

I. GENERAL INTRODUCTION

1.1 Plant-Derived Drugs

Natural products once served humankind as the source of all drugs, and higher plants provided most of these therapeutic agents. Today, natural products (and their derivatives and analogs) still represent over 50% of all drugs in clinical use, with higher plant-derived natural products representing *ca.* 25% of the total.¹ The World Health Organization estimates that 80% of the people in developing countries of the world rely on traditional medicine for their primary health care, and about 85% of traditional medicine involves the use of plant extracts. This means that about 3.5 to 4 billion people in the world rely on plants as sources of drugs.²

In the United States plant-derived drugs represent about 25% of the prescription drugs market, and in 1991 this equated to a retail value of approximately \$15.5 billion.³ From 1983 to 1994 39% of the New Approved Drugs were of natural origin, including original natural products, products derived semisynthetically from natural products, and synthetic products based on natural product models.⁴

Further evidence of the importance of natural products is provided by the fact that almost half of the world's 25 best selling pharmaceuticals in 1991 were either natural products or their derivatives.⁵

Conservative estimates suggest that there are more than 250,000 species of higher plants existing on this planet, and only a very small percentage of plants have been

¹ Balandrin, N. F., Kinghorn, A. D., Farnsworth, N. R. in *Human Medicinal Agents from Plants* Kinghorn, A. D., Balandrin, M. F., Eds., ACS Symposium Series 534, **1993**, pp. 2-12

² Farnsworth, N. R. in *Biodiversity* Wilson, E. O. Ed., National Academy Press Washington, DC **1988**, pp. 83 -97

³ Pezzuto, J. M. *Biochemical Pharmacology* **1997**, *53*, 121-133

⁴ Cragg, G. M., Newman, D. J., Snader, K. M. *J. Nat. Prod.*, **1997**, *60*, 52 - 60

⁵ O'Neill, M. J., Lewis, J. A. in *Human Medicinal Agents from Plants* Kinghorn, A. D., Balandrin, M. F., Eds., ACS Symposium Series 534, **1993**, pp. 48 - 55

exhaustively studied for their potential value as a source of drugs. Obviously natural products will continue to be extremely important as sources of medicinal agents. In addition to the natural products which have found direct medicinal application as drug entities, many others can serve as chemical models or templates for the design, synthesis, and semisynthesis of novel substances for treating humankind's diseases. Although there are some new approaches to drug discovery, such as combinatorial chemistry and computer-based molecular modeling design, none of them can replaced the important role of natural products in drug discovery and development.

1.1.1 Natural Products as Anticancer Agents

Cancer may be the most feared disease of our time. It is second only to heart disease as a leading cause of death in the United States and it is estimated that about one out of every three Americans will develop cancer at some point during his or her life. Currently, although intensive research and some major advances in treatment are attempting to reduce this figure, cancer claims the life of nearly one out of every four Americans. About 1 million cases of cancer are diagnosed every year in this country, and about 500,000 Americans die annually of the disease. Moreover, the number of cancer deaths continues to increase steadily. For example, about 514,000 Americans died of cancer in 1991. The corresponding number was about 510,000 in 1990, and 502,000 in 1989.⁶

In 1971 President Richard M. Nixon signed the National Cancer Act to declare the start of the so-called War on Cancer. Over the past 25 years, the United States government, through the National Cancer Institute, has expended a total of approximately \$30 billion, and undeniable progress has been made.³

⁶ Cooper, G. M. *The Cancer Book* Jones and Bartlett Publishers, Boston, MA 1993, p. 7

The most important progress that has been made is our understanding of cancer at the cellular and molecular levels. The discoveries of oncogenes and tumor suppressor genes have afforded a conceptual framework to understand the mechanisms that control normal cell growth and differentiation, and the ways in which breakdown of these normal cellular controls leads to the development of cancer.

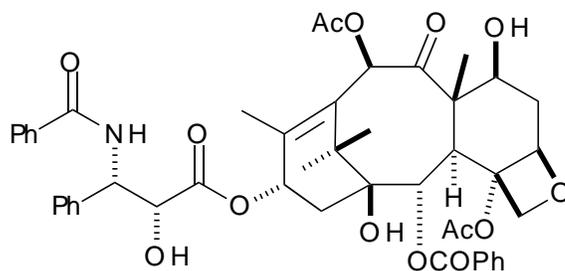
Significant progress has also been made in identifying the causes of several cancers, as well as in detecting some cancers at early, readily treatable stages. If current recommendations for cancer prevention and early detection were put into general practice, they would result in about a two-fold reduction in total cancer mortality.⁶

Progress has also been made in the treatment of some cancers. Certain cancers can be effectively controlled by drug combinations, such as acute lymphocytic leukemia, Hodgkin's disease, some non-Hodgkin's lymphomas, and testicular cancer. Relative to 1971, the consequences of chemotherapy are managed with greater efficacy through the use of antiemetics and immunostimulants.

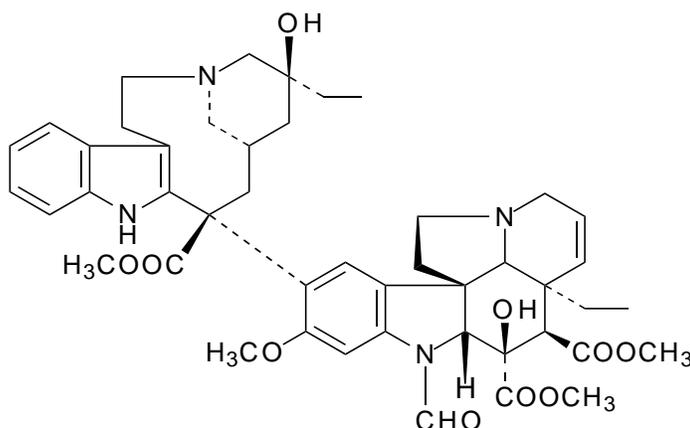
In the War on Cancer, and through the history of combating the disease of cancer, natural products have played an important role in the development of contemporary cancer chemotherapy. Between 1960 and 1982 the National Cancer Institute screened around 114,000 extracts from an estimated 35,000 plant samples for anticancer activity. They initiated a new natural products program with a new *in vitro* human cancer cell line screen in 1987, and as of December, 1991, 28,800 plant samples had been collected from over 20 countries to screen for anticancer activity.⁷ As a result of this ongoing research a number of clinically useful and market-approved drugs are available. A recent survey showed that of the 87 approved anticancer drugs over the past ten years, 62% are of natural origin or are modeled on natural product parents.⁴ Among those clinically useful

⁷ Cragg, G. M., Boyd, M. R., Cardellina II, J. H., Grever, M. R., Schepartz, S. A., Snader, K. M., Suffness M. in *Human Medicinal Agents from Plants* Kinghorn, A. D., Balandrin, M. F., Eds., ACS Symposium Series 534, **1993**, pp. 81- 95

drugs include paclitaxel (Taxol®) (**1.1**),⁸ vincristine (Oncovin®) (**1.2**),⁹ podophyllotoxin (a natural product precursor) (**1.3**),¹⁰ and camptothecin (a natural product precursor for water-soluble derivatives) (**1.4**).¹¹ These substances embrace some of the most exciting new chemotherapeutic agents currently available for use in a clinical setting.



Paclitaxel (**1.1**)



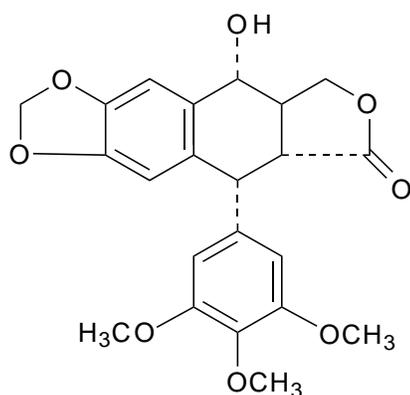
Vincristine (**1.2**)

⁸ Kingston, D. G. I. in *Human Medicinal Agents from Plants* Kinghorn, A. D., Balandrin, M. F., Eds., ACS Symposium Series 534, **1993**, pp. 138 - 148

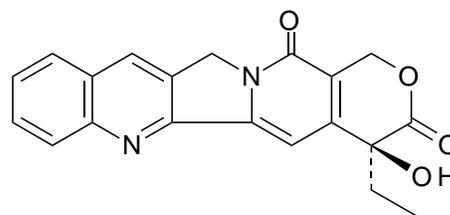
⁹ Gerzon, K. in *Anticancer agents Based on Natural Product Models* Cassady, J. M., Douros J. D., Eds., Academic Press, **1980**, pp.271 - 317

¹⁰ Jardine, I. in *Anticancer agents Based on Natural Product Models* Cassady, J. M., Douros J. D., Eds., Academic Press, **1980**, pp.319 - 351

¹¹ Wall, M. E., Wani, M. C. in *Human Medicinal Agents from Plants* Kinghorn, A. D., Balandrin, M. F., Eds., ACS Symposium Series 534, **1993**, pp. 149 - 169



Podophyllotoxin (1.3)



Camptothecin (1.4)

Although significant progress has been made in cancer chemotherapy, current drugs are ineffective against many common cancers and are often very toxic. Also at the current time, it is apparent that drug-based therapeutic strategies will predominate into the 21st century. For these reasons the discovery of new drugs effective against resistant solid tumors is an important and necessary strategy in improving chemotherapy. Undoubtedly more effort is needed to search for new cancer drugs with the aid of better screening methods from plants and other natural sources.

With regards to the selection and collection of material from natural sources for the discovery of naturally occurring anticancer drugs, various methods may be employed. There is the random method, where complete collections of plants found in a given area are screened. With this method, large numbers of species can be collected in a short period of time. Another strategy is to target plant families which are known to be rich in biologically active compounds. A third and most fascinating approach is an ethnobotanical method, where a local people's knowledge about the medicinal uses of the indigenous plants is taken into consideration when making plant selections.

In the screening of plant material and subsequent fractionation, the approach generally regarded as most practical for drug discovery is referred to as bioassay-directed fractionation

1.2 BIOASSAY-GUIDED FRACTIONATION

1.2.1 General Consideration

In a natural products drug discovery program, bioassay plays an important role. A bioassay will be applied to large numbers of initial samples to determine whether or not they have any bioactivity of the desired type (prescreen). A bioassay will be used to select materials for detailed individual study (screen). And a bioassay will be used to guide fractionation of a crude material towards isolation of the pure bioactive compounds (monitor). For these purposes, bioassay tests must be simple, rapid, reliable, reproducible, sensitive, meaningful and, most importantly, predictive.

In the area of anticancer drug discovery, a large number of bioassay systems are available. Generally *in vitro* assays can be divided into two groups: cellular assays and mechanism-based assays (molecular assays). Cellular assays use intact cells while mechanism-based assays look for activity using isolated systems such as enzymes, receptors, DNA, etc..

Cellular assays can also be divided into cytotoxicity assays and other assay types (including morphological). The simplest cytotoxicity assay is to measure the concentration of sample needed to inhibit cell growth by 50% against a single cell line. In 1956 the NCI selected the L1210 mouse leukemia as a main screen and it was used until 1971, when it was replaced by the P388 lymphocytic leukemia. This cell line, which is somewhat more sensitive but retain characteristics similar to those of the L1210 cell line, was used as the primary screen through 1985.¹²

Since cytotoxicity is an activity that is consistent with antitumor activity, cytotoxicity-based assays have one major advantage: all potential mechanisms concerning

¹² Suffness M. in *Biologically Active Natural Products* Hostettmann K., Lea, P. J., Eds., Oxford University Press, Oxford, **1987**, pp.85 - 104

cellular proliferation are monitored simultaneously. However, cytotoxicity is neither necessary nor sufficient for antitumor activity, and in many cases the active materials are simply toxic. These “active” materials could include heavy metals, detergents, various other protein denaturants, indiscriminate DNA-damaging agents, agents poisoning cellular energetics such as mitochondrial poisons, sodium or calcium channel poisons, non-specific oxidants, etc. More importantly, almost all agents detected by the L1210 and P388 models were only effective against rapidly growing tumors and had little or no activity against slow growing tumors.

In 1987 the National Cancer Institute initiated a program wherein the cytotoxic potential of compounds or extracts was evaluated with a new primary *in vitro* screening system which comprised a panel of 60 human cancer cell lines obtained from eight organ systems, including those of lung, colon, breast, melanoma and other refractory, solid tumor, disease types.¹³ Based on the concept of the presence of a cell-specific receptor that differentiates one tumor type from another, it is conceivable that an agent capable of demonstrating selective cytotoxic activity with a cell line derived from a single type of primary human tumor could be useful in the treatment of a specific type of cancer. This assay system is proving to be effective, but it is rather complex and time consuming.

With a deeper understanding of cell biology and molecular pharmacology, mechanism-based assays have become increasingly important. Due to their selectivity and sensitivity combined with good reproducibility and high sample throughput, this type of assay is given preference for large-scale screening programs in industry or in a collaborative setting.

Mechanism-based assays can be designed by analogy with the types of molecular responses mediated by known (clinically effective) antitumor agents and utilization of these assay systems should aid in the procurement of unique natural products. Since

¹³ McLaughlin, J. L. in *Assays for Bioactivity* Hostettmann, K., Ed., *Methods in Plant Biochemistry* Vol. 6, Academic Press, 1991, pp. 1 - 32

However, there are some specific limitations of mechanism-based assay drug discovery procedures. These include an inability to detect compounds with unknown mechanisms of action, and the fact that non-specific interactions may lead to false positives. In order to establish the true efficacy of promising anticancer agents, subsequent evaluation in more advanced testing systems is required, followed by (pre) clinical trials.

1.2.2 Yeast-Based Bioassay for DNA-Damaging Agents

An important difference between cancer cells and normal cells is that cancer cells are undergoing a more rapid proliferation than normal cells. Because of this they are more susceptible than normal cells to agents that cause DNA damage. Cancer cells may also have defects in their ability to repair damage to DNA as compared with normal cells. These facts suggest that agents which cause damage to DNA might be effective anticancer agents.

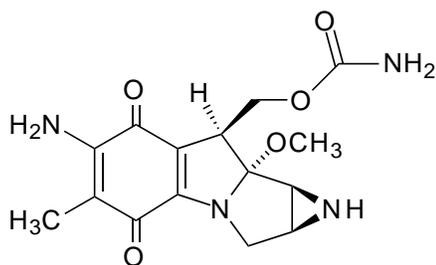
This logic is supported by the fact that many effective anticancer drugs do indeed operate by damaging DNA in some way. Thus DNA can be damaged by alkylation by mitomycin C (**1.7**),¹⁵ by oxidation by bleomycin (**1.6**),¹⁶ by inhibition of DNA topoisomerase I by camptothecin (**1.4**) and its analogs,¹⁷ and by inhibition of topoisomerase II by drugs as doxorubicin (**1.8**).¹⁸

¹⁵ Remers, W. A., in *Anticancer agents Based on Natural Product Models* Cassady, J. M., Douros J. D., Eds., Academic Press, **1980**, pp.131 - 146

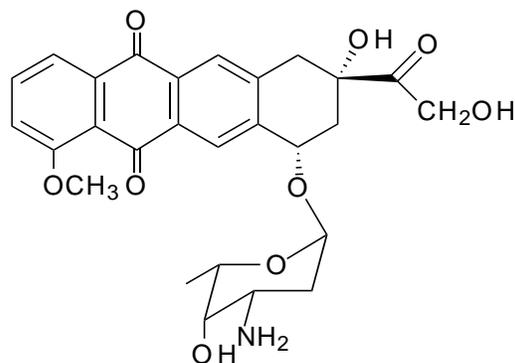
¹⁶ Zunino, F., Capranico, G. in *Cancer Therapeutics* Teicher, B. A. Ed., Humana Press, **1997**, p. 199

¹⁷ Li, S., Adair, K. T., *Xi Shu* The Tucker Center, **1994**, p. 16

¹⁸ Pommier, Y. in *Cancer Therapeutics* Teicher, B. A. Ed., Humana Press, **1997**, p. 159



Mitomycin C (1.7)



Doxorubicin (1.8)

The mechanism-based assay employed by the Kingston group uses genetically modified yeast to detect agents which cause DNA damage. Investigators studying cellular response to DNA damage have used the yeast *Saccharomyces cerevisiae* for many years, and extensive genetic analysis of DNA repair in yeast cells began in the 1970's.¹⁹ Investigations of sensitive yeast strains have allowed DNA repair pathways to be defined. Three major DNA repair pathways have been defined in yeast, and they are known as the *rad3*, *rad6*, and *rad52* pathways. The *rad3* pathway is associated with excision repair, the *rad6* pathway is the error-prone pathway, and *rad52* is the recombinational pathway associated with the repair of double-strand breaks and meiotic recombination.²⁰ Yeasts deficient in each of these repair pathways and also having increased cell membrane permeability have been used to screen for potential anticancer agents.

The yeast-based bioassay that we have been using is based on the differential response of DNA repair-deficient and DNA repair-proficient yeast strains to the test sample. The assay is carried out by measuring the growth inhibition of a repair-deficient yeast mutant, usually *rad52*, in comparison with the wild-type yeast, RAD+, having the

¹⁹ Friedberg, E. C. *Microbiological Review*, **1988**, 52, 70

²⁰ Game, J. C. in *Yeast Genetics: Fundamental and Applied Aspects* Spencer, J. F. T., Spencer, D. M., Smith, A. R. W., Eds., Springer Verlag, New York, **1983**, pp. 109 - 137

same permeability mutation. A mutant lacking one of the repair pathways will be more sensitive than the wild-type yeast to DNA damage repaired predominantly by that pathway, and thus agents which cause DNA damage can be selectively detected. Assay results are reported as IC₁₂ values, which represent the concentration (in µg/mL) required to produce an inhibition zone of 12 mm diameter around a 100 µL “well” in an agar gel coated with the yeast strain in question. In order to consider a compound to be selectively active its IC₁₂ in one or more repair-deficient yeast must be less than one third that of its IC₁₂ in the wild-type yeast.

A mutant *rad52* repair-deficient strain, *rad52. top1*, with the additional deletion of the DNA topoisomerase I gene is also available and can detect agents that produce DNA damage specifically by interacting with DNA topoisomerase II. Thus an agent which exhibits greater activity towards the *rad52.top1* strain than to the *rad52* strain, with a differential of at least 3, most probably mediates its activity through inhibition of DNA topoisomerase II. Conversely, greater toxicity towards *rad52* implies the presence of a DNA topoisomerase I inhibitor.

In the search for potential anticancer agents from natural sources, this mechanism-based yeast bioassay has been employed to screen over 5,000 extracts derived from bryophytes to higher plants, and the DNA damaging agents encountered have had diverse structures ranging from sterols, sesquiterpenoids, limonoids, pterocarpanes, naphthoquinones, oxoaporphines, piperidines, and coumarins.²¹

²¹ Gunatilaka, A. A. L., Kingston, D. G. I., Johnson, R. K. *Pure & Appl. Chem.* **1994**, *66*, 2219 - 2222

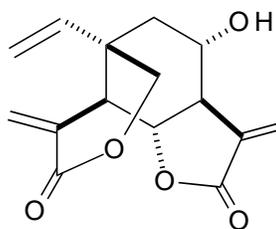
II SESQUITERPENOIDS FROM *CHILOSCYPHUS RIVULARIS*

2.1 INTRODUCTION

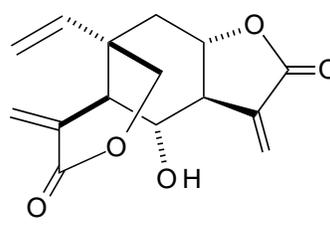
2.1.1 Occurrence of Bioactive Sesquiterpenoids

The sesquiterpenoids are a widespread group of substances occurring in many different organisms, and form the largest class of terpenoids. Over 200 skeletal types are known, and several thousand compounds of the class have been isolated and identified. Among the sesquiterpenoids the sesquiterpenoid lactones is one of the largest groups, with 3,200 known members by 1987.²² It is also one of the largest groups of sesquiterpenoids that demonstrates cytotoxic and antitumor activity.

Some of the first sesquiterpenoids with potential antitumor activity were vernolepin (**2.1**) and vernomenin (**2.2**), which were isolated as tumor inhibitors from *Vernonia hymenolepis* by Kupchan and colleagues in 1968. They showed cytotoxicity (ED₅₀) against KB cell culture at 2 and 20 µg/mL, respectively, and vernolepin also showed significant inhibitory activity against the Walker intramuscular carcinosarcoma 256 in the rat at 12 mg/kg.²³



Vernolepin (**2.1**)



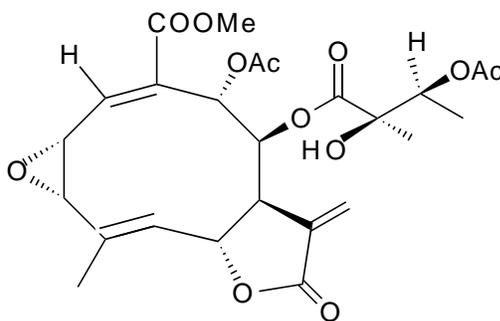
Vernomenin (**2.2**)

²² Hill, R. A. in *The Chemistry of Natural Products*; Thomson, R. H., Ed. Blackie Academic & Professional, **1993**, 106 - 139

²³ Kupchan, S. M., Hemingway, R. J., Werner, D., Karim, A. *J. Am. Chem. Soc.* **1968**, *90*, 3596 - 3597

The discovery of vernolepin and of its antitumor properties was the impetus for a decade of intensive searching for cytotoxic and anti-cancer active sesquiterpenoid lactones during the 1970s. A large number of active agents was isolated from plants, primarily from plants in the family Compositae (Asteraceae). A majority of the hundreds of compounds evaluated were cytotoxic, and a small number have shown activity *in vivo* against P-388 leukemia and other tumor systems.

Melampodin A (**2.3**), isolated in 1976 by N. H. Fischer and colleagues,²⁴ is the major constituent in a number of populations of the yellow-rayed species *Melampodium americanum* from Mexico. It showed inhibitory activity *in vivo* against lymphocytic leukemia P-388, exhibiting an optimum % T/C 140 at 12 mg/kg.²⁵ Melampodin A was determined to be a sesquiterpenoid lactone, and its structure was confirmed by X-ray crystallographic study.



