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Synthetic studies on phenazine antibiotics and antheridic acid

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Abstract; In order to synthesize phenazine antibiotics with complex N-alkyl substituents, various types of activated alkylating reagents with or without catalysts were examined to alkylate 1-trialkylsiloxyphenazine **4**. Among them, halides-silver triflate and *in situ* prepared triflates from alcohol and triflic anhydride were reagents of choice. Lavanducyanin **2** was synthesized via this procedure. The synthesis of benthocyanin A **10** is in progress. Starting from a chiral bicyclic building block **18**, enantioselective synthesis of a pentacyclic antheridan skeleton was developed and the total synthesis of antheridic acid **17** is under investigation.

We discuss herein the synthetic studies on phenazine antibiotics with complex N-alkyl derivatives and antheridic acid, antheridium inducing factor of fern.

SYNTHESIS OF N-ALKYL-1-PHENAZINONES

Phenazines are divided into two groups and the major one is that without N-alkyl substituent. The minor group is N-substituted phenazine and the simplest analog is pyocyanin **1** with weak antibiotic activity. Recently, phenazinones with more complex side chains were isolated and they show remarkable bioactivities. Lavanducyanin (2) or WS-9659A **2** (3) was isolated from *Streptomyces* species and has interesting antitumor and enzyme-inhibiting activities. Phenazinomycin **3** was also isolated from a *Streptomyces* and has antileukemic activity (4). Both of these phenazines contain mono- and sesquiterpenes. Because of their unique structures and bioactivities, we studied the synthesis of them and related analogs to evaluate their biological functions.

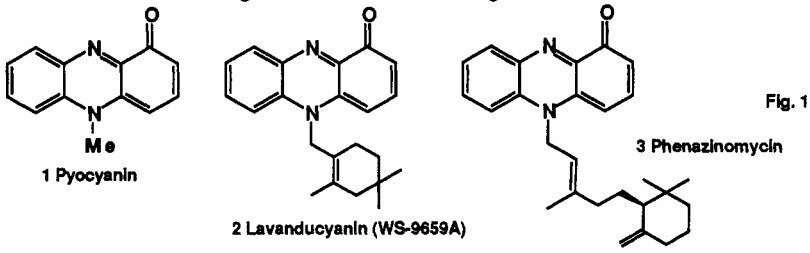
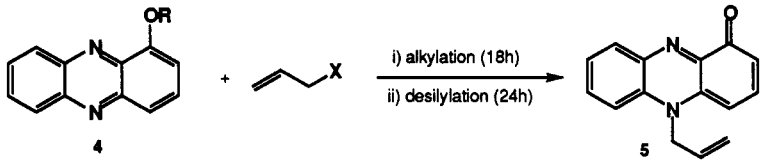


Fig. 1

Direct alkylation of 1-hydroxyphenazine or its derivative was examined and in order to promote alkylation either catalysts was added to activate halides or reactive leaving group like triflate was employed. Phenolic OH is protected with silyl group to prevent O-alkylation. Treatment of TES ether **4a** with allyl bromide-silver triflate (entry 1) did not give any phenazinone **5**. Reaction of TBS ether **4b** with triflate *in situ* prepared from allyl alcohol and triflic anhydride (entry 2), however, afforded the desired phenazinone **5**. Although the yield was extremely low, it was realized that direct alkylation is promising route to afford N-substituted phenazinones under mild condition. Unreacted hydroxyphenazine was recovered almost quantitatively. The yield of **5** greatly improved up to 23% by using TASF instead of TBAF (entry 3).

