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DITERPENE ACIDS FROM *HELIANTHUS* SPECIES AND THEIR MICROBIOLOGICAL CONVERSION BY *GIBBERELLA FUJIKUROI*, MUTANT B1-41a

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Key Word Index—*Helianthus* sp.; Compositae; diterpene acids; ent-12,16-cyclokauranoic acid (trachylobanic acid); ent-13(*S*)-angeloxyatisenoic acid; ent-13(*S*)-hydroxyatisenoic acid; ent-12β-acetoxykaurenoic acid; metabolism; 12,16-cyclogibberellins A_9 and A_{12}.

Abstract—The diterpene acid content in 10 species of *Helianthus* has been investigated. Ent-12,16-cyclokauranoic acid, isolated from *H. annuus*, is converted into a series of 12,16-cyclogibberellins by cultures of *Gibberella fujikuroi*, mutant B1-41a, and 12,16-cyclogibberellins A_9 and A_{12} have been isolated. Ent-12β-acetoxykaurenoic acid and ent-13(*S*)-angeloxyatisenoic acid have been isolated from *H. decapetalus*; the metabolism of ent-13(*S*)-hydroxyatisenoic acid and atisenoic acid by B1-41a is also described.

INTRODUCTION

The enzymes of *Gibberella fujikuroi* which catalyse the biosynthesis of gibberellins (GAs) have been shown to possess low substrate specificity. For example, *G. fujikuroi*, mutant B1-41a, which is blocked for GA-biosynthesis at the step before ent-kaur-16-en-19-oic acid (**1**) has been shown to convert analogues of ent-kaurenoic acid (**1**) into the corresponding analogues of the fungal GAs [1, 2]. Continuing our studies to define the limits of this substrate non-specificity we have examined the metabolism of the following ring C/D isomers of ent-kaurenoic acid by cultures of the mutant B1-41a: ent-12,16-cyclokauran-19-oic acid (trachylobanic acid) (**9**) and ent-atis-16-en-19-oic acid (**10**). We also report on the metabolism of ent-13(*S*)-hydroxy-atis-16-en-19-oic acid (**14**) by cultures of the mutant B1-41a. A preliminary report on the metabolism of ent-12,16-cyclokauranoic acid (**9**) has been published [3] and Hanson *et al.* [4] have reported the conversion of ent-7α-hydroxyatis-16-en-19-oic acid (**13**) to atisaGA_{12} (**27**) and atisaGA_{14} (**28**) by *G. fujikuroi*, blocked for GA-biosynthesis with AMO-1618.

RESULTS AND DISCUSSION

Acquisition of substrates

The substrates were obtained from *Helianthus* sp. Ent-kaurenoic acid (**1**) and ent-12,16-cyclokauranoic acid (**9**) co-occur in *Helianthus annuus* [5, 6] but the mixture is not readily separated. In the present study, the mixture was oxidized by sodium periodate–osmium tetroxide and the unreacted ent-12,16-cyclokauranoic acid (**9**) was then separated from ent-16-oxo-17-norkauranoic acid (**2**) by chromatography.

In a survey of other *Helianthus* sp., extracts of flower heads at full bloom were examined by GC/MS. The major diterpenes identified are shown in Table 1. Ent-12β-acetoxykaur-16-en-19-oic acid (**3**) and ent-13(*S*)-angeloxyatis-16-en-19-oic acid (**11**) were unknown at the time of this survey. Their isolation and structure determination were simultaneously and independently reported by Bohlmann *et al.* [7] and by us [8]. The ethyl acetate portion of a methanol extract of flower heads *H. decapetalus* was subjected to droplet-countercurrent chromatography to yield the pure esters **3** and **11**. In larger scale preparations, the free alcohols **5** and **14** were more conveniently obtained by separation of the petrol-soluble portion, from the methanol extract of the aerial parts of *H. decapetalus*, into angelate-rich and acetate-rich fractions by chromatography on a Si gel column. Chromatographic purification of the hydrolysates of these fractions gave the pure alcohols **5** and **14**.

The alcohol (**5**), obtained from the acetate (**3**), was identical to ent-12β-hydroxykaur-16-en-19-oic acid (**5**) synthesized by Lewis and MacMillan [9], and the methyl ester (**6**) was de-oxygenated to methyl ent-kaur-16-en-19-oate (**7**) by reduction [10] of the thiobenzoate (**8**) with Bu_3SnH.

The alcohol (**14**) from the angelate (**11**) was shown to have the ent-atisane skeleton by reduction of the methyl ester thiobenzoate (**15**) to methyl ent-atisenoate (**16**) with Bu_3SnH. Although methyl ent-kaurenoate (**7**) and methyl ent-atisenoate (**16**) are not separable by GC or TLC, the mass spectrum of the latter, but not the former, contains a characteristic $[M - 28]^+$ ion. The mass spectrum of the TMSi derivative of the methyl ester (**17**) of the alcohol (**14**) from the angelate (**11**) contained a base peak $[M - 116]^+$ indicating that the hydroxyl in the parent alcohol was at C-13 or C-14. Oxidation of the methyl ester (**17**) gave a ketone (**18**) with UV λ_{max} 286 nm (ε 288). The high extinction coefficient indicated a β, γ-ketone thus placing the hydroxyl group at C-13. This location was confirmed by base-catalysed epimerization of the nor-

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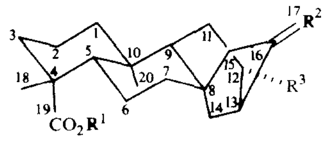
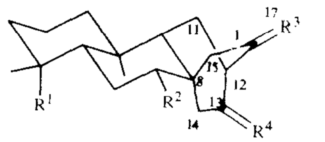
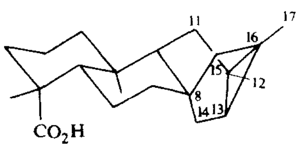


	R^1	R^2	R^3
1	H	CH_2	H
2	H	O	H
3	H	CH_2	OAc
4	Me	CH_2	OAc
5	H	CH_2	OH
6	Me	CH_2	OH
7	Me	CH_2	H
8	Me	CH_2	OCSPh



	R^1	R^2	R^3	R^4
10	CO_2H	H	CH_2	H_2
11	CO_2H	H	CH_2	H, α—OAng
12	CO_2Me	H	CH_2	H, α—OAng
13	CO_2H	OH	CH_2	H_2
14	CO_2H	H	CH_2	H, α—OH
15	CO_2Me	H	CH_2	H, α—OCSPh
16	CO_2Me	H	CH_2	H_2
17	CO_2Me	H	CH_2	H, α—OH
18	CO_2Me	H	CH_2	O
19	CO_2Me	H	O	H, α—OH
20	CO_2Me	H	O	H, β—OH
21	Me	H	O	H, β—OH
22	CO_2Me	H	CH_2	H, β—OH
23	CO_2Me	H	O	H_2
24	CO_2Me	H	O	H, α—OCSPh
25	CO_2Me	H	O	H, β—Cl
26	CO_2Me	H	CH_2	H, β—Cl

