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THE CONSTITUTION OF CLEROCIDIN
A NEW ANTIBIOTIC ISOLATED FROM OIDIODENDRON TRUNCATUM

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Abstract. The antibiotic, clerocidin (PR 1350) is derived from a novel oxygenated diterpene ($3 \leftrightharpoons 4 \leftrightharpoons 5$) of the clerodane class (1).

The isolation of clerocidin, previously called PR 1350, from the fungus, Oidiodendron truncatum, has been reported.[1] In view of the wide range of activities shown by clerocidin against Gram-positive and Gram-negative microorganisms, as well as its activity against P 388 leukemia in mice, structural studies have been carried out. These studies are now reported.

Clerocidin gives complex n.m.r. spectra in solution which are associated with equilibration between monomers involving hydroxy-aldehyde and hemiacetal forms and dimers. However, crystallisation of clerocidin from methanol gave the crystalline monomeric O-methyl derivative (PR 1381)[1] $C_{21}H_{32}O_6$, m.p. 139-141°C. Reduction of clerocidin with sodium borohydride gave hexahydroclerocidin (PR 1383),[1] $C_{20}H_{34}O_5$, and this transformation was eventually interpreted as involving the reduction of one aldehyde group, one ketone group, and one αβ-unsaturated aldehyde group. Similar reduction of clerocidin with sodium borodeuteride followed by treatment with water gave trideuteriohexahydroclerocidin ($C_{21}H_{31}D_3O_5$).

Fermentation of Oidiodendron truncatum in the presence of [1-^{13}C]-acetate yielded clerocidin which, from its ^{13}C n.m.r. spectrum, was shown to contain eight ^{13}C-enriched carbon atoms per C_{20}-skeleton. The eight positions of ^{13}C-enrichment were identified and this suggested that clerocidin was an oxygenated diterpene with a clerodane C_{20}-skeleton (1).[1] This hypothesis received good support from ^{1}H n.m.r., ^{13}C n.m.r., and mass spectral studies. The FAB mass spectra, kindly determined by Dr. R. Misra[2] on clerocidin, established the molecular formula, $C_{40}H_{56}O_{10}$ (M = 696) [Found: positive ion mode, m/z 697 $(M+H)^+$; negative ion mode, m/z 695 $(M-H)^-$, and 347.5 $(M-H)^{2-}$]. The field ionisation mass spectrum of clerocidin showed fragment ions at m/z 366 ($C_{20}H_{30}O_6^+$) and m/z 205 ($C_{14}H_{21}O^+$). This fragmentation pattern matched the mass spectra reported[3] for numerous clerodane derivatives which are usually dominated by C(9)-C(11) bond cleavage and the formation of C_{14}-fragment ions associated with rings A and B. The mass spectrum of hexahydroclerocidin (PR 1383) shows a fragment ion at m/z 189 ($C_{14}H_{21}^+$), whereas trideuteriohexahydroclerocidin shows

a fragment ion at m/z 190 ($C_{14}H_{20}D^+$). This evidence was compatible with the association of one carbonyl group of clerocidin with rings A and B of the clerodane skeleton (1). The question could now be addressed whether this carbonyl group belonged to the αβ-unsaturated aldehyde group present (Table, fragment A) in clerocidin.

Detailed examination of the ^{1}H n.m.r. and ^{13}n.m.r. spectra of the three compounds [(i) $NaBH_4$ reduction product of clerocidin (ii) $NaBD_4$ reduction product of clerocidin, and (iii) $NaBH_4$ reduction product of ^{13}C-biosynthetically enriched clerocidin] and their corresponding tetra-acetates, established (Table) the presence of the fragments B, C, D, and E in hexahydroclerocidin (PR 1383). Clearly fragment E is formed from fragment A during the reduction (clerocidin → hexahydroclerocidin). The placing of the fragments B, C, D, and E (Table) of hexahydroclerocidin on the clerodane skeleton (1) could now be examined.

