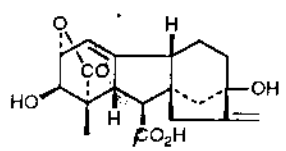
Partial Syntheses of some Isomers of Gibberellin A_1 and Gibberellin A_3 Methyl Ester

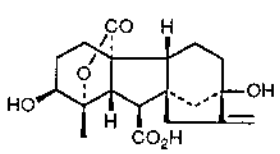
Braulio M. Fraga,* Clemente D. Gómez, Melchor G. Hernández, Emilio de Paz, and Fernando G. Tellado
Instituto de Productos Naturales Orgánicos, CSIC, La Laguna, 38206-Tenerife, Canary Islands, Spain
Aurea A. Perales
Departamento de Rayos X, Instituto Rocasolano, CSIC, Serrano 119, 28006-Madrid, Spain

The partial synthesis of an isomer of gibberellic acid methyl ester, with a lactone between C-6 and C-10 and an esterified acid group at C-19, is described. The overall yield from gibberellic acid was 46%. The preparation of 6-*epi*-GA_1 has also been carried out. Its structure was confirmed by an X-ray analysis of its 1β-iodo derivative.

Highly active gibberellins possess a 19→10 lactone function whose spatial disposition with respect to the D-ring seems to be important in determining the 'fit' of the hormone at the active site. Thus, rearranged gibberellins with the stereochemical configuration of the D-ring opposite to that normally encountered are found to be devoid of biological activity.[1] On the other hand, the role of the γ-lactone in such activity is unclear. Whilst isomerization of the 19→10 lactone ring in GA_3 (**3**) to the 19→2 position of iso-GA_3 (**1**) affects the activity surprisingly little,[2] isomerization to the 20→4 position in the 19-norgibberellin analogue (**2**) results in total loss of biological activity.[3]



(1)



(2)

In the light of these features, we devised the relocation of the 19→10 lactone to the 7→10 position to test the real importance of the position of the lactone in GA_3 (**3**)[4] in relation to the biological activity of this compound. The transformation of GA_3 (**3**) into the methyl esters of the isomers of GA_3 and GA_1, (**12**) and (**14**) respectively, was made by following a methodology outlined in the Scheme, which also permits access to 6-*epi* C-19 gibberellins.

The key step in the strategy is the effective trapping of the base-generated at C-6α carboxy group by forming the internal anhydride (**8**).[5] The precursors of the gibberellin analogues were obtained either by regioselective opening of the anhydride (**8**) and iodolactonization (gibberellin isomers) or by hydrolysis of the anhydride and regioselective iodolactonization of the resulting diacid (**15**) (6-*epi*-gibberellins).

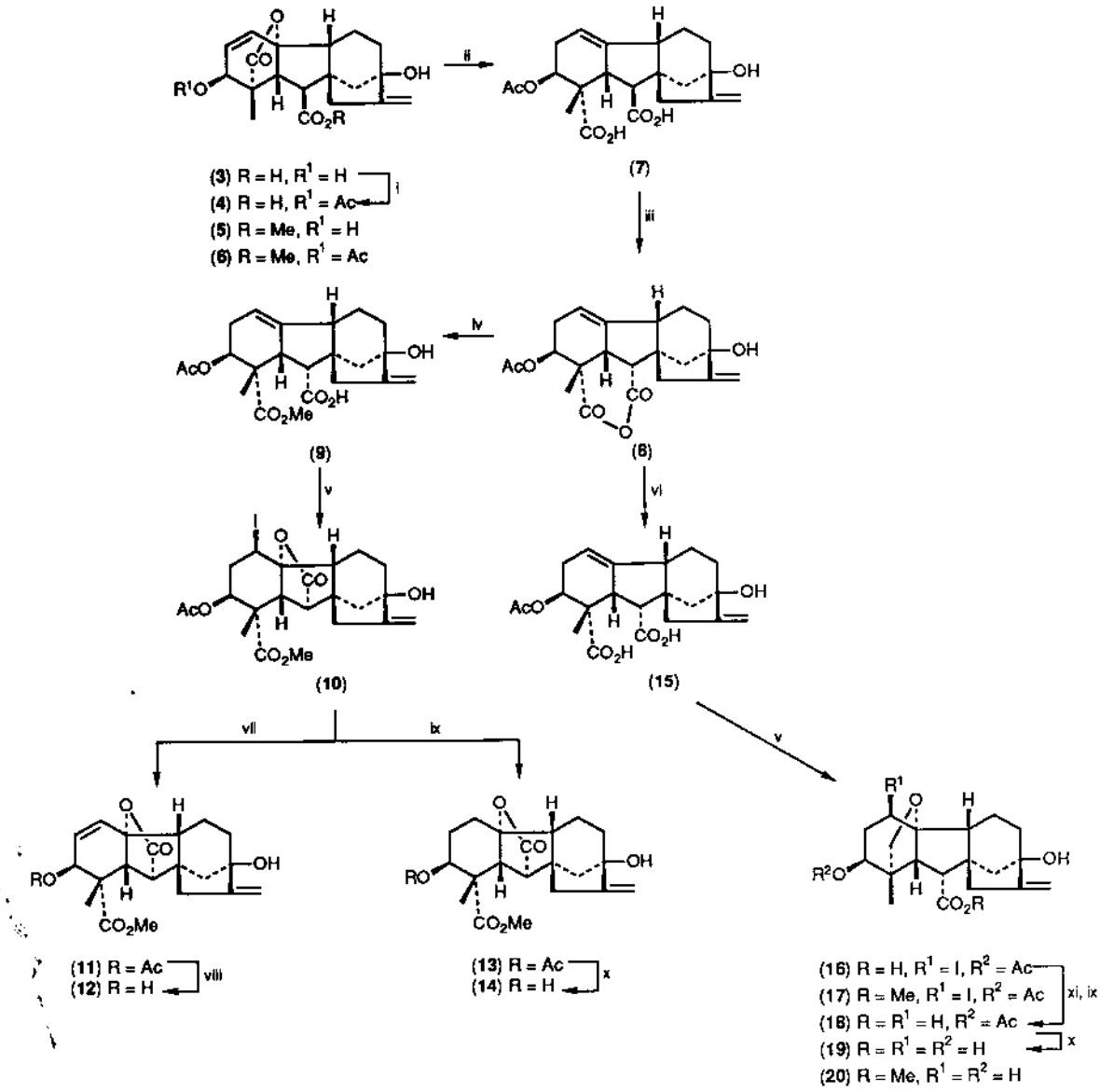
The anhydride (**8**) was obtained in 60% yield from gibberellic acid (**3**) by selective acetylation at C-3, followed by hydrogenation (H_2, 10% Pd on $CaCO_3$, MeOH–Py)[6] and condensation of the crude resulting diacid (**7**) with dicyclohexylcarbodi-imide in triethylamine.[5] This condensation was also made, but in lower yield and with a longer reaction time, by treatment of the diacid (**7**) with tosyl chloride in tetrahydrofuran–triethylamine.[7] Methanolysis of the anhydride gave the monomethyl ester (**9**), which was treated with iodine in dichloromethane–tetrahydrofuran and aqueous sodium hydrogen carbonate to afford the iodolactone (**10**) (64%).[8] The yield in the iodolactonization is usually variable and depends on the reaction time, stirring, *etc.* No attempts were made to improve it. The iodolactone (**10**) has IR absorption at 1 780 cm^{-1}. Its ^1H NMR spectrum shows the hydrogen geminal to the iodine as a doublet at δ 4.45 (*J* 4.3 Hz), and the 5- and 6-H as two singlets at δ 3.04 and 2.50, respectively, in accordance with the near 90° angle observed in these hydrogens in Dreiding models of the molecule. In this assignment we have assumed that the 6-H resonates at a higher field than 5-H, as occurs in the C_{19} gibberellins hydroxylated at C-3(β). A conventional 2D ^1H-^{13}C correlation experiment supported the 5- and 6-C resonance assignments (Table 1).

