31. Revised Structure of the Fungal Germination Self-Inhibitor Gloeosporone

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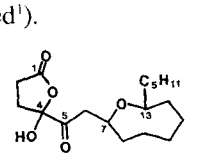
(5.XI.86)

Gloeosporone, the germination self-inhibitor from the fungus *Colletotrichum gloeosporioides* f. sp. *jussiaea*, is shown by spectroscopic data and X-ray analysis to have the constitution and relative configuration as shown in *Formula* **2** (either (1*S*,6*R*,12*R*)-1-hydroxy-6-pentyl-5,15-dioxabicyclo[10.2.1]pentadecan-4,13-dione or its enantiomer), rather than the previously assigned constitution **1**.

Introduction. – Spores of the fungus *Colletotrichum gloeosporioides* (PENZ.) SACC. f. sp. *jussiaea* germinate readily when dispersed, but poorly when crowded. A metabolite which inhibits germination was isolated from the spores by *Lax et al.* [1], and was given the trivial name gloeosporone. In 1983, *Meyer et al.* [2] proposed the constitution **1** for this substance, based on IR, NMR, and MS evidence, with the relative and absolute configurations undefined[1]).



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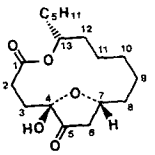

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In the course of subsequent research directed toward total synthesis of gloeosporone [3] [4][2]), information inimical to constitution **1** began to accumulate. Consequently, a collaborative program was undertaken to obtain additional evidence regarding the structure of this intriguing natural product. Results of this research, including X-ray crystal structure analysis led to the correct constitution and relative configuration as given by **2**, with only the absolute configuration still undetermined.

Derivation of Constitution 1. – The salient spectral features which led to constitution **1** were as follows [2]. MS indicated a molecular formula of $C_{18}H_{30}O_5$. IR and ^{13}C-NMR

[1]) Formulae **1**–**6** are numbered and discussed as derivatives of octadecanoic acid for clarity in comparison, even though this is not always in accord with correct IUPAC nomenclature.

[2]) We are grateful to Prof. *A. B. Holmes* [4] for informing us about his research on synthesis of gloeosporone and for permission to cite some of his results.

showed the presence of two C=O groups, one a ketone and the other an ester group. There is no additional unsaturation (^{13}C-NMR), so the compound is bicyclic. The remaining O-atoms made up one acetal or hemiacetal (^{13}C-NMR), two secondary CH units bonded to an ether or ester O-atom (CH–O; ^{1}H- and ^{13}C-NMR), and one tertiary OH group (3.65 ppm, converted by (D_6)DMSO to a sharp *s* at 5.37 ppm). The OH must be part of the hemiacetal system, because the only other oxygenated C-sites would make it a secondary rather than a tertiary alcohol.

The 13 non-oxygenated C-atoms comprised 1 CH_3 and 12 CH_2 groups (^{13}C-NMR), so the C-skeleton is unbranched. Among other important MS features (see *Exper. Part*), several series of large hydrocarbon ions culminating in $C_{13}H_{24}^+$ required the presence of a C_{13} chain which does not contain C=O or hemiacetal C-atoms. Fragmentation to ions such as $C_{13}H_{19}O_5^+$ ($M^+ - C_5H_{11}$) and $C_{13}H_{17}O_4^+$ ($M^+ - C_5H_{11} - H_2O$) but none derived by simple loss of C_4H_9 or C_6H_{13} demonstrated the presence of a terminal pentyl group attached at a point of preferential cleavage, undoubtedly one of the oxygenated C-atoms.

^{1}H-NMR spin coupling (*Table 1*) showed that the C-atoms of both CH–O moieties are attached to two CH_2 groups, *i.e.* $CH_2CH(O-)CH_2$. Three of these CH_2 resonances were concealed in an unresolved envelope at 1.7–1.2 ppm, so further information about them was obscured. However, the fourth CH_2 must be attached to a C=O or the hemiacetal C-atom, since its diastereotopic protons resonate at 2.73 and 2.04 ppm and are coupled only to each other and a vicinal CH–O at 4.43 ppm. There is also an isolated CH_2CH_2 segment which must be terminated at each end by C=O or the hemiacetal, because these protons are downfield of 2 ppm and coupled mutually but not further.

A 2D-H,H-COSY-NMR spectrum confirms these relations. It unequivocally demonstrates that the isolated CH_2CH_2 protons are coupled only to one another, and it gives

Table 1. *^{1}H-NMR Properties of Gloeosporone*[a])

Protons	δ [ppm][a])	Number of H	Multiplicity	J [Hz][a])	Assignment[b])
a	5.06 (4.96)	1	*dddd*	9.1 (7.4); 7.6 (7.3); 5.5 (5.6); 2.8 (3.1)	H–C(13)
b	4.43 (4.44)	1	*dddd*	J(b,i) = 8.3 (7.8); J(b,d) = 6.2 (6.6); 9.6 (9.2); 1.7 (1.9)	H–C(7)
c[c])	3.65 (5.37)	1	*s*		OH
d	2.73 (2.75)	1	*dd*	J(d,i) = −18.7 (−18.8); J(b,d) = 6.3 (6.6)	H^{Re}–C(6)
e	2.44 (2.35)	1	*ddd*	J(e,h) = 8.5 (8.7); J(e,f) = 4.1 (2.5); J(e,g) = −15.4 (−16.0)	H^{Re}–C(2)
f	2.35 (2.39)	1	*ddd*	J(f,g) = 9.1 (10.4); J(e,f) = 4.1 (2.5); J(f,h) = −14.6 (−14.6)	H^{Si}–C(3)
g	2.28 (2.07)	1	*ddd*	J(f,g) = 9.1 (10.4); J(g,h) = 3.8 (2.3); J(e,g) = −15.4 (−16.0)	H^{Si}–C(2)
h	2.10 (2.12)	1	*ddd*	J(e,h) = 8.5 (8.7); J(g,h) = 3.8 (2.3); J(f,h) = −14.6 (−14.6)	H^{Re}–C(3)
i	2.04 (1.99)	1	*dd*	J(d,i) = −18.7 (−18.8); J(b,i) = 8.2 (7.8)	H^{Si}–C(6)
j	1.7–1.2	18	*m*	Unresolved	
k	0.88	3	*t*	7.0	CH_3

