Tetrahedron. Vol. 30, pp. 3631 to 3640. Pergamon Press 1974. Printed in Great Britain

GIBBERELLIN METABOLITES FROM *ENT*-KAURA-2,16-DIEN-19-OL AND ITS SUCCINATE IN *GIBBERELLA FUJIKUROI*[a]

H. J. BAKKER, I. F. COOK, P. R. JEFFERIES* and J. R. KNOX

School of Chemistry, The University of Western Australia, Nedlands, Western Australia, 6009

(*Received in the UK* 3 *May* 1974; *Accepted for publication* 7 *June* 1974)

Abstract—Exposure of *ent*-kaura-2,16-dien-19-ol (**1**) or its succinate (**2**) to resuspended mycelia of *G. fujikuroi* has produced a complex mixture of acids which after methylation gave the esters of two C_{19} (**24**) and (**30**) and five C_{20} gibberellins (**4**, **11**, **20**, **32** and **33**). The triester (**32**) and the lactone ester (**24**) have been prepared before from the esters of gibberellin A_{13} (**8**) and gibberellin A_4 (**26**) respectively. The structures of the other metabolites were assigned on spectroscopic data and by chemical transformations. Thus the lactone diester (**4**) has been converted to the known keto triester (**6**). The epoxide (**11**) has been related to gibberellin A_{14} (**14**) and the aldehyde (**33**) has been related to gibberellin A_{13} trimethyl ester (**8**) by way of the triol (**34**). Selective de-epoxidation of the 16,17-epoxy function in diepoxides has provided a route from the dienes (**20** and **24**) to the epoxides (**11** and **30**) respectively, but not from the ester of gibberellin A_5 (**23**) to that of gibberellin A_6 (**29**). On the other hand the latter can be obtained by epoxidation of gibberellin A_5 methyl ester trifluoroacetate. Backfeeding experiments carried out with the epoxy diacid (**12**), the diene diacid (**21**) and the derived diol (**39**) indicate pathways connecting the various metabolites. The natural gibberellins A_5 and A_6 were shown to be formed in some of the backfeeding experiments.

INTRODUCTION AND DISCUSSION

A bioassay system developed by Phinney[3] involves the use of dwarf mutant strains of *Zea mays* which show enhanced growth in response to treatment with gibberellins. This bioactivity is also shown by a group of diterpenes,[4,5] most of which have been found to be converted[6] to gibberellic acid (**3**) by the mould *Gibberella fujikuroi*. The observation[7] of activity in the dienol (**1**) was exceptional since the other active diterpenes have the A ring saturated and this is dehydrogenated apparently only at a late stage[8] in the formation of gibberellic acid (**3**). If the activity is due to bioconversion to a gibberellin we expected that the mould would reflect the plant metabolic pathways and so we chose this simpler vehicle to study metabolism of the dienol (**1**). It was evident that the dienol might be converted circuitously to normal mould gibberellins or unnatural gibberellins might be formed through enzyme systems of low specificity. It was also evident that the plant gibberellins A_5 (**22**) and A_6 (**28**) could be produced in this way even though they are not natural products of the mould. In the early experiments the dienol (**1**) labelled with ^{14}C or ^{3}H at C17 was fed directly to resuspended *G. fujikuroi* and although sufficient of the major metabolites could be obtained for structural work the efficiency of conversion was poor in that even at low substrate loading much starting material was recovered. Later we observed highly efficient oxygenation of *ent*-kaurenes which were blocked by a 19-succinoyloxy group[9] and so the dienol (**1**) was converted to its succinate (**2**) and the metabolism studied. Suprisingly[9] the succinate residue proved very labile and the product consisted mainly of a mixture equivalent to that obtained from the dienol itself. However the succinate gave a much higher percentage metabolism than the dienol and so it was adopted for further work. Traces of succinates remaining after metabolism were hydrolysed with base before partitioning into neutral and acidic compounds. The latter were separated by repeated chromatography of the methyl esters which gave members of both the *ent*-kaurane and *ent*-gibberellane series. The former group will be described elsewhere[10] and we now detail our results with the gibberellins. These consist of the esters of two C_{19} gibberellins, 13-deoxygibberellin A_5 (**22**) and 13-deoxygibberellin A_6 (**30**) along with the lactone diester (**4**) the epoxy diester (**11**), the diene diester (**20**), the triester (**32**) and the aldehyde diester (**33**). Apart from 13-deoxygibberellin A_5 which was isolated only in very small quantity all the gibberellins were shown to be metabolites of the labelled dienol (**1**) or its succinate (**2**) by isolation in a radiochemically pure form.

[a] Some of the results reported have been published as short communications[1,2].

The most polar gibberellin metabolite encountered (**4**) was also the most abundant and was available in adequate quantity from the dienol metabolisms. The IR spectrum revealed absorption for γ-lactone (1780 cm^{-1}), ester (1725 cm^{-1}), hydroxyl (3620 cm^{-1}) and the exocyclic methylene group (3080, 1650 cm^{-1}). The mass spectrum provided the molecular formula $C_{22}H_{28}O_7$ and was dominated by fragments associated with two methoxycarbonyl (M-32, M-60, M-120), hydroxyl (M-18) and lactone groups (M-44, M-46)[11]. The NMR spectrum (Table) includes resonances for the two methoxycarbonyls, a tertiary methyl and the exocyclic methylene group and these together with the lactone function provide five pendant carbon atoms required of a C_{20}-gibberellin. The NMR spectrum also shows a broad singlet (δ 4·65) expected for an equatorial proton at the ether oxygen terminus of the γ-lactone. The AB quartet, δ_A 2·66, δ_B 3·61, J_{AB} 12 Hz is consistent with the characteristic pattern for C5-H and C6-H in the gibberellins.[12] The position (δ 3·66) for the C6-H corresponds[13,14] to a C_{20}-gibberellin with CO groups axial at C4 and at C10. The resonance for C5β-H* (δ 2·66) corresponds to a 3β-hydroxygibberellin[14,15] and consistently it is shifted downfield to δ 3·01 in pyridine.[15] The presence of a secondary alcohol was confirmed by oxidation of the lactone diester with Jones reagent which gave a keto lactone (**5**) which showed the expected spectral properties. Thus the multiplicity of the C2-H resonance was simplified to a doublet (J 7 Hz) supporting the vicinal substitution at C3. The simplest way of interrelating the keto lactone with a compound of established constitution evidently involved reductive fission of the lactone link to C2 leading after methylation to the keto triester (**6**) which is available from gibberellin A_{13}[14] through its triester (**8**). In view of the expected sensitivity of the keto lactone (**5**) to acids and bases this reduction was carried out using chromous acetate in aqueous-THF and after methylation the product was identified as the keto triester (**6**).[14] This result confirmed the spectral conclusions but did not distinguish between the structures (**4**) and the isomer (**9**) in which lactonisation is between C19 and C2.

The method chosen to make this distinction was based on the observation that reduction of the keto triester (**6**) with borohydride gives the δ-lactone (**10**) directly[14] and the selectivity of the reaction allows a distinction between the ester groups attached to C4 and C10. Accordingly the product of the chromous acetate reduction was ethylated and the ethyl dimethyl ester (**7**) reduced with borohydride. Lactonisation occurred on heating in acetic acid to give the lactone diester (**10**) which was identical with an authentic sample prepared from the keto triester (**6**).

