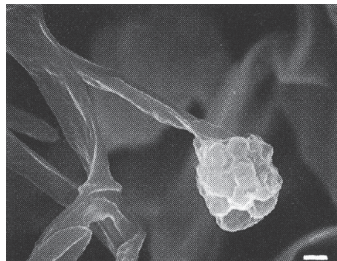


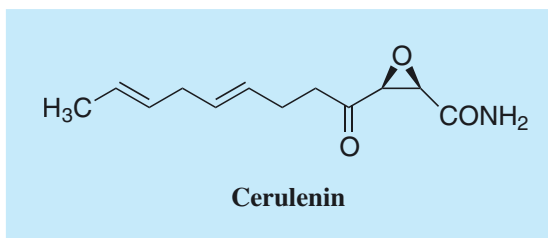
Cerulenin[©]

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*-dodecadien-oylamide. 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 μ M



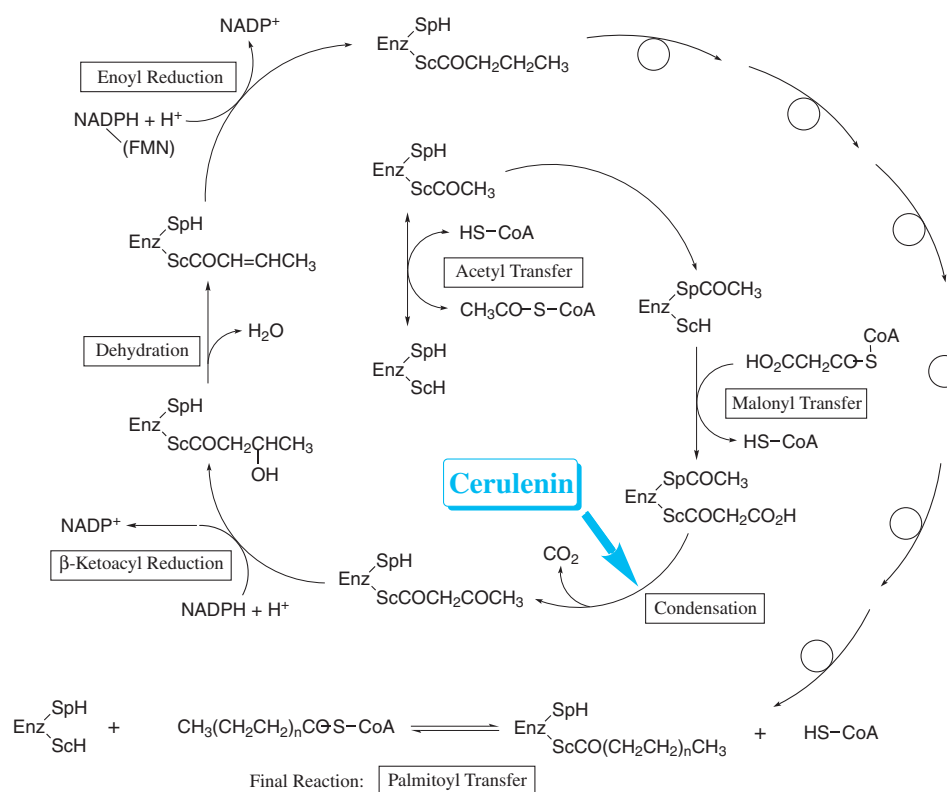
2. Physical data¹⁾

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 synthesis⁶⁻⁸⁾

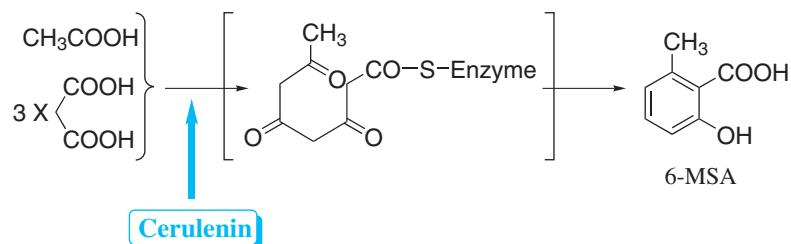
Cerulenin is a potent inhibitor of fatty acid synthase (FAS). It inhibits all known types of FASs; both multifunctional enzyme complexes (Type I) (from yeast, rat liver, mammalian cells, and certain bacteria) and unassociated enzymes (Type II) (from most bacteria, and higher plants).



