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# NEW METABOLITES OF *GIBBERELLA FUJIKUROI*—XVI[1]

## CYCLONERODIOL

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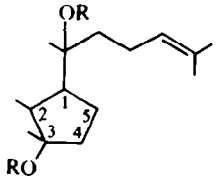
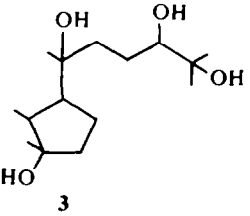
**Abstract**—A sesquiterpene diol produced by *G. fujikuroi* has been shown to be identical with cyclonerodiol. Additional evidence in support of the structure **1** of cyclonerodiol is described.

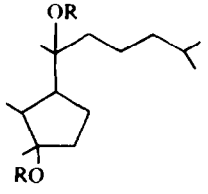
IN CONTINUATION of our work on the metabolites* of *Gibberella fujikuroi* we have isolated and examined an optically active liquid diol. Although the diol gave unsatisfactory elemental analyses, and gave no molecular ion in its mass spectrum, its



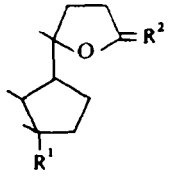
**1**: R = H
**2**: R = OC·$C_6H_3(NO_2)_2$



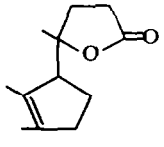
**3**



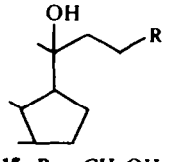
**4**: R = H
**5**: R = OC·$C_6H_3(NO_2)_2$



| | $R^1$ | $R^2$ |
|---|---|---|
| **6** | OH | O |
| **7** | OH | H, OH |
| **8** | H | O |
| **9** | OH | α-H, β-OMe |
| **10** | OH | α-OMe, β-H |
| **11** | OH | α-H, β-OEt |
| **12** | OH | α-OEt, β-H |
| **13** | H | $H_2$ |



**14**



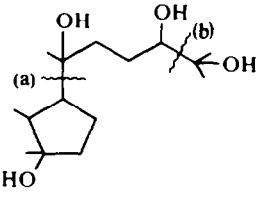
**15**: R = $CH_2OH$

* See previous parts in this series.

formula was established as $C_{15}H_{28}O_2$ by analysis of its crystalline bis-3,5-dinitrobenzoate **2** and its osmylation product $C_{15}H_{30}O_4$ **3**. When our work was nearing completion, the isolation of cyclonerodiol **1** from a *Trichothecium* sp. was reported.[2] Comparison of the *G. fujikuroi* metabolite with cyclonerodiol* by optical rotation, GLC, IR, and mass spectra showed that the two compounds were identical. The isolation of cyclonerodiol from *G. fujikuroi* is of interest since although over thirty metabolites are known to be produced by this fungus,† including many diterpenoids,[3] this is the first occasion on which a sesquiterpene has been found.

Our work on cyclonerodiol, which followed different degradative pathways to those used by the Japanese workers[2] and provides further evidence for the structure of the diol, is described here.

The NMR and mass spectra of cyclonerodiol provide strong evidence[2] in support of structure **1**. In agreement with this structure the mass spectrum of the derived tetraol **3** (see Scheme) showed strong ions at *m/e* 143 and 125 corresponding to




SCHEME

fission at (a) together with the loss of one and two molecules of water respectively from the side chain. Fragmentation of the α-diol system at (b) with loss of one and two molecules of water gave ions of *m/e* 197 and 179 respectively. thus providing evidence for the presence of the group Me.C(OH).Me in the tetraol and therefore of $Me_2C=$ in cyclonerodiol. The presence of the latter grouping was confirmed by the isolation of acetone, as its semicarbazone, from the products obtained by oxidation of cyclonerodiol with osmium tetroxide-sodium metaperiodate. The other products from the oxidation corresponded in $R_F$ to the lactone **6** and the hemi-acetal **7** (see below).

