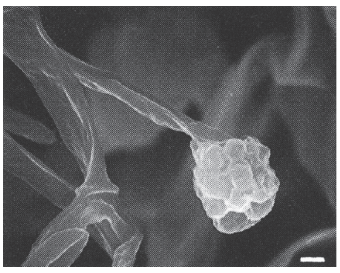
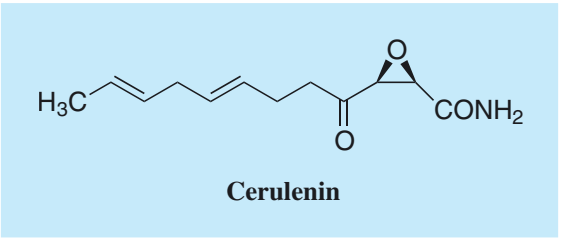
Cerulenin©

1. Discovery[1,2], producing organism[1,2,55] and structure[3,4,24]

Cerulenin was originally isolated from the culture broth of *Sarocladium oryzae* KF-140 as an antifungal antibiotic with a structure of (2*R*,3*S*)-2,3-epoxy-4-oxo-7,10-*trans*, *trans*-dodecadienoylamide. The first paper on the total synthesis of cerulenin was reported by Boeckman *et al*.[5] (See Appendix I).



Cephalosporium caerulens KF-140^T
(*Sarocladium oryzae* KF-140)
Bar: 5 μM



Cerulenin

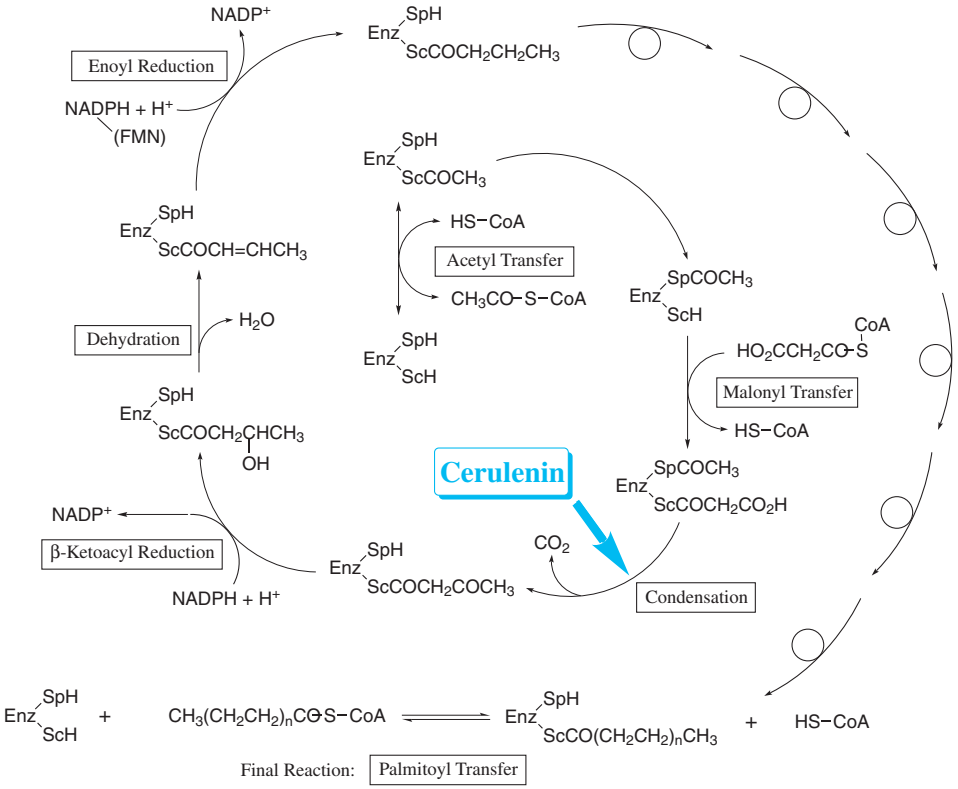
2. Physical data[1]

White powder. $C_{12}H_{17}NO_3$; MW 223.12. Sol. in EtOH, acetone, benzene. Slightly sol. in H_2O. Practically insol. in petroleum ether.