The structure of the epoxy diester (**11** was assigned on spectral evidence. Thus the NMR spectrum (Table) included signals expected for two methyl ester, two tertiary methyl groups and an exocyclic methylene. The lack of other vinylic proton signals together with a doublet at δ 3·49 (C3-H) and a multiplet at δ 3·28 (C2-H) indicated that the 2-ene was epoxidised. Support for this view came from the high resolution mass spectrum which gave $C_{22}H_{30}O_5$ for the molecular ion and the IR spectrum which lacked hydroxyl absorption. The configuration of the epoxide link was assigned β as in **11** since INDOR spectroscopy provided $J_{1,2}$ values of 5·5 and 1 Hz which correspond better for the β-epoxide in which the C1-H_2, C2-H dihedrals are −20° and 100°, than the α-epoxide which shows these angles both near 60°. There is evidence indicating that fungi which introduce axial OH groups in steroids can transform the unsaturated analogues to the correspondingly oriented epoxides.[16] Since all C3 hydroxylated fungal gibberellins possess the β (axial) OH group the same configuration would be predicted for the epoxide oxygen in **11**. We hoped to determine the structure of the epoxy diester (**11**) by hydride reduction to give the triol (**13**)[17] which has been prepared from gibberellin A_{14} (**14**). In the absence of special effects hydride reduction of the epoxide (**11**) should give only the 3β-hydroxy compound.[18] However the α-face in these compounds is hindered and if normal reduction is slow a C4 axial hydroxymethyl group could be formed more rapidly and this could complex with metal hydride and direct hydride attack on the epoxide ring. This process is expected to favour substitution of hydride at C3[19] with formation of the undescribed 2β-hydroxy compound. The best model we had available to study this reduction was the epoxykaurenol (**15**) which had been required in another project, since abandoned, and had been prepared by selective de-epoxidation of the 16,17-epoxy function in the diepoxide (**16**). Of the procedures[20] available for elimination of epoxy oxygen the method developed by Culvenor[21] using selenocyanate is convenient and proved selective when applied to the diepoxide (**16**). The spectra of the monoepoxide product showed regeneration of the exocyclic methylene group and demonstrated the structure **15**.

When this epoxide was reduced with LAH two isomeric diols were produced in similar quantity. One of these was the 3β,19-diol (**17**)[19] corresponding to normal diaxial opening and the isomer was identified as the anomalous 2β,19-diol (**18**) since oxidation with Jones reagent and hydride reduction of the resulting ketoaldehyde gave the known[19] 2α,19-diol (**19**). The formation of a reasonable proportion of 3β,19-diol (**17**) justified submitting the epoxy diester (**11**) to hydride reduction which in the

*Stereochemical designations refer to the structural representations[9].

event gave the known[17] triol (**13**) in good yield without significant admixture with other material. The difference between this reduction and that of the epoxykaurenol may reflect differences in the rate of attack on epoxide and ester functions in **11** or it may simply be the result of more subtle stereoelectronic factors.

The structure of the diene diester (**20**) was also evident from its spectra. Thus the NMR spectrum (Table) showed retention of both olefinic centres of the dienol and included signals for two tertiary methyl and two methoxycarbonyl groups. The latter showed the characteristic fragmentation in the mass spectrum. Structure proof by interrelation with the epoxy diester (**11**) was sought by application of the di-epoxidation, selenocyanate de-epoxidation sequence which had been successful with the dienol (**1**). Thus the diene diester (**20**) was treated with excess *m*-chlorperbenzoic acid and then with potassium selenocyanate which gave the epoxy diester (**11**) in good yield thus linking the diene diester (**20**) with gibberellin A_{14} (**14**).

The methyl ester of 13-deoxygibberellin A_5 (**24**) was indicated in a mixed fraction by intense MS peaks at *m/e* 284 (M-44) and *m/e* 224 (M-60–44) corresponding to loss of the lactone and methyl ester groups.[11] It was identified by GLC and GLC-MS comparison with an authentic sample[22] prepared from gibberellin A_4 (**27**). Proof for its formation from the dienyl succinate (**2**) followed from dilution of the metabolic mixture with cold material and crystallisation to constant specific activity. In view of the co-occurrence of the diene diacid (**21**) and epoxy diacid (**12**) in the metabolic mixture it seemed probable that the A-ring epoxy analogue (**31**) of 13-deoxygibberellin A_5 (**25**) was also present. The compound was in fact isolated as its ester (**30**) in small yield and its structure was assigned on spectroscopic data. Thus the MS determined the molecular formula $C_{20}H_{24}O_5$ and fragmentation leading to ions M-32, M-60, M-44 suggested a methoxycarbonyl and the bridging lactone function of the C_{19} gibberellins. The IR spectrum supported the presence of ester and γ-lactone groups and the absence of OH. Finally the proposed structure was fully supported by the NMR spectrum (Table).

Although the epoxide has not been described it seemed clear that it should be readily available by diepoxidation and selenocyanate treatment of the ester of 13-deoxygibberellin A_5 (**24**). Using the conditions employed on the previous occasions with the diepoxidation product from **24** both epoxy groups were lost. However, short exposure to selenocyanate was selective and gave the requried monoepoxide (**30**) which was identical with the sample separated from the metabolic mixture.

The presence of the triester (**32**) in crude fractions was evident from its mass spectrum which showed fragmentations for the three methoxycarbonyl groups. Its isolation was established by comparison with an authentic sample[14] prepared from gibberellin A_{13} trimethyl ester (**8**) by tosylation and halide-catalysed elimination.

One other relatively weakly polar gibberellin which is a metabolite of the dienyl succinate (**2**) has been assigned the structure **33**. This was indicated by the MS which gave $C_{22}H_{28}O_5$ for the molecular ion and characteristic fragments for the loss of aldehyde (M-28) and two methoxycarbonyl groups. The NMR spectrum (Table) showed an unsplit signal for the aldehyde proton and the resonances expected for two methoxy-carbonyls, one tertiary methyl, the A-ring and exocyclic olefins together with the AB pattern for C5-H and C6-H. Most of the structural detail was confirmed by hydride reduction to the triol (**34**) which was identical with a reference sample obtained by similar reduction of the triester (**32**).[14] The formation of the triol (**34**) from the aldehyde leaves only the choice between C10 and C4-α for the positions of a methoxycarbonyl and the aldehyde function. We prefer the arrangement **33** since the C4-Me resonance appears at δ 1·32 which is in agreement with the range δ 1·22–1·32 we have observed for C4-axial esters in the *ent*-kaur-2-ene[10] and *ent*-gibberell-2-ene series. On the other hand in the *ent*-kaur-2-ene series the C4-α aldehyde[19] shows this methyl resonance near δ 1·00. Further, the available evidence suggests that B-ring contraction in gibberellin biosynthesis only occurs at the carboxyl level of oxidation of the C4-α group.[23]

Backfeeding experiments. The structural relationships between the gibberellin metabolites obtained from the dienyl succinate (**2**) indicate various pathways linking their formation. To provide more evidence on these links, samples of the free acids were required for backfeeding experiments. After repeated chromatography of the acidic metabolite mixture, the diene diacid (**21**), the epoxy diacid (**12**) and the lactone diacid (**36**) were separated and were identified as the methyl esters. Bioassay data for these compounds will be reported elsewhere. Radioactively-labelled samples of **12** and **21** were obtained by repetition of the separation using the metabolite mixture obtained from [17-^{14}C]-labelled dienyl succinate with added unlabelled samples of the two acids.

A sample of the [17-^{14}C]-epoxy diacid (**12**) thus obtained was fed to resuspended *G. fujikuroi*. Methylation of the acidic fraction and radiochromatography gave four main radioactive areas. The least polar and most abundant component corresponded to the ester (**11**) of the unchanged substrate (~50%) and this was overlapped by a zone corresponding to the ester of 13-deoxygibberellin A_6 (**30**, 15%). The identifications were confirmed by separation of the mixed zone and GLC analysis. A more polar zone (~10%) had R_f identical to the lactone diester (**4**). After dilution

with cold material this diester was separated by TLC, oxidised with Jones reagent to the ketone (5) which was crystallised to constant specific activity. The most polar component (~ 15%) which was also separated by TLC co-chromatographed with the ester of gibberellin A_6 (29) and the fraction showed mass-spectral fragmentation for this compound.[11] The transformations observed for the epoxy diacid (12) are shown in the scheme which also provides a picture of the results from the further backfeedings described below.