Table 1. ^{13}C NMR resonances of gibberellin analogues.

Carbon	(8)	(10)	(11)	(13)	(17)
1	113.20	13.46	124.50	21.68	15.23
2	28.44	31.95	133.31	22.79	35.27
3	70.12	70.44	68.76	71.74	73.49
4	50.54	43.65	43.50	44.75	51.15
5	48.22[a]	53.96	51.93[a]	54.00[a]	49.11[a]
6	46.42[a]	52.10	51.63[a]	53.05[a]	51.25[a]
7	166.61[b]	174.53	174.74	175.81	176.08
8	43.56	44.41	44.77	44.04	51.88
9	39.09[a]	55.67	53.79[a]	54.64[a]	51.56[a]
10	138.60	90.43	85.89	89.53	95.81
11	18.34	14.94	16.31	16.20	17.02
12	37.25	38.87	39.22	39.24	38.00
13	79.02	78.08	78.03	78.03	78.96
14	44.48	43.51	43.56	43.63	47.25
15	40.59	42.06	41.81	41.72	42.93
16	152.80	156.98	157.28	157.47	156.33
17	107.29	108.52	108.52	108.32	106.92
18	20.93	23.48	20.71	21.15	22.09
19	168.84[b]	172.88	172.58	173.02	172.29

[a,b] Signals can be interchanged.

The iodolactone (**10**) was treated with DBU in toluene under reflux to give the β-elimination compound (**11**). Hydrolysis of (**11**) with sodium carbonate in methanol afforded iso-GA_3 methyl ester (**12**). The ^1H NMR spectrum of (**12**) showed a system of signals for the A-ring double bond composed of a doublet (*J* 10 Hz) at δ 5.98 (1-H) and a double doublet (*J* 5.4 and 10 Hz) at δ 6.43 (2-H). Whilst the multiplicities were those expected, the resonance positions were changed with regard to the methyl esters of GA_3 (**5**) (1-H, δ 6.32; 2-H, δ 5.90) and 6-*epi*-GA_3 (**22**) (1-H, δ 6.27; 2-H, δ 5.83).[9] This observed inversion in the resonance positions could be related with the different



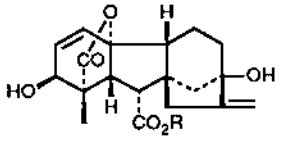
Scheme. *Reagents and conditions:* i, Ac_2O, Py; ii, H_2, 10% Pd on $CaCO_3$, MeOH, Py; iii, DCC, Et_3N, THF; iv, MeOH, Py; v, I_2, CH_2Cl_2, THF, $NaHCO_3$ (1:1:1.5); vi, H_2O, Py; vii, DBU, toluene; viii, satd. aq. $NaHCO_3$–MeOH (1:4); ix, Bu_3SnH, AIBN, toluene; x, Na_2CO_3–MeOH; xi, $(Bu_3Sn)_2O$, toluene.

anisotropy of the lactone carbonyl group and with the molecular torsion introduced in the A-ring by the 7,10 lactone bridge.

The iso-GA_1 methyl ester derivative (**14**) was obtained from the iodolactone (**10**) in 71% yield by a reductive deiodination with tributyltin hydride in toluene and subsequent careful hydrolysis of the resulting acetate (**13**).

The attempts to transform both iso-GA_1 and iso-GA_3 methyl esters, (**14**) and (**12**), into the free acids were unsuccessful. Thus, treatment of these methyl esters with sodium ethanethiolate in hexamethylphosphoramide afforded the desired acids in very low yield and with a high proportion of their corresponding C-3 epimers. We also tried to introduce other acid protective groups which could be more easily deprotected than the methyl ester. Unfortunately, such attempts were also unsuccessful. The pyridine-catalyzed nucleophilic opening of the anhydride (**8**) with 2-(trimethylsilyl)ethanol or *p*-nitrobenzyl alcohol afforded a 1:1 mixture of both 7- and 19-monoesters in <20% yield. These results can be related with the larger volume and lower nucleophilic strength of these alcohols with regard to methanol (see above). This feature leads to a slower opening of the anhydride which competes with a protective group migration from one acid group to another, also catalyzed by the base.