—OAng = —OCO(Me) $\overset{t}{=}$ CHMe



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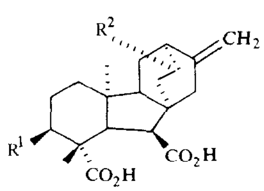


	R^1	R^2
27	H	H
28	OH	H
29	H	OH

ketone methyl ester (**19**) of the alcohol (**17**) to the epimer **20**. This retro-alcohol equilibration via the intermediate aldehyde (**30**) to give uniquely the epimer **20** defines the ent-13(*R*)-hydroxy stereochemistry in **20** and, therefore, the ent-13(*S*)-hydroxy stereochemistry in the norketone (**19**) and in the parent alcohol (**14**). This conclusion follows from the results of Kelly *et al.* [11] who found that deketalization of **31**, with concurrent formation, and condensation of the intermediate aldehyde (**30**) gave only the ent-13(*R*)-hydroxyketone (**21**). The stereochemical assignments at C-13 are supported by the ^1H NMR chemical shifts of the C-20 methyl protons. In the methyl ester (**17**) of the alcohol (**14**), derived from the natural angelate, these protons occur at δ 0.88 and are deshielded compared to those at 0.78 in methyl atisenoate (**16**). In the 13-ketone (**18**) these protons are shielded and occur at 0.65. Similarly the C-20 protons in the epimeric norketones **19** and **20** occur at 0.94 and 0.78, respectively, compared with 0.85 for the C-20 protons of methyl ent-atisenoate norketone (**23**).

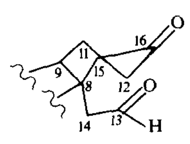
Ent-atis-16-en-19-oic acid (**10**) was prepared for the metabolic studies by hydrolysis, with NaSEt in HMPA, of methyl ent-atisenoate (**16**) which in turn was prepared either by reduction of the thiobenzoate (**15**) with Bu_3SnH or by similar reduction of the thiobenzoate (**24**) of the norketone (**19**), followed by Wittig reaction of the reduction product with methylenetriphenylphosphorane.

In preparations of the thiobenzoate (**24**) of the norketone (**19**) traces of another product were observed. This compound became the major product when *iso*-Pr_2EtN was added to the reaction and it was identified as the ent-13(*R*)-chloroketone (**25**). Full characterization of compound **25** was carried out after Wittig methylenation to the ent-13(*R*)-chloro-olefin (**26**), the mass spectrum of which showed a molecular ion at *m/z* 350 with the correct isotope ratios for a molecule containing one chlorine atom. The ent-13(*R*) stereochemistry is expected from an S_N2 displacement of an ent-13(S)-OC(Ph) = $\overset{+}{N}Me_2$ group

Table 1. Diterpenes identified by GC/MS in the extracts of flower heads of various *Helianthus* sp.

Species	Diterpene
H. decapetalus	Ent-kaurenoic acid
	Ent-12β-acetoxykaurenoic acid*
	Ent-13(*S*)-angeloxyatisenoic acid*
H. decapetalus (var. multiflorus)	Ent-12β-acetoxykaurenoic acid*
H. rigidus	Trachylobanic acid
	Ent-kaurenoic acid*
	Ent-12β-acetoxykaurenoic acid
H. debilis	Trachylobanic acid
H. annuus	Ent-kaurenoic acid*
H. grosse-serratus	Ent-9, 11-didehydrokaurenoic acid
H. giganteus	Trachylobanic acid
	Ent-kaurenoic acid*
H. maximilliani	Ent-9, 11-didehydrokaurenoic acid*
H. nuttallii	Ent-kaurenoic acid*
H. tomentosus	Trachylobanic acid
H. hirsutus	An isomer of ent-kaurenoic acid*
	Trachylobanic acid
	Ent-kaurenoic acid
	Ent-12β-acetoxykaurenoic acid

*Main constituent.



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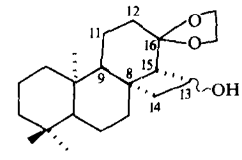

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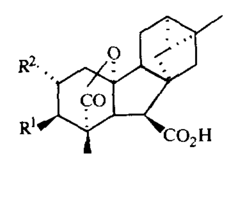
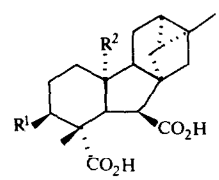


32 $R^1 = R^2 = H$

33 $R^1 = OH, R^2 = H$

34 $R^1 = R^2 = OH$

35 $R^1 = H, R^2 = OH$



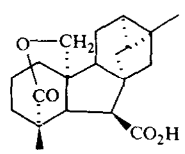
36 $R^1 = H, R^2 = Me$

37 $R^1 = OH, R^2 = Me$

38 $R^1 = H, R^2 = CHO$

39 $R^1 = H, R^2 = CO_2H$

40 $R^1 = OH, R^2 = CO_2H$



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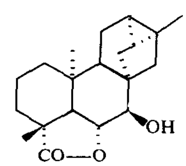

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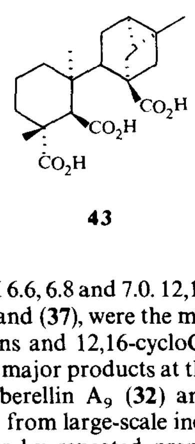

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by chloride and is supported by the ^1H NMR chemical shift of the C-20 protons in the chloroketone (**25**) (δ 0.80) and the chloro-olefin (**26**) (δ 0.73).

Metabolism of substrates

Substrates were incubated in shake-flask cultures of pigmented mycelium of *G. fujikuroi*, mutant B1-41a, resuspended in 0%N ICI medium [12] either at the natural pH 4.7 or buffered at the specified pH. The products were methylated (Me) and trimethylsilylated (MeTMSi) then analysed by GC/MS using packed columns of 2% SE-33 or 2% QF-1. The structures of the metabolites were deduced from their mass spectra except for 12,16-cycloGA$_9$ (**32**) and 12,16-cycloGA$_{12}$ (**36**) which were isolated and fully characterized.

