The Synthesis of 2,2-Dialkylgibberellins

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An improved synthesis of 2,2-dimethylgibberellin A_4 (3), the highly active plant growth promoter, is described. The methods used were adapted to the synthesis of both radio- and stable-isotope labelled (3). The route was applied, with limited success, to the synthesis of 2,2-diethylgibberellin A_4 (6). Incubation of 2,2-dimethylgibberellin A_4 (3) with cultures of *Gibberella fujikuroi*, mutant B1-41a, gave 2,2-dimethylgibberellin A_1 (20) on a preparative scale.

In a recent study of structure–biological activity relationships in the gibberellin (GA) family of plant hormones we described the synthesis [1] and bioassay [2] of several C-2 and C-3 substituted derivatives of GA_9 (1) and GA_4 (2). Of the compounds prepared, 2,2-dimethylGA_4 (3) showed exceptionally high biological activity in certain bioassays. Indeed in the d_5-maize assay, 2,2-dimethylGA_4 (3) was the most active compound known at that time with an activity of 100 times that of GA_4 and 50 times that of gibberellic acid (GA_3). In order to examine further the biological properties of this compound, we required a more efficient chemical synthesis. We now report, in detail,[3] an alternative synthesis of (3) and the application of the method to the synthesis of the isotopically labelled derivatives (4) and (5) as well as 2,2-diethylgibberellin A_4 (6).

The previous synthesis [1] of 2,2-dimethylgibberellin A_4 (3) proceeded *via* alkylation of the keto-ester (7) with an excess of potassium t-butoxide and methyl iodide to give the 2,2-dimethylketo-ester (8), containing *ca.* 10% of the enol-ether (21). Reduction of (8) gave the alcohol (9) [and its epimer (10)], hydrolysis of which, by sodium hydroxide, required protection of the 3β-hydroxy as a tetrahydropyranyl ether in order to avoid the known [4] base-catalysed epimerisation of 3β-hydroxy-C_{19} gibberellins. The aim of this work was to devise a synthesis where protection and deprotection of the 3β-hydroxy group could be avoided.

Results and Discussion

Our initial stratagem was to examine the preparation and 2,2-dialkylation of the tri-n-butyltin ester (12). Trialkyltin esters have been used to protect carboxylic acids in the penicillin [5] and amino acid [6] areas. They are easily prepared from the carboxylic acid and bis(tri-n-butyltin) oxide and they can be cleaved by treatment with mild acid or with bases such as hydroxide and methoxide. We have previously observed [7] the formation of a gibberellin-7-tri-n-butyltin ester in the reaction of the carboxylic acid with tri-n-butyltin hydride and also noted its easy hydrolysis when chromatographed on silica gel.

Reaction of the keto-acid (11) with bis(tri-n-butyltin) oxide in refluxing benzene with azeotropic removal of water gave the ester (12). Treatment of the ester (12) with methyl iodide and either potassium t-butoxide and 18-crown-6-ether in t-butyl alcohol–benzene or potassium hydride and 18-crown-6-ether in tetrahydrofuran gave the dimethyl ketone methyl ester (8). However, treatment of (12) with an excess of lithium di-isopropylamide (LDA) in tetrahydrofuran and then with methyl iodide, followed by work-up with acetic acid, gave the required dimethyl-keto-acid (13). The addition of 5 equivalents of hexamethylphosphoramide (HMPA) to this reaction resulted in rapid formation of the dimethylketone methyl ester (8). Large-scale alkylation of (12) with LDA–methyl iodide gave the 2,2-dimethylketo-acid (13) in 30% isolated yield. No other products were detected in this reaction.

We subsequently achieved the C-2 alkylation of the keto-acid (11) directly with LDA–methyl iodide in similar yield. Addition of HMPA again caused methyl ester formation. Thus it appears that the tri-n-butyltin ester is hydrolysed by potassium hydride, potassium t-butoxide, or LDA to give the carboxylate anion. Whether this anion reacts with methyl iodide to form the methyl ester depends on the counter ion and the medium. With K^+ and 18-crown-6-ether the ester is obtained. With Li^+ the ester is not formed unless HMPA is present.