Once the isogibberellin analogues were obtained, we addressed our attention to the synthesis of 6-*epi*-gibberellin analogues using the diacid (**15**) as a suitable precursor of both 6-*epi*-GA_1 (**19**) and 6-*epi*-GA_3 (**21**). We thought that the direct iodolactonization of diacid (**15**) would be selective towards the



(**21**) R = H
(**22**) R = Me

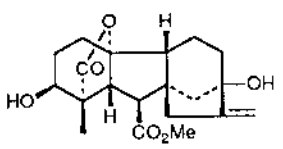
iodolactone (**16**) because access to C-10 from C-19 is thermodynamically more favourable than from C-7. The above-mentioned resonances of 5- and 6-H appearing as two singlets in the iodolactone (**10**) point to a greater structural distortion in

Table 2. Atomic co-ordinates ($\times 10^4$) and equivalent isotropic temperature factors (Å $\times 10^3$) for compound (**17**) with esds in parentheses.

Atom	*x*	*y*	*z*
I	7 695(1)	11 864(0)	9 687(1)
C(1)	6 950(9)	10 000(9)	9 147(10)
C(2)	7 669(9)	9 274(10)	8 350(10)
C(3)	7 137(9)	9 323(9)	6 600(10)
O(3)	7 520(7)	10 441(6)	6 051(8)
C(4)	5 694(9)	9 341(8)	5 906(9)
C(5)	5 246(8)	10 446(7)	6 576(9)
C(6)	3 862(8)	10 847(8)	6 040(9)
C(7)	2 910(9)	10 133(11)	4 768(10)
O(7)	2 846(8)	9 057(8)	4 613(8)
O(8)	2 073(8)	10 924(10)	3 878(9)
C(8)	3 451(8)	10 808(7)	7 476(8)
C(9)	4 690(9)	10 683(8)	8 853(9)
C(10)	5 568(8)	10 022(7)	8 232(9)
O(10)	5 213(6)	8 705(5)	8 017(6)
C(11)	4 551(10)	10 056(11)	10 250(11)
C(12)	3 302(10)	10 289(11)	10 408(11)
C(13)	2 172(9)	10 304(10)	8 869(10)
O(13)	1 189(6)	9 621(8)	9 097(8)
C(14)	2 550(8)	9 804(9)	7 565(10)
C(15)	2 715(8)	11 952(14)	7 559(9)
C(16)	1 844(9)	11 611(8)	8 377(11)
C(17)	0 955(11)	12 264(11)	8 550(14)
C(18)	5 256(11)	9 218(9)	4 162(10)
C(19)	5 285(9)	8 299(8)	6 719(9)
O(19)	5 124(8)	7 237(6)	6 356(8)
C(21)	1 003(13)	10 337(21)	2 677(16)
C(22)	8 515(10)	10 401(10)	5 620(12)
O(22)	9 210(8)	9 562(10)	5 910(12)
C(23)	8 616(13)	11 568(11)	4 813(15)

this molecule than in GA_3 (**3**) and related compounds and, thus, a higher energy level. Hydrolysis of the anhydride (**8**) with water–pyridine at reflux gave the diacid (**15**) in quantitative yield. This compound, in its crude form, was iodolactonized, under the same conditions as for (**9**), to give iodolactone (**16**) as the sole product (89%). Its methyl ester derivative (**17**) showed a ^{1}H NMR spectrum completely different from that of the iodolactone (**10**), the 5- and 6-H signals now appearing as two doublets (*J* 10.8 Hz) at δ 3.74 and 3.21, respectively, while the methoxy group resonates at δ 3.65 (δ 3.60 in the iodolactone isomer), in total accordance with the values observed for the dimethyl ester of the diacid (**15**) at δ 3.63 and 3.61. This dimethyl ester was identical with that obtained by methylation of the monoacid (**9**).

The reductive deiodination of (**16**), achieved by forming the tributyltin ester and subsequent treatment with tributyltin hydride, gave (**18**), which was carefully hydrolysed with sodium carbonate in methanol to afford the target 6-*epi*-GA_1 (**19**) (83%). Its methyl ester (**20**) shows a ^{1}H NMR spectrum different to that of the GA_1 methyl ester (**23**). Thus, while the 5- and 6-H protons appear as doublets (*J* 10.7 Hz) at δ 3.13 and 3.06, respectively, and the methoxy group resonates at δ 3.64, in GA_1 methyl ester (**23**) the 5- and 6-H protons appear as two doublets (*J* 12 Hz) at δ 3.21 and 2.69, and the methoxy group at δ 3.71.



(**23**)

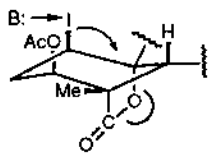


Figure 1.

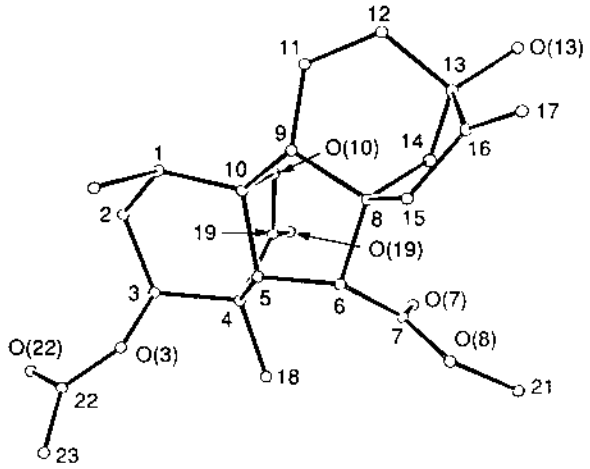


Figure 2. Perspective view of the molecule (**17**)

Although 6-*epi*-GA_3 (**21**) has been prepared previously by Adam *et al.*,[10] we decided to prepare it by dehydrohalogenation of iodolactone (**16**). Unfortunately, our attempts were unsuccessful. Treatment of (**16**) with DBU in refluxing toluene afforded an intractable mixture of compounds. When the methyl ester (**17**) was used instead of the free acid, the same treatment gave the 3β-acetate of GA_3 methyl ester (**6**), by β-elimination of the halogen with concomitant epimerization at C-6 by the base. When pyridine (80 °C) was used instead of DBU, the iodolactone (**16**) was converted into an intractable mixture of compounds. Treatment of the methyl ester derivative (**17**) with collidine (110 °C), a more steric demanding base, gave after methylation the dimethyl ester of the diacid (**7**). This apparently 'surprising' result may be rationalized in terms of steric impedance for the necessary capture of the 2α proton by the base in the β-elimination. Under the forced reaction conditions, the base promotes a halogen abstraction with concomitant lactone ring fission and 1,2-double bond formation (see Figure 1).