Investigations were first made with ent-12,16-cyclokauran-19-oic acid (**9**). The following exploratory small-scale experiments were conducted: (a) with 2 mg substrate per 25 ml resuspension medium at pH 3.0 for 1, 3 and 5 days and at pH 7.0 for 5 days; (b) with 2, 10 and 10 mg substrate in 25, 25 and 125 ml resuspension medium, respectively, at pH 7.0; and (c) with 10 mg substrate in 25 ml resuspension medium at pH 6.6, 6.8 and 7.0. In all experiments, the 12,16-cyclo-analogues of GA$_4$(**33**), GA$_9$(**32**), GA$_{14}$(**37**), GA$_{24}$(**38**), GA$_{25}$(**39**), GA$_{47}$(**34**), 7β-hydroxykaurenolide (**42**), the tri-acid (**43**), and a trihydroxy derivative of the substrate were identified. An unidentified metabolite was later identified as 12,16-cycloGA$_{40}$(**35**) by GC/MS comparison with a compound, isolated by Dr. J. R. Hanson from the incubation of ent-trachylobane with *G. fujikuroi* and shown by him to have the structure **35** by X-ray crystallography. Traces of 12,16-cycloGA$_{13}$(**40**) were detected in the pH 3.0 incubations and 12,16-cycloGA$_{12}$(**36**), 12,16-cycloGA$_{15}$ (**41**) and ent-7α-hydroxy- and ent-6α,7α-dihydroxy-12,16-cyclokaurenoic acids (**44** and **45**) were detected in the incubations at pH 6.6, 6.8 and 7.0. 12,16-Cyclogibberellins A$_4$ and A$_{14}$, (**33**) and (**37**), were the major products of the pH 3.0 incubations and 12,16-cycloGA$_9$ and GA$_{12}$ (**32**) and (**36**) were the major products at the higher pH values.

12,16-Cyclogibberellin A$_9$ (**32**) and 12,16-cycloGA$_{12}$ (**36**) were isolated from large-scale incubations at pH 6.6, 6.8 and 7.0 either by repeated prep. TLC of the total product or, more effectively, by ethyl acetate extraction of the mixture of total products, dissolved in phosphate buffer pH 6.5, followed by prep. TLC of the extract. A mixture containing 12,16-cycloGA$_4$ (**33**) and 12,16-cycloGA$_{14}$(**37**) was isolated from the experiments but isolation of the pure compounds could not be achieved.

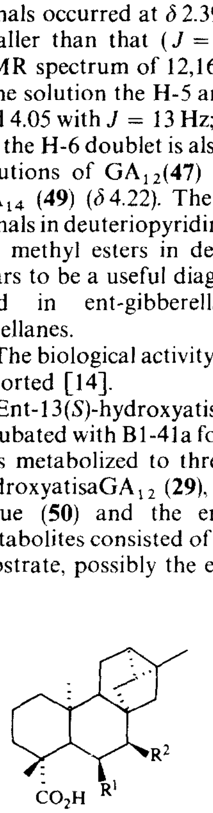
12,16-Cyclogibberellins A$_9$ (**32**) and A$_{12}$(**36**) were characterized by their spectroscopic properties. In the IR spectrum of chloroform solutions, the former showed γ-lactone absorption at 1760 cm^{-1} and carboxyl carbonyl absorption at 1700 cm^{-1} and the latter showed carboxyl carbonyl absorption at 1695 cm^{-1}. The ^{13}C NMR spectra showed no olefinic carbon signals; signals at δ 25.6 for 12,16-cycloGA$_9$ (**32**) and at 25.4 for 12,16-cycloGA$_{12}$ (**36**) were assigned to C-16, the corresponding signal for ent-12,16-cyclokauranoic acid (**9**) occurring at 22.4 (cf. ref. [13]). The ^{1}H NMR spectra of **32** in deuteriochloroform solution and **36** in deuteriopyridine solution contained no vinylic proton signals, two cyclopropyl protons in the range δ 0.5–0.9, two methyl singlets for (**32**) and three methyl singlets for **36**. In the ^{1}H NMR spectrum of 12,16-cycloGA_9 (**32**) in deuteriochloroform the H-5 and H-6 signals occurred at δ 2.39 and 2.55; the J value of 8 Hz is smaller than that (J = 12 Hz) in GA_9 (**46**). In the ^{1}H NMR spectrum of 12,16-cycloGA_{12} (**36**) in deuteriopyridine solution the H-5 and H-6 signals occurred at δ 2.26 and 4.05 with J = 13 Hz; the unexpectedly low field signal for the H-6 doublet is also present in the deuteriopyridine solutions of GA_{12}(**47**) (δ 4.08), GA_{13} (**48**) (δ 5.04) and GA_{14} (**49**) (δ 4.22). The shift to lower field of the H-6 signals in deuteriopyridine solutions, compared to that for the methyl esters in deuteriochloroform solutions, appears to be a useful diagnosis of the presence of a 19-oic acid in ent-gibberellanes and ent-12,16-cyclogibberellanes.

The biological activity of 12,16-cycloGA_9 (**32**) has been reported [14].

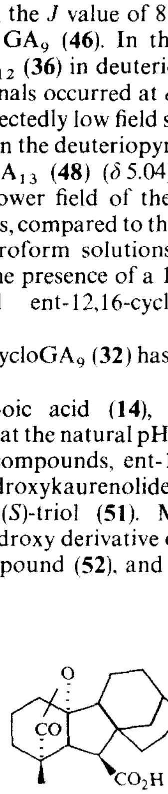
Ent-13(*S*)-hydroxyatis-16-en-19-oic acid (**14**), when incubated with B1-41a for five days at the natural pH (4.7), was metabolized to three major compounds, ent-13(*S*)-hydroxyatisaGA_{12} (**29**), the 7β-hydroxykaurenolide analogue (**50**) and the ent-6α,7α,13(*S*)-triol (**51**). Minor metabolites consisted of a monohydroxy derivative of the substrate, possibly the ent-7α compound (**52**), and three isomeric dihydroxy substrates. No analogues of the C_{19}-GAs were observed and ent-13(*S*)-hydroxyatisaGA_{12} (**29**) was the only C_{20}-GA analogue obtained.

Ent-atis-16-en-19-oic acid (**10**) similarly gave no C_{19}-GA analogues. Only three metabolites could be identified by GC/MS. They were the seco-B-diacid (**53**), atisaGA_{14}(**28**) and an isomer of **28** which was the major metabolite. AtisaGA_{14} was identified by direct comparison of its mass spectrum as the MeTMSi derivative with that of a sample provided by Dr. J. R. Hanson. The position of the hydroxy group in the isomer could not be deduced from its mass spectrum but it may, by analogy, be 2α.

The failure of the atisene derivatives to undergo oxidation at C-20 to give C-19 gibberellin analogues has also been observed by Hanson *et al.* [4] whose incubations of ent-7α-hydroxyatisenoic acid (**13**) gave only atisaGA_{12} (**27**) and atisaGA_{14} (**28**).



44 R^1 = H, R^2 = OH
45 R^1 = R^2 = OH



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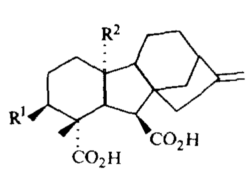
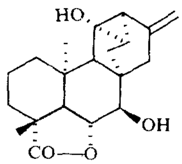


47 R^1 = H, R^2 = Me
48 R^1 = OH, R^2 = CO_2H
49 R^1 = OH, R^2 = Me



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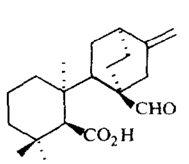

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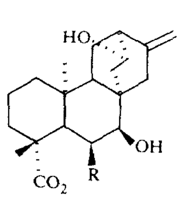

51 R = OH
52 R = H

EXPERIMENTAL

For general procedures see ref. [15].

