The Conformation of Some Site-Specific Inhibitors of the Ring Contraction Step in Gibberellin Plant Hormone Biosynthesis

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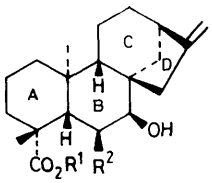
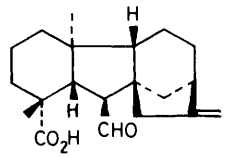
The *X*-ray crystal structures are reported for methyl *ent*-7α-hydroxykaur-16-en-19-oate (**3**) and for two inhibitors of the ring contraction of the corresponding acid (**1**) to gibberellin A_{12} 7-aldehyde (**4**) by *Gibberella fujikuroi*. The superimposition of these structures is described and the possible relationship of this to the steric requirements for the biosynthetic ring contraction are discussed.

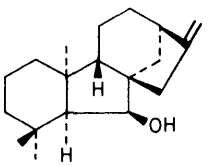
The gibberellin plant growth hormones possess a unique diterpenoid perhydrofluorene carbon skeleton. In studies on the biosynthesis of the gibberellins by the fungus, *Gibberella fujikuroi*, we[1] and others[2] have shown that their carbon skeleton is formed by the unusual oxidative ring contraction of *ent*-7α-hydroxykaur-16-en-19-oic acid (**1**) to gibberellin A_{12} 7-aldehyde (**4**). Using stereospecifically tritiated mevalonates [*pro*-5(*R*) and *pro*-5(*S*)] and *ent*-[6α-^{3},17-^{14}C]-7α-hydroxykaur-16-en-19-oic acid, we have shown[1,3] that the rearrangement involves the removal of the 6β-hydrogen. However, the 6β,7β-diol (**2**) is not an intermediate and consequently an oxidative mechanism, shown in the Scheme, has been proposed for the ring contraction. The enzyme system, which is responsible for the ring contraction in *G. fujikuroi*, is not particularly substrate-specific[4,5] and it will accept a variety of substrate analogues. Consequently it is a suitable target for active site directed inhibitors.



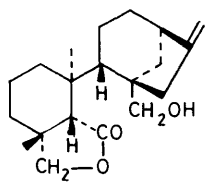
	R^1	R^2
(**1**)	H	H
(**2**)	H	OH
(**3**)	Me	H



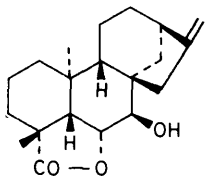
(**4**)



(**5**)

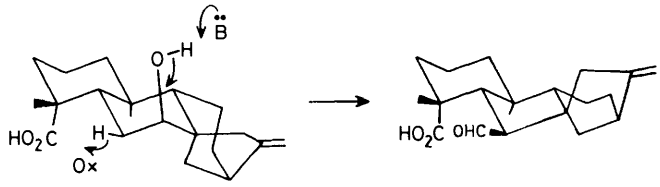


(**6**)



(**7**)

In an effort to develop selective inhibitors of gibberellin plant hormone biosynthesis which might lead to novel plant growth regulators, we have prepared[6,7] a number of compounds which, whilst they resemble the substrate *ent*-7α-hydroxykaur-16-en-19-oic acid (**1**) for this step, lack essential biosynthetic structural features. This has led to a number of gibberellin biosynthesis inhibitors. In this paper, we report the *X*-ray crystal structure of some of these compounds and their comparison with that of the natural substrate. Preliminary studies[8] kindly carried out through the courtesy of ICI Plant Protection Ltd., with data derived from the acid (**5**) and a structure of xylopic acid[9] suggested that overlap of ring D also juxtaposed the 7-hydroxy groups and provided the optimum close fit.



Scheme.

The *X*-ray crystal structure of the natural substrate, *ent*-7α-hydroxykaur-16-en-19-oic acid (**1**), was determined as its methyl ester (**3**) (see Figure 1). The methyl ester gave crystals which, unlike the parent acid, were suitable for *X*-ray measurements. Although molecular models suggest that there are significant interactions between C-19 and C-20 and between C-14 and C-20, the *X*-ray structure revealed that rings A and B remain in the chair conformation with the internal torsion angles lying in the range 52—55° for ring A and 46—60° for ring B. Hence ring B showed only a relatively small deformation compared, for example, to the kaurenolides (*vide infra*). Ring C revealed some flattening, internal torsion angles 36—70°, presumably due to interactions with C-20 and the constraints imposed by the bicyclo[3.2.1]octane system. Ring D adopts an envelope conformation. Similar conclusions were made in the earlier work on xylopic acid.[9] Subsequent to our work an independent structure determination was reported.[10]

We have shown[6] that *ent*-6α-hydroxy-5β(*H*)-7-norgibberell-16-en-19-oic acid (**5**) and the corresponding 6,19-diol are effective inhibitors of gibberellin biosynthesis at the ring contraction stage. The inhibition was dependent on the stereochemistry of the ring B alcohols and it was shown that the 6α-epimers were not inhibitors. We have determined the *X*-ray crystal structure of the alcohol (see Figure 2). A 'best-fit' of the structure with that of the natural substrate (see Figure 3) revealed the similarity in conformation of the ring B hydroxy groups relative to ring D. Not unexpectedly in view of the difference in the A/B ring junction, there was little coincidence in ring A.