In conclusion, in this work, gibberellic acid is transformed into the isogibberellin analogues (**12**) and (**14**), by a relocation of the naturally occurring 19→10 lactone to the 7→10 position, and into 6-*epi*-GA_1 (**19**) by an epimerization of the acid group at C-6 from its naturally occurring β-orientation to the less favoured α-disposition.

The crystallographic conformation of molecule (**17**) is illustrated in Figure 2, which also shows the atom numbering and the absolute stereochemistry. The latter was not determined since the absolute stereochemistry of gibberellins is well known.[11] Ring A of (**17**) adopts a distorted chair conformation, while the five-membered ring B is envelope conformed, having the flap at C-9. Ring C has a conformation between a twist and a boat. The dominant symmetry in rings A and C is the mirror symmetry.[12] The lactonic bridge between C-4 and C-10 is almost perpendicular to ring A.

The bond lengths observed are not significantly different from those of other similar compounds.[13] The molecular packing as viewed along the *b* axis is shown in Figure 3. A hydrogen bond of the O–H · · · O type links the molecules together, showing elongated distances to the acceptor O(22), but it may still be

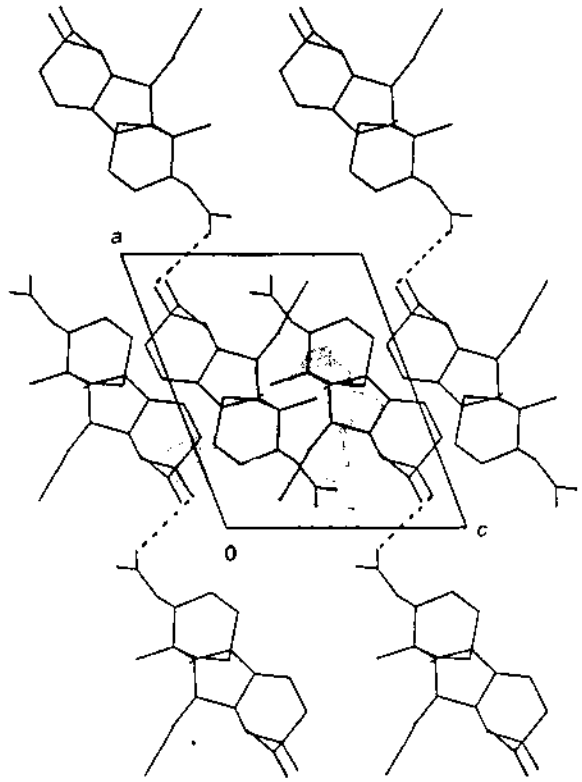


Figure 3. A projection of the crystal structure along the *b* axis. Broken lines show the hydrogen bonds.

considered as a hydrogen bond: O(13)–HO(13) · · · O(22) = 3.04(1) Å; with O(13)–HO(13) = 0.944 Å, HO(13) · · · O(22) = 2.22 Å; and O(13)–HO(13) · · · O(22) angle of 144.6°, with the symmetry operation: $(1 + x, y, z)$. There are two other intermolecular contacts, which correspond to Van der Waals' interactions: C(5) · · · O(19) = 3.28(1) Å and C(6) · · · O(19) = 3.26 Å, with the symmetry operation: $1 - x, \frac{1}{2} + y, 1 - z$.

Experimental

M.p.s were taken on a Kofler hot-plate apparatus and are uncorrected. ^1H (200 MHz) and ^{13}C (50.32 MHz) NMR spectra were recorded on a Bruker WP 200 SY spectrometer, and $CDCl_3$ was used as the solvent. IR spectra were obtained on a Perkin-Elmer 681 spectrophotometer. Unless otherwise indicated, $CHCl_3$ was used as solvent. Silica for flash chromatography was Merck Kiesegel 60 (230 mesh ASTM). Light petroleum refers to the fraction boiling in the range 60–80 °C. TLC was carried out on silica gel Schleicher-Schull plates (F 1 500, LS 254). Dry solvents were distilled before use from an appropriate drying agent. Methanol was refluxed on magnesium and distilled; tetrahydrofuran and toluene were distilled from sodium and benzophenone; triethylamine was refluxed on calcium hydride and distilled; pyridine was refluxed on potassium hydroxide and distilled; collidine was refluxed on barium oxide and distilled. Dicyclohexylcarbodi-imide (DCC) was high vacuum bulb-to-bulb distilled prior to use.

ent-3α-*Acetoxy*-13-*hydroxy*-20-*nor*-6-epi-*gibberella*-1(10),16-*diene*-7,19-*dioic Acid* 7,19-*Anhydride* (**8**).—Crude GA_3 (**3**) (2.0 g) in acetic anhydride (10 ml) and pyridine (10 ml) was stirred for 2 h at 0 °C. The reaction mixture was poured into acid–water and extracted with ethyl acetate. The organic layers were collected and washed with 2M hydrochloric acid and brine, dried (Na_2SO_4), filtered, and concentrated to give the crude compound (**4**), which was purified by crystallization from ethyl acetate–light petroleum (2.06 g, 5.3 mmol).