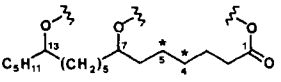
[a]) 500-MHz and 300-MHz spectra in $CDCl_3$; multiplicities from 500-MHz spectra. Values in parentheses are from 300-MHz spectra in $CDCl_3$/(D_6)DMSO *ca.* 8:1. All δ and J are from 2nd-order analysis using LAOCN3 [21].

[b]) Numbering as in Formula **2**. *Re* and *Si* designations refer to the absolute configuration shown in *Formula* **2**; they reverse if gloeosporone is the other enantiomer. Protons d–i are assigned to geminal pairs on the basis of their mutual large J and to individual protons within the pairs on the basis of their NOE interactions with the OH and/or H–C(7).

[c]) δ at 300 MHz: broad in $CDCl_3$, sharp in $CDCl_3$/(D_6)DMSO.

positive evidence for coupling of the CH–O at 5.06 and 4.43 ppm to protons in the 1.7- to 1.2-ppm envelope as well as for coupling of the CH–O at 4.43 to protons at 2.73 and 2.04 which had been demonstrated earlier by spin-decoupling irradiation at 4.43 ppm [2].

Two C=O, one hemiacetal, an isolated CH_2CH_2, and a C_{13} chain devoid of C=O and hemiacetal C-atoms account for all 18 C-atoms. Accordingly, the terminal pentyl group and both $CH_2CH(O-)CH_2$ units must be part of the C_{13} chain. The only arrangement for that chain which places the pentyl group adjacent to an oxygenated C-atom, as required by MS, and attaches one $CH_2CH(O-)CH_2$ to a C=O or the hemiacetal, as required by ^{1}H-NMR, is $C_5H_{11}CH(O-)(CH_2)_5CH(O-)CH_2C^*$ in which C* represents either a C=O or the hemiacetal. Only two combinations of this C_{14} segment and the isolated CH_2CH_2 can incorporate an ester, a ketone, and a hemiacetal. These are the systems **3** and **4**, in which one C* holds the ketonic O-atom and the other carries the OH and one of the disubstituted O-atoms already shown, in the form of a hemiacetal ring (a pseudo acid if this is the ester O-atom). The other two disubstituted O-atoms must be the same atom, part of an ether or lactone ring. This allows 12 constitutions for gloeosporone, six derived from **3** and six from **4**.



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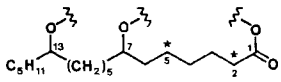

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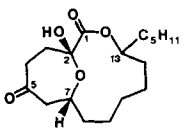
Three of these constitutions are untenable because they contain a 3- or 4-membered cyclic hemiacetal or pseudo acid and would not exist as such a strained tautomer. The IR spectrum of gloeosporone was initially used to select among the others [2]. One C=O absorbs at 1770 cm^{-1} in $CHCl_3$. This is in accord with a γ-lactone (see **1**), and would also be satisfied by a cyclopentanone in which the normal 5-ring $\tilde{\nu}$ (C=O) is shifted higher by the electrostatic effect of two α-hemiacetal O-atoms (see **2**). That two O(α) atoms might shift absorption of an unstrained lactone this far is less likely, but not impossible (see **5** and **6**). However, no other constitution derivable from **3** or **4** is in even remote agreement with a 1770 cm^{-1} absorption.



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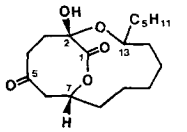

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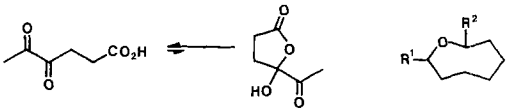
Absorption of the other C=O was observed at 1710 cm^{-1}, characteristic of an unstrained ketone, but too low in frequency for an ester. This militated against **2**. No precedent could be found for shift of an unstrained-ester absorption to higher than 1755 cm^{-1} by α-oxygenation, so **5** and **6** were considered unlikely. Only constitution **1** seemed to accommodate all of these data [2].

Revised Formulation as 2. – Even at the time of the original assignment [2], two bits of evidence were disquieting. First, in **1** it would be H–C(13) which resonates at 5.06 ppm. This is too far downfield for the CH–O of a simple cyclic ether and could only be

rationalized by assuming that additional deshielding results from a special juxtaposition of the lactol ring 'vis-à-vis' to that proton. Second, a potential model for the lactol moiety of **1**, 4,5-dioxohexanoic acid (**7**) [5], was synthesized by *Michael* addition of *tert*-butyl acetoacetate to *tert*-butyl acrylate, de-esterification, and treatment of the resulting 2-acetylglutaric acid with excess HNO_2. Absence of IR absorption at 1770 cm^{-1} and ^{13}C-NMR hemiacetal resonance together with the presence of three ^{13}C-NMR C=O signals show that in $CHCl_3$ this 4,5-diketo acid does *not* exist as the tautomeric pseudo acid **8**[3]). Nonetheless, these anomalies could be rationalized in terms of **1**, and structures **2**, **5**, and **6** seemed to have even more serious IR drawbacks.