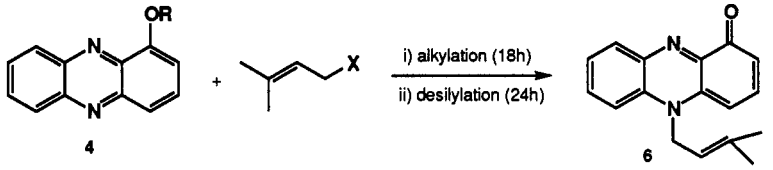


4 5

Fig. 2

Entry	R	X	Reagent for Alkylation	Temp.	Desilylation	Yield
1	TES	Br	CF_3SO_3Ag/CH_2Cl_2	-70°C~rt	-------	~0%
2	TBS	OH	Tf_2O, 2,6-t-Bu_2-4-MeC_5H_2N CH_2Cl_2	-70°C~rt	TBAF	3.2%
3	TBS	OH	Tf_2O, 2,6-t-Bu_2-4-MeC_5H_2N CH_2Cl_2	-70°C~rt	TASF	23%

Next, prenylation was examined (Fig. 3). Treatment of TBS ether **4b** with prenyl bromide and two equivalents of silver triflate (entry 1) or with silver fluoroborate (entry 2) gave prenylphenazinone **6**. When TES ether **4a** was used as a substrate, both methods of triflic anhydride-alcohol and silver triflate-bromide gave **6** in slightly higher yield (entry 3, 4). In the case of entry 5, reaction temperature was fixed at -25℃ and the yield was improved. Major reaction pathway of alkylating reagent might be elimination. Optimization of alkylation step using this model system to suppress elimination and to promote alkylation is under investigation.

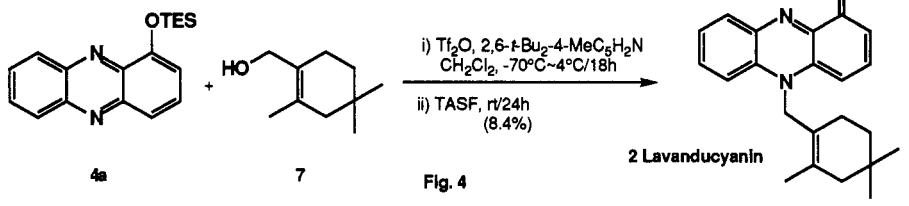


4 6

Fig. 3

Entry	R	X	Reagent for Alkylation	Temp.	Desilylation	Yield
1	TBS	Br	CF_3SO_3Ag/CH_2Cl_2	-70°C~rt	-------	9.7%
2	TBS	Br	$AgBF_4/CH_2Cl_2$	-70°C~rt	TBAF	9.7%
3	TES	Br	CF_3SO_3Ag/CH_2Cl_2	-70°C~rt	-------	15%
4	TES	OH	Tf_2O, 2,6-t-Bu_2-4-MeC_5H_2N CH_2Cl_2	-70°C~rt	TASF	11%
5	TES	OH	Tf_2O, 2,6-t-Bu_2-4-MeC_5H_2N CH_2Cl_2	-25°C	TASF	17%

Synthesis of lavanducyanin was then executed. Treatment of TES ether **4b** with cyclolavandulol and triflic anhydride gave lavanducyanin **2** in 4.5% yield. IR and NMR spectra of authentic and synthetic lavanducyanin are essentially identical. Thus we completed concise synthesis of lavanducyanin via direct alkylation under mild condition, although the yield of alkylation step should be improved.



4a 7

Fig. 4

SYNTHESIS OF N-ALKYLPHENAZINES WITH ANTIOXIDANT ACTIVITY

Benthocyanins A~C **8~10** are the other type of N-alkylphenazines, which were isolated from *Streptomyces prunicolor* as potent antioxidant and free radical scavenger and they are 30 to 70 times stronger than tocopherol (5). Interestingly, it was shown that these phenazines enhance interleukin 2 activity of macrophage. Unfortunately, original *Streptomyces* does not produce these phenazine antibiotics any more, because of mutation. Thus, the synthesis of these phenazines to afford substantial amount of materials for further biological evaluation was investigated.

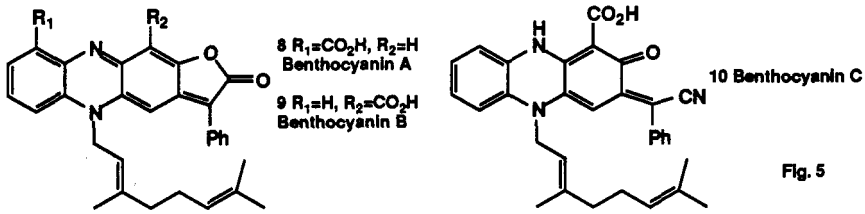


Fig. 5

We found direct formation of phenazine **14** just by refluxing an equivalent amount of bromobenzoic acid **11** and *m*-amino phenol with base in pentanol (6). Probably, initially formed nitro-amine **13a** spontaneously cyclized because of the presence of reactive phenoxide ion to give phenazine N-oxide **13b**, which was reduced to afford phenazine **13c** via disproportionation. Phenolic OH must have played key role again.

