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Stereoselective synthesis of daunomycinone and its analogues

Fu, Xiaoping, Ph.D.

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U·M·I
300 N. Zeeb Rd.
Ann Arbor, MI 48106

STEREOSELECTIVE SYNTHESIS OF DAUNOMYCINONE AND ITS ANALOGUES

by

Xiaoping Fu

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Stereoselective Synthesis of Daunomycinone

and its Analogues

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Xiaoping Fu

SUPERVISORY COMMITTEE:

	APPROVED	DATE
Signature	Desmond M. S. Wheeler	Feb 1 1993
Typed Name	Dr. Desmond M. S. Wheeler	
Signature	Carolyn Price	2/1/93
Typed Name	Dr. Carolyn Price	
Signature	Henry Baumgarten	2/1/93
Typed Name	Dr. Henry Baumgarten	
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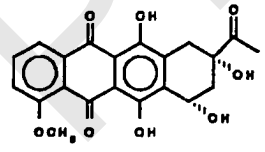
by XIAOPING FU, Ph.D

University of Nebraska, 1993

Advisor : DESMOND M. S. WHEELER

Total synthesis of doxorubicin has attracted the attention of many synthetic chemists. Daunomycinone (**1**), a key fragment of doxorubicin, has been synthesized in many different ways. The basic strategies of these approaches involve (1) anthraquinone approaches with ring A synthesis (DCBA), (2) juglone approaches with ring B formation (DCAB), (3) tetralone approaches with construction of ring C (ABDC) and (4) formation of ring D (ABCD).

Wheeler and co-workers have worked on the synthesis of daunomycinone for more than a decade. This work has involved a synthesis using the DCAB approach with a stereochemically fixed ring A and a stepwise connection of ring B in controlling the stereo- and regiochemistry in the total synthesis.



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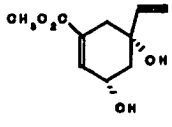

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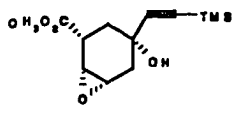

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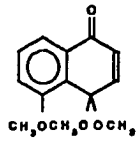

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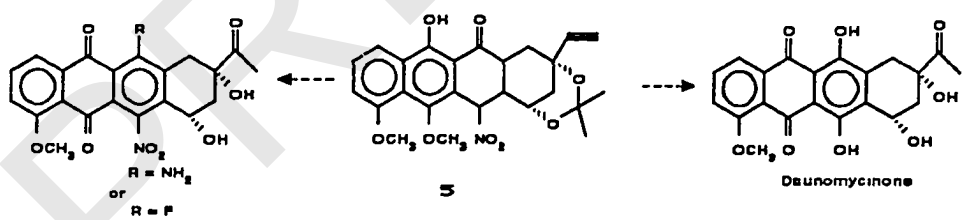
Stereoselective synthesis of ring A (**2**) was completed in 5 steps and 70% overall yield from *m*-anisic acid. The key reaction involved in the synthesis of ring A was the

Sharpless epoxidation of **3**. Use of molecular sieves reduced a number of by-products from the epoxidation and improved the reaction yield. It was also found that the addition of nitromethane to ring A precursor (**2**) was catalyzed by hydrogen bond acceptors, such as DMSO, Me_3NO and Ph_3PO.

Regioselective synthesis of the ring CD precursor (**4**) was completed in 3 steps and 50% overall yield from 1,5-dihydroxynaphthalene. This synthesis involved as a key step an oxidation of 5-methoxynaphthol by iodobenzene diacetate.

The tetracyclic system (**5**) was synthesized from (**4**) and the acetonide of the adduct of **2** and nitromethane by a stepwise Michael addition, Dieckmann cyclization and demethanolation. The Nef reaction of transforming the nitro group to ketone failed. Alternatively, oxidative aromatization of **5** and manipulation of functional groups gave the daunomycinone in 13 steps and 6.4% overall yield. The oxidative aromatization by cerium ammonium nitrate (CAN) and a catalytic amount of dichlorodicyanoquinone (DDQ) provided a new feature in which the organic oxidizing reagent was regenerated by an inorganic oxidizing reagent (e.g. CAN) *in-situ*.



Daunomycinone and several of its analogues were synthesized from **5**. 6-Desoxy-6-nitrodaunomycinone was synthesized in 12 steps from *m*-anisic acid. We have also converted **5** into 6,11-diamino-6,11-didesoxy, 11-amino-6,11-didesoxy-6-nitro and 6,11-didesoxy-11-fluoro-6-nitrodaunomycinones.