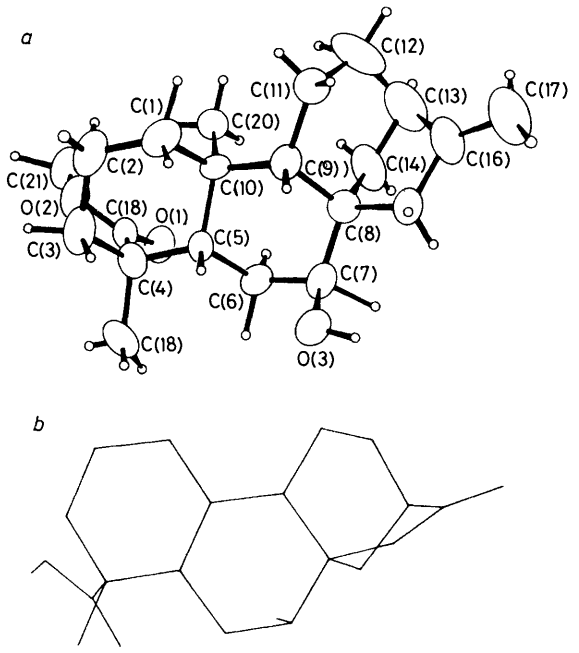


Figure 1. (*a*) Molecular structure of methyl *ent*-7α-hydroxykaur-16-en-19-oate (**3**) and (*b*) line drawing of the ester (**3**) for superimposition

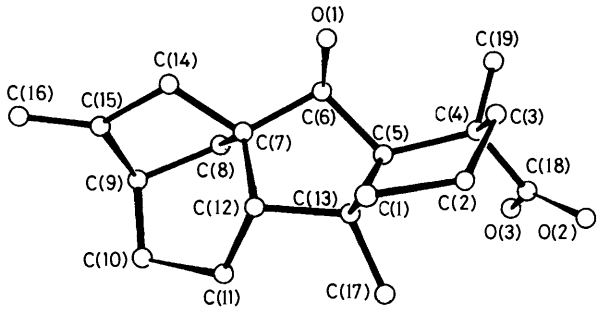


Figure 2. Molecular structure of *ent*-6α-hydroxy-5β(*H*)-7-norgibberell-16-en-19-oic acid (numbering scheme as for crystallographic data)

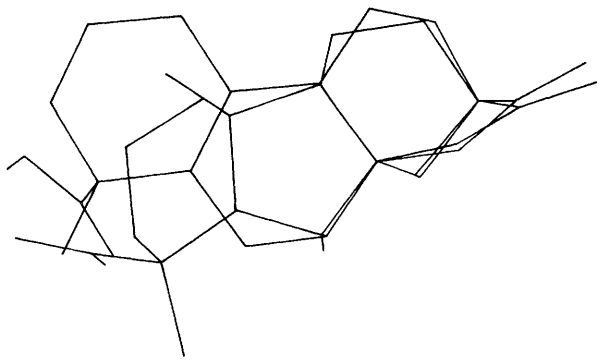


Figure 3. Superimposition of compounds (**3**) and (**5**)

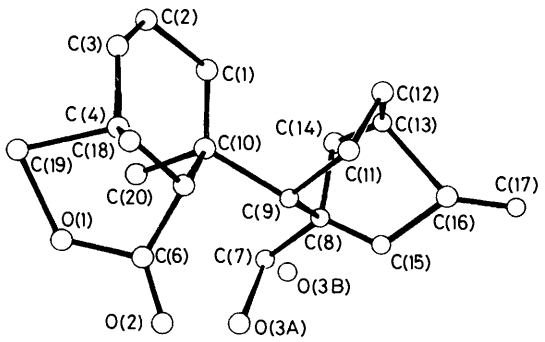


Figure 4. Molecular structure of *ent*-7,19-dihydroxy-6,7-secokaur-16-en-6-oic acid 6,19 lactone

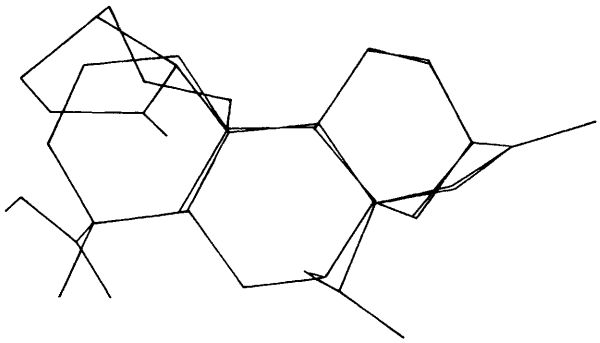


Figure 5. Superimposition of compounds (**3**) and (**6**)