Oxidation of cyclonerodiol with potassium permanganate afforded the lactone **6** which was also obtained by ozonolysis of cyclonerodiol followed by oxidative work-up with Jones reagent (*cf* Ref 2). Our samples of lactone formed polymorphs, m.p. 82–83° or 190–193°, $[\alpha]_D$ −38° whereas Nozoe *et al.* report[2] m.p. 66–68°, $[\alpha]_D$ −52·5°. Nevertheless the spectroscopic data for our sample of lactone (Experimental) is in full agreement with structure **6**. Dehydration of the lactone **6** gave the unsaturated lactone **14** in which the structure of the 5-membered ring was established by its NMR spectrum which showed two vinylic Me groups and the 1-proton as a 1-H multiplet at τ 7·08. The structure of lactone **14** was confirmed by hydrogenation to the saturated lactone **8** whose NMR spectrum showed two secondary Me groups as overlapping doublets at τ 9·05, whilst the 1-proton had moved upfield into the methylene envelope. On

* We are indebted to Professor S. Nozoe for a generous sample of cyclonerodiol.
† See previous parts in this series.

reduction with LAH the lactone **8** afforded the diol **15** which was shown to contain the grouping $CH_2.CH_2OH$ by its NMR spectrum (triplet at $\tau$ 6·35). It follows that there is a $CH_2$ group $\alpha$ to the CO group in the lactone **6**. Treatment of the diol **15** with thionyl chloride in pyridine afforded the tetra-hydrofuran **13**.

Oxidation of the tetraol **3** with sodium metaperiodate in aqueous methanol afforded a mixture which was separated by chromatography. The least polar fraction showed two peaks on GLC analysis which are believed to be due to the epimeric acetals (**9** and **10**) because the NMR spectrum of the mixture showed a OMe peak at $\tau$ 6·66 and the acetal proton at $\tau$ 5·02 and its mass spectrum contained an M-1 ion but no molecular ion.[4] Furthermore after treatment of the acetal with dilute sulphuric acid at room temperature a OMe peak was no longer present in the NMR spectrum of the product. The more polar product was the hemi-acetal **7** (*cf* Ref 2). When a solution of the hemi-acetal in ethanol was allowed to stand, the product, which showed peaks due to OEt in the NMR and two peaks on GLC, was a mixture of the ethyl acetals (**11** and **12**).

## EXPERIMENTAL

Details of chromatographic materials and conditions used for the determination of physical data, etc., have been reported.[5] Mass spectra were determined with an A.E.I. MS 902 instrument and optical rotations were measured with a Perkin–Elmer 141 Polarimeter in $CHCl_3$ solns. A Pye Argon Chromatograph with a 5 foot column was used for GLC.

*Isolation of cyclonerodiol*

*Gibberella fujikuroi* ACC.917 was grown in stirred aerated culture (62·1) on a glucose-ammonium tartrate medium.[6] The crude neutral products (12·5 g) were isolated in the usual manner[6] and chromatographed on silica gel-Celite[6,7] (1:2) (1000 g). Elution with increasing concentrations of EtOAc in $CHCl_3$ (% of EtOAc in parentheses) gave the following results: Fractions (1–4) (7·5%) gave fujenal (205 mg), fractions (5–7) (10%) afforded 7-hydroxykaurenolide (386 mg), fractions (8 and 9) (12·5%) gave a gum (466 mg) consisting mainly of cyclonerodiol but containing some 7-hydroxykaurenolide. Later fractions (25–30%) yielded 7,18-dihydroxykaurenolide (1·37 g).

The gum was chromatographed on Kieselgel G (200 g). Elution with EtOH–benzene (1:9) and collection of 15 ml fractions gave *cyclonerodiol* (**1**) in fractions (19–28) as a colourless oil (250 mg) shown to be homogeneous by GLC, $[\alpha]_D$ −21·6° (*c* 1·485) (Found: *m/e* 222·19836. $C_{15}H_{28}O_2$ requires *M*-18, 222·19835). (Satisfactory analytical data could not be obtained) $\nu_{max}$ 3520 vs and 845 ($R^1R^2C = CHR^3$) $cm^{-1}$, *m/e* 225 (2%), 222 (15), 204 (4), 157 (2·5), 139 (30), 127 (8), 121 (5), 113 (2), 109 (100), 96 (20), 95 (21), 83 (15), and 82 (80), *m** 187·46 ($204^2/222$), *ca* 105·4 ($121^2/139$) and 93·6 ($109^2/127$).

Its *bis*-3,5-*dinitrobenzoate* (**2**) was prepared in pyridine at room temp during 5 days. Chromatography on silica gel and elution with 8% of EtOAc in light petroleum followed by crystallisation from acetone-light petroleum gave needles, m.p. 141–143° (Found: C, 55·9; H, 5·3; N, 8·8. $C_{29}H_{32}N_4O_{12}$ requires: C, 55·6; H, 5·1; N, 8·95%), $\nu_{max}$ 1720 $cm^{-1}$, $\tau$ 8·69 (3H, d, *J* 6·5 Hz, CHMe), 8·34, 8·27 and 8·23 (12H, 4x Me), 7·07 (2H, m, allylic $CH_2$), 4·83 (1H, m, =CH) and 0·9 (6H, m, aromatic H).

