5.451 F2005
Polyketide Biosynthesis
Type II Polyketide Biosynthesis: Aromatic Polyketides

Aromatic polyketides were at the start of the biosynthetic field
One of the first:

- **biomimetic synthesis (Collie, 1907)**
- **isotopic labeling studies (Birch, 1955)**
- **gene cluster sequence (Hopwood, 1984)**

Figures removed due to copyright reasons.

Please see: Fig.2A-D in *Top Curr Chem* 209 (2000): 1-51.

5.451 F2005
Polyketide Biosynthesis
Type II Polyketide Biosynthesis: Aromatic Polyketides

We now know that there are many variations of the polyketide pathway

Type I PKS: Catalytic domains are linked together on same polypeptide
Each catalytic domain is used once- "noniterative"
Recognized 1991

Type II PKS: Catalytic domains are individual proteins
A Catalytic domain is used for multiple claisen condensations "iterative"
Recognized 1984

Type III PKS: Similar to Type II
No ACP; all substrates are acyl-CoA
Called chalcone synthases and frequently found in plants
Recognized 1999

Iterative Type I PKS: Catalytic domains are linked together on same polypeptide and used iteratively
Recognized in last 2-3 years

Figure removed due to copyright reasons.

Please see Fig. 1 in *Current Opinion in Chemical Biology* 7 (2003): 285-295.

5.451 F2005
Polyketide Biosynthesis
Type II Polyketide Biosynthesis: Aromatic Polyketides

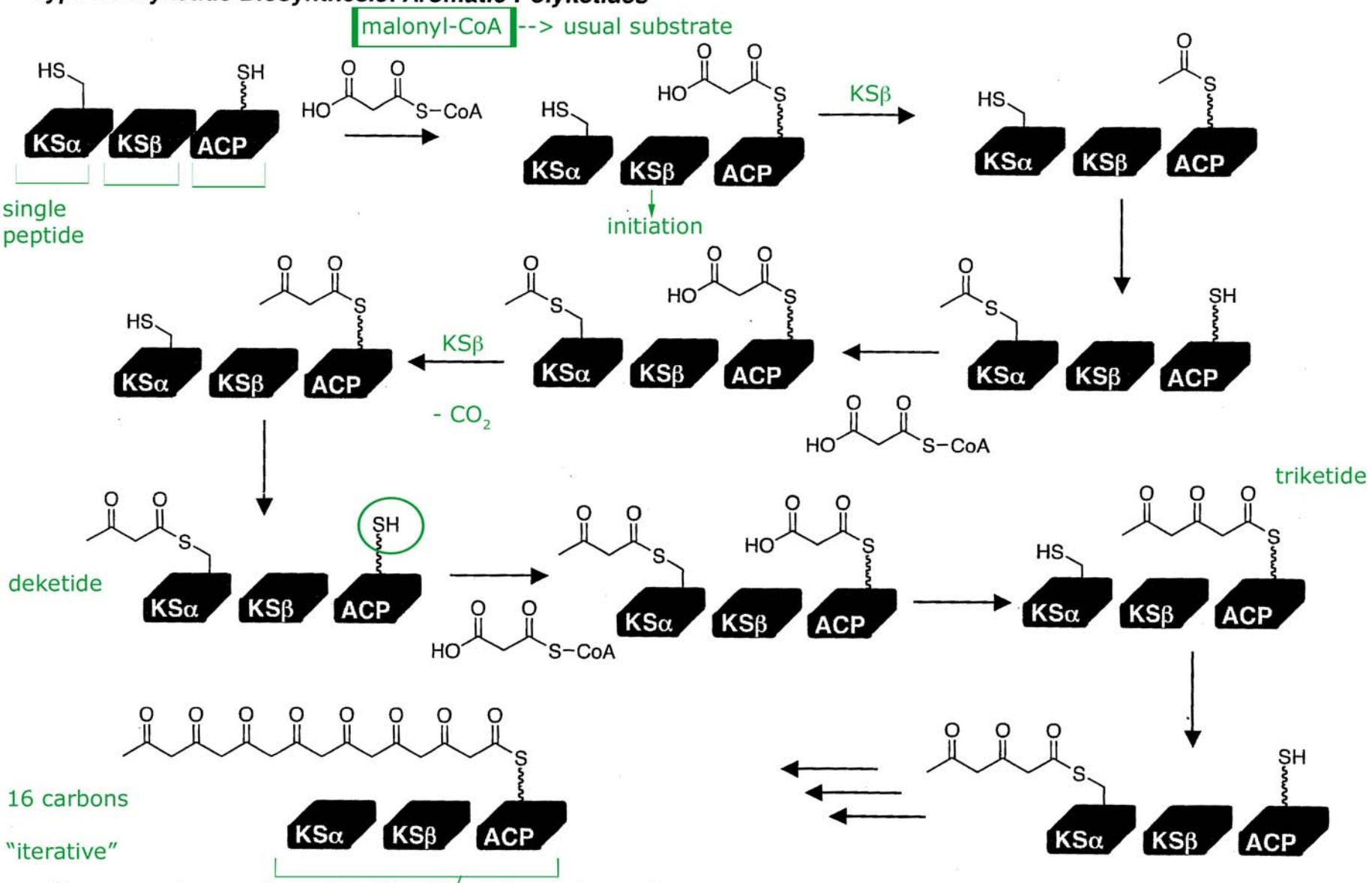
DNA of Type II PKS pathway Actinorhodin was sequenced in mid 1980's:

Inspired the search for the genes and was accomplished in 1984 by Hopwood
(this in turn inspired the search for the erythromycin genes)

Although the genes are known it is not completely clear how the enzymes work
(shown on next pages)- Enzymes observed were:
"Minimal" PKS: ACP, KS, KSβ chain length factor (CLF)
KR (ketoreductase) --> reduces carbonyl to hydroxyl
CYC (cyclase) like dehydratases
ARO (aromatase) CYC and ARO very similar --> same type of protein

Questions??
No AT domain (loading by either fatty acid AT, by KS or by ACP)
No TE domain
Regioselectivity of reduction/cyclization
Unknown function for second KS domain that lacks active site cysteine (KSβ or CLF (Chain Length Factor))
What controls the chain length?

5.451 F2005
Polyketide Biosynthesis
Type II Polyketide Biosynthesis: Aromatic Polyketides



Enzymes of aromatic polyketide biosynthesis bacteria separate proteins; fungi single protein

minimal polyketide synthase module

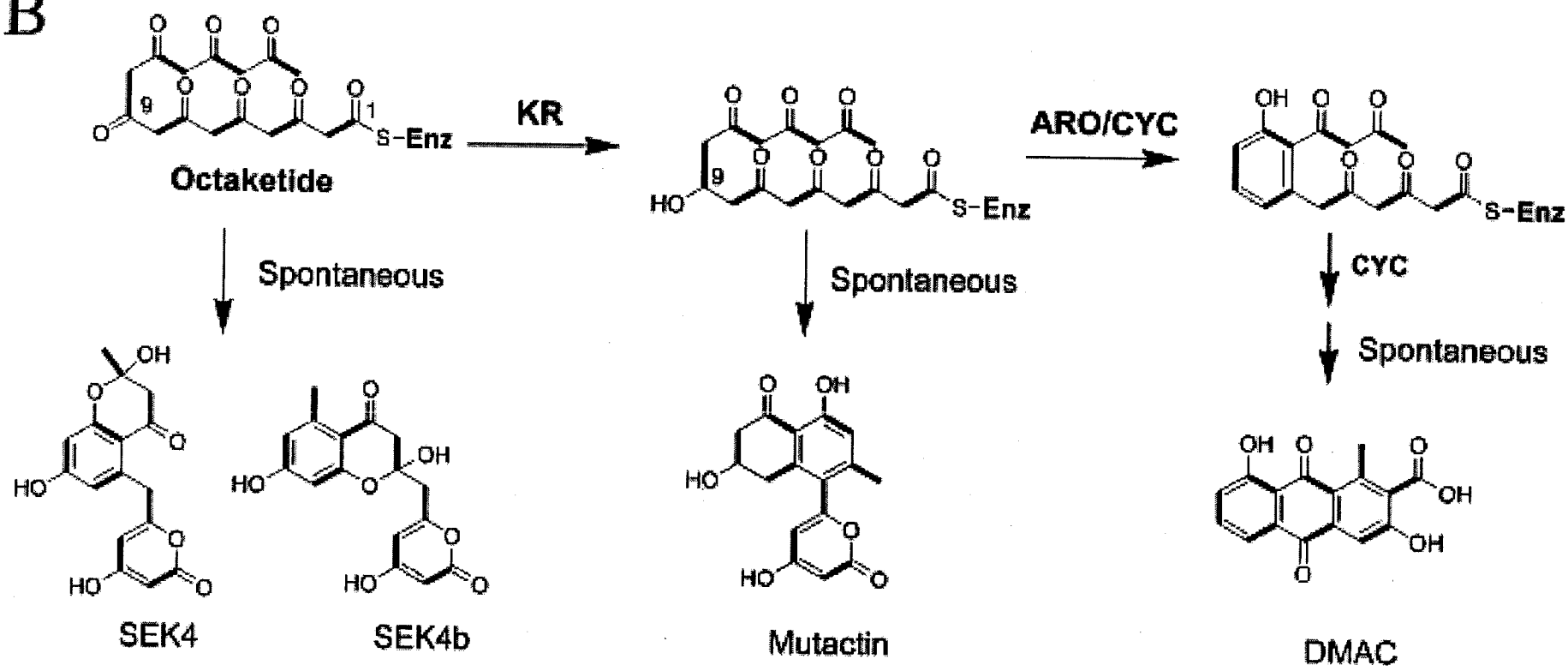
type II ACP domains have AT activity

Figure removed due to copyright reasons.
Please see Fig. 44 in *Nat Prod Rep* 18 (2001): 380-416.