Although the large amount of unmetabolised epoxy diacid may be due to transport problems it suggests that the epoxide is to some extent a dead end in the dienyl succinate (2) metabolism and provides only a minor path to the lactone diacid (36) which is the major metabolite from 2. The efficient formation of deoxygibberellin A_6 (31) and gibberellin A_6 (28) suggests that the epoxy diacid may provide a route to the minor amounts of deoxygibberellin A_6 obtained from 2 but the formation of 3β-hydroxylated gibberellins as normal *G. fujikuroi* metabolites is considered to involve late-stage C7-aldehyde intermediates and an analogous pathway to 31 cannot be excluded in the present case.

We next sought evidence on the epoxidation stage by carrying out a backfeeding experiment with the [17-^{14}C]-diene diacid (21). The acidic products which were identified by radiochromatography of the esters consisted largely of the aldehyde diacid (37, ~25%) and the triacid (38, ~30%) with only a small amount of unmetabolised material (~10%). These results suggest that although oxidation of C20 in the diacid (21) occurs readily, the formation of C_{19} gibberellins is not favoured. The metabolites from the epoxy diacid (12) on the other hand contain a high proportion of C_{19} gibberellins. This situation is comparable to the transformations of normal *G. fujikuroi* metabolites. Thus although the A-ring saturated gibberellin A_{12} (35) appears to be metabolised largely to C_{20} gibberellins[17,24] its 3β-hydroxy derivative gibberellin A_{14} (14) provides a facile path to the C_{19} gibberellins via gibberellin A_4 (27).[17,24] This sequence is closely comparable with the route shown in the scheme from the epoxy diacid (12)→13-deoxygibberellin A_6 (31)→gibberellin A_6 (28) and suggests that the 3β-hydroxyl and the epoxide oxygen are favourable interchangeable binding sites for the enzyme system involved in removal of C20. Earlier evidence[25] indicating that C20 is eliminated at the aldehyde level has not been supported by direct feeding experiments[24] and so we refrain from indicating C10 formyl compounds as intermediates to the C_{19} gibberellins. The products isolated also suggest that epoxidation of the diene diacid and its metabolites is slow since the lactone diacid (36), gibberellin A_6 (28) and the epoxy diacid (12) represent only a small part of the products. This apparent slow rate of epoxidation for the diene diacid (21) is reminiscent of the hindered 3β-hydroxylation of the saturated A-ring analogue (35) particularly in view of work in the steroid field[16] which suggests that the same enzyme system would be involved for 3β-hydroxylation of 35 and β-epoxidation of 21.

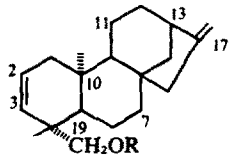
The 3β-hydroxylation leading to the formation of gibberellin A_{14} (14) is recognized to occur best at the C7-aldehyde oxidation level[17,26] and we were interested to determine if epoxidation of the Δ^2-gibberellins would also take place more rapidly at the same oxidation state. Although the required aldehyde (40) is not readily available it can be assumed that it would arise from metabolism of the diol (39) which can be prepared from the diene diester (20) by reduction with LAH. Metabolism of a sample of [17-^{14}C]-labelled diol gave only acidic metabolites. Radiochromatography of the derived methyl ester mixture suggested that the acidic products corresponded to the epoxy diacid (12, 10%), the triacid (38, 10%), the lactone diacid (36, ~25%) and gibberellin A_5 (22, ~20%), as well as two unidentified peaks (~10% each) one of which corresponded to the unknown component of the diacid feeding described above. The presence of gibberellin A_5 was confirmed by dilution with cold material, separation by TLC and crystallization to constant specific activity.

About a third of the identified products evidently arise through epoxidation of the 2-ene and the facilitation of this step in comparison with the diene diacid metabolism suggests that it occurs best for an aldehyde oxidation state of C7, thus extending the analogy with the 3β-hydroxylating system of *G. fujikuroi*. The efficient production of the lactone diacid (36) and the absence of a significant quantity of gibberellin A_6 (28) supports the earlier indication of another route to this lactone (36) not involving the epoxy-diacid (12). The large proportion of gibberellin A_5 from the diol feed compares sharply with its minor contribution to the metabolites of the diene diacid and this suggests that the elimination of C20 is also favoured by the aldehyde oxidation level of C7. Gibberellin A_5 and gibberellin A_6 were not observed as metabolites of the dienyl succinate (2) under the conditions employed whereas they were obtained from the backfeeding experiments. This is apparently due to the slow rate of 13-hydroxylation by the mould limiting the further transformation of the observed 13-deoxy compounds (25 and 31) in the time used for the dienyl succinate feedings. However it is noteworthy that 13-deoxygibberellin A_5 (25) might be expected to be a more important metabolite of the dienyl succinate if this were the sole factor involved.

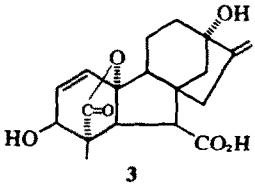
It was of interest to determine whether the dienol was metabolically converted to normal *G. fujikuroi* metabolites. The major fungal metabolite, gibberellic acid (3), was isolated as the methyl ester after feeding the [17-^{14}C]dienol and gibberellin A_4, a

precursor of 3, was isolated as the methyl ester of the 3-epimer after feeding the [17-^{14}C]dienyl succinate. Both materials were devoid of radioactivity. Furthermore there was no sign of radioactivity corresponding to methyl gibberellate on radiochromatograms of methyl esters from the various backfeeding metabolisms. This excludes mechanisms for biosynthesis of the normal metabolites which invoke the dienol or its metabolites as intermediates. Previous feeding experiments with doubly-labelled mevalonic acid[27] had excluded hydroperoxidation of a 2-ene intermediate as a mechanistic pathway to gibberellic acid.

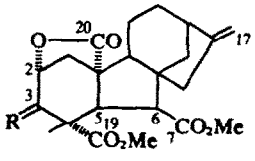
The metabolic transformations of the dienol (**1**) in the mould are of interest in that they may parallel some plant metabolic pathways. The manner in which gibberellins A_5 and A_6 are derived in plant systems is obscure but the experiments we have described show that the presence of the A-ring double bond at the *ent*-kaurene level cannot be excluded. In view of the known bioactivity of this pair, their formation may also account in part for the growth response of dwarf maize mutants to



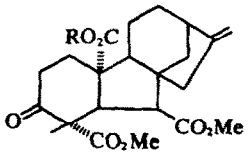
1: R = H
2: R = $CO(CH_2)_2CO_2H$



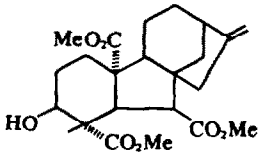
3



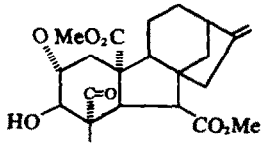
4: R = α-H,β-OH
5: R = O



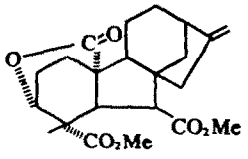
6: R = Me
7: R = Et



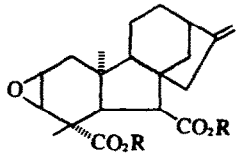
8



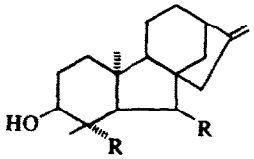
9



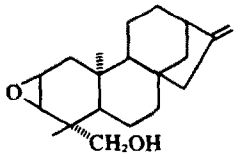
10



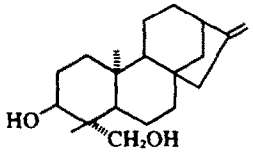
11: R = Me
12: R = H



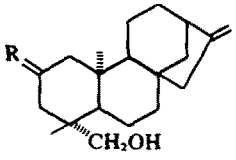
13: R = CH_2OH
14: R = CO_2H



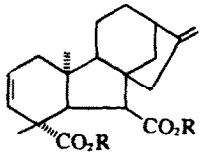
15:
16: 16,17-epoxy



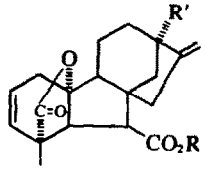
17



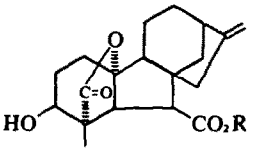
18: R = β-OH, α-H
19: R = α-OH, β-H



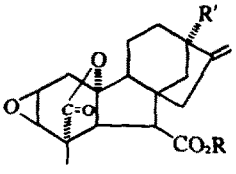
20: R = Me
21: R = H



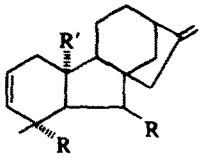
22: R = H, R' = OH
23: R = CH_3, R' = OH
24: R = CH_3, R' = H
25: R = H, R' = H