*Osmylation of cyclonerodiol*

Cyclonerodiol (305 mg) in pyridine (4 ml) was treated with $OsO_4$ (300 mg) for 70 hr. Water (15 ml), pyridine (7·5 ml), and $NaHSO_3$ (0·9 g) were added and the soln was left to stand for 45 min. The product was recovered in EtOAc and crystallised from acetone-light petroleum to give the *tetraol* (**3**) as needles (110 mg), m.p. 149–150°. (Found: C, 65·9; H, 11·05. $C_{15}H_{30}O_4$ requires: C, 65·7; H, 11·0%) $\nu_{max}$ 3500 vs $cm^{-1}$, $\tau$ (pyridine) 6·29 (m, CHOH), *m/e* 241 (4%), 223 (5), 197 (5), 179 (10), 161 (10), 157 (5), 143 (100), 139 (37), 121 (12), 109 (8), 96 (18) and 95 (18).

*Hydrogenation of cyclonerodiol*

Cyclonerodiol (130 mg) in EtOAc (15 ml) was added to 25% Pd— charcoal (50 mg), previously reduced in EtOAc (5 ml). It took up 12·8 ml (0·97 mol) of hydrogen in 2 hr. The dihydrocyclonerodiol (**4**) was a colour-

less oil (123 mg), $\nu_{max}$ 3510 $cm^{-1}$, $\tau$ 9·17, 9·1, 8·93, 8·84 and 8·75 (methyls), *m/e* 227 (3%), 224 (2), 209 (10), 206 (4), 191 (4), 157 (20), 139 (100), 129 (28), 121 (15), 111 (37), 96 (100), 95 (30), 85 (12) and 81 (85).

Its *bis*-3,5-*dinitrobenzoate* (**5**), prepared as in the case of cyclonerodiol (above), crystallised from benzene-light petroleum in needles, m.p. 140–142°, $[\alpha]_D^{25}$ −10° (*c* 0·9 in acetone) (Found: C, 55·5, 55·6; H, 5·8, 6·0; N, 8·6, 9·1. $C_{29}H_{34}N_4O_{12}$ requires: C, 55·2; H, 5·45; N, 9·1%) $\nu_{max}$ 1730 $cm^{-1}$.

*Oxidation of cyclonerodiol with potassium permanganate*

Cyclonerodiol (535 mg), in acetone (30 ml), was stirred with $KMnO_4$ (497 mg) at room temp for 2 hr. $NaHSO_3$ followed by dil HCl were added and the product was recovered in EtOAc. Chromatography on alumina (10 g) and elution with EtOAc-light petroleum (1:9 → 1:4) gave a product (124 mg) shown by TLC to be a mixture. PLC in AcOH-di-isopropyl ether (5:95) and recovery of the band of lower $R_F$ afforded the *lactone* (**6**) which crystallised from ether-light petroleum (b.p. 40–60°) in needles (25 mg), m.p. 87–88°, $[\alpha]_D^{20}$ −38° (*c* 0·45) (Found: C, 68·25; H, 9·45. $C_{12}H_{20}O_3$ requires: C, 67·9; H, 9·5%) $\nu_{max}$ ($CHBr_3$) 3600 and 1762 $cm^{-1}$, $\tau$ 8·97 (3H, d, *J* 6 Hz, CHMe), 8·74 (3H, s, Me), 8·62 (3H, s, Me) and 7·4 (2H, m, $CH_2.CH_2.CO$), *m/e* 212 (4%), 197 (5), 194 (10), 183 (4), 179 (9), 154 (45), 99 (100) and 95 (30).

A sample of the lactone which was 2·5 years old had changed into a polymorph, m.p. 190–193°, which was identical [IR ($CHBr_3$) and mass spectra] with a new sample, m.p. 82–83°. Nozoe *et al.* report[2] m.p. 66–68°.