Melampodin A (**2.3**)

Other antitumor-active sesquiterpenoid lactones include the following compounds with different skeletal types: helenalin (**2.4**),²⁶ eupatolide (**2.5**),²⁷ eupatoriopicrin (**2.6**),²⁷ parthenin (**2.7**),²⁸ parthenolide (**2.8**),²⁹ and tenulin (**2.9**).³⁰ In general, an α -methylene- γ -

²⁴ Fischer, N. H., Wiley, R. A., Perry, D. L., Haegle, K. D. *J. Org. Chem.*, **1976**, *41*, 3956 - 3959

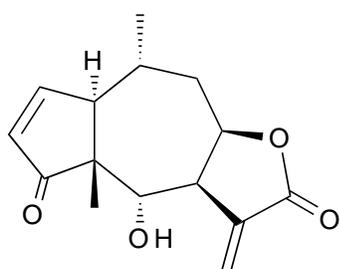
²⁵ Malcolm, A., Difeo, D., Fischer, N. H. *Phytochemistry*, **1982**, *21*, 151 - 155

²⁶ Herz, W., Vivar, A. R. D., Romo, J., Viswanathan, N. *J. Am. Chem. Soc.* **1963**, *85*, 19-26

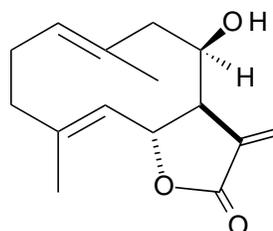
²⁷ Doskotch, R. W., El-Ferally, F. S. *J. Org. Chem.*, **1970**, *35*, 1928-1936

²⁸ Herz, W., Watanabe, H., Miyazaki, M., Kishida, Y. *J. Am. Chem. Soc.* **1962**, *84*, 2601-2610

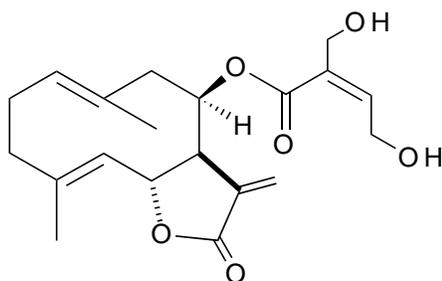
butyrolactone or cyclopentenone group is a necessary and usually sufficient condition for activity. Activities are generally enhanced by the presence of further alkylating groups (epoxides, α,β -unsaturated ketones and esters), which represent reactive receptor sites for biological nucleophiles, in particular thiol and amino groups. In spite of the large number of anti-tumor active sesquiterpenoid lactones, none of them have been considered for clinical testing, which is partly due to their high toxicity.³¹



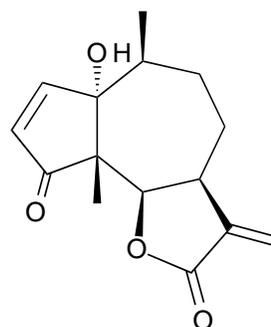
Helenalin (2.4)



Eupatolide (2.5)



Eupatoriopicrin (2.6)

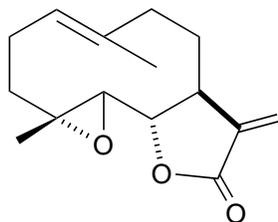


Parthenin (2.7)

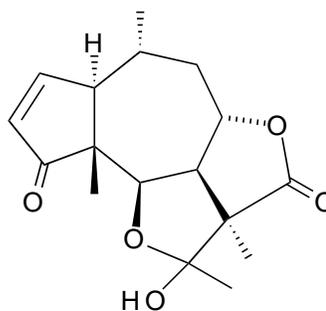
²⁹ Govindachari, T. R., Joshi, B. S., Kamat, V. K. *Tetrahedron* **1965**, *21*, 1509-1519

³⁰ Herz, W., Rohde, W. A., Rabindran, K., Jayaraman, P., Viswanathan, N. *J. Am. Chem. Soc.* **1962**, *84*, 3857-3870

³¹ Fischer, N.H. in *Terpenoids*; Charlwood, B. V., Banthorpe, D. V., Eds., *Methods in Plant Biochemistry* Vol. 7, Academic Press, **1991**, pp.187 - 211



Parthenolide (2.8)



Tenulin (2.9)

Some other classes of sesquiterpenoid also show antitumor activity. The glycoside phyllanthoside (2.10), obtained from *Phyllanthus acuminatus*, has a marked antineoplastic activity against the NCI murine B-16 melanoma.³²

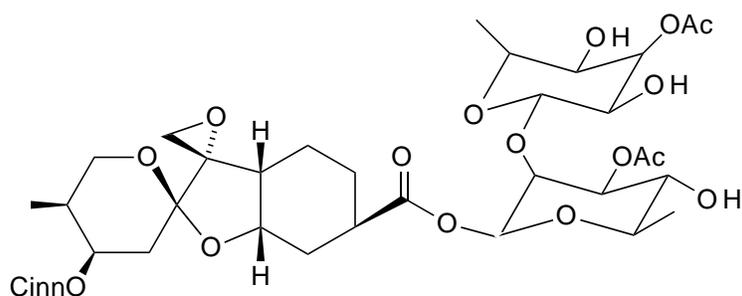
Besides cytotoxic and antitumor activity, sesquiterpenoids exhibit a rich variety of other biological properties. The endoperoxide artemisinin (qinghaosu) (2.11),³³ isolated from the Chinese herb *Artemisia annua*, has been employed for the treatment of malaria. The main sesquiterpenoid mycotoxins are the trichothecenes (2.12),³⁴ which are associated with a wide variety of human and animal toxic effects. They are also phytotoxic compounds and potent anticancer agents, and some have antibacterial activity, such as verrucarins (2.13). The drimane sesquiterpenoids warburganal (2.14) and polygodial (2.15), isolated from *Warburgia stuhlmannii*, are examples of insect antifeedant substances.³⁵

³² Kupchan, S. M., LaVoie, E. J., Branfman, A. R., Fei, B. Y., Bright, W. M., Bryan, R. F. *J. Am. Chem. Soc.* **1977**, *99*, 3199-3201

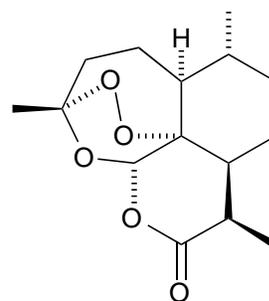
³³ Klayman, D. L. *Science* **1985**, *228*, 1049-1055

³⁴ Jarvis, B. B., Pavanadasivam, G., Bean, G. A. in *Trichothecenes and Other Mycotoxins* Lacey, J., Ed., John Wiley & Sons Ltd., **1985**, pp. 221-231

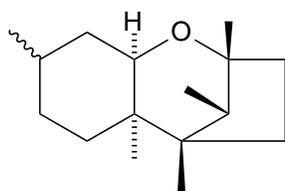
³⁵ Fraga, B. M. in *Terpenoids*; Charlwood, B. V., Banthorpe, D. V., Eds., *Methods in Plant Biochemistry* Vol. 7, Academic Press, **1991**, pp.145 - 185



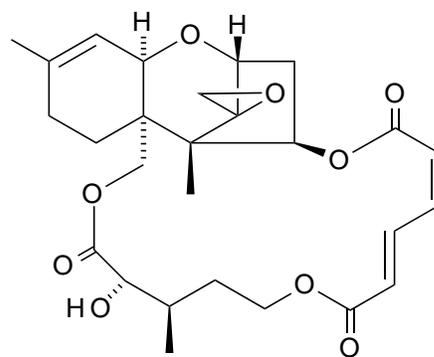
Phyllanthoside (2.10)



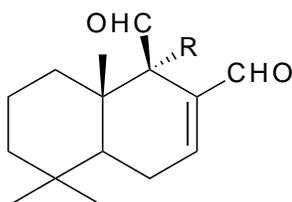
Qinghaosu (2.11)



Trichothecanes (2.12)



Verrucarin (2.13)



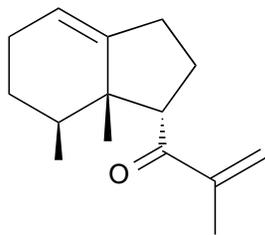
Warburganal R = OH (2.14)
Polygodial R = H (2.15)

2.1.2 Previous Chemical Investigation of the Genus *Chiloscyphus*

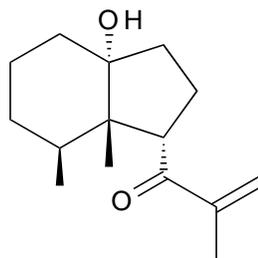
Chiloscyphus is a genus of liverworts, a group of small, green, nonvascular plants of the family Lophocoleaceae within the class Hepaticae in the division Bryophyta. Members of this family were believed to be able to cure diseases of the liver in ancient times, hence their name.

Previous work on liverworts of the *Chiloscyphus* genus has yielded an assortment of sesquiterpenoids and other compounds. Perhaps the most interesting compound

isolated to date is the sesquiterpenoid chiloscypnone (**2.16**), obtained initially from a Japanese collection of *C. polyanthos*.³⁶ Originally assigned a *cis*-decalin structure, its structure was reassigned based on synthetic and spectroscopic studies to the novel ring-contracted structure **2.16**, and it has given its name to the class of chiloscypnone sesquiterpenoids. Other compounds reported from *Chiloscyphus* sp. include chiloscypnone (**2.17**),³⁷ 11,12-epoxychiloscypholone (**2.18**), and *ent*-(5*R*,6*S*,9*R*)-4a-hydroxyoppositan-10-one (**2.19**)³⁸ from *C. pallescens*, (+)- α -selinene (**2.20**)³⁹ (enantiomeric with the α -selinene from *Acorus calamus*), the *ent*-7,8-eudesmanolides diplophyllolide (**2.21**), 7 α -hydroxydiplophyllolide (**2.22**), diplophyllin (**2.23**), 3-oxodiplophyllin (**2.24**),⁴⁰ small amounts of other sesquiterpenoids, and carotenoids from *C. polyanthos*, and (*E*)-dec-2-enal from *C. pallidus*. The only previous work reported on *C. rivularis* is a study on its photosynthetic characteristics and the observation that its thalli had high concentrations of chlorophylls a and b and carotenoids.⁴¹



Chiloscypnone (**2.16**)



Chiloscypnone (**2.17**)

³⁶ Hayashi, S., Matsuo, A., Matsuura, T. *Tetrahedron Lett.* **1969**, 1599 - 1600

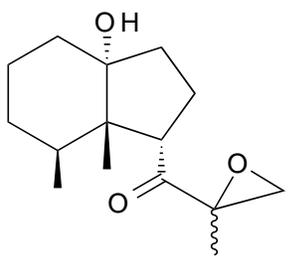
³⁷ Connolly, J. D., Harrison, L. J., Rycroft, D. S. *J. Chem. Soc., Chem. Commun.* **1982**, 1236 - 1238

³⁸ Harrison, L. J., Asakawa, Y. *Phytochemistry* **1991**, *30*, 3806 - 3807

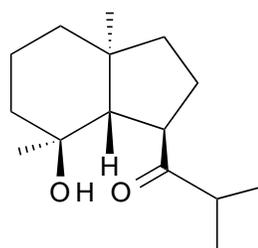
³⁹ Andersen, N. H., Shank, B., Costin, C. R. *Experientia* **1973**, *29*, 645 - 647

⁴⁰ Asakawa, Y., Toyota, M., Takemoto, T., Suire, C. *Phytochemistry* **1979**, *18*, 1007 - 1009

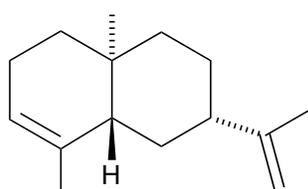
⁴¹ Farmer, A. M., Boston, H. L., Adama, M. S. *Verh.-Int. Ver. Theor. Angew. Limnol.* **1988**, *23*, 1912 - 1915



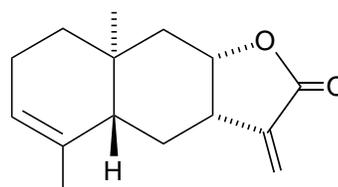
11, 12-Epoxychiloscypholone (**2.18**)



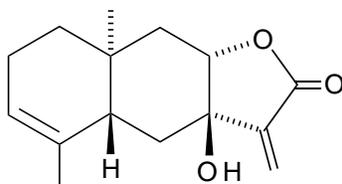
ent-(5*R*, 6*S*, 9*R*)-4 α -hydroxyoppositan-10-one (**2.19**)



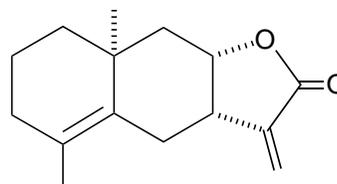
(+)- α -Selinene (**2.20**)



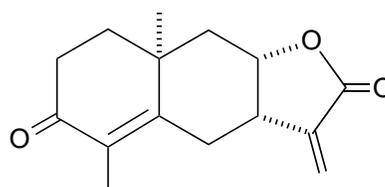
Diplophyllolide (**2.21**)



7- α -Hydroxydiplophyllolide (**2.22**)



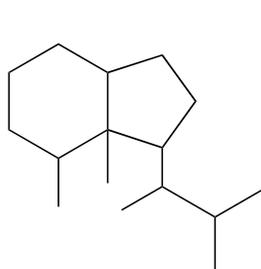
Diplophyllin (**2.23**)



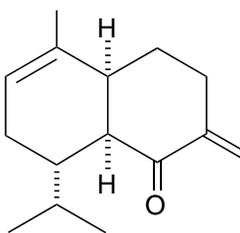
3-Oxidiplophyllin (**2.24**)

2.1.3 Chiloscyphane Sesquiterpenoids

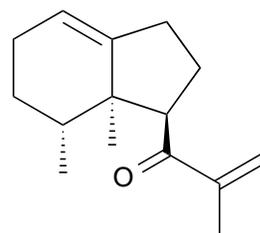
The chiloscyphane sesquiterpenoids comprise a small group of sesquiterpenoids with the basic structure of octahydro-7,7a-dimethyl-1-(2-methylpropyl)-1H-indene (**2.25**). Previously only three members of this class of sesquiterpenoids have been found and all of them were isolated from the liverwort *Chiloscyphus* sp..



2.25



2.26



2.27

The first chiloscyphane sesquiterpenoid, chiloscyphone (**2.16**), was isolated *from* *C. polyanthos* by Hayashi et al. in 1969³⁶ and was later proposed to be the cadinane sesquiterpenoid **2.26**.⁴² However, a subsequent synthesis of racemic **2.26** by Gras revealed that the original structural proposals were incorrect.⁴³ In 1982, the structure of chiloscyphone was revised by Connolly and his associates.⁴⁴ They showed that chiloscyphone is a sesquiterpenoid with the novel carbon skeleton of **2.27**; this skeleton gave its name to the group of chiloscyphane sesquiterpenoids. Later, based on synthetic work and circular dichroism studies, Tori et al. concluded that **2.27** also represents the correct absolute configuration of this sesquiterpenoid.⁴⁵ This deduction also turned out

⁴² Matsuo, A. *Tetrahedron* **1972**, 28, 1203 - 1209

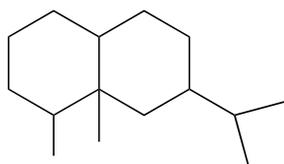
⁴³ Gras, T. L. *J. Org. Chem.* **1981**, 46, 3738 - 3741

⁴⁴ Connolly, J. D., Harrison, L. J., Rycroft, D. S. *J. Chem. Soc., Chem. Commun.* **1982**, 1236 - 1238

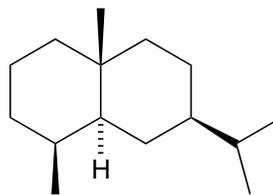
⁴⁵ Tori, M., Hasebe, T., Asakawa, Y. *Bull. Chem. Soc. Jpn.* **1990**, 63, 1706-1712

to be incorrect and the fact that chiloscypnone possesses the absolute configuration shown in **2.16** was finally determined by an X-ray crystallographic study.⁴⁶

No biosynthetic scheme has been proposed for the carbon skeleton of the chiloscypnone sesquiterpenoids. However they may be regarded as ring-contracted eremophilanes (**2.28**). Some eudesmane sesquiterpenoids such as logiborneol (**2.29**) which co-occur with chiloscypnone sesquiterpenoids are attractive candidates for ring-contraction and methyl migration processes which would lead to the chiloscypnone carbon skeleton with the correct stereochemistry.



2.28



2.29

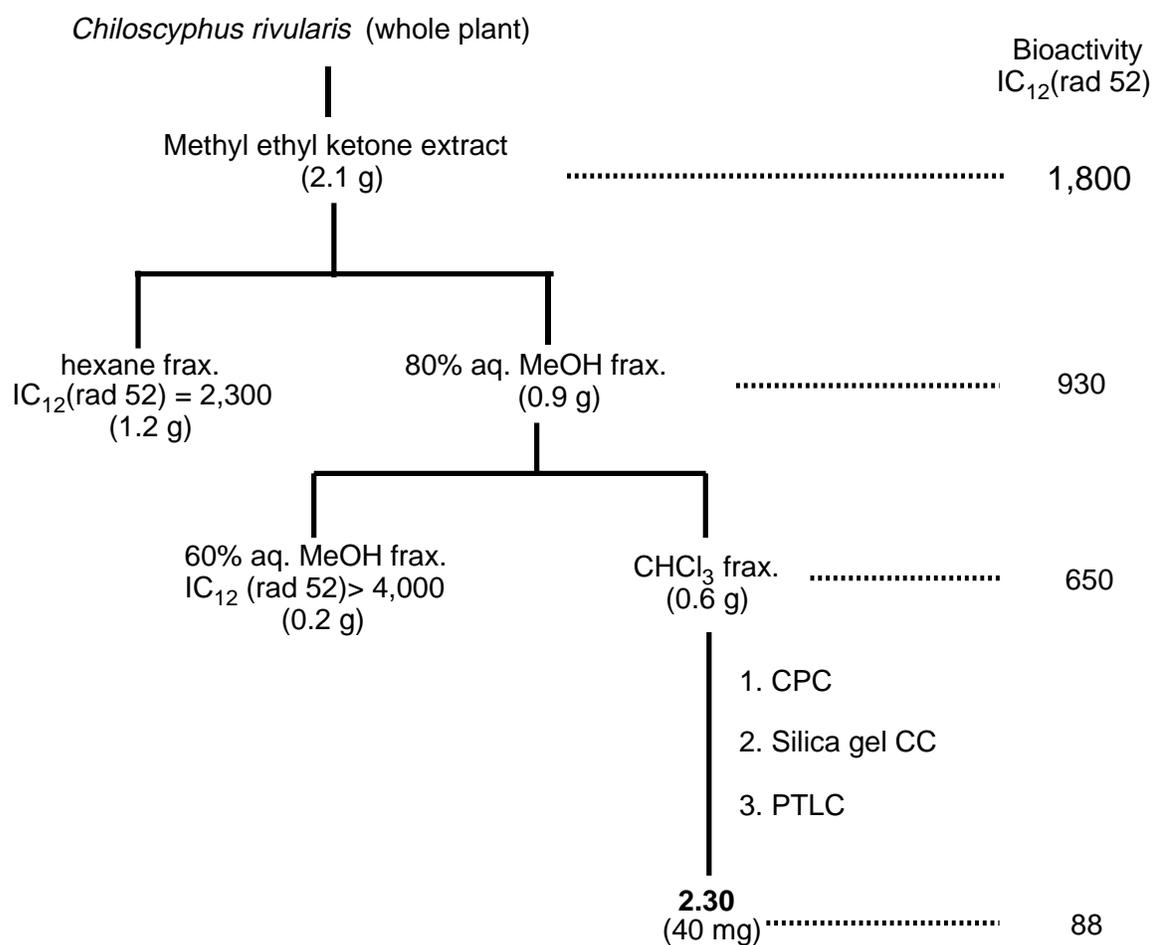
⁴⁶ Tori, M., Hasebe, T., Asakawa, Y., Ogawa, K., Yoshimura, S. *Bull. Chem. Soc. Jpn.* **1991**, *64*, 2303 - 2306

2.2 RESULTS AND DISCUSSION

2.2.1 Isolation of Sesquiterpenoids from *Chiloscyphus rivularis*

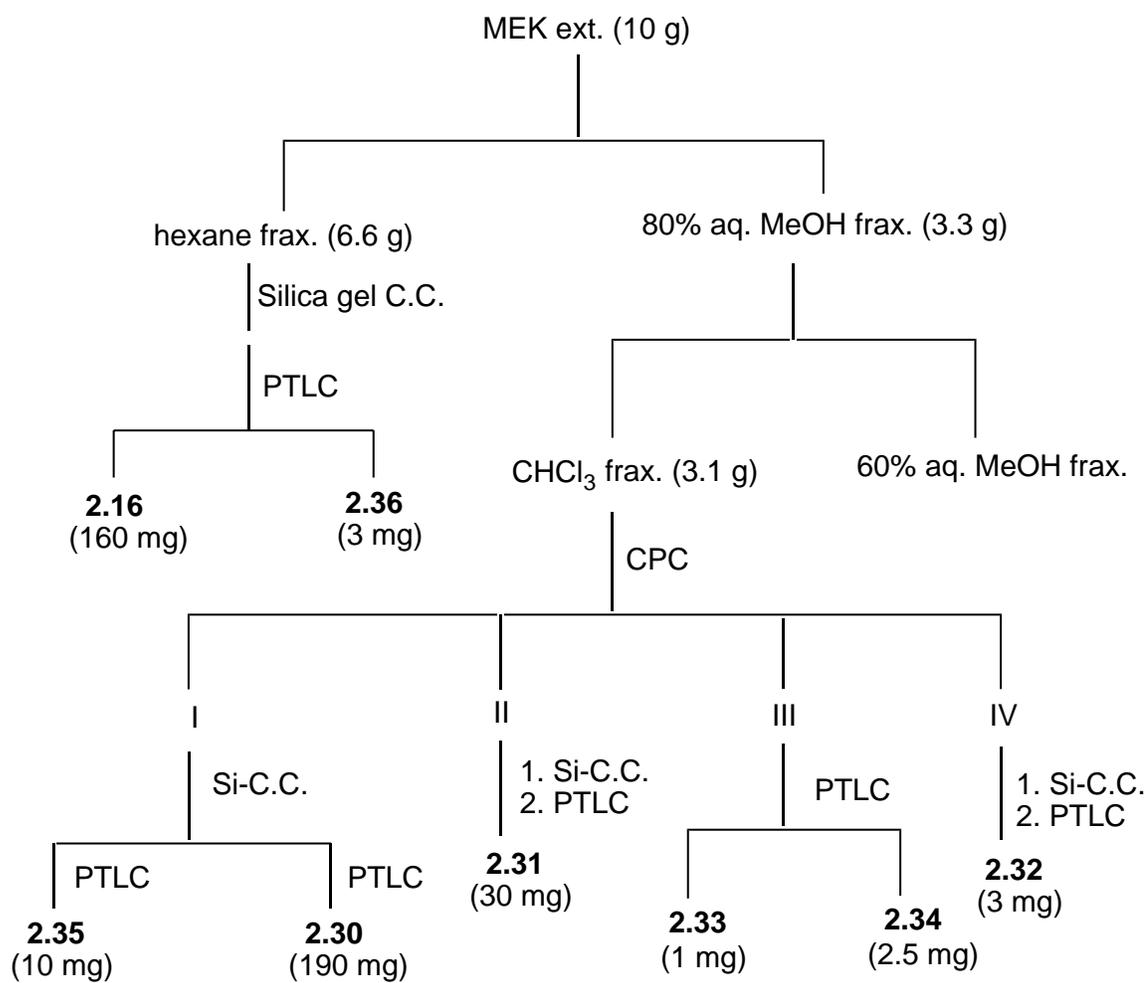
As part of a systematic study of plants for antitumor activity, the methyl ethyl ketone (MEK) extract of *Chiloscyphus rivularis* (Hepaticae, Lophocoleaceae) was found to show selective activity in the yeast-based bioassay with IC_{12} values of 1,800 against *rad52* and greater than 8,000 against RAD+.

An MEK extract was subjected to solvent-solvent partition between hexanes and 80% aqueous methanol. The aqueous methanol fraction showed increased activity (IC_{12} *rad52* = 930) and the hexanes fraction gave lower activity (IC_{12} *rad52* = 2,300). The aqueous methanol fraction was diluted to 60% aqueous MeOH and extracted thoroughly with chloroform to give a chloroform fraction in which the activity was concentrated. Further bioassay-directed fractionation of the chloroform fraction by centrifugal partition chromatography, silica gel chromatography, and PTLC afforded bioactive compound **2.30**, with an IC_{12} value of 88 $\mu\text{g/mL}$ against *rad52* (Scheme 1).



Scheme 1. Bioassay-directed Isolation of Active Sesquiterpenoid **2.30**

In order to obtain more active compound and other related compounds, a large-scale isolation was carried out. In addition to compound **2.30**, fractionation of the chloroform fraction also gave compounds **2.31-2.35**. While fractionation of the hexane fraction yielded chiloscyphone (**2.16**) and compound **2.36** (Scheme 2).



Scheme 2. Isolation Procedure of Sesquiterpenoids from *C. rivularis*

2.2.2 Structure Elucidation of Novel Sesquiterpenoids

2.2.2.1 Sesquiterpenoid **2.30**

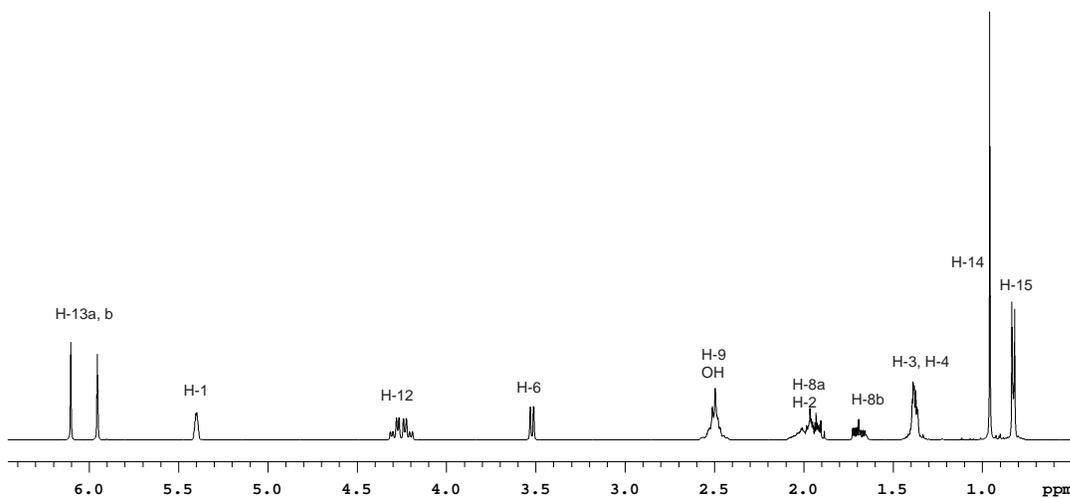
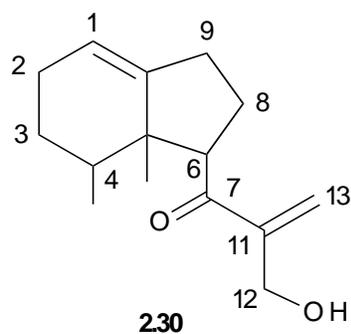
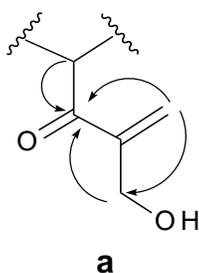


Figure 1. ^1H NMR Spectrum of **2.30**

Compound **2.30** had the composition $\text{C}_{15}\text{H}_{22}\text{O}_2$ as determined by HREIMS. UV and IR spectra indicated the presence of an α,β -unsaturated ketone (λ_{max} 224 nm and ν_{max} 1659 cm^{-1}). Its ^1H NMR spectrum showed signals for two olefinic methylene protons (δ 5.95, 6.10), a methine proton α to a carbonyl group (δ 3.52, $J = 7.6\text{ Hz}$), and two proton (δ 4.21, dd, $J = 15.6, 6.0$; δ 4.29, dd, $J = 15.6, 6.0$) suggestive of a CH_2OH group. A DQCOSY spectrum indicated that these latter signals were coupled only to a

multiplet at $\delta 2.50$, and when the spectrum was obtained in the presence of added D_2O , the eight-line pattern was simplified to an AB quartet ($J = 15.6\text{Hz}$). The presence of an OH group was supported by the observation of a ^{13}C NMR signal for a CH_2 group at $\delta 63.1$, and was confirmed by an absorption at 3558 cm^{-1} in the IR spectrum. These data thus indicated the presence of structural unit **a** in compound **2.30**, and this was confirmed by an HMBC spectrum, which gave the correlations shown (arrows).