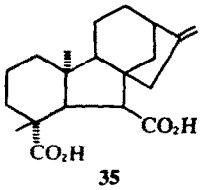
26: R = CH_3,
27: R = H



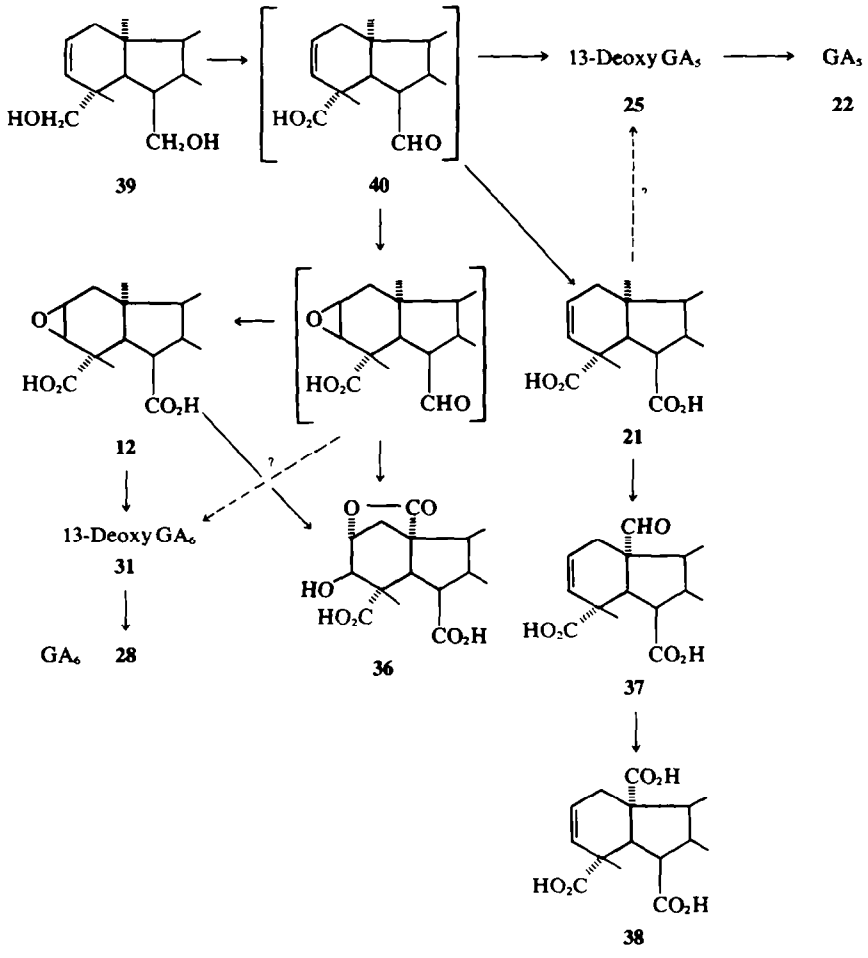
28: R = H, R' = OH
29: R = Me, R' = OH
30: R = Me, R' = H
31: R = H, R' = H



32: R,R' = CO_2Me
33: R = CO_2Me, R' = CHO
34: R,R' = CH_2OH



35



treatment with the dienol (**1**). Further bioresponse may be attributed to the lactone diacid and the other *ent*-gibberellane metabolites which however have not previously been observed as natural metabolites.

As yet no successful preparation of gibberellin A_6 from readily available materials has been reported and it is only available in small quantity from plant sources.[28] Our need for a reference sample in the foregoing work prompted us to attempt a preparation by application of the diepoxidation sequence to gibberellin A_5, which is available from gibberellic acid[29]. However, exhaustive exposure of gibberellin A_5 methyl ester (**23**) to *m*-chlorperbenzoic acid and treatment of the product with selenocyanate afforded only trace of gibberellin A_6 methyl ester (**29**). Nevertheless it was noted, predictably[30], during epoxidation of gibberellin A_5 methyl ester

Table 1. NMR Spectra of gibberellin metabolites from the succinate (**2**) in $CDCl_3$ (δ)

Compound	18-CH_3	20-CH_3	CO_2Me	2-H	3-H	5-H	6-H	17-H_2	$J_{5,6}$ Hz
Lactone diester (**4**)	1·26	—	3·65, 3·70	4·65	obsc.	2·66	3·61	4·88	12
Epoxy diester (**11**)	1·34	0·73	3·69, 3·72	3·28	3·49	2·19	3·28	4·81, 4·89	12
Diene diester (**20**)	1·29	0·73	3·67, 3·73	5·65		2·12	3·35	4·88	12·5
13-Deoxy GA_5 (**24**)	1·22	—	3·71	5·72		2·67, 2·79		4·86, 4·98	10
13-Deoxy GA_6(**30**)	1·31	—	3·71	3·15		2·68	3·08	4·74, 4·97	10·5
Diene triester (**32**)	1·29	—	3·57, 3·68 3·76	5·82		2·44	3·92	4·90	12·5
Aldehyde diester (**33**)	1·32	C20-H 9·58	3·65, 3·77	5·67		2·45	3·82	4·90	13

that the rate of attack on the 16-ene relative to the 2-ene had dropped markedly in comparison with the 13-deoxy analogue (**24**). Since esterification of allylic alcohols further reduces[30] the rate of epoxidation of the olefinic group we expected that after esterification of the hydroxyl group gibberellin A_5 should undergo selective epoxidation of the 2-ene. In view of the lability of the A-ring function of the gibberellins any ester would need to be prone to facile hydrolysis and to maximise inhibition of epoxidation the ester should apparently have a high-I effect. The trifluoroacetate group fits both these criteria and so gibberellin A_5 methyl ester (**23**) was treated with trifluoroacetic anhydride and the crude product epoxidised directly with *m*-chlorperbenzoic acid. After chromatography gibberellin A_6 methyl ester (**29**) was obtained in modest yield.

EXPERIMENTAL

General. M.ps (uncorrected) were determined on a Koffler block. Microanalyses were carried out by the Australian Microanalytical Service. NMR spectra were determined for $CHCl_3$ or $CDCl_3$ solutions unless stated otherwise, using a Varian A60 spectrometer and a Bruker spectrospin spectrometer (90 MHz). Chemical shifts are δ values. Optical rotations were determined for $CHCl_3$ solution in 1 dm tubes at r. t. using a Perkin-Elmer model 141 polarimeter. IR spectra were recorded using a Perkin-Elmer model 337 spectrometer for CS_2 solutions. MS were measured with a Varian MAT CH7 Mass Spectrometer using ionisation energies of 20 or 70 ev with inlet temperatures of 60–280°. High Resolution MS were determined through the courtesy of Dr. J. K. McLeod, Australian National University. Analytical GLC was carried out with a Varian Aerograph 1400 Gas Chromatograph using the following 1·5 mm i.d. glass columns (1). 1·5 m containing 2% SE-30 on Gas-Chrom. Q (85–100 mesh); (2). 2 m containing 4% QF-1 on Gas-Chrom. Q (60–85 mesh) and (3). 0·5 m containing 2% carbowax 20M on NAW Chromosorb W (100–120 mesh). Oven temperatures ranged from 190–200°C.

