論文内容の要旨

応用生命化学　専攻
平成 15 年度博士課程　入学
氏名　Md. Sadequl ISLAM
指導教員名　渡邉　秀典

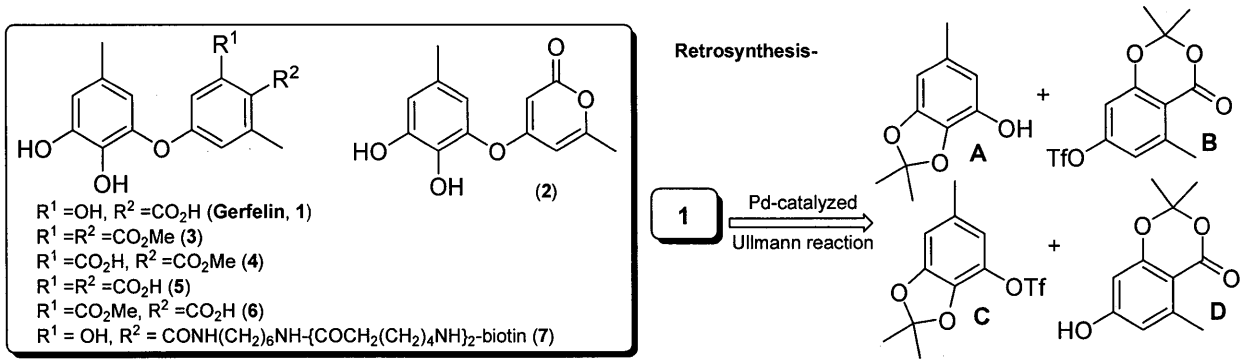
論文題目

Synthetic Studies on Bioactive Phenolic Polyketides
(生物活性を有するフェノール性ポリケチドの合成研究)

Polyketides are structurally a very diverse family of natural products with an extremely broad range of biological activities and pharmacological properties. In the first chapter, synthesis of **Gerfelin** and its analogous compounds; whereas in the second chapter, synthesis of **(+)-Ramulosin**, **(−)-Mellein** and some of related naturally occurring compounds will be discussed.

1. Synthesis of gerfelin and related analogous compounds

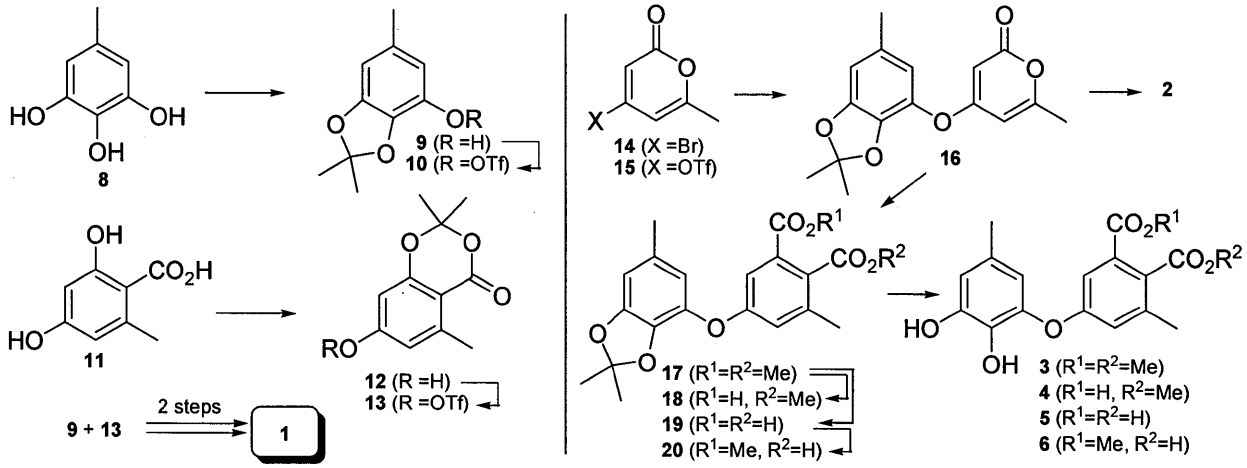
In the course of screening for an inhibitor of geranylgeranyl diphosphate (GGPP) synthase, Imoto and his co-workers have isolated gerfelin (**1**) from a fungal strain, *Beauveria felina* QN22047. For the biological activity they performed an *in vitro* GGPP synthase assay and reported that gerfelin inhibited GGPP synthase activity with an IC_{50} of 3.5 μg/ml. It also inhibits the farnesyl diphosphate (FPP) synthesizing activity of GGPP synthase. I felt interest in this simple structure with an interesting bioactivity and started the synthesis of **1** and its analogs to know the structure-activity relationship (SAR).



