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NEW METABOLITES OF *GIBBERELLA FUJIKUROI*—XIII

TWO GIBBANE 1 → 3-LACTONES[1]

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Abstract Two new compounds have been isolated from culture filtrates of *Gibberella fujikuroi*, one of which is shown to have structure II (R = H) whilst the other is provisionally assigned structure XI (R = H, R' = CH_2). The preparation of some 2,3-epoxygibberellin derivatives is described.

THE search for possible precursors of gibberellic acid has led to a thorough examination of the culture filtrates of *Gibberella fujikuroi*. In previous papers we have described the isolation and structure of some new gibberellins[2] and of other related diterpenoids.[2] This paper describes some new metabolites which are assigned gibbane 1 → 3-lactone structures.

Chromatography on silica gel:celite of the weakly acidic material from a large scale *Gibberella fujikuroi* ACC 917 fermentation led to the isolation of gibberellins A_{10}[3] and A_{15}.[1] Examination of the fraction eluted with 15% ethyl acetate in light petroleum by TLC on silica gel in di-isopropyl ether:acetic acid (19:1) revealed the presence of five components. Repeated chromatography of this fraction followed by careful crystallization enabled the separation and purification of four of these to be achieved. Two of the components were identified as gibberellin A_9[4] and fujenal.[5] The latter frequently occurs in acidic fractions due to hydrolysis and subsequent dehydration.

The third compound to be isolated was an acid, m.p. 180–182°, which, with ethereal diazomethane, gave a monomethyl ester, m.p. 176–178°. Analytical data of the acid and its ester indicated that the acid had the formula $C_{19}H_{22}O_4$. The IR spectra of the acid [2740–2650 (br) (OH of CO_2H), 1755 (γ-lactone), 1700 (CO_2H) cm^{-1}] and the ester [1762 (γ-lactone), 1726 (CO_2Me) cm^{-1}] showed the oxygen functions to be a γ-lactone and carboxyl group. Microhydrogenation of the ester indicated the presence of two double bonds which, from the olefinic absorption of the acid (1678, 1663, 1655, 880 and 850 cm^{-1}) and its ester (1679, 1655, 890 and 851 cm^{-1}), could be accounted for by a terminal methylene group and a trisubstituted double bond. These assignments were supported by the NMR spectrum of the ester (Table), which contained a peak at $\tau = 5{\cdot}18$ assigned to the terminal methylene protons,

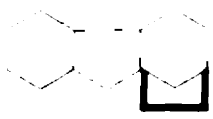
[1] J. R. Hanson, *Tetrahedron* **23**, 733 (1967).

[2] See earlier parts in this series.

[3] J. R. Hanson, *Tetrahedron* 701 (1966).

[4] B. E. Cross, R. H. B. Galt and J. R. Hanson, *Tetrahedron* **18**, 451 (1962).

[5] B. E. Cross, R. H. B. Galt and J. R. Hanson, *J. Chem. Soc.* 5052 (1963).



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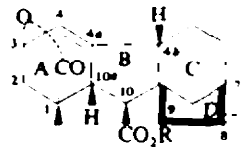

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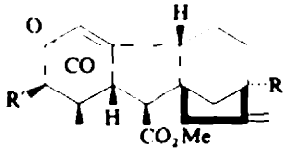

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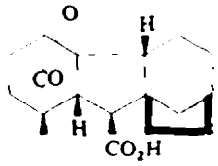

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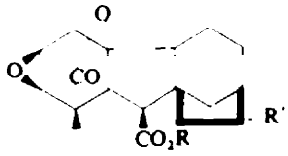

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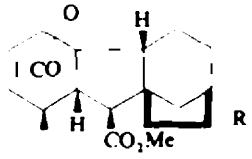

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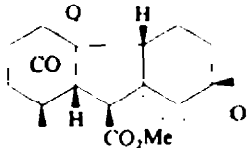

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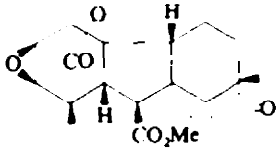

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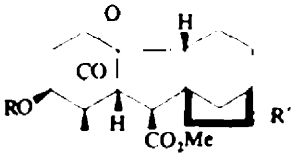

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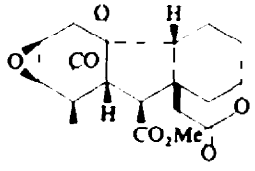