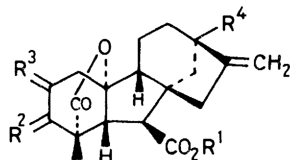
Table. Reduction of the 2,2-dimethylketo-acid (13)

Reagents	Conditions	% β-ol (3) [a]	% α-ol (14) [a]
$NaBH_4$–Pr^iOH	1 h, 20 °C	11	89
$NaBH_4$–MeOH	4 h, 20 °C	16	84
$NaBH_4$–DME	1 h, 85 °C	26	74
$Li(Bu^s_3)BH$–THF	22 h, 20 °C	19	81
$LiAlH_4$–THF	1 h, 0 °C	10	90
$Na(Pr^iO)_3BH$–Pr^iOH	1 h, 82 °C	38	62
$Na(Bu^tO)_3BH$–Bu^tOH	1 h, 83 °C	26	74
$Li(Bu^tO)_3AlH$–THF	1 h, 0 °C	22 [b]	78 [b]

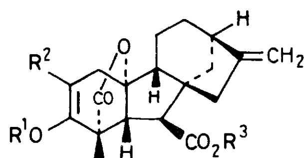
[a] By g.l.c. of $Me,SiMe_3$ derivatives on 2% SE33 at 220 °C. [b] Contains unchanged ketone (48% of total product).

In the previous synthesis we observed [1] that sodium borohydride reduction of the 2,2-dimethylketo-ester (8) was solvent dependent. In tetrahydrofuran–methanol the 3α-alcohol (10) was the major product, whereas in refluxing 1,2-dimethoxyethane (9) and (10) were formed in equal amounts. The results of a similar exploratory study of the reduction of the 2,2-dimethylketo-acid (13) are shown in the Table. For large-scale preparations reduction of (13) with sodium isopropoxyborohydride in refluxing isopropyl alcohol was chosen. 2,2-DimethylGA_4 (3) was obtained in 22% isolated yield together with the 2,2-dimethyl-3α-alcohol (14) in 54% yield. Similar reductions of (13) were carried out with [^{2}H] and [^{3}H] sodium isopropoxyborohydride to give the labelled 2,2-dimethylgibberellin A_4 (4) and (5).

3-Hydroxy-C_{19}-gibberellins are known [4] to undergo retro-aldol equilibration. When the 2,2-dimethyl-3α-alcohol (14) was refluxed in potassium carbonate and aqueous methanol g.l.c. indicated that epimerisation had proceeded to give a mixture containing *ca.* 30% β-alcohol (3) and 70% α-alcohol (14). Thus the 3α-isomer (14) was recycled to increase the yield of 2,2-dimethylgibberellin A_4 (3).



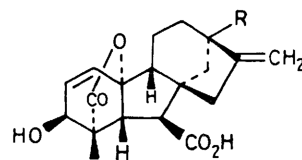
	R^1	R^2	R^3	R^4
(1)	H	H_2	H_2	H
(2)	H	β-OH,H	H_2	H
(3)	H	β-OH,H	Me_2	H
(4)	H	β-OH,^{2}H	Me_2	H
(5)	H	β-OH,^{3}H	Me_2	H
(6)	H	β-OH,H	Et_2	H
(7)	Me	O	H_2	H
(8)	Me	O	Me_2	H
(9)	Me	β-OH,H	Me_2	H
(10)	Me	α-OH,H	Me_2	H
(11)	H	O	H_2	H
(12)	$SnBu_3$	O	H_2	H
(13)	H	O	Me_2	H
(14)	H	α-OH,H	Me_2	H
(15)	Me	O	Et_2	H
(16)	Me	β-OH,H	Et_2	H
(17)	Me	α-OH,H	Et_2	H
(18)	H	α-OH,H	Et_2	H
(19)	H	β-OH,H	H_2	OH
(20)	H	β-OH,H	Me_2	OH



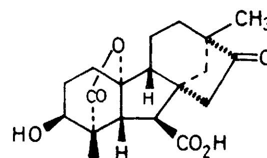
	R^1	R^2	R^3
(21)	Me	Me	Me
(22)	Et	H	Me
(23)	Et	Et	Me
(24)	Et	Et	H

This new synthesis (three steps from GA_4) represents a considerable improvement and now makes 2,2-dimethylgibberellin A_4 easily obtainable. The obvious progression was to prepare other 2,2-dialkylgibberellins by this route. However, treatment of the keto-acid (11) with an excess of LDA and ethyl iodide gave no reaction, even at reflux temperature. Ethyl iodide is known [8] to be some 37 times less reactive than methyl iodide towards enolate anions. The addition of HMPA resulted in C-2 ethylated products but also caused, as expected, formation of the ethyl ester. As esterification could not be avoided, a large-scale ethylation with LDA–HMPA–ethyl iodide was carried out on the methyl ester of the ketone (7). The product, which was one spot by analytical t.l.c., consisted not only of the required 2,2-diethyl ketone (15) but also contained considerable amounts of the *O*-alkylated products (22) and (23) which were detected by g.l.c. and g.c.–m.s. The ratio of the products was 26% of (15), 21% of (22), and 53% of (23). The *O*-ethylated products were easily recognisable by the strong loss of 44 a.m.u. (CH_3–CH=O) in their mass spectra. The large proportion of *O*-ethylation is undoubtedly due to the presence of HMPA which is well known [9] to promote *O*-alkylation.