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 activity²⁾

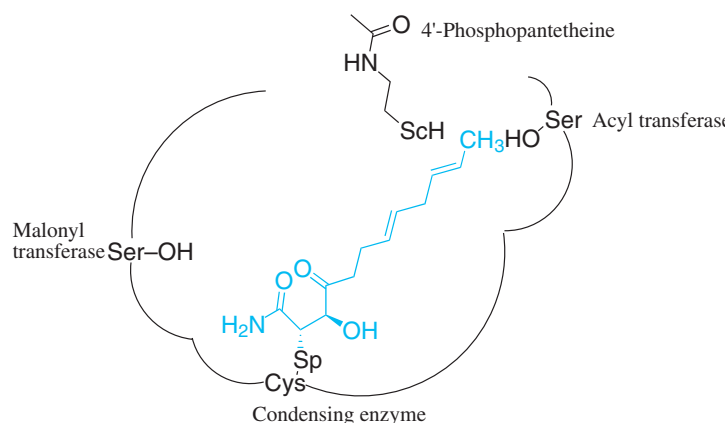
Cerulenin has a wide range of antimicrobial activity as shown below. Most notably, the drug significantly inhibits growth of yeast like fungi, such as *Candida*, *Saccharomyces* and *Cryptococcus*.

Test Organism	MIC (µg/ml)	Test Organism	MIC (µg/ml)
<i>Staphylococcus aureus</i> FDA 209p	100	<i>Alternaria kikuchiana</i>	50
<i>Micrococcus luteus</i> PCI 1001	25	<i>Aspergillus flavus</i>	100
<i>Streptococcus haemolyticus</i>	100	<i>Aspergillus fumigatus</i>	50
<i>Escherichia coli</i> NIHJ	12.5	<i>Botrytis cryptoneriae</i>	12.5
<i>Salmonella typhosa</i>	50	<i>Candida albicans</i>	1.5
<i>Shigella dysenteriae</i> Shiga	25	<i>Cephalosporium caeruleum</i>	100
<i>Shigella flexineri</i> E-20	50	<i>Cryptococcus neoformans</i>	1.5
<i>Bacillus subtilis</i> PCI 219	12.5	<i>Penicillium crysogenum</i>	12.5
<i>Corynebacterium diphtheriae</i>	50	<i>Penicillium notatum</i>	12.5
<i>Haemophilus influenzae</i>	100	<i>Pyricularia oryzae</i>	6.25
<i>Mycobacterium avium</i> F	1.5	<i>Pullularia pullulans</i>	25
<i>Mycobacterium smegmatis</i>	3.7	<i>Rhizopus nigricans</i>	25
<i>Mycobacterium tuberculosis</i>	100	<i>Saccharomyces cerevisiae</i>	0.8
<i>Nocardia asteroides</i>	1.5	<i>Trichophyton asteroides</i>	6.2
<i>Streptomyces griseus</i> SN-15	3.1	<i>Trichophyton interdigitale</i>	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 below¹⁸⁾.

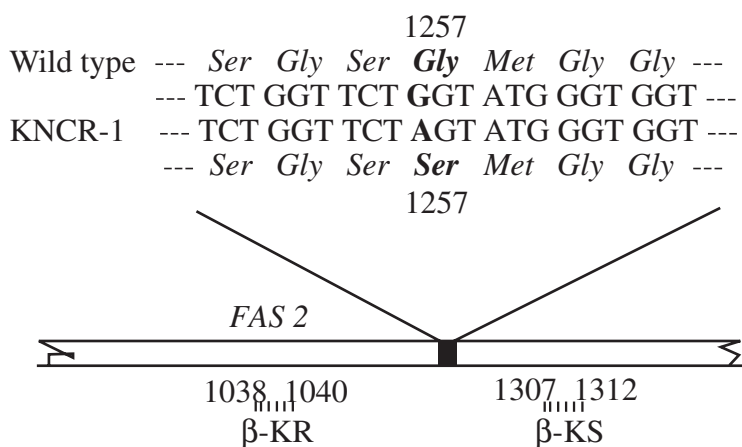


Proposed model for cerulenin binding to the active center of the cysteine residue in the condensation reaction

2) Mechanism of cerulenin-resistance in a cerulenin producer¹⁴⁻¹³⁾ and mutant yeast¹⁷⁾

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 FAS¹³⁻¹⁵⁾.