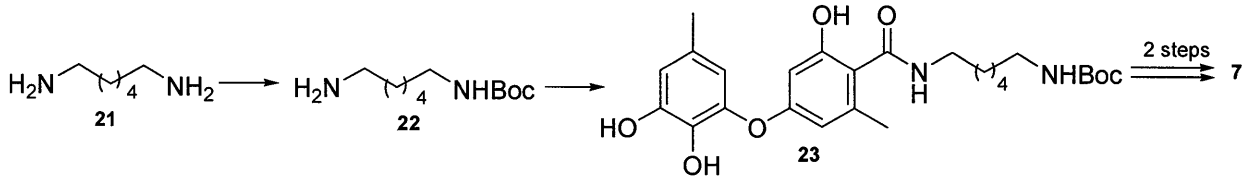
It would be possible to form an ether linkage by the Pd-catalyzed Ullmann diaryl ether synthesis between segments **A** and **B** (or **C** and **D**) which is shown in the retrosynthesis of gerfelin. For the preparation of the left-hand segment, 3,4,5-trihydroxytoluene (**8**) was converted into acetonide

phenol **9** and also into triflate **10**. On the other hand, the right-hand segments (**12** and **13**) were prepared from the known 2,2-dihydoxy-6-methylbenzoic acid (**11**) by acetalization and triflation.

With both the left- and right-hand segments in hand, firstly I investigated coupling between **9** (≡**A**) and **13** (≡**B**). The use of bulkier ligand, 2-(di-*tert*-butylphosphino)biphenyl instead of triphenylphosphine for Pd catalyzed diaryl ether reaction dramatically improved the yield of coupled product. On the other hand, another combination of the triflate and phenol [**10** (≡**C**) and **12** (≡**D**)] afforded no desired product even when bulkier ligand was used. Finally, treatment of coupled product with aq. TFA afforded gerfelin (**1**).



To synthesize some related analogs (**2**–**6**) of gerfelin, I treated bromide (**14**) or triflate (**15**) with **9** under the basic condition for Michael addition-*β*-elimination to afford **16**. Diels-Alder-decarboxylated product **17** was obtained by reaction of **16** with dimethyl acetylenedicarboxylate. Dimethylester **17** was also converted to half methyl ester **18**, diacid **19** and another half ester **20**. Acid catalyzed hydrolysis of the acetonide groups of **16**–**20** afforded **2**–**6**, respectivly. To synthesize biotinylated derivative of gerfelin (**7**), I adopted diamine **21** as a starting material. Boc-protected amine **22** was coupled with gerfelin to give **23** from which amine-protecting group was removed and reacted with biotinylating agent [Biotin-{$NH(CH_2)_4CH_2CO$}$_2$-SE] to afford gerfelin-biotin conjugate (**7**).