5.451 F2005
Polyketide Biosynthesis
Type II Polyketide Biosynthesis: Aromatic Polyketides

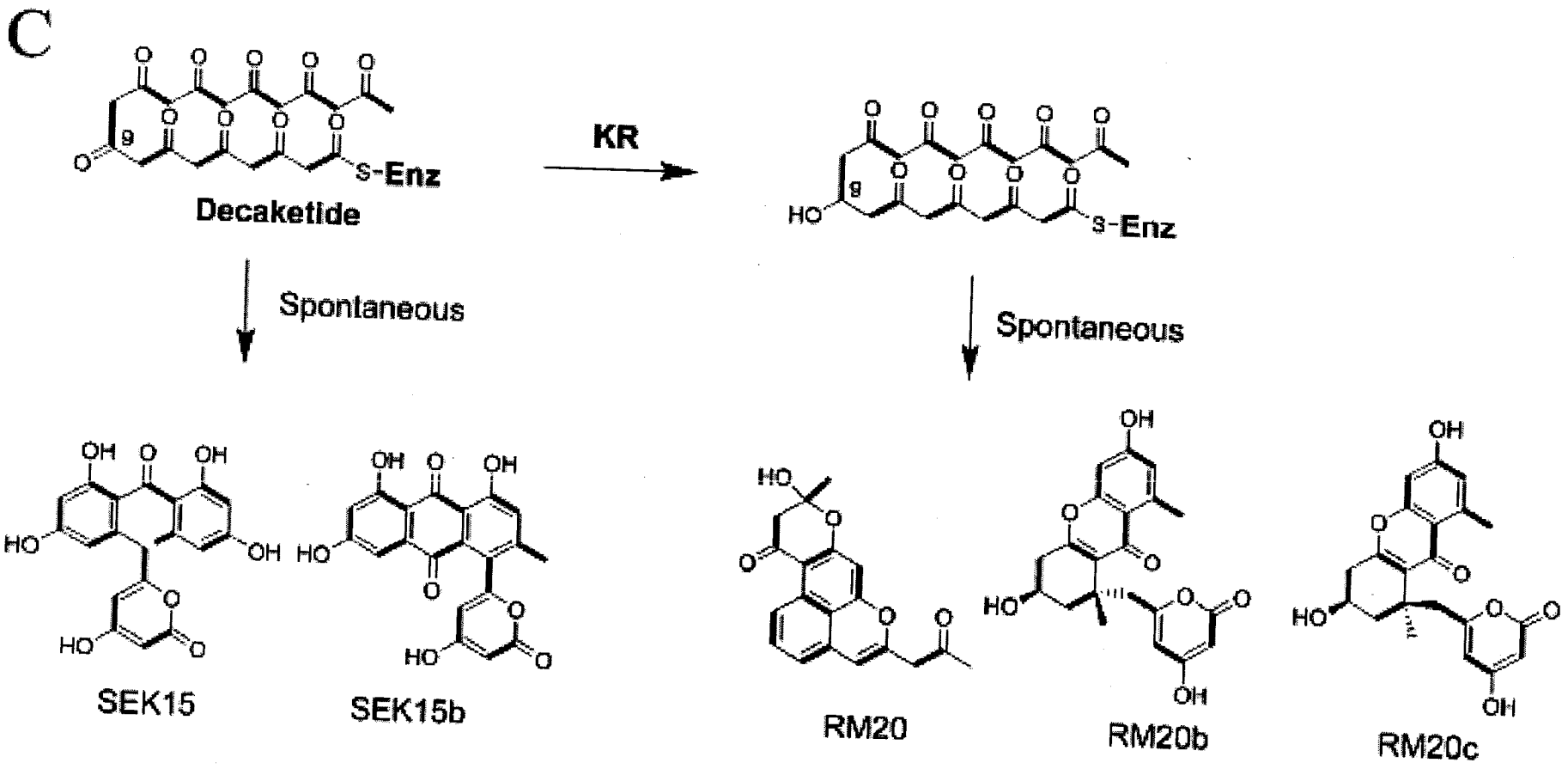
PLOS Biology 2004 2 0228

B



5.451 F2005
Polyketide Biosynthesis
Type II Polyketide Biosynthesis: Aromatic Polyketides

C



PLOS Biology 2004 2 0228

KR / reduction

changes way PK folds on itself

5.451 F2005
Polyketide Biosynthesis
Type II Polyketide Biosynthesis: Aromatic Polyketides

1. Where is AT domain?

   ACP has some endogenous AT activity, though perhaps not enough to support in vivo biosynthesis
   likely that an AT from FAS synthesis or elsewhere is recruited (Biochemistry (1998) 37, 2084-2088)

2. What controls chain length?

   KS forms a heterodimer: second protein called KSβ or CLF (Chain Length Factor)
   proof that this controls chain length

Crystal structure and mutagenesis studies suggest that a channel formed between the KS and CLF control the length of the polyketide

In vitro: mutate G116T mutation changes from 20 length to >65% C16

Figure removed due to copyright reasons.
Please see Fig. 1 in *JACS* 125 (2003): 12708-12709.

5.451 F2005
Polyketide Biosynthesis
Type II Polyketide Biosynthesis: Aromatic Polyketides

Where is the thioesterase?

self cleavage from the thioester (see novobiocin biosynthesis from NRPS section)

A set of "design rules" were proposed in 1995 (Nature (1995) 375, 549-554).
May be outdated

"Minimal PKS" sets the chain length- can switch out an ACP without affecting length but a KS and KSb from same cluster are required for consistent chain lengths K

A KR domain reduces carboyl to the hydroxyl (act reduces C9)

First ring formation appears to be formed from the minimal PKS (or is not enzymatically catalyzed)

Position of redution affects regioselectivity of cyclization
reduction at C9 cyclization at C7/C12; if reduce at C7 cyclize at C5/C10
(regiospecificity less well defined when reduction does not occur)

Aromatization domains will only recognize specific carbon length
act ARO recognizes only 16 carbon chains

KR --> each KR has own regioselectivity
--> timing of KR. --> sometimes before ARO/CYC
sometimes acts after ARO/CYC

Figure removed due to copyright reasons.
Please see Fig. 11 in *Top Curr Chem* 209 (2000): 1.