Isolation of 12,16-*cyclokauran*-19-*oic acid* (**9**). Flower heads minus seeds (4 kg) of *Helianthus annuus* were chopped-up and covered with MeOH. After 18 hr, the MeOH extract was collected. This process was repeated twice more and the combined MeOH extract was evaporated *in vacuo*. The aq. residue was extracted with EtOAc to yield a gum (18.8 g) which was chromatographed on a column of Si gel (550 g). After elution with petrol (1 l.), petrol–EtOAc (99:1, 1 l.), petrol–EtOAc (97:3, 1 l.), petrol–EtOAc (93:7, 1 l.), a mixture (*ca* 2:1 by GC) of ent-kaur-16-en-19-oic acid (**1**) and ent-12,16-cyclokauran-19-oic acid (**9**) was eluted with petrol–EtOAc (85:15, 1 l. 7.6 g), petrol–EtOAc (8:2, 1 l., 1.36 g) and petrol–EtOAc (7:3, 1 l., 0.48 g).

The mixture of **1** and **9** (2.66 g) in THF (20 ml) and H_2O (20 ml) was stirred at room temp. overnight with $NaIO_4$ (9 g) and a small crystal of OsO_4. After addition of H_2O, the THF was removed under vacuum and the residual aq. soln, after acidification to pH 3.5 with HCl, was extracted with EtOAc. Recovery from the EtOAc extract gave a gum (2.6 g) containing (TLC and GC) a mixture of ent-12,16-cyclokauranoic acid (**9**) and ent-16-oxo-17-norkauranoic acid (**2**).

The oxidation product (2.6 g) was chromatographed on a column of Si gel (150 g) eluted with petrol–EtOAc (10:1). Fractions (10 ml) were monitored by TLC using petrol–EtOAc–HOAc (70:30:1). Fractions 49–80 contained ent-12,16-cyclokauranoic acid (**9**, 0.52 g) which, after purification by TLC on Si gel using petrol–EtOAc–HOAc (70:30:1) had mp 92–93°; IR ν_{max} cm^{-1}: 3500–2500, 1690 and 1260; ^{1}H NMR ($CDCl_3$): δ 0.90, 1.15, 1.23 (each *s*, 3 × Me); ^{13}C NMR ($CDCl_3$): δ 12.4 (*q*, C-20), 18.7 (*t*, C-2), 19.8 (*t*, C-6), 20.6 (*d*, C-12), 20.6 (*q*, C-17), 21.8 (*t*, C-11), 22.4 (*s*, C-16), 24.3 (*d*, C-13), 28.9 (*q*, C-18), 33.1 (*t*, C-14), 37.8 (*t*, C-3), 38.9 (*s*, C-10), 39.4 (*t*, C-1), 39.4 (*t*, C-7), 40.8 (*s*, C-8), 43.7 (*s*, C-4), 50.4 (*t*, C-15), 52.8 (*d*, C-9), 57.0 (*d*, C-5), 184.9 (*s*, C-19); EIMS (Me ester) *m/z* (rel. int.): 316 [M]$^+$ (79), 301 (32), 284 (25), 273 (34), 260 (63), 257 (63), 245 (20), 241 (35), 121 (100).

The column was then eluted with petrol–EtOAc (5:1, 800 ml) to give ent-16-oxo-17-norkauranoic acid (**2**, 1.23 g), identified by GC, TLC and MS.

Droplet counter-current chromatography (*DCCC*) *of* Helianthus decapetalus *extract*. The instrument used was a Tokyo Rikakikai DCCA containing 350 tubes [40 × 0.2 (i.d.) cm]. Fractions (*ca* 7 ml) were collected. The EtOAc-soluble portion (1 g) of a MeOH extract of the flower heads of *H. decapetalus* was subjected to DCCC using the solvent system petrol–EtOH–H_2O–EtOAc (5:4:1:2) in the ascending mode (normal phase).

Fractions 40–48 (310–380 ml) contained ent-13(*S*)-angeloxyatis-16-en-19-oic acid (**11**) (87 mg), characterized as its methyl ester (**12**). (Found: M^+, 414.276. $C_{26}H_{38}O_4$ requires M^+, 414.277.) IR $\nu_{max}^{CHCl_3}$ cm^{-1}: 1710, 1650, 975, 913, 887 and 848; ^{1}H NMR ($CDCl_3$): δ 0.83 (*s*, Me-20), 1.19 (*s*, Me-18), 1.93 (*q*, *J* = 1 Hz, Me-2'), 2.02 (*dq*, *J* = 7, 1 Hz, Me-3'), 2.52 (*br*, A-12), 3.67 (*s*, CO_2Me), 4.73 and 4.94 (each *br*, H_2-17), 5.03 (*dt*, *J* = 10, 2Hz, H-13), 6.12 (*q*, *J* = 7 Hz, H-3'); ^{13}C NMR ($CDCl_3$): δ 13.0 (*q*, C-20), 15.6 (*q*, Me-2'), 18.7 (*t*, C-2), 20.3 (*t*, C-6), 20.7 (*q*, Me-3'), 22.1 (*t*, C-11), 28.7 (*q*, C-18), 34.2 (*s*, C-8), 36.8 (*t*, C-14), 38.1 (*t*, C-3), 38.4 (*s*, C-10), 39.6 (*t*, C-1), 39.9. (*t*, C-7), 41.4 (*d*, C-12), 43.8 (*s*, C-4), 46.8 (*t*, C-15), 51.2 (*d*, C-9), 51.2 (*q*, OMe), 56.8 (*d*, C-5), 72.0 (*d*, C-13), 108.3 (*t*, C-17), 128.1 (*d*, C-2'), 137.9 (*d*, C-3'), 147.5 (*s*, C-16), 167.5 (*s*, C-1'), 177.9 (*s*, C-19). EIMS *m/z* (rel. int.): 414 [M]$^+$ (8), 370 (7), 314 (51), 299 (8), 259 (22), 255 (18), 83 (100).