Radioactivity measurements were made with a Nuclear Chicago Scintillation Counter Model 720. The samples were dissolved in 10 ml of scintillator solution prepared from 2,5-diphenyloxazole (0·7 g), 2-*p*-phenylenebis(5-phenyloxazole) (0·05 g), naphthalene (5 g) in dioxan (100 ml). Radioscanning of chromatograph plates was carried out using a Packard Model 7201 Scanner with plate attachment. Light petroleum had bp 55–65°. For chromatography neutral alumina activity III was used, prepared by the method of Djerassi[31] and standardised by the method of Brockman and Schodder.[32] Silica gel GF-254 (Merck) was used for TLC. Esters were separated using appropriate mixtures of benzene-acetone and diisopropyl ether-light petroleum. General metabolic procedures have been described.[9] ent - 19 - (3 - *Carboxypropionyloxy*) - *kaura* - 2,16 - *diene* (**2**). The dienol[19] (**1**, 4·5 g) in pyridine (100 ml) was set aside with succinic anhydride (7·0 g) for 6 days. Isolation of the product gave the *succinate* (**2**) which crystallized from light petroleum as prisms, m.p. 120–122°, $[\alpha]_D$-108° (*c*, 1·2) (Found: C, 74·5; H, 9·0. $C_{24}H_{34}O_4$ requires: C, 74·6; H, 8·9%); ν_{max}: 3065 (=CH_2); 3010 (CH=CH); 1715 cm^{-1} (acid); NMR spectrum: s, 1·09 (6H, tertiary Me); s, 2·71 (-$COCH_2CH_2CO_2H$); AB, δ_A 4·03, δ_B 4·25, *J* 10·5 Hz (C 19-H_2); br s 4·82, $W_{h/2}$ 8 Hz (C 17-H_2); m, 5·67 (C 2-H, C 3-H); br s, 10·4 (CO_2H). MS: *m/e* (%), 386 (M^+, 13); 371 (2); 286 (1); 268 (81); 255 (86); 187 (46); 119 (91); 95 (100). [17-^{14}C]- and [17-^{3}H]-dienyl succinate were prepared as above from the labelled dienol.[10]

Metabolism of the dienyl succinate (**2**). Preliminary experiments with [17-$^3H_2$]-dienyl succinate (**2**) and resuspended mycelia of *G. fujikuroi* showed that optimum conversion to acidic products occurred at pH 5·7 during 4 days at a substrate loading of 100 mg/400 ml. The dienyl succinate (**2**, 6·82 g, 1919 dpm/mg) was then exposed to suspended mycelia under these conditions. The products were isolated from the filtrate with EtOAc, hydrolysed by treatment with 5% KOH in water for 2 hr at 25° and separated into neutral (260 mg) and acidic fractions (3·23 g) with EtOAc. The latter fraction was methylated with diazomethane and chromatographed on alumina. The less polar fractions (1·78 g) were separated by thick LC and then prep. TLC to give the hydroxylated kaurenes to be described elsewhere[10] and in order of polarity (1) *Methyl* ent-*gibberella*-2,16-*diene*-7,19-*dioate* (**20**, 52 mg, 1965 dpm/mg) as a resin homogeneous to GLC ν_{max}: 3050, 875 (=CH_2); 3010 (CH=CH); 1725 cm^{-1} (CO_2Me). MS: *m/e* (%) 358 (M^+, 15); 326 (73); 298 (100); 283 (18); 267 (15); 266 (7); 239 (7); 238 (37); 223 (41); 206 (50); 164 (37); 135 (48); 119 (37); 107 (35); 105 (63); 91 (79); 79 (49); 77 (33); 65 (115); 51 (5). (Found: M^+ 358·214. $C_{22}H_{30}O_4$ requires: 358·214).

(2) Compound **24** which was shown to be present in a fraction (16 mg) by GLC and GLC-MS comparison with an authentic sample prepared[22] from gibberellin A_4 (**27**).

(3) *Methyl* ent-20-*oxogibberella*-2,16-*diene*-7,19-*dioate* (**33**). 4·6 mg, 1907 dpm/mg) as a resin, homogeneous to GLC. ν_{max}: 3050, 880 (=CH_2); 3010 (CH=CH); 2785, 1710 (CHO); 1730 (CO_2Me). MS: *m/e* (%) 372 (M^+, 18); 344 (38); 340 (27); 312 (95); 284 (98); 280 (49); 253 (71); 252 (38); 223 (100); 205 (100); 119 (71); 107 (50); 105 (100); 91 (98); 79 (74); 77 (37). (Found: M^+ 372·195. $C_{22}H_{28}O_5$ requires: 372·194).

(4) *Methyl* ent-2α,3α-*epoxygibberell*-16-*ene*-7,19-*dioate* (**11**, 26 mg, 1939 dpm/mg) was obtained as a resin homogeneous to GLC. ν_{max}: 3060 and 875 (=CH_2); 1730 cm^{-1} (CO_2Me). MS: *m/e* (%) 374 (M^+ 6); 342 (100); 314 (9), 299 (24); 282 (8); 281 (9); 258 (11); 255 (18); 254 (12); 239 (14); 237 (23); 221 (9); 185 (20); 155 (17); 142 (18); 119 (15); 107 (9); 105 (18); 91 (26); 79 (20); 77 (12); 65 (3); 51 (3). (Found: M^+, 374·210. $C_{22}H_{30}O_5$ requires: 374·209).

(5) *Methyl* ent-2α,3α-*epoxy*-20-*norgibberell*-16-*en*-7-*oate* 19,10-*lactone* (**30**, 4·8 mg, 1903 dpm/mg) which crystallized from benzene-light petroleum as prisms, m.p. 135–136°alone or mixed with the authentic sample described below. ν_{max}: 3060, 1640, 880 (=CH_2); 1770 (γ-lactone); 1730 cm^{-1} (ester). MS: *m/e* (%) 344 (M^+, 18); 312 (100); 300 (3); 284 (4); 240 (57); 222 (30); 155 (34); 119 (8); (Found: M^+ 344·162. $C_{20}H_{24}O_5$ requires: 344·162).

(6) Methyl *ent*-gibberella-2,16-dien-7,19,20-trioate (**32**) crystallized from light petroleum as prisms (6 mg, 1924 dpm/mg) m.p. 129–131° alone or mixed with a sample prepared from gibberellin A_{13} via the trimethyl ester[14] (**8**).

(7) *Methyl* ent-3α-*hydroxygibberell*-16-*ene*-7,19-*dioate* 20,2β-*lactone*(**4**) was obtained as a resin. ν_{max} ($CHCl_3$): 3620 (OH); 3080, 1650 (=CH_2); 1780 (γ-lactone); 1725 cm^{-1} (ester); NMR spectrum (C_5H_5N): s, 1·51 (tertiary Me); s, 3·63, 3·70 (methoxycarbonyls); AB, δ_A 3·01, δ_B 4·05, *J* 12 Hz (C 5-H, C 6-H); m, 4·92 (C3-H, C 17-H_2). MS: *m/e* (%), 404 (M^+, 5); 402 (2); 386 (10); 372 (26); 360 (2); 358 (1);

344 (10); 312 (100); 298 (8); 284 (17) (Found: M^+, 404·175; $M^+ - CH_4O$, 372·157; $M^+ - C_3H_8O_3$, 312·134; $C_{22}H_{28}O_7$ requires: 404·184; $C_{21}H_{24}O_6$ requires: 372·157; $C_{19}H_{20}O_4$ requires: 312·136). A further sample of this lactone (49 mg) was available from the metabolism of the dienol (1) described elsewhere.[10]

(8) The methyl ester of the C 3-epimer of gibberellin A_4 (33 mg) m.p. 169–170° alone or mixed with a sample from base treatment of gibberellin A_4 (27).[33]

Methyl gibberellate. The methyl ester fraction (129 mg) from metabolism of the ^{14}C-dienol (1, 800 mg, 470 cpm/mg) was fractionated by prep. TLC to give crude methyl gibberellate (Me ester of 3, 23 mg) which after two crystallizations from light petroleum-EtOAc had m.p. 207–208° alone or mixed with an authentic sample.[34] The sample showed only background radioactivity.