The remaining signals in the ^1H -NMR spectrum of **2.30** were less easily assigned, consisting of complex multiplets in the $\delta 1.4 - 2.5$ region, together with a signal for a vinyl proton at 5.40 (1H, m) and for two methyl groups at 0.96 (3H, s) and 0.83 (3H, d, $J = 6.4$). The presence of an additional double bond was confirmed by the ^{13}C -NMR spectrum, which gave signals for two olefinic carbons at $\delta 117.4$ and 146.2, in addition to the carbons of the unsaturated ketone system at $\delta 207.2$, 147.8, and 124.8. The presence of three unsaturations demanded that compound **2.30** be bicyclic.

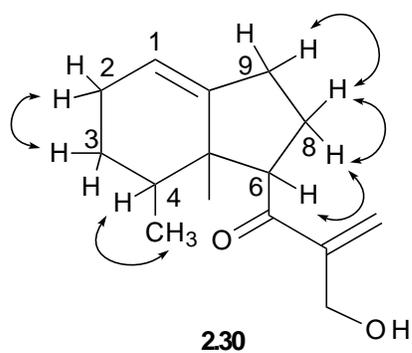
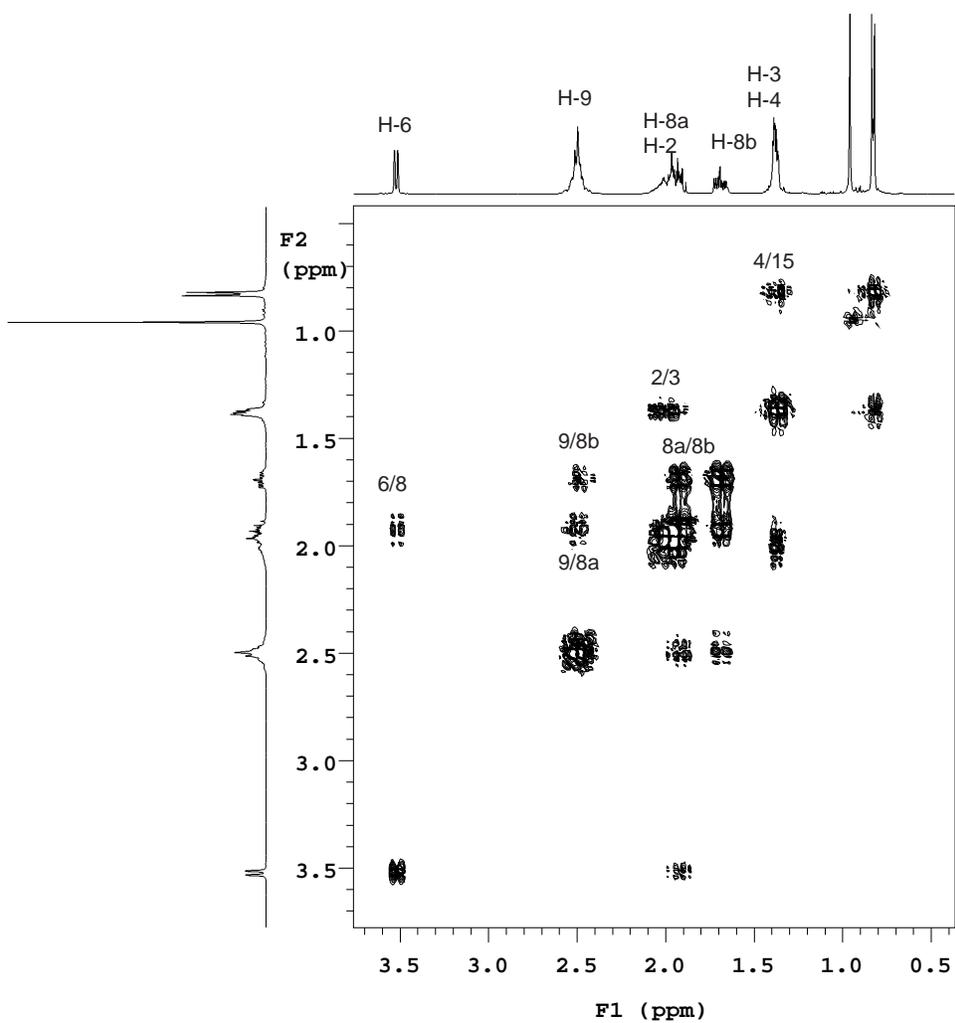
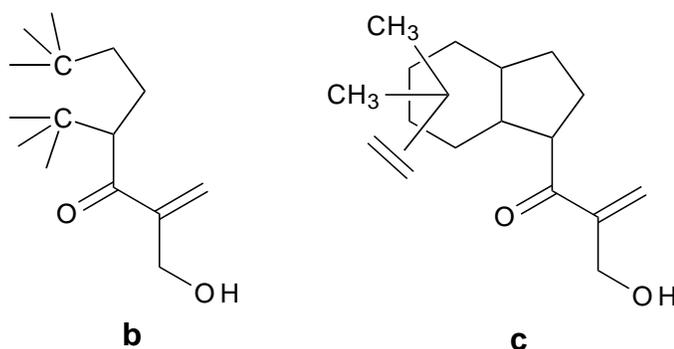


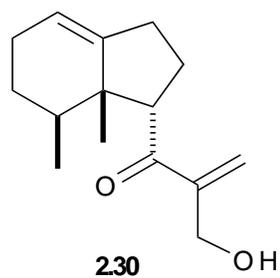
Figure 2. Expanded DQCOSY Spectrum of **2.30**

The structure could be elucidated further starting from the methine proton at 3.52 ppm α to the carbonyl group. Because this proton appeared as an apparent doublet, it must be flanked by a quaternary carbon and by a carbon carrying one or possibly two

protons. The DQCOSY spectrum showed a correlation between the proton at 3.52 ppm and a proton at 2.00 ppm, and the latter proton was correlated with a proton at 1.69 ppm and a signal at 2.50 ppm. A combination of HETCOR and DEPT spectra showed clearly that both sets of protons at 1.69/2.00 and 2.50 ppm were due to methylene groups, and the COSY spectrum showed no additional couplings to the protons at 2.50 ppm. These data thus allow the expansion of structural unit **a** to unit **b**, where both terminal carbons are quaternary. This fact, together with the presence of two methyl groups and an HMBC correlation between H-6 and C-10, demands the expansion of the partial structure to **c**, where two methyl groups and one double bond remain to be located.



Of the two methyl groups, the carbon of the one giving rise to a singlet at 0.96 ppm in the $^1\text{H-NMR}$ spectrum showed an HMBC correlation with the methine proton at 3.52 ppm; this evidence thus demands the location of this carbon at C-5. The other methyl group appeared as a doublet at 0.83 ppm, and its protons gave an HMBC correlation to the quaternary carbon at C-5; this methyl group is thus located at C-4. The double bond must be located at the 1(10) position to account for the chemical shift of the H-9 protons.



The above data thus indicate that compound **2.30** has the structure shown, excluding stereochemistry. The conclusion is confirmed by the HMBC correlations given in Table 1 and shown in Figure 3. The mass spectral fragmentation of **2.30** offers strong corroborative evidence for this conclusion (Scheme 3).

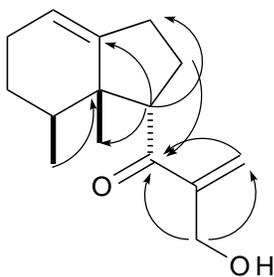
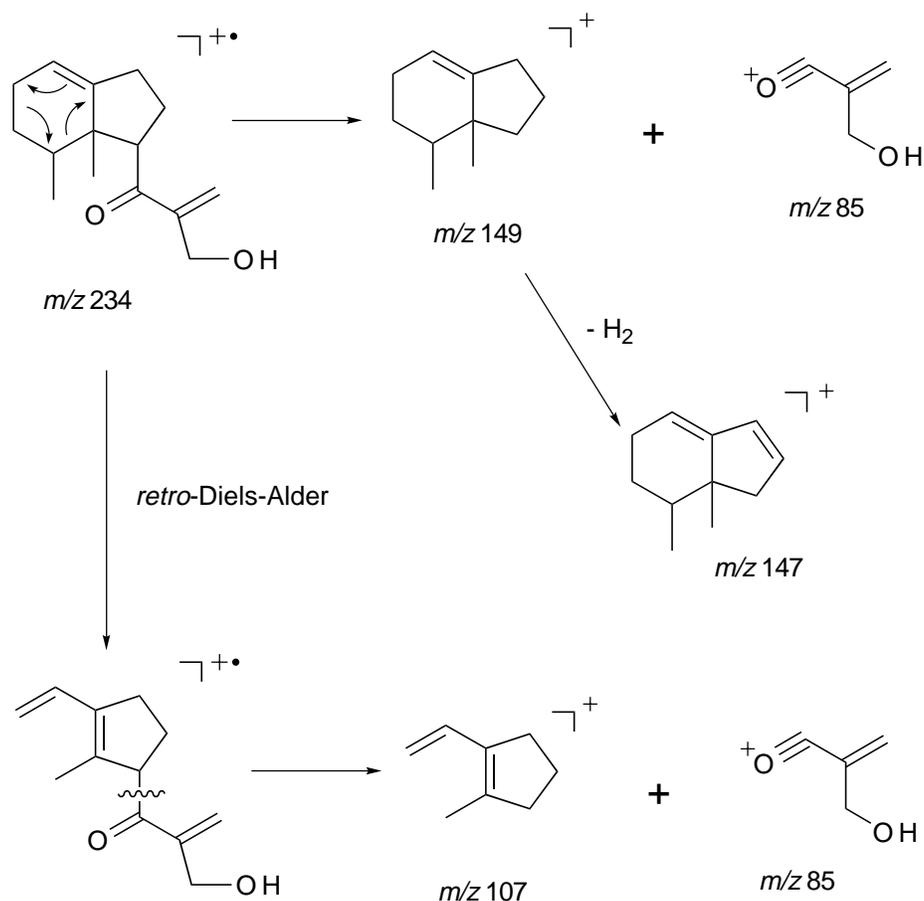


Figure 3. Selected HMBC Correlations for Compound **2.30**



Scheme 3. Mass Spectrum Fragmentation of **2.30**

The stereochemistry of **2.30** was assigned with the aid of a NOESY spectrum, which showed NOE correlations between the protons of H₃-14 and H-6 and between the protons of H₃-15 and H-6. These correlations require that H-6, CH₃-14 and CH₃-15 all be *cis* to each other, and thus *trans* to the C-6 side chain. The absolute stereochemistry of **2.30** was assigned as that of chiloscypone (**2.16**) on the basis of their very similar CD spectra, with both showing negative Cotton effects at about 348 nm. The structure and absolute stereochemistries of **2.30** can be thus be assigned as those of 12-hydroxylchiloscypone. Complete ¹H and ¹³C-NMR assignments for **2.30** were made by

a combination DQCOSY, HETCOR, and HMBC experiments and are given in Table 1.

Table 1. ^1H - and ^{13}C -NMR Data for Compound **2.30**^a

position	δ_{H}^b	$\delta_{\text{C}}^{c,d}$	COSY	HMBC
1	5.40 m	117.4 (1)	H-2	
2a, b	1.94 m	25.3 (2)	H-1, H-3	
3a, b	1.38 m	26.9 (2)	H-2	
4	1.36 m	32.9 (1)	H-15	
5		50.0 (0)		
6	3.52 d 7.6	52.8 (1)	H-8a	C-5, C-7, C-9, C-10, C-14
7		207.2 (0)		
8a	2.00 m	26.0 (2)	H-6, H-8b, H-9	C-7
8b	1.69 m		H-8a, H-9	C-7
9a, b	2.50 m	29.0 (2)	H-8a, H-8b	
10		146.2 (0)		
11		147.8 (0)		
12a	4.21 dd 15.6, 6.0	63.1 (2)	12b, 12-OH	C-7, C-11, C-13
12b	4.29 dd 15.6, 6.0	63.1 (2)	12a, 12-OH	
13a	6.10 br, s	124.8 (2)		C-7, C-12
13b	5.95 br, s			C-7, C-12
14	0.96 (3H) s	20.6 (3)	H-4	
15	0.83 (3H) d 6.4	17.6 (3)	H-12	C-3, C-4, C-5
12-OH	2.50 m			

^a Data recorded in CDCl_3 at 400MHz (^1H) and 100 MHz (^{13}C). ^b Multiplicities and coupling constants (in Hz) are listed. ^c Assignments were determined by HETCOR and DEPT experiments. ^d Carbon type as determined by DEPT spectra: 0 = quaternary, 1 = methine, 2 = methylene, 3 = methyl.

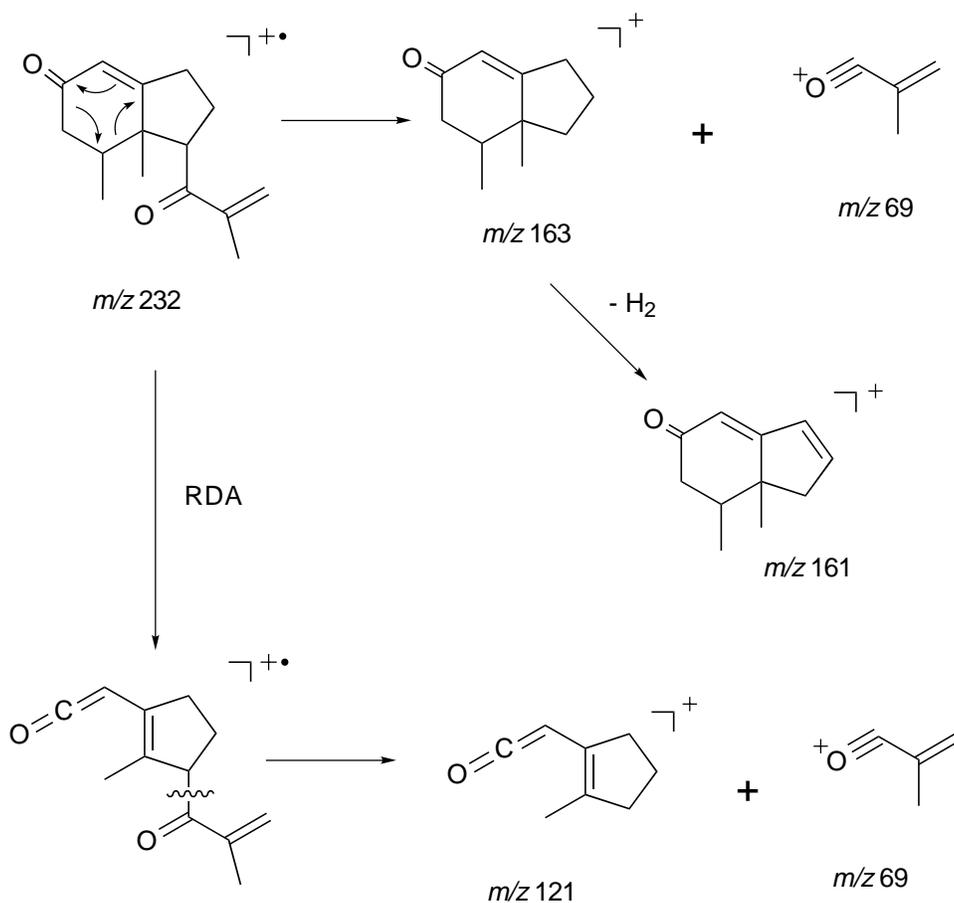
2.2.2.2 Sesquiterpenoid **2.31**

Compound **2.31** was assigned the molecular formula $C_{15}H_{20}O_2$ from its HREIMS. Its NMR spectra, which showed some similarities to those of **2.30**, indicated the presence of an α,β -unsaturated ketone system. The signals at 1.33 ppm and 0.99 ppm in its 1H NMR spectrum indicated the presence of two methyl groups, and the signal at 1.86 ppm gave evidence of the presence of a vinyl methyl group.

One major difference between the spectra of **2.30** and **2.31** was that in the spectra of **2.31** the signals for the $-CH_2OH$ group of **2.30** (δ_H 4.25, δ_C 63.1) were replaced by the signals for a vinyl methyl group (δ_H 1.86, 3H; δ_C 17.6). A second major difference was that the ^{13}C -NMR spectrum of **2.31** contained signals for two carbonyl carbons (δ_C 177.9 and 204.7).

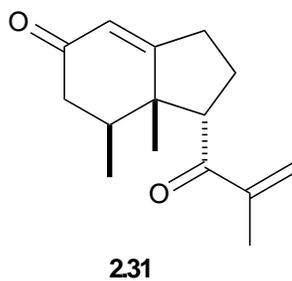
The first carbonyl group of **2.31** was clearly still conjugated to a double bond in the side chain, because the H_{2-13} protons had the downfield shift (δ_H 5.84, 5.88) characteristic of β -protons in an α,β -unsaturated carbonyl system. The other carbonyl group was assigned to C-2 because the 1H -NMR signal of H-1 changed from a multiplet of 5.40 ppm in **2.30** to a sharp singlet at 6.01 ppm in **2.31**.

The mass spectrum gave similar fragmentation processes to compound **2.30**, which also supported the conclusion drawn from NMR spectra.



Scheme 4. Mass Spectrum Fragmentation of **2.31**

These data indicated that compound **2.31** had the structure shown.



The stereochemistry of **2.31** was determined by the observation of an NOE correlation between H₃-15 and H-6, showing that it had the same relative stereochemistry

as **2.30**, and also on the basis of their similar CD spectra. The structure and stereochemistry of **2.31** were thus assigned as chiloscypa-2,7-dione, a new sesquiterpenoid.

¹H NMR and ¹³C NMR data for **2.31** are given in Tables 2 and 3.

Table 2. ¹H-NMR Data for Compounds **2.31**, **2.32** and **2.33**^{a, b}

position	2.31	2.32	2.33
1	6.01 s	5.88	6.41 s
3	2.18 (2H) m	2.22 (2H) m	2.33 (2H) m
4	2.16 m	2.22 m	2.16 m
6	3.68 d 7.4	3.66 d 8.0	3.98 d 7.6
8a	2.15 m	2.18 m	2.73 dd 18.4, 7.6
8b	1.82 m	1.86 m	2.43 d 18.4
9	a 2.85 m b 2.79 m	2.83 (2H) m	
12	1.86 (3H) s	4.31 (2H) br, s	1.88 (3H) s
13a	5.88 br, s,	6.18 br, s	6.13 br, s
13b	5.84 br, s,	6.08 br, s	5.98 br, s
14	1.13 (3H) s	1.15 (3H) s	1.28 (3H) s
15	0.90 (3H) d 6.0	0.91 (3H) d 5.6	1.02 (3H) d 6.7
12-OH		2.22 m	

^a Data recorded in CDCl₃ at 400MHz. ^b Multiplicities and coupling constants (in Hz) are listed.

Table 3. ^{13}C -NMR Data for Compounds **2.31**, **2.32** and **2.33**^{a, b}

position	2.31	2.32	2.33
1	124.9	121.5	122.9
2	199.0	199.0	199.4
3	41.2	41.2	41.9
4	33.4	33.4	35.2
5	51.0	51.2	48.3
6	51.8	52.2	47.1
7	204.7	205.1	204.3
8	26.7	26.7	41.1
9	29.9	29.9	203.6
10	177.9	177.6	160.0
11	145.2	147.4	145.0
12	17.6	62.5	17.3
13	121.4	125.6	126.7
14	18.2	18.3	18.7
15	16.6	16.8	16.0

^a Data recorded in CDCl_3 at 100 Mhz. ^b Assignment made with the aid of HETCOR and DEPT spectra.

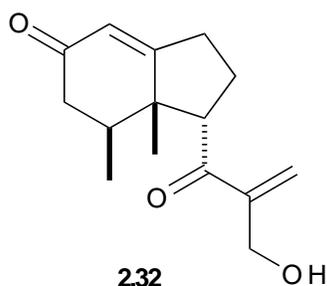
2.2.2.3 Sesquiterpenoid **2.32**

Compound **2.32** had the composition $\text{C}_{15}\text{H}_{20}\text{O}_3$ as determined by HREIMS, and it had NMR spectra that showed some similarities to both **2.30** and **2.31**.

The ^1H NMR and ^{13}C NMR spectra of **2.32** indicated the presence of a $-\text{CH}_2\text{OH}$ group (δ_{H} 4.31, 2H, δ_{C} 62.5), two methyl groups (δ_{H} 1.15, 3H, and δ_{H} 0.91, 3H) and also an α,β -unsaturated carbonyl system.

The ^{13}C NMR spectrum showed the presence of two carbonyl carbons (δ_{C} 177.6 and 205.1). Besides the carbonyl group conjugated to a double bond in the side chain, the other carbonyl group could be assigned to C-2 since the H-1 proton appeared as a sharp singlet at 5.88 ppm.

Based on comparison with compounds **2.30** and **2.31**, compound **2.32** could be assigned as the new sesquiterpenoid 12-hydroxychiloscyph-2,7-dione. Since it was isolated from the same plant, had a similar structure, and gave similar value of specific rotation with **2.30** and **2.31**, its stereochemistry was assigned as shown.



2.2.2.4 Sesquiterpenoid **2.33**

Compound **2.33** had the composition $\text{C}_{15}\text{H}_{18}\text{O}_3$ as determined by HRCIMS. Its NMR spectra (Table 2 and 3) indicated that it had a very similar structure to compound **2.31**.

The ^1H NMR and ^{13}C NMR spectra of **2.33** indicated the presence of a vinyl methyl group (δ_{H} 1.88, 3H, δ_{C} 17.3), two methyl groups (δ_{H} 1.28, 3H, and δ_{H} 1.02, 3H) and also an α,β -unsaturated carbonyl system.

The major difference between **2.32** and **2.33** was that **2.33** contained a third carbonyl group (δ_{C} 203.6) in place of a methylene group. The carbonyl group was

assigned to C-9 on the basis of the coupling observed in a DQCOSY spectrum between H-6 and H₂-8; the latter protons appeared downfield at 2.43 and 2.73 ppm, providing further evidence of the adjacent carbonyl group. Compound **2.33** could be assigned as chiloscyp-2,7,9-trione (**2.33**), a new sesquiterpenoid. By the comparison of specific rotation value with **2.30** and **2.31** its stereochemistry was assigned as shown.

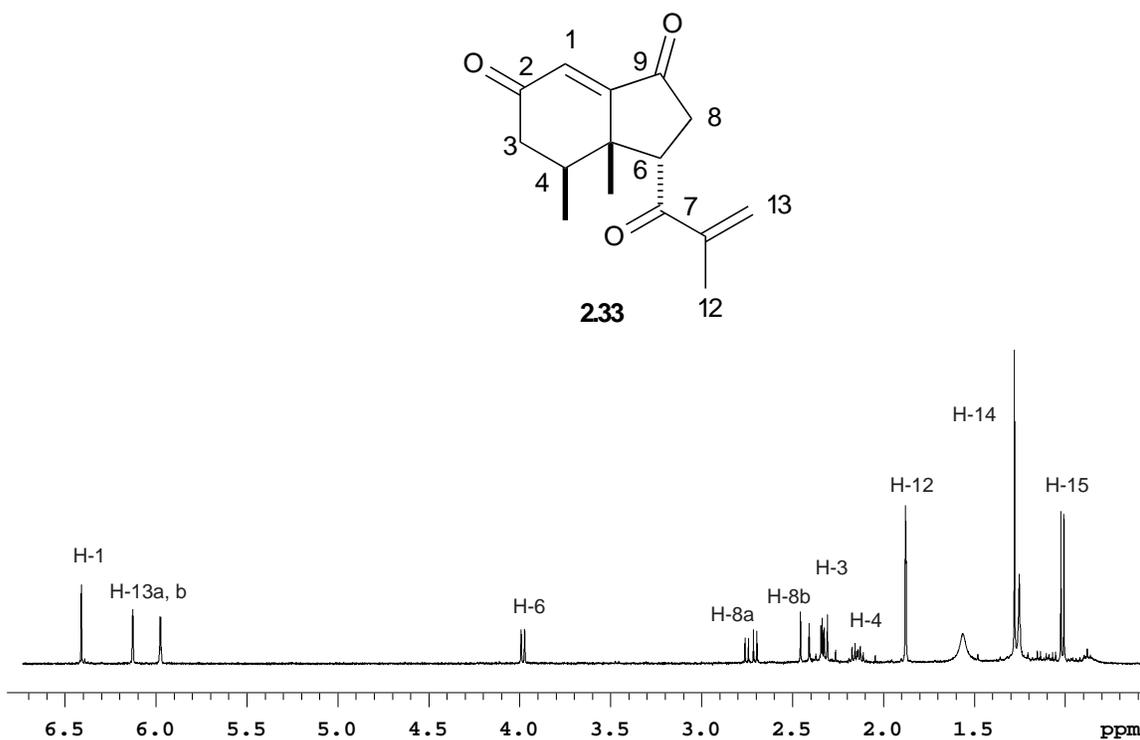


Figure 4. ¹H NMR Spectrum of Compound **2.33**

2.2.2.5 Sesquiterpenoid **2.34**

Compound **2.34** had the composition C₁₂H₁₈O₃ as determined by HREIMS. Its ¹³C NMR spectrum did not show any signals for an olefinic carbon but did have a signal

for one carbonyl carbon. The IR spectrum showed a carbonyl absorption at 1770 cm^{-1} , suggesting the presence of a γ -lactone.

All the signals in its ^1H NMR spectrum occurred in the region of 0.8 ppm - 2.7 ppm except one at 4.18 ppm (dd, 1H). Analysis of its HMQC spectrum indicated the presence of two methyl groups, four methylene groups and three methine groups.