The monoacetate (**4**) (2.06 g, 5.3 mmol) in methanol (100 ml) and pyridine (4 ml) was stirred with 10% palladium on calcium carbonate (47 mg) under an atmosphere of hydrogen for 8 h. The mixture was filtered and the solvents were distilled off. The last pyridine traces were removed by azeotropic distillation with toluene. The crude residue dissolved in dry tetrahydrofuran (90 ml) and dry triethylamine (5.5 ml, 39.6 mmol) was refluxed with dicyclohexylcarbodi-imide (1.3 g, 6.3 mmol) for 13 h. The solvents were partially evaporated off and the crystalline cyclohexylurea separated by filtration. The filtrate was diluted with ethyl acetate and washed with aqueous saturated sodium hydrogen carbonate and brine, dried (Na_2SO_4), and evaporated. The residue was flash chromatographed (ethyl acetate–light petroleum, 3:7 v/v) to afford the *anhydride* (**8**) (1.2 g, 3.2 mmol) (Found: M^+, at 372.1581. $C_{21}H_{24}O_6$ requires M, 372.1513); ν_{max} 3 580, 1 800, 1 755, 900, and 855 cm^{-1}; δ_H 5.47 (1 H, d, J 3.45 Hz, 1-H), 5.36 (1 H, br s, 3-H), 5.17 (1 H, t, J 2.7 Hz, 17-H), 5.04 (1 H and, br s, 17-H), 5.30 (1 H, d, J 10.8 Hz, 5-H), 2.07 (3 H, s, OAc), and 1.33 (3 H, s, 18-H); *m/z* 372 (M^+, 0.24%), 312 (3.03), 284 (2.66), 240 (4.22), and 224 (100).

ent-3α-*Acetoxy*-10,13-*dihydroxy*-1α-*iodo*-20-*nor*-6-epi-*gibberell*-16-*ene*-7,19-*dioic Acid* 19-*Methyl Ester* 7,10-*Lactone* (**10**).—The anhydride (**8**) (530 mg, 1.43 mmol) in methanol (30 ml) and pyridine (6 ml) was refluxed for 10 h. The solvents were evaporated off and the last pyridine traces were azeotroped off with toluene. The resulting residue was dissolved in dichloromethane (10 ml), tetrahydrofuran (10 ml), and aqueous saturated sodium hydrogen carbonate (pH 9) (15 ml) and vigorously stirred with iodine (406 mg, 1.6 mmol) at room temperature for 75 min. Ethyl acetate was added and the organic layer decanted off. The aqueous phase was further extracted with ethyl acetate and the organic layers were combined and washed with sodium thiosulphate and brine, dried (Na_2SO_4), and evaporated to dryness. The oil residue was flash chromatographed. Elution with ethyl acetate–light petroleum (3:7, v/v) gave the *iodolactone* (**10**) (490 mg, 0.92 mmol) (Found: M^+, at 530.0807. $C_{22}H_{27}IO_7$ requires M, 530.0801); ν_{max} 3 590, 1 780, 1 735, and 895 cm^{-1}; δ_H 5.43 (1 H, t, J 2.9 Hz, 3-H), 5.35 and 5.05 (each 1 H, br s, 17-H), 4.45 (1 H, br d, J 4.3 Hz, 1-H), 3.63 (3 H, s, OMe), 3.04 (1 H, s, 5-H), 2.50 (1 H, s, 6-H), 2.16 (3 H, s, OAc), and 1.32 (3 H, s, 18-H); *m/z* 530 (M^+, 4.32%), 470 (2.49), 403 (6.40), 343 (19.27), 325 (3.96), 311 (6.79), 283 (7.69), 239 (18.24), 211 (7.55), and 43 (100).

Dehydrohalogenation of the Iodolactone (**10**).—The iodolactone (**10**) (200 mg, 0.37 mmol) in toluene (5 ml) was refluxed with 1,8-diazabicyclo[5.4.0]undec-7-ene (DBU) (0.2 ml, 1.3 mmol) for 0.5 h. The solvent was evaporated off and the resulting residue was flash chromatographed. Elution with ethyl acetate–light petroleum (4:6, v/v) gave the β-elimination compound, ent-3α-*acetoxy*-10,13-*dihydroxy*-20-*nor*-6-epi-*gibberella*-1(2),16-*diene*-7,19-*dioic acid* 19-*methyl ester* 7,10-*lactone* (**11**) (120 mg, 0.29 mmol), as a crystalline solid, m.p. 174–175 °C (ether–light petroleum) (Found: M^+, at 402.1655. $C_{22}H_{26}O_7$ requires M, 402.1676); ν_{max} 3 580, 1 780, 1 765, 1 735, and 890 cm^{-1}; δ_H 6.36 (1 H, dd, J 5.5 and 10 Hz, 2-H), 6.05 (1 H, d, J 10 Hz, 1-H), 5.65 (1 H, d, J 5.5 Hz, 3-H), 5.34 (1 H, br s, 17-H), 3.60 (3 H, s, MeO), 2.91 (1 H, s, 5-H), 2.49 (1 H, s, 6-H), 2.08 (3 H, s, OAc), and 1.30 (3 H, s, 18-H); *m/z* 402 (M^+, 0.42%), 384 (1.54), 360 (2.52), 342 (8.06), 314 (3.59), 298 (3.13), 283 (10.16), 266 (9.26), 255 (16.70), 239 (32.21), 237 (8.96), 221 (8.03), 78 (100), and 43 (98.71).

Hydrolysis of Compound (**11**).—The 3β-acetate derivative

(**11**) (80 mg, 0.20 mmol) in methanol (4 ml) was stirred with saturated aqueous potassium carbonate (1 ml) for 45 min at room temperature. The reaction mixture was poured into water and extracted with ethyl acetate. The organic fractions were combined and washed with brine, dried (Na_2SO_4), and concentrated to dryness to give ent-3α,10,13-*trihydroxy*-20-*nor*-6-epi-*gibberella*-1(2),16-*diene*-7,19-*dioic acid* 19-*methyl ester* 7,10-*lactone* (**12**) (65 mg, 0.18 mmol), m.p. 255–257 °C (ethyl acetate–light petroleum) (Found: C, 66.9; H, 6.85. $C_{20}H_{24}O_6$ requires C, 66.65; H, 6.71%); ν_{max}(Nujol) 3 500, 3 440, 3 360, 1 790, and 1 720 cm^{-1}; δ_H 6.43 (1 H, dd, *J* 5.4 and 10 Hz, 2-H), 5.98 (1 H, d, *J* 10 Hz, 1-H), 5.32 (1 H, br s, 17-H), 5.00 (1 H, br s, 17-H), 4.48 (1 H, t, *J* 5 Hz, 3-H), 3.59 (3 H, s, MeO), 2.89 (1 H, s, 5-H), 2.56 (1 H, s, 6-H), and 1.40 (3 H, s, 18-H); *m/z* 360 (M^+, 8.30%), 342 (7.46), 328 (12.07), 300 (8.05), 283 (9.56), 255 (20.37), 239 (19.87), 225 (46.19), 224 (41.16), 209 (29.17), 165 (44.68), 149 (56.38), and 136 (100).