Treatment of the three-component mixture of (15), (22), and (23) with acidic ion-exchange resin in methanol resulted in hydrolysis of (22) to the ketone (7) which was separable by chromatography from the unchanged (15) and (23). The failure of (23) to undergo acid hydrolysis was surprising.



(25) R = H
(26) R = OH



(27)

Under harsher conditions (refluxing with toluene-*p*-sulphonic acid) endomerisation of the 16,17-double bond occurred but the enol–ether function remained unaffected.

Fractional crystallisation of the mixture of (15) and (23) gave some pure (23) which was hydrolysed by sodium hydroxide in aqueous methanol to the acid (24). The material from the mother liquors was reduced with sodium tri-isopropoxyborohydride in refluxing isopropyl alcohol to give the 3β- and 3α-alcohols (16) and (17) in approximately equal amounts along with unchanged enol-ether (23). The three components were separated by chromatography. Finally the 3β-alcohol (16) was demethylated using sodium ethanethiolate in HMPA to give 2,2-diethylgibberellin A_4 (6), accompanied by a small amount of its 3-epimer (18) which was removed by chromatography.

Both 2,2-dimethylGA_4 (3) and 2,2-diethylGA_4 (6) are very hygroscopic compounds. Each crystallises from aprotic solvents but microanalysis of both show the inclusion of one molecule of water. The water could be removed by heating over P_2O_5 *in vacuo*, but the dried compound rapidly gained weight on exposure to air. Indeed in the *X*-ray crystal structure [3,10] of 2,2-dimethylgibberellin A_4 (3) a water molecule is present bridging from the C-7 carboxy function to the 3β-hydroxy group.

Incubations of 2-alkylated GAs with *Gibberella fujikuroi*, mutant B1-41a were also investigated. Gibberellin A_4 (2) is metabolised [11] by this organism to GA_7 (25) which, in turn, is converted into GA_3 (26), the terminal metabolite; GA_1 (19) is a minor product of GA_4 (2) metabolism. In 2,2-dimethylGA_4 (3) C-1/C-2 dehydrogenation is not possible and, therefore, it was anticipated that metabolism would proceed solely by C-13 hydroxylation to give 2,2-dimethylgibberellin A_1 (20). Indeed 2,2-dimethylGA_1 (20) is the major metabolite when 2,2-dimethylGA_4 (3) is incubated with mutant B1-41a, providing a preparative route to (20) which was isolated from the culture extract by chromatography and preparative h.p.l.c.

Experimental

All solvents were redistilled before use. Light petroleum refers to the fraction boiling at 60—80 °C. T.l.c. and p.l.c. were carried out on Merck Kieselgel 60 HF254 and visualised with iodine vapour or by spraying with 5% H_2SO_4 in EtOH followed by heating to 120 °C for 5 min. Flash chromatography [12] was carried out on Merck Kieselgel 60 (40—63 μm) or Woelm silica (32—63 μm). N.m.r. chemical shifts are relative to internal tetramethylsilane. G.l.c. and g.c.–mass spec.

analyses were on capillary glass WCOT OV-1 columns with derivatisation, where necessary, as Me esters $SiMe_3$ ethers.

Work-up refers to addition to water, acidification with HCl to pH 4, and recovery in ethyl acetate.

3-Ketogibberellin A_4 (11). This was prepared as previously described.[1]

Lithium di-isopropylamide (*LDA*). This was prepared by the method of House *et al.*;[13] butyl-lithium (110 ml; 1.6M-solution in hexane) was injected into a rubber septum sealed round-bottomed flask under nitrogen. n-Pentane (150 ml) (distilled from phosphorus pentaoxide) was added. Di-isopropylamine (20.75 g) (distilled from potassium hydroxide) was added dropwise with stirring during 40 min. The resultant, slightly cloudy solution was allowed to settle overnight. The concentration of LDA was determined by the method of Gall and House[14] and was typically 0.5—0.6M.

Treatment of the Tri-n-butyltin Ester (12) *with Lithium Di-isopropylamide and Methyl Iodide*.—The keto-acid (11) (10.3 g) in benzene (500 ml) with bis(tri-n-butyltin) oxide (9.29 g) was refluxed in a Dean and Stark apparatus for 1 h. The benzene was removed under reduced pressure and replaced with dry oxygen-free tetrahydrofuran (1 l). LDA solution (280 ml, *ca.* 5 equiv.) was transferred to the ice-cold stirred solution of the tri-butyltin ester (12) by means of a double-ended needle under positive N_2 pressure. After the addition was complete the cooling bath was removed and dry methyl iodide (20 ml, from phosphorus pentaoxide) was added and the reaction stirred at room temperature for 5.5 h.