Fractions 64–74 (500–590 ml) contained ent-12β-acetoxykaur-16-en-19-oic acid (**3**, 87 mg), characterized as its methyl ester (**4**), mp 126–128° (found: M^+ 374.246; $C_{23}H_{34}O_4$ requires M^+ 374.246); IR $\nu_{max}^{CHCl_3}$ cm^{-1}: 1720 and 895; ^{1}H NMR ($CDCl_3$): δ0.91 (*s*, Me-20), 1.19 (*s*, Me-18), 2.03 (*s*, OCOMe), 2.76 (*t*, H-13), 3.66 (*s*, COOMe), 4.75 (*t*, *J* = 4Hz, H-12), 4.83 and 4.94 (each *br*, H_2-17); ^{13}C NMR ($CDCl_3$): δ 13.3 (*q*, C-20), 19.0 (*t*, C-2), 21.6 (*q*, COMe), 21.8 (*t*, C-6), 23.3 (*t*, C-11), 28.9 (*q*, C-18), 33.9 (*t*, C-14), 37.9 (*t*, C-3), 38.5 (*s*, C-10), 40.6 (*t*, C-1), 41.1 (*t*, C-7), 43.3 (*s*, C-4), 43.9 (*s*, C-8), 48.1 (*d*, C-13), 49.0 (*t*, C-15), 51.2 (*q*, OMe), 55.3 (*d*, C-9), 56.8 (*d*, C-5), 73.8 (*d*, C-12), 106.4 (*t*, C-17), 150.9 (*s*, C-16), 170.3 (*s*, COMe), 178.0 (*s*, C-19); EIMS *m/z* (rel. int.): 374 [M]$^+$ (3), 314 (100), 299 (32), 255 (52), 239 (29), 146 (56), 121 (74).

Large scale isolation of the hydroxy acids **5** *and* **14**. Aerial parts of *H. decapetalus* (2.7 kg) were extracted with MeOH. The extract was concd to *ca* 2 l., diluted with H_2O to 10% aq. and extracted with petrol. Evaporation of the extract gave a gum (30.5 g). The aq. MeOH was evaporated, diluted with H_2O and extracted with EtOAc. Evaporation of the EtOAc gave a gum (35.3 g).

CC of the petrol extract on Si gel (1.1 kg, 80 × 6 cm), eluted with increasing amounts of EtOAc in petrol, gave with 15% EtOAc 'angelate-containing' fractions (8.7 g) and with 20–25% EtOAc 'acetate-containing' fractions (4.3 g).

Similar chromatography of the EtOAc extract gave 'angelate-containing' fractions (0.5 g) and 'acetate-containing' fractions (1.8 g).

The combined 'angelate' fractions (9.2 g) in 5% KOH in MeOH (500 ml) were refluxed for 2 hr. The MeOH was evaporated and the residue partitioned between H_2O and EtOAc. The aq. layer was then acidified with conc. HCl and extracted with EtOAc to give a gum (3.9 g). Chromatography on Si gel (250 g), eluted with increasing amounts of EtOAc in petrol gave, at 20–30% EtOAc, ent-13(*S*)-hydroxyatisenoic acid (**14**, 799 mg). The methyl ester (**17**), prepared with CH_2N_2, was a gum. (Found: M^+ 332.234; $C_{21}H_{32}O_3$ requires M^+ 332.235.) IR $\nu_{max}^{CHCl_3}$ cm^{-1}: 3600, 3450, 1715, 885; ^{1}H NMR ($CDCl_3$): δ 0.88 (*s*, Me-20), 1.18 (*s*, Me-18), 3.64 (*s*, CO_2 Me), 4.0 (*dt*, *J* = 10, 2 Hz, H-3) and 4.65 and 4.83 (each *br*, H_2-17); EIMS *m/z* (rel. int.): 332 [M]$^+$ (9), 330 (10), 314 (30), 299 (15), 288 (69), 273 (18), 265 (17), 255 (38), 239 (22), 121 (100), 109 (66), 105 (78).

The combined 'acetate' fractions (6.1 g) were hydrolysed as above with 5% KOH in MeOH (300 ml). Work-up as before and chromatography of the EtOAc-soluble acids gave at 20–25% EtOAc in petrol, ent-12β-hydroxykaurenoic acid (**5**, 750 mg), mp 229–231°. The methyl ester (**6**) had M^+ 332.235 ($C_{21}H_{32}O_3$ requires M^+ 332.235); IR $\nu_{max}^{CHCl_3}$ cm^{-1}: 3610, 3450, 1715, 1655, 980; ^{1}H NMR ($CDCl_3$): δ 1.0 (*s*, Me-20), 1.18 (*s*, Me-18), 2.64 (*t*, *J* = 4 Hz, H-3), 3.66 (*s*, CO_2 Me), 3.80 (*t*, *J* = 4 Hz, H-12), 4.78 and 4.84 (each *br*, H_2-17); EIMS *m/z* (rel. int.): 332 [M]$^+$ (66), 317(6), 314 (57), 299 (23), 288 (7), 273 (17), 255 (32), 239 (21), 121 (56), 107 (71), 91 (100) and was identical (TLC, NMR and GC/MS) with a synthetic sample [9].

Deoxygenation of methyl ent-13(S)-hydroxyatisenoate (**17**). The alcohol **17** (5 mg) in dry THF (0.5 ml) was treated with a 0.6 M soln of $PhCCl{=}\overset{+}{N}Me_2\overset{-}{C}l$ in CH_2Cl_2 (0.5 ml) prepared as in ref. [10] at room temp. overnight. Pyridine (100 μl) and H_2S gas were added. After 5 min H_2O was added and the product recovered in EtOAc. After evaporation the residue in PhMe (1 ml) was treated with Bu_3SnH (50 μl) and 2,2'-azo-bis(2-methylpropionitrile) (one crystal), at reflux for 1 hr. The solvents were evaporated and the product analysed by GC/MS and shown to be methylatisenoate (**16**), identical with an authentic sample; EIMS *m/z* (rel. int.): 316 [M]$^+$ (41), 301 (69), 288 (4), 284 (2), 273 (24), 261 (4), 257 (96), 241 (48), 234 (8), 147 (34), 121 (100).

Oxidation of methyl ent-13(S)-hydroxyatisenoate (**17**). The alcohol (**17**, 20 mg) in pyridine (0.7 ml) was treated with a soln of CrO_3 (40 mg) in pyridine (0.7 ml) at room temp. for 1.5 hr. H_2O was added and the product recovered in EtOAc to give the ketone **18** (15 mg) as a gum. (Found: M^+ 330.220; $C_{21}H_{30}O_3$ requires M^+ 330.220.) UV λ_{max}^{MeOH} nm: 286 (ε 288), IR $\nu_{max}^{CHCl_3}$ cm^{-1}: 1717, 1250, 1090, 893; ^{1}H NMR ($CDCl_3$): δ 0.65 (*s*, Me-20), 1.22 (*s*, Me-18), 3.69 (*s*, CO_2Me) 4.84 and 4.98 (each *br*, H_2-17), EIMS *m/z* (rel. int.): 330 [M]$^+$ (100), 315 (7), 312 (9), 298 (6), 286 (66), 271 (66), 263 (56), 253 (19), 121 (96), 109 (64), 105 (69).