Reductive Dehalogenation of the Iodolactone (**10**).—The iodolactone (**10**) (67 mg, 0.12 mmol) in toluene (5 ml) was added dropwise during 1 h to a refluxing solution of tributyltin hydride (0.070 ml, 0.26 mmol) and azoisobutyronitrile (AIBN) (traces) in toluene (5 ml) under a nitrogen atmosphere. The reaction mixture was refluxed for a further 1 h and the solvent was evaporated off. The residue was dissolved in acetonitrile and washed with light petroleum. The acetonitrile was distilled off and the resulting residue flash chromatographed. Elution with ethyl acetate–light petroleum (1:1 v/v) gave the reduced compound, ent-3α-*acetoxy*-10,13-*dihydroxy*-20-*nor*-6-epi-*gibberell*-16-*ene*-7,19-*dioic acid* 19-*methyl ester* 7,10-*lactone* (**13**) (42 mg, 0.104 mmol) (Found: M^+, 404.1815. $C_{22}H_{28}O_7$ requires *M*, 404.1796); ν_{max} 3 560, 1 775, 1 740, and 890 cm^{-1}; δ_H 5.34 (1 H, br s, 3-H), 5.32 (1 H, br s, 17-H), 5.01 (1 H, br s, 17-H), 3.63 (3 H, s, MeO), 2.97 (1 H, s, 5-H), 2.14 (1 H, s, 6-H), 2.08 (3 H, s, OAc), and 1.24 (3 H, s, 18-H); *m/z* 404 (M^+, 1%), 344 (10), 269 (12), 209 (13), 136 (37), 121 (21), and 43 (100).

Hydrolysis of (**13**).—The 3β-acetate derivative (**13**) (42 mg, 0.1 mmol) dissolved in a homogeneous solution of potassium carbonate in aqueous methanol (5 ml) was stirred at room temperature for 6 h. The reaction mixture was acidified to *ca.* pH 3–4 and partially concentrated under reduced pressure. The resulting mixture was diluted with water and extracted with ethyl acetate. The organic fractions were combined and washed with saturated aqueous sodium hydrogen carbonate and brine, dried (Na_2SO_4), and concentrated to give ent-3α10,13-*trihydroxy*-20-*nor*-6-epi-*gibberell*-16-*ene*-7,19-*dioic acid* 19-*methyl ester* 7,10-*lactone* (**14**) (30 mg, 0.082 mmol), m.p. 236–240 °C (methanol–ethyl acetate) (Found: M^+, 362.1726. $C_{20}H_{26}O_6$ requires *M*, 362.1720); ν_{max}(Nujol) 3 520, 3 500, 1 785, 1 730, and 890 cm^{-1}; δ_H 5.31 (1 H, br s, 17-H), 4.99 (1 H, br s, 17-H), 4.15 (1 H, br s, 3-H), 3.61 (3 H, s, MeO), 2.92 (1 H, s, 5-H), 2.24 (1 H, s, 6-H), and 1.24 (3 H, s, 18-H); *m/z* 362 (M^+, 21%), 344 (52), 316 (28), 312 (20), 298 (17), 284 (19), 227 (95), 226 (83), and 43 (100).

Hydrolysis of the Anhydride (**8**).—The anhydride (**8**) (500 mg, 1.74 mmol) in water (10 ml) and pyridine (5.5 ml) was refluxed for 3 h. Benzene was added and the water and pyridine distilled off by means of a Dean–Stark system. Traces of pyridine were eliminated by further distillation with toluene to give quantitatively ent-3α-*acetoxy*-13-*hydroxy*-20-*nor*-6-epi-*gibberella*-1(10),16-*diene*-7,19-*dioic acid* (**15**) (Found: $M^+ - 78$, 312.1379. $C_{19}H_{20}O_4$ requires *M*, 312.1377); δ_H 5.49 1 H, br s, 1-H), 5.23 (1 H, br s, 3-H), 5.19 (1 H, br s, 17-H), 4.95 (1 H, s, 17-H), 2.98 (1 H, d, *J* 9.3 Hz, 5-H), 2.74 (1 H, br s, 6-H), 2.02 (3 H, s, OAc), and 1.37 (3 H, s, 18-H); *m/z* 312 ($M^+ - 78$, 3%), 286 (2), 284 (2), 268 (3), 250 (2), 240 (4), 135 (59), and 43 (100).

The diacid was methylated to give the corresponding *dimethyl ester* (Found: $M^+ - 30$, at 388.1933. $C_{22}H_{28}O_6$ requires *M*, 388.1884); δ_H 5.43 (1 H, dd, *J* 5.3 and 7.7 Hz, 1-H), 5.20–5.27 (2 H, m, 3-H and 17-H), 4.96 (1 H, br s, 17-H), 3.63 and 3.61 (each 3 H, s, 2 × OMe), 2.87 (1 H, d, *J* 9 Hz, 5-H), 2.77 (1 H, d, *J* 9 Hz, 6-H), 2.01 (3 H, s, OAc), and 1.37 (3 H, s, 18-H); *m/z* 388 ($M^+ - 30$, 1.1%), 358 (13.8), 326 (28.3), 298 (37.6), 267 (9.4), 239 (100), and 221 (29.7). This dimethyl ester was identical with that obtained by methylation of (**9**) with diazomethane.