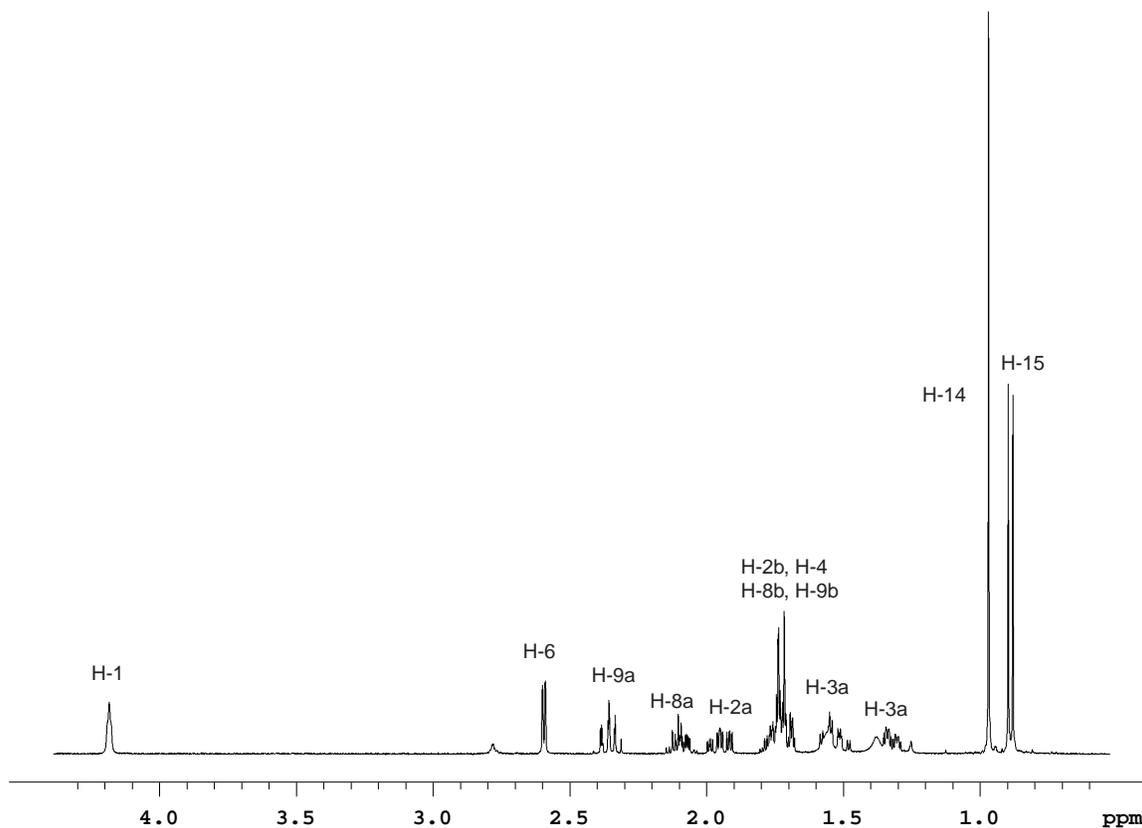
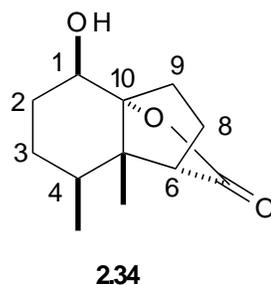


Figure 5. ^1H NMR Spectrum of 2.34

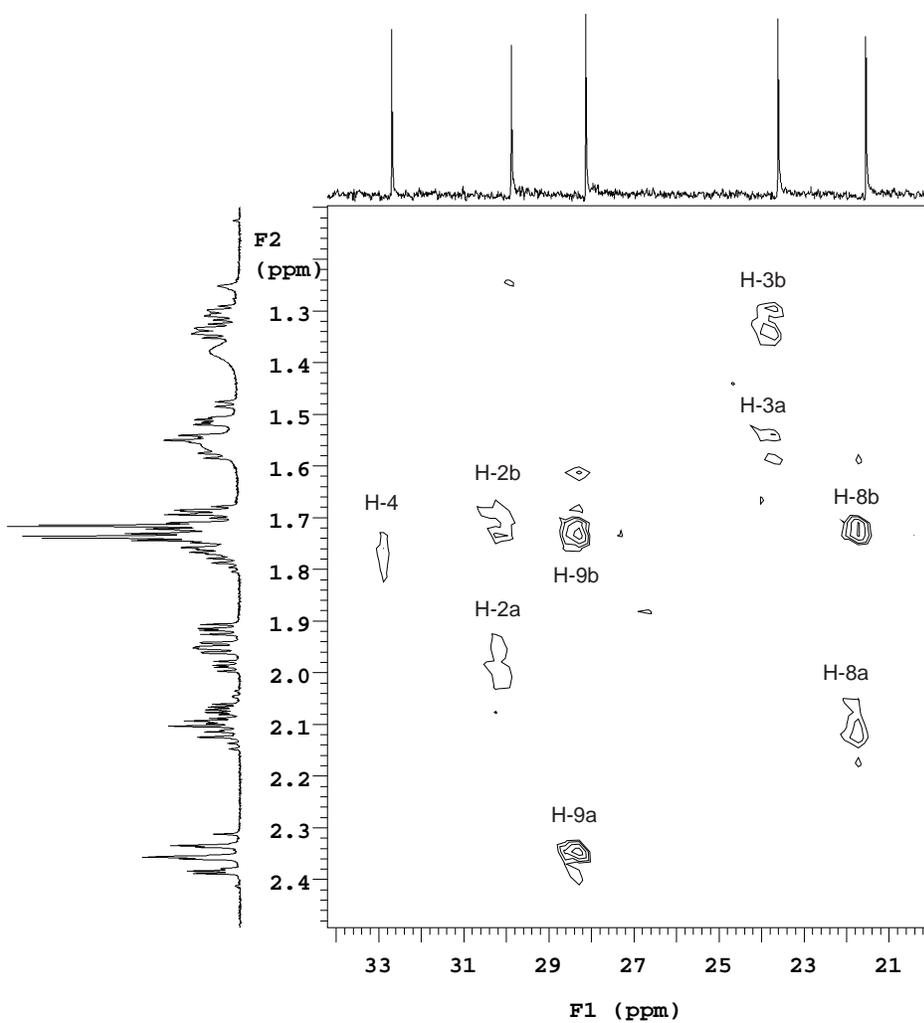
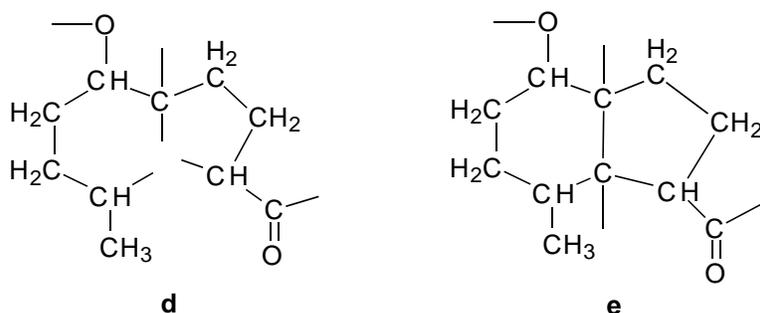


Figure 6. Expanded HMQC Spectrum of **2.34**

With the help of a DQCOSY NMR spectrum, two $-\text{CH}_2-\text{CH}_2-$ units could be assigned. One of these units was bounded by a quaternary carbon at one end and by a methine group α to a carbonyl group at the other end; the quaternary carbon had an unusually large chemical shift of 92.5 ppm for an sp^3 carbon. The other $-\text{CH}_2-\text{CH}_2-$ group showed coupling to a methine group at each end. One of these groups was oxygen bearing (δ_{H} 4.18, δ_{C} 66.5) and was also connected to the same quaternary carbon with the

unusually large chemical shift. The other methine group (δ_{H} 1.73) carried a methyl group (δ_{H} 0.89, $J = 6.7$ Hz) and a quaternary carbon (δ_{C} 51.5). These data led to structural unit

d.



Because of the four unsaturations the compound must be bicyclic, so structure unit **d** could be connected to give structure unit **e**. The lactone must form on the quaternary carbon with the unusually large chemical shift, and the remaining methyl group could only be assigned to the other quaternary carbon to give structure **2.34**, an unusual trisnorsesquiterpenoid of the chiloscyphane class; the key HMBC correlations that confirm this structure are shown in Figure 7.

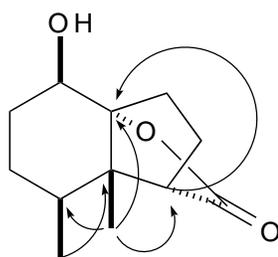


Figure 7. Selected HMBC Correlations for Compound **2.34**

The unusual downfield shift of C-10 is explicable based on its substitution by an acyloxy function and by the presence of a β -hydroxyl group.

A NOESY spectrum of **2.34** showed NOE correlations between the protons of H₃-14 and H-6 and between the protons of H₃-15 and H-6, indicating that H-6, CH₃-14 and CH₃-15 all are *cis* to each other, just as were the corresponding atoms or groups in compound **2.30**. The stereochemistry at C-1 was assigned by a consideration of the coupling constants of H-1, which appeared as a doublet of doublets with $J = 2.3$ and 2.6 Hz. These data indicated that H-1 must be equatorial, and thus the C-1 OH group must be β -axial. Compound **2.34** thus has the structure and stereochemistry shown, and is given the name rivulalactone.

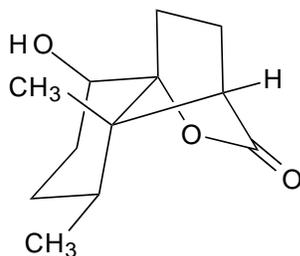


Figure 8. Conformational Structure of Compound **2.34**

Table 4. ¹H- and ¹³C-NMR Data for Compound **2.34**^a

position	δ_{H}^b	$\delta_{\text{C}}^{c,d}$	COSY	HMBC
1	4.18 dd 2.3, 2.6	66.5(1)	H-2a, b	
2a	1.95 m	29.9(2)	H-1, H-2b, H-3	
2b	1.73 m		H-1, H-2a, H-3	
3a	1.53 m	23.6(2)	H-2a, b, H-3b	
3b	1.32 m		H-2a, H-3a	
4	1.73 m	32.7(1)	H-15	
5		51.5(0)		
6	2.59 d 3.8	51.8(1)	H-8a	C-10
7		177.8(0)		
8a	2.10 m	21.5(2)	H-6, H-8b, H-	C-7
8b	1.73 m		9a	
9a	2.53 m	28.1(2)	H-8a, H-9a	C-6
9b	1.73 m		H-8a, H-9b	
10		92.5(0)	H-9a	
14	0.97(3H) s	17.1(3)		C-4, C-6, C-10
15	0.89(3H) d 6.7	11.3(3)	H-4	C-3, C-4, C-5

^a Data recorded in CDCl₃ at 400MHz (¹H) and 100 MHz (¹³C). ^b Multiplicities and coupling constants (in Hz) are listed. ^c Assignments were determined by HETCOR and DEPT experiments. ^d Carbon type as determined by DEPT spectra: 0 = quaternary, 1 = methine, 2 = methylene, 3 = methyl.

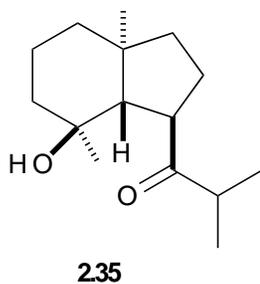
2.2.3 Identification of Known Sesquiterpenoids

2.2.3.1 Sesquiterpenoid **2.16**

Compound **2.16** was isolated as one of the major components in the MEK extract of *C. rivularis*. Its NMR spectra and MS indicated it to be the known compound chiloscyphone.⁴⁷

2.2.3.2 Sesquiterpenoid **2.35**

Based on its spectral data, compound **2.35** was identified as 4-hydroxyoppositan-7-one.³⁸



Its spectral data may be found in the experimental section.

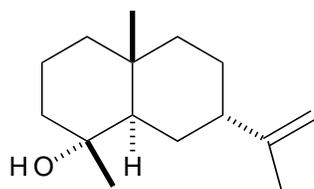
2.2.3.3 Sesquiterpenoid **2.36**

Another known sesquiterpenoid was isolated in small amount, and from its NMR spectra and MS it was assigned as either isointermedeol⁴⁸ or intermedeol,⁴⁹ a pair of

⁴⁷ Harrison, L. J., Asakawa, Y. *Phytochemistry* **1991**, *30*, 3806 - 3807

⁴⁸ Thappa, R. K., Dhar, K. L., Atal, C. K. *Phytochemistry* **1979**, *18*, 671-672

enantiomeric compounds. In the literature⁵⁰ the specific optical rotation values are +2° (*c.* 3.3, MeOH) for the former, and +18° (*c.* 2.8, CHCl₃), or +10.7° (*c.* 0.95, EtOH) for the latter. Since the specific optical rotation values for **2.36** are -16.2° (*c.* 0.49, MeOH), -10.7° (*c.* 0.58, EtOH), and -11.2° (*c.* 0.27, CHCl₃), its structure must be isointermedeol, the enantiomer of intermedeol. There is presumably an error with the specific optical rotation value for intermedeol in the literature.



2.36

2.2.4 Synthetic Chemistry Studies of **2.30** and **2.34**

2.2.4.1 Semisynthesis of **2.34**

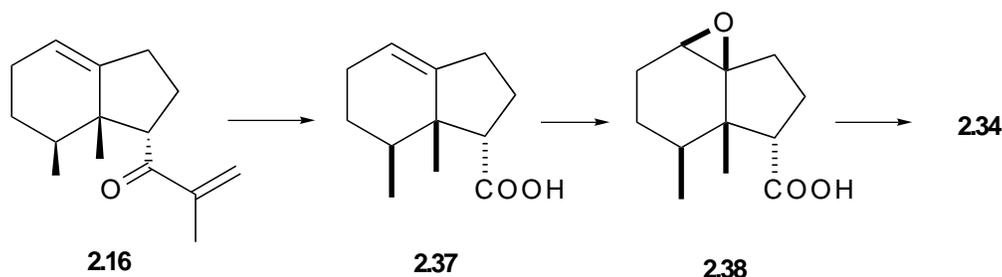
The structure of rivulalactone (**2.34**) appeared to be derivable, both synthetically and possibly biosynthetically, from chiloscypnone (**2.16**), and it was of interest to carry out this conversion.

Conversion of chiloscypnone (**2.16**) to rivulalactone (**2.34**) was proposed to proceed through the steps of side-chain degradation to the trisnor acid **2.37**, followed by

⁴⁹ Feliciano, A. S., Medarde, M., Rey, B. D., Corral, J. M., Barrero, A. F. *Phytochemistry* **1990**, *29*, 3207-3211

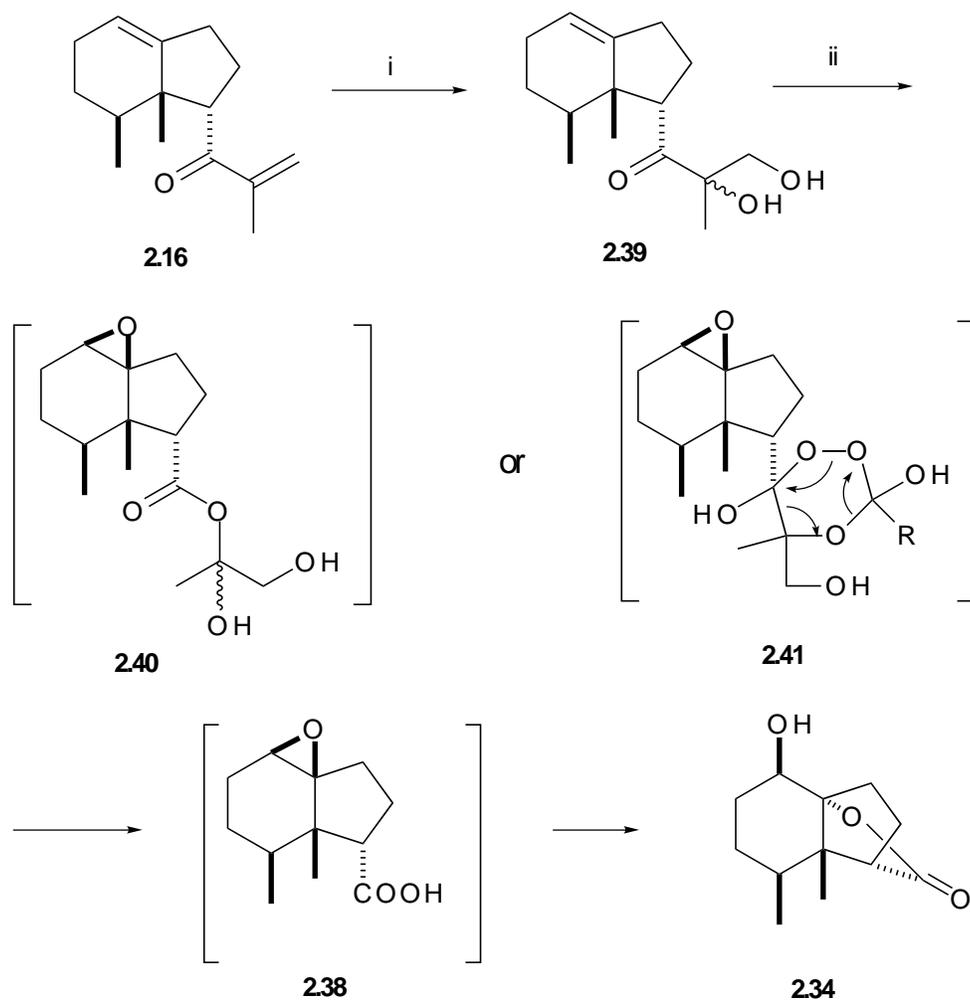
⁵⁰ Connolly, J. D., Hill, R. A. *Dictionary of Terpenoids* Vol. 1, Chapman & Hall, **1991**, pp. 332-333

epoxidation to the epoxyacid **2.38**, which would be expected to undergo intramolecular cyclization to **2.34**.



In the event, epoxidation and side-chain degradation could be carried out in a single step. Chiloscypnone was first selectively hydroxylated with osmium tetroxide in Me_2CO at -10 to -20°C to yield the diol **2.39** by reaction of the less hindered of the two double bonds. Treatment of **2.39** with *m*-chloroperbenzoic acid then converted **2.39** in a clean reaction into rivulalactone (**2.34**), identical with the natural product in all respects. This conversion of chiloscypnone (**2.16**), with a known structure and stereochemistry, into **2.34** offers independent confirmation of the structure and stereochemistry of rivulalactone (**2.34**); in particular, the stereochemistry of the C-1 hydroxyl group is established by this synthesis.

The mechanism of the conversion of the diol **2.39** to **2.34** is of some interest. Presumably the double bond is epoxidized in the normal way, to the β -epoxide, while the α -hydroxycarbonyl group of the side chain is also attacked by the peracid. The resulting Baeyer-Villiger reaction product (**2.40**) would then give an unstable hemiacetal intermediate that would fall apart to the epoxyacid **2.38** and thence to rivulalactone (**2.34**). Alternatively, oxidative cleavage could occur via a cyclic peroxide intermediate **2.41**.



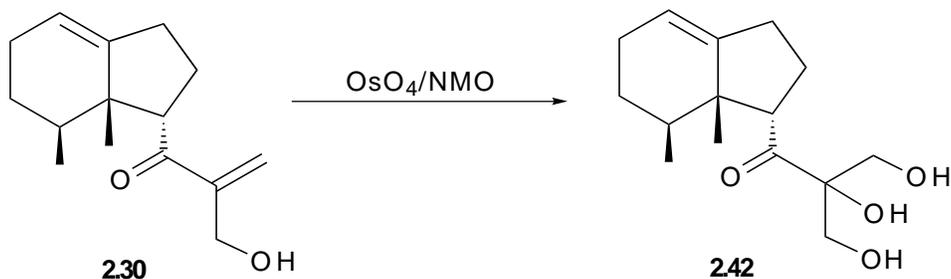
i OsO_4/NMO , acetone: H_2O (8:1), -10 - -20°C, 60 min
 ii *m*-CPBA/ CH_2Cl_2 , RT, 60 min

Scheme 5. Semi-synthesis of rivulalactone (2.34) and possible mechanisms of side chain degradation

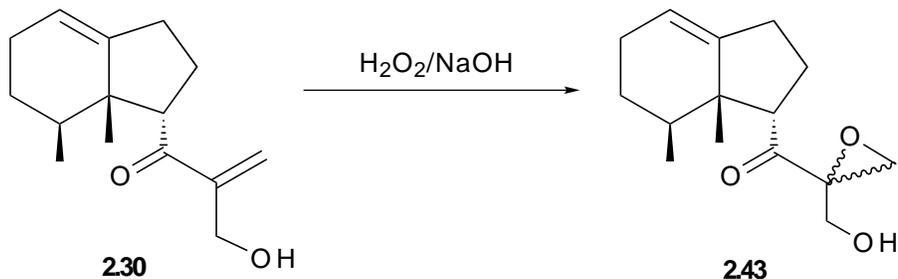
2.2.4.2 Modification of **2.30**

Because the bioactivity of **2.30**, as compared with the related sesquiterpenes **2.16** and **2.31-2.34**, appeared to be related in part to the presence of the hydroxyl group at C-12, it was of interest to determine the effect of additional oxidation on the bioactivity of compound **2.30**.

Treatment of **2.30** with OsO_4/NMO gave compound **2.42** and the structure was determined to be 11,12,13-trihydroxychiloscyphone by the spectroscopic data in the Experimental Section.



Compound **2.30** was treated with H_2O_2 in basic condition, and epoxidation occurred selectively on the conjugated double bond to give 11,13-epoxy-12-hydroxychiloscyphone (**2.43**), with undetermined stereochemistry at the new chiral center.



2.2.5 Biological Evaluation of Compounds

All the compounds isolated were tested against the *rad52* yeast strain, and compound **2.30** was also tested against the RAD+ and *rad52.top1* strains. Compound **2.30** had IC₁₂ values of 88 and 75 µg/mL against the DNA repair - deficient strains *rad52* and *rad52.top1*, respectively, but was inactive (IC₁₂ > 1000 µg/mL) in the repair-proficient strain RAD+. These data are characteristic of a selective DNA-damaging agent that does not act as a topoisomerase I or topoisomerase II inhibitor. Compound **2.30** was cytotoxic to the human lung carcinoma A-549 cell line with an IC₅₀ of 1.95 µg/mL.

Although several of the other compound isolated were structurally very similar to compound **2.30**, surprisingly none of them showed any activity against the repair-deficient yeast strain *rad52* (IC₁₂ values all greater than 500 µg/mL). The two semisynthetic analogues **2.42** and **2.43** were also inactive in this assay. It seems the structure unit CH₂=C-CH₂OH is essential to its bioactivity, and any modification on this unit would destroy its activity. The addition of one carbonyl group on the ring also causes the loss of its activity.

2.3 EXPERIMENTAL

General Experimental Procedures. Mp's were determined on a Kofler hot-stage apparatus and are uncorrected. Optical rotations were taken in CHCl_3 solution with a Perkin-Elmer Model 241 polarimeter, and CD spectra were obtained on a JASCO J720 spectropolarimeter. The ^1H - and ^{13}C -NMR spectra were recorded on a Varian Unity 400 spectrometer at 400 and 100.57 MHz, respectively. ^1H - ^1H COSY, DEPT, and ^1H - ^{13}C HETCOR NMR experiments were performed on the same spectrometer, using standard Varian pulse sequences. Mass spectra were taken on a VG 7070 E-HF instrument. Chromatography was performed using Si gel Merck G60(230-400 mesh), preparative TLC with Si gel GF254 plates (Analtech, 500 mm, 20x20 cm), and reversed-phase preparative TLC with Whatman PLKC18F linear K reversed-phase (500 mm, 20x20 cm) plates.

Plant Material. *Chiloscyphus rivularis* (5 kg) was collected in June 1994, on submersed rocks in Oregon as SPJ-13165B (WBA-2731). A voucher specimen is preserved at the U. S. National Herbarium.

Bioassay. In this report, assay results are reported in the form of an IC_{12} value, where IC_{12} is the concentration required to show a inhibition of growth zone of 12 mm diameter around a 100 μL well in an agar plate overlaid with the yeast strain used. Four appropriate doses are selected, bracketing the anticipated IC_{12} value. The test sample is applied to the plate in a 50:50 mixture of MeOH-DMSO. After a 48 hour incubation period, the zones of inhibition are measured and a linear regression analysis is run to arrive at the IC_{12} value which is recorded. Camptothecin is used as a positive control in doses of 5 and 200 μl for the *rad52* and RAD+, respectively.

Isolation of Sesquiterpenoids. Plant material (500 g) was extracted with MEK, and the crude MEK extract (10.3 g) was partitioned between hexane and 80% aqueous methanol, the hexane removed, and water was added until a 60% aq. MeOH mixture was achieved. This was extracted thoroughly with chloroform. The chloroform extract was dried under vacuum to yield 3.1 g of active material. This extract was chromatographed using centrifugal partition chromatography (CPC) with hexanes-ethyl acetate-methanol-water (1:1:1:1) as a solvent system.

The active fraction 3 from CPC was loaded onto a silica gel column and eluted with a gradient of ethyl acetate in hexane. Fraction 3 obtained from above column was found to be the most active and was further purified with PTLC to give the pure compound **2.30** (190 mg). Fraction 2 from this column was re-chromatographed with PTLC to give compound **2.35** (10 mg).