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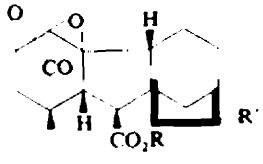


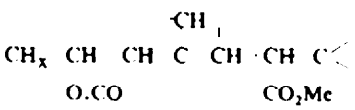
XI

as well as a tertiary C-Me singlet at $\tau = 8{\cdot}77$. Thus spectroscopic analysis has shown the presence of four extranuclear carbon atoms which together with the C_{19} formula implies a gibbane carbon skeleton (I). With this assumption, a detailed analysis of the NMR spectrum of the ester led to the structure II (R = H) for the acid. Comparison with the spectra of the rearrangement products (III; R = H, R′ = OH)[6] derived from gibberellin A_7, and (III; R = OH, R′ = OAc)[7] and (III; R = R′ = OAc)[8] derived from gibberellic acid clarified the assignments.

TABLE 1. τ VALUES OF NMR SPECTRA AT 100 Mc IN $CDCl_3$

Substituent	II; R = Me	III; R = H, R′ = OH	III; R = OH, R′ = OAc	III; R = R′ = OAc
1-Me	8·77	8·85	8·83	8·84
2-H		5·84	5·08	5·10
2-OAc			7·94	7·94
3-H	5·20	5·32	4·91	4·91
J (c/s)		6		
4-H	4·14	4·30	4·35	4·35
J (c/s)	6, 3, 3	6, 3, 3	6, 3, 3	6, 3, 3
4b-H		7·40	7·40	7·25
7-OAc				8·08
8 =CH_2	5·18	5·18	5·10	5·10
10-H	7·48	7·52	7·48	7·48
J (c/s)	6·5	6·5	6·5	6·5
10a-H	6·85	6·80	6·76	6·75
J (c/s)	6·5, 3	6·5, 3	6·5, 3	6·5, 3

The key to structure II (R = Me) is a single-proton quintuplet at $\tau = 4{\cdot}14$. Double-irradiation experiments showed this proton was coupled to a proton in the multiplet system centred at $\tau = 5{\cdot}20$ ($J = 6$ c/s) and to a proton at $\tau = 6{\cdot}85$ ($J = 3$ c/s). Further double irradiation experiments showed that the proton at $\tau = 7{\cdot}48$ was coupled to the proton at $\tau = 6{\cdot}85$ ($J = 6{\cdot}5$ c/s). These features require the partial structure



which is accommodated in rings A and B of the structure II (R = Me).

The isolation from the fermentation of the acid II (R = H), which is probably an isomerization product of the as yet unknown Δ^3-dehydrogibberellin A_9 (IV), led to a search for the latter. A crude fraction [ν_{max} 3040 (OH of CO_2H), 1769 (γ-lactone), 1694 (CO_2H), 1657 and 885 ($>C{=}CH_2$) cm^{-1}] was isolated with the anticipated

[6] D. C. Aldridge, J. R. Hanson and T. P. C. Mulholland, *J. Chem. Soc.* 3539 (1965).
[7] B. E. Cross, J. F. Grove and A. Morrison, *J. Chem. Soc.* 2498 (1961).
[8] D. F. Jones, J. F. Grove and J. MacMillan, *J. Chem. Soc.* 1835 (1964).

TLC characteristics, but owing to the shortage of material it could not be further purified.

The fifth component of the mixture was an acid, m.p. 242–244°, $[\alpha]_D$ + 11°, which showed weak activity in the pea seedling test.[9] Analytical data for the acid and its methyl ester together with the mass spectrum of its methyl ester nor-ketone were consistent with the formula $C_{19}H_{22}O_5$ for the acid. The IR spectra of the acid [3150 (br) (OH of CO_2H), 1771 (γ-lactone), 1730 (CO_2H) cm^{-1}] and its ester [1765 (γ-lactone) and 1729 (CO_2CH_3) cm^{-1}] enabled four of the oxygen atoms to be accounted for as γ-lactone and carboxyl groupings. The absence of absorption due to OH or further carbonyl groups suggested that the remaining oxygen atom was present as an ether link. Microhydrogenation of the methyl ester revealed the presence of one double bond. The IR spectrum of the acid (ν_{max} 1658 and 896 cm^{-1}) and its ester (ν_{max} 1659 and 886 cm^{-1}) indicated that this was a terminal methylene group. Confirmation was provided by ozonolysis of the ester which led to the formation of a nor-ketone, $C_{19}H_{22}O_6$ (mol. ion 346). The IR spectrum of the latter contained a new cyclopentanone carbonyl absorption (ν_{max} 1748 cm^{-1}). With these features in mind a 7-deoxygibberellin A_6 structure V (R = H, R′ = CH_2) was originally suggested for the acid which was at that time (1962) named gibberellin A_{11}.