3. Biological Activity[2,6,8-10]

1) Inhibition of fatty acid synthesis[6-8]

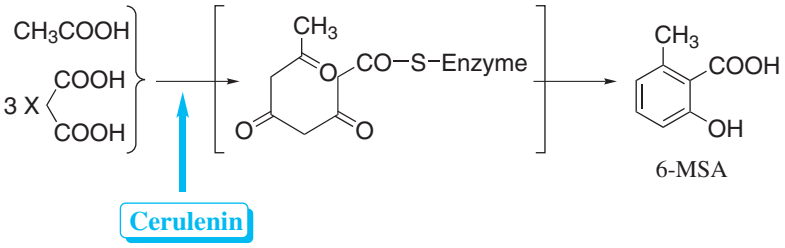
Cerulenin is a potent inhibitor of fatty acid synthase (FAS). It inhibits all known types of FASs; both multifunctional enzyme complexes (Type I) (from yeast, rat liver, mammalian cells, and certain bacteria) and unassociated enzymes (Type II) (from most bacteria, and higher plants).



ScH and SpH are abbreviations for central and peripheral SH groups indicating different functions in the enzymatic reaction.

2) Inhibition of polyketide synthesis[9,10)]

Cerulenin blocks the synthesis of polyketides in a wide variety of organisms, including actinomycetes, fungi and higher plants. In addition, cerulenin is suggested to inhibit the condensation step in polyketide synthesis as well as fatty acid synthesis.



Biosynthesis of 6-methylsalicylic acid (6-MSA)

3) Antimicrobial activity[2)]

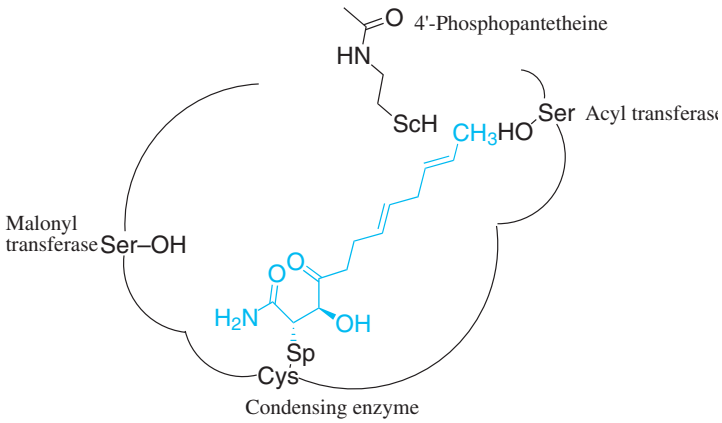
Cerulenin has a wide range of antimicrobial activity as shown below. Most noteably, the drug significantly inhibits growth of yeast like fungi, such as *Candida*, *Saccharomyces* and *Cryptococcus*.

Test Organism	MIC (μg/ml)	Test Organism	MIC (μg/ml)
Staphylococcus aureus FDA 209p	100	*Alternaria kikuchiana*	50
Micrococcus luteus PCI 1001	25	*Aspergillus flavus*	100
Streptococcus haemolyticus	100	*Aspergillus fumigatus*	50
Escherichia coli NIHJ	12.5	*Botrytis cryptoneriae*	12.5
Salmonella typhosa	50	*Candida albicans*	1.5
Shigella dysenteriae Shiga	25	*Cephalosporium caerulen*	100
Shigella flexineri E-20	50	*Cryptococcus neoformans*	1.5
Bacillus subtilis PCI 219	12.5	*Penicillium crysogenum*	12.5
Corynebacterium diphtheriae	50	*Penicillium notatum*	12.5
Haemophilus influenzae	100	*Pyricularia oryzae*	6.25
Mycobacterium avium F	1.5	*Pullularia pullulans*	25
Mycobacterium smegmatis	3.7	*Rhizopus nigricans*	25
Mycobacterium tuberculosis	100	*Saccharomyces cerevisiae*	0.8
Nocardia asteroides	1.5	*Trichophyton asteroides*	6.2
Streptomyces griseus SN-15	3.1	*Trichophyton interdigitale*	12.5

4. Mode of Action[7,8,11-19,25-29)]

1) Inhibition of the condensing enzyme of FAS[7,8,11-13)]

The inhibition of FAS activity by cerulenin is based on its covalent binding to the cysteine residue (SpH) in the condensation reaction domain. Cerulenin was initially thought to take a lactam form in order to react with the SpH,[12)] however X-ray crystallographic analysis of the cerulenin-condensing enzyme complex revealed that cerulenin itself is covalently attached in a hydrophobic cavity of the active site as illustrated below[18)].