Iodolactonization of the Diacid (**15**).—The diacid (**15**) (500 mg, 1.28 mmol) in dichloromethane (10 ml), tetrahydrofuran (10 ml), and saturated aqueous sodium hydrogen carbonate (15 ml) was stirred with iodine (330 mg, 1.35 mmol) at room temperature for 90 min. Ethyl acetate was added and the aqueous phase decanted, and extracted with ethyl acetate again. The combined organic fractions were washed with saturated aqueous sodium hydrogen carbonate and the combined aqueous basic solution carefully acidified with 2M hydrochloric acid. The resulting acid solution was exhaustively extracted with ethyl acetate and the combined organic fractions were dried (Na_2SO_4) and concentrated to dryness to give ent-3α-*acetoxy*-10,13-*dihydroxy*-1α-*iodo*-20-*nor*-6-epi-*gibberell*-16-*ene*-7,19-*dioic acid* 19,10-*lactone* (**16**) (614 mg, 1.19 mmol) as a crystalline solid, m.p. 132–134 °C (ethyl acetate) (Found: $M^+ - 127$, 389.1596. $C_{21}H_{25}O_7$ requires *M*, 389.1598); ν_{max}(Nujol) 3 350br, 1 775, 1 720, 1 710, and 885 cm^{-1}; δ_H 5.25 (1 H, br s, 17-H), 4.97 (1 H, br s, 17-H), 4.88 (1 H, d, *J* 2.6 Hz, 3-H), 4.43 (1 H, d, *J* 5.1 Hz, 1-H), 3.68 (1 H, d, *J* 10.6 Hz, 5-H), 3.20 (1 H, d, *J* 10.6 Hz, 6-H), 2.16 (3 H, s, OAc), and 1.21 (3 H, s, 18-H); *m/z* 516 (M^+, 1%), 475 (1), 389 (1), 371(4), 329 (4), 312 (8), and 254 (100). The *methyl ester* derivative (**17**) was obtained as a colourless crystal, m.p. 192–194 °C (ethyl acetate–light petroleum) (Found: M^+, 530.0800. $C_{22}H_{27}O_7I$ requires *M*, 530.0800); δ_H 5.24 (1 H, br s, 17-H), 4.97 (1 H, br s, 17-H), 4.91 (1 H, d, *J* 3.8 Hz, 3-H), 4.437 (1 H, br d, *J* 5.8 Hz, 1-H), 3.73 (1 H, d, *J* 10.8 Hz, 5-H), 3.65 (3 H, s, MeO), 3.21 (1 H, d, *J* 10.8 Hz, 6-H), 2.16 (3 H, s, OAc), and 1.14 (3 H, s, 18-H); *m/z* 530 (M^+, 2.3%), 471 (6.5), 403 (5.1), 371 (8.6), 343 (14.5), 311 (10.7), 135 (35.4), and 43 (100).

ent-3α,10β,13-*Trihydroxy*-20-*nor*-6-epi-*gibberell*-16-*ene*-7,19-*dioic Acid* 19,10-*Lactone* (6-epi-GA_1) (**19**).—The iodo lactone (**15**) (250 mg, 0.41 mmol) in dry toluene was refluxed with bis(tributyltin) oxide (0.2 ml, 0.39 mmol) in a Dean–Stark apparatus for 1 h under a nitrogen atmosphere. The Dean–Stark was replaced by a condenser and tributyltin hydride (0.3 ml, 1.1 mmol) and azoisobutyronitrile (1 mg) were added. The resulting solution was refluxed for 1 h and the solvent was evaporated. The residue was dissolved in a saturated methanolic sodium carbonate (20 ml) and stirred for 8 h. The reaction mixture was acidified to pH 2 with 2M hydrochloric acid and partially concentrated under reduced pressure. Acidic water was added and the mixture exhaustively extracted with ethyl acetate. The organic fractions were combined and washed with brine, dried (Na_2SO_4), and concentrated to dryness to give crude 6-epi GA_1 (**19**) which crystallized from acetone–light petroleum (120 mg, 0.34 mmol), m.p. 214–218 °C (Found: M^+, 348.1571. $C_{19}H_{24}O_6$ requires *M*, 348.1573); δ_H [*Methyl ester* (**20**)] 5.21 (1 H, dd, *J* 2 and 3 Hz, 17-H), 4.93 (1 H, br s, 17-H), 3.82 (1 H, br s, 3-H), 3.64 (3 H, s, MeO), 3.13 (1 H, d, *J* 10.7 Hz, 5-H), 3.06 (1 H, d, *J* 10.7 Hz, 6-H) and 1.18 (3 H, s, 18-H); *m/z* 348 (M^+, 2.20%), 330 (3.54), 302 (4.08), 291 (6.52), 284 (4.42), 192 (7.95), 185 (3.61), 173 (3.79), 163 (3.25), 152 (4.13), 143 (4.86), 135 (12.04), and 43 (100).

Attempts to obtain ent-3α,10β,13-*Trihydroxy*-20-*nor*-6-epi-

gibberella-1,16-*diene*-7,19-*dioic Acid* 7-*Methyl Ester* 19,10-*Lactone* (**21**).—(*a*) The iodolactone (**16**) (79 mg, 0.15 mmol) in dry toluene (2 ml) was refluxed with DBU (0.160 ml, 1.07 mmol) for 75 min under a nitrogen atmosphere. Removal of the solvent under reduced pressure gave a gum residue formed by an intractable mixture of polar compounds.

(*b*) The iodolactone 7-methyl ester (**17**) (40 mg, 0.073 mmol) in dry toluene (1 ml) was refluxed with DBU (0.060 ml, 0.4 mmol) for 35 min. The reaction mixture was transferred to a decantation funnel with the aid of ethyl acetate and the resulting brown solution was washed with 2M hydrochloric acid, saturated aqueous sodium hydrogen carbonate and brine, dried (Na_2SO_4), and concentrated to dryness to give a gum residue which was flash chromatographed.

Elution with ethyl acetate–light petroleum (4:6, v/v) gave 3β-acetate of GA_3 methyl ester (**22**) (24 mg, 0.062 mmol).

(*c*) The iodolactone (**16**) (10 mg, 0.026 mmol) in pyridine (1 ml) was heated at 80 °C for 9 h and for a further 8 h at 90 °C. The pyridine was distilled off with the aid of toluene to give an intractable mixture of compounds.