The reaction mixture was then added with stirring and ice cooling to ethyl acetate (1.5 l) containing acetic acid (60 ml). This solution was then diluted with water and the layers separated. The aqueous layer was further extracted (× 2) with ethyl acetate and the combined organic layers evaporated under reduced pressure with the addition of toluene to assist removal of residual acetic acid by azeotropic distillation.

The product was combined with that from a second reaction using the keto-acid (7.5 g), bis(tri-n-butyltin) oxide (6.7 g), LDA solution (220 ml), and methyl iodide (18 ml). The combined products were chromatographed on silica gel (1 kg), with increasing amounts of ethyl acetate in light petroleum as eluant. After elution of the tri-butyltin residues in the early fractions, elution with 25—35% ethyl acetate gave an oil (13 g) which was rechromatographed on silica gel (500 g) under the same conditions to give at 25—30% ethyl acetate the ent-*2,2-dimethyl-10-hydroxy-3-oxo-20-norgibberell-16-ene-17,19-dioic acid 19,10-lactone* (13) (5.4 g) which crystallised from ethyl acetate–light petroleum, m.p. 216—218 °C (Found: C, 70.5; H, 7.0. $C_{21}H_{26}O_5$ requires C, 70.4; H, 7.3%) $\nu_{max.}$ (Nujol) 1 780, 1 730, 1 700, 1 660, and 880 cm^{-1}; δ ($CDCl_3$) 1.25 (s, 3 × CH_3), 2.86 (d, *J* 10 Hz, 5-H), 3.04 (d, *J* 10 Hz, 6-H), and 4.90 and 5.01 (each br, s, 17-H_2); *m/z* 358 (M^+, 13%), 340 (2), 314 (8), 312 (4), 274 (100), 246 (13), 229 (14), and 201 (12); δ (Me ester, $CDCl_3$) 1.18 (s, 1 × CH_3), 1.23 (s, 2 × CH_3), 2.83 (d, *J* 10 Hz, 5-H), 3.07 (d, *J* 10 Hz, 6-H), 3.72 (s, CO_2Me), and 4.88 and 5.00 (each br, s, 17-H_2).

Treatment of the Keto-acid (10) *with LDA–Methyl Iodide*.—To the keto-acid (10) (475 mg) in dry THF (50 ml) was added LDA solution (15 ml). After 15 min, dry methyl iodide (1.0 ml) was added and the reaction stirred for 5 h. The mixture was added to ethyl acetate (100 ml) containing acetic acid (20 ml). The mixture was filtered and evaporated with toluene. The residue was partitioned between water and ethyl acetate and the resultant gum was chromatographed as above to give the dimethylketo-acid (13) (124 mg), identical with the material prepared above.

Reduction of the 2,2-Dimethylketo-acid (13).—Sodium borohydride (750 mg) in propan-2-ol (100 ml) was refluxed under nitrogen for 1 h. After cooling of the mixture to room temperature the 2,2-dimethylketo-acid (13) (5 g) in propan-2-ol (150 ml) was added during 20 min. Then the reaction mixture was refluxed for a further 1 h. After concentration under reduced pressure to *ca.* 75 ml the solution was poured into water. Work-up gave a gum (4.7 g) which on trituration with ethyl acetate–light petroleum (1 : 1; 2 × 20 ml) gave the pure α-alcohol (14) (1.5 g) (see later for data). The filtrate from the trituration contained a mixture (3.2 g, *ca.* 1 : 1) of the α- and β-alcohols (14) and (3) which were separated by flash chromatography (16 × 4 cm). Elution with ethyl acetate–light petroleum–acetic acid (40 : 60 : 0.5, 3 000 ml) in 100-ml fractions gave the faster running β-alcohol (3) (1.1 g) followed by a mixture (3) and (14), (350 mg) and then the α-alcohol (14) (1.2 g).

The β-alcohol, ent-*2,2-dimethyl-3α,10-dihydroxy-20-norgibberell-16-ene-7,19-dioic acid 19,10-lactone* (*2,2-dimethylgibberellin* A_4) (3) crystallised from ethyl acetate–light petroleum and was hygroscopic, rapidly forming a hydrate on exposure to air after being dried *in vacuo* at 80 °C. The hydrate had m.p. 214—216 °C (Found: C, 66.6; H, 7.9. $C_{21}H_{28}O_5 \cdot H_2O$ requires C, 66.6; H, 8.0%); $\nu_{max.}$ (Nujol) 3 375, 1 755, 1 693, 1 660, and 880 cm^{-1}; δ (C_5D_5N) 1.26, 1.28, and 1.64 (3 s, 18-H_3, 2α and 2β CH_3), 3.12 (d, *J* 10 Hz, 6-H), 3.68 (s, 3-H), 3.82 (d, *J* 10 Hz, 5-H), and 4.93 and 5.04 (each br, s, 17-H_2); *m/z* 360 (M^+, 4%), 342 (10), 328 (6), 324 (10), 315 (50), 298 (100), 296 (30), 286 (35), 283 (36), 270 (26), 269 (20), 258 (25), 253 (28), 243 (47), 242 (42), 237 (59), 230 (49), and 214 (36).