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Concern over the 5.06 ppm chemical shift of H–C(13) deepened when it was found that CH–O protons of numerous synthetic 2,8-disubstituted oxocanes (H–C(2) and H–C(8) in **9**) do not have chemical shifts nearly that far downfield [3] [4] [6] [7], and a mounting number of examples made it more and more unlikely that differences in the nature of substituents or their *cis*/*trans* arrangement on the oxocane ring would bring about such deshielding in either *cis* or *trans* **1**.

Resonances from the OH proton, the two CH–O protons (5.06 and 4.43 ppm), and the downfield member of the C(=O)CH_2CH(O–) methylene (2.73 ppm) are sufficiently separated from other signals to allow ^{1}H,^{1}H-NOE experiments.

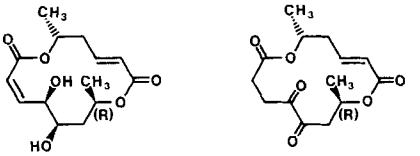
Irradiation of the OH leads to significant enhancement of the signals at 4.43 ppm (CH–O; 2%) and two of the protons of the isolated CH_2CH_2 unit, but no enhancement is observed for the signals of the other two protons of CH_2CH_2 and the resonance at 5.06 ppm (CH–O). Irradiation at 4.43 ppm intensifies resonances in the backbone envelope near 1.7 (6%) and 1.3 (4%) ppm and produces a 6% enhancement of the 2.73-ppm resonance of one CH_2C=O proton, but does not enhance the other one (2.04 ppm) nor the 5.06-ppm resonance (methine). Likewise, irradiation at 5.06 ppm shows no NOE at 4.43 ppm, but only near 1.7 (12%) and 1.3 (6%) ppm. Finally, irradiation at 2.73 ppm enhances the signals at 4.43 (4%) and 2.04 (12%) as well as the OH signal (5%).

The NOE data cast additional doubt on structure **1**, because with free rotation around the bonds between C(4) and C(7) it is unlikely that the OH and H–C(7) (= CH–O at 4.43 ppm) would maintain sufficient proximity to undergo mutual relaxation, and even more improbable that H–C(7) would cross-relax strongly with *one* of the adjacent CH_2C=O protons and not at all with the other. On the other hand, spatial relationships required by the NOE results *are* consistent with structures **2** and **5**, and perhaps also **6**, provided relative configurations are as shown at centers other than C(13). In addition, **2** and **5** are in good accord with the 5.06-ppm resonance of H–C(13), because their O–C(13) is an ester rather than an ether. The major shortcomings of **5** and **6** lie in the high-frequency C=O absorption and MS formation of an abundant $C_4H_7O_4^+$ ion. Barring an extraordinary rearrangement, this would have to contain the two lactone O-atoms and the two hemiacetal O-atoms. Such a fragmentation mechanism can be formulated, but it is not

[3]) Prof. *A. B. Holmes* has also made this observation [4].

probable enough to account for such an intense peak. Furthermore, a 5.06 ppm chemical shift for H–C(13) would be as difficult to rationalize for **6** as it is for **1**.

Structure **2** accommodates all data except the 1710 cm^{-1} IR absorption. This would have to be from the lactone, but with few exceptions [8], 14-ring macrolides and diolides show normal ester frequencies near 1730 cm^{-1} [9]. Structure **2** is also biogenetically attractive. Like **1**, it has the oxygenation pattern of several macrodiolides such as colletodiol (**10**) [10] and grahamimycin A_1 (**11**) [11], and even the same 7-oxy-4,5-diketone



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oxidation level as the latter. Indeed, the only differences between **2** and **1** are in the loci of the C–O–C bonds. Both are progeny of 7,13-dihydroxy-4,5-dioxooctadecanoic acid. In **1**, the lactone O-atom connects to C(4) and O–C(7) to C(13) rather than *vice versa*. Structure **2**, with only the absolute configuration and the relative configuration at C(13) unassigned, therefore, became the strongest contender for a revised formulation of

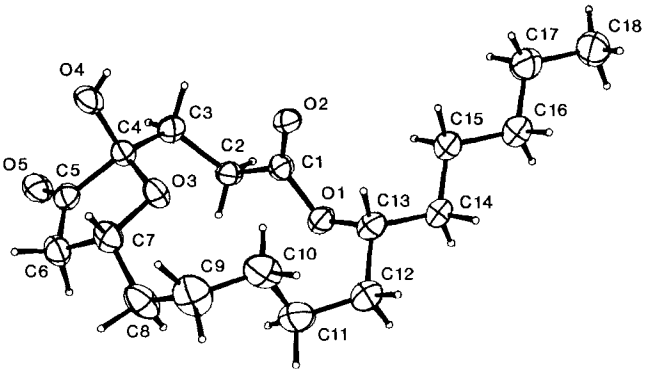

Fig. 1. *ORTEP* [22] *drawing of gloeosporone*. Arbitrary numbering as in *Formula* **2**. Radius of H-atoms arbitrary; other atoms shown as thermal ellipsoids at the 30% probability level.