*Oxidation of cyclonerodiol with osmium tetroxide-sodium periodate*

$NaIO_4$ (280 mg) was added in portions during 1 hr to a soln of cyclonerodiol (140 mg) in THF (12 ml) and water (12 ml) at 0°. The soln was left overnight at room temp and then the organic solvent was distilled off *in vacuo* and collected in a trap cooled in liquid $N_2$. Semicarbazide hydrochloride (120 mg), AcONa (90 mg), and water (0·5 ml) were added to the distillate. The mixture was allowed to stand for 10 min and then most of the THF was removed *in vacuo* and the product (15 mg) was collected by filtration. It crystallised from EtOH in prisms, m.p. 183–184°. (Found: *m/e* 115. Calc. for $C_4H_9N_3O$: *M*, 115) identical (IR spectrum) with authentic acetone semicarbazone.

Extraction of the aqueous residue from the oxidation with EtOAc followed by evaporation gave a gum (75 mg), $\nu_{max}$ 1760 and 1722 $cm^{-1}$, which showed 3 spots on TLC, two of which corresponded in $R_F$ to the lactone (**6**) and the hemiacetal (**7**).

*Ozonolysis of cyclonerodiol*

The diol (517 mg) in EtOAc (60 ml) was treated with excess of ozone at −70°. The solvent was removed *in vacuo* at 0° and the residue in acetone (100 ml) was oxidised with the 8N $CrO_3$ reagent[8] (2·0 ml) at room temp for 15 min. MeOH was added, the soln was evaporated *in vacuo*, water was added to the residue, and the product was recovered in EtOAc. Chromatography on Kieselgel G in EtOH–benzene (1:9) gave the lactone (**6**) (246 mg) which crystallised from ether-light petroleum (b.p. 40–60°) in needles, m.p. 82–83°, identical (IR and mass spectra) with the sample prepared above.

*Oxidation of cyclonerodiol bis-dinitrobenzoate* (**5**)

$NaIO_4$ (400 mg) was added in portions during 1 hr to an ice-cold soln of the dinitrobenzoate (145 mg) and $OsO_4$ (10 mg) in THF (12 ml) and water (6 ml). The soln was left to stand overnight and then concentrated *in vacuo*. Recovery in EtOAc followed by elution from a column of silica gel (14 g) with EtOAc-light petroleum (15:85) gave a 3,5-*dinitrobenzoate* which crystallised from EtOAc-light petroleum with m.p. 144–146° (dec) (Found: C, 58·05; H, 6·0; N, 7·2. $C_{19}H_{22}N_2O_7$ requires: C, 58·45; H, 5·7; N, 7·2%) $\nu_{max}$ 2720 and 1720 $cm^{-1}$, $\tau$ 8·76 (s, Me), 8·75 (d, *J* 6·5 Hz, CHMe), 8·34 (s), 8·26 (s), 0·9 (3H, aromatic protons) and 0·14 (1H, t, *J* 1Hz. $CH_2$. $C\underline{H}O$).

*Dehydration of the lactone* (**6**)

The lactone (190 mg) in pyridine (10 ml) was treated with thionyl chloride (3 ml) at 0° for 1 hr. The mixture was poured into dil HCl at 0° and the product was recovered in EtOAc. Elution from a column of silica gel (5 g) with EtOAc-light petroleum (1:9) gave the *lactone* (**14**) as a gum (130 mg) (Found: *m/e* 194·13150. $C_{12}H_{18}O_2$ requires: *M*, 194·13067) $\nu_{max}$ (film) 1777 $cm^{-1}$, $\tau$ 8·70 (3H, s, Me), 8·39 (6H, s, 2 C=C.Me), 7·5 (2H, m, $CH_2.C\underline{H}_2.CO$), 7·08 (1H, br, =C(Me).C$\underline{H}$.C.O) and no vinylic protons.

*Hydrogenation of the lactone* (**14**)

The lactone (60 mg) in AcOH (10 ml) was shaken in $H_2$ with Adams catalyst (110 mg) until uptake ceased. After recovery the partially hydrogenated product was again reduced in AcOH (10 ml) with Adams catalyst

(120 mg) until no more $H_2$ was absorbed. (Total uptake 6·5 ml of $H_2$; 0·97 mol). Recovery gave the dihydrolactone (**8**) as an oil, $\nu_{max}$ (film) 1774 $cm^{-1}$, $\tau$ 9·05 (6H, overlapping d, $J \sim 6Hz$, 2 CHMe), 8·62 (3H, s, Me), 7·4 (2H, m, $CH_2.C\underline{H}_2.CO$).

