Tetrahedron Letters Vol. 21, pp 5079 - 5082 0040-4039/80/1222-5079$02.00/0

ENGLEROMYCIN, A NEW CYTOCHALASAN FROM ENGLEROMYCES GOETZEI HENNINGS

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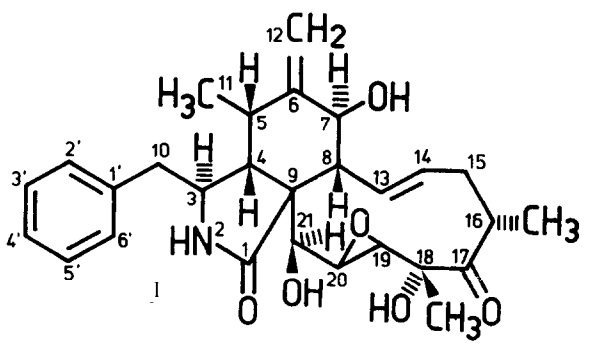
Abstract: The structure of engleromycin isolated from Engleromyces goetzei Hennings has been established as 1 by spectral techniques.

Engleromycin (1) is a new cytochalasan[1] which we have isolated from the fungus Engleromyces goetzei Hennings. It is a neutral substance, $C_{28}H_{35}NO_6$,[2] crystallizing in colourless needles from ethanol-H_2O, mp 226-228°, $[\alpha]_D^{25}$ + 64°(EtOH). On standing in pyridin and acetic anhydride 1 formed a diacetyl derivative.[2] Hydrogenation at atmospheric pressure (10 % Pd/C) gave a tetrahydro derivative.[2]

The structure of 1 was assigned mainly on the basis of spectral evidence. Fig. 1 shows the ^1H NMR spectrum and in Table 1 the chemical shifts are given. The spectrum is close to being identical to that published for cytochalasin D[3] (2) (cf. Table 1) with a few exceptions caused by the following structural differences: 1 contains no acetyl group, only two double bonds, but three hydroxy groups, identified by the strong temperature dependence of their chemical shifts (10°



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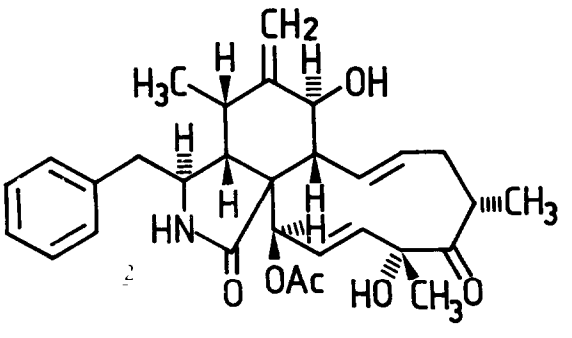

2

corresponds to 50 Hz). The OH proton at 7.14 ppm must be strongly hydrogen bonded as seen from its low field chemical shift and the observed coupling constant. In addition to the mentioned differences the other distinct deviation from the spectrum of cytochalasin D (2) is the resonances at 3.996, 4.270 and 4.297 ppm, which are missing in the spectrum of 2. These shift values indicate that hydrogen is bonded through carbon to strongly electronegative atoms like oxygen. Decoupling experiments show that the proton at 4.270 ppm is coupled to the other two (tables 1 and 2) and that the proton at 4.297 ppm additionally is coupled to the OH proton at 7.14 ppm. The following moiety HO-CH-CH(O-)-CH(O-)- is thus indicated. The presence of three hydroxy groups and two carbonyl groups as observed in the ^{13}C NMR spectrum (Fig. 2 and Table 1) leaves, when taking into consideration that 1 contains six oxygen atoms, the following formulation for the moiety HO-CH-CH-CH-. A positive thiosulfate test confirmed the presence of an epoxide.[4] Due to the close spectral similarity of 1 and 2 and taking into consideration that 1 lacks an acetyl group and contains an epoxide at C(19)-C(20) in stead of the double bond of 2, makes it possible to formulate the structure of engleromycin as 1.

It is evident that it is the C(19)=C(20) double bond which is missing due to the observed coupling from the olefinic proton on C(13) to the proton on C(7). Since all cytochalasans isolated sofar[1] possess a C(19)=C(20) double bond it seems very reasonable to place the epoxy group at this position. But as an alternative it is not possible from the spectral data to exclude a structure with the epoxide group placed between C(20) and C(21), although this possibility from biogenetic reasons seems highly unlikely.