Fragment B belongs to an oxygenated ethyl group, so it must be placed at the terminus of the side chain of the clerodane skeleton (1) at C(14) and C(15). The formation of the fragment ions ($C_{14}H_{21}^+$) and ($C_{14}H_{20}D^+$) from hexahydroclerocidin and trideuteriohexahydroclerocidin respectively, must be associated with dehydration during mass spectral fragmentation. Only fragment E, which already contains one olefinic double bond could participate in a monodehydration process and give rise to a bicyclic fragment ion ($C_{14}H_{21}^+$) containing two olefinic double bonds. Thus fragment E must be associated with the C_{14} bicyclic A/B system of the clerodane skeleton (1). By exclusion, the two fragments C and D must be associated with C(11), C(12), C(13), and C(16) of the side chain. Furthermore, fragment C must be associated with C(11) and C(12): the secondary hydroxyl group of fragment C must be placed at C(12) because it is this carbon atom which is not ^{13}C-enriched in biosynthetic ^{13}C-labelled clerocidin.[1] Finally, the epoxide fragment D must be placed at C(13) and C(16). Fragment E must be located either at C(3)-C(4)-C(18) or at C(7)-C(8)-C(17). The decision between these two possibilities followed from additional ^{1}H spin decoupling studies. C(1) of fragment E (Table) was shown to be directly bonded to an allylic methylene group (multiplet centred at δ 2.41). Irradiation of the vinyl proton (δ 5.54) (H_A of fragment E) showed some sharpening of the multiplet (~ δ 2.41), but no sign of further simplification to yield an AB quartet was observed. On this evidence, the allylic methylene group could not be placed at C(6) so fragment E of hexahydroclerocidin had to be placed at C(3)-C(4)-C(18) with the allylic methylene at C(2).

These results establish the constitution of hexahydroclerocidin (2a) and trideuterohexahydroclerocidin (2b). These two structures correspond with the monomeric hydroxy-triketo-structure (3) and this leads to the dimeric structure (5) for clerocidin ($C_{40}H_{56}O_{10}$, M=696). There is ample precedent for the equilibration between monomeric α-hydroxycarbonyl compounds and dimeric 2,5-dihydroxy-1,4-dioxans[4]. Mechanistically analogous equilibrations are involved in the observed transformations: (i) the dimeric clerocidin (5) in

methanol solution yields the crystalline monomeric O-methyl derivative (6) and (ii) the monomeric O-methyl derivative (6) yields dimeric clerocidin (5) when dissolved in either chloroform, benzene, methyl cyanide, or dimethyl sulphoxide. Although clerocidin is represented by the dimeric structure (5), it is clear that clerocidin can equilibrate with monomeric equivalents such as (3), the hemi-acetal (4), and the corresponding hydrates in suitable solvent environments.

(1)

(2)

(2a) X = H
(2b) X = D

(3)

(4)

(5)

(6)

Table*

Fragment		Data
FRAGMENT A	^{1}H data** :	a; 6.63 bt; c; 9.28 s
	^{13}C data& :	1; 152.2 d, 2; 152.4 s, 3; 193.6 d
FRAGMENT B R=H or Ac	R = H	
	^{1}H data+ :	a; 3.99 m J=6.0, 4.4, b; 3.66 m J=4.4, 11.5, c; 3.74 m J=6.0, 11.5
	^{13}C data& :	1; 72.3 d, 2; 63.8 t
	R = OAc	
	^{1}H data** :	a; 5.21 m, b; 3.99 m, c; 4.49 m
FRAGMENT C R=H or Ac	R = H	
	^{1}H data+ :	a; 1.39 m J=2.4, 14.4, b; 1.73 m J=8.6, 14.4, c: 3.92 m J=2.4, 8.6
	^{13}C data& :	1; 41.1 t, 2; 68.9 d
	R = OAc	
	^{1}H data** :	c; 5.01 m
FRAGMENT D	^{1}H data&& :	a; 2.88 d J=4.9, b; 2.74 d J=4.9
	^{13}C data& :	1; 48.7 t, 2; 63.1 s
FRAGMENT E R=H or Ac	R = H	
	^{1}H data+ :	a; 5.51 t, b+c; 4.09 bs
	^{13}C data& :	1; 120.1 d, 2; 148.7 s, 3; 62.1
	R = OAc	
	^{1}H data** :	a; 5.54 t, b+c; 4.52 bs

*Bold letters indicate incorporation of ^{13}C or ^{2}H. Positions b and c in fragments B and E are only partially deuterated giving a total of one deuterium in each methylene group. All NMR data in ppm δ scale, all coupling constants given as numerical values. TMS reference. Instruments: JEOL FX 100 and Bruker HX 270 Solv.: **$CDCl_3$, 100 MHz. &$(CD_3)_2CO$, 25 MHz. +$CDCl_3$, 270 MHz. &&$(CD_3)_2CO$, 100 MHz.

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