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——郑板桥

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Chapter One

Introduction to Anthracyclines

1. DISCOVERY

The first anthracycline was discovered in 1939 by the Russian scientists Krassilhikov and Koveniako, who designated these red antibiotics as the mycetin-violarin group. Little progress was made with the chemistry of these antibiotics until the 1950s; when the basic structures of the aglycones were determined by Brockmann[1]. He later named the compounds as anthracyclines and the aglycones anthracyclinones.

In 1963 an important anthracycline was discovered independently at Farmitalia[2] and Rhone-Poulenc[3]. The Farmitalia group prepared their product by aerobic fermentation of *Streptomyces peucetius via. caesius* followed by solvent extraction and chromatographic purification and named the compound daunomycin. The Rhone-Poulenc group under Du Bost made its isolation from *Streptomyces coerublerubidus* and gave this compound the name rubidomycin. When the identity of these two antibiotics was established, the name daunorubicin was chosen to reflect the dual origin. In 1974, the U.S. Food and Drug Administration (FDA) approved daunorubicin as a clinical antibiotic.

Daunorubicin (**1b**) is a glycoside antibiotic whose structure and stereochemistry

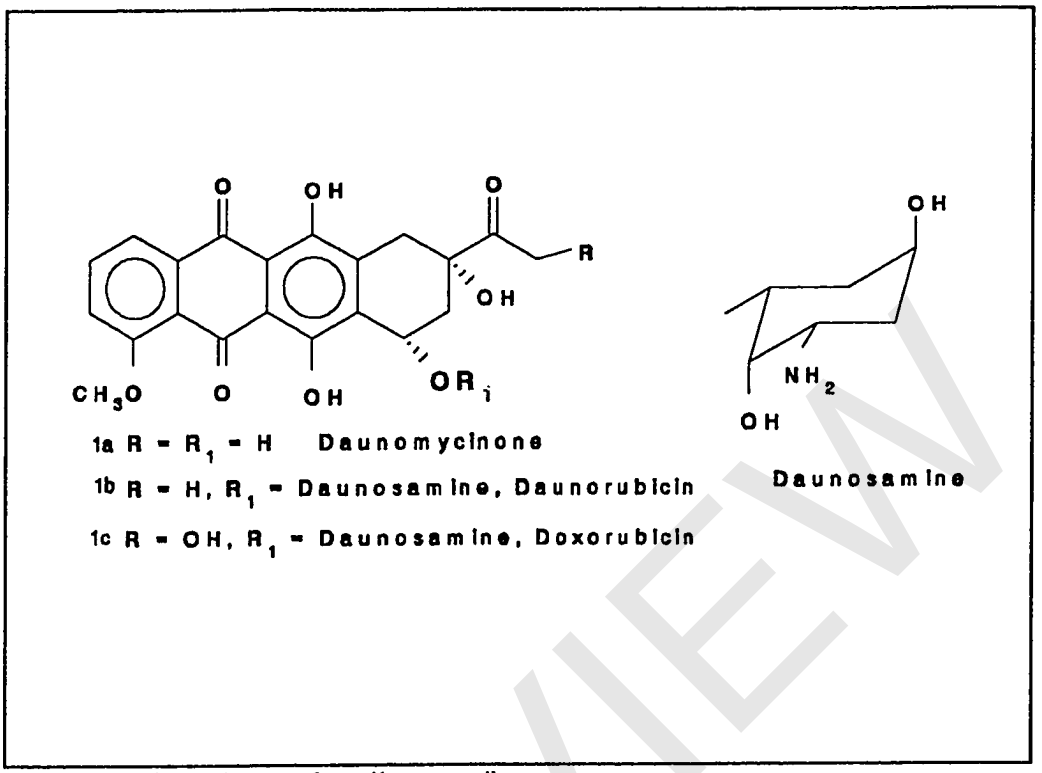

Figure 1 Structure of anthracyclines

are represented in figure **1.** It consists of the tetracyclic quinone aglycone daunomycinone linked to the aminosugar daunosamine. The chemical name for daunorubicin is: (7S:9S)-9-acetyl-4-methoxy-7,8,9,10-tetrahydro-6,7,9,11-tetrahydroxy-7-O-(2',3',5'-trideoxy-3'-amino-α-L-lyxohexopyranosyl)-5-12-naphthacenedione (**1b**). The difference between the daunorubicin (1b) and doxorubicin (**1c**) is that doxorubicin contains a hydroxyl group at C-14. Doxorubincin (**1c**) was also isolated by Farmitalia scientists and its structure was determined[4]. It was approved for clinical use in 1975. It has a broader spectrum of activities than any other anticancer drug in the market. Doxorubicin (**1c**) ranks first in sales of all anticancer drugs and 29th of all drugs in 1985.