Owing to shortage of material no further degradative work could be carried out on the acid, $C_{19}H_{22}O_5$. Consequently it was decided to synthesize the nor-ketone methyl ester (V; R = Me, R′ = O) corresponding to structure V (R = H, R′ = CH_2) for the acid. This synthesis required gibberellin A_4, which is difficult to obtain in quantity, and involved the epoxidation of the 2,3-double bond in the gibberellin A_4 derivative VI (R = O). Since such double bonds in the gibberellin series are known to be resistant to epoxidation,[10] some model experiments were carried out on the gibb-2-ene (VII). This was prepared from gibberellic acid by the literature method.[11, 12] The difficulty in epoxidizing gibb-2-ene 1 → 4a-lactones is presumably due to interaction of the π electrons of the carbon–carbon double bond with those of the carbonyl group of the lactone. That such interaction occurs is shown by the UV absorption (λ_{max} 223 mμ, ε = 1230) of VII (cf. Ref. 12) and related compounds (see below and Refs 12, 13). However, prolonged treatment of the gibb-2-ene (VII) with excess *m*-chloroperbenzoic acid in boiling acetone gave the required epoxide (VIII). The structure of the epoxide followed from its formula ($C_{20}H_{24}O_6$) and spectroscopic data. Thus, in the UV a peak at λ_{max} 290 mμ (ε = 41), due to the ring D cyclopentanone, showed that Baeyer Villiger oxidation of the 8-ketone had not taken place. Its NMR spectrum showed no vinylic protons but contained a peak at τ = ~6·77, which was incompletely resolved from the 10a-proton doublet at τ = 6·88 (*J* = 6·5 c/s), and which was assigned to the epoxide protons; the 10-H proton appeared as a doublet at τ = 7·34 superimposed upon the 4b-proton at τ 7·23. The shift to low field of the 10a-proton in the epoxide, relative to its position (τ = 7·17, *J* = 6·5 c/s) in the gibb-2-ene (VII), established the β-orientation of the epoxide ring.[14] The

[9] P. W. Brian and H. G. Hemming, *Physiol. Plant.* **8**, 669 (1955).
[10] J. MacMillan, personal communication.
[11] B. E. Cross, *J. Chem. Soc.* 3022 (1960).
[12] J. MacMillan, J. C. Seaton and P. J. Suter, *Tetrahedron* **11**, 60 (1960).
[13] J. R. Hanson and T. P. C. Mulholland, *J. Chem. Soc.* 3550 (1965).
[14] J. R. Hanson, *J. Chem. Soc.* 5036 (1965).

small 10,10a coupling constants in the epoxide VIII and the gibb-2-ene (VII) are in agreement with those previously reported[15] for 7α-gibbanes. Although chromatography of the crude product from the peracid oxidation revealed the presence of a minor component, it seems unlikely that Baeyer-Villiger oxidation of the ring D ketone took place to any considerable extent.

The synthesis of the epoxy-nor-ketone (V; R = Me, R′ = O) required the gibb-2-ene (VI; R = O). This was prepared from gibberellin A_4 by the route of Cross *et al.*[4] except that gibberellin A_4 methyl ester (IX; R = H, R′ = CH_2) was converted into its nor-ketone by sodium periodate in the presence of a catalytic amount of osmium tetroxide.[16] On another occasion, the methane sulphonate (IX; R = SO_2CH_3, R′ = CH_2)[13] of gibberellin A_4 methyl ester was converted into its nor-ketone (IX; R = SO_2CH_3, R′ = O) by oxidation with osmium tetroxide followed by sodium periodate. With refluxing collidine the nor-ketone (IX; R = SO_2CH_3, R′ = O) gave the required gibb-2-ene (VI; R = O).

