Tetrahedron Letters,Vol.24,No.26,pp 2673-2676,1983 0040-4039/83 $3.00 + .00
Printed in Great Britain

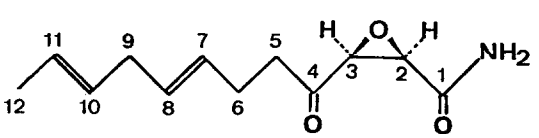
A MODEL STUDY ON THE MECHANISM OF FATTY ACID SYNTHETASE INHIBITION BY ANTIBIOTIC CERULENIN

Hiroshi Funabashi, Shigeo Iwasaki* and Shigenobu Okuda
Institute of Applied Microbiology, The University of Tokyo, Bunkyo-ku, Tokyo, Japan
Satoshi Ōmura
Kitazato University and Kitazato Institute, Minatoku, Tokyo, Japan

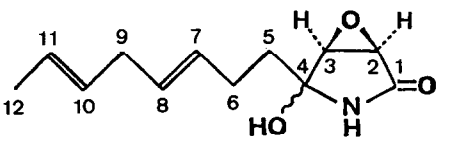
Summary In relation to the mechanism of fatty acid synthetase inhibition by cerulenin (1) the reaction between this antibiotic and thiols were studied. The structures of the propanethiol adduct 2 and of the cysteine methyl ester adduct 3 were determined to be respective (2*S*, 3*R*)-2-alkylmercapto-3-hydroxy derivatives forming the hydroxylactam structures in their 4-oxo-amide moiety.

An antibiotic cerulenin, (2*R*, 3*S*)-2,3-epoxy-4-oxo-7,10-*trans*, *trans*-dodecadienamide (1), was shown to be a potent inhibitor of fatty acid synthetase systems isolated from various organisms[1]. This antibiotic specifically blocks the activity of ß-ketoacyl thioester synthetase (condensing enzyme) irreversibly[1, 2, 3]. Since this enzyme is known to have a cysteine thiol group as the enzyme active site, the nucleophilic charactor of this SH group has been expected to interact with cerulenin molecule[2]. The structure of cerulenin, on the other hand, is characterized by two unique features; a C_8 side chain with a *trans, trans*-1,4 diene system and a 4-oxo-2,3-epoxyamide moiety. The former may play a role in the specific hydrophobic affinity of this compound to the enzyme cavity and the latter may act for modification of the enzyme SH group in somewhat non-specific fashion to form a covalently bound adduct. Having interested in this regard, we have studied the reaction of ceruleinin with thiols.

It has already been suggested from its ^{1}H-NMR spectra that cerulenin is equilibrated almost to the acyclic structure 1a in the aprotic solvents such as chloroform and to the hydroxylactam structure 1b in the protic solvents such as methanol[4] (see also table 1). This was supported also by its ^{13}C-NMR spectra in which the signal due to a ketonic carbon (C-4, at δ = 202.0 ppm) appearing in $CDCl_3$ was not shown in CD_3OD but instead a new signal due to an amidal carbon was exhibited at δ = 87.1 ppm. All the other carbon signals were shown at the comparable chemical shifts in the both solvents (table 3).



1a



1b

Cerulenin was incubated with equimolar amount of propanethiol or thiophenol in chloroform at 40°, and the reaction mixtures were analyzed by silica gel thin layer chromatography (t.l.c.) as well as by ^{13}C-NMR spectroscopy (in $CDCl_3$). Cerulenin was inactive to the thiols under this condition and no conversion of the starting materials was observed. Cerulenin in

Table 1. ^{1}H-NMR Data of Cerulenin[a)]

Proton	Cerulenin in $CDCl_3$ 1a	Cerulenin in CD_3OD 1b
H-2	3.74 (1H) d J_{2-3}=5.0	3.82 (1H) d J_{2-3}=3.0
H-3	3.88 (1H) d J_{3-2}=5.0	3.59 (1H) d J_{3-2}=3.0
H-5	2.66 (2H) m	1.78 (2H) m
H-6	2.32 (2H) dt J_{6-5}=7.0 J_{6-7}=7.0	2.24 (2H) m
H-7, H-8, H-10, H-11	5.42 (4H) m	5.46 (4H) m
H-9	2.66 (2H) m	
H-12	1.66 (3H) d J_{12-11}=5.0	1.64 (2H) d J_{12-11}=5.0
H-N	6.30 (1H) & 5.42 (1H)	-

a) These spectra were measured on JEOL JNM FX-400 instrument

Table 2. ^{1}H-NMR Data of Cerulenin-Thiol Adducts[a)]