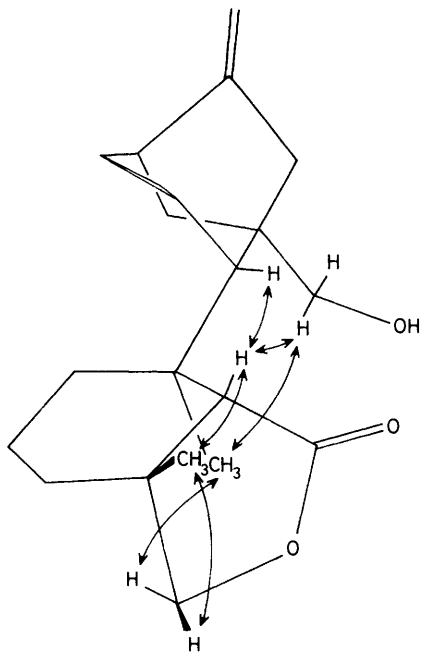


Figure 6. N.O.e. Effects

The 6,7-seco-kaurene (**6**) has also been shown[7] to be an effective inhibitor of this stage in the biosynthesis. Its *X*-ray crystal structure was determined (see Figure 4). It showed some disorder in the crystal and there were two positions for the C-7 oxygen. Nevertheless, one of these again showed a similarity in the relative conformation of the C-7 alcohol and rings C and D with that of the natural substrate (see Figure 5). There is a possibility of free-rotation about the C(9)–C(10) bond. However, a series of ^{1}H n.m.r. nuclear Overhauser enhancement (5—10%) studies, summarized in Figure 6, revealed that this compound retained this conformation in solution. A similar situation has been observed[11,12] with relatives of enmein and fujenal.

Table 1. The O(7)–C(7)–C(8)–C(15) torsion angle and calculated O(7) oxygen–oxygen distances between substrate and substrate analogues

Compound	Torsion angle (°)	O···O Distance (Å)	
(3)	+51		
(5)	+33	0.3	Inhibitor
(6)	+71	0.1	Inhibitor
(7)	−49	3.6	Inactive

Table 2. Fractional atomic co-ordinates ($\times 10^4$) with estimated standard deviations in parentheses for compound (3)

	x	y	z
O(1)	4 714(5)	3 571(3)	3 774(2)
O(2)	6 881(5)	3 787(3)	4 464(2)
O(3)	6 394(5)	−42(3)	2 316(2)
C(1)	10 496(7)	2 226(5)	3 334(3)
C(2)	10 193(7)	2 449(6)	4 024(3)
C(3)	8 610(8)	1 770(5)	4 275(2)
C(4)	6 822(7)	1 976(4)	3 916(2)
C(5)	7 196(6)	1 739(4)	3 224(2)
C(6)	5 535(6)	1 746(5)	2 798(2)
C(7)	5 976(7)	1 160(4)	2 192(2)
C(8)	7 502(6)	1 782(4)	1 853(2)
C(9)	9 164(7)	1 956(4)	2 279(2)
C(10)	8 780(6)	2 453(4)	2 943(2)
C(11)	10 713(7)	2 555(6)	1 925(2)
C(12)	10 175(11)	3 485(7)	1 458(4)
C(13)	8 516(13)	3 176(6)	1 119(3)
C(14)	6 906(9)	2 958(5)	1 561(2)
C(15)	8 119(7)	1 093(5)	1 271(2)
C(16)	8 770(10)	1 979(5)	821(2)
C(17)	9 399(13)	1 788(7)	253(3)
C(18)	5 394(9)	1 114(5)	4 159(3)
C(19)	6 027(6)	3 186(4)	4 030(2)
C(20)	8 409(7)	3 781(4)	2 938(2)
C(21)	6 232(9)	4 968(5)	4 578(3)

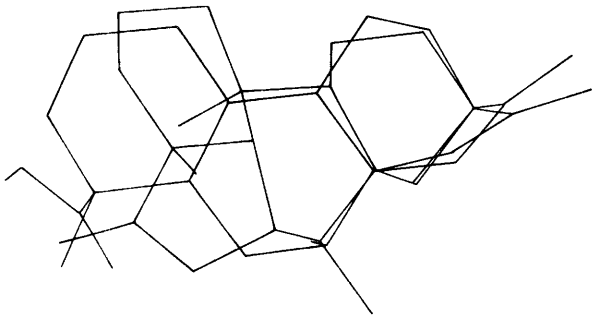


Figure 7. Superimposition of compounds (3) and (7)

Finally the fungus, *Gibberella fujikuroi*, produces a series of kaurenolide lactones [*e.g.* (7)]. Superficially these possess the same 7β-hydroxy group and ring C/D system as the natural substrate for the ring contraction. However, 7β-hydroxykaurenolide (7) has no effect on gibberellin biosynthesis. We have previously shown by ^{1}H n.m.r. studies[13] and by an *X*-ray crystallographic study[14] of the 7-*p*-bromobenzenesulphonate, that ring B adopts a twisted boat conformation. Deletion of the bromobenzenesulphonate and superimposition of this structure with that of methyl *ent*-7α-hydroxykaur-16-en-19-oate (see Figure 7) revealed the effect of the different conformation of ring B which places the 7-hydroxy group in a very different position relative to ring D. A comparison between the natural substrate, the inhibitors, and the inactive kaurenolide is given in Table 1 in terms of the C(15)–C(8)–C(7)–O(7) torsion angle and the O···O distance.