The acetate of gibberellin A_4 methyl ester nor-ketone (IX; R = $COCH_3$, R′ = O) readily undergoes Baeyer-Villiger oxidation with perbenzoic acid,[4] hence it was expected that the gibb-2-ene (VI; R = O) would behave similarly. However, since the 7α-gibb-2-ene (VII) did not undergo Baeyer–Villiger oxidation with *m*-chloroperbenzoic acid (see above) the effect of *m*-chloroperbenzoic acid on the gibb-2-ene VI (R = O) was investigated. In refluxing acetone the epoxy-δ-lactone (X) was formed in good yield; no other product was isolated. Structure X for the epoxy-δ-lactone was supported by its NMR spectrum which showed a broad peak at τ = 6·81 assigned to the epoxide protons and a broad multiplet at τ = 5·2 attributed to the 7-proton. The 10,10a AB quartet was partly obscured on the low field side by the peak due to the epoxide protons and on the other side by the peak at τ = 7·56 ascribed to the 9-protons. Nevertheless, the high field half of the doublet associated with the 10a-proton could be seen at τ = 7·01, thus establishing the β-configuration for the epoxide function [in VI (R = CH_2) the 10a-proton is found at τ = 7·42[14]].

The reason why the 7α-gibb-8-one (VII), unlike the gibb-8-one (VI; R = O), fails to undergo the Baeyer-Villiger reaction is not clear, but it is presumably dependent upon the stereochemistry of ring D.

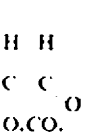
To prevent the Baeyer-Villiger oxidation, the gibb-8-one (VI; R = O) was reduced with sodium borohydride to the alcohol VI (R = H, OH). Treatment of the alcohol VI (R = H, OH) with excess *m*-chloroperbenzoic acid in boiling acetone gave, as the main product, the gummy epoxy-alcohol V (R = Me, R′ = H, OH), which was not characterized, and a small amount of a compound subsequently identified as the epoxy-nor-ketone (V; R = Me, R′ = O). Oxidation of the epoxy-alcohol with chromium trioxide in pyridine gave the epoxy-nor-ketone (V; R = Me, R′ = O; mol. ion 346), ν_{max} (in $CHBr_3$) 1778 (γ-lactone) and 1740 cm^{-1} (ester and cyclopentanone). IR and mass spectroscopy showed that the ester V (R = Me, R′ = O) was not the same as the methyl ester nor-ketone of the acid $C_{19}H_{22}O_5$. The acid does not therefore have structure V (R = H, R′ = CH_2).

However, the 100 Mc NMR spectra of the esters of the acid $C_{19}H_{22}O_5$ and its nor-ketone do permit a tentative location of the functional groups. The spectrum of the ester, which contained a three-proton system between τ 4·9 and 5·15, replaced

[15] D. C. Aldridge, J. F. Grove, R. N. Speake, B. K. Tidd and W. Klyne, *J. Chem. Soc.* 143 (1963).
[16] R. Pappo, D. S. Allen, R. U. Lemieux and W. S. Johnson, *J. Org. Chem.* **21**, 478 (1956).

in the nor-ketone by one-proton at $\tau = 4{\cdot}98$, confirmed the presence of the terminal methylene group and indicated the presence of the fragment

The proton in this fragment was coupled to a single proton appearing as a doublet ($J = 3$ c/s) at $\tau = 6{\cdot}59$ in the ester and $\tau = 6{\cdot}61$ in the nor-ketone. This coupling was confirmed by double-irradiation experiments. In the absence of hydroxylic absorption, the resonance at $\tau = 6{\cdot}59$ can be assigned to the single proton of a trisubstituted epoxide. Hence the partial structure



is present. The NMR spectra also contain a pair of doublets at $\tau = 7{\cdot}12$ and $7{\cdot}33$ ($J = 5$ c/s) (ester) and $\tau = 7{\cdot}09$ and $7{\cdot}28$ ($J = 5$ c/s) (nor-ketone), reminiscent of the 10,10a quartet in the gibberellins, but with a coupling constant nearer that (6·5 c/s) of the gibbane 1 → 3-lactones described above (see Table). The presence of a tertiary C-Me group is indicated by a sharp singlet at $\tau = 8{\cdot}83$ (ester) and $\tau = 8{\cdot}84$ (nor-ketone). On this basis, structure XI (R = H, R′ = CH_2) is tentatively proposed for the acid $C_{19}H_{22}O_5$. Since the 1 → 3-lactone ring system is usually associated with artefacts it seems desirable to discard the name gibberellin A_{11} for this acid. Biogenetically the acid $C_{19}H_{22}O_5$ could arise by epoxidation of the ring A double bond in the acid II (R = H) which was isolated from the same fermentation (see above).