Proton	Propanethiol Adduct in $CDCl_3$ 2	Cysteine Methyl Ester Adduct in $CDCl_3$ 3
H-2	3.49 (1H) d J_{2-3}=8.0	3.60 (1H) d J_{2-3}=8.0
H-3	4.00 (1H) dd J_{3-2}=8.0 J_{3-OH}=8.0	4.00 (1H) d J_{3-2}=8.0
H-5	1.88 (2H) t J_{5-6}=8.0	1.82 (2H) m
H-6	2.20 (2H) m	2.19 (2H) m
H-7, H-8, H-10, H-11	5.50 (4H) m	5.45 (4H) m
H-9	2.68 (2H) br.t	5.45 (4H) br.t
H-12	1.67 (3H) m J_{12-11}=5.0	1.66 (3H) d J_{12-11}=5.0
H-N	7.03 (1H) s	6.56 (1H) s
HO-3	3.39 (1H) d J_{OH-3}=8.0	
HO-4	4.09 (1H) s	
H-1'	2.74 (2H) t $J_{1'-2'}$=7.0	3.31 & 2.96 2dd $J_{1'-2'}$=5.0 & 4.0 $J_{1'-1'}$=15.0
H-2'	1.67 (2H) m	3.98 (1H) dd $J_{2'-1'}$=4.0 & 5.0
H-3'	1.02 (3H) t $J_{3'-2'}$=8.0	
H_3C-O		3.78 (3H) s

a) These spectra were measured on JEOL JNM FX-400 instrument

Table 3. ^{13}C-NMR Data of Cerulenin and Cerulenin-Thiol Adducts[a)]

carbon	Cerulenin in $CDCl_3$ 1a	Cerulenin in CD_3OD 1b	Propanethiol Adduct($CDCl_3$) 2	Cysteine Methyl Ester Adduct ($CDCl_3$) 3
C-1	167.3 s	174.4 s	173.9 s	172.4 s
C-2	55.4 d	59.0 d	50.7 d	53.3 d
C-3	58.4 d	53.2 d	77.9 d[b)]	79.3 d[b)]
C-4	202.0 s	87.1 s	86.3 s	85.7 s
C-5	40.9 t	36.6 t	38.3 t	38.3 t
C-6	26.0 t	28.0 t	26.6 t	26.7 t
C-7	130.7 d[c)]	131.1 d	130.3 d	130.1 d
C-8	129.2 d[c)]	130.8 d	129.2 d	130.0 d
C-10	127.8 d[c)]	130.6 d	129.2 d	129.3 d
C-11	125.8 d[c)]	126.4 d	125.9 d	125.4 d
C-9	35.5 t	36.6 t	35.5 t	35.5 t
C-12	17.9 q	18.1 q	17.9 q	17.9 q
C-1'			33.4 t	37.0 t
C-2'			22.8 t	54.3 t
C-3'			13.5 q	173.1 s
CH_3-O				52.7 q

a) These spectra were measured on JEOL JNM FX-400 instrument
b) Long-range coupling with H_2-5 was observed
c) Each of C-7, 8, 10 and 11 signals were assigned in the ^{13}C enriched cerulenin
J. Awaya et al; J. Antibiot., 28 824 (1975)

methanolic or in aqueous solutions, however, was found to be reactive to these thiols giving several spots of reaction products on t.l.c. This fact would suggest that the hydroxylactam form 1b should be more susceptible to the substitution by nucleophiles than the acyclic structure 1a.

Since the reaction of cerulenin with propanethiol in 0.1 M potassium phosphate buffer solution (pH 6.5) gave the cleanest result in the preliminary experiment, cerulenin (10 mg) was treated with propanethiol (6.13 µl) in the buffer solution (6.7 ml) at 40° for 4 days. A crude chloroform extract (13 mg) from the reaction solution was separated by silica gel column chromatography eluted with $CHCl_3$/MeOH 19:1 to afford a pure cerulenin-propanethiol adduct 2 as a colorless oil (6 mg), IR ($CHCl_3$); 3400, 2920, 1710, 1090 and 970 cm^{-1}, ms; m/z 300 (M^++1), 281 (M^+-18). Its mass spectrum as well as ^{1}H- and ^{13}C-NMR spectra (table 2 and 3) indicated that the product was a cerulenin-propanethiol 1:1 adduct. The fact that the signal due to C-4 was exhibited at δ = 86.3 ppm demonstrated that the product is also forming a hydroxylactam structure as in the case of cerulenin in protic solvents (1b).