Table 3. Intramolecular distances (Å) and angles (°) with estimated standard deviations in parentheses for compound (3)

(*a*) Bonds

O(1)–C(19)	1.201(6)	O(2)–C(19)	1.323(6)
O(2)–C(21)	1.449(7)	O(3)–C(7)	1.428(6)
C(1)–C(2)	1.534(8)	C(1)–C(10)	1.547(7)
C(2)–C(3)	1.504(8)	C(3)–C(4)	1.551(8)
C(4)–C(5)	1.549(7)	C(4)–C(18)	1.534(8)
C(4)–C(19)	1.517(7)	C(5)–C(6)	1.536(7)
C(5)–C(10)	1.550(6)	C(6)–C(7)	1.508(7)
C(7)–C(8)	1.521(7)	C(8)–C(9)	1.548(7)
C(8)–C(14)	1.544(7)	C(8)–C(15)	1.554(7)
C(9)–C(10)	1.572(7)	C(9)–C(11)	1.538(7)
C(10)–C(20)	1.536(7)	C(11)–C(12)	1.517(10)
C(12)–C(13)	1.472(12)	C(13)–C(14)	1.547(10)
C(13)–C(16)	1.518(9)	C(15)–C(16)	1.484(8)
C(16)–C(17)	1.334(9)		
O(3)–H(03)	0.89	H(03)···O(1)	2.12
O(03)···O(1)	2.96		

(*b*) Angles

C(19)–O(2)–C(21)	116.4(4)	C(2)–C(1)–C(10)	112.8(4)
C(1)–C(2)–C(3)	112.4(5)	C(2)–C(3)–C(4)	113.7(4)
C(3)–C(4)–C(5)	107.9(4)	C(3)–C(4)–C(18)	108.5(4)
C(3)–C(4)–C(19)	112.6(4)	C(5)–C(4)–C(18)	110.1(4)
C(5)–C(4)–C(19)	112.6(4)	C(18)–C(4)–C(19)	105.0(4)
C(4)–C(5)–C(6)	116.0(4)	C(4)–C(5)–C(10)	115.1(4)
C(6)–C(5)–C(10)	111.3(4)	C(5)–C(6)–C(7)	110.4(4)
O(3)–C(7)–C(6)	107.9(4)	O(3)–C(7)–C(8)	112.1(4)
C(6)–C(7)–C(8)	112.0(4)	C(7)–C(8)–C(9)	111.0(4)
C(7)–C(8)–C(14)	113.0(4)	C(7)–C(8)–C(15)	112.0(4)
C(9)–C(8)–C(14)	111.1(4)	C(9)–C(8)–C(15)	108.4(4)
C(14)–C(8)–C(15)	100.8(4)	C(8)–C(9)–C(10)	116.6(4)
C(8)–C(9)–C(11)	110.4(4)	C(10)–C(9)–C(11)	115.6(4)
C(1)–C(10)–C(5)	108.4(4)	C(1)–C(10)–C(9)	107.1(4)
C(1)–C(10)–C(20)	108.3(4)	C(5)–C(10)–C(9)	107.9(4)
C(5)–C(10)–C(20)	112.6(4)	C(9)–C(10)–C(20)	112.3(4)
C(9)–C(11)–C(12)	116.6(5)	C(11)–C(12)–C(13)	112.6(6)
C(12)–C(13)–C(14)	111.6(6)	C(12)–C(13)–C(16)	108.9(7)
C(14)–C(13)–C(16)	102.4(6)	C(8)–C(14)–C(13)	100.0(5)
C(8)–C(15)–C(16)	106.6(4)	C(13)–C(16)–C(15)	106.8(5)
C(13)–C(16)–C(17)	125.6(6)	C(15)–C(16)–C(17)	127.5(6)
O(1)–C(19)–O(2)	121.6(5)	O(1)–C(19)–C(4)	124.7(4)
O(2)–C(19)–C(4)	113.6(4)		
O(3)–H(03)···O(1)	156		

In conclusion, the structural resemblance of the fragment of the inhibitors containing the ring B hydroxy group and ring D to the similar portion of the natural substrate suggests that this fragment may be involved in determining the binding to the ring contraction enzyme system. The difference in structure such as the lack of a centre equivalent to C-6 or the C(6)–C(7) bond then block the ring contraction. Since these compounds act as gibberellin biosynthesis inhibitors at concentrations that are comparable with those of established plant growth regulators, this suggests a number of simple synthetic target structures which might be examined as potential plant growth regulators.

Experimental

Crystallographic Structure Determinations.—(*a*) *Methyl* ent-7α-*hydroxykaur*-16-*en*-19-*oate* (3). *Crystal data.* $C_{21}H_{32}O_3$, M = 332.5, orthorhombic, space group $P2_12_12_1$, a = 7.388(2), b = 11.379(1), c = 21.680(2) Å, U = 1 822.5 Å^3, Z = 4, D_c =

Table 4. Final atomic co-ordinates ($\times 10^4$) with estimated standard deviations in parentheses for compound (**5**)