EXPERIMENTAL

M.ps were determined on a Kofler block and were corrected. Unless otherwise stated, IR and UV spectra were determined as KCl discs and in EtOH soln respectively. NMR spectra were measured in $CDCl_3$ soln on Varian Associates A-60 and HA100 spectrometers and had TMS as internal standard. Light petroleum refers to the fraction b.p. 60–80°. The term "worked-up as usual" means washed successively with dil. HCl, water, $NaHCO_3$ aq, and water, dried over anhyd Na_2SO_4 and evaporated *in vacuo*. The course of reactions and the progress of column chromatograms was followed by TLC on silica gel G (Merck).

Isolation of the acids. The crude "neutral" fraction from a large-scale *Gibberella fujikuroi* ACC 917 fermentation was carefully extracted with $NaHCO_3$ aq. This extract was acidified and the organic material recovered in EtOAc. These "weak acids" were then chromatographed on silica:celite (1:2) by elution with increasing amounts of EtOAc in light petroleum. Elution with 15% EtOAc in light petroleum gave a gum (A).

A portion (1·2 g) of the gum (A) was rechromatographed on silica gel (1·5 × 22 cm). The fraction eluted with 10% EtOAc–light petroleum gave fujenal[3] (60 mg) identified by its IR spectrum. The fraction eluted with 15% EtOAc–light petroleum gave gibberellin A_9[4] (210 mg) identified by its IR spectrum. The crude fraction eluted with 20% EtOAc–light petroleum eventually gave II (R = H; 100 mg) after repeated crystallization. The crude mother liquor on TLC in di-isopropyl ether–AcOH (19:1) showed 3 spots at R_f 0·66 (gibberellin A_9), 0·61 (? Δ^3-gibberellin A_9) and 0·56 (acid II; R = H). Repeated chromatography and crystallization eventually gave a fraction [ν_{max} (Nujol) 3040 (br), 1769, 1694, 1657, 885 cm^{-1}] comprising predominantly the spot of R_f 0·61.

The fraction eluted with 30% EtOAc–light petroleum gave XI (R = H, R′ = CH_2; 80 mg).

3α-Hydroxy-1β-methyl-8-methylenegibb-4-ene-1α, 10β-dicarboxylic acid 1 → 3α-lactone (II; R = H)

The *acid* crystallized from acetone–light petroleum as needles, m.p. 180–182°. (Found: C, 72·5; H, 7·0.

$C_{19}H_{22}O_4$ requires: C, 72·5; H, 7·05%.) ν_{max} (Nujol) 2740–2650 (br), 1755, 1700, 1678, 1663, 1655, 880 and 850 cm^{-1}. The *methyl ester*, prepared with diazomethane in ether, crystallized as needles from acetone–light petroleum, m.p. 176–178°. (Found: C, 73·0; H, 7·45. $C_{20}H_{24}O_4$ requires: C, 73·1; H, 7·4%.) ν_{max} (Nujol) 3070, 1762, 1726, 1679, 1662, 1655, 1028, 938, 890 and 851 cm^{-1}. On microhydrogenation it took up 1·93 mol. hydrogen.

4,4a-*Epoxy*-3α-*hydroxy*-1β-*methyl*-8-*methylenegibbane*-1α,10β-*dicarboxylic acid* 1α → 3α-*lactone* (XI; R = H; R′ = CH_2)

The *acid* crystallized as needles from acetone light petroleum, m.p. 242–244° (dec). (Found: C, 69·3; H, 6·8. $C_{19}H_{22}O_5$ requires: C, 69·1; H, 6·7%.) ν_{max} (Nujol) 3150, 3060, 1777, 1730, 1658 and 896 cm^{-1}, $[\alpha]_D$ +11° (*c*, 0·3).

The *methyl ester*, prepared with diazomethane in ether, crystallized from acetone–light petroleum as needles, m.p. 179–181°. (Found: C, 70·5; H, 7·1. $C_{20}H_{24}O_5$ requires: C, 69·75; H, 7·0%.) ν_{max} (Nujol) 3070, 1765, 1729, 1659, 886 cm^{-1}. Microhydrogenation resulted in the uptake of 1 mole H_2.