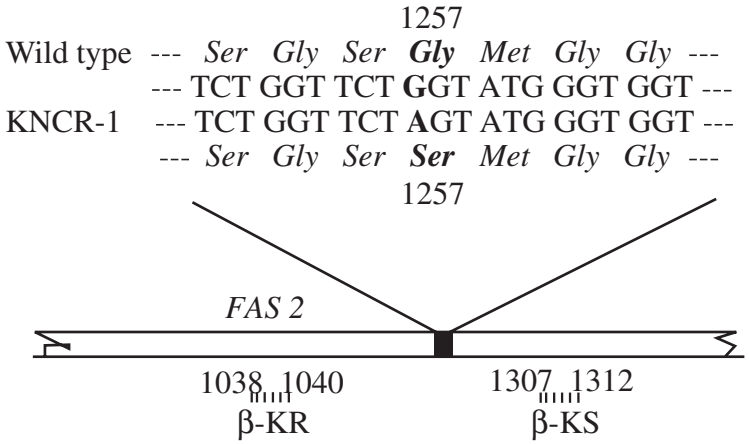


Proposed model for cerulenin binding to the active center of the cysteine residue in the condensation reaction

2) Mechanism of cerulenin-resistance in a cerulenin producer[14-13)] and mutant yeast[17)]

The cerulenin-producing fungus *Acremonium* (*Cephalosporium*) *caerulens* KF-140 was resistant to cerulenin. The mechanism of self-resistance revealed that cerulenin cannot bind to the significantly altered condensation reaction domain of the producer FAS[13-15)].

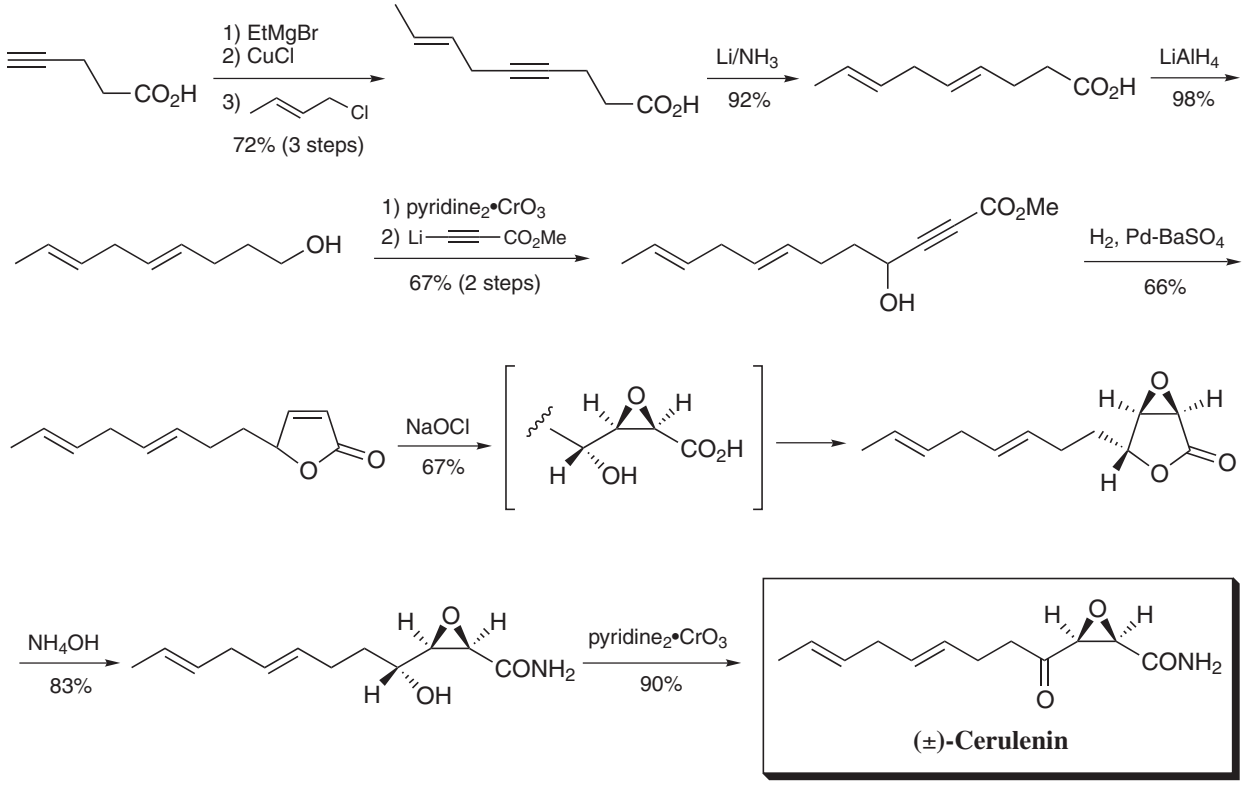
In a cerulenin resistant mutant (KNCR-1) (a 30-fold increase) of *Saccharomyces cerevisiae*, the GGT codon encoding *Gly*-1257 of *FAS 2*, (the gene encoding the α subunit of the fatty acid synthase) was changed to AGT, resulting in the codon for *Ser*[16)]. As predicted, Gly-1257 is incorporated in the hydrophobic cavity of the active site to fit cerulenin[18)]. Therefore, the change causes steric hindrance to cerulenin binding, leading to cerulenin resistance in mutant yeast.