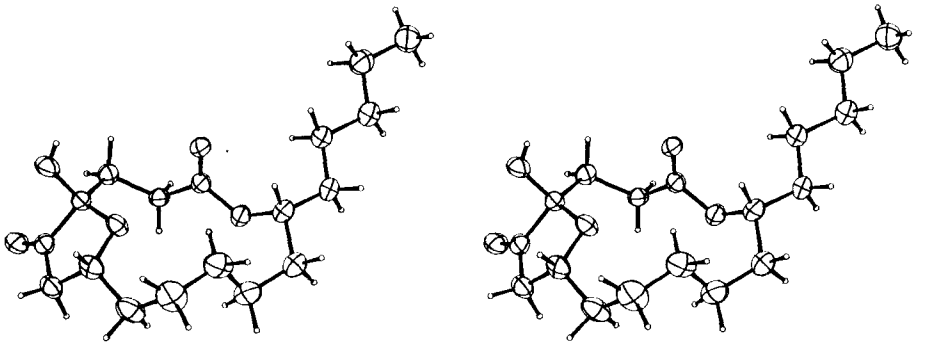

Fig. 2. *Stereoscopic view of gloeosporone*

Table 2. *Fractional Atomic Coordinates and Displacement Parameters for* **2**. E.s.d. are given in parenthesis.

Atom	x	y	z	U_{11}	U_{22}	U_{33}	U_{23}	U_{13}	U_{12}
C(1)	0.1300(5)	0.235(1)	0.2146(3)	0.050(3)	0.058(4)	0.040(3)	0.006(3)	0.004(3)	0.005(3)
C(2)	0.0238(6)	0.029(1)	0.2082(3)	0.048(3)	0.042(3)	0.057(4)	−0.001(3)	0.005(3)	0.005(3)
C(3)	−0.0915(6)	0.052(1)	0.2612(3)	0.042(3)	0.058(4)	0.054(3)	0.001(3)	0.000(3)	−0.003(3)
C(4)	−0.0417(5)	−0.013(1)	0.3419(3)	0.056(3)	0.045(3)	0.050(3)	0.004(3)	0.010(3)	0.002(3)
C(5)	−0.0626(6)	−0.279(1)	0.3640(3)	0.062(3)	0.049(4)	0.052(3)	0.004(3)	0.017(3)	0.002(3)
C(6)	0.0703(7)	−0.348(1)	0.4143(3)	0.082(4)	0.058(4)	0.056(4)	0.012(3)	0.011(3)	0.002(4)
C(7)	0.1626(7)	−0.125(1)	0.4199(3)	0.076(4)	0.070(5)	0.044(4)	0.006(3)	−0.004(3)	0.003(4)
C(8)	0.3219(7)	0.185(2)	0.4159(5)	0.084(5)	0.071(5)	0.087(5)	0.020(5)	0.021(4)	−0.001(4)
C(9)	0.4270(9)	0.021(2)	0.4246(5)	0.081(5)	0.118(7)	0.093(6)	0.014(6)	−0.031(4)	−0.013(6)
C(10)	0.4384(8)	0.179(2)	0.3555(5)	0.066(4)	0.079(6)	0.109(6)	0.004(5)	−0.019(4)	−0.006(4)
C(11)	0.4871(8)	0.044(2)	0.2898(5)	0.067(5)	0.087(6)	0.126(7)	0.013(6)	0.012(5)	0.016(5)
C(12)	0.5064(7)	0.200(2)	0.2222(5)	0.056(4)	0.097(6)	0.115(6)	0.025(6)	0.019(4)	0.009(5)
C(13)	0.3732(6)	0.342(1)	0.1925(4)	0.047(3)	0.071(4)	0.090(5)	0.006(4)	0.017(3)	0.005(4)
C(14)	0.3899(7)	0.458(2)	0.1173(5)	0.063(4)	0.079(5)	0.098(5)	0.021(5)	0.039(4)	0.012(4)
C(15)	0.2648(7)	0.605(1)	0.0854(3)	0.065(4)	0.063(4)	0.074(4)	0.002(4)	0.023(3)	0.003(4)
C(16)	0.2911(8)	0.744(2)	0.0151(4)	0.073(5)	0.080(5)	0.079(5)	0.002(4)	0.024(4)	0.003(4)
C(17)	0.1657(8)	0.894(2)	−0.0170(4)	0.073(5)	0.097(6)	0.082(6)	0.013(5)	0.014(4)	0.014(5)
C(18)	0.192(1)	1.038(2)	−0.0844(6)	0.079(5)	0.113(8)	0.100(6)	0.031(6)	0.005(5)	0.008(6)
O(1)	0.2522(3)	0.1741(0)	0.1848(2)	0.054(2)	0.055(3)	0.075(3)	−0.002(2)	0.021(2)	−0.001(2)
O(2)	0.1092(4)	0.4279(9)	0.2412(2)	0.055(2)	0.048(3)	0.071(3)	−0.003(2)	0.020(2)	0.005(2)
O(3)	0.1086(3)	0.0253(9)	0.3571(2)	0.057(2)	0.053(3)	0.058(2)	0.015(2)	−0.007(2)	−0.005(2)
O(4)	0.1218(6)	0.111(1)	0.3926(3)	0.100(4)	0.041(3)	0.067(3)	0.002(3)	0.022(2)	0.012(3)
O(5)	0.1656(4)	−0.399(1)	0.3444(2)	0.070(2)	0.044(2)	0.094(3)	0.002(2)	0.013(2)	−0.003(2)