Ozonolysis of the ester (XI; R = Me, R′ = CH_2). The ester (40 mg) in glacial AcOH (5 ml) was treated with excess ozonized O_2 for 2 min. The soln was neutralized with $NaHCO_3$aq and the organic product recovered in EtOAc. Chromatography on Al_2O_3 gave, in the fraction eluted with EtOAc light petroleum (1:1), the *norketone* (XI; R = Me, R′ = O) which crystallized from acetone light petroleum as small needles, m.p. 179–180°. (Found: C, 65·2; H, 6·4. $C_{19}H_{22}O_6$ requires: C, 65·9; H, 6·4%.) ν_{max} (Nujol) 1770, 1748 and 1725 cm^{-1}, (in $CHBr_3$) 1778, 1740 and 1734 cm^{-1}.

Epoxidation of the unsaturated keto-ester (VII). The keto-ester[12] (100 mg), λ_{max} 223 mμ ε, 1230) and *m*-chloroperbenzoic acid (400 mg) in acetone (20 ml) were refluxed for 21 hr, then a further amount of the peracid (800 mg) in acetone (5 ml) was added in portions, and the mixture refluxed until all the ketoester had been consumed (84 hr). Evaporation of the acetone *in vacuo* gave a white solid which was dissolved in EtOAc, washed successively with dil Na_2SO_3aq, dil $NaHCO_3$aq and water and dried. Recovery gave a solid residue (95 mg), which on TLC revealed the presence of two components. It was chromatographed on silica gel (4 gm; 9·5 × 1·0 cm).

Elution with 25% EtOAc in light petroleum gave the *epoxyketo-ester* (V; R = Me, R′ = O; 66 mg), which crystallized from acetone light petroleum as prisms (42 mg), m.p. 218–220°. (Found: C, 67·0; H, 6·8. $C_{20}H_{24}O_6$ requires: C, 66·65; H, 6·7%.) ν_{max} 1775 (γ-lactone), 1734 (cyclopentanone), 1723 (ester) and 823 (epoxide) cm^{-1}, λ_{max} 218 mμ (ε, 300) and 290 mμ (ε, 41), τ = 8·97 (7-Me), 8·63 (1-Me), 7·81 (–CH_2–CO–), 7·34 and 6·88 (doublets, J = 6·5 c/s; 10 and 10α protons respectively), 7·23 (4b-H), ~6·77 (2 and 3 protons) and 6·24 (OMe).

Elution with 45% EtOAc in light petroleum gave the minor component as a glass (10 mg), which crystallized from EtOAc-light petroleum in prisms (5 mg), m.p. 220–230°, ν_{max} 1783, 1739 and 1730 cm^{-1}.

Preparation of gibberellin A_4 *methyl ester nor-ketone* (IX; R = H, R′ = O)

Compound IX (R = H, R′ = CH_2, 50 mg, 0·14 mmole) and OsO_4 (1 mg) in THF (3 ml) and water (3 ml), were cooled to 0° with stirring, and this temp was maintained whilst powdered $NaIO_4$ (70 mg, 0·3 mmole) was added during 15 min. The mixture was stirred overnight at room temp, and the THF was evaporated *in vacuo*. The product was taken up into EtOAc and worked-up as usual, giving a residue (44 mg) which crystallized from acetone light petroleum in prisms of IX (R = H, R′ = O;[18] 42 mg), m.p. 194–204°. This high yield (84%) was not reproduced when working on a larger scale [1·4 g of gibberellin A_4 methyl ester gave only a 52% yield of IX (R = H, R′ = O)].

Preparation of the nor-ketone (IX; R = SO_2CH_3, R′ = O)

The methanesulphonate IX (R = SO_2CH_3, R′ = CH_2, 500 mg), prepared as a colourless glass by the method of Hanson and Mulholland,[13] was mixed with OsO_4 (350 mg) in dry pyridine (3 ml) and left to stand at room temp for 2·5 days. Water (11·5 ml), pyridine (8 ml), and sodium metabisulphite (600 mg) were added. The mixture was left to stand for 1 hr, and then triturated with water. The aqueous mixture was extracted with EtOAc and worked-up as usual, giving the glycol IX (R = SO_2CH_3, R′ = OH, CH_2OH) as a gum that would not crystallize.

The glycol in MeOH (100 ml) was treated with aqueous $NaIO_4$ (8·5 ml, 0·25 M) and left overnight at room temp. The MeOH was evaporated *in vacuo*, and the residue triturated with water. The ppt consisted

[13] B. E. Cross, R. H. B. Galt and J. R. Hanson, *J. Chem. Soc.* 3783 (1963).