*Reduction of the lactone* (**8**) *with lithium aluminium hydride*

The lactone (70 mg) in ether (25 ml) was refluxed with excess of LAH for 1·5 hr. EtOAc (2 ml) and sodium potassium tartrate (20%; 20 ml) were added and the *diol* (**15**) was recovered in ether as an oil (70 ml) (Found: *m/e* 185·15518. $C_{12}H_{24}O_2$ requires *M*-15, 185·15415) $\nu_{max}$ 3400 $cm^{-1}$, $\tau$ 9·1 (6H, overlapping d, 2 CHMe), 8·83 (3H, s, Me), 7·67 (2H, s, 2 OH) and 6·35 (2H, t, *J* 5·5 Hz, $CH_2.C\underline{H}_2.OH$), *m/e* 185 (4%), 182 (1), 167 (3), 149 (3), 141 (40), 123 (40), 103 (45), 97 (30), 95 (25) and 85 (100).

*Dehydration of the diol* (**15**)

The diol (60 mg) in pyridine (5 ml) was treated with thionyl chloride (1 ml) at 0° for 10 min. The soln was poured into iced dil HCl and the *tetrahydrofuran* (**13**) was recovered in ether as an oil (50 mg) which distilled at 70° (bath)/$10^{-1}$ mm (Found: C, 79·05; H, 12·2. $C_{12}H_{22}O$ requires: C, 79·1; H, 12·2%) $\nu_{max}$ No OH band, $\tau$ 9·1 (6H, m, 2 CHMe), 8·73 (s, Me) and 6·22 (2H, m, $CH_2.O$), *m/e* 182 (2%), 167 (6), 149 (4), 141 (9), 123 (15), 97 (20), 95 (50) and 85 (100).

*Oxidation of the tetraol* (**3**) *with periodate*

The tetraol (100 mg) in MeOH (10 ml) was treated with $NaIO_4$ (106 mg) in water (5 ml) for 3 days. The MeOH was removed *in vacuo* and the product was recovered in EtOAc and chromatographed on silica gel (5 g). Elution with EtOAc-light petroleum (1:4) gave an oil (41 mg) which on GLC [5% Carbowax 20M on 80/90 mesh Anakrom at 148° (argon 46 ml/min)] showed two peaks believed to be due to the epimeric *acetals* (**9** and **10**), at retention times of 9·5 and 11·0 min in the relative intensity of 1:3 respectively (Found: *m/e* 227·16473. $C_{13}H_{24}O_3$ requires: *M*-1, 227·16471) $\nu_{max}$ (film) 3500 $cm^{-1}$, $\tau$ 6·66 (3H, s, OMe), 5·02 (1H, m, O.CH.O), *m/e* 227 (1%), 213 (2), 210 (0·5), 209 (0·5), 197 (3), 196 (4), 179 (4), 161 (4), 129 (32), 115 (100) and 83 (23).

Elution of the column with EtOAc-light petroleum (2:3) gave the *hemiacetal* (**7**) as an oil (44 mg) (Found: 199·13331. $C_{12}H_{22}O_3$ requires: *M*-15, 199·13341) $\nu_{max}$ 3450 $cm^{-1}$, $\tau$ 4·55 (m, O.CH.O), *m/e* 199 (3%), 196 (10), 181 (10), 179 (5), 178 (5), 139 (22), 129 (10) and 101 (100).

The acetal from another oxidation showed two peaks on GLC corresponding to equal proportions of the two epimers.

Treatment of the acetal (40 mg) in THF (10 ml) and 0·1 N $H_2SO_4$ (5 ml) for 7 days at room temp gave an oil which showed several spots on TLC. Its NMR spectrum contained no OMe signal.

The hemi-acetal (40 mg) was allowed to stand in EtOH (0·5 ml) for 7 days. Removal of the solvent *in vacuo*, followed by distillation of the residue under reduced pressure, gave an oil which on GLC showed two peaks believed to be due to the epimeric *ethyl acetals* (**11** and **12**) at retention times of 9·7 and 10·2 mins [5% Carbowax 20 M on 80/90 mesh Anakrom at 135° (argon 47 ml/min)] (Found: C, 69·7; H, 10·6. $C_{14}H_{26}O_3$ requires: C, 69·4; H, 10·8%) $\nu_{max}$ (film) 3520 $cm^{-1}$. $\tau$ 8·83 (t, *J* 7 Hz, $O.CH_2.C\underline{H}_3$), 8·73 (s, methyls), 6·45 (2H, m, $O.C\underline{H}_2.CH_3$) and 4·95 (1H, m, O.CH.O), *m/e* 227 (3%), 224 (0·5), 209 (2), 197 (3), 196 (3), 179 (7), 161 (5), 129 (100) and 101 (20).

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