Fraction #4 from CPC was chromatographed on silica gel column and eluted with a gradient of ethyl acetate in hexane. The purification of fraction 2 obtained from this column with PTLC gave compound **2.31** (30 mg).

Fraction #6 from CPC was purified by PTLC and RP-PTLC to give compound **2.33** (1 mg) and **2.34** (2.5 mg).

The stationary phase from CPC was loaded onto a silica gel column and eluted with a gradient of acetone in chloroform. Fraction 5 from this column was further purified by PTLC to yield compound **2.32** (3 mg).

The hexane fraction (6.0 g) from the first partition was chromatographed on silica gel column with a gradient of ethyl acetate in hexane. Fraction #1 obtained from this column was re-chromatographed with PTLC and gave compound **2.16** (160 mg). After purification by PTLC and RP-PTLC, fraction 3 from the same column yielded compound **2.36** (3 mg).

12-Hydroxychiloscyphone (2.30): colorless liquid; $[\alpha]_D = -31.3^\circ$ (c. 0.3, CHCl₃); CD (MeOH) $\lambda_{\max} (\Delta\epsilon)$ 348 (-1.11) nm; UV (MeOH) $\lambda_{\max} (\log\epsilon)$: 224 (3.65) nm; IR(CHCl₃) ν_{\max} : 3558, 3019, 2967, 2926, 2842, 1659, 1459, 1433, 1373, 1231, 1200, 1075, 1029, 992, 947 cm⁻¹; ¹H NMR: see Table 1; ¹³C NMR: see Table 2; EIMS m/z 234 (M⁺), 216 (14), 149 (69), 147 (93), 107 (100), 85 (49); HREIMS m/z 234.1619 (M⁺, calcd for C₁₅H₂₂O₂, 234.1620).

Chiloscyph-2,7-dione(2.31): white crystals; mp 98°C; $[\alpha]_D = -73.3^\circ$ (c.0.3, CHCl₃); UV(MeOH) $\lambda_{\max}(\log \epsilon)$: 240 (4.23) nm; ¹H NMR: see Table 1; ¹³C NMR: see Table 2; EIMS m/z 232 (M⁺), 163 (66), 161 (100), 136 (57), 121 (53), 97 (21), 93 (16), 69 (50); HREIMS m/z 232.1461(M⁺, calcd for C₁₅H₂₀O₂, 232.1464).

12-Hydroxychiloscyph-2,7-dione (2.32): oil; $[\alpha]_D = -40.5^\circ$ (c.0.24, CHCl₃); UV (MeOH) $\lambda_{\max}(\log \epsilon)$: 204 (3.50), 240 (3.75) nm; ¹H NMR: see Table 1; ¹³C NMR: see Table 2; EIMS m/z 230 (3), 163 (44), 161 (32), 121 (76), 93 (23), 91 (43), 85 (20), 77 (43), 55 (100); HRCIMS m/z 249.1498 (M+1⁺) (calcd for C₁₅H₂₁O₃, 249.1490).

Chiloscyph-2,7,9-trione (2.33): white crystals; mp179 - 181°C; $[\alpha]_D = -110.1^\circ$ (c. 0.13, CHCl₃); UV (MeOH) $\lambda_{\max} (\log \epsilon)$: 228 (3.72), 270 (4.03); ¹H NMR: see Table 1; ¹³C NMR: see Table 2; EIMS m/z 246 (M⁺, 8), 177 (13), 175 (21), 149 (57), 135 (51), 122 (73), 107 (53), 91 (52), 79 (100); HRCIMS m/z 247.1342 (M+1⁺) (calcd for C₁₅H₁₉O₃, 247.1334).

Rivulalactone (2.34): white crystals; mp97-99°C; $[\alpha]_D = +20.4^\circ$ (c. 0.23, CHCl₃); UV (MeOH) λ_{\max} (log ϵ): 206 (3.17), 274 (2.83), 314 (2.62) nm; IR (CHCl₃) ν_{\max} : 3450, 3153, 2927, 1770, 1652, 1471, 1386, 1297 cm⁻¹; ¹H NMR: see Table 1; ¹³C NMR: see Table 2; EIMS m/z 210 (M⁺, 5), 195 (4), 192 (3), 182 (13), 122 (28), 107 (47), 95 (54), 81 (100), 67 (98); HREIMS m/z 210.1254 (M⁺, calcd for C₁₂H₁₈O₃, 210.1256).

4-Hydroxyoppositan-7-one (2.35): white crystals; mp46-48°C; $[\alpha]_D = +84^\circ$ (c.0.31, CHCl₃); ¹H NMR(CDCl₃) δ 0.88 (3H, s), 1.11 (6H, d, $J = 7$), 1.13 (3H, s), 1.21 (2H, m), 1.52 (5H, m), 1.63 (1H, m), 1.72 (1H, s, br), 1.80 (1H, m), 1.88 (1H, d, $J = 11$), 2.02 (1H, tt, $J = 2.0, 11.0$), 2.77 (1H, septet, $J = 7.0$), 3.09 (1H, dt, $J = 6, 11$); ¹³C NMR(CDCl₃) δ 18.1, 18.2, 20.1, 21.2, 21.6, 27.0, 38.5, 41.7, 42.1, 43.6, 43.7, 46.1, 60.5, 72.4, 220.4; EIMS m/z 238 (M⁺), 195, 177, 167, 149.

Chiloscyphone (2.16): oil; $[\alpha]_D = -15.7^\circ$ (c. 0.91, CHCl₃); ¹H NMR(CDCl₃) δ 0.83 (3H, d, $J = 6.4$), 0.95 (3H, s), 1.33-1.38 (3H, m), 1.68 (3H, m), 1.82 (3H, s, br), 1.87-2.02 (3H, m), 2.50 (2H, m), 3.56 (1H, dd, $J = 7.2, 1.6$), 5.39 (1H, dd, $J = 4.0, 2.4$), 5.71 (1H, s, br), 5.92 (1H, s); ¹³C NMR(CDCl₃) δ 17.5, 17.7, 20.6, 25.3, 26.0, 27.0, 29.1, 33.0, 49.8, 52.6, 117.3, 123.7, 146.0, 146.4, 206.5; EIMS m/z 218 (M⁺), 203, 175, 161, 149, 133, 122, 107, 93, 79, 69.

Isointermedeol (2.36): white solid (hexanes-EtOAc); mp36-38°C; $[\alpha]_D = -11.2^\circ$ (c. 0.27, CHCl₃), - 16.2° (MeOH), - 10.7° (EtOH); ¹H NMR(CDCl₃) δ 0.93 (3H, s), 1.09 (3H, s), 1.13 (1H, m), 1.25 - 1.55 (10H, m), 1.70 (2H, m), 1.75 (3H, s, br), 2.05 (1H, m), 2.43 (1H, m), 4.87 (1H, s, br), 4.92 (1H, s, br); ¹³C NMR (CDCl₃) δ 18.4, 20.1,

22.3, 22.7, 22.8, 23.5, 35.3, 39.3, 40.3, 41.3, 43.5, 49.2, 72.1, 110.8, 146.9; EIMS m/z 222 (M^+), 204, 189, 161, 149, 107.

Oxidation of chiloscypnone (2.16). To the solution of chiloscypnone (**2.16**, 10 mg) in a mixture of MeOH and H₂O (8:1, 1.0 ml), Osmium tetroxide (2.5 wt % solution in 2-methyl-2-propanol, 0.08 mL) and 4-methylmorpholine N-oxide (97%, 4 mg) were added at -10° - -20°. After one hour the reaction was stopped by adding sodium sulfide. Excess EtOAc was added and the solution was washed with water, dried, and evaporated to dryness to yield the crude product which was purified by prep. TLC (Si gel, 20% acetone in chloroform) to give 11,13-dihydroxichiloscypnone (**2.39**, 4.6 mg). Data for **2.39**: UV (MeOH) λ_{max} (log ϵ): 210 (3.13) nm; IR (CHCl₃) ν_{max} : 3426, 2945, 1701, 1456, 1350, 1040 cm⁻¹; ¹H NMR(CDCl₃) δ 0.85 (3H, d, J = 6.7), 0.93 (3H, s), 1.36-1.56 (2H, m), 1.60-1.62 (1H, m), 1.83-1.86 (1H, m), 1.94-2.10 (4H, m), 2.32-2.43 (2H, m), 3.18 (1H, d, J = 6.8), 3.29 (1H, d, J = 9.6), 3.79 (1H, d, J = 9.6), 4.28 (1H, s), 5.39 (1H, s, br); ¹³C NMR(CDCl₃) δ 17.8 (CH₃-15), 20.3 (CH₃-14), 21.4 (CH₃-12), 25.3 (CH₂-2), 25.6 (CH₂-1), 27.1 (CH₂-3), 28.0 (CH₂-9), 31.8 (CH-4), 49.0 (C-5), 52.6 (CH-6), 67.1 (CH₂-13), 79.7 (C-11), 116.8 (CH-1), 146.1 (C-10), 214.7 (C-7).

Conversion of 2.37 to rivulalactone (2.34). *m*-CPBA (57-86%, 20 mg) was added to a solution of **2.37** (4 mg) in CH₂Cl₂ (1 ml). After 1 hour (TLC control) excess EtOAc was added and the solution was washed with satd. aq. NaHCO₃, dried, and evaporated to afford the crude product. Separation of this by prep. TLC (Si. gel, 30% acetone in CHCl₃) gave the synthetic rivulalactone **2.34** (2.5 mg). Data for **2.34**: white crystals; mp 203-5°C; $[\alpha]_D = + 22.8^\circ$ (*c.* 0.19, CHCl₃). All spectral data are identical with the natural product **2.34**.

Oxidation of 12-hydroxychiloscyphone (2.30). To the solution of 12-hydroxychiloscyphone (**2.30**, 6 mg) in a mixture of Me₂CO and H₂O (8:1, 1.0 mL), osmium tetroxide (2.5 wt % solution in 2-methyl-2-propanol, 0.1 mL) and 4-methylmorpholine N-oxide (97%, 6 mg) were added. After one hour the reaction was stopped by adding sodium sulfide. The reaction solution was diluted with water and extracted with EtOAc 3 times. The combined extracts was washed with water, dried, and evaporated to dryness to yield the crude product which was purified by prep.TLC (Si gel, 20% methanol in chloroform) to give 11, 12, 13-trihydroxychiloscyphone (**2.42**, 3.8 mg). Data for **2.42**: UV (MeOH) λ_{\max} (log ϵ): 210 (3.23) nm; IR (CHCl₃) ν_{\max} : 3394, 2944, 1698, 1460, 1360, 1058 cm⁻¹; ¹H NMR (CD₃OD) δ 0.93 (3H, d, J = 6.8), 0.93 (3H, s), 1.40-1.45 (2H, m), 1.63-1.69 (1H, m), 1.77-1.82 (1H, m), 1.93-2.03 (4H, m), 2.32 (1H, m), 2.49 (1H, m), 3.57 (2H, dd, J = 6, 6), 3.65-3.71 (3H, m), 5.27 (1H, br, s); ¹³C NMR (CD₃OD) δ 18.0 (CH₃-15), 20.8 (CH₃-14), 26.3 (CH₂-2), 26.6 (CH₂-8), 28.3 (CH₂-3), 29.2 (CH₂-9), 33.3 (CH-4), 50.1 (C-5), 54.0 (CH-6), 64.9 (CH₂-12), 65.9 (CH₂-13), 84.3 (C-11), 117.4 (CH-1), 148.3 (C-10), 218.7 (C-7). CIMS m/z 269 (M+1⁺), 149, 107.

Epoxidation of 12-hydroxychiloscyphone (2.30). To the solution of 12-hydroxychiloscyphone (**2.30**, 3.9 mg) in CH₂Cl₂, H₂O₂ (30%, aq. solution, 0.08mL) and NaOH (1 N aq. solution, 0.05 mL) were added. After 2 hours the reaction solution was diluted with water and extracted with EtOAc 3 times. The combined extracts was washed with water, dried, and evaporated to dryness to yield the crude product which was purified by prep.TLC (Si gel, 30% EtOAc in hexane) to give 11, 13-epoxy-12-hydroxychiloscyphone (**2.43**, 2.2 mg). Data for **2.43**: UV (MeOH) λ_{\max} (log ϵ): 210 (3.27) nm; IR (CHCl₃) ν_{\max} : 3380, 3018, 2926, 1697, 1215 cm⁻¹; ¹H NMR (CDCl₃) δ 0.79 (3H, d, J = 7.2), 0.89 (3H, s), 1.40-1.46 (2H, m), 1.60-1.68 (1H, m), 1.83-1.88 (1H,

m), 2.00-2.14 (3H, m), 2.35 (1H, m), 2.53 (1H, m), 2.86 (2H, d, $J = 8$), 3.02-3.07 (2H, m), 3.83-3.97 (2H, m), 5.37 (1H, br, s); ^{13}C NMR (CDCl_3) δ 17.6 (CH_3 -15), 20.3 (CH_3 -14), 25.4 (CH_2 -2), 26.7 (CH_2 -8), 27.9 (CH_2 -3), 31.3 (CH_2 -9), 33.4 (CH -4), 47.4 (CH -6), 48.7 (CH_2 -13), 50.7 (C -5), 61.2 (C -11), 62.2 (CH_2 -12), 116.4 (CH -1), 146.5 (C -10), 211.2 (C -7); CIMS m/z 251 ($\text{M}+1^+$), 177, 149, 147.

III. SYNTHESIS OF POTENTIAL ANTITUMOR DNA INTERCALATORS--- FURANONAPHTHOQUINONES WITH HYDROXYAMINO SIDE CHAINS

3.1 INTRODUCTION

3.1.1 Antitumor DNA Intercalators

A drug may interfere with the role of DNA as a template in replication and transcription (a) directly, by reacting with it to form a complex, or (b) indirectly, by causing structural alterations such as strand breakage, removal of bases, or formation of cross-links. Among drugs which bind directly to DNA, some react covalently to form an essentially irreversible complex, but the most important examples of drugs which act at this level are those which form a strong, but reversible, non-covalent complex with DNA.⁵¹ The process of complex formation is called intercalation.

The acridines, the earliest antibacterial and antimalarial agents, stand as the archetypes among intercalating compounds. Their toxicity to bacteria and the malarial parasite is associated with inhibition of DNA and RNA synthesis.⁵² Acridines are also important as potential antitumor agents, especially members of the acridinylmethane-sulphonanilide series which are active against a number of experimental tumors in animals as well as man.⁵³

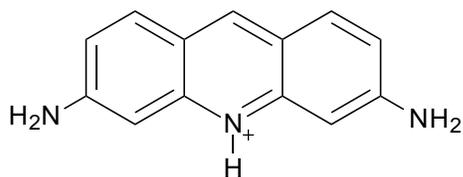
Proflavine (3,6-diaminoacridine) (**3.1**) forms complexes with single-stranded polynucleotides, including RNA as well as heat-denatured DNA and naturally occurring single-stranded DNA.⁵⁴

⁵¹ Gale, E. F., Cundliffe, E., Reynolds, P. E., Richmond, M. H., Waring, M. J. *The Molecular Basis of Antibiotic Action* 2nd Ed. John Wiley & Sons, 1981, p. 273

⁵² Acheson, R. M. Ed., *Acridines* 2nd Ed., Wiley, London, **1973**

⁵³ Legha, S. S., Gutterman, J. U., Hall, S. W., Benjamin, R. S., Burgess, M. A., Valdivieso, M., Bodey, G. P. *Cancer Res.*, **1978**, *38*, 3712

⁵⁴ Blake, A., Peacocke, A. R. *Biopolymers*, **1968**, *6*, 1225



Proflavine (3.1)

Based on his observations on the proflavine-DNA complex, Lerman first described an intercalation model in 1961.⁵⁵ He proposed that the flat aromatic proflavine molecule becomes inserted (i.e. intercalated) between adjacent base-pairs of the double helix. The base-pairs remain perpendicular to the helix axis, but they are moved apart to accommodate the 3.4 Å thick acridine molecule which lies in van der Waals contact with the base-pairs above and below, and thus the proflavine occupies the same space as an extra base-pair. The intimate contact between the π -orbitals of the drug molecule and the base pairs would help to stabilize the complex via hydrophobic and charge-transfer forces. The intercalation model clearly requires that the plane of the polycyclic aromatic chromophore of the bound drug molecule be parallel to that of the DNA base-pairs, i.e. more or less perpendicular to the helix axis.⁵⁶ The presence of the amino group adds stability to the complex, and there is evidence that the character of DNA-binding by drugs which lack one or both of the amino groups is significantly different.⁵⁷

Besides acridines, a great variety of substances, including several agents of importance in chemotherapy, are now known to bind DNA by intercalation.⁵⁸ A few well-investigated examples with antitumor activity include ellipticine (3.2),⁵⁹ daunomycin

⁵⁵ Lerman, L. S. *J. Mol. Biol.* **1961**, *3*, 18

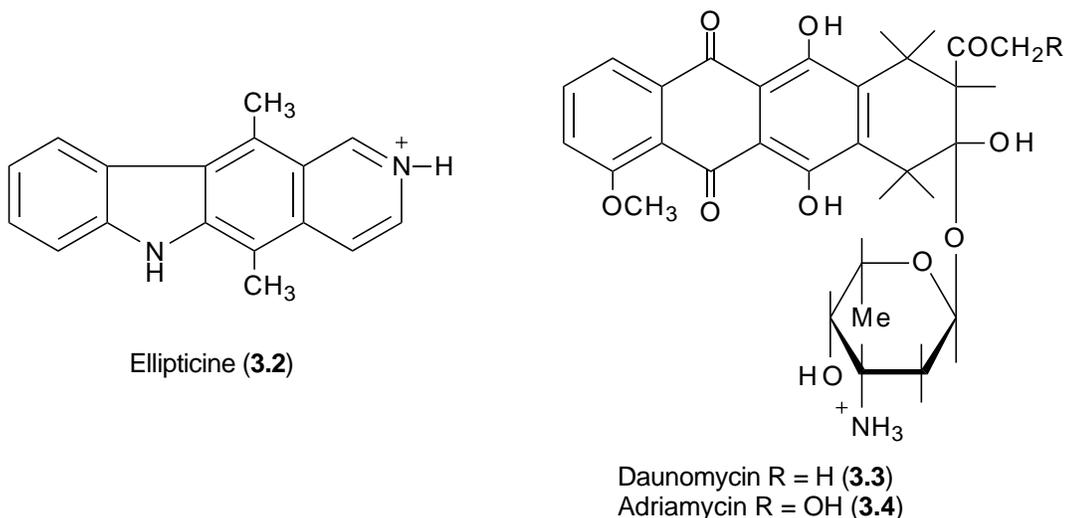
⁵⁶ Gale, E. F., Cundliffe, E., Reynolds, P. E., Richmond, M. H., Waring, M. J. *The Molecular Basis of Antibiotic Action* 2nd Ed. John Wiley & Sons, **1981**, p. 283

⁵⁷ Kindelis, A., Aktipis, S. *Biopolymers*, **1978**, *17*, 1469

⁵⁸ Gale, E. F., Cundliffe, E., Reynolds, P. E., Richmond, M. H., Waring, M. J. *The Molecular Basis of Antibiotic Action* 2nd Ed. John Wiley & Sons, **1981**, p. 307

⁵⁹ Kohn, K. W., Waring, M. J., Glaubiger, D., Friedman, C. A. *Cancer Res.*, **1975**, *35*, 71

(3.3),⁶⁰ and adriamycin (3.4).⁶¹ The later two compounds belong to anthracyclines, the first class of intercalating antitumor agents proven to have clinical antitumor activity. One of them, doxorubicin, remains one of the most widely used antitumor drugs.⁶²



Biophysical studies on a large variety of carbocyclic polycyclic aromatic derivatives have shown that two structural elements contribute to the interaction of the molecule with DNA. The ring system that intercalates with DNA must contain at least three fused planar rings. The second structural component required for good DNA binding with these compounds is a pendant side chain containing an amine group, which binds electrostatically to DNA. Optimal DNA binding occurs when the first amine group is one carbon atom removed from the aromatic ring.⁶³

In order to design new types of antitumor intercalators, K.W. Bair and colleagues began a systematic study of the interactions between DNA and polycyclic aromatic

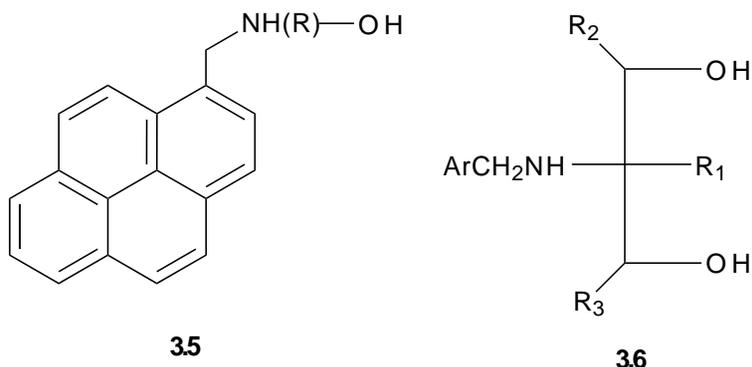
⁶⁰ Waring, M. J. *J. Mol. Biol.*, **1970**, *54*, 247

⁶¹ Waring, M. J. in *Topics in Infectious Diseases, Vol. 1, Drug-Receptor Interactions in Antimicrobial Chemotherapy* Drews, J., Hahn, F. E., Eds., Springer-Verlag, New York, **1975**, pp. 77-90

⁶² Zunino, F. in *Cancer Therapeutics* Teicher, B. A. Ed., Humana Press, **1997**, p. 197

⁶³ Bair, K. W., Tuttle, R. L., Knick, V. C., Cory, M., McKee, D. D. *J. Med. Chem.* **1990**, *33*, 2385 - 2393

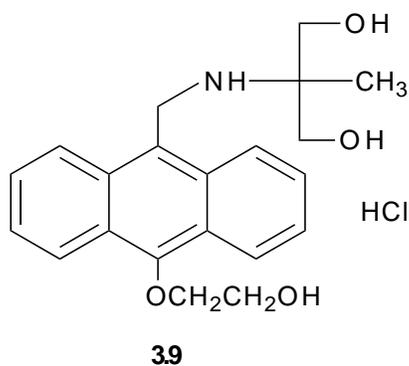
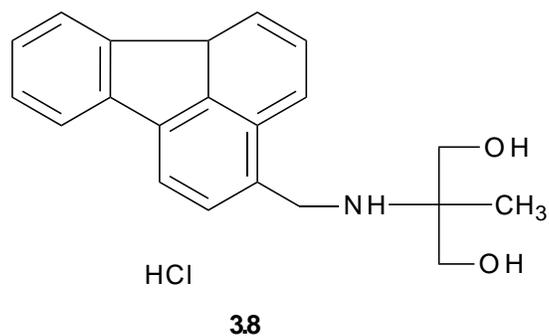
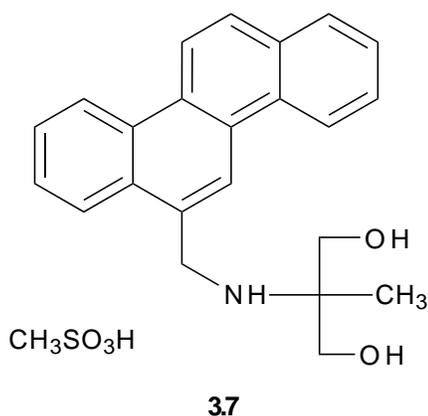
derivatives containing polar side chains. Antitumor activity was discovered in a series of (1-pyrenylmethyl)amino alcohol derivatives (**3.5**) by using a standard murine lymphocytic leukemia screen.



The P388 screening results showed that derivatives of the secondary amine 1-pyrenylmethylamine containing a simple alkyl group or more than one amine in the side chain showed little antitumor activity. The first traces of activity were seen with congeners possessing an OH group in the side chain and the optimal arrangement of the OH group was found to be two carbon atoms from the NH group. Optimal antitumor activity was seen for 2-[(arylmethyl)amino]propanediols (AMAPs) of general structure **3.6**. The side chain on these secondary amines contains two OH groups, each of which is two carbon atoms away from the NH group.