Atom	x	y	z	U	Atom	x	y	z	U
H–O(4)	−0.139(6)	0.22(1)	0.382(3)	0.03(2)	H(112)	0.433(7)	−0.09(1)	0.283(4)	0.10(3)
H(021)	0.024(4)	0.03(1)	0.152(3)	0.07(2)	H(121)	0.541(5)	0.12(1)	0.181(3)	0.07(2)
H(022)	0.072(4)	−0.106(8)	0.213(2)	0.02(1)	H(122)	0.588(5)	0.34(1)	0.239(3)	0.08(2)
H(031)	−0.172(4)	−0.042(9)	0.245(2)	0.04(1)	H(13)	0.355(5)	0.48(1)	0.231(3)	0.06(2)
H(032)	0.131(5)	0.21(1)	0.261(3)	0.06(2)	H(141)	0.419(6)	0.36(1)	0.086(3)	0.07(2)
H(061)	0.050(5)	−0.40(1)	0.461(3)	0.07(2)	H(142)	0.481(6)	0.57(1)	0.123(3)	0.11(2)
H(062)	0.120(5)	0.47(1)	0.388(3)	0.07(2)	H(151)	0.171(5)	0.49(1)	0.073(3)	0.08(2)
H(07)	0.157(5)	−0.04(1)	0.466(3)	0.06(2)	H(152)	0.236(5)	0.74(1)	0.122(3)	0.07(2)
H(081)	0.336(7)	−0.28(1)	0.367(3)	0.10(2)	H(161)	0.321(5)	0.65(1)	−0.021(3)	0.07(2)
H(082)	0.341(6)	−0.30(1)	0.461(3)	0.08(2)	H(162)	0.374(6)	0.87(1)	0.029(3)	0.10(2)
H(091)	0.376(9)	0.08(2)	0.470(5)	0.19(5)	H(171)	0.070(6)	0.78(1)	−0.026(3)	0.11(2)
H(092)	0.528(6)	−0.03(1)	0.438(3)	0.09(2)	H(172)	0.146(6)	0.99(1)	0.023(3)	0.08(2)
H(101)	0.531(6)	0.31(1)	0.371(3)	0.09(2)	H(181)	0.101(7)	1.10(1)	−0.101(3)	0.11(3)
H(102)	0.353(7)	0.26(1)	0.340(3)	0.10(3)	H(182)	0.235(9)	0.93(2)	0.116(4)	0.17(5)
H(111)	0.579(6)	−0.05(1)	0.307(4)	0.12(3)	H(183)	0.265(9)	1.14(2)	−0.071(4)	0.14(4)

gloeosporone when two independent syntheses of *cis*-**1** [3] [4] and one of *trans*-**1** [4] unequivocally excluded that constitution.

Single-crystal X-ray analysis of gloeosporone confirms that the structure is **2**, with the relative configurations of C(4) and C(7) *u* and those of C(7) and C(13) *l* (see *Figs. 1–2* and *Table 2*). The ester unit is essentially normal in geometry, with the (*Z*)-configuration, the usual bond lengths and angles, and deviating from coplanarity by only 6° in torsion at the C(1)–O(1) bond. Packing in the crystal *(Fig. 3)* utilizes H-bonds from the OH of one molecule to the ketonic C=O of its neighbor (O(4)···O(5′) distance 2.864 Å; O(4)–H–O(4)···O(5′) angle 171.1°). This focuses the hydrophilic ends of the relatively

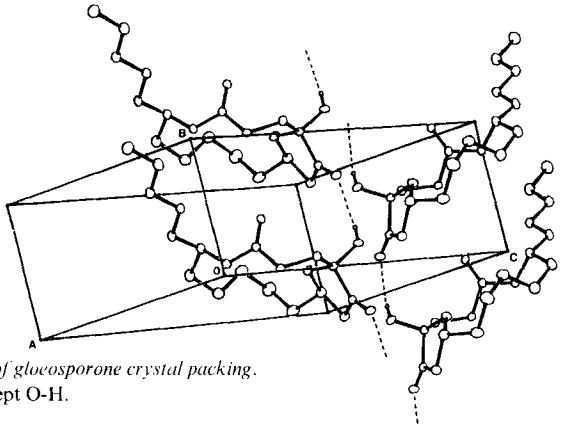

Fig. 3. *ORTEP* [22] *drawing of gloeosporone crystal packing.* All H-atoms are omitted except O-H.

flat macrocyclic rings toward the center of the helical packing axis, with the lipophilic ends oriented outward to contact corresponding ends of neighbor molecules. The crystal conformation orients O-atoms of the lactone C=O, the OH, and the hemiacetal in relatively close proximity on one face of the macrocycle (O(2)···O(4) = 4.037 Å; O(2)···O(3) = 3.054 Å; O(3)···O(4) = 2.343 Å). This raises the intriguing possibility that these sites might complex with certain metal ions and that the transport of such ions might be involved in the inhibition of germination which gloeosporone induces, as has been proposed for the activity of some other fungal germination self-inhibitors [12].

It was IR absorption at 1710 cm^{-1}, interpreted as a ketone rather than an ester, which led to initial selection of constitution **1** [2]. The X-ray structure shows no distortion of the ester which could engender abnormal absorption. A higher-resolution spectrum reveals that in CCl_4, the ester band is in fact composed of two peaks, one at 1731 cm^{-1} and a second of greater intensity at 1712 cm^{-1}, with the ketone absorbing at 1772 cm^{-1}. The 1731-cm^{-1} peak was an unresolved and undetected shoulder in the original spectrum. The origin of the misleading 1712-cm^{-1} absorption is clarified by a spectrum in $CHCl_3$/DMSO 4:1. Here, there are only two sharp C=O bands, 1762 and 1723 cm^{-1}, corresponding to normal ketone and ester frequencies shifted by *ca.* 10 cm^{-1} by the change in solvent polarity [13]. In CCl_4 and $CHCl_3$, a substantial fraction of the molecules must have an intramolecular H-bond between OH and the lactone C=O which shifts their ester absorption to 1712 cm^{-1}, while those which are not H-bonded absorb at 1731 cm^{-1}. In the presence of DMSO, the intramolecular H-bonds are replaced by H-bonds to DMSO, so the C=O frequency no longer shows the influence of association.