The α-alcohol, ent-*2,2-dimethyl-3β,10-dihydroxy-20-norgibberell-16-ene-7,19-dioic acid 19,10-lactone* (14) crystallised from acetone–light petroleum, m.p. 246—248 °C (Found: C, 69.7; H, 8.2. $C_{21}H_{28}O_5$ requires C, 70.0; H, 7.8%); $\nu_{max.}$ (Nujol) 3 450, 1 765, 1 697, 1 660, and 880 cm^{-1}; δ (C_5D_5N) 1.28, 1.46, 1.68 (3 s, 18-H_3, 2α and 2β CH_3), 3.03 (d, *J* 10 Hz, 5-H), 3.24 (d, *J* 10 Hz, 6-H), 3.88 (s, 3-H), and 4.94 and 5.04 (each br, s, 17-H_2); *m/z* 360 (M^+, 1%), 342 (22), 332 (10), 314 (100), 298 (9), 296 (26), 286 (34), 271 (22), 270 (23), 258 (38), 243 (34), 242 (31), 230 (44), and 214 (23).

Epimerisation of the 2,2-Dimethyl-3α-alcohol (14).—The α-alcohol (14) (2.74 g) in 10% potassium carbonate in methanol–water (4 : 1) was refluxed for 1 h. After removal of the methanol under reduced pressure, work-up gave a mixture (3 : 7 by g.l.c.) of the β- and α-alcohols. This mixture was combined with the mixture (350 mg) from the above experiment and separated by flash chromatography as before to give the β-alcohol (3) (500 mg), a mixture (3) and (14) (160 mg) and the α-alcohol (14) (2.2 g).

[3-^{2}H]-*2,2-Dimethylgibberellin* A_4 (4).—Sodium [$^2H_4$]borohydride (36 mg) in isopropyl alcohol (25 ml) was refluxed for 1 h. After the mixture had been cooled to 40 °C the keto-acid (13, 100 mg) in isopropyl alcohol (5 ml) was added and the solution refluxed for a further 1.5 h. Work-up gave an oil (95 mg) which was fractionated by flash chromatography as above to give 2,2-dimethyl[3-^{2}H]gibberellin A_4 (4) (18 mg), M^+ 361 with isotope composition 86.5% d_1, 13.5% d_0.

2,2-Dimethyl[3-^{3}H]*gibberellin* A_4 (5).—The keto-acid (13) (100 mg) was reduced with sodium [^{3}H]borohydride (37 mg, 100 mCi) as above to yield after chromatography 2,2-dimethyl-[3-^{3}H]gibberellin A_4 (5) (13 mg) with a specific activity of 7.56×10^8 Bq/mmol.

Preparation of 2,2-*Diethylgibberellin* A_4 (6).—*Ethylation of the keto-ester* (7). The keto-ester (7) (6.3 g) in tetrahydrofuran (500 ml) and hexamethylphosphoramide (10 ml; distilled from calcium hydride) was stirred with LDA solution (60 ml) and ethyl iodide (3 ml distilled from potassium) at 20 °C for 19 h. The reaction mixture was poured into ethyl acetate (400 ml) and acetic acid (50 ml) and then washed successively with saturated aqueous sodium hydrogen carbonate (400 ml) and water (400 ml). The solvents were removed under reduced pressure to give a yellow gum. This gum was chromatographed on a column (40 cm × 45 mm) of silica gel (350 g) with increasing proportions of ethyl acetate in light petroleum as eluant. Elution with 15% ethyl acetate afforded a gum (2.5 g) which appeared as one spot on analytical t.l.c. G.l.c. on a column of 2% SP-2100 at 215 °C revealed that the gum was comprised of three products which were identified by combined g.l.c.–mass spec. as the following products, in the ratio 0.8 : 1 : 2 and in order of increasing retention times:

(a) Methyl *ent*-19-carboxy-3-ethoxy-10-hydroxy-20-norgibberella-2,16-dien-7-oate 19,10-lactone (22); *m/z*, 372 (M^+, 1%), 328 (60), 326 (35), 313 (9), 296 (10), 282 (7), 267 (40), 253 (28), 239 (25), 223 (36), 175 (28), 162 (72), 151 (56), and 44 (100).