These compounds are derivatives of 2-amino-1,3-propanediol, and possess a broad spectrum activity against both murine and human tumors in various *in vitro* and *in vivo* systems. From the large number of AMAPs synthesized, three congeners were chosen for development. Compounds **3.7**, **3.8**, and **3.9** are currently in clinical trials.⁶⁴

⁶⁴ Bair, K. W., Tuttle, R. L., Knick, V. C., Cory, M., McKee, D. D. *J. Med. Chem.* **1990**, *33*, 2385 - 2393

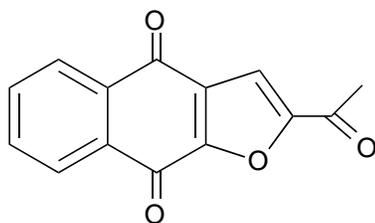


3.1.2 Furanonaphthoquinones with Hydroxyamino Side Chain, Potential Antitumor DNA Intercalators?

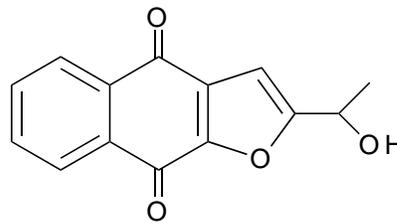
There are many natural furanonaphthoquinones which have been isolated from various plants,⁶⁵ and a number of these compounds show interesting biological activity. From an alcoholic extract of the stem bark of *Tabebuia cassinoides*, three cytotoxic furanonaphthoquinones were isolated, 2-acetyl-naphtho[2,3-b]furan-4,9-dione (**3.10**), 2-(1-hydroxyethyl)-naphtho[2,3-b]furan-4,9-dione (**3.11**), and 5 (or 8)-hydroxy-2-(1-hydroxyethyl)-naphtho[2,3-b]furan-4,9-dione (**3.12a** or **3.12b**), showing ED₅₀ values in

⁶⁵ Tisler, M. *Heterocyclic Quinones* in *Advances in Heterocyclic Chemistry*, Vol. 45, Katritzky, A. R., Ed., Academic Press, Inc., **1989**, pp. 38 - 150

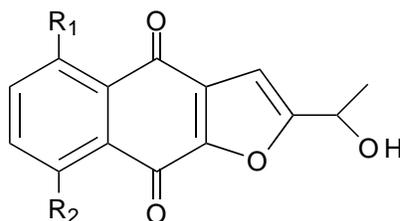
the KB cell culture assay of 1.0, 2.0, and 1.0 $\mu\text{g/mL}$, respectively.⁶⁶ Some other examples are compounds **3.13** and **3.14**, isolated from *Crescentia cujete*. They showed selective DNA-damage activity against the repair-deficient *rad52* yeast strain. They also showed cytotoxicity to Vero cells with IC_{50} values 3.7 and 4.7 $\mu\text{g/mL}$, respectively.⁶⁷



3.10

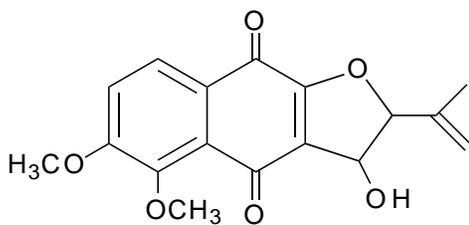


3.11

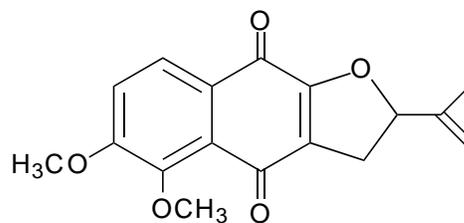


3.12a $\text{R}_1 = \text{OH}$, $\text{R}_2 = \text{H}$

3.12b $\text{R}_1 = \text{H}$, $\text{R}_2 = \text{OH}$



3.13



3.14

⁶⁶ Rao, M. M., Kingston, D. G. I. *J. Nat. Prod.* **1982**, *45*, 600 - 604

⁶⁷ Heltzel, C. E., Gunatilaka, A. A. L., Glass, T. E., Kingston, D. G. I., Hofmann, G., Johnson, R. K. *J. Nat. Prod.* **1993**, *56*, 1500 - 1505

These cytotoxic furanonaphthoquinones possess three fused planar rings, suggesting that intercalation into DNA may be involved in the mechanism of cytotoxicity.⁶⁸ It may thus be possible to improve their activity by connecting a side chain with an amine group, which binds to DNA electrostatically. Based on Bair's studies mentioned above, 2-[(arylmethyl)amino]propanediol system gave optimal DNA binding ability and optimal antitumor activity, so 2-amino-2-methyl-1,3-propanediol was chosen as the hydroxyamino side chain in this experiment.

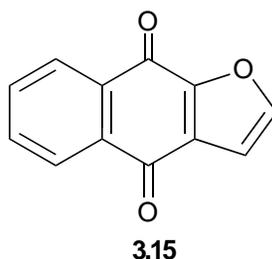
⁶⁸Gunatilaka, A. A. L., Kingston, D. G. I., Johnson, R. K. *Pure & Appl. Chem.* **1994**, *66*, 2219 - 2222

3.2 RESULTS AND DISCUSSION

3.2.1 Synthesis of Furanonaphthoquinone Derivatives

In order to evaluate the effect of the hydroxyamino side chain on the activity, a few furanonaphthoquinone derivatives were synthesized to measure their activity.

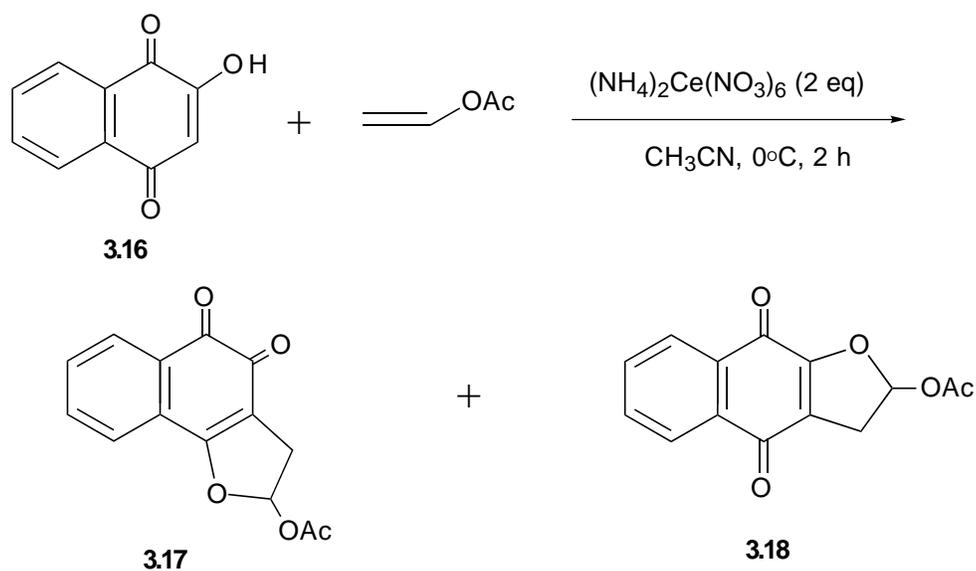
3.2.1.1 Synthesis of naphtho[2,3-*b*]furan-4,9-dione (**3.15**)



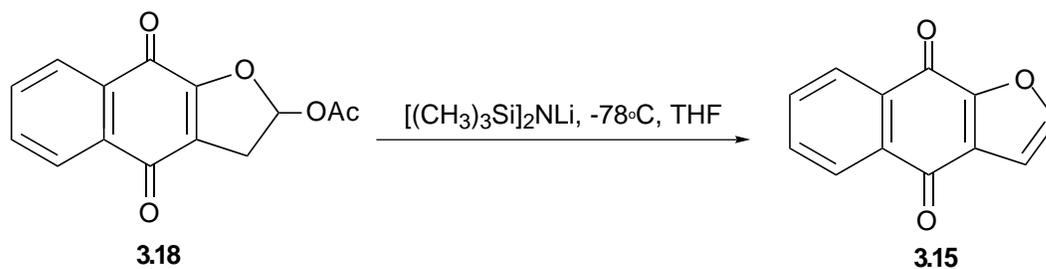
Many strategies have been used for the construction of the naphtho[2,3-*b*]furan-4,9-dione skeleton.⁶⁹ In 1996 K. Kobayashi and colleagues reported a new one-step synthesis of 2,3-dihydronaphtho[2,3-*b*]furan-4,9-dione by cycloaddition of hydroxyquinones with alkenes.⁷⁰ This method was chosen for our synthesis.

The acetonitrile solution of 2-hydroxy-1,4-naphthoquinone (**3.16**) and vinyl acetate was treated with ceric ammonium nitrate at 0°C. A usual work up followed by column chromatography afforded two products, which proved to be the furo-*o*-quinone derivative 2-acetoxy-2,3-dihydro-naphtho[1,2-*b*]furan-4,5-dione (**3.17**) (24% yield) and furo-*p*-quinone derivative 2-acetoxy-2,3-dihydro-naphtho[2,3-*b*]furan-4,9-dione (**3.18**) (35% yield).

⁶⁹ Koyanagi, J., Yamamoto, K., Nakayama, K., Tanaka, A. *J. Heterocyclic Chem.* **1994**, *31*, 1303-1304



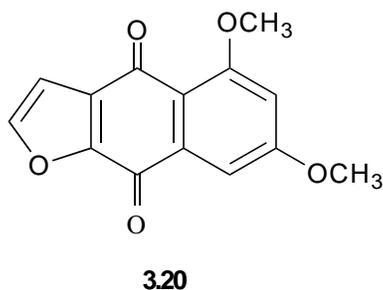
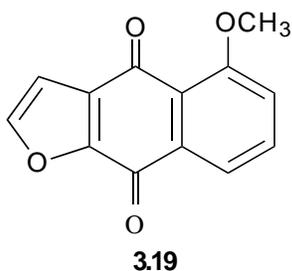
The desired product **3.18** was converted to naphtho[2,3-b]furan-4,9-dione (**3.15**) by elimination of acetic acid. Potassium *tert*-butoxide⁷¹ was chosen as the base first, but the yield was quite low (20%). A better yield (65%) of **3.15** was achieved by the treatment of **3.17** with lithium bis(trimethylsilyl)amide.



3.2.1.2 Synthesis of 5-Methoxynaphtho[2,3-b]furan-4,9-dione (**3.19**) and 5,7-Dimethoxy naphtho[2,3-b]furan-4,9-dione (**3.20**)

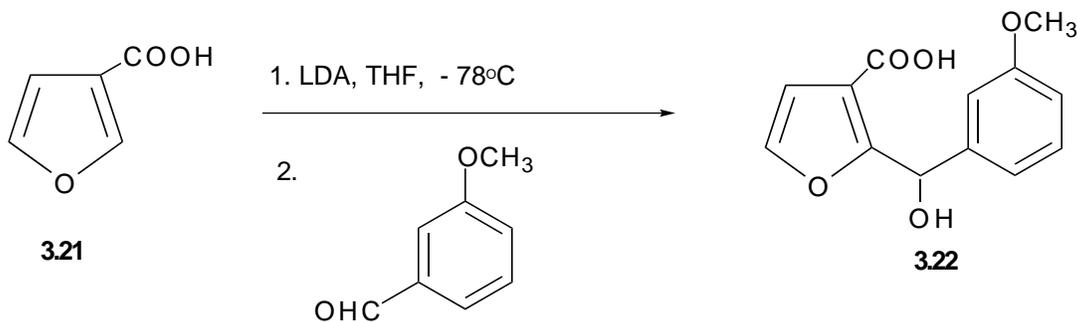
⁷⁰ Kobayashi, K., Mori, M., Uneda, T., Morikawa, O., Konishi, H. *Chem. Lett.* **1996**, 451 - 452

⁷¹ Kobayashi, K., Shimizu, H., Sasaki, A., Sugimoto, H. *J. Org. Chem.* **1991**, 56, 3204 - 3205



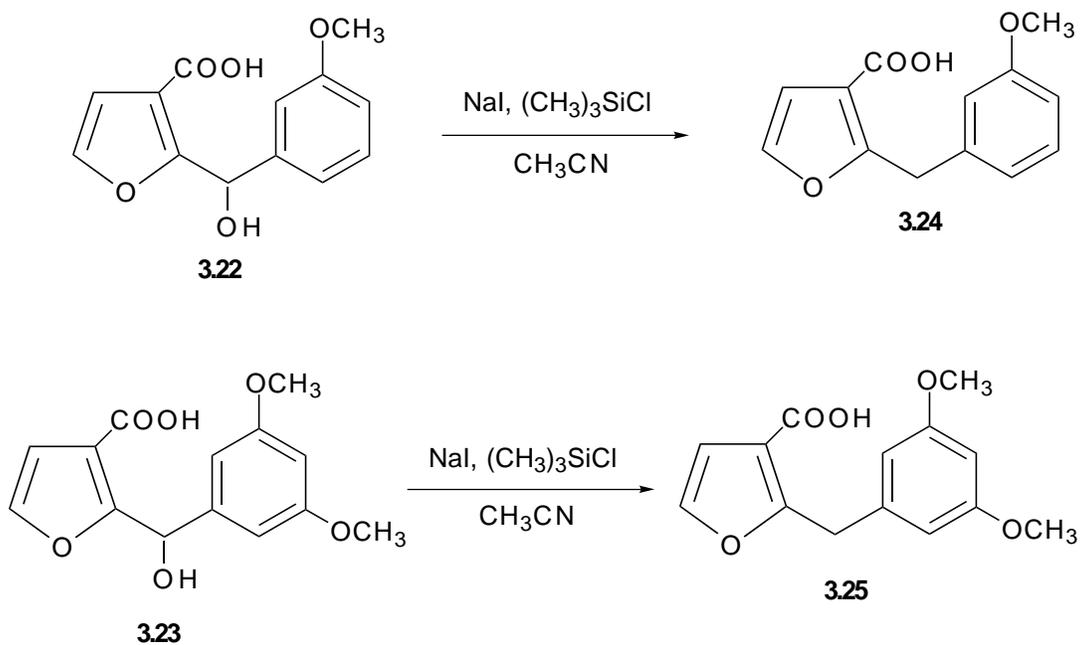
Compound **3.20** was synthesized by P. J. Perry and colleagues in 1995.⁷² The same route was followed to synthesize compounds **3.19** and **3.20**.

Regiospecific metallation of 3-furoic acid **3.21** at the 2-position using lithium diisopropylamide at -78°C and subsequent treatment with either 3-methoxybenzaldehyde or 3,5-dimethoxybenzaldehyde afforded the hydroxymethylfuroic acids **3.22** (82% yield) and **3.23** (76% yield).



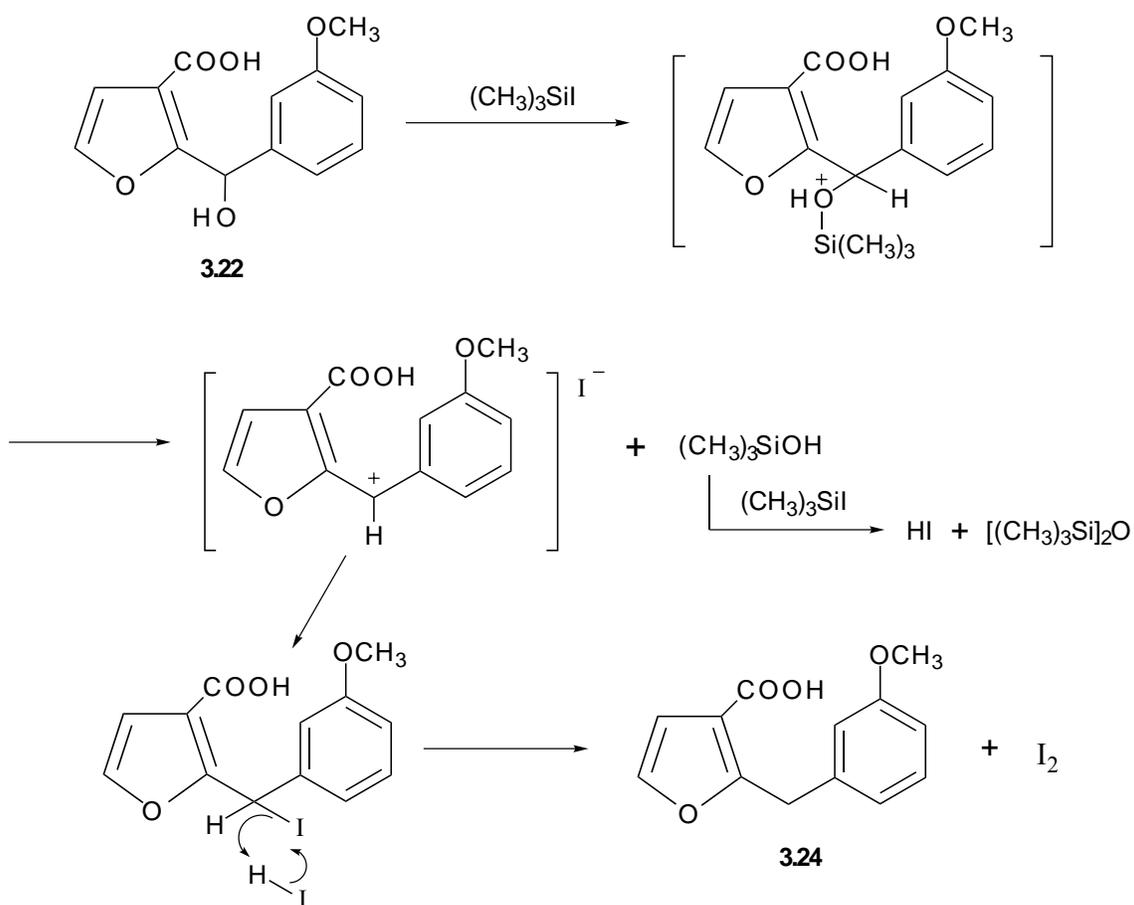
Reduction of **3.22** and **3.23** with iodotrimethylsilane generated *in situ* gave the benzylfuroic acids **3.24** (85% yield) and **3.25** (80% yield) respectively.

⁷² Perry, P. J., Pavlidis, V. H., Hadfield, J. A., Coutts, I. G. C. *J. Chem. Soc. Perkin Trans. I* **1995**, 1085 - 1087



Although the mechanism of this reaction is not fully determined, it is believed proceed via an initially formed oxonium ion to the intermediate iodide. A proposed mechanism is shown in Scheme 6.⁷³

⁷³ Perry, P. J., Pavlidis, V. H., Coutts, I. G. *Synth. Commun.* **1996**, *26*, 101 - 111

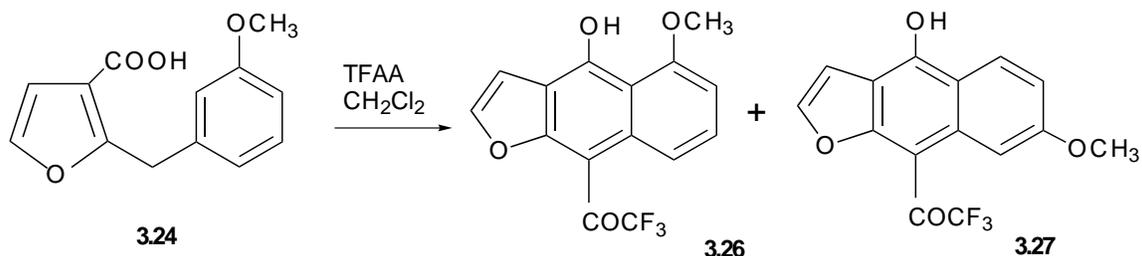


Scheme 6. Possible Mechanism for the Formation of **3.24**

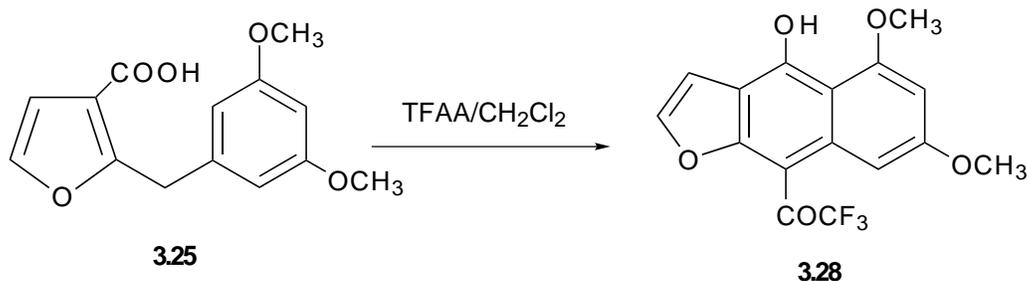
In Friedel-Crafts cyclisation of **3.24**, ring closure may occur *ortho* or *para* to the methoxy substituent. The literature reported that Friedel-Crafts cyclisation of **3.24** with trifluoroacetic anhydride in dry dichloromethane only gave the 5-methoxy naphthol **3.26**, the *ortho* ring closure product.⁷⁴ In our hands the Friedel-Crafts cyclisation afforded 4-hydroxy-5-methoxy-9-trifluoroacetylnaphtho[2,3-b]furan (**3.26**) in 32% yield as well as a mixture of **3.26** and a similar compound **3.27**, which was difficult to purify with regular chromatography methods. Evidence provided later indicates that **3.27** must have the

⁷⁴ Perry, P. J., Pavlidis, V. H., Hadfield, J. A. *Tetrahedron* **1997**, *53*, 3195-3204

structure shown, and must thus be formed by cyclization para to the methoxy group of **3.24**.

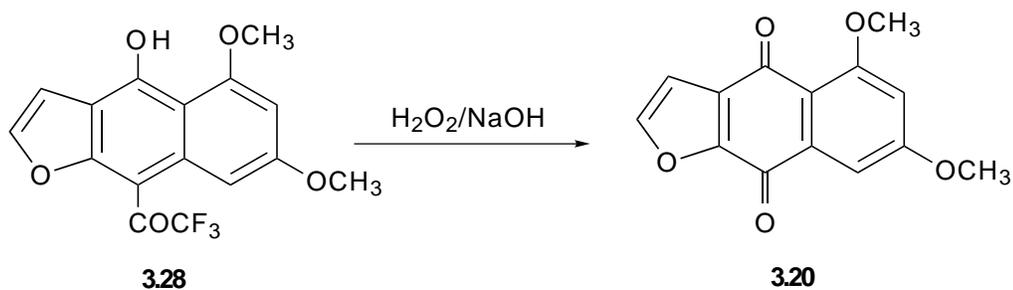
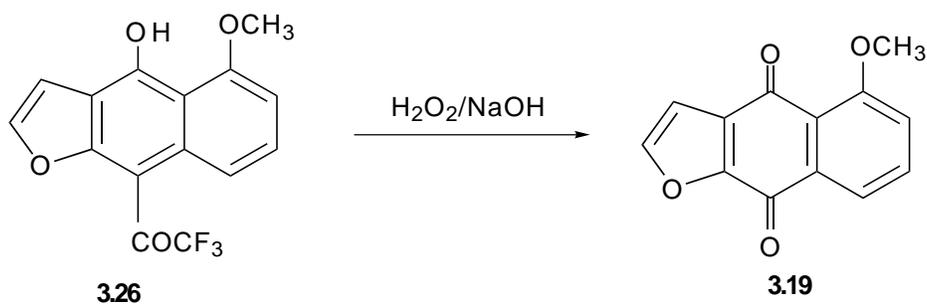


2-(3,5-Dimethoxybenzyl)-3-furoic acid **3.25** underwent cyclization under the same conditions to yield 4-hydroxy-3,5-dimethoxy-9-trifluoroacetylnaphtho[2,3-b]furan (**3.28**) in 64% yield.

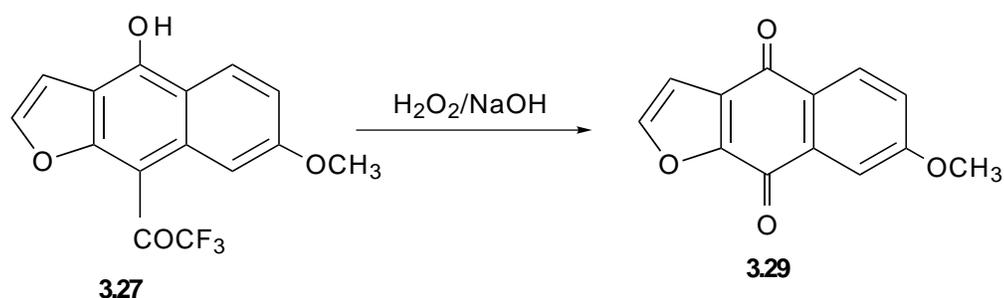


The mechanism of the conversion of **3.24** to **3.26** or of **3.25** to **3.28** presumably involves an initial Friedel-Crafts reaction to give the ring closure product followed by the conversion to its aromatic enol form. Compounds **3.26** and **3.28** are then formed by the acylation at the highly activated 9-position by the trifluoroacetyl carbocation.

Hydrogen peroxide oxidation of **3.26** and **3.28** in aqueous base smoothly gave 5-methoxy-4,9-dihydronaphtho[2,3-b]furan-4,9-dione **3.19** in 69% yield and 3,5-dimethoxy-4,9-dihydro-naphtho[2,3-b]furan-4,9-dione **3.20** in 92% yield.



The mixture produced from the cyclisation of **3.26** was treated with hydrogen peroxide in aqueous base to give **3.19** and a compound with similar structure. The aromatic region of the ^1H NMR spectrum of **3.29** exhibited a set of two furan protons (δ_{H} 6.98, 7.76) and a set of three protons characteristic of a 1, 2, 4-trisubstituted benzene ring rather than a 1, 2, 3-trisubstituted one as with **3.19**, indicating that the methoxy group must be assigned to the 7 position. So this compound is 7-methoxy-4,9-dihydro-4,9-dioxo-1H-naphtho[2,3-b]furan (**3.29**), and its isolation provides evidence that ring closure also occurred *para* to the methoxy substituent to give 4-hydroxy-7-methoxy-9-trifluoroacetylnaphtho [2,3-b]furan (**3.27**).



3.2.1.3 Biological Activity of Furanonaphthoquinone Derivatives

The activity data for furanonaphthoquinone derivatives **3.15**, **3.19**, **3.20**, and **3.29** in the yeast bioassay, and cytotoxicity data for selected compounds, are given in Table 5. The derivatives without any substituted group or with a methoxy group at the 5 position showed moderate but selective activity against the repair-deficient *rad52.top1* yeast strain, indicating that they act as DNA-damaging agents, and probably as topoisomerase II inhibitors. But the derivatives with a methoxy group at the 7 position did not show activity to the yeast strains. However, they did give similar cytotoxicity against H4IIE rat hepatoma cell as those without a substituted group at the 7 position.