5.451 F2005
Polyketide Biosynthesis
Type II Polyketide Biosynthesis: Aromatic Polyketides

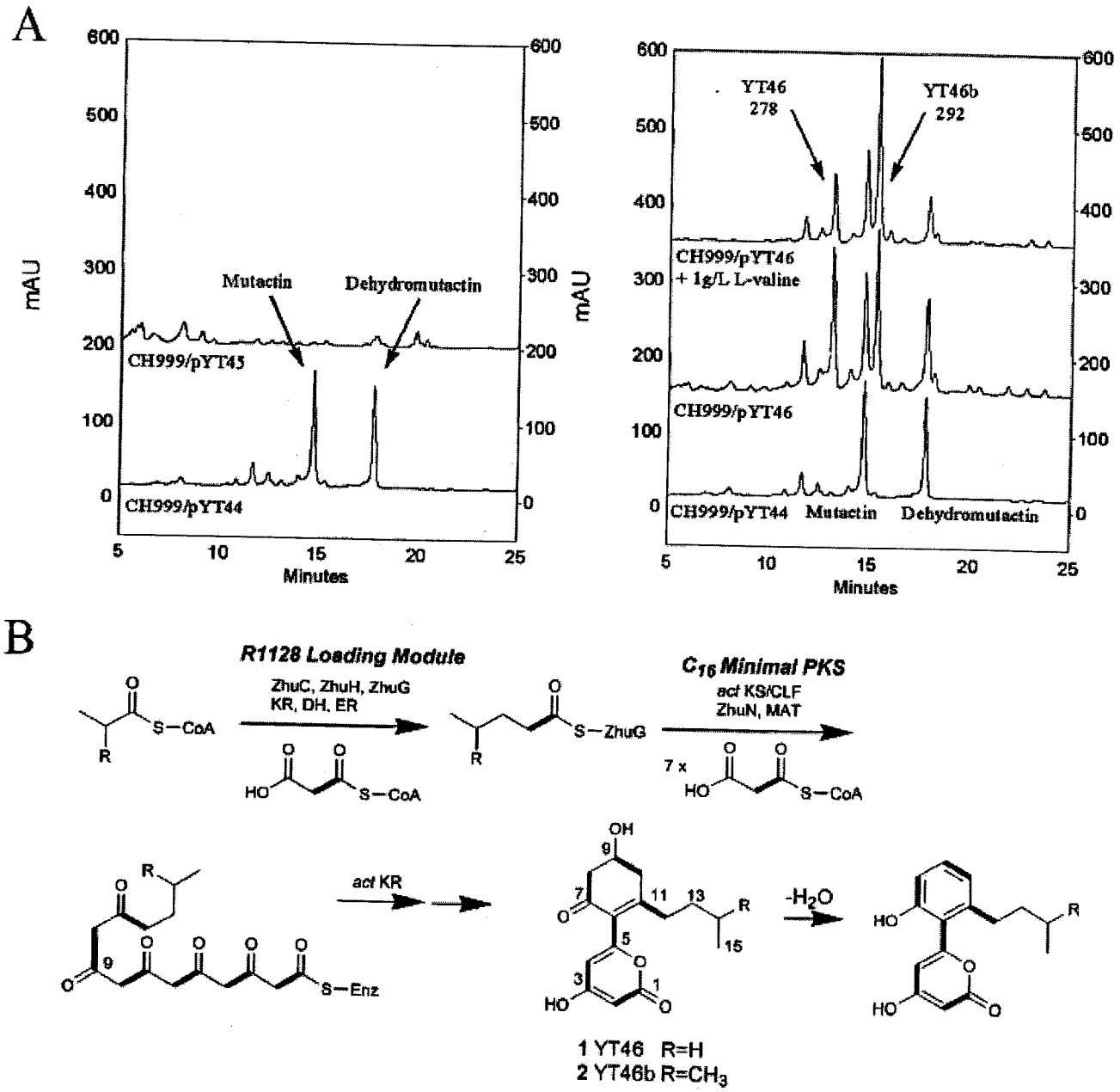
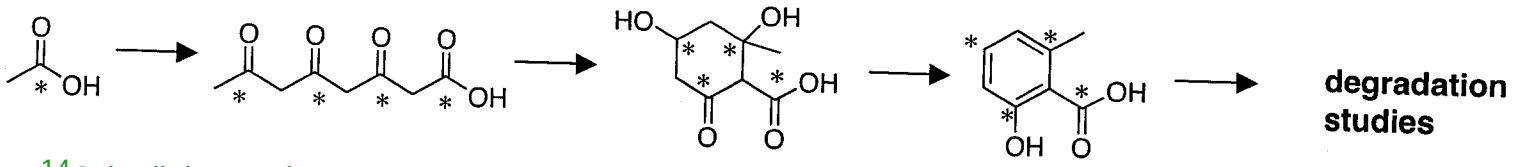


Figure courtesy of:
Tang, Yi, Taek Soon Lee, and Chaitan Khosla.
"Engineered Biosynthesis of Regioselectively Modified Aromatic Polyketides
Using Bimodular Polyketide Synthases." *PLoS Biology* 2, no.2 (2004): 0227-0238.

5.451 F2005
Polyketide Biosynthesis
Type II Polyketide Biosynthesis: Aromatic Polyketides

Birch (1954) recognized that polyketones can be made by condensation of acetates
What happens if *Penicillium patulum* are fed isotopically labeled acetates?