(b) Methyl *ent*-19-carboxy-2,2-diethyl-10-hydroxy-20-nor-3-oxogibberell-16-en-7-oate 19,10-lactone (15); *m/z*, 400 (M^+, 1%), 354 (4), 340 (5), 326 (10), 312 (8), 288 (100), 267 (20), 229 (35), and 201 (26).

(c) Methyl *ent*-19-carboxy-2-ethyl-3-ethoxy-10-hydroxy-20-norgibberella-2,16-dien-7-oate 19,10-lactone (23); see later for data.

Acid hydrolysis of the ethylation product. The above product (2.5 g) in methanol (80 ml) was stirred with Amberlite IR120 (H^+) ion exchange resin (4.0 g) at 20 °C for 2 d. The resin was filtered off and the solvent removed under reduced pressure. Analytical t.l.c. showed two spots, one of which was coincident with the starting material and the other, at a lower R_F, was coincident with the keto-ester (7). G.l.c. on a 2% SP-2100 column at 210 °C, showed that two of the starting mixture peaks were unaffected while one, the peak corresponding to the 3-ethoxy-2,3-olefin (22), had moved to a shorter retention time, and was now co-eluted with the keto-ester (7).

The product was subjected to flash chromatography, in two batches, on a 16 × 3 cm diameter column eluted with ethyl acetate–light petroleum (18 : 82). Early fractions contained the two diethylated products (15) and (23); later fractions contained further diethylated material contaminated with the keto-ester (7). These late fractions were purified by preparative t.l.c. in ethyl acetate–light petroleum (25 : 75), and the identity of the recovered keto-ester (7) was confirmed by n.m.r. spectroscopy.

The binary mixture of the 2,2-diethylketo-ester (15) and the 2-ethyl-3-ethoxy-olefin (23) (1.33 g, 1 : 2 by g.l.c.) was crystallised from methanol to give *methyl* ent-19-*carboxy*-2-*ethyl*-3-*ethoxy*-10-*hydroxy*-20-*norgibberella*-2,16-*dien*-7-*oate*-19,10-*lactone* (23), needles (0.6 g), m.p. 154—157 °C (Found: C, 72.9; H, 7.9. $C_{24}H_{32}O_5$ requires C, 72.0; H, 8.1%); $\nu_{max.}$ ($CHCl_3$) 2 980, 2 950, 1 775, 1 735, 1 655, 1 390, 1 105, and 1 035 cm^{-1}; δ ($CDCl_3$), 0.98 (t, *J* 8.5 Hz, 2-C–CH_3), 1.25 (s, 18-CH_3), 1.29 (t, *J* 7 Hz, 3-O–C–CH_3), 2.67 (d, *J* 9 Hz, 5-H), 2.89 (d, *J* 9 Hz, 6-H), 3.71 (m, 2-O–CH_2), 3.75 (s, CO_2Me), 4.86 (br, s, 17-H), and 5.00 (br, s, 17-H); *m/z* 400 (M^+, 38%), 369 (10), 356 (80), 327 (25), 281 (52), 267 (100), 251 (51), and 190 (28).

The mother liquors yielded a gum (700 mg) consisting of the 2,2-diethylketo-ester (15) and the 2-ethyl-3-ethoxy-olefin (23) in the ratio *ca.* 2 : 1 (by g.l.c.).

Reduction of the diethylated mixture (15) *and* (23). Sodium borohydride (110 mg) was refluxed in isopropyl alcohol (80 ml) for 1 h. The material from the mother liquors from the above experiment (650 mg) in isopropyl alcohol (40 ml), was added and the mixture refluxed for 4 h. After cooling of the reaction mixture, work-up gave a yellow oil.

Flash chromatography, on a 16 × 3 cm diameter column eluted with ethyl acetate–light petroleum (3 : 7), followed by preparative t.l.c., with ethyl acetate–light petroleum (4 : 6), yielded the following. (a) *Methyl* ent-19-*carboxy*-2,2-*diethyl*-3α,10-*dihydroxy*-20-*norgibberell*-16-*en*-7-*oate* 19,10-*lactone* (16) (96 mg), m.p. 239—242 °C (from chloroform–light petroleum) (Found: C, 71.2; H, 8.8. $C_{24}H_{34}O_5$ requires C, 71.6; H, 8.5%); δ ($CHCl_3$) 0.80 (m, 2 × CH_3), 1.14 (s, 18-CH_3), 2.68 (d, *J* 11 Hz, 6-H), 3.16 (d, *J* 11 Hz, 5-H), 3.47 (s, 3-H), 3.68 (s, CO_2Me), 4.86 (br, s, 17-H), and 4.98 (br, s, 17-H); *m/z* 402 (M^+, 0.8%), 384 (8), 374 (3), 370 (8), 356 (20), 328 (28), 311 (40), and 251 (100).