2. BIOLOGICAL ACTIVITY[5]

The anthracycline antibiotics, doxorubicin (C_{14}-hydroxy derivative of daunorubicin) and daunorubicin, have attracted much attention in recent years because of their biological activity. They are important clinical antineoplastic agents. They have been used in the treatment of a broad spectrum of human cancers, such as blood cancers, and many solid tumors. The clinical data indicate that the tumor size of breast cancer was reduced to 60% after a week of treatment with adriamycin. It has been found that anthracycline antibiotics inhibit both DNA and RNA synthesis. There is much evidence to support the view that their biological effects are attributable to the binding of the antibiotics to DNA. Observations utilizing the fluorescence of daunorubicin revealed that it penetrated into cells and became fixed in the nucleus. Studies with tritiated daunorubicin further showed that it bonded to nucleoproteins and uptake into DNA was found to be maximal during replication. Both daunorubicin and adriamycin were shown to inhibit the incorporation of adenine-8-^{14}C into nuclear DNA of He La cells and Yoshida ascites hepatoma cells. The degree of inhibition increased as the concentration of daunorubicin was increased, and this inhibition was decreased by adding DNA.

Experiments on the effects of daunorubicin on synchronously dividing populations of He La cells and rat fibroblast cells in culture revealed that daunorubicin was more effective in inhibiting DNA synthesis and more lethal to the cells in the late S-phase than in G_1 or G_2. These observations led Di Marco[6] to suggest that, since strong binding by daunorubicin occurs only with double-stranded DNA, this drug can

find access to DNA resulting in mitotic blockage and serious chromosomal damage due to physicochemical changes in the DNA such as lengthening and stiffening of the chain.