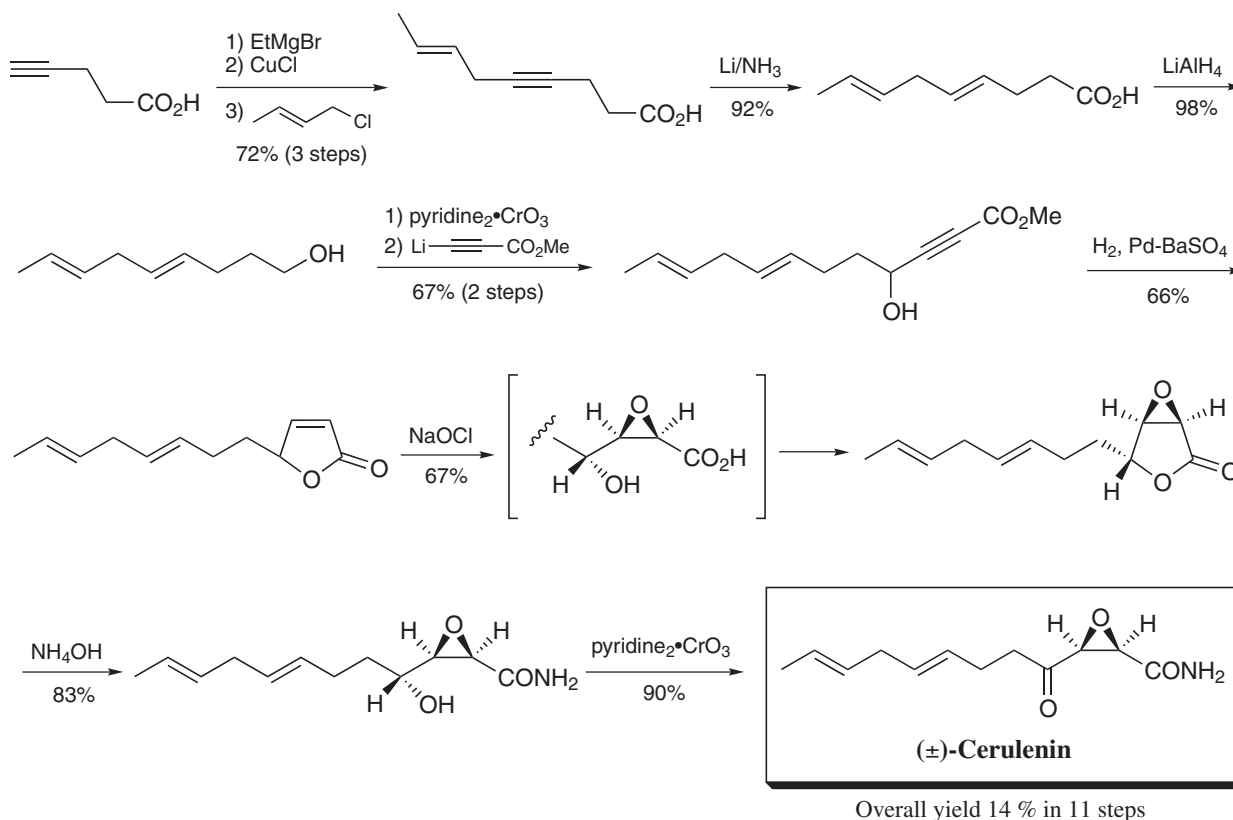
In a cerulenin resistant mutant (KNCR-1) (a 30-fold increase) of *Saccharomyces cerevisiae*, the GGT codon encoding Gly-1257 of FAS 2, (the gene encoding the α subunit of the fatty acid synthase) was changed to AGT, resulting in the codon for Ser¹⁶⁾. As predicted, Gly-1257 is incorporated in the hydrophobic cavity of the active site to fit cerulenin¹⁸⁾. 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 synthesis²⁰⁾

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



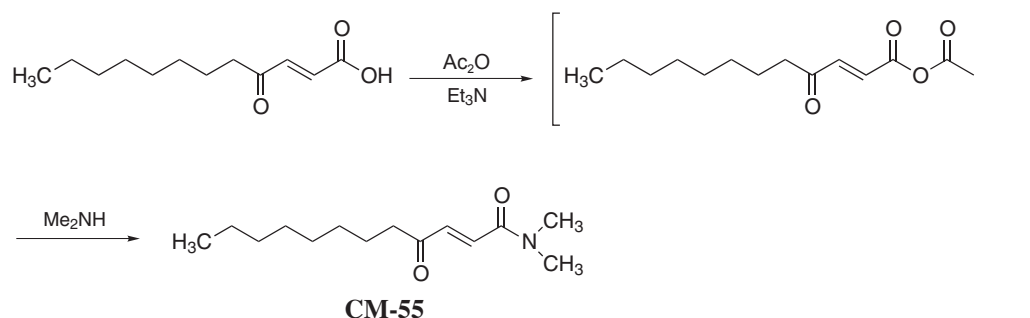
6. Research reagent as a chemical probe^{30-47,50-54)}

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 structure²¹⁾

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



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 $\mu\text{g/ml}$, see below)²¹⁾. The mode of action appears to be attributed to inhibition of acetyl-CoA by incorporation into the non-saponifiable fraction²²⁾.

Antimicrobial activity

Test organism	MIC ($\mu\text{g/ml}$)
<i>Bacillus subtilis</i> PCI 219	6.25
<i>Staphylococcus aureus</i> FDA 209P	3.12
<i>Mycobacterium smegmatis</i> ATCC607	6.25
<i>Escherichia coli</i> NIHJ	3.12
<i>Pseudomonas aeruginosa</i> P-3	>100
<i>Candida albicans</i>	12.5
<i>Pyricularia oryzae</i>	6.25
<i>Trichophyton rubrum</i>	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-55²³⁾.

8. Biosynthesis⁴⁹⁾

Biosynthesis of cerulenin is closely related with fatty acid synthesis.

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