(b) *Methyl* ent-19-*carboxy*-2,2-*diethyl*-3β,10-*dihydroxy*-20-*norgibberell*-16-*en*-7-*oate* 19,10-*lactone* (17) (70 mg), m.p. 178—181 °C (from chloroform–light petroleum) (Found: C, 71.3; H, 8.9. $C_{24}H_{34}O_5$ requires C, 71.6; H, 8.5%); $\nu_{max.}$ ($CHCl_3$) 3 500, 2 950, 1 770, 1 735, 1 660, 1 390, and 1 180 cm^{-1}; δ ($CDCl_3$) 0.94 (m, 2 × CH_3), 1.20 (s, 18-Hz), 2.56 (d, *J* 9 Hz, 5-H), 2.82 (d, *J* 9 Hz, 6-H), 3.74 (s, CO_2Me and 3-H), 4.90 (br, s, 17-H), and 5.00 (br, s, 17-H); *m/z* 402 (M^+, 7%), 384 (39), 374 (43), 356 (100), 342 (75), 328 (97), 319 (67), 258 (62), 244 (83), and 230 (78).

(c) The 2-ethyl-3-ethoxy-olefin (23) was also recovered, coinciding, in its physical data (n.m.r., m.p.), with material obtained above.

ent-2,2-*Diethyl*-3α,10-*dihydroxy*-20-*norgibberell*-16-*ene*-7,19-*dioic Acid* 19,10-*Lactone* (6).—Sodium hydride (480 mg), as a 50% suspension in oil, was washed with light petroleum. Freshly distilled hexamethylphosphoramide (10 ml) was added *via* a syringe under N_2. The flask was cooled in ice–water and ethanethiol (1.3 ml) was added dropwise with stirring. The reagent was stirred for 1 h and then allowed to settle.

A portion (4.5 ml) of the supernatant sodium ethanethiolate–hexamethylphosphoramide solution was added to 2,2-diethylgibberellin A_4 methyl ester (16, 100 mg) and the solution set aside at 20 °C for 4 h. The reaction mixture was then poured into 2M-hydrochloric acid (50 ml) and extracted with ethyl acetate (2 × 40 ml). The combined organic washings were extracted with aqueous sodium hydrogen carbonate (2 × 50 ml). Acidification to pH 3 of the combined basic layers with concentrated hydrochloric acid and subsequent extraction with ethyl acetate, was followed by washing with water and removal of the solvents under reduced pressure to give a yellow oil. Analytical t.l.c. revealed one major product and a very minor, more polar product.

The product was flash chromatographed on a column (16 × 2 cm) eluted with ethyl acetate–light petroleum–acetic acid (30 : 70 : 6) to give 2,2-*diethylgibberellin* A_4 (6) (20 mg), m.p. 224—226 °C (from chloroform–light petroleum) (Found: C, 67.6; H, 8.8. $C_{23}H_{32}O_5 \cdot H_2O$ requires C, 68.0; H, 8.4%), M^+, 370.2157 ($C_{23}H_{32}O_5$ requires M^+, 370.2144); δ [$(CD_3)_2CO$], 0.76 (t, *J* 7.5 Hz, CH_3), 0.82 (t, *J* 7.5 Hz, CH_3), 1.13 (s, 18-CH_3), 3.15 (d, *J* 10.5 Hz, 5-H), 3.40 (s, 3-H), 4.78 (br, s, 17-H), and 4.91 (br, s, 17-H); *m/z* 388 (M^+, 2%), 352 (13), 342 (47), 326 (64), 314 (29), 297 (100), 286 (28), 270 (34), 251 (46), and 242 (31).

ent-2-*Ethyl*-3-*ethoxy*-10-*hydroxy*-20-*norgibberella*-2,16-*diene*-7,19-*dioic Acid* 19,10-*Lactone* (24).—The 2-ethyl-3-ethoxy-olefin (23) (50 mg), in methanol (25 ml) and aqueous 2M-sodium hydroxide solution (20 ml) was refluxed for 20 h. Work-up gave a gum which, by analytical t.l.c. consisted of

four spots. The gum was heated in a stream of dry nitrogen at 80 °C for 2 h and inspected, again by analytical t.l.c., which revealed the presence of two spots, one of which was unchanged methyl ester (23).