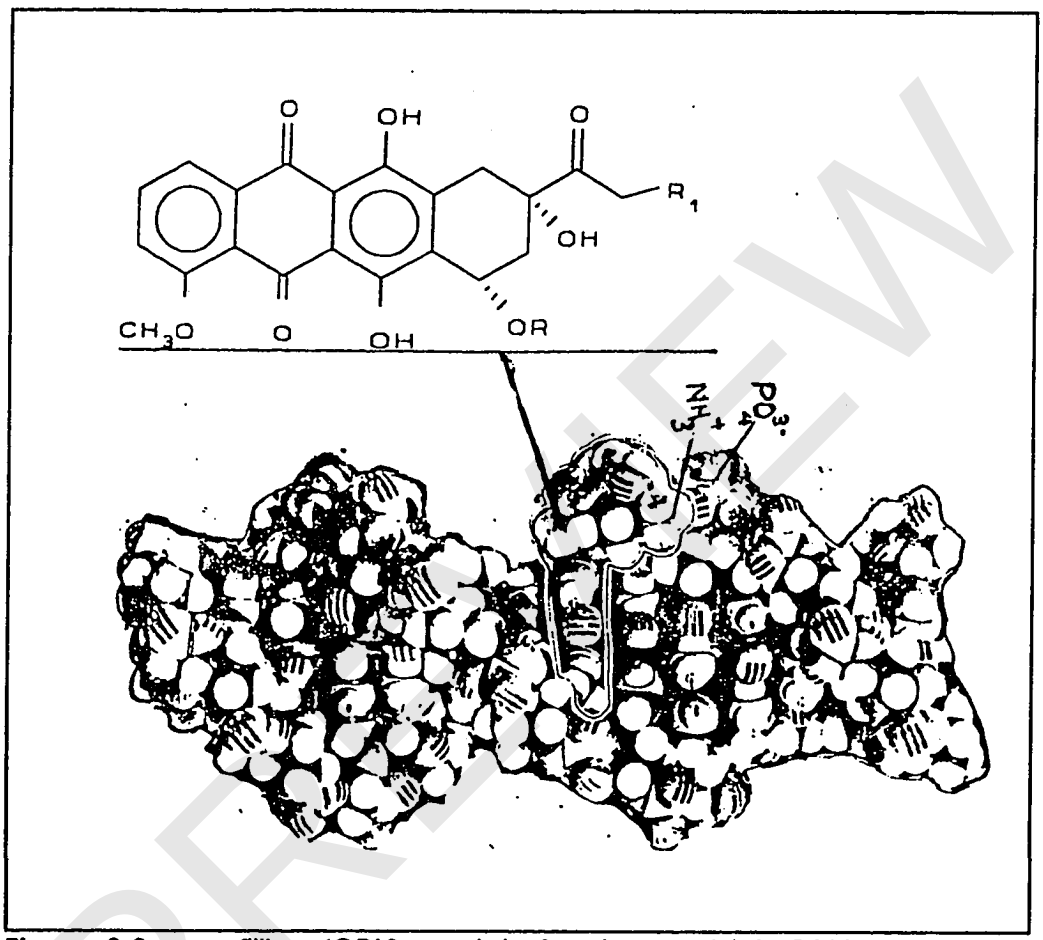

Figure 2 Space-filling (CPK) model of a daunorubicin-DNA intercalation complex. The antibiotic molecule has been outlined

The molecular model[7] (figure 2) for daunorubicin-DNA interaction was proposed during the early 70s. It was based principally on the results of fibre X-ray diffraction analysis. The three co-planar aromatic rings of the chromophore are intercalated between adjacent base-pairs in a fairly classical fashion while the puckered A ring (cyclohexene) with its attached daunosamine substituent projects into the wide

groove of the helix. Additional stabilization is provided by strong electrostatic interaction between the ionized amino group and a nearby DNA phosphate grouping. The gross unwinding angle compared to normal β-DNA is 12^0. This model is consistent with the known requirement for the aminosugar as witnessed by the diminished bonding seen with the aglycones of daunorubicin and its derivatives. Various proposals have been made for hydrogen bond formation which might stabilize the conformation of the antibiotic itself as well as its complex with DNA, including one involving the additional C_{14} hydroxyl group peculiar to the doxorubicin.

Doxorubicin and daunorubicin are called "red devil" due to their color and toxicity. It has been found that they are cardiotoxic and cause bone marrow depression. The risk of heart failure from the cumulative, dose-dependent side effect limits the use of these antibiotics; the lifetime dosage for patients is 550 mg/m^2 or 750 mg for an average male.[8] There is uncertainty in the literature regarding the precise mechanism of the toxicity involved. Reactive oxygen species[9], including the superoxide anion radical, hydrogen peroxide, and the hydroxyl radical, which are generated *in vivo* during metabolism, are suspected as agents. Evidence that anthracyclinols (**1f**) accumulate in the heart by *in situ* reduction suggests that their intemediates may also play a role in cardiotoxicity. It appears that the mode of action of anthracyclines as antitumor agents is mechanistically distinct from their mode of action as cardiotoxic agents, which means that the design of different anthracyclines may result in promising anticancer drugs that are high in anticancer activity but have low toxicity (see chapter three).