Methyl ent-13(S)-hydroxy-16-oxo-17-noratisenoate (**19**). The alcohol (**17**, 190 mg) in dioxan–H_2O (1:1, 20 ml) was treated with OsO_4 (one crystal) and $NaIO_4$ (320 mg) at room temp. overnight. The dioxan was removed *in vacuo* and the residual H_2O extracted with EtOAc to give the norketone (**19**, 173 mg). (Found: M^+ 334.214; $C_{20}H_{30}O_4$ requires M^+ 334.214.) IR ν_{max}^{Nujol} cm^{-1}: 3390, 1720, 1705; ^{1}H NMR ($CDCl_3$): δ 0.95 (*s*, Me-20), 1.20 (*s*, Me-18), 3.60 (*s*, CO_2 Me), 4.22 (*dt*, *J* = 10, 2 Hz, H-13); EIMS *m/z* (rel. int.): 334 [M]$^+$ (22), 316 (28), 306 (4), 302 (4), 291 (12), 284 (17), 275 (88), 274 (100), 257 (25), 256 (16), 242 (31), 215 (28).

Methyl ent-13(R)-hydroxy-16-oxo-17-noratisenoate (**20**). The preceding ent-13 (*S*)-hydroxyketone (**19**, 41 mg) in 10% NaOH in MeOH (10 ml) was refluxed for 1 hr under N_2. The MeOH was removed *in vacuo* and the residue partitioned between dil. HCl and EtOAc. Recovery from the EtOAc gave the ent-*13*(*R*)-hydroxyketone (**20**, 33 mg), mp 230–233°. (Found: M^+ 334.212; $C_{20}H_{30}O_4$ requires M^+ 334.214.), ^{1}H NMR ($CDCl_3$): δ0.78 (*s*, Me-20), 1.19 (*s*, Me-18), 3.63 (*s*, CO_2Me), 4.09 (*dt*, *J* = 10, 4 Hz, H-13); EIMS *m/z* (rel. int.): 334 [M]$^+$ (24), 316 (27), 291 (21), 284 (24), 275 (91), 274 (100), 257 (26), 242 (28), 215 (38).

Conversion of the hydroxyketone (**19**) *into methyl atisenoate* (**16**). The ent-13 (*S*)-hydroxyketone (**19**, 60 mg) in THF (2 ml) was treated with 0.6 M $PhCCl{=}\overset{+}{N}Me_2\overset{-}{Cl}$ in CH_2Cl_2 (2 ml) in the normal way [10]. After 16 hr pyridine (0.5 ml) and H_2S gas were added. After 10 min the solvents were evaporated and purification of the residue by prep. TLC on Si gel (EtOAc–petrol, 1:1) gave the yellow thiobenzoate (**24**, 30 mg); ^{1}H NMR ($CDCl_3$): δ 0.86 (*s*, Me-20), 1.22 (*s*, Me-18), 3.64 (*s*, CO_2Me), 5.86 (*dt*, *J* = 10, 2 Hz, H-13), 7.37 (*m*, 3 × ArH) and 8.6 (*m*, 2 × ArH).

The thiobenzoate (**24**, 30 mg) in PhMe (5 ml) with 2,2'-azo-bis(2-methylpropionitrile) (1 mg), $(Bu_3Sn)_2O$ (90 μl) and poly-methylhydrogen siloxane (30 μl) was refluxed for 1 hr. Evaporation of the solvent, followed by prep. TLC on Si gel gave methyl-16-oxo-17-noratisenoate (**23**, 22 mg); ^{1}H NMR ($CDCl_3$): δ 0.85 (*s*, Me-20), 1.20 (*s*, Me-18), 3.65 (*s*, CO_2Me); EIMS *m/z* (rel. int.): 318 [M]$^+$ (20), 303 (3), 290 (8), 286 (12), 259 (100), 258 (17), 245 (20), 241 (9), 216 (6), 203 (6), 189 (18), 121 (42), 109 (50).

The above norketone (**23**, 22 mg) was treated with the supernatant ylide soln (4 ml) prepared from $Ph_3\overset{+}{P}Me\overset{-}{B}r$ (3.2 g), NaH (740 mg of 60% oil dispersion, washed with petrol) and THF (25 ml) at room temp. overnight. After 2 hr Me_2CO was added and the soln evaporated. The residue in EtOAc–petrol (1:1) was passed through a short column of Si gel and then purified by prep.

TLC to give methylatisenoate (**16**, 6 mg), mp 119–121°; ^{1}H NMR ($CDCl_3$): δ 0.78 (*s*, Me-20), 1.17 (*s*, Me-18), 3.64 (*s*, CO_2Me), 4.56 and 4.92 (each *br*, H_2-17); EIMS *m/z* (rel. int.): 316 [M]$^+$ (41), 301 (69), 288 (4), 284 (2), 273 (24), 261 (41), 257 (96), 241 (48), 234 (8), 147 (34), 121 (100), identical with an authentic sample.

Methyl ent-13(R)-chloroatis-16-en-19-oate (**26**). The ent-13 (*S*)-hydroxyketone (**19**, 134 mg) in dioxan (3 ml) was treated with iso-Pr_2EtN (300 μl) and 0.6 M $PhCCl=\overset{+}{N}Me_2\bar{C}l$ in CH_2Cl_2 (3 ml) overnight at room temp. Addition of pyridine and H_2S followed by work-up and prep. TLC as above gave the ent-13(*R*)-chloroketone (**25**, 70 mg); ^{1}H NMR ($CDCl_3$): δ 0.80 (*s*, Me-20), 1.20 (*s*, Me-18), 3.66 (*s*, CO_2Me) and 4.28 (*q* of *d*, *J* = 10, 6, 3 Hz, H-13).

The above chloroketone (**25**, 40 mg) was treated with methyltriphenylphosphorane soln (7 ml) prepared as above. After 3 hr the soln was evaporated and the residue in EtOAc–petrol (1:1) was passed through a short column of Si gel and purified by prep. TLC to give methyl ent-13(R)-chloroatis-16-en-19-oate (**26**, 22 mg), mp 150–152°. (Found: M$^+$ 350.202; $C_{21}H_{31}O_2{}^{35}Cl$ requires M$^+$ 350.201.) ^{1}H NMR ($CDCl_3$): δ0.73 (*s*, Me-20), 1.16 (*s*, Me-18), 3.64 (*s*, CO_2Me), 4.16 (*q* of *d*, *J* = 10, 6, 3 Hz, H-13), 4.88 (*br*, CH_2-17); EIMS *m/z* (rel. int.): 352 [M + 2]$^+$ (16), 350 [M]$^+$ (50), 335 (35), 315 (27), 293 (37), 291 (100), 275 (32), 255 (17).