Table 5. Biological Activity of Compounds **3.15**, **3.19**, **3.20** and **3.29**

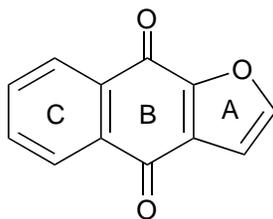
Compound	Yeast Strain			Cytotoxicity to rat hepatoma cells ^b
	rad 321 ^a	rad 52 ^a	rad + ^a	
3.15	17	83	78	5
3.19	44	152	135	7
3.20	>500	>500	>500	5
3.29	>500	>500	>500	NT ^c

^a Results are expressed as IC₁₂ values in µg/mL (concentration required to produce an inhibition zone of 12 mm around a 100 µl well in the yeast strain)

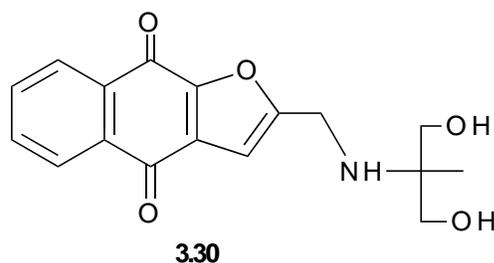
^b Results are expressed as IC₅₀ values (µM)

^c Not tested

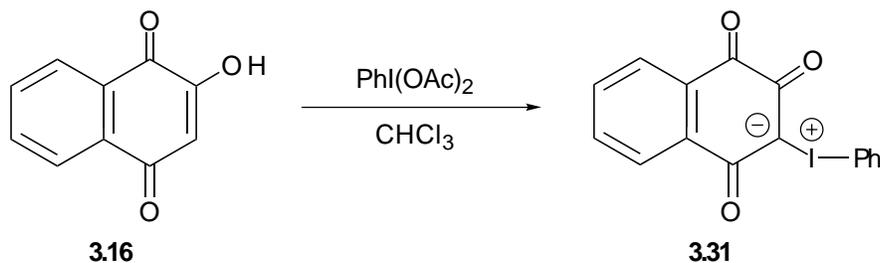
3.2.2 Synthesis of Furanonaphthoquinones with a Hydroxyamino Side Chain on the Furan Ring (A ring)



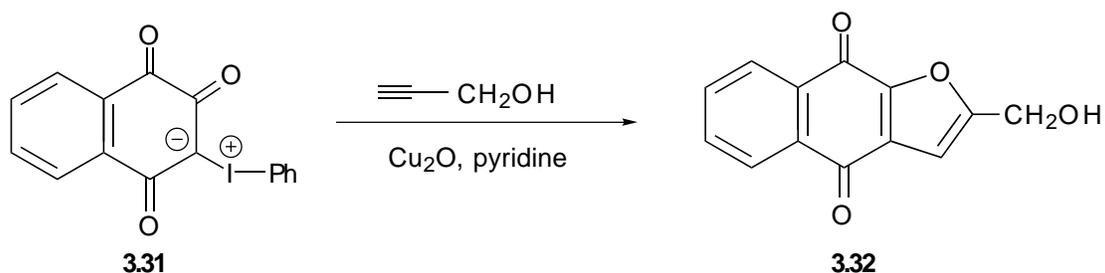
3.2.2.1 Synthesis of 2-Methyl-2-[2'-(4',9'-dihydronaphtho[2',3'-b]furan-4',9'-dionyl-methyl)amino]-1,3-propanediol (**3.30**)



The synthetic scheme began with 2-hydroxy-1,4-naphthoquinone (**3.16**), which was treated with (diacetoxyiodo)benzene in chloroform for four hours to give 3-phenyliodonio-1,2,4-trioxo-1,2,3,4-tetrahydronaphthalenide (**3.31**)⁷⁵ in 88% yield.



Next step was the coupling/ring closure of **3.31** with propargyl alcohol to give 2-hydroxymethyl-naphtho[2,3-b]furan-4,9-dione (**3.32**).⁷⁶ It was achieved by treatment of **3.31** with propargyl alcohol in pyridine in the presence of a molar amount of cuprous oxide at 80°C for 2 hours.

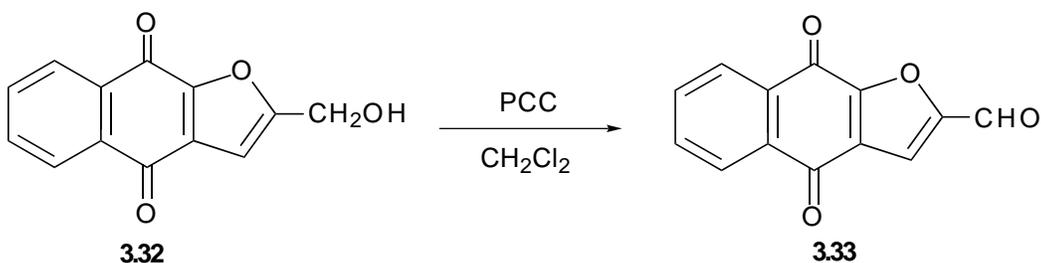


⁷⁵ Hatzigrigoriou, E., Spyroudis, S., Varvoglis, A. *Liebigs Ann. Chem.* **1989**, 167 - 170

⁷⁶ Kobayashi, K., Uneda, T., Kawakita, M., Morikawa, O., Konishi, H. *Tetra. Lett.* **1997**, 38, 837 - 840

This reaction gave only 25% yield of product **3.32** primarily because of difficulty in the purification of the product.

In order to oxidize the alcohol **3.32** to the corresponding aldehyde **3.33**, a variety of different reagents was employed. Oxidation with manganese dioxide only gave a 40% yield, and pyridinium dichromate oxidation also gave a yield less than 50%. The best method was the treatment of **3.32** with pyridinium chlorochromate for 20 hours, which afforded a 70% yield of **3.33**.

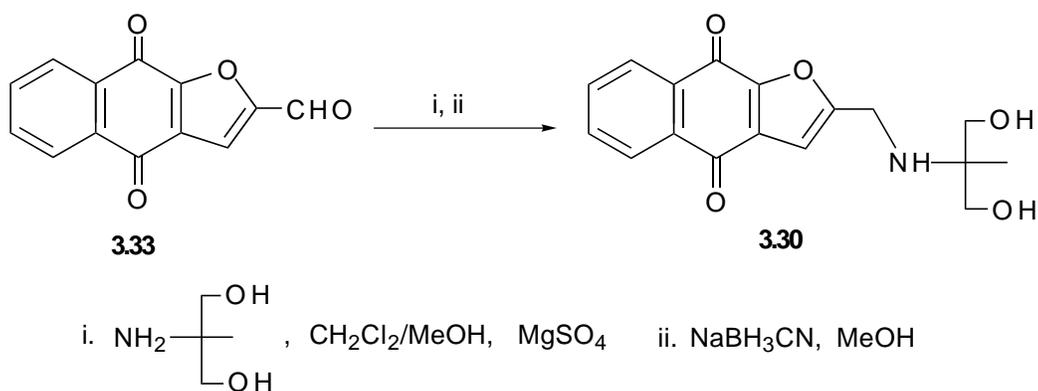


The ¹H and ¹³C NMR of **3.33** indicated the presence of the aldehyde resonance (δ_{H} 9.96, δ_{C} 179.1), and the absence of the methylene protons of **3.32** at δ 4.69.

In the last step of this synthesis the hydroxyamino side chain was affixed to **3.33** *via* reductive amination.⁷⁷ Taking magnesium sulfate as the drying agent,⁷⁸ treatment of **3.33** with of 2-amino-2-methyl-1,3-propanediol in dichloromethane, followed by reduction with sodium cyanoborohydride afforded **3.30** in over 60% yield.

⁷⁷ Borch, R. F., Bernstein, M. D., Durst, H. D. *J. Amer. Chem. Soc.*, **1971**, 93, 2897-2904

⁷⁸ Ojima, I., Habus, I., Zhou, M., Zucco, M., Park, Y. H., Sun, C. M., Brigaud, T. *Tetrahedron* **1992**, 48, 6985-7012

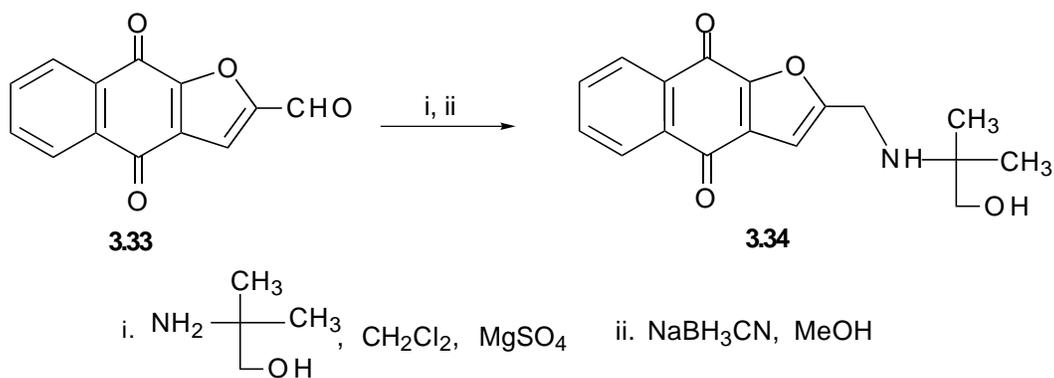


The NMR spectra of **3.30** gave the expected signals for the side chain. The FAB mass spectrum of **3.30** gave the expected protonated molecular ion $(M+1)^+$ at m/z 316, and its EI mass spectrum showed significant peaks at m/z 226 ($M^+ - \text{C}_4\text{H}_9\text{O}_2$), and 197 ($M^+ - \text{C}_5\text{H}_{12}\text{NO}_2$).

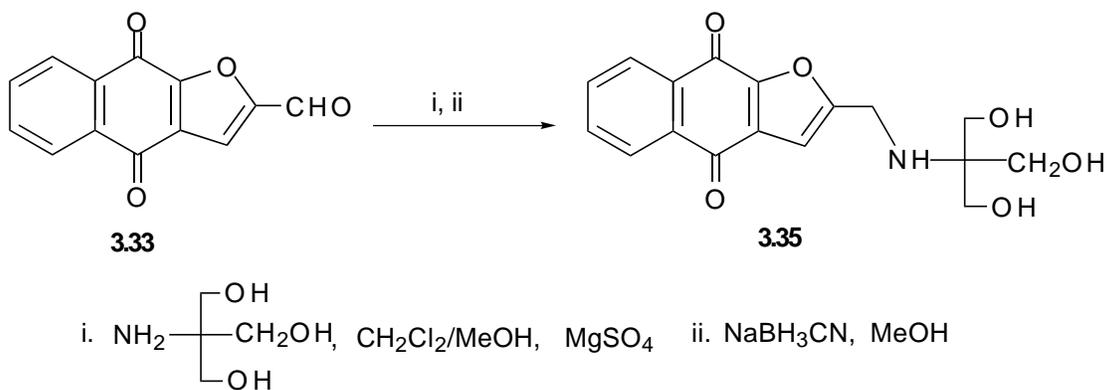
3.2.2.2 Preparation of Analogs of **3.30**

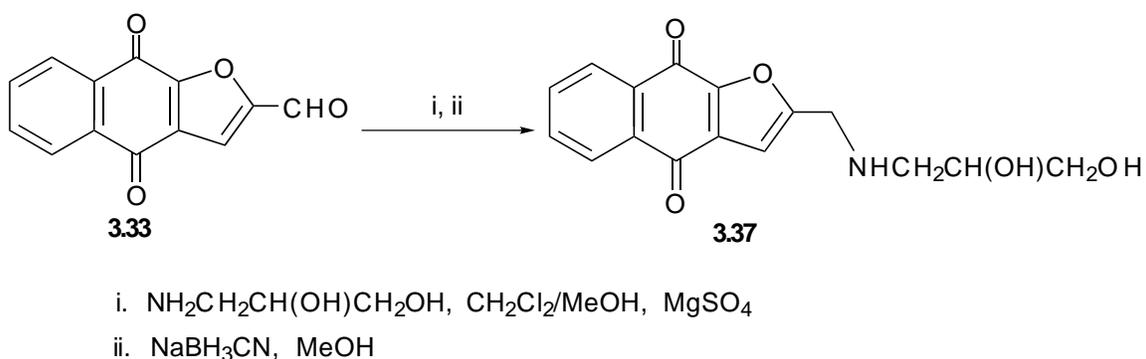
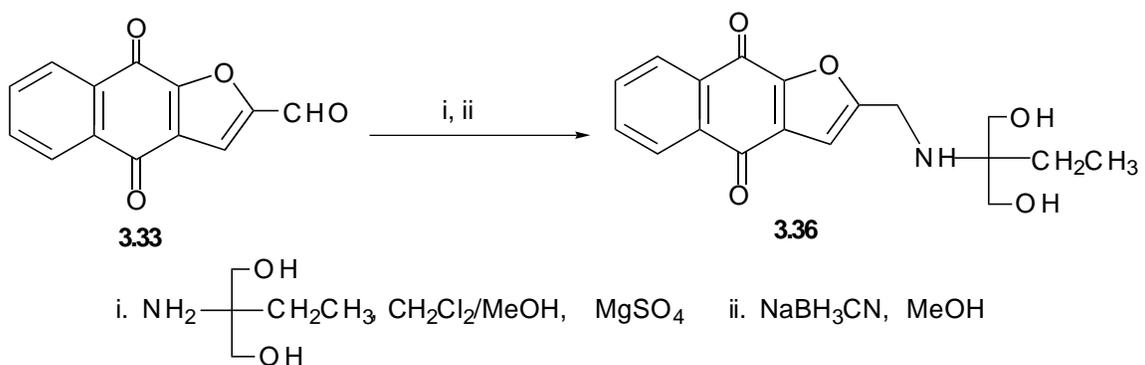
In order to compare the effect of different side chains on the activity of furanonaphthoquinones, some analogs of **3.30** with different hydroxyamino side chains were prepared.

2-Methyl-2-[2'-(4',9'-dihydronaphtho[2',3'-b]furan-4',9'-dionylmethyl)amino]-1-propanol **3.34** was prepared in over 80% yield by treating **3.33** with 2-amino-2-methylpropanol and magnesium sulfate in dichloromethane for 20 hours, followed by reduction with sodium cyanoborohydride.



With the same procedure 2-hydroxymethyl-2-[2'-(4',9'-dihydronaphtho[2',3'-b]furan-4',9'-dionylmethyl)amino]-1,3-propanediol **3.35**, 2-ethyl-2-[2'-(4',9'-dihydronaphtho[2',3'-b]furan-4',9'-dionylmethyl)amino]-1,3-propanediol **3.36**, and 1-[2'-(4',9'-dihydronaphtho[2',3'-b]furan-4',9'-dionylmethyl)amino]-2,3-propanediol **3.37** were prepared in over 80% yield.





3.2.2.3 Bioassay of **3.30** and Its Analogs

Bioactivity data for compounds with different amino side chains in the yeast bioassay and in a cytotoxicity assay are given in Table 6. Surprisingly none of these compounds showed any significant activity against the repair-deficient yeast strain *rad52.top1* and *rad52*. However all of them, except **3.35**, showed cytotoxicity against the H4IIE rat hepatoma cell. Compound **3.30** gave better activity than its parent compound **3.15**.

Table 6. Biological Activity of **3.30** and Its Analogs

Compound	Yeast Strain		Cytotoxicity to rat hepatoma cells ^b
	<i>rad52.top1</i> ^a	<i>rad52</i> ^a	
3.30	>500	>500	2
3.34	500	>500	4
3.35	>500	>500	> 100
3.36	>500	>500	5
3.37	>500	>500	2

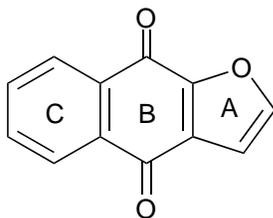
^a Results are expressed as IC₁₂ values in µg/mL (concentration required to produce an inhibition zone of 12 mm around a 100 µl well in the yeast strain)

^b Results are expressed as IC₅₀ values (µM)

Considering these data, it appears that these furanonaphthoquinones with a amino side chain act by a different mechanism of action than their parent furanonaphthoquinone derivatives. It is thus difficult to determine whether or not the activity of other furanonaphthoquinone derivatives could be improved by affixing a hydroxyamino side chain at the 2-position. More investigation is needed to know if they act by intercalation to DNA.

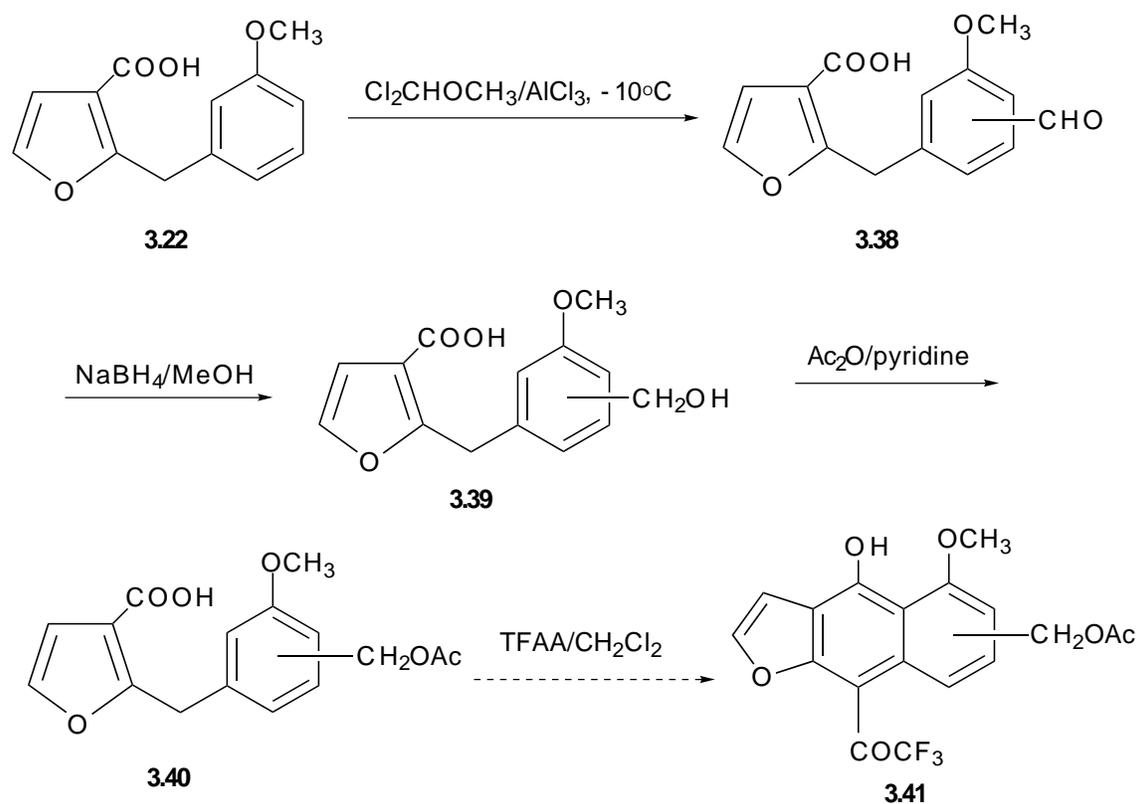
No clear evidence showed the effect of different side chains on their activity, although compound **3.35** with three hydroxy groups was not active.

3.2.3 Attempted Synthesis of Furanonaphthoquinones with Hydroxyamino Side Chain at C-Ring (benzene ring)



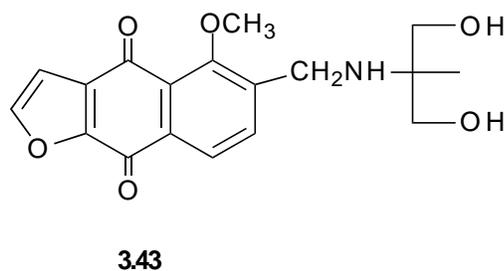
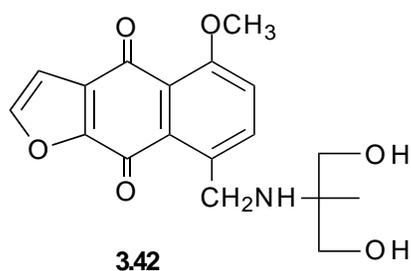
3.2.3.1 Synthetic Strategy

The synthetic strategy was based on the synthesis of 5-methoxynaphtho[2,3-b]furan-4,9-dione (**3.19**) mentioned in 3.2.1.2. The first scheme tried began with the formylation of benzylfuroic acids **3.24**. Since the benzene ring was more activated than the furan ring the formylation mainly occurred on the benzene ring and not on the furan ring. Then the resulting aldehyde **3.38** was then reduced to alcohol **3.39**. Unfortunately Friedel-Crafts cyclization of this alcohol or its acetate **3.40** failed to give the desired cyclized product **3.41** under different conditions.



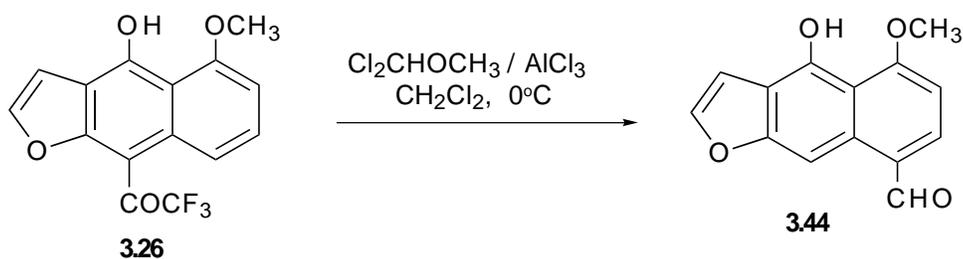
Scheme 4. First Scheme to Synthesize Compound **3.41**

The scheme was thus modified to one in which the cyclisation of **3.24** was carried out as previously described. The cyclized product **3.26** would then be subjected to formylation reaction to yield the aldehyde, which would be oxidized to the desired furanonaphthoquinone. Finally reductive amination of this furanonaphthoquinone derivative with an aldehyde group on the C ring would convert it to desired product **3.42** or **3.43**.



3.2.3.2 Synthetic Scheme

The scheme began with the formylation of 4-hydroxy-5-methoxy-9-trifluoroacetylnaphtho[2,3-b]furan (**3.26**), which was obtained from the Friedel-Crafts cyclisation of **3.24** as previously described. Compound **3.26** was treated with α,α -dichloromethyl methyl ether in dichloromethane in the presence of aluminum chloride.⁷⁹ Work up with aqueous sodium hydroxide solution and repeated chromatographic separation gave one major product **3.44** in 25% yield.



⁷⁹ Rieche, A., Gross, H., Hoft, E. *Chem. Ber.* **1960**, *93*, 88-94

The ^1H - and ^{13}C - NMR of product **3.44** indicated the presence of an aldehyde group (δ_{H} 10.11, δ_{C} 179.9). The aromatic region of the ^1H NMR spectrum of this compound exhibited a set of two furan protons (δ_{H} 7.01, δ_{H} 7.63) and a set of two protons characteristic of a 1, 2, 3, 4-tetrasubstituted benzene ring rather than the 1, 2, 3-trisubstituted one of **3.26**, indicating that the aldehyde group must be assigned either to the 6- or the 8- position. Since a NOESY spectrum of **3.44** showed NOE correlations between the protons in the methoxy group and one aromatic proton the aldehyde group was assigned to the 8- position. Among the three proton signals in the region of 9.0 ppm -11.0 ppm, one was the aldehyde proton and one was the hydroxy proton, which was exchangeable with D_2O . The remaining proton was assigned to be the proton at the 9- position, based on the fact that ^{13}C and ^{19}F NMR did not give any signals for the COCF_3 group. The unusual low-field position for this proton could be explained by the deshielding effect of the $\text{C} = \text{O}$ function on a neighboring proton which lies in the nodal region of the π -bond.⁸⁰ The structure of **3.44** could thus be assigned as 4-hydroxy-5-methoxy-8-formyl-naphtho[2,3-b]furan. It was confirmed by its MS spectrum, which gave a molecular ion at m/z 242. The removal of the COCF_3 group may be caused by an *ipso* substitution reaction,⁸¹ in which the attacking electrophile was H^+ .

In addition to the major product **3.44**, several minor products were isolated but not purified. The production of many side products may be the reason for this reaction giving only 25% yield of the desired product.