	x	*y*	*z*
C(1)	3 050(6)	2 862(4)	7 625(6)
C(2)	4 626(6)	2 589(4)	6 452(7)
C(3)	4 574(6)	1 965(4)	5 171(6)
C(4)	3 910(5)	853(4)	5 926(5)
C(5)	2 372(5)	1 091(4)	7 222(5)
C(6)	883(6)	1 729(4)	6 538(6)
C(7)	−312(5)	2 034(4)	8 094(4)
C(8)	−1 246(7)	1 097(5)	8 995(9)
C(9)	−2 441(7)	1 694(5)	10 296(9)
C(10)	−1 626(7)	1 730(6)	11 683(7)
C(11)	120(7)	1 519(5)	11 112(6)
C(12)	624(5)	2 170(4)	9 272(5)
C(13)	2 389(5)	1 770(3)	8 525(5)
C(14)	−1 652(6)	3 138(5)	7 698(8)
C(15)	−2 914(6)	2 965(5)	9 216(8)
C(16)	−4 212(7)	3 748(6)	9 640(10)
C(17)	3 375(6)	998(4)	9 823(6)
C(18)	5 085(6)	−182(4)	6 791(6)
C(19)	3 653(8)	414(5)	4 498(7)
O(1)	1 102(5)	2 758(4)	5 229(5)
O(2)	6 446(4)	−99(3)	6 634(5)
O(3)	4 609(4)	−1 128(3)	7 584(5)
C(1A)	−1 903(6)	5 842(5)	3 739(6)
C(2A)	−2 719(7)	5 425(5)	2 793(7)
C(3A)	−1 699(6)	5 167(4)	1 158(6)
C(4A)	−945(4)	6 189(4)	52(5)
C(5A)	−230(5)	6 690(3)	1 068(5)
C(6A)	1 356(5)	5 988(4)	1 472(5)
C(7A)	1 598(5)	6 571(4)	2 730(5)
C(8A)	2 464(6)	7 554(5)	1 899(6)
C(9A)	2 687(7)	7 865(5)	3 436(6)
C(10A)	1 107(7)	8 543(4)	4 279(7)
C(11A)	−249(7)	8 371(5)	3 759(7)
C(12A)	−79(5)	7 079(4)	3 664(5)
C(13A)	−1 243(5)	6 922(4)	2 750(5)
C(14A)	2 653(6)	5 774(4)	3 963(7)
C(15A)	3 150(6)	6 642(5)	4 561(6)
C(16A)	3 838(7)	6 391(6)	5 859(8)
C(17A)	−2 619(6)	8 008(4)	2 491(6)
C(18A)	−2 135(6)	7 203(4)	−854(6)
C(19A)	266(7)	5 761(4)	−1 417(6)
O(1A)	1 413(5)	4 742(3)	2 117(5)
O(2A)	−3 407(5)	7 063(3)	−905(5)
O(3A)	−1 676(4)	8 165(3)	−1 628(5)

1.21 g cm^{-3}, Cu-K_α radiation (Ni filter), λ = 1.5418 Å, μ = 5.9 cm^{-1}.

A crystal *ca.* 0.5 × 0.3 × 0.2 mm was mounted on an Enraf–Nonius CAD 4 diffractometer. Intensities of unique reflections with $2 < \theta < 50°$ were measured by an $\omega - 2\theta$ scan with a maximum scan time of 1 min. No correction was made for absorption. Out of 1 197 reflections measured, 1 048 with $|F^2| > \sigma(F^2)$ were used in the refinement, where $\sigma(F^2) = [\sigma^2(I) + 0.04(I)^2]^{\frac{1}{2}}/Lp$.

The structure was solved by direct methods using MULTAN and refined with full-matrix least-squares with anisotropic temperature factors. Hydrogen atoms were held at fixed positions taken from a difference map with a common B_{iso} of 6.0 Å^2. Refinement converged at R = 0.047, R' = 0.079 when the weighting scheme was $\omega = 1/\sigma^2(F)$ and with the atomic co-ordinates consistent with the known absolute configuration. All calculations were done on a PDP 11/34 computer using the Enraf–Nonius SDP-Plus progam package. Fractional atomic co-ordinates and selected bond lengths and angles are given in Tables 2 and 3. Hydrogen atom co-ordinates, torsion angles,

Table 5. Intramolecular distances (Å) and angles (°) with estimated standard deviations in parentheses for compound (**5**)

(*a*) Bonds

C(1)–C(2)	1.534(6)	C(1A)–C(2A)	1.494(10)
C(1)–C(13)	1.528(6)	C(1A)–C(13A)	1.528(7)
C(2)–C(3)	1.512(9)	C(2A)–C(3A)	1.518(7)
C(3)–C(4)	1.538(7)	C(3A)–C(4A)	1.539(7)
C(4)–C(5)	1.556(6)	C(4A)–C(5A)	1.542(8)
C(4)–C(18)	1.531(6)	C(4A)–C(18A)	1.543(6)
C(4)–C(19)	1.550(9)	A(4A)–C(19A)	1.542(6)
C(5)–C(6)	1.525(7)	C(5A)–C(6A)	1.522(6)
C(5)–C(13)	1.564(7)	C(5A)–C(13A)	1.539(6)
C(6)–C(7)	1.541(6)	C(6A)–C(7A)	1.531(8)
C(6)–O(1)	1.428(6)	C(6A)–O(1A)	1.435(5)
C(7)–C(8)	1.524(8)	C(7A)–C(8A)	1.522(8)
C(7)–C(12)	1.546(8)	C(7A)–C(12A)	1.574(6)
C(7)–C(14)	1.547(6)	C(7A)–C(14A)	1.530(7)
C(8)–C(9)	1.522(9)	C(8A)–C(9A)	1.534(9)
C(9)–C(10)	1.565(11)	C(9A)–C(10A)	1.547(7)
C(9)–C(15)	1.542(7)	C(9A)–C(15A)	1.513(7)
C(10)–C(11)	1.514(8)	C(10A)–C(11A)	1.509(10)
C(11)–C(12)	1.544(6)	C(11A)–C(12A)	1.538(8)
C(12)–C(13)	1.563(6)	C(12A)–C(13A)	1.559(8)
C(13)–C(17)	1.522(6)	C(13A)–C(17A)	1.538(6)
C(14)–C(15)	1.500(8)	C(14A)–C(15A)	1.513(10)
C(15)–C(16)	1.326(8)	C(15A)–C(16A)	1.324(9)
C(18)–O(2)	1.251(7)	C(18A)–O(2A)	1.240(7)
C(18)–O(3)	1.281(6)	C(18A)–O(3A)	1.287(6)