Demethylation of methyl atisenoate (**16**). The ester (**16**, 6 mg) was treated with the supernatant soln (2 ml), prepared from HMPA (5 ml), NaH (250 mg, 50% oil dispersion, washed with petrol) and EtSH (250 μl), at room temp. for 3 hr. After the addition of H_2O the soln was washed with EtOAc and then acidified with conc. HCl. Recovery in EtOAc followed by chromatography on a short Si gel column eluted with increasing amounts of EtOAc in petrol gave, at 20–30% EtOAc, atisenoic acid (**10**, 3 mg).

Culture conditions. The mutant B1-41a of *Gibberella fujikuroi* was grown on 40%N ICI medium and resuspended in 0%N ICI medium as previously described [12]. The resuspension medium was either at the natural pH of 4.7 or adjusted to pH 3.0 by the addition of aq. H_2SO_4 or to pH 6.6, 6.8 or 7.0 by the addition of aq. KOH. To Erlenmeyer flasks (100 or 250 ml) containing hot sterilized H_2O (5 or 25 ml), substrates (2 or 10 mg) were added in Me_2CO (100 or 300 μl). The Me_2CO was allowed to evaporate, resuspended mycelium (20 or 100 ml) was added and the flasks were shaken at 25° for 1, 3, 4 or 5 days.

Product analysis. For extraction, derivatization and GC/MS conditions see refs. [1, 2, 12].

Metabolites from ent-12, 16-cyclokauran-19-oic acid (**9**). Metabolites, identified by GC/MS only, were as given below.

12,16-Cyclogibberellin A_4 (**33**), (MeTMSi derivative) *m/z* (rel. int.): 418 [M]$^+$ (12), 400 (5), 382 (15), 358 (8.5), 328 (12), 289 (61), 284 (66), 225 (36.5), 129 (46.5), 75 (57), 73 (100).

12,16-Cyclogibberellin A_{13} (**40**), (MeTMSi derivative) *m/z* (rel. int.): 492 [M]$^+$ (3), 477 (12), 460 (19), 436 (5), 432 (20), 400 (27.5), 392 (14), 342 (19), 310 (31), 303 (18.5), 283 (23), 282 (25.5), 223 (10.5), 160 (10.5), 129 (100), 73 (50).

12,16-Cyclogibberellin A_{14} (**37**), (MeTMSi derivative) *m/z* (rel. int.): 448 [M]$^+$ (12.5), 433 (25.5), 416 (78), 388 (55), 326 (17.5), 318 (12), 298 (41), 287 (100), 259 (98), 231 (88), 129 (100), 73 (91.5).

12,16-Cyclogibberellin A_{15} (**41**), (Me ester) *m/z* (rel. int.): 344 [M]$^+$ (37.5), 326 (10.5), 312 (46.5), 298 (26.5), 285 (42), 284 (37.5), 239 (100).

12,16-Cyclogibberellin A_{24} (**38**), (Me ester) *m/z* (rel. int.): 374 [M]$^+$ (8.5), 359 (1.5), 356 (3), 346 (5), 342 (35.5), 324 (10.5), 314 (73), 296 (15), 286 (100), 282 (26), 255 (26) 254 (22), 236 (36.5), 227 (47), 226 (30).

12,16-Cyclogibberellin A_{25} (**39**), (Me ester) *m/z* (rel. int.): 404 [M]$^+$ (9.5), 372 (39), 344 (42.5), 312 (90), 284 (100), 253 (16.5), 225 (59).

12,16-Cyclogibberellin A_{47} (**34**), (MeTMSi derivative) *m/z* (rel. int.): 506 [M]$^+$ (100), 491 (1.5), 488 (2), 474 (8.5), 459 (12.5), 447 (4.5), 431 (5.5), 416 (5.5), 217 (27), 147 (19.5), 75 (41), 73 (89).

12,16-Cyclogibberellin A_4 (**35**), (MeTMSi derivative) *m/z* (rel. int.): 418 [M]$^+$ (22), 386 (50), 371 (65), 343 (100), 299 (44), 295 (60), 284 (48), 225 (55), 224 (49), 223 (94), 143 (43), 75 (> 100), 73 (64).

Ent-7α-hydroxy-12,16-cyclokauran-19-oic acid (**44**), (MeTMSi derivative) *m/z* (rel. int.): 404 [M]$^+$ (5.5), 389 (4.5), 362 (4.5), 330 (4.5), 314 (95.5), 299 (24.5), 254 (50.5), 133 (54.5), 75 (100), 73 (25).

Ent-6α,7α-dihydroxy-12,16-cyclokauran-19-oic acid (**45**), (MeTMSi derivative) *m/z* (rel. int.): 492 [M]$^+$ (absent), 477 (13), 402 (16), 344 (6.5), 312 (12), 269 (100), 209 (21.5).

Ent-*x*,6α,7α-trihydroxy-12,16-cyclokauran-19-oic acid, (MeTMSi derivative) *m/z* (rel. int.): 580 [M]$^+$ (absent), 565 (13.5), 490 (71), 475 (10.5), 458 (5), 446 (8), 431 (5), 400 (57.5), 385 (8), 357 (42.5), 341 (37.5), 310 (26), 75 (26), 73 (100).

Ent-6,7-seco-12,16-cyclokauran-4,7,19-trioic acid (**43**), *m/z* (rel. int.): 406 [M]$^+$ (1.5), 391 (4), 374 (5.5), 359 (12), 346 (1.5), 315 (4.5), 299 (3.5), 286 (4.5), 272 (5.5), 239 (2.5), 228 (22.5), 227 (21.5), 195 (81.5), 167 (76.5), 107 (100).

Ent-6α,7α-trihydroxy-12,16-cyclokauran-19-oic acid 19,6-lactone (**42**), (MeTMSi derivative) *m/z* (rel. int.): 476 [M]$^+$ (2), 461 (5.5), 446 (14.5), 433 (3.5), 386 (7), 356 (13), 343 (10), 296 (6.5), 281 (19), 268 (15), 251 (16), 224 (21.5), 103 (52), 75 (32), 73 (100).

Isolation of 12,16-cyclogibberellins A_9 (**32**) *and A_{12}* (**36**). Three large scale 5-day cultures were conducted: (a) ent-12,16-cyclokauranoic acid (**9**, 220 mg), distributed between 110 conical flasks (100 ml) containing resuspension cultures (25 ml), adjusted to pH 7.0; (b) ent-12,16-cyclokauranoic acid (**9**, 240 mg), distributed between 24 conical flasks (100 ml) containing resuspension culture (25 ml), adjusted to pH 6.8; and (c) ent-12,16-cyclokauranoic acid (**9**, 230 mg), distributed between 23 conical flasks (100 ml) containing resuspension cultures (25 ml) adjusted to pH 6.6.