Various attempts to oxidize the phenol **3.44** with pyridinium dichromate,⁸² pyridinium chlorochromate,⁸³ Fremy's salt⁸⁴ and Triton B - O_2 ⁷² failed to give

⁸⁰ Gunther, H. *NMR Spectroscopy* 2nd Ed. John Wiley & Sons, **1995**, p 83

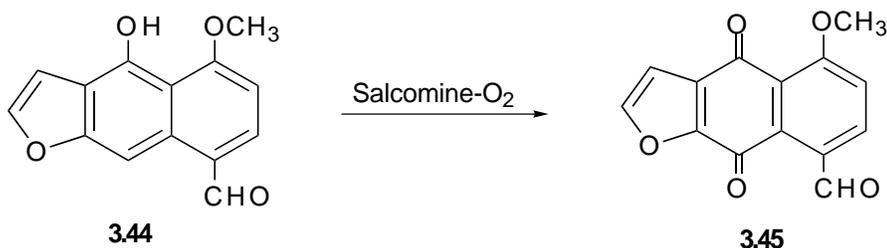
⁸¹ Carey, F. A., Sundberg, R. J. *Advanced Organic Chemistry* Part A, 3rd Ed., Plenum Press, **1990**, p 577

⁸² Czernecki, S., Georgoulis, C., Stevens, C. L., Vijayakumaran, K. *Tetra. Lett.* **1985**, 26, 1699-1702

⁸³ Brown, H. C., Rao, C. G., Kulkarni, S. U. *J. Org. Chem.*, **1979**, 44, 2809-2810

⁸⁴ Zimmer, H., Lankin, D. C., Horgan, S. W. *Chem. Reviews* **1971**, 71, 229-246

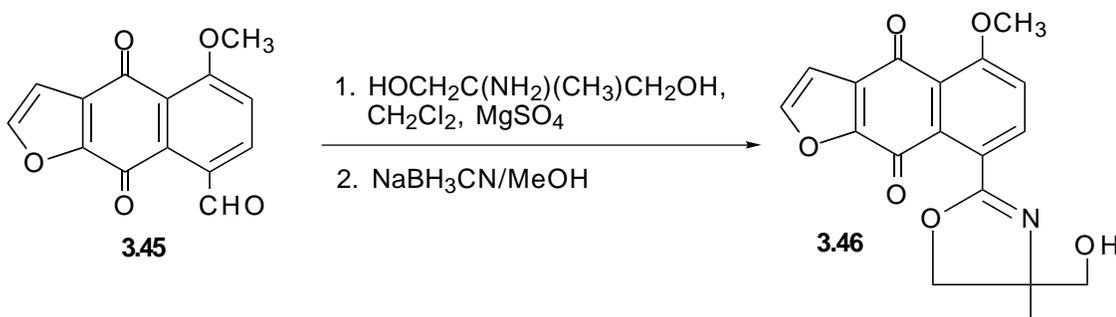
corresponding quinone **3.45**. But success was achieved by oxidizing the phenol **3.44** with salcomine-O₂ as the oxidation system⁸⁵ to yield the quinone **3.45** in 58% yield.



The ¹H NMR spectrum of **3.45** revealed the absence of hydroxy protons and the 9-proton in **3.44**, and its ¹³C NMR spectrum showed two more carbonyl carbon signals than that of **3.44**.

With the quinone **3.45** in hand, the expected final product **3.42** was expected to be formed by the reductive amination reaction discussed previously.

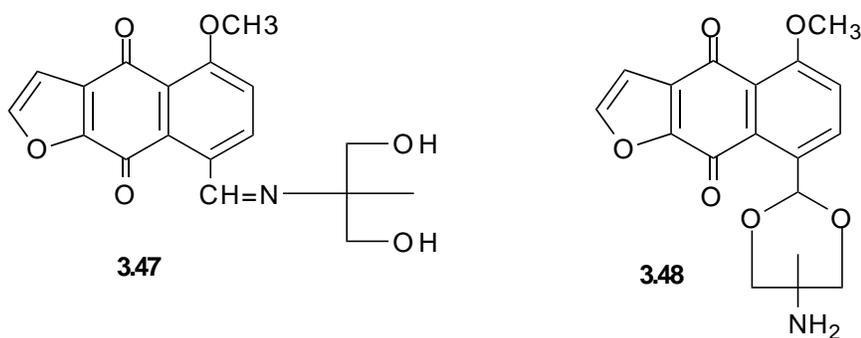
Compound **3.45** was treated with 2-amino-2-methyl-1,3-propanediol and magnesium sulfate in dichloromethane for 20 hours. The reaction solution was evaporated to remove dichloromethane and the residue was dissolved in methanol. The resulting solution was adjusted to pH 6-7 and treated with sodium cyanoborohydride. Usual work up and chromatography afforded a yellow solid **3.46** in 50% yield.



⁸⁵ Wakamatsu, T., Nishi, T., Ohnuma, T., Ban, Y. *Syn. Comm.* **1984**, *14*, 1167-1173

Further investigation revealed that no reduction had occurred in the above procedure, since treatment of **3.45** with 2-amino-2-methyl-1,3-propanediol and magnesium sulfate in dichloromethane, in the absence of sodium cyanoborohydride, afforded the identical product **3.46**.

The HRFABMS of **3.46** indicated its composition to be $C_{18}H_{16}NO_6$, which was four hydrogens fewer than the expected product **3.42**. This excluded the possibility of its structure being the imine **3.47** or the cyclic acetal **3.48**, since both of them would have composition $C_{18}H_{18}NO_6$.



The 1H and ^{13}C NMR spectra of **3.46** did show signals for the hydroxyamino side chain with one methyl group (δ_H 1.43, δ_C 23.2), and two oxygen bearing methylene groups (δ_H 3.56 and 3.72, δ_C 68.7; δ_H 4.17 and 4.55, δ_C 72.9). These two methylene groups were thus located in quite different chemical environments. Further evidence came from acetylation of **3.46**. The 1H NMR spectrum of the acetate of **3.46** showed only one acetate signal (δ_H 2.14, 3H), and two oxygen bearing methylene groups. One of them gave peaks at 4.24 and 4.51 ppm, close to the values of the unacetylated starting material, but the other gave peaks at 4.20 and 4.27 ppm, 0.6 ppm downfield compared with those in **3.46**. This evidence indicated that only one free hydroxy group exists in the molecule.

Since no signal was found for the protons on the carbon connecting the benzene ring and the nitrogen, one of the hydroxy groups in the side chain must be bound to that carbon.

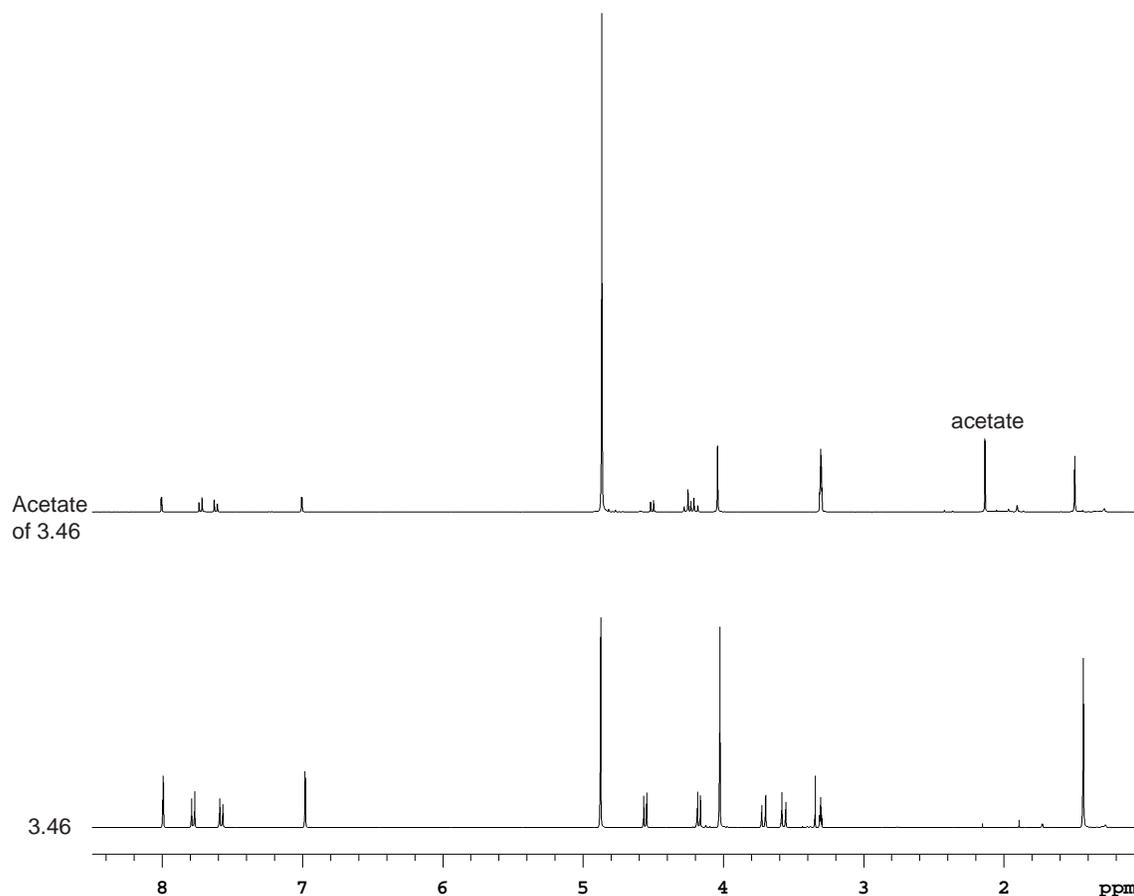


Figure 9. ^1H NMR Spectra of **3.46** and Its Acetate

The HMBC spectrum of **3.46** showed correlations between H-2' and C-1', and H-7 and C-1' (Figure 11), indicating that C-1' was the carbon connecting the benzene ring and the side chain.

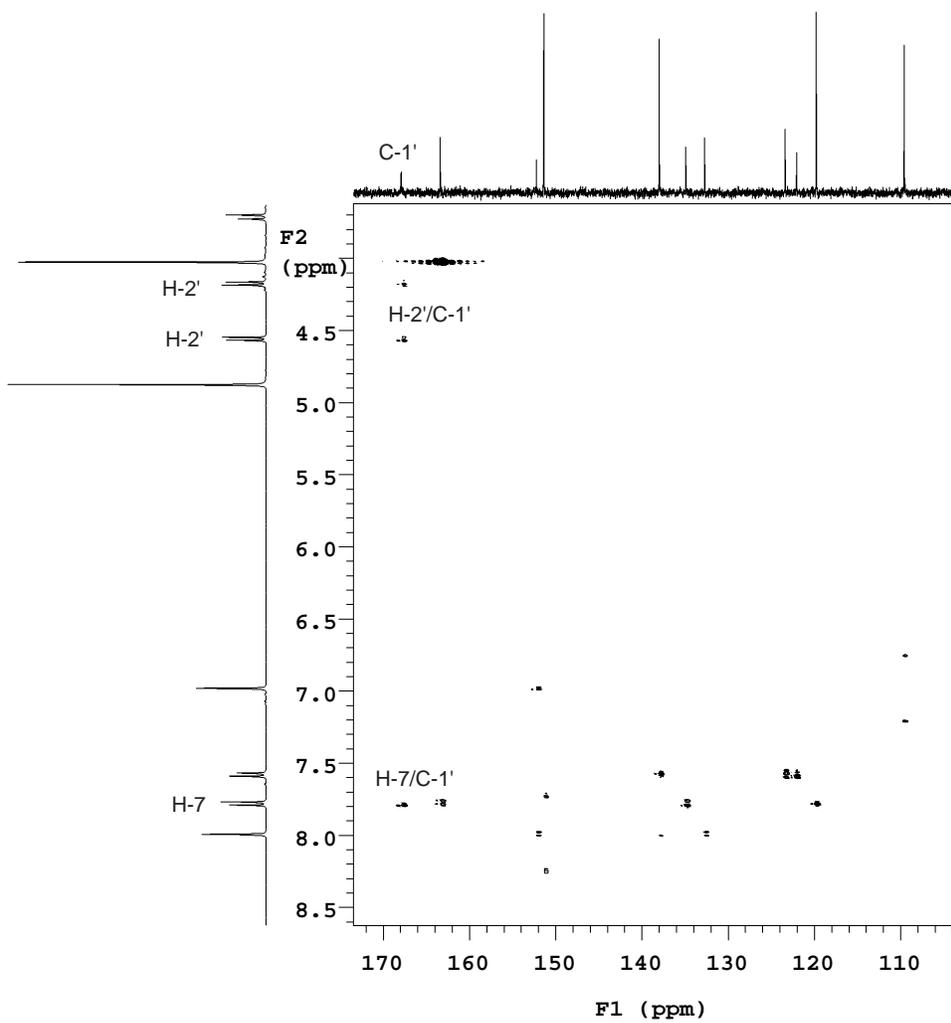
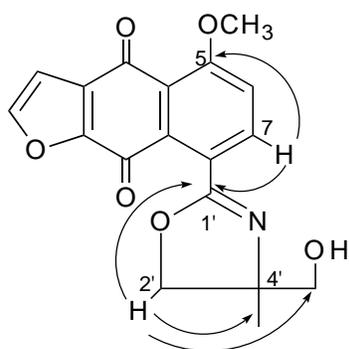


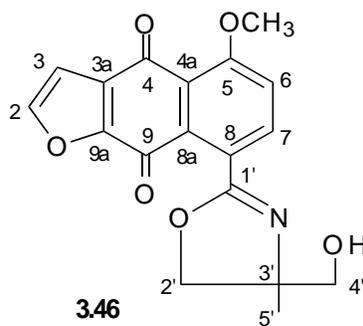
Figure 10. Expanded HMBC Spectrum of **3.46**



3.46

Figure 11. Selected HMBC Correlations for Compound **3.46**

Based on these data **3.46** was assigned the structure shown.



3.46

^1H NMR and ^{13}C NMR data for **3.46** are showed in Table 7.

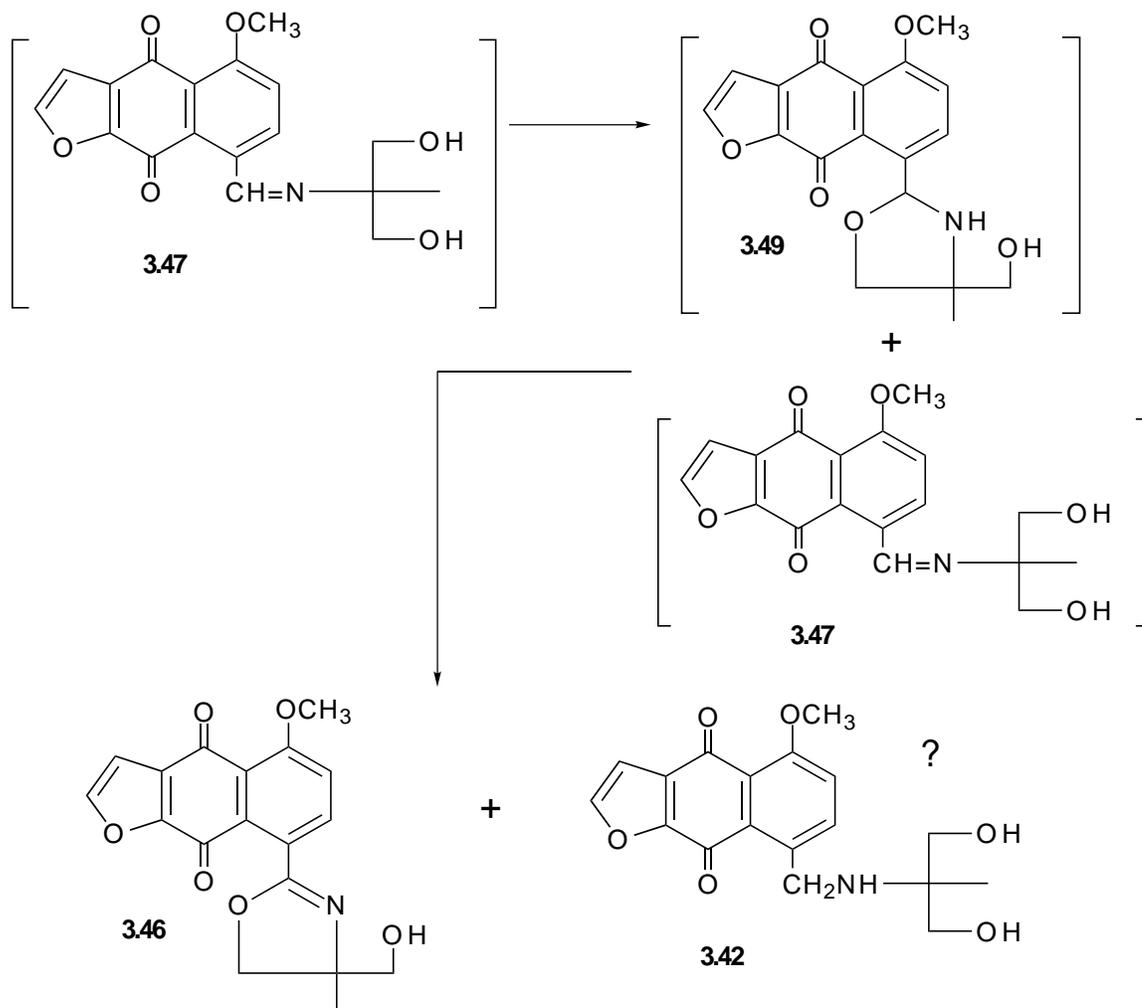
Table 7. ¹H and ¹³C NMR Data for Compound **3.46**

position	δ_C^b	δ_H^c	HMBC
2	151.3	7.99(1H, d, J=2.0)	C-3, C-3a, C-9a
3	109.6	6.98(1H, d, J=2.0)	C-2, C-9a
4	173.5		
5	163.3		
6	119.7	7.57(1H, d, J=8.8)	C-8, C-4a
7	138.0	7.78(1H, d, J=8.8)	C-1', C-5, C-8a
8	122.0		
9	180.9		
3a	132.7		
4a	123.3		
8a	134.9		
9a	152.2		
OCH ₃	57.2	4.02(3H, s)	C-5
1'	167.9		
2'	77.4	a. 4.55(1H, d, J=8.4) b. 4.17(1H, d, J=8.4)	C-1', C-4', C-5'
3'	72.9		
4'	68.7	a. 3.71(1H, d, J=10.8) b. 3.57(1H, d, J=10.8)	C-2', C-3', C-5'
5'	23.2	1.43(3H, s)	C-2', C-3', C-4'

^a Data recorded in CD₃OD at 400MHz (¹H) and 100 MHz (¹³C). ^b Assignments were determined by HETCOR and HMBC experiments. ^cMultiplicities and coupling constants (in Hz) are listed.

The cyclized product might be caused by the nucleophilic attack of the hydroxy group onto the -C=N- group. Transfer of hydrogen from this resulting intermediate **3.49**

to another mole of the imine⁸⁶ would then result in the formation of the product **3.46** and the unstable amine **3.42**, which will be discussed later.

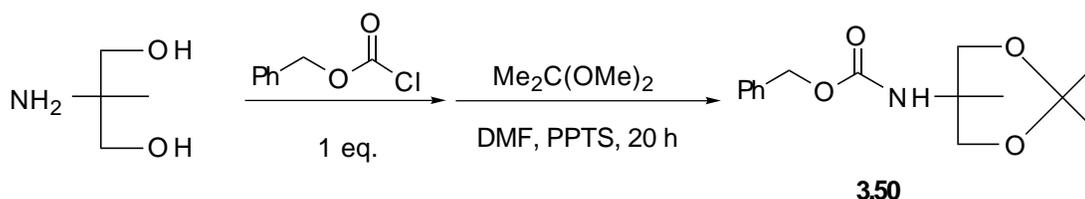


Scheme 8. Possible Mechanism for Formation of **3.46**

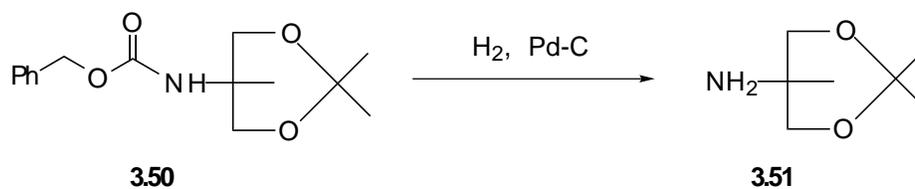
In order to prevent such an undesired cyclisation reaction from occurring, it was decided to protect the side chain before amination. This was achieved by protection of the NH_2 group with benzyl chloroformate, protection of the OH groups as an acetonide,

⁸⁶ March J. *Advanced Organic Chemistry* John Wiley & Sons, 4th Ed., 1992, p. 1194

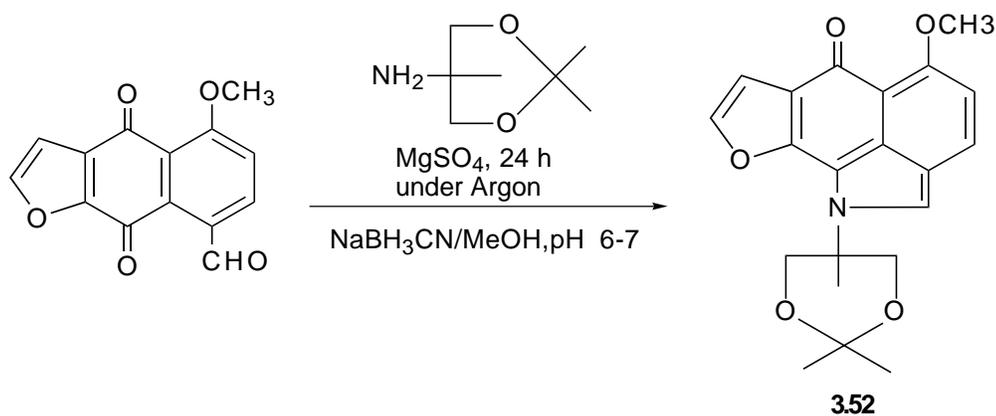
and then deprotection of the NH₂ group by hydrogenolysis. Thus 2-amino-2-methyl-1,3-propanediol was treated with benzyl chloroformate in *N,N*-dimethylformamide for 4 hours with stirring. 2,2-Dimethoxypropane and pyridinium *p*-toluenesulfonate were then added to the reaction solution and stirred for 20 hours. Usual work up and crystallization afforded the N, O- diprotected substrate **3.50** in 70% yield.



Deprotection of the NH₂ group of **3.50** was carried out by hydrogenolysis with Pd-C as catalyst. This reaction gave a 90% yield of the protected side chain amine **3.51**.



Reductive amination was repeated under argon by the same procedure mentioned previously but using the protected side chain **3.51** as the amine instead of 2-amino-2-methyl-1,3-propanediol. Usual work up and preparative TLC gave the amination product **3.52** in 46% yield.



The NMR spectra of **3.52** showed the expected signals for the protected side chain: three methyl groups (δ_{H} 1.25, 1.58, 1.76, δ_{C} 19.4, 21.4, 28.6), and two methylene groups (δ_{H} 4.45, 4.69, δ_{C} 67.7). However, no signal was found for the methylene group which should have connected the benzene ring and nitrogen in the expected structure **3.42**. Instead, the ^1H NMR spectrum gave a singlet at 8.38 ppm in addition to the signals of the two furan protons (δ_{H} 7.06, 7.66) and the two protons characteristic of a 1, 2, 3, 4-tetrasubstituted benzene ring.

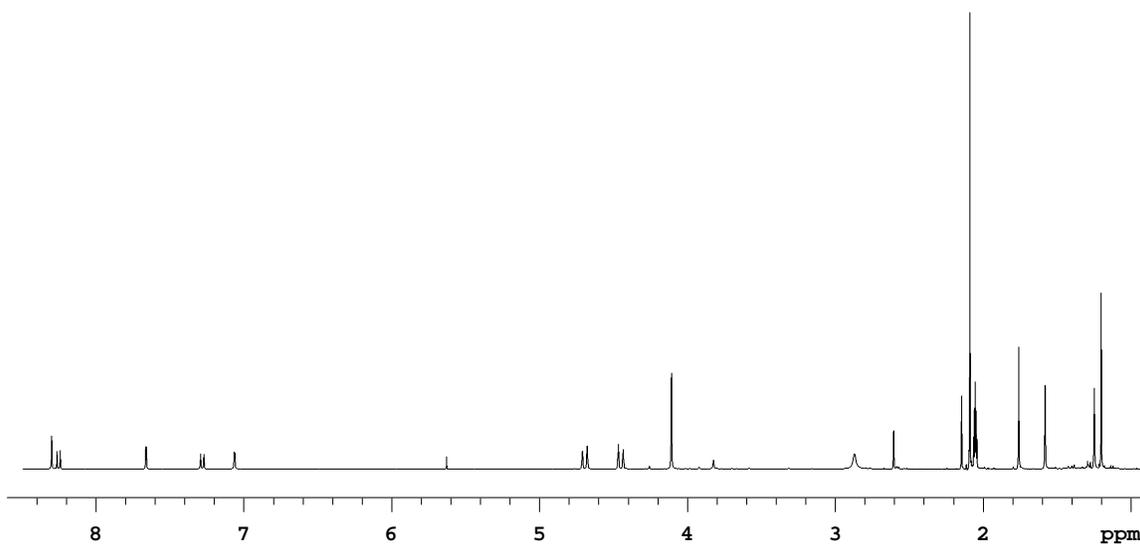
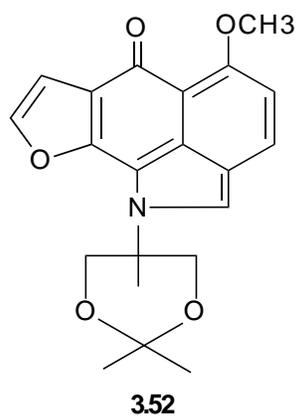


Figure 12. ¹H NMR spectrum of Compound 3.52

Its HMBC spectrum revealed that this proton had correlations with C-8, C-9 and C-3', indicating that it was the proton of a methine connecting the benzene ring and the side chain.

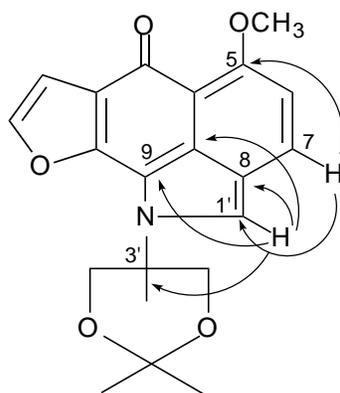


Figure 13. Selected HMBC Correlations for Compound **3.52**