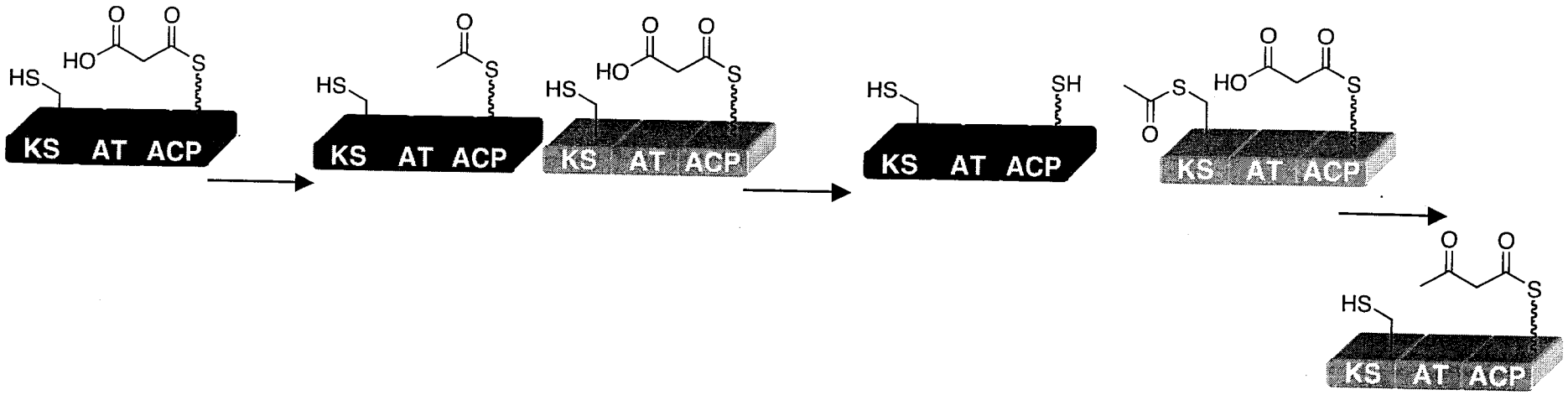
* = ^{14}C (radioisotope)

6-methyl salicylic acid

degradation studies

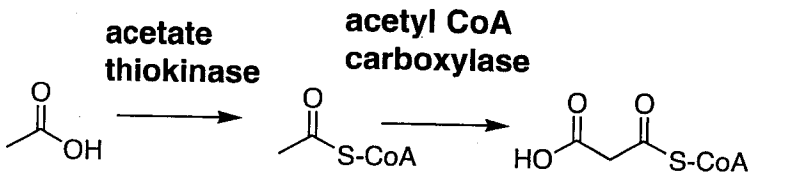
Support for Collie's 1907 hypothesis that aromatic compounds were made from polyketone structures in the cell

5.451 F2005
Polyketide Biosynthesis
Type II Polyketide Biosynthesis: Aromatic Polyketides



Labeled acetates can be converted into malonyl-CoA

starting material



immediate precursor

"Primary metabolism"

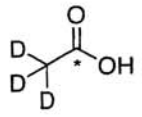
5.451 F2005
Polyketide Biosynthesis
Type II Polyketide Biosynthesis: Aromatic Polyketides

Labeling with 13C isotopes facilitated NMR studies

How does labeling work?
- **Indicates the starting material**
- **Provides structural information**

α field signal increases

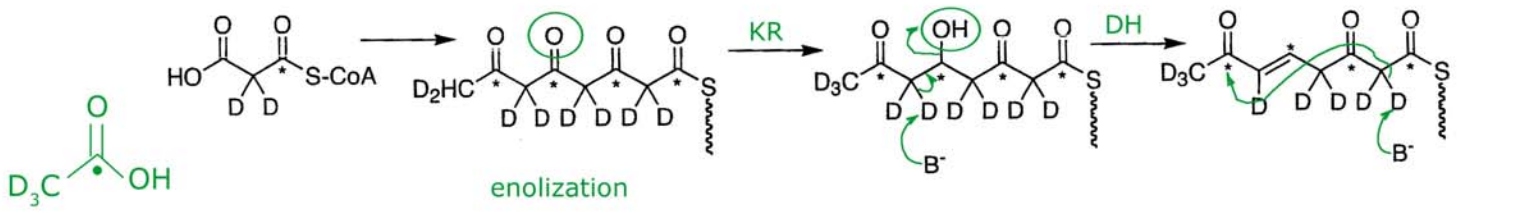
Assign unlabeled compound- then see which signals increase
Use non- decoupled 13C spectra to observe splitting from the protons