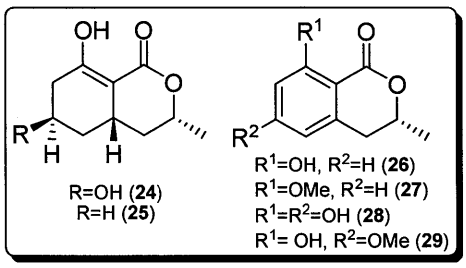


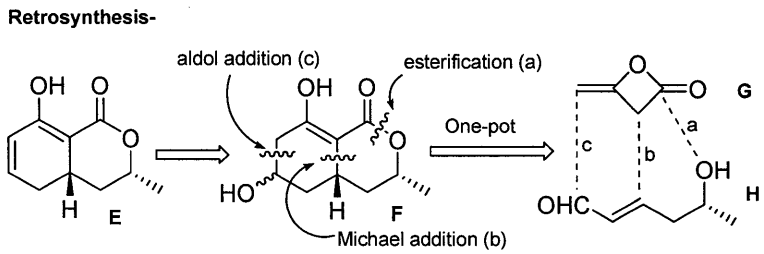
Inhibitory activities of the synthetic gerfelin and its analogs (**2-5**) were estimated by *in vitro* geranylgeranyl diphosphate synthase assay. Synthetic gerfelin showed similar bioactivity to the natural one; and analogs **3-5** were found to be two to four times less active than gerfelin but **2** exhibited no bioactivity. Although the activity of biotinylated gerfelin analog **7** has not been performed yet, the intermediate gerfelin-amine **23** showed interesting results of activity.

2. Synthesis of (+)-ramulosin, (–)-mellein and related natural compounds

(+)-Ramulosin (**25**) and (+)-6-hydroxyramulosin (**24**) were isolated from *Pestalotia ramulosa*, *Botrytis sp.* and also from some other sources. Their bioactivity is reported as an inhibition of germination of seeds and spores of microorganisms and antimicrobial activity. (−)-Mellein (**26**) was isolated from different source including many fungi and several insects after it's first isolation from *Aspergillus melleus* by Nishikawa in 1933. It is the part of defense secretions in ants and termites. Isolation of (–)-mellein methyl ether (**27**) was reported from *Septoria nodorum*.

(–)-6-Hydroxymellein (**28**) was isolated from a wide range of natural source including *Daucus carota*, *Azadirachta indica*, *Ceratocystis minor* etc. and (−)-6-methoxymellein (**29**) was first isolated by Sondheimer, E. in 1957 from carrots which had developed a bitter taste during storage. Coxon *et al.* in 1973 and Superchi *et al.* in 1993, described a differential effect of the dihydroisocoumarins (**28** and **29**) to the brine shrimp (*Artemia salina*) and Chinese hamster cells, respectively. ED_{50} values of **28** and **29** were 0.66 and 0.43 mM for *Artemia salina* & 0.66 and 0.46 mM for Chinese hamster cells. To investigate the differences in the toxicity as well as in understanding the physiological and metabolic correlation between **28** and **29** to the producing carrot cells, Marinelli *et al.* in 1996, reported that ED_{50} values for these were 0.07 and 0.032 mM, respectively.