From (a), the culture filtrate was adjusted to pH 2.5 and extracted with EtOAc to give a crude product (293 mg) which was fractionated by prep. TLC on Si gel with EtOAc–petrol–HOAc (50:50:1). The material (26 mg), recovered from the band at R_f 0.55, was further purified by prep. TLC on Si gel with EtOAc–petrol–HOAc, followed by crystallization to give 12,16-cyclogibberellin A_9 (**32**) mp 114–116° (see analytical data below). The material (35 mg) from R_f 0.46 was further purified as for 12,16-cyclogibberellin A_9, to give 12,16-cyclogibberellin A_{12} (**36**) mp 237–240° with change of crystal form at 210–220° (see analytical data below). The material (32 mg) from R_f 0.22 contained (by GC/MS) an intractable mixture of 12,16-cyclogibberellins A_4 (**33**) and A_{14} (**37**).

From (b), the same work-up and purification as in (a) gave a crude product (157 mg) from which 12,16-cyclogibberellin A_9 (**32**) (15 mg) and 12,16-cyclogibberellin A_{12} (**36**) (22 mg) were isolated.

From (c), the crude product (130 mg) was dissolved in 6 M NaOH (2 ml) and H_2O (8 ml). The pH of the soln was adjusted to 6.5 with a mixture (1:1) of 5 M HCl and 1.5 M KH_2PO_4. The soln was then extracted with CH_2Cl_2 to yield a gum. Prep. TLC of this gum on Si gel with EtOAc–petrol–HOAc (70:30:1) gave 12,16-cyclogibberellin A_9 (**32**, 11 mg) and 12,16-cyclogibberellin A_{12} (**36**, 6 mg).

12,16-Cyclogibberellin A_9 (**32**), mp 114–116°. (Found: M$^+$, 316.167; $C_{19}H_{24}O_4$ requires M$^+$ 316.167.) IR $\nu_{max}^{CHCl_3}$ cm^{-1}: 3500–2700, 1760, 1700; ^{1}H NMR ($CDCl_3$): δ 0.5–0.9 (*m*, 2 × cyclopropyl-H), 1.17 and 1.20 (both *s*, 2 × Me), 2.39 (*d*, *J* = 8 Hz, H-5), and 2.55 (*d*, *J* = 8 Hz, H-6); ^{13}C NMR (C_5D_5N): δ 17.5 (*q*, C-18), 18.4 (*q*, C-17), 19.0 and 19.6 (both *t*, C-2, C-11), 20.8 (*d*, C-12), 22.00 (*d*, C-13), 25.6 (*s*, C-16), 29.5 (*t*, C-1), 35.5 (*t*, C-

3), 36.1 (*t*, C-14), 44.9 (*t*, C-15), 48.7 and 49.8 (both *s*, C-4, C-8), 51.2 and 51.9 (both *d*, C-6, C-9), 59.9 (*d*, C-5), 92.5 (*s*, C-10), 175.7 (*s*, C-7), 179.1 (*s*, C-19); EIMS *m/z* (rel. int.): 316 $[M]^+$ (100), 298 (65), 272 (82), 270 (95), 227 (88), 204 (43), 183 (42), 119 (63), 105 (70), 91 (86); *m/z* (Me ester) 330 $[M]^+$ (14), 298 (72), 270 (100), 227 (36), 226 (48), 225 (31).

12,16-Cyclogibberellin A_{12} (**36**), mp 237–240° (change of crystalline form at 210–220°). (Found: M^+ 332.198; $C_{20}H_{28}O_4$ requires M^+ 332.199.) IR ν_{max}^{Nujol} cm^{-1}: 3500–2500, 1695; ^{1}H NMR (C_5D_5N): δ 0.5–0.9 (*m*, 2 × cyclopropyl-H), 1.14 and 1.16 (both *s*, 2 × Me), 1.61 (*s*, Me-18), 2.26 (*d*, J = 13 Hz, H-5), and 4.05 (*d*, J = 13 Hz, H-6); ^{13}C NMR (C_5D_5N): δ 13.7 (*q*, C-20), 19.2 (*q*, C-17), 19.2 and 20.4 (both *t*, C-2, C-11), 21.1 (*q*, C-18), 21.8 (*d*, C-12), 25.4 (*s*, C-16), 29.2 (*d*, C-13), 35.9 (*t*, C-14), 38.7 (*t*, C-3), 39.3) (*t*, C-1), 43.5 (*s*, C-8) 44.6 (*s*, C-10), 47.2 (*t*, C-15), 47.6 (*s*, C-4), 50.2 (*d*, C-6), 57.8 and 58.9 (both *d*, C-5, C-9), 178.0 (*s*, C-7), 180.0 (*s*, C-19); EIMS *m/z* (rel. int.): 332 $[M]^+$ (23), 314 (100), 286 (97), 271 (79), 241 (23), 185 (36), 171 (55), 150 (57), 137 (76), 119 (61), 109 (88), 105 (85), 91 (87); *m/z* (diMe ester) 360 $[M]^+$ (4), 328 (23), 300 (100), 285 (62), 241 (53), 164 (76).

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REFERENCES

1. Bearder, J. R., Frydman, V. M., Gaskin, P., MacMillan, J., Wels, C. M. and Phinney, B. O. (1976) *J. Chem. Soc. Perkin Trans.* 1, 173.
2. Lunnon, M. W., MacMillan, J. and Phinney, B. O. (1977) *J. Chem. Soc. Perkin Trans.* 1, 2308.
3. Bearder, J. R., MacMillan, J. and Matsuo, A. (1979) *J. Chem. Soc. Chem. Commun.* 650.
4. Hanson, J. R., Sarah, F. Y., Fraga, B. M. and Hermandez, M. G. (1979) *Phytochemistry* **18**, 1875.
5. Pyrek, J. St. (1970) *Tetrahedron* **26**, 5029.
6. Achmatowicz, O., Ejchart, A., Kozerski, L. and Pyrek, J. St. (1971) *J. Chem. Soc. Chem. Commun.* 98.
7. Bohlmann, F., Jakupovic, J., King, R. M. and Robinson, H. (1980) *Phytochemistry* **19**, 863.
8. Matsuo, A., Bearder, J. R. and MacMillan, J. (1980) *IUPAC 12th Int. Symp. Chem. Nat. Prod.* Teneriffe, 1980.
9. Lewis, N. J. and MacMillan, J. (1980) *J. Chem. Soc. Perkin Trans.* 1, 1270.
10. Barton, D. H. R. and McComie, S. W. (1975) *J. Chem. Soc. Perkin Trans.* 1, 1574.
11. Kelly, R. B., Eber, J. and Hung, H. K. (1973) *Can. J. Chem.* **51**, 2534.
12. Bearder, J. R., MacMillan, J. and Wels, C. M. (1975) *Phytochemistry* **14**, 1741.
13. Cory, R. H. and Stothers, J. B. (1978) *Org. Magn. Reson.* **11**, 252.
14. Hoad, G. V., Phinney, B. O., Sponsel, V. M. and MacMillan, J. (1981) *Phytochemistry* **20**, 703.
15. Beale, M. H. and MacMillan, J. (1980) *J. Chem. Soc. Perkin Trans.* 1, 877.