13C, 2H labeled acetate

upfield shift when a deuterium is attached to a C13 (alpha effect) or is one carbon removed (beta effect)

more pronounced — less pronounced



4 enriched signals
→ **8 = s**inglet
2 = d
4 = d
6 = d

6-methyl salicylic acid

BH $^{-}$A

A = acid, B = base

5.451 F2005
Polyketide Biosynthesis
Type II Polyketide Biosynthesis: Aromatic Polyketides

Double label enables observation of 13C-13C coupling
Isotopically labeled substrate becomes diluted- only one 13C acetate gets incorporated per structure
Therefore, only observe coupling between 2 carbons that belong to the same acetate

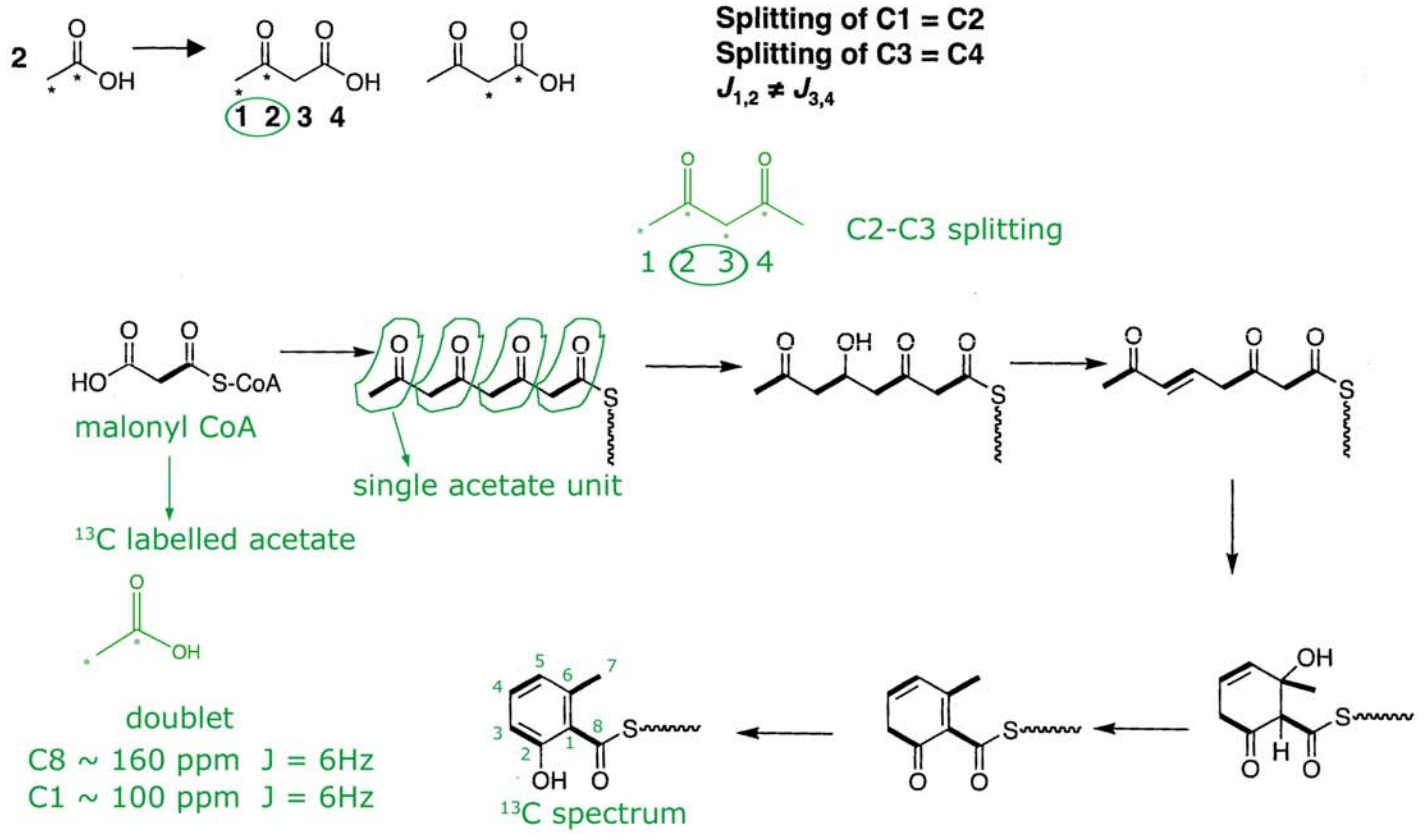


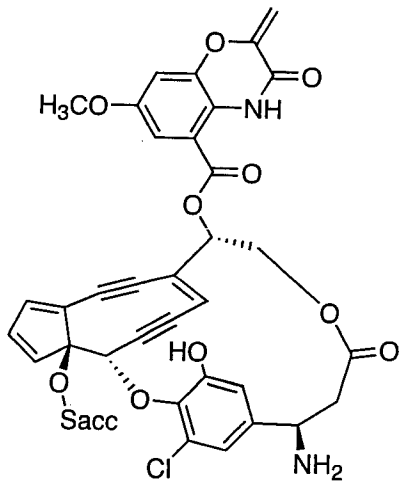
Figure removed due to copyright reasons.
Please see Fig. 8 in *Nat Prod Rep* 18 (2001): 380-416.

Content removed due to copyright reasons.

Please see: Mann, John. *Chemical Aspects of Biosynthesis*. New York, NY: Oxford University Press, 1994, pp. 22-23, 26-27.

5.451 F2005
Polyketide Biosynthesis
Type II Polyketide Biosynthesis: Aromatic Polyketides