(*b*) Angles

C(13)–C(1)–C(2)	113.7(4)	C(13A)–C(1A)–C(12A)	115.0(4)
C(3)–C(2)–C(1)	112.2(5)	C(3A)–C(2A)–C(1A)	112.1(5)
C(4)–C(3)–C(2)	114.4(4)	C(4A)–C(3A)–C(2A)	113.7(4)
C(5)–C(4)–C(3)	111.8(4)	C(5A)–C(4A)–C(3A)	112.3(4)
C(18)–C(4)–C(3)	110.7(4)	C(18)A–C(4A)–C(3A)	110.9(4)
C(18)–C(4)–C(5)	108.8(3)	C(18A)–C(4A)–C(5A)	109.4(4)
C(19)–C(4)–C(3)	109.8(4)	C(19A)–C(4A)–C(3A)	109.4(4)
C(19)–C(4)–C(5)	110.6(5)	C(19A)–C(4A)–C(5A)	111.7(4)
C(19)–C(4)–C(18)	104.9(4)	C(19A)–C(4A)–C(18A)	102.8(4)
C(6)–C(5)–C(4)	117.6(4)	C(6A)–C(5A)–3(4A)	116.8(4)
C(13)–C(5)–C(4)	115.5(4)	C(13A)–C(5A)–C(4A)	116.0(4)
C(13)–C(5)–C(6)	105.5(3)	C(13A)–C(5A)–C(6A)	105.4(4)
C(7)–C(6)–C(5)	103.6(4)	C(7A)–C(6A)–C(5A)	104.0(3)
O(1)–C(6)–C(5)	110.8(4)	O(1A)–C(6A)–C(5A)	111.7(4)
O(1)–C(6)–C(7)	112.8(4)	O(1A)–C(6A)–C(7A)	113.4(4)
C(8)–C(7)–C(6)	113.3(5)	C(8A)–C(7A)–C(6A)	113.1(4)
C(12)–C(7)–C(6)	106.1(4)	C(12A)–C(7A)–C(6A)	105.6(4)
C(12)–C(7)–C(8)	110.8(4)	C(12A)–C(7A)–C(8A)	110.3(4)
C(14)–C(7)–C(6)	114.6(4)	C(14A)–C(7A)–C(6A)	116.1(4)
C(14)–C(7)–C(8)	99.9(4)	C(14A)–C(7A)–C(8A)	99.8(4)
C(14)–C(7)–C(12)	112.3(4)	C(14A)–C(7A)–C(12A)	112.0(4)
C(9)–C(8)–C(7)	101.8(5)	C(9A)–C(8A)–C(7A)	101.4(4)
C(10)–C(9)–C(8)	109.6(5)	C(10)A–C(9A)–C(8A)	109.1(5)
C(15)–C(9)–C(8)	100.6(5)	C(15A)–C(9A)–C(8A)	101.5(5)
C(15)–C(9)–C(10)	109.0(6)	C(15A)–C(9A)–C(10A)	109.4(4)
C(11)–C(10)–C(9)	111.9(5)	C(11A)–C(10A)–C(9A)	112.3(5)
C(12)–C(11)–C(10)	113.4(4)	C(12A)–C(11A)–C(10A)	113.9(4)
C(11)–C(12)–C(7)	111.7(4)	C(11A)–C(12A)–C(7A)	110.8(4)
C(13)–C(12)–C(7)	107.9(4)	C(13A)–C(12A)–C(7A)	106.5(4)
C(13)–C(12)–C(11)	114.7(3)	C(13A)–C(12A)–C(11A)	115.7(4)
C(5)–C(13)–C(1)	110.2(3)	C(5A)–C(13A)–C(1A)	109.6(4)
C(12)–C(13)–C(1)	109.8(3)	C(12A)–C(13A)–C(1A)	110.8(4)
C(12)–C(13)–C(5)	102.6(4)	C(12A)–C(13A)–C(5A)	103.5(3)
C(17)–C(13)–C(1)	107.7(4)	C(17A)–C(13A)–C(1A)	107.8(4)
C(17)–C(13)–C(5)	112.3(3)	C(17A)–C(13A)–C(5A)	112.0(3)
C(17)–C(13)–C(12)	114.3(3)	C(17A)–C(13A)–C(12A)	113.1(4)
C(15)–C(14)–C(7)	103.7(4)	C(15A)–C(14A)–C(7A)	104.0(4)
C(14)–C(15)–C(9)	108.2(4)	C(14A)–C(15A)–C(9A)	107.9(5)
C(16)–C(15)–C(9)	123.7(5)	C(16A)–C(15A)–C(9A)	125.6(6)
C(16)–C(15)–C(14)	128.0(5)	C(16A)–C(15A)–C(14A)	126.5(5)
O(2)–C(18)–C(4)	120.3(4)	O(2A)–C(18A)–C(4A)	121.5(4)
O(3)–C(18)–C(4)	116.1(5)	O(3A)–C(18A)–C(4A)	115.4(5)
O(3)–C(18)–O(2)	123.5(4)	O(3A)–C(18A)–O(2A)	123.0(4)