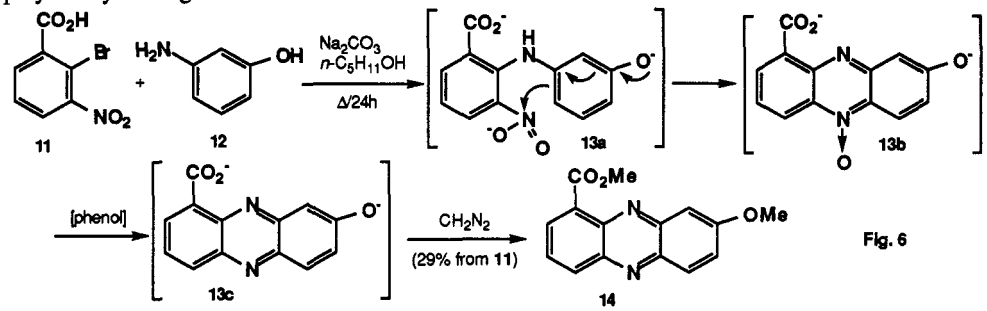


Fig. 6

Heating the phenazine **14** with methyl phenylbromoacetate in the presence of excess aluminum chloride and the following diazomethane treatment gave the diester **15** exclusively. ^{1}H-NMR showed three singlet peaks and therefore the product **15** should have 1,2,4,5-tetrasubstituted aromatic system. Hydrolysis of the diester with concentrated hydrobromic acid gave the lactone-ester **12** with whole skeleton. The final alkylation step via silyl ketene acetal to afford benthocyanin A **8** is underway.

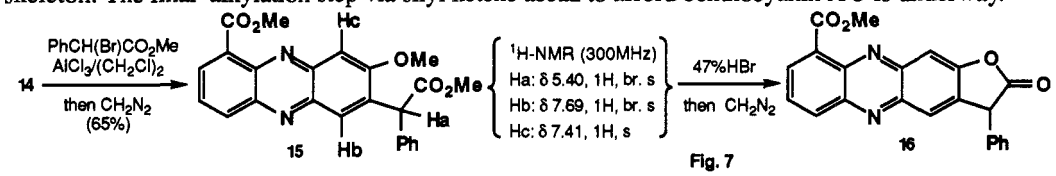
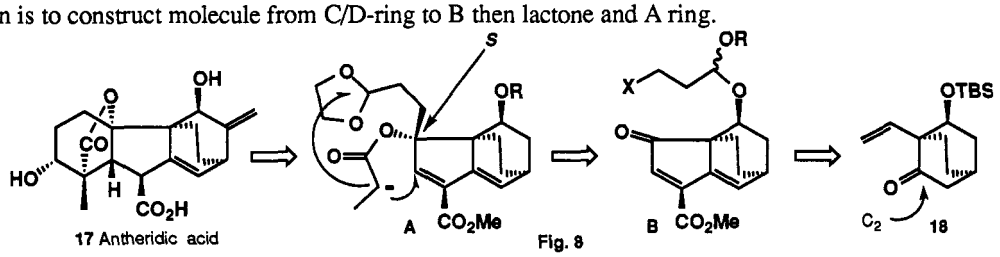


Fig. 7

SYNTHETIC STUDIES ON ANTHERIDIC ACID

Antheridiogen is a substance to induce germination and development of male sex organ, antheridium, to spores of fern. In 1971, Nakanishi and co-workers first identified antheridic acid (antheridiogen-An) **17** from *Anemia phyllitidis*. (7). Its structure was revised partly as shown below by Corey's first total synthesis of (±)-**17** (8, 9). We have been studying the enantioselective synthesis of **17**. Synthetic plan is to construct molecule from C/D-ring to B then lactone and A ring.