Purification of the product by preparative t.l.c. with ethyl acetate–light petroleum–acetic acid (40 : 60 : 1) afforded *2-ethyl-3-ethoxy-2,3-dehydrogibberellin* A_9 (24) (35 mg), m.p. 178—180 °C (from chloroform–light petroleum); M^+, 386.2087 ($C_{23}H_{30}O_5$ requires *M*, 386.2093); δ ($CHCl_3$), 0.98 (t, *J* 7.7 Hz, 2-C–CH_3), 1.27 (s, 18-CH_3), 1.26 (m, O–C–CH_3), 2.68 (d, *J* 10.3 Hz, 6-H), 2.82 (d, *J* 10.3 Hz, 5-H), 3.54 (m), 3.83 (m, 3-O–CH_2), 4.86 (br, s, 17-H), and 4.98 (br, s, 17-H); *m/z* 386 (M^+, 65%), 342 (100), 313 (27), 281 (39), 267 (69), 251 (27), and 190 (28).

Microbiological Conversion of 2,2-*Dimethylgibberellin* A_4 (3) *into* 2,2-*Dimethylgibberellin* A_1 (20).—A 1-l conical flask containing 40% ICI solution (500 ml) was inoculated with *Gibberella fujikuroi* mutant B1-41a culture (5 ml) and maintained under the usual conditions [15] for 6 d. The mycelium was obtained by filtration under sterile conditions and resuspended in 0% ICI solution [16] (500 ml) containing 2,2-dimethylgibberellin A_4 (3) (15 mg) which had been added in acetone (0.5 ml). The culture was maintained for 8 days, then filtered and washed with water (200 ml). The filtrate was acidified to pH 2 with 2M-hydrochloric acid and extracted with ethyl acetate (3 × 500 ml). The extract was washed with water (100 ml) and the solvent removed under reduced pressure. The product was taken up in water (pH 8; 100 ml) and extracted with ethyl acetate (3 × 100 ml). The organic extract was washed with water (30 ml) and then the solvent was removed under reduced pressure. The aqueous solution was acidified to pH 2 with 2M-hydrochloric acid, extracted with ethyl acetate (3 × 150 ml), washed with water (40 ml), and the organic solvent removed under reduced pressure.

Examination of both extracts by g.l.c. on a column of 2% SP-2100 programmed from 180 to 240 °C at 4 °C min^{-1} showed that the neutral extract contained a small amount of 2,2-dimethylgibberellin A_4 (3) and the acidic extract contained a mixture of 2,2-dimethylgibberellin A_4 (7) and 2,2-dimethylgibberellin A_1 (41) (2 : 3). The acidic extract was separated by flash chromatography with ethyl acetate–light petroleum–acetic acid (50 : 50 : 1). Fractions 6—8 gave impure 2,2-dimethylgibberellin A_4 (3) and fractions 14—17 gave a yellow gum containing 2,2-dimethylgibberellin A_1 (24). This product was examined by combined g.l.c.–mass spec. as the Me, $SiMe_3$ derivative showing, *m/z* 534 (M^+, 100%), 519 (9), 448 (9), 235 (11), 221 (5), 207 (24), 159 (13), 157 (29), 144 (12), 75 (39), and 73 (35).

The product was purified by h.p.l.c. on a C_{18} reverse-phase Spherisorb ODS column (25 cm × 4.5 mm) using methanol–1% aqueous phosphoric acid (1 : 1) eluting at 1 ml min^{-1} and monitoring the u.v. absorption at 210 nm. The eluant (20 ml) containing 2,2-dimethylgibberellin A_1 was added to water (30 ml) and extracted with ethyl acetate (3 × 50 ml). The organic layer was washed with water (20 ml) and the solvent removed under reduced pressure giving a gum (10 mg).

Examination of the gum by g.l.c., under the same conditions as before, showed two compounds (1 : 1) identified as 2,2-dimethylgibberellin A_1 (20) and a less polar compound, the C- and D-rings rearranged product (27). Crystallisation from ethyl acetate gave 2,2-dimethylgibberellin A_1 (20) (4 mg), m.p. 208—210 °C; M^+, 358.1794 [$C_{21}H_{26}O_5$ (*M*, H_2O) requires 358.1780]; δ [$(CD_3)_2CO$], 1.03 (s, CH_3), 1.07 (s, CH_3), 1.15 (s, CH_3) 2.57 (d, *J* 10 Hz, 6-H), 3.17 (d, *J* 10 Hz, 5-H), 3.29 (s, 3-H), 4.86 (br, s, 17-H), and 5.19 (br, s, 17-H); *m/z* 376 (M^+, 28%), 358 (100), 330 (23), 312 (31), 289 (40), 163 (13), 135 (17), 91 (11), 44 (22), and 43 (20).

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