Table 6. Fractional atomic co-ordinates ($\times 10^4$) with estimated standard deviations in parentheses for compound (**6**)

	x	*y*	*z*
O(1)	2 078(25)	2 503(12)	1 982(6)
O(2)	−408(25)	2 422(11)	2 749(5)
O(3A)	514(38)	2 242(17)	4 155(9)
O(3B)	1 979(64)	2 779(27)	5 055(16)
C(1)	4 667(34)	5 083(15)	3 041(8)
C(2)	6 000(35)	5 005(18)	2 466(11)
C(3)	4 594(38)	5 160(18)	1 918(10)
C(4)	2 844(34)	4 327(16)	1 874(8)
C(5)	1 720(31)	4 073(13)	2 542(8)
C(6)	956(30)	2 939(15)	2 532(9)
C(7)	2 157(45)	2 838(19)	4 463(10)
C(8)	1 649(35)	4 116(16)	4 314(8)
C(9)	1 366(32)	4 409(15)	3 627(8)
C(10)	3 132(33)	4 187(14)	3 124(8)
C(11)	629(30)	5 586(14)	3 620(7)
C(12)	1 838(37)	6 381(16)	4 043(8)
C(13)	2 063(39)	5 848(16)	4 644(9)
C(14)	3 271(37)	4 794(16)	4 630(9)
C(15)	−339(38)	4 322(18)	4 695(9)
C(16)	−82(34)	5 462(16)	4 892(8)
C(17)	−1 408(41)	6 041(17)	5 262(10)
C(18)	1 123(37)	4 652(18)	1 441(9)
C(19)	3 627(32)	3 181(15)	1 669(8)
C(20)	4 403(34)	3 164(17)	3 197(9)

and anisotropic temperature factors have been deposited at the Cambridge Crystallographic Data Centre.*

(*b*) ent-6α-*Hydroxy*-5β(H)-7-*norgibberell*-16-*en*-19-*oic Acid* (**5**). *Crystal data.* $C_{19}H_{28}O_3$, M = 304.5, triclinic, space group P_1, a = 9.172(1), b = 12.109(2), c = 8.478(1) Å, α = 72.83(1), β = 74.91(1), γ = 72.88(1)°, U = 844.1 Å^3, Z = 2, D_c = 1.20 g cm^{-3}, $F(000)$ = 332, Mo-K_α radiation λ = 0.7107 Å, μ = 0.44 cm^{-1}.

A crystal of *ca.* 0.3 × 0.3 × 0.3 mm was used for data collection on a Hilger and Watts Y290 diffractometer. Accurate cell parameters were derived from the setting angles for 12 reflections. Intensities for $h \pm k$, ± 1 reflections with $2 < \theta < 25°$ were measured by an $\omega/2\theta$ step scan using Mo-K_α radiation. Three standard reflections monitored every 100 reflections showed no significant variation. After correction for Lorentz and polarisation effects but not for absorption, 3 026 non-zero reflections were used in the structure solution and 2 349 with $|F^2| > 3\sigma(F^2)$ were used in the structure refinement. The application of the non-centrosymmetric direct methods routine in the SHELX program revealed only a six-atom fragment. Use of the tangent formula phase recycling procedure was not successful. A series of weighted Fourier maps were used to successfully extend the structure from the starting fragment. The carbon and oxygen atoms were then refined with anisotropic temperature factors by full-matrix least-squares, with the parameters for the two independent molecules refined in alternate cycles. A difference map revealed the positions of 53 of the hydrogen atoms which were then included in the structure factor calculations with a common U_{iso} of 0.08 Å^2. Continued refinement with hydrogen atom parameters fixed converged at R = 0.054, R' = 0.078 where $\omega = 1/[\sigma^2(F) + 0.006F^2]$ and the maximum shift/error was 0.2. A final difference map was everywhere < 0.25 e Å^{-3}. No attempt was made to determine the absolute configuration. Atomic co-ordinates and selected bond lengths and angles are given in Tables 4 and 5. Hydrogen atom co-ordinates, torsion angles, and temperature factors have been deposited with the Cambridge Crystallographic Data Centre. The intermolecular contacts indicate that the molecules are associated in pairs linked by hydrogen bonds between the carboxy group of molecules 1 and 2, since O(2) · · · O(3A) is 2.63 Å and O(3) · · · O(2A) is 2.64 Å. The two independent molecules in the unit cell have identical conformations.

(*c*) ent-7,19-*Dihydroxy*-6,7-*secokaur*-16-*en*-6-*oic acid* 6,19-*lactone* (**6**). *Crystal data.* $C_{20}H_{30}O_3$, M = 3.18.5, orthorhombic, space group $P2_12_12_1$, a = 6.384(2), b = 12.616(3), c = 21.990(7) Å, U = 1 771.1 Å^3, Z = 4, D_c = 1.19 g cm^{-3}, monochromated Mo-K_α radiation, λ = 0.710 69 Å, μ = 0.8 cm^{-1}. A crystal of *ca.* 0.4 × 0.22 × 0.18 mm was mounted on an Enraf–Nonius CAD 4 diffractometer. Intensities of unique reflections with $2 < \theta < 20°$ were measured by a $\theta/2\theta$ scan with a scan width of $\Delta\theta = (0.8 + 0.35 \tan\theta)°$ and a maximum scan time of 1 min. The data were corrected for *Lp* effects but not for absorption. 567 Reflections with $|F^2|\sigma(F^2)$ were used in the refinement where $\sigma(F^2) = [\sigma^2(I) + (0.02I)^2]^{\frac{1}{2}}/Lp$.