Methyl ent-3-*oxogibberell*-16-*ene*-7,19-*dioate* 20,2β-*lactone* (5). The diester (4, 49 mg) in acetone (15 ml) was oxidised with Jones reagent (0·13 ml). After 1 hr the product was isolated and separated by prep. TLC to give the *keto diester* (5, 25 mg) which crystallized from light petroleum-benzene as needles m.p. 142–144°, $[\alpha]_D$-76° (*c*, 0·48). ν_{max}: 1785 (γ-lactone), 1725 cm^{-1} (cyclohexanone and ester). NMR spectrum: s, 1·50 (tertiary methyl); s, 3·65, 3·70 (methoxycarbonyls); AB, δ_A 2·55, δ_B 3·93, J 12 Hz (C 5-H, C 6-H); d, 4·73, J 7 Hz (C 2-H); m, 4·86 and 5·00 (C 17-H_2). MS: *m/e* (%), 402 (M^+, 14%); 370 (100); 358 (10); 356 (2); 342 (23); 310 (83); 298 (15); 282 (21) (Found: M^+ 402·166; $M^+ - CH_4O$, 370·142; $M^+ - C_2H_4O_2$, 342·145. $C_{22}H_{26}O_7$ requires: 402·168; $C_{21}H_{22}O_6$ requires; 370·142; $C_{20}H_{22}O_5$ requires: 342·147).

Methyl ent-3-*oxogibberell*-16-*ene*-7,19,20-*trioate* (6). The diester (5, 9 mg) in THF (2 ml) was added to chromous acetate (75 mg) in 10% aqueous THF (15 ml) under CO_2. After 16 h the acidic product was separated in the usual way with ether and methylated (diazomethane). After prep. TLC the keto triester (6, 3·2 mg) was isolated and identified by comparison of TLC, NMR, MS and IR spectra with an authentic sample.

Methyl ent-*gibberell*-16-*ene*-7,19-*dioate* 20,3β-*lactone* (10). The reduction of the lactone (5, 35 mg) with Cr $(OAc)_2$ was repeated and the product ethylated by heating with EtI and K_2CO_3 in acetone. After purification by prep. TLC the *keto triester* (7, 18 mg) was obtained as a gum. ν_{max}: 1730, 1725 cm^{-1} (CO_2R) NMR spectrum: s, 1·26 (tertiary Me); t, 1·33, q, 4·10, J 7 Hz (ethoxycarbonyl); AB, δ_A 2·45, δ_B 3·95, J 12 Hz (C 5-H, C 6-H); 3·66, 3·73 (methoxycarbonyl); m, 4·90 (C 17-H_2). MS: *m/e* (%), 432 (M^+, 27%); 404 (41); 400 (71); 386 (18); 372 (44); 344 (13); 340 (100); 294 (31); 266 (12). (Found: $M^+ - CH_4O$; 400·187. $C_{23}H_{28}O_6$ requires: 400·188).

The triester (7, 18 mg) was treated with $NaBH_4$ (100 mg) in MeOH (3 ml) at r.t. overnight. The product was purified by prep. TLC to give a gum (4·1 mg) which showed ν_{max}: 3550 (OH); 1725 and 1705 cm^{-1} (esters). MS: *m/e* (%), 434 (6); 416 (71); 406 (49); 402 (21); 388 (16); 374 (34); 370 (100); 356 (86); 346 (41); 342 (27); 314 (31); 296 (89); 268 (39). This material, in acetic acid (3 ml), was heated on the steam bath for 24 h and the products separated by prep. TLC to give the lactone (10) as a gum (2·5 mg) which showed identical TLC, NMR, IR, and MS behaviour with an authentic sample.[14]

ent-2α,3α-*Epoxykaur*-16-*en*-19-*ol* (15). (With W. Schockoff). *ent*-Kaura-2,16-dien-19-ol (1, 4·3 g) was epoxidised with perbenzoic acid (10 g) in $CHCl_3$ (200 ml) at 0° for 5 days. The neutral product crystallized from benzene-light petroleum as prisms of the *diepoxide* (16, 4·0 g). (Found: C, 75·7; H, 9·6. $C_{20}H_{30}O_3$ requires: C, 75·4; H, 9·5%); ν_{max}: 3045, 825 (epoxide); 3624 cm^{-1} (OH); NMR spectrum: s, 1·04, 1·21 (tertiary Me); d, 2·78 *J*, 2 Hz (C 17-H_2); m, 3·15 (C 2-H, C 3-H); AB, δ_A 3·53, δ_B 3·86 *J* 11 Hz (C 19-H_2).

The diepoxide (16, 4·5 g) was boiled with KSeCN (18·0 g) in MeOH (30 ml) for 1·5 h. Isolation with ether and chromatography on alumina gave 15 (1·8 g) which crystallized from benzene-light petroleum as prisms, m.p. 153–154° (Found: C, 79·1; H, 10·0. $C_{20}H_{30}O_2$ requires: C, 79·4; H, 10·0%) ν_{max}: 3060, 780 (=CH_2); 825 cm^{-1} (epoxide); NMR spectrum: s, 1·01, 1·19 (tertiary Me); br s 2·62 (C 13-H); m, 3·20 (C 2-H, C 3-H); AB, δ_A 3·55, δ_B 3·85, *J* 11 Hz (C 19-H_2); br s 4·74 (C 16-H_2). A more polar component (0·43 g) from the chromatogram is formulated as ent-2α,3α-*epoxykaur*-15-*ene*-17,19-*diol* and separated from benzene as flakes, m.p. 189–191° $[\alpha]_D$-30° (*c*, 1·3 in C_5H_5N) (Found: C, 75·4; H, 9·65. $C_{20}H_{30}O_3$ requires: C, 75·4; H, 9·5%).

Lithium aluminium hydride reduction of ent-2α,3α-*epoxykaur*-16-*en*-19-*ol* (15) The epoxide (15, 250 mg) in THF (6 ml) was boiled with LAH (780 mg) for 6 h. Isolation with EtOAc and chromatography on Al_2O_3 gave a less polar fraction which crystalized from acetone to give 17 (98 mg) m.p. and mixed[19] m.p. 201–202°. The more polar fraction gave ent-*kaur*-16-*ene*-2α,19-*diol* (18, 125 mg) which crystallized from $CHCl_3$ as needles, m.p. 220–221°, $[\alpha]_D$-91° (*c*, 1·05 in EtOH) (Found: C, 78·7; H, 10·5. $C_{20}H_{32}O_2$ requires: C, 78·9; H, 10·6%); ν_{max} (nujol): 3500 (OH); 1655, 870 cm^{-1} (=CH_2); NMR spectrum (C_5D_5N): s, 1·08, 1·23 (tertiary Me); AB, δ_A 3·63, δ_B 3·94, *J* 11 Hz (C 19-H_2); m, 4·26, $W_{h/2}$ ~27 Hz (C 2-H); m, 4·87 (C 17-H_2) MS: *m/e* (%) 304 (M^+, 18); 286 (44); 273 (27); 271 (29); 255 (100). The diol (18, 54 mg) was oxidised with Jones reagent (0·08 ml) for 15 min when the keto aldehyde was obtained; ν_{max}: 1725 (CHO), 1705 (C=O); NMR spectrum: s, 0·87, 1·22 (tertiary Me); m, 4·83 (C 17-H_2); s, 9·77 (CHO). The keto aldehyde (18 mg) was reduced as above with LAH and the product separated by prep. TLC to give *ent*-kaur-16-ene-2β,19-diol[19] (10 mg) m.p. and mixed m.p. 174–175°. A more polar component (4 mg) was identified as 18.

ent-3α,7,19-*Trihydroxygibberell*-16-*ene* (13). The epoxydiester (11, 13 mg) in THF (4 ml) was refluxed with LAH (30 mg) for 3 h. The product, isolated with EtOAc crystallized from EtOH-light petroleum as needles of 13 (8·7 mg), m.p. 185–186° (lit.[17] 182–184°). ν_{max} (Nujol): 3250 (OH); 1655, 875 cm^{-1} (=CH_2). The spectrum was identical with a copy supplied by Dr. B. E. Cross. NMR spectrum (C_5D_5N): s, 1·08, 1·79 (tertiary Me); AB, δ_A 3·85, δ_B 4·25, *J* 11·5 Hz (C 19-H_2); d, 3·98, *J* 8 Hz (C 7-H_2); m, 3·97, $W_{h/2}$ ~7 Hz (C 3-H); m, 4·83, m, 5·00 (C 17-H_2). MS: *m/e* (%), 302 (M-18, 43); 284 (93); 271 (86); 253 (100). (Found: $M^+ - 18$, 302·225. $C_{20}H_{30}O_2$ requires: 302·225).