Several pieces of evidence indicate that the conformation in the crystal is not completely duplicated in $CHCl_3$. Intramolecular H-bonding shown by IR is impossible in the crystal conformation; the C=O···O–H distance is too great (O(2)···O(4) = 4.04 Å). In addition, vicinal H,H coupling constants between CH_2(2) and CH_2(3) (*Table 1*) are not in reasonable accord with those predicted by the *Karplus* relationship [14] for the crystal dihedral angle (C(1)–C(2)–C(3)–C(4) = –76.9°). Finally, in the conformation of the crystal, it would be the two H–C(3) which show an NOE upon irradiation of OH, but the two which are in fact enhanced (2.44 and 2.35 ppm) are a vicinal pair rather than a geminal pair (see *Table 1*). Molecular models of a conformation which brings the OH and lactone C=O close enough for H-bonding also change the C(1)–C(2)–C(3)–C(4) torsion angle to bring one H–C(2) and only one H–C(3) near the OH.

It thus appears that in the absence of a favorable intermolecular H-bond acceptor, two ring conformers are about equally populated. One of them resembles that in the solid state but without H-bonds, while the other has quite different C(1)–C(2)–C(3)–C(4) torsional geometry and is intramolecularly H-bonded. When intermolecular H-bonding is possible, as in the crystal or in the presence of DMSO, the former conformer dominates.

^{1}H-NMR spectra in $CDCl_3$/(D_6)DMSO support this conclusion (see *Table 1*). The most obvious result of replacing intramolecular H-bonds by H-bonds to DMSO is a large downfield shift of the OH resonance. However, the CH_2(2),CH_2(3) vicinal couplings also change from their values in $CDCl_3$ to a set of values which are in reasonable accord with *Karplus* predictions [14] for the crystal geometry. Furthermore, while chemical shifts of three of those protons undergo only small changes, the fourth shifts dramatically upfield (2.28 to 2.07 ppm). This is attributed to H–C(2) which is directly below the ketone C=O in the crystal. A conformation which allows intramolecular H-bonding moves this proton well away from that shielded area, so its relative chemical shifts are in excellent accord with a crystal-like conformation in the presence of DMSO, but a mixture of that form and a different one in its absence.

The areas of C(7) and C(13) are also open to conformational scrutiny by ^{1}H-NMR. Chemical shifts and coupling constants of CH_2(6) and H–C(7) differ very little between $CDCl_3$ and $CDCl_3$/DMSO solutions, and all of these J fit the *Karplus* expectation for the crystal structure. Apparently, this part of the molecule is similar in both forms. The situation at C(13) is less clear because couplings can only be observed in the resonance of H–C(13), and our data do not indicate which are to CH_2(12) and which to CH_2(14). The best we can say is that three of the four J are about the same in both solvents and the fourth is significantly different (9.1 *vs.* 7.3 or 7.4 Hz). Neither set fit *Karplus* predictions for the crystal structure very well. In solution, this part of the molecule is probably a mobile mixture of two or more forms which differ in torsional arrangement around C(12)–C(13) or C(13)–C(14) or both.

We presently have no evidence to assign the absolute configuration of gloeosporone. However, on two independent biogenetic counts, the (4*S*,7*R*,13*R*) configuration shown in **2** is more probable than the one of its enantiomer. First, all 14-ring macrolides for which absolute configurations have been determined have the 13*R* configuration (the *Celmer* stereochemical model [15]). Second, all known 14- to 18-ring macrolides and macrodiolides which have the 4,7 or 4,5,7 oxygenation pattern of gloeosporone (colletodiol [10], grahamimycin A_1 [11], colletoketol (grahamimycin A) [10] [16], grahamimycin B [16], pyrenophorin [16], vermiculin [16], albocycline [17]) have the C(7) configuration corresponding to *R* in this system[4]). It would be remarkable, if gloeosporone proved to be the first exception to both of these striking regularities of nature. Confirmation of this prediction must await comparison of the levorotatory natural product with a synthetic sample of known configuration.

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[4]) All are 7*R* except albocycline and vermiculin, but they are 7*S* only because other substituents change the CIP priorities of the carboxyl and methyl ends of their hydroxy-acid chains.

Experimental Part

General. Optical rotation: *Perkin-Elmer-241* polarimeter (ETH). IR: *Perkin-Elmer 283*. FT-IR: *Nicolet 5SX* (Yale). ^{1}H-NMR: 500 MHz, *Bruker HX-500* (Yale Chemical Instrumentation Center and National Center for Toxicological Research, Jefferson, AR); 300 MHz, *Bruker WM-300* (ETH); 250 MHz, *Bruker WM-250* (Yale); 90 MHz, *JEOL FX90Q* (Arkansas); chemical shifts in ppm rel. to internal $SiMe_4$ (= 0 ppm), with coupling constants *J* in Hz. The COSY experiment was performed at 500 MHz (Yale) using the *Bruker* program COSY. ^{1}H,^{1}H-NOE experiments were performed at 250 and 500 MHz (Yale) with the *Bruker* program 12.5 (NOE difference, direct A–B FID accumulation) and used the following parameters: relaxation delay 10 s, NOE generation 5 s (approx. 2–3 T_1), repetitive cycling with accumulation of 8 scans on and off resonance and 2 dummy scans to assure saturation. Homogated irradiation was used to eliminate spurious noise, with irradiating power of < 0.5 W and accumulation of 128 to 640 scans. Exponential multiplication of 0.3 to 2 Hz was used for data processing. NOE enhancements are reported in % relative to the irradiation signal. NOE and COSY experiments were done in '100 atom-% D' $CDCl_3$ *(Aldrich)* which was passed through basic Al_2O_3 before use; samples were degassed by a minimum of 3 freeze/thaw cycles under high vacuum and flushed with N_2. ^{13}C-NMR: 22.5 MHz, *JEOL FX90Q* (Arkansas); 20 MHz, *Varian CFT-20* (ETH); multiplicities from ^{1}H single-frequency off-resonance decoupled spectra. MS: high-resolution, *Kratos MS80* (Midwest Center for Mass Spectrometry, Lincoln, NE); low resolution, *Perkin-Elmer Hitachi RMU-6M* (ETH); in *m/z* (high-resolution intensity, low-resolution intensity, composition).