17 Antheridic acid

Fig. 8

Our synthesis was started from a bicyclic chiral building block **18** derived via the asymmetric reduction with baker's yeast (10). Ethoxyethynylalaneadded efficiently to the enone to give a diene-ester via acid rearrangement, which was transformed to a precursor **19** for cyclization. Base treatment gave the conjugated ester **20**. Epoxidation and the following mild acid treatment with either deutero

chloroform or 0.01M solution of hydrochloric acid in chloroform gave allylic alcohol, which was converted to the desired hydroxy-dienone **21**.

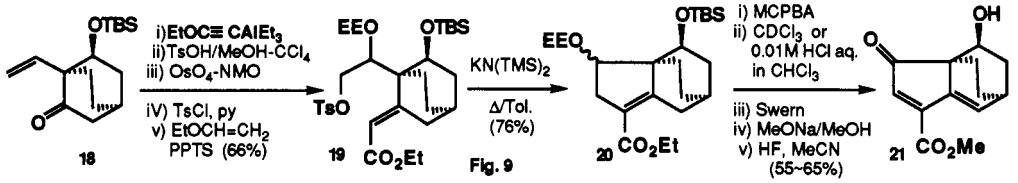


Fig. 9

After unsuccessful C3-extension, 6-membered ring formation was examined. Intramolecular Reformatsky reaction of bromoacetate **22** by heating with activated magnesium afforded the expected δ-lactone **23** as a sole product, which afforded the precursor **24** for radical cyclization in 3 steps. Heating **24** with tributyltin hydride in benzene gave γ-lactone **25**. Acetal cleavage via thioacetal and the Wittig reaction underwent smoothly to give thiovinyl ether **26**, which was converted to homologous aldehyde **27**.Basic treatment of the aldehyde under deoxygenated medium gave the desired pentacyclic system **28**. Although this equilibrium tends to be as open form, we reached to the antheridane skeleton. Stepwise functional group modification gave the pentacyclic ketone **29**, which is a double bond regio-isomer of Corey's intermediate. Completion of the synthesis is now in progress.

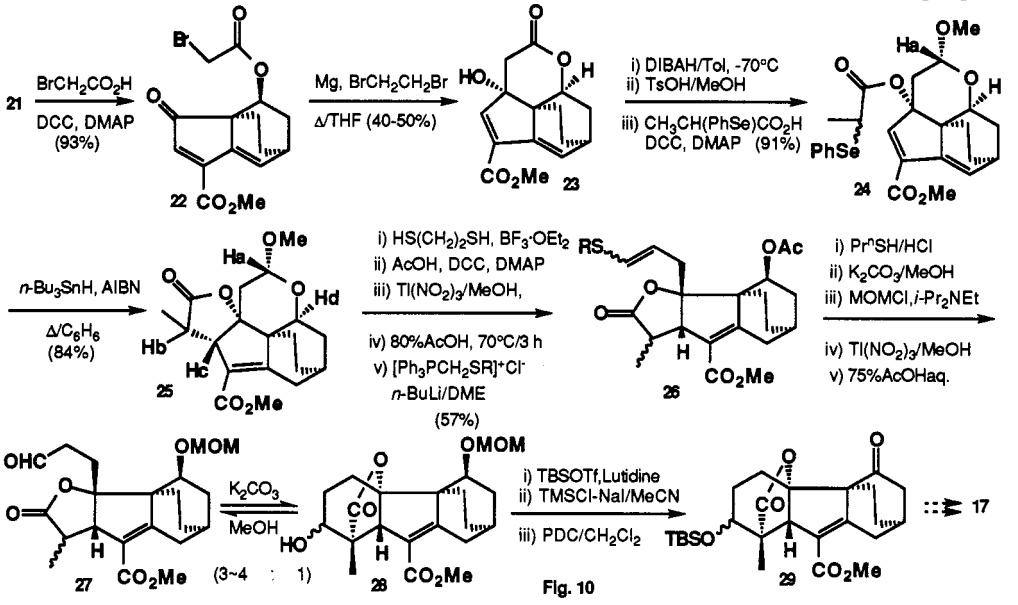


Fig. 10

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