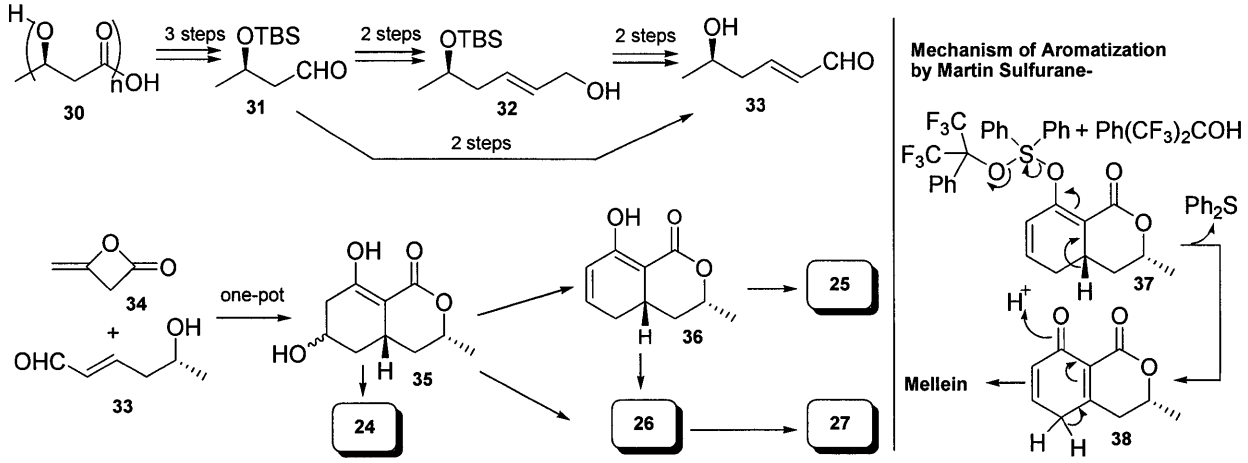




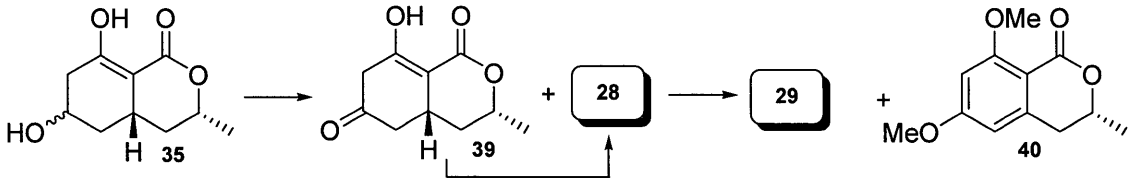
In the present study, I employed one-pot esterification-Michael addition-aldol reaction as shown in the retrosynthesis. To synthesize the one-pot component (*R*,*E*)-5-hydroxy-2-hexenal **33** (≡**H**), I have treated poly-(*R*)-3-hydroxybutyrate (**PHB**, **30**) for sequential reactions; ethanolysis, TBS protection of the hydroxyl group and then DIBAL reduction to afford aldehyde **31**. Reaction of **31** for Wittig olefination ($Ph_3P{=}CHCO_2Me$), and then DIBAL reduction, MnO_2 oxidation and finally removal of TBS gave **33**. To shorten the steps, I applied aldehyde **31** for Wittig olefination ($Ph_3P{=}CHCHO$) and then removal of TBS to afford **33**.

The next approach was one-pot reaction to the desired bicyclic diol **35** (≡**F**), which was successful through esterification-Michael addition-aldol reaction of (*R*,*E*)-5-hydroxyhex-2-enal (**33**) and diketene (**34**). Performing a dilution-controlled one-pot reaction (0.028 M solution of **33** in

benzene) afforded the best yield (74%) for **35** and result is reproducible. PTLC separation of the diastereomers (**35**) afforded (+)-6-hydroxyramulosin (**24**). To dehydrate **35**, I treated it with a various dehydrating agents but best yield of the intermediate diene **36** (≡**E**) was obtained in high yield (74%) by Martin sulfurane. Selective reduction or aromatization of **36** affored (+)-ramulosin (**25**) or (−)-mellein (**26**), respectively. The conversion of phenolic OH group of mellein to methoxy group afforded (−)-mellein methyl ether (**27**).



Interestingly, when **35** was treated with excess amount of Martin sulfurane (~3 eq.) it afforded (−)-mellein (72%) just by one-step. Therefore, I felt interest to know what is happening for aromatized. To elucidate the mechanism, I have treated the diene **36** with Martin sulfurane which successfully afforded (−)-mellein. I have isolated and characterize the byproduct diphenyl sulfide which result strongly suggested the mechanism shown in the scheme.



Jones oxidation of **35** gave mixture of ketone (**39**) and (−)-6-hydroxymellein (**28**). The conversion of 6-OH group of **28** to a methyl ether afforded (−)-6-methoxymellein (**29**) along with an overmethylated compound (**40**).

3. Summary

Gerfelin (**1**), a novel inhibitor of geranylgeranyl diphosphate synthase, was synthesized for the first time and in a convenient feasible way (3 and 4 steps) where overall yield was 43.6% (total 5 steps). For the SAR study, I have also synthesized six analogous compounds of gerfelin (**2-7**). In chapter 2, I have described convenient synthetic routes for six naturally occurring 3,4-dihydroisocoumarins (**24-29**). Overall yields were as following: 49% (5 steps) for **24**, 29.3% (7 steps) for **25**, 35.2% (7 steps) for **26**, 35.2% (8 steps) for **27**, 3.9% (6 steps) for **28**, 3.5% (7 steps) for **29**.