Gloeosporone (= (1S,6R,12R)-1-Hydroxy-6-pentyl-5,15-dioxabicyclo[10.2.1]pentadecan-4,13-dione or its Enantiomer; **2**). Isolation and purification as described in [1]. M.p. 108–110°. FT-IR (CCl_4): 3578*w*, 3450 (br.), 1772*s*, 1731*m*, 1712*s*. FT-IR ($CHCl_3$/DMSO 4:1): 1762*s*, 1723*s*. ^{1}H-NMR: *Table 1*. ^{13}C-NMR ($CDCl_3$): 209.0 (*s*); 174.4 (*s*); 99.0 (*s*); 74.4 (*d*); 73.5 (*d*); 40.4 (*t*); 34.6 (*t*); 32.3 (*t*); 32.1 (*t*); 31.7 (*t*); 30.0 (*t*); 29.5 (*t*); 26.0 (*t*); 25.3 (*t*); 24.9 (*t*); 22.5 (*t*); 21.2 (*t*); 14.0 (*q*). MS: 326 (0, 1, $C_{18}H_{30}O_5$), 308.1989 (2, 23, $C_{18}H_{28}O_4$), 264.2091 (1, 12, $C_{17}H_{28}O_2$), 255.1225 (3, 11, $C_{13}H_{19}O_5$), 237.1122 (3, 8, $C_{13}H_{17}O_4$), 209.1176 (6, 10, $C_{12}H_{17}O_3$), 183.1745 (6, 8, $C_{12}H_{23}O$), 180.1873 (10, 29, $C_{13}H_{24}$), 165.1638 (8, 10, $C_{12}H_{21}$), 152.1561 (11, 22, $C_{11}H_{20}$), 138.1405 (9, 17, $C_{10}H_{18}$), 127.0392 (35, 11, $C_6H_7O_3$), 119.0341 (41, 100, $C_4H_7O_4$), 110.1084 (29, 24, C_8H_{14}), 109.1014 (60, 31, C_8H_{13}),

Table 3. *Bond Lengths and Angles.* Standard deviations in parentheses.

Bond lengths [Å]					
C(1)–C(2)	1.502(8)	C(5)–C(6)	1.505(8)	C(11)–C(12)	1.52(1)
C(1)–O(1)	1.347(6)	C(5)–O(5)	1.188(6)	C(12)–C(13)	1.517(8)
C(1)–O(2)	1.194(6)	C(6)–C(7)	1.502(9)	C(13)–C(14)	1.521(9)
C(2)–C(3)	1.510(7)	C(7)–C(8)	1.525(9)	C(13)–O(1)	1.455(7)
C(3)–C(4)	1.522(7)	C(7)–O(3)	1.451(6)	C(14)–C(15)	1.486(9)
C(4)–C(5)	1.544(7)	C(8)–C(9)	1.50(1)	C(15)–C(16)	1.521(8)
C(4)–O(3)	1.412(5)	C(9)–C(10)	1.53(1)	C(16)–C(17)	1.499(9)
C(4)–O(4)	1.413(7)	C(10)–C(11)	1.51(1)	C(17)–C(18)	1.49(1)
Bond angles [°]					
C(2)–C(1)–O(1)	110.7(5)		C(8)–C(7)–O(3)	110.4(5)	
C(2)–C(1)–O(2)	125.2(5)		C(6)–C(7)–O(3)	105.5(5)	
O(1)–C(1)–O(2)	124.1(5)		C(7)–C(8)–C(9)	117.0(7)	
C(1)–C(2)–C(3)	113.1(5)		C(8)–C(9)–C(10)	116.5(6)	
C(2)–C(3)–C(4)	114.7(4)		C(9)–C(10)–C(11)	114.0(8)	
C(3)–C(4)–C(5)	115.9(5)		C(10)–C(11)–C(12)	114.7(8)	
C(3)–C(4)–O(3)	110.5(4)		C(11)–C(12)–C(13)	114.8(6)	
C(3)–C(4)–O(4)	112.0(4)		C(12)–C(13)–C(14)	112.3(5)	
C(5)–C(4)–O(3)	103.9(4)		C(14)–C(13)–O(1)	109.4(6)	
O(3)–C(4)–O(4)	112.1(5)		C(12)–C(13)–O(1)	107.3(5)	
C(5)–C(4)–O(4)	102.0(4)		C(13)–C(14)–C(15)	115.4(5)	
C(4)–C(5)–C(6)	106.2(5)		C(14)–C(15)–C(16)	114.4(5)	
C(6)–C(5)–O(5)	128.7(6)		C(15)–C(16)–C(17)	114.7(6)	
C(4)–C(5)–O(5)	125.0(5)		C(16)–C(17)–C(18)	115.1(6)	
C(5)–C(6)–C(7)	105.2(5)		C(1)–O(1)–C(13)	118.4(5)	
C(6)–C(7)–C(8)	111.6(6)		C(4)–O(3)–C(7)	108.9(4)	