Methyl ent-2α,3α-*epoxygibberell*-16-*ene*-7,19-*dioate* (11) *from methyl* ent-*gibberella*-2,16-*diene*-7,19-*dioate* (20) The diester (20, 23 mg) in $CHCl_3$ (5 ml) was treated with *m*-chlorperbenzoic acid (87 mg) for 2 days at 0°. Recovery of the neutral product gave the crude diepoxide (25 mg) which was dissolved in MeOH and boiled with KSeCN (390 mg) for 4 h. Isolation with ether and prep. TLC yielded the epoxy diester (11, 7·5 mg) identical in all respects with the metabolic sample above. A polar band gave another crop (5·4 mg) on further treatment with KSeCN.

13-*Deoxygibberellin* A_6 *methyl ester* (30) *from* 13-*deoxygibberellin* A_5 *methyl ester* (24). The ester[22] (30,

52 mg) in $CHCl_3$ (3 ml) was epoxidised with *m*-chlorperbenzoic acid (179 mg) for 5 days at 20°. Isolation of the neutral product and prep. TLC gave the crude diepoxide (**34** mg) which was dissolved in MeOH (2 ml) and boiled with KSeCN (150 mg) for 30 min. Isolation with ether and prep. TLC gave **30** (17·4 mg) identical with the sample from the metabolism described above.

ent-7,19,20-*Trihydroxygibberella*-2,16-*diene* (**34**). (a) The aldehyde diester (**33**, 4·6 mg) was reduced in THF with LAH at reflux for 4 h. The triol (**34**) isolated with EtOAc crystallized from acetone-light petroleum as needles (2 mg) m.p. 146–148° alone or mixed with the sample described below (b). ν_{max} (nujol): 3350 (OH); 3060, 1650, 875 (=CH_2); 3010 (HC=CH). NMR spectrum (C_5D_5N): s, 1·49 (tertiary Me); s, 4·28 (C 20-H_2); d, 4·08, *J* 8 Hz (C 7-H_2); AB δ_A 3·93, δ_B 4·35, *J* 11 Hz (C 19-H_2); m, 4·83, m, 5·00 (C 17-H_2). MS: *m/e* (%), 318 (M^+, 6); 300 (6); 288 (19); 282 (13); 270 (38); 269 (38), 257 (31); 251 (18); 239 (100) (Found: M^+, 318·219. $C_{20}H_{30}O_3$ requires: 318·220). (b) The triester (**32**, 15 mg) was reduced with $LiAlH_4$ as in (a) to give the triol (**34**) identical in all respects with the sample described in (a).

Separation of the gibberellin acids from metabolism of ent-*kaura*-2,16-*dienyl succinate* (**2**). Metabolism of **2** (2·2 g) as described above gave an acidic extract (1·03 g) which was chromatographed on silicic acid and the fractions batched in groups on the basis of TLC. The required gibberellins were identified as the esters in the bulk fractions by TLC of methylated aliquots and the appropriate fractions were submitted to prep. TLC using multiple development in light petroleum-diisopropyl ether-acetic acid (45:50:5). In this way a homogeneous fraction (15 mg) of **21** was obtained as a gum. NMR spectrum: s, 0·79, 1·38 (tertiary Me); m, 4·83, 4·93 (C 17-H_2); m, 5·63 (C 2-H, C 3-H); 10·77 (CO_2H). Methylation with diazomethane gave the ester (**20**) homogeneous to TLC and GLC. A more polar fraction (97 mg) was separated similarly to give the epoxy diacid (**12**, 13 mg) as a gum, which was identified as its homogeneous (TLC, GLC) methyl ester (**11**). The major fraction (445 mg) gave, after multiple development TLC using acetic acid in diisopropyl ether, the lactone acid (**36**, 60 mg) a portion of which was methylated to give the ester (**4**) identical with the sample described above. The procedure was repeated using [17-^{14}C]-labelled dienyl succinate (**2**, 136 mg, $8·1 \times 10^5$ dpm/mg) and resuspended mycelia of *G. fujikuroi* at pH 5·7. After 4 days the acidic products (100 mg) were diluted with cold diene diacid (**21**, 5 mg) and epoxy diacid (**12**, 5 mg) and the mixture separated to give the [17-^{14}C]-epoxy diacid (4·7 mg) and the diene diacid (7·0 mg). The samples were homogeneous on the basis of the radioscans of their TLC plates in either light petroleum/diisopropyl ether/acetic acid (45:50:5) or, 3% acetic acid in diisopropyl ether. The remaining acidic material was methylated (diazomethane), 13-deoxygibberellin A_5 methyl ester (**24**, 7 mg) added and recovered by prep. TLC. After two crystallisations the sample had constant specific activity (1800 dpm/mg) m.p., and mixed m.p. 152–153°.

Incubation of ent-[17-^{14}*C*]-2α,3α-*epoxygibberell*-16-*ene* 7,19-*dioic acid* (**12**). The [17-^{14}C]-epoxy diacid (**12**, 6·3 mg, $1·27 \times 10^5$ dpm/mg) was fed to resuspended *G. fujikuroi* at pH 5·7 for 4 days and the acidic metabolites were isolated. Radioscan revealed 4 zones, corresponding to the epoxy diester (**11**, ~50%) overlapping with 13-deoxygibberellin A_5 ester (**24**, 15%), the lactone diester (**4**, ~10%) and gibberellin A_6 methyl ester (**29**, 10%). To the mixture cold lactone diester (**4**, 9·7 mg) was added and the three main zones separated by TLC. The least polar band was shown to contain the epoxy diester (**11**) and the ester of **30** by GLC analysis. The second band (10·5 mg) corresponded to the lactone diester (**4**). It was diluted with more material (7·8 mg), dissolved in acetone (10 ml) and oxidised with Jones reagent (0·07 ml) for 1 h. The product (**5**) separated by prep. TLC was recrystallized from benzene-light petroleum to constant specific activity (3·2 mg, 2972 dpm/mg). The most polar band was radiochemically homogeneous to TLC using 3% acetic acid–diisopropyl ether, diethyl ether–benzene and diethyl ether–light petroleum. The MS showed intense peaks for gibberellin A_6.

Metabolism of ent-*gibberella*-2,16-*diene*-7,19-*dioic acid* (**21**). The diacid (**21**, 6·7 mg, $6·78 \times 10^4$ dpm/mg) was fed to the mould as for the epoxide and the acidic products methylated. Radioscans of TLC plates and GLC analysis indicated the diester (**20** ~5%) the aldehyde diester (**33**, 25%) and the triester (**32**, 30%). Minor peaks (~5% each) were assigned provisionally to esters of gibberellin A_5 (**23**), -A_6 (**29**) and the diene diester (**20**).

ent-7,19-*dihydroxygibberella*-2,16-*diene* (**39**). The diester (**20**, 20 mg) in THF (13 ml) was refluxed with LAH (10 mg) for 3 h. The neutral product crystallized from acetone-light petroleum to give the diol (**39**, 16 mg) as needles, m.p. 147–148° ν_{max} (Nujol): 3350, 3450 (OH); 3050, 1645, 870 (=CH_2) and 3010 cm^{-1} (HC=CH); NMR spectrum: s, 0·88, 1·18 (tertiary Me); AB, δ_A 3·47, δ_B 3·93, *J* ~ 11·5 Hz (C 19-H_2); d, 3·78, *J* 5·5 Hz (C 7-H_2); m, 4·80, 4·93 (C 17-H_2); m, 5·46 (C 2-H, C 3-H). MS *m/e* (%), 302 (M^+9); 284 (32); 271 (69); 268 (29); 253 (100); 241 (11); 119 (77); 107 (74); 105 (86); 91 (74); 79 (20); 77 (29). (Found: M^+, 302·225. $C_{20}H_{30}O_2$ requires: 302·225). A sample of [17-^{14}C]-diol m.p. 147–148° (3·3 mg, $1·68 \times 10^5$ dpm/mg) was prepared similarly from [17-^{14}C]-diene diester (**20**).