[18] J. F. Grove, J. MacMillan, T. P. C. Mulholland and W. B. Turner, *J. Chem. Soc.* 3049 (1960).

of small prisms (130 mg), m.p. 207–211°, which, on recrystallization from EtOAc–light petroleum gave the pure *nor-ketone* (IX; R = SO_2CH_3, R' = O), m.p. 217·5–218·5°. (Found: C, 56·4; H, 5·9. $C_{20}H_{26}O_6S$ requires: C, 56·3; H, 6·1%.) ν_{max} 1767 (γ-lactone, 1735 (cyclopentanone) and 1722 (ester) cm^{-1}. Extraction of the aqueous filtrate with EtOAc followed by the usual work-up and crystallization from EtOAc–light petroleum gave more prisms (10 mg) of IX (R = SO_2CH_3, R' = O), m.p. 201–210°.

Preparation of the unsaturated keto-ester (VI; R = O)

(a) *From the hydroxy-ketone* (IX; R = H, R' = O). Using the method of Cross *et al.*,[4] IX (R = H, R' = O) was treated with toluene-*p*-sulphonyl chloride in pyridine, and the crude IX (R = *p*-$CH_3C_6H_4SO_2$, R' = O) boiled with collidine to give the required keto-ester VI (R = O), m.p. 159·5–160°.

(b) *From the methanesulphonate* (IX; R = SO_2CH_3, R' = O). The methanesulphonate (140 mg) was refluxed with collidine (10 ml) for 6 hr. The soln was cooled, poured into a mixture of HCl and water (1:1, 50 ml), and the product (98 mg) recovered in EtOAc. Chromatography on silica gel 5 gm; 17 × 1·0 cm) and elution with 25% EtOAc in light petroleum gave the unsaturated keto-ester VI (R = O) as prisms (44 mg), m.p. 158·5–160°, ν_{max} 1774 (γ-lactone), 1741 (cyclopentanone), 1729 (ester) and 686 (C=C) cm^{-1}, λ_{max} 222 (ε, 1490) and 291 (ε, 39) mμ.

Epoxidation of the unsaturated keto-ester (VI; R = O). The keto-ester (85 mg) was refluxed in acetone (10 ml) with *m*-chloroperbenzoic acid (1·30 g) until all the starting material had been consumed (3 hr). The soln was refluxed for a further 1 hr and then the acetone was evaporated *in vacuo*. The residue was dissolved in EtOAc, and washed successively with dil. Na_2SO_3 aq, dil. Na_2CO_3 aq and sat. NaCl solns and dried over Na_2SO_4. Recovery gave a crude product (132 mg) which showed the presence of 3 components on TLC in benzene–EtOH (3:1). It was chromatographed on silica gel (6·6 gm; 8·5 × 1·5 cm). Elution with 5% EtOAc in light petroleum gave *m*-chlorobenzoic acid (44 mg). Elution with 40% EtOAc in light petroleum gave the *epoxy-lactone* (X) as a gum (69 mg), which crystallized from EtOAc–light petroleum as prisms (55 mg), m.p. 158–163°. Recrystallization raised the m.p. to 161·5–163·5°. (Found: C, 62·65; H, 6·05. $C_{19}H_{22}O_5$ requires: C, 63·0; H, 6·1%.) ν_{max} ($CHBr_3$) 1776 (γ-lactone), 1737 (δ-lactone) and 1727 (ester) cm^{-1}, λ_{max} 224 mμ (ε, 580), τ = 7·67 (singlet; >C Me), 7·56 (singlet; 9-protons), 6·81 (multiplet; epoxide protons), 6·27 (singlet; $-CO_2Me$), 5·2 (multiplet; 7-proton).

The third component of the crude reaction product could not be isolated.

Reduction of the keto-ester (VI; R = O). The keto-ester (150 mg) in dry MeOH (15 ml) was treated with excess powdered $NaBH_4$ (250 mg) at 0° for 90 min. The soln was acidified with dil. HCl (10 ml) and concentrated *in vacuo*, giving a crystalline ppt, which was filtered off, washed with water and dried (140 mg). Recrystallization from acetone–light petroleum gave the *unsaturated hydroxy-ester monohydrate* (VI; R = H, OH) as small plates, m.p. 154–154·5°. (Found: C, 65·15; H, 7·5. $C_{19}H_{24}O_5$, H_2O requires: C, 65·1; H, 7·5%.) ν_{max} 3560, 3350 (br) (OH), 1756 (γ-lactone), 1734 (ester) and 1655 (H_2O bending) cm^{-1}. λ_{max} 222 mμ (ε, 1450). Extraction of the aqueous filtrate with EtOAc, and work-up as usual, gave an intractable gum (7 mg) which was shown by TLC to consist mainly of the above alcohol.