Gene clusters of calicheamycin and C-1027 have been shown to made up of polyketide synthases
Feeding studies in 1989 established an acetate starting material
(Science (2002) 297, 1170, 1173)



C-1027

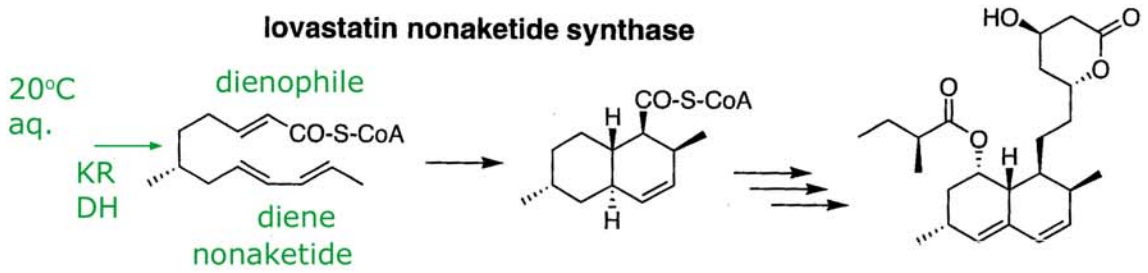
Figure removed due to copyright reasons.
Please see Fig. 23 in *Nat Prod Rep* 18 (2001): 380-416.

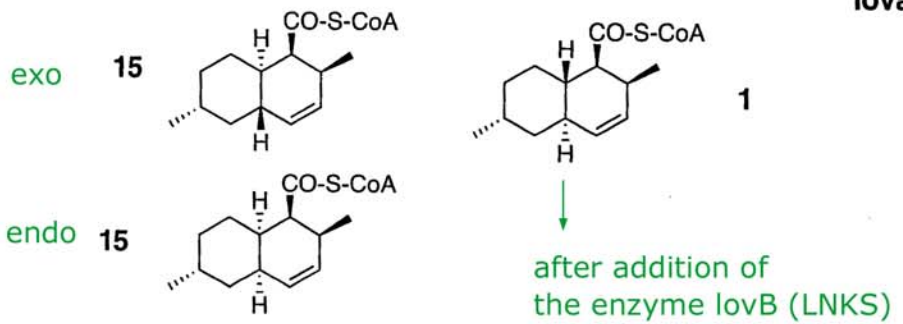
5.451 F2005
Polyketide Biosynthesis
Type II Polyketide Biosynthesis: Aromatic Polyketides

Diels Alderase?

Has nature discovered the Diels-Alder reaction??

lovastatin nonaketide synthase





after addition of
the enzyme lovB (LNKS)

Rate enhancement
without $t_{1/2}$ 60h
with k_{cat} ~$4s^{-1}$

ChemBioChem (2001) 2, 873-875
JACS (2000) 122 11519-11520

Figure removed due to copyright reasons.
Please see Scheme 1 in *J Biol Chem* 275 (2000): 38393-38401.

Figure removed due to copyright reasons.
Please see Scheme 1 in *ChemBioChem 4* (2003): 713-715.

Figure removed due to copyright reasons.
Please see Fig. 6-13 in *Nat Prod Rep 18 (*2001): 380-416.