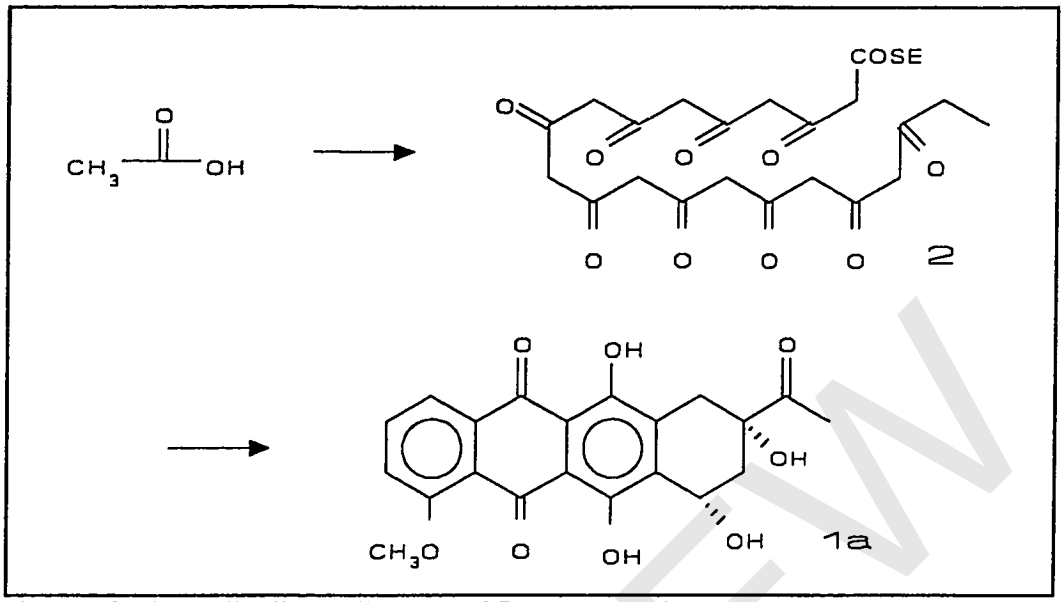

Figure 3 Biosynthetic pathway of Daunomycinone

3 BIOSYNTHESIS[10]

In nature, the anthracyclines are synthesized from propionate as the starter unit and nine malonate extenders to give the polyketides (**2**) which then cyclize and then aromatize to daunomycinone *via* several steps. It is postulated that a series of Claisen condensations gives long chain molecules with β-polyketone functionalities (polyketides, **2**), whose intramolecular condensation and enolization construct the aromatic nuclei.This hypothesis was supported by radioactive label studies[11].

4 METABOLISM[12]

It is believed that there are four major metabolic transformations of anthracyclines: reduction of ketone, anaerobic reductive deglucosylation, aerobic redox cycling and glucuronide conjugate formation.

Daunorubicin is first metabolized to daunorubincinol (**1f**), which is the only

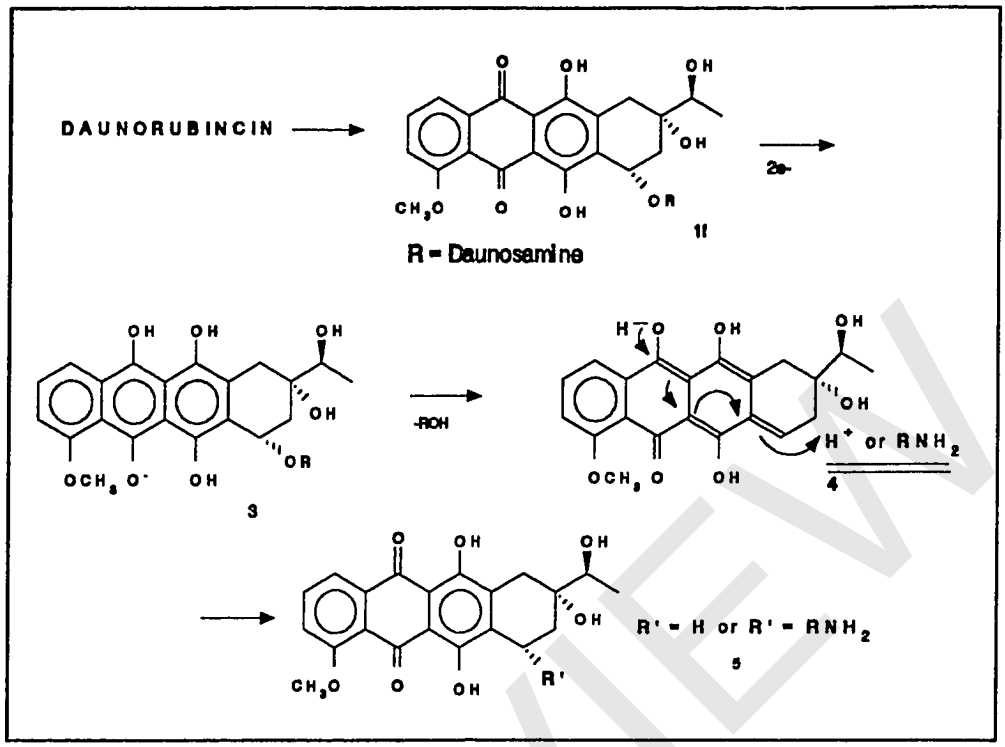

Figure 4 Metabolism of daunorubicin

antitumor active species among the metabolites of daunorubicin[13]. Daunorubicinol (**1f**) is then reduced by enzymes to its hydroquinone (**3**). In the absence of oxygen, glycoside loss from this hydroquinone anion yields the quinone methide (**4**). The role of that these compounds play in the therapeutic action of the anthracycline is not well understood[14]. It appears that the vinylogous quinone methide that is formed in this manner alkylates a biomolecule such as DNA. The issue of whether reductive deglycosylation is a significant metabolic pathway remains unresolved[15]. Likewise, the question as to whether the quinone methide expresses antitumor activity by a Michael addition of the RNH_2 or RSH in DNA to C_{6a}-C_7 of **4** is as yet without direct evidence. Nevertheless, it is believed that such quinone methides are likely upon bioreductive alkylation to bind to nitrogen and sulfur nucleophile sites in protein and, possibly,