a) *Methanolysis.* The anhydride (5 mg) in methanolic 10% potassium hydroxide (5 ml) was refluxed for 13 h. Work-up gave a monomethyl ester which was trimethylsilylated to give a product, *m/e* (g.l.c.–m.s.) 420 (M^+, 3%), 405 (2), 361 (14), 304 (60), 303 (100), 271 (16), 243 (100), 229 (21), 225 (19), and 109 (19); the methylation product of the monomethyl ester had *m/e* (g.l.c.–m.s.) 362 (M^+, 11%), 342 (24), 303 (100), 271 (6), 243 (56), 235 (26), 225 (8), and 109 (16).

(b) *Reduction.* The anhydride (1 mg) in tetrahydrofuran (5 ml) was refluxed with lithium aluminium anhydride (20 mg) for 12 h. Work-up in the usual way gave a diol, homogeneous by g.l.c. The trimethyl silylation product had *m/e* (g.l.c.–m.s.) 450 (M^+, 17%), 347 (100), 277 (15), 257 (5), 243 (8), and 103 (57).

(c) *With acetyl tosylate.* The anhydride (1 mg) in dry acetonitrile (100 μl) was treated at 65° for 17 h with acetyl tosylate (10 mg). Water was added and the solution was extracted with ethyl acetate. The recovered product

contained one major product which had *m/e* (g.l.c.–m.s.) 358 (M^+, 53%), 343 (7), 340 (4), 315 (8), 286 (78), 271 (100), 243 (12), 203 (25), 178 (29), 161 (37), 135 (25), 125 (55), and 107 (53).

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