Incubation of ent-[17-^{14}*C*]-*gibberella*-2,16-*diene*-7,19-*diol* (**39**). The radioactive diol (**39**, 3·3 mg, $1·68 \times 10^5$ dpm/mg) was fed to resuspended *G. fujikuroi* as for the epoxy diacid. There was no radioactivity in the neutral fraction. Radioscans of TLC plates and GLC of the methylated acids indicated the epoxy diester (**11**, ~10%), the triester (**32**, ~10%), the lactone diester (**4**, ~20%) and gibberellin A_5 methyl ester (**23**, 20%) along with two unidentified components the less polar of which had the same R_f as the unknown component (A) from the diacid feeding. To one tenth of the ester mixture cold gibberellin A_5 methyl ester (**23**, 5 mg) was added and this ester recovered by prep. TLC and diluted with another portion (6·8 mg) of cold material. Recrystallization from acetone–light petroleum to constant specific activity gave **23**, (0·3 mg; 451 dpm/mg) m.p. 192–194°.

Gibberellin A_6 *methyl ester* (**29**). Gibberellin A_5 methyl ester (**23**) prepared from gibberellin A_1 via the tosylate methyl ester[29] was treated with $(CF_3CO)_2O$ (0·4 ml) in pyridine (2 ml) for 1·5 h at 0°. The crude product (116 mg) isolated with ether contained ~80% trifluoracetate as judged by the NMR spectrum. This material was treated with *m*-chlorperbenzoic acid (308 mg) in dioxane (6 ml) and benzene (3 ml) for 5 days at r.t. The product was separated by prep. TLC to give in order of polarity gibberellin A_5 methyl ester (12 mg) and then **29** (12 mg) mp 133–135° (lit[28] 136–138°). The MS was identical with the published spectrum.[11] NMR spectrum: s, 1·29 (tertiary Me); AB, δ_A 2·63, δ_B 3·04, *J* 10 Hz (C 5-H, C 6-H); m, 3·15 (C 2-H, C 3-H); s, 3·70 (methoxycarbonyl); m, 4·93, 5·22

(C 17-H_2). Two polar components were considered to be the 16,17-epoxide (16 mg) and the 2,3; 16,17-diepoxide (12 mg) of gibberellin A_5 methyl ester as judged by NMR spectra.

Acknowledgements—This work was supported in part by a grant from the Australian Research Grants Committee. Grateful acknowledgement is made to Dr. J. R. MacMillan for GLC-MS analysis of 13-deoxygibberellin A_5, Dr. C. A. West for a sample of gibberellin A_{13}, Dr. J. Broadbent for gibberellin A_4, Dr. B. E. Cross for spectral data and Dr. R. H. B. Galt for a sample of the keto triester (**6**).

REFERENCES

[1]I. F. Cook, P. R. Jefferies and J. R. Knox, *Tetrahedron Letters* 2157 (1971)
[2]H. J. Bakker, P. R. Jefferies and J. R. Knox, *Ibid.* 2723 (1972)
[3]B. O. Phinney, Plant Growth Regulation p. 489. Iowa State Press, Ames, Iowa (1961)
[4]M. Ruddat, A. Lang and E. Mosettig, *Naturwiss.* **50**, 23 (1963); M. Katsumi, B. O. Phinney, P. R. Jefferies and C. A. Henrick, *Science* **144**, 849 (1964); *Plant Physiol.* Suppl. No. 39 (1964)
[5]B. E. Cross, J. C. Stewart and J. L. Stoddart, *Phytochemistry*, **6**, 1475 (1967)
[6]B. E. Cross, R. H. B. Galt and J. R. Hanson, *J. Chem. Soc.* 295 (1964); J. E. Graebe, D. T. Dennis, C. D. Upper and C. A. West, *J. Biol. Chem.* **240**, 1847 (1965); A. J. Verbiscar, G. Cragg, T. A. Geissman and B. O. Phinney, *Phytochemistry* **5**, 933 (1966); **6**, 807 (1967). J. R. Hanson and A. F. White, *Chem. Comm.* 410 (1969)
[7]M. Katsumi, B. O. Phinney, P. R. Jefferies and E. J. Middleton, Unpublished data
[8]D. W. Pitel, L. C. Vining and G. P. Arsenault, *Can. J. Biochem.* **49**, 194 (1971)
[9]P. R. Jefferies, J. R. Knox and T. Ratajczak, *Tetrahedron Letters* 3229 (1970); *Phytochemistry* in press
[10]I. F. Cook, P. R. Jefferies and J. R. Knox, *Tetrahedron* submitted
[11]R. Binks, J. MacMillan and R. J. Pryce, *Phytochemistry* **8**, 271 (1969)
[12]N. Sheppard, *J. Chem. Soc.* 3040 (1960)
[13]K. Koshimizu, H. Fukui, M. Inui, Y. Ogawa and T. Mitsui, *Tetrahedron Letters* 1143 (1968)
[14]R. H. B. Galt, *J. Chem. Soc.* 3143 (1965)
[15]J. R. Hanson, *Ibid.* 5036 (1965)
[16]B. M. Bloom and G. M. Shull, *J. Am. Chem. Soc.* **77**. 5767 (1955)
[17]B. E. Cross, K. Norton and J. C. Stewart, *J. Chem. Soc.* (C), 1054 (1968)
[18]D. H. R. Barton, D. A. Lewis and J. F. McGhie, *Ibid.* 2907 (1957)
[19]E. L. Ghisalberti, P. R. Jefferies and E. J. Middleton, *Aust. J. Chem.* **22**, 455 (1969)
[20]A. Rosowsky, *Heterocyclic Compounds* (Edited A. Weissberger) Vol. 19, Part I, pp 181–228, Interscience, New York (1964)
[21]C. C. J. Culvenor, W. Davies and K. H. Pausacker, *J. Chem. Soc.* 1050 (1946); C. C. J. Culvenor, *Aust. J. Chem.* **17**, 233 (1964)
[22]J. R. Hanson and T. P. C. Mulholland, *J. Chem. Soc.* 3550 (1965)
[23]J. MacMillan, *Aspects of Terpenoid Chemistry and Biochemistry* (Edited T. W. Goodwin) Academic Press, London and New York (1971)
[24]J. R. Bearder, P. Hedden, J. MacMillan, C. M. Wels and B. O. Phinney, *Chem. Comm.* 777 (1973)
[25]J. R. Hanson and A. F. White, *J. Chem. Soc.* (C), 981 (1969)
[26]J. R. Bearder, J. MacMillan and B. O. Phinney, *Phytochemistry* **12**, 2173 (1973)
[27]J. R. Hanson and A. F. White, *Chem. Comm.* 1071 (1969)
[28]J. MacMillan, J. C. Seaton and P. J. Suter, *Tetrahedron* **18**, 349 (1962)
[29]J. MacMillan, J. C. Seaton and P. J. Suter, *Ibid.* **11**, 60 (1960)
[30]H. B. Henbest and R. A. L. Wilson, *J. Chem. Soc.* 1958 (1957)
[31]C. Djerassi, C. H. Robinson and D. B. Thomas, *J. Am. Chem. Soc.* **78**, 5685 (1956)
[32]H. Brockmann and H. Schodder, *Chem. Ber.* **74**, 73 (1941)
[33]D. C. Aldridge, J. R. Hanson and T. P. C. Mulholland, *J. Chem. Soc.* 3539 (1965)
[34]J. F. Grove, P. W. Jeffs and T. P. C. Mulholland, *Ibid*, 1236 (1958)