For epoxides the H-H cis coupling is 4.5 Hz, whereas the corresponding trans coupling is 3.1 Hz. In 1 the measured coupling is 2.5 Hz indicating a trans substitution. The coupling from the proton on C(20) to the proton on C(21) is very small, 0.8 Hz, indicating that the dihedral angle has to be close to 90^{o}. This conformation is consistent with a conformation having a strongly hydrogen bonded C(21) OH group to the C(17) carbonyl group forming an eight-membered ring and, therefore, in agreement with placing the epoxy group at C(19) and C(20). The remaining coupling constants as given in Table 2 are very similar to those given for 2.[3]

The ^{13}C NMR spectrum (Fig. 2) shows all 28 resonances. The off-resonance decoupled spectrum gives multiplets as indicated in Table 1 corresponding to 31

Table 1

NMR Results for Engleromycin[5]

Structure	^{1}H NMR			^{13}C NMR		
Position	Chem. Shift	([a])	Mult.	Chem. Shift	([a])	Mult.
C(1)				177.00	(174.9)	1
N(2)	8.660	(8.92)	broad			
C(3)	3.6	(3.54)	broad	54 24	(54.00)	2
C(4)	2.7-2 9	(2.43)	broad	52.07	(50.00)	2
C(5)	3.2	(2.7)	broad	33.61	(33.12)	2
C(6)				152.33	(151.4)	1
C(7)	4.428	(4.36)	2	71.61[b]	(71.20)	2
C(8)	3.500	(3.34)	3	46.58	(47.80)	2
C(9)				54.80	(54.37)	1
C(10)	2,7-2.9	(2.92)	broad	45.59	(45.49)	3
C(11)	0.934	(0.97)	2	13.86	(13.65)	4
C(12)	5.275(A)	(5.42)	2	111.78	(112.2)	3
	5.089(B)	(5.07)	2			
C(13)	6.592	(6.23)	4	131.79	(132.1)	2
C(14)	5.964	(5.64)	7	133.10	(132.7)	2
C(15)	2.7-2.9(A)	(2.7)	broad	38.47	(38.58)	3
	2.024(B)	(2.7)	4			
C(16)	3.2	(2.0)	broad	42.67	(42.45)	2
C(17)				217.00	(210.7)	1
C(18)				77.72	(78.32)	1
C(19)	3.996	(5.55)	2	60.90[c]		2
C(20)	4.270	(6.79)	4	56.38[d]		2
C(21)	4.297	(5.98)	2	74.93[e]		2
C(1')				138.69	(138.3)	1
C(2',6')	~7.2	(7 24)	broad	130.08	(129.9)	2
C(3',5')				128.83	(128.7)	2
C(4')				126.89	(126.8)	2
C(16)CH_3	1.079	(1.09)	2	19.17	(19.44)	4
C(18)CH_3	1.672	(1.54)	1	23.08	(24.64)	4
C(21)OH	7.14		2			
C(7)OH	5.2					
C(18)OH	5.6					

a) The chemical shifts in parenthesis are the corresponding literature values for cytochalasin D.[3] b),c),d),e) The carbon resonances are determined by selective decoupling of the protons to C(7), C(19), C(20) and C(21), respectively.

Table 2

Proton-proton Coupling Constants for Engleromycin

H_i	H_j	J_{ij}
7	8	10.7
8	13	10.7
11	5	6.7
12A	12B	~1.4
13	14	15.5
14	15A	9.9
14	15B	5.6
15A	15B	12.6
15B	16	~2.5
16	16-Me	6.8
19	20	2.1
20	21	~0.8
21	21-OH	4.2

i and j refer to the numbering of the carbon atoms in 1.