101.0233 (100, 93, $C_4H_5O_3$), 97.1011 (21, 31, C_7H_{13}), 96.0936 (36, 44, C_7H_{12}), 95.0861 (37, 36, C_7H_{11}), 83.0857 (31, 38, C_6H_{11}), 82.0777 (35, 39, C_6H_{10}), 81.0701 (40, 38, C_6H_9), 73.0291 (13, 20, $C_3H_5O_2$), 69.0703 (37, 40, C_5H_9), 68.0625 (26, 26, C_5H_8), 67.0551 (36, 34, C_5H_7), 55.0563 (58, 69, C_4H_7), 55.0200 (24, 69, C_3H_3O). $[\alpha]_{589}^{r.t.} = -14°$, $[\alpha]_{578}^{r.t.} = -15°$, $[\alpha]_{546}^{r.t.} = -18°$, $[\alpha]_{436}^{r.t.} = -45°$, $[\alpha]_{365}^{r.t.} = -127°$ ($c = 0.28$, $CHCl_3$).

*Di(*tert*-Butyl) 2-Acetylglutarate.* A procedure for the synthesis of the diethyl ester was modified [18]. A mixture of 7.9 g (50 mmol) of *tert*-butyl acetoacetate, 6.4 g (50 mmol) of *tert*-butyl acrylate, and 350 mg of KO(*t*-Bu) was heated at 120° for 18 h, diluted with 100 ml of Et_2O, and washed with 10% HOAc soln., H_2O, and 10% $NaHCO_3$ soln. Removal of solvent and bulb-to-bulb distillation (170°/0.05 Torr) gave a yellowish distillate which was flash chromatographed (pentane/Et_2O 4:1) to afford 7.7 g (66%) of a colorless oil suitable for use. ^{1}H-NMR (90 MHz, $CDCl_3$): 3.42 (*t*, $J = 7$, 1 H); 2.21 (*s*, 3 H); 2.2–1.9 (*m*, 4 H); 1.48 (*s*, 9 H); 1.44 (*s*, 9 H).

4,5-Dioxohexanoic Acid (**7**). A 2.0-g (7.0-mmol) sample of the foregoing product was stirred at 0° with 10 ml of CF_3COOH for 50 min, diluted with 3 ml of H_2O, and treated slowly at 0° with 568 mg (8.2 mmol) of $NaNO_2$ in 2 ml of H_2O. Strong gas evolution ceased after 30 min, and the mixture was stirred at r.t. for 2 h, cooled to 0°, treated with 487 mg (7.1 mmol) of $NaNO_2$ in 2 ml of H_2O, and stirred at 0° for 2 h and r.t. for 2 d. Solvent was removed and the residue was taken up in EtOAc, filtered, and evaporated to leave a yellow oil which was flash chromatographed (Et_2O/pentane/HOAc 3:6.5:0.5) to afford 200 mg of 5-oxohexanoic acid and 114 mg (11%) of **7** as yellow crystals which were recrystallized from CCl_4. M.p. 76–78° ([5]: 75°). IR ($CHCl_3$): 3500*w*, 3300–2500*m*, 1710*s*. ^{1}H-NMR (90 MHz, $CDCl_3$): 8.72 (br. *s*, 1 H); 3.10–2.50 (*m*, 4 H); 2.36 (*s*, 3 H). ^{13}C-NMR (22.5 MHz, $CDCl_3$): 197.0, 196.8, 178.1, 30.6, 27.5, 23.6.

X-Ray Structure Analysis of (**2**). $C_{18}H_{30}O_5$, monoclinic space group $P2_1$, $Z = 2$; $a = 9.289(3)$, $b = 5.535(2)$, $c = 18.013(3)$ Å; $\alpha = \gamma = 90°$, $\beta = 95.77(2)°$. X-Ray measurements were made with an *Enraf-Nonius-CAD4* diffractometer equipped with graphite monochromator (MoK_α radiation, $\lambda = 0.71069$ Å) at 25°. The structure was solved by direct methods with SHELX86 [19]. Of the 2005 unique reflections, 965 with $I > 3\sigma(I)$ were used for the refinement with SHELX76 [20]. At an intermediate stage, H-atoms were included in the full-matrix-least-squares analysis and refined isotropically (other atoms anisotropically). Refinement converged at $R = 0.03$ using unit weights. Atomic coordinates are shown in *Table 2* and bond distances and angles in *Table 3*. Data have been deposited at the *Cambridge Crystallographic Data Centre*.

W. L. M. is grateful to Prof. *J. F. Hinton* (Arkansas) and Drs. *R. Hässig* and *C. Schregenberger* (ETH) for ^{13}C-NMR spectra, Dr. *Fred Evans* (National Center for Toxological Research, Jefferson, AR) for 500-MHz, and Ms. *B. Brandenberg* and Mr. *F. Fehr* (ETH) for 300-MHz ^{1}H-NMR spectra, Prof. *J. Seibl* (ETH) for the low-resolution-MS data, and Prof. *A. W. Cordes* and Mr. *S. Craig* (Arkansas) for education in use of ORTEP. Financial support for *S. L. S.* from a *National Science Foundation Presidential Young Investigator Award* and *Pfizer, Inc.*, is gratefully acknowledged. *S. L. S.* and *S. E. K.* are grateful to *Tarek Sammakia* for stimulating discussions and experimental assistance.

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