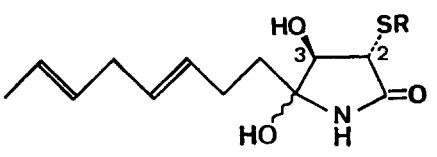
Epoxide opening in the product 2 by propanethiol addition was clearly indicated by the shifts of its NMR signals due to H-2, H-3, C-2 and C-3 as well as by the change of coupling constant between H-2 and H-3 comparing with those of cerulenin in CD_3OD. Substitution of the propylthio group at C-2 position was elucidated by excluding C-3 as this position as follows: A

methine carbon signal at δ = 77.9 ppm was assigned to C-3 based on its long-range coupling with H_2-5. On the other hand the substitution of a hydroxy group on this carbon was indicated by its chemical shift value in its ^{13}C-NMR as well as by the spin-spin coupling (J = 8.0) between a hydroxy proton (doublet at δ = 3.37 ppm) and the proton attached to this carbon (double doublet at δ = 4.00 ppm). (2*S*, 3*R*)-Configuration in the product 2 was elucidated essentially based on the mechanistic requirement for the epoxide opening by the S_N2 type of reaction.

Since the cysteine residue is involved in the active site of the target enzyme of cerulenin, the reaction of cerulenin with a cysteine derivative should provide a more plausible model for the enzyme inhibition mechanism. Cerulenin (15 mg) was thus treated with cysteine methyl ester hydrochloride (17 mg) in a 0.1 M potassium phosphate buffer solution (pH 7.0, 10 ml) at 25°. This reaction proceeded very rapidly and after 2 hours incubation all cerulenin was consumed and a spot of a main product was shown on the t.l.c. plate. A crude chloroform extract from the reaction solution (18 mg) was separated by silica gel column chromatography eluted with $CHCl_3$/MeOH 9:1 affording a pure cerulenin-cysteine methyl ester adduct 3 (ca 8 mg) as colorless oil, IR ($CHCl_3$); 3400, 2900, 1710, 1200, 1019 and 970 cm^{-1}, ms; m/z 340 (M^+-18). The structure of the adduct was determined from its spectral data, especially by comparison of its NMR data with those of the cerulenin-prpanethiol adduct 2 (see table 2 and 3). The NMR data demonstrated that the product 3 is also forming a hydroxylactam structure with the alkyl-mercapto substitution at the 2 position.

Although the respective configurations at 4 position in the adduct 2 and 3 have so far been undetermined, both were equilibrated almost to one diastereomer each.

The result recorded in this paper suggests that the enzyme inhibition by cerulenin should also be caused by alkylating the cysteine residue in the enzyme active site at the 2 position of cerulenin molecule presumably in a hydroxylactam form as shown in the structure 4.



2: R= $CH_2^{1'}CH_2^{2'}CH_3^{3'}$

3: R= $CH_2^{1'}CH^{2'}(NH_2)C^{3'}(=O)OCH_3$

4: R= Enzyme

Acknowledgement This work was supported by Grants-in-Aid from the Ministry of Education, Sciences and Culrure, Japan, which is gratefully acknowledged.

References

1) D. Vance, I. Goldberg, O. Mitsuhashi, K. Bloch, S. Ōmura and S. Nomura, Biochem. Biophys, Res. Commun., 48, 649 (1972)

2) G. D'Agnolo, I. S. Rosenfeld, J. Awaya, S. Ōmura and P. R. Vagelos, Biochim. Biophys. Acta, 326, 155 (1973)

3) A. Kawaguchi, H. Tomoda, S. Nozoe, S. Ōmura and S. Okuda, J. Biochem. 92, 7 (1982)

4) A. A. Jakubowski, F. S. Guziec, Jr., M. Sugiura, C. C. Tam, M. Tishler and S. Ōmura, J. Org. Chem., 47, 1221 (1982)

(Received in Japan 19 March 1983)