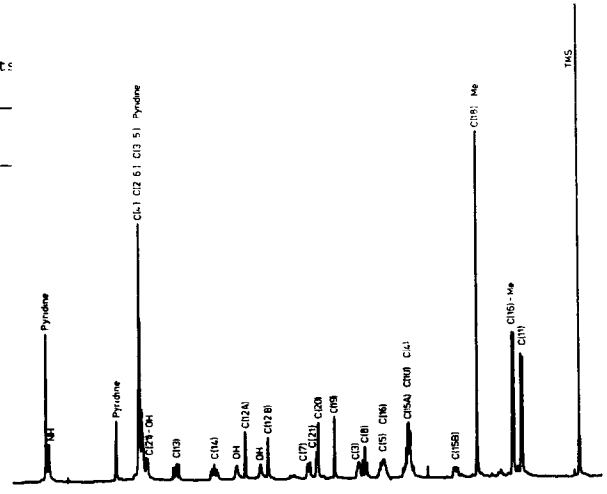

Fig. 1. ^{1}H NMR Spectrum of Engleromycin

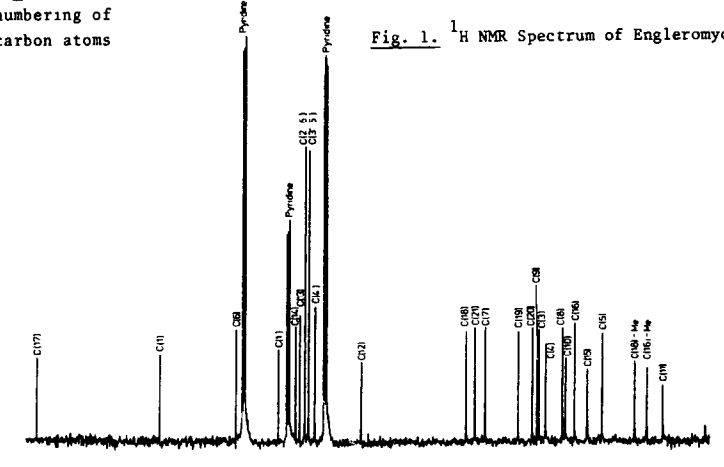

Fig. 2. ^{13}C NMR Spectrum of Engleromycin

protons attached directly to carbon. This together with the three OH groups and the NH group give the correct number of carbon and hydrogen ($C_{28}H_{35}$). All resonances except the ones at 60.90, 56.38 and 74.93 ppm relative to TMS can be assigned unambiguously from the chemical shifts given for 2 together with the multiplet structure in the off-resonance decoupled spectrum. Three lines were assigned by selective decoupling at the protons at C(19), C(20) and C(21).

In conclusion the structure of engleromycin (1), with the uncertainty existing for the absolute configuration of the trans-substituted epoxide ring, can be formulated as (7S,16S,18R,21S)-7,18,21-trihydroxy-19R,20S-epoxy-16,18-dimethyl-10-phenyl-[11]cytochalasa-6(12),13-diene-1,17-dione or its 19S,20R-epoxy isomer.

Acknowledgement: The authors are indebted to Professor J. O. Kokwaro, University of Nairobi, Kenya, and to Professor O. Hedberg, University of Uppsala, Sweden, for supplying the fungus, and to The Danish Natural Science Research Council for permission to use the Bruker HX-270 spectrometer.

References and Notes

1. M. Binder and C. Tamm, Angew. Chem. 85, 369 (1973) and references cited therein.
2. The compound was adequately characterized by spectral methods (IR, NMR and MS) and gave satisfactory high resolution mass spectral and/or combustion analytical data.
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4. G. G. Freman, J. E. Gill and W. S. Waring, J. Chem. Soc. 1128 (1959) and G. Büchi, Y. Kitaura, S.-S. Yuan, H. E. Wright, J. Clardy, A. L. Demain, T. Glinsukon, N. Hurt and G. N. Wogan, J. Am. Chem. Soc. 95, 5424 (1973).
5. The sample consisted of 15 mg of engleromycin in 0.4 ml pyridine-d_5 in a 5 mm sample tube. The sample was sealed and degassed by the usual freeze-pump-thaw technique. The spectra were recorded at 313 K and all chemical shifts are given at this temperature relative to TMS as internal standard. The spectra were measured on a Bruker HX-270 spectrometer with Fourier-transform system and a Nicolet 1180 computer.

   The ^1H NMR spectra are an average of 10 scans, using 32K data points and a spectral range of 5000 Hz which corresponds to a digital resolution of 0.3 Hz. The ^{13}C NMR spectra were obtained as an average of approximately 6000 scans using 32K data points and a spectral width of 17000 Hz giving a digital resolution of 1 Hz. The repetition time was 0.95 sec. and pulse width 4 μsec, corresponding to 60°. The decoupling power used in the selective decoupled spectra was adjusted to the minimum power necessary to observe the effect of the decoupling on C(11).

(Received in UK 13 October 1980)