Epoxidation of the unsaturated hydroxy-ester (VI; R = H, OH). The hydroxy-ester (135 mg) and *m*-chloroperbenzoic acid (2·0 gm) in acetone (10 ml) were refluxed until all starting material had been consumed (3 hr). The acetone was evaporated *in vacuo*, the residue dissolved in EtOAc, and the soln washed successively with dil. Na_2SO_3 aq, dil. Na_2CO_3 aq (thoroughly) and water. Recovery gave a residue (188 mg) which was chromatographed on silica gel (9·4 gm; 13 × 1·5 cm). Elution with 5% EtOAc in light petroleum gave *m*-chlorobenzoyl peroxide (62 mg), which crystallized from light petroleum as prisms (50 mg), m.p. 120·5–121·5°. (Lit.[19] m.p. 122–123° dec.) (Found: C, 53·9; H, 2·85; Cl, 22·35. Calc. for $C_{14}H_8O_4Cl_2$: C, 54·0; H, 2·6; Cl, 22·8%.) ν_{max} 1787 and 1762 cm^{-1}. Elution with 25% EtOAc in light petroleum gave a crystalline mixture (22 mg), which was rechromatographed on silica gel (2·2 gm; 8 × 1·0 cm). 25% EtOAc in light petroleum eluted a gum (3 mg), which crystallized from EtOAc–light petroleum as prisms (1 mg), m.p. 166–174°, ν_{max} 1777 (γ-lactone), 1730 and 1723 (ester) cm^{-1}. This IR spectrum was different from that of the epoxy-lactone X. 30% EtOAc in light petroleum eluted a gum (6 mg), which on crystallization gave rosettes of needles (3 mg), m.p. 178–181·5°, shown to be the epoxy-ketone V (R = Me, R' = O) (see below) by their IR spectrum.

Elution with 40% EtOAc in light petroleum gave a gum (7 mg) which crystallized from EtOAc–light petroleum as rosettes of needles, m.p. 178–179·5° shown to be the epoxy-ketone V (R = Me, R' = O) by their IR spectrum. Elution with 80% EtOAc in light petroleum gave the epoxy-alcohol V (R = Me, R' = H, OH) as a gum (82 mg), ν_{max} (film) 3580–3480 (br) (OH), 1775 (γ-lactone) and 1732 (ester) cm^{-1}.

[19] C. G. Swain, W. H. Stockmayer and J. T. Clarke, *J. Am. Chem. Soc.* 72, 5426 (1950).

Oxidation of the epoxy-alcohol (V; R = Me, R' = H, OH). The alcohol (80 mg) in pyridine (0·8 ml) was added to CrO_3 (30 mg) in pyridine (0·3 ml). The soln was stirred at room temp (15 min), then left to stand overnight, added to water, and extracted with EtOAc. The extract was washed with water, dil. Na_2CO_3 and sat. NaCl soln, dried (Na_2SO_4), and evaporated *in vacuo*, giving a gum (59 mg), which was chromatographed on silica gel (4·6 gm; 1·5 × 1·1 cm). Elution with 40% EtOAc in light petroleum gave the *epoxy-ketone* V (R = Me, R' = O), which crystallized as rosettes of needles, m.p. 180–181·5°. (Found: C, 65·8; H, 6·5. $C_{19}H_{22}O_6$ requires: C, 65·9; H, 6·4%.) ν_{max} 1776 (γ-lactone), 1743 (cyclopentanone) and 1718 (ester) cm^{-1}, (in $CHBr_3$) 1778 and 1740 cm^{-1}. The mass spectrum showed a parent ion peak at *m e* 346. The IR spectrum was identical to that of the crystals, m.p. 178–181·5°, isolated in the preceding experiment as a by-product of the epoxidation of the hydroxy-ester VI (R = H, OH).

The soln IR and mass spectra were different from those of the methyl ester nor-ketone of the acid $C_{19}H_{22}O_5$, m.p. 242–244° (dec).

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