The structure was solved by direct methods using the MULTAN program. Refinement was by full-matrix least-squares and because of the relatively small number of significant reflections only isotropic temperature factors were used.

Table 7. Intramolecular distances (Å) and angles (°) with estimated standard deviations in parentheses for compound (**6**)

(*a*) Bonds

O(1)–C(6)	1.51(1)	O(1)–C(19)	1.48(1)
O(2)–C(6)	1.19(1)	O(3A)–C(7)	1.46(2)
O(3B)–C(7)	1.31(2)	C(1)–C(2)	1.53(2)
C(1)–C(10)	1.51(2)	C(2)–C(3)	1.52(2)
C(3)–C(4)	1.54(2)	C(4)–C(5)	1.67(2)
C(4)–C(18)	1.51(2)	C(4)–C(19)	1.60(2)
C(5)–C(6)	1.51(2)	C(5)–C(10)	1.57(2)
C(7)–C(8)	1.68(2)	C(8)–C(9)	1.57(1)
C(8)–C(14)	1.51(2)	C(8)–C(15)	1.54(2)
C(9)–C(10)	1.60(2)	C(9)–C(11)	1.56(2)
C(10)–C(20)	1.53(2)	C(11)–C(12)	1.57(2)
C(12)–C(13)	1.49(2)	C(13)–C(14)	1.54(2)
C(13)–C(16)	1.55(2)	C(15)–C(16)	1.51(2)
C(16)–C(17)	1.38(2)		

(*b*) Angles

C(6)–O(1)–C(19)	119(1)	C(2)–C(1)–C(10)	114(1)
C(1)–C(2)–C(3)	109(1)	C(2)–C(3)–C(4)	113(1)
C(3)–C(4)–C(5)	113(1)	C(3)–C(4)–C(18)	113(1)
C(3)–C(4)–C(19)	114(1)	C(5)–C(4)–C(18)	107(1)
C(5)–C(4)–C(19)	102(1)	C(18)–C(4)–C(19)	107(1)
C(4)–C(5)–C(6)	108(1)	C(4)–C(5)–C(10)	117(1)
C(6)–C(5)–C(10)	106(1)	O(1)–C(6)–O(2)	118(1)
O(1)–C(6)–C(5)	102(1)	O(2)–C(6)–C(5)	139(1)
O(3A)–C(7)–O(3B)	112(2)	O(3A)–C(7)–C(8)	105(1)
O(3B)–C(7)–C(8)	103(2)	C(7)–C(8)–C(9)	116(1)
C(7)–C(8)–C(14)	109(1)	C(7)–C(8)–C(15)	102(1)
C(9)–C(8)–C(14)	113(1)	C(9)–C(8)–C(15)	113(1)
C(14)–C(8)–C(15)	103(1)	C(8)–C(9)–C(10)	123(1)
C(8)–C(9)–C(11)	106(1)	C(10)–C(9)–C(11)	112(1)
C(1)–C(1)–C(5)	110(1)	C(1)–C(10)–C(9)	114(1)
C(1)–C(1)–C(20)	107(1)	C(5)–C(10)–C(9)	100(1)
C(5)–C(10)–C(20)	108(1)	C(9)–C(10)–C(20)	117(1)
C(9)–C(11)–C(12)	117(1)	C(11)–C(12)–C(13)	107(1)
C(12)–C(13)–C(14)	115(1)	C(12)–C(13)–C(16)	112(1)
C(14)–C(13)–C(16)	100(1)	C(8)–C(14)–C(13)	99(1)
C(8)–C(15)–C(16)	103(1)	C(13)–C(16)–C(15)	107(1)
C(13)–C(16)–C(17)	126(1)	C(15)–C(16)–C(17)	127(1)
O(1)–C(19)–C(4)	100.5(9)		

* For details of the Supplementary publications scheme, see Instructions for Authors (1988), *J. Chem. Soc., Perkin Trans. 1*, 1988, Issue 1.

Hydrogen atoms, except for those on C(7) and O(3), were included at idealised positions (C–H, 1.08 Å) with $B_{iso} = 6.0$ Å^2 and held fixed. There are two alternative sites for O(3) and initially refinement of their occupancies with equal temperature factors gave values of 0.6 for O(3A) and 0.4 for O(3B). The occupancies of the two sites were then fixed during the subsequent refinement. Refinement converged at $R = 0.11$, $R' = 0.12$ when the maximum shift/error was 0.01 and the weighting scheme was $\omega = 1/\sigma^2(F)$. A final difference map was featureless.

The structure solution and refinement were carried out on a PDP 11/34 computer using the Enraf–Nonius SDP-Plus program package. Fractional atomic co-ordinates are given in Table 6 and selected bond lengths and angles in Table 7. Hydrogen atom positions, torsion angles, and temperature factors have been deposited with the Cambridge Crystallographic Data Centre.

A FORTRAN computer program was written to calculate on the PDP 11/34 the best fit between a selected set of equivalent atoms in two molecules and then to draw the molecules superimposed on one another. In all cases, the carbon atoms of the D ring were used for the fit.

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