(*d*) The iodolactone 7-methyl ester (**17**) (22 mg, 0.041 mmol) in collidine (0.5 ml) was heated at 110 °C for 8 h. The reaction mixture was allowed to reach room temperature and then cooled to 0 °C (ice bath). Methanol (2 ml) was added and the resulting solution was treated with ethereal diazomethane (0.5 ml). The excess of reagent was destroyed with acetic acid, the solvent evaporated, and the residue flash chromatographed. Elution with ethyl acetate–light petroleum (1:1, v/v) gave the dimethyl ester derivative of the diacid (**15**) (15 mg, 0.037 mmol).

Crystallographic Data for Compound (**17**).—The X-ray intensity data for compound (**17**) were collected on a Philips PW-1100 automatic four-circle diffractometer using graphite-Cu-K_α radiation (λ = 1.5418 Å), with a crystal of dimensions 0.31 × 0.20 × 0.15 mm. The unit cell was determined by least-squares with 2θ values of 28 high-angle reflections: a = 11.456(1), b = 10.930(1), c = 9.399(1) Å, β = 111.085(2)°. All accessible independent reflections collected with a range $2\theta \leqslant 65°$ and systematic absences (only $0\ k\ 0$ when $K = 2n + 1$ was observed) are consistent with the space group $P2_1$. Two reflections were monitored periodically and every 90 reflections showed no variation in intensity, with 2θ/ω scan, scan speed 0.060, and scan width 1.50. The intensities of 1 968 reflections were collected, 1 831 reflections were considered in accordance with the criterion $I \geqslant 2\sigma(I)$, and they were used for structure determination and refinement. Corrections for Lorentz and polarization effects were applied, but not for absorption and secondary extinction.

The structure was solved using direct methods, DIRDIF,[14] and refined by full-matrix least-squares on F_o with anisotropic thermal parameters for non-hydrogen atoms. H-Atoms located from difference Fourier synthesis were included as fixed contributors with isotropic thermal parameters. Function minimized $\Sigma w(|F_o| - |F_c|)^2$, where w was empirically calculated to obtain flat dependence in $\langle w\Delta^2 F \rangle$ *vs.* $\langle F_o \rangle$ and *vs.* $\langle \sin\theta/\lambda \rangle$. Convergence was reached at R = 4.2% and R_w = 5.3%. The final difference Fourier map showed values not exceeding 0.09 eÅ^3. All calculations were performed on a Vax 750/11 computer using a package of crystallographic programs.[15] The atomic scattering factors have been obtained from International Tables for X-ray Crystallography.[16] The final positional parameters are listed in Table 2. Bond distances and angles, and thermal parameters are available on request from the Cambridge Crystallographic Data Centre.*

Acknowledgements

We thank Abbott Laboratories, Chicago, USA, for samples of gibberellic acid, and the CICYT, Madrid, for financial support. One of us (E. P.) thanks 'Caja Canarias' for a fellowship.

References

1 J. L. Stoddart and M. A. Venis, in 'Encyclopedia of Plant Physiology,' vol. 9, 'Hormonal Regulation of Development I,' ed. J. MacMillan, Springer-Verlag, Berlin, 1980, p. 478.
2 J. E. Graebe and H. J. Ropers, in 'Phytohormones and Related Compounds: A Comprehensive Treatise,' vol. 1, 'The Biochemistry of Phytohormones and Related Compounds,' eds. D. S. Letham, P. B. Goodwin, and T. J. V. Higgins, Elsevier/North-Holland Biomedical Press, Amsterdam, 1978, p. 138.
3 J. R. Bearder and J. MacMillan, *J. Chem. Soc., Chem. Commun.*, 1976, 421.
4 V. M. Frydman and J. MacMillan, *Planta*, 1975, **125**, 181.
5 E. J. Corey, R. L. Danheiser, S. Chandrasekaran, G. E. Keck, B. Golapagan, S. D. Larsen, P. Siret, and J. L. Grass, *J. Am. Chem. Soc.*, 1978, **100**, 8034.
6 D. F. Jones and P. McCloskey, *J. Appl. Chem.*, 1963, 13.
7 B. M. Fraga, M. G. Hernández, and F. G. Tellado, *J. Chem. Soc., Perkin Trans. 1*, 1986, 21.
8 N. Murofushi, I. Yamaguchi, H. Ishigooka, and N. Takahashi, *Agr. Biol. Chem.*, 1976, **40**, 2471.
9 M. Lischewski and G. Adam, *Tetrahedron Lett.*, 1980, **21**, 1627.
10 M. Lischewski, *Z. Chem.*, 1982, **22**, 311.
11 W. Klyne and J. Buckingham, 'Atlas of Stereochemistry,' Chapman and Hall, London, 1974, vol. 1, p. 111; 1978, vol. 2, p. 59.
12 W. L. Duax, C. M. Weeks, and D. C. Roher, in 'Topics in Stereochemistry,' eds. N. L. Allinguer and E. L. Eliel, Wiley, New York, 1976, vol. 9, pp. 271–383.
13 B. M. Fraga, I. González-Collado, M. G. Hernández, F. G. Tellado, and A. Perales, *J. Chem. Soc., Perkin Trans. 1*, 1987, 1955.
14 P. T. Beurskens, W. P. Bosman, H. M. Doesburg, R. D. Gould, T. E. M. Van der Hark, P. A. J. Prick, J. H. Noordick, G. Beursquens, and V. Parthasarathi, 'Direct Methods for Difference Structures,' Crystallography Laboratory, Toernooiveld, 6525 ED, Nijmegen, Netherlands.
15 J. M. Stewart, P. A. Machin, C. W. Dickinson, H. L. Ammont, H. Heck, and H. Flack, 'The X-Ray System,' Tech. Rep. 446, Computer Science Center, University of Maryland, College Park, Maryland, U.S.A., 1976.
16 'International Tables for X-Ray Crystallography,' Kynoch Press, Birmingham, 1974, vol. 4.

* For details see the 'Instructions for Authors (1990)', *J. Chem. Soc., Perkin Trans. 1*, 1990, Issue 1.

Paper 9/03050F
Received 18*th July* 1989
Accepted 13*th November* 1989