Comparison of the DNA sequence of the *FAS 2* gene.

5. Total synthesis[20)]

The total synthesis of cerulenin has been reported by many groups (See Appendix-I). Below is S. Ōmura's approach.



Overall yield 14 % in 11 steps

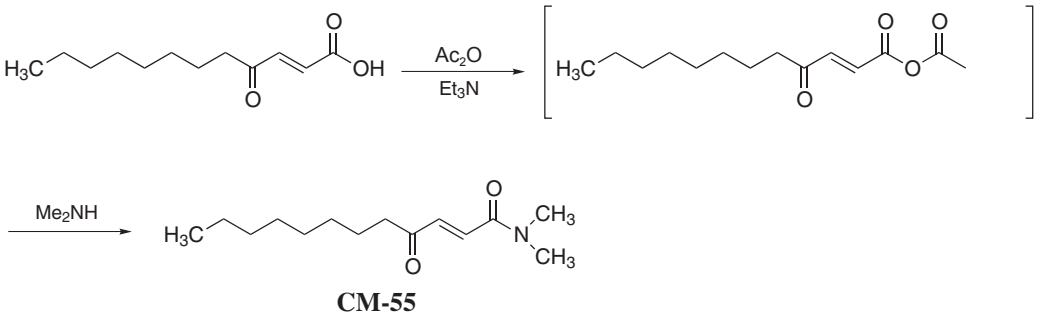
6. Research reagent as a chemical probe[30-47,50-54)]

Cerulenin is commercially available as a biochemical reagent for widely use in the field of obesity, cancer biology, posttranslational protein modification system, drug discovery research and so on. Until now, Cerulenin is a typical chemical probe to research fatty acid synthesis in many biological mechanisms.

7. Synthetic derivative CM-55

1) Synthesis and structure[21)]

CM-55 was synthesized as an analog of antilipogenic antibiotic cerulenin (See also "Cerulenin"). The general procedure for the synthesis is shown below.



CM-55

2) Biological activity[21-23,48)]

i) Antimicrobial activity[21,22,48)]

CM-55 shows antifungal and antimicrobial activity with rather low MIC values (6.25-12.5 μg/ml, see below)[21)]. The mode of action appears to be attributed to inhibition of acetyl-CoA by incorporation into the non-saponifiable fraction[22)].

Antimicrobial activity

Test organism	MIC (μg/ml)
Bacillus subtilis PCI 219	6.25
Staphylococcus aureus FDA 209P	3.12
Mycobacterium smegmatis ATCC607	6.25
Escherichia coli NIHJ	3.12
Pseudomonas aeruginosa P-3	>100
Candida albicans	12.5
Pyricularia oryzae	6.25
Trichophyton rubrum	6.25

ii) CM-55 was found to inhibit epithelial transport of sodium ions across the toad urinary bladder and to inhibit hydro-osmotic response of the bladder to vasopressin after 2 hours of pretreatment with CM-55[23)].

8. Biosynthesis[49)]

Biosynthesis of cerulenin is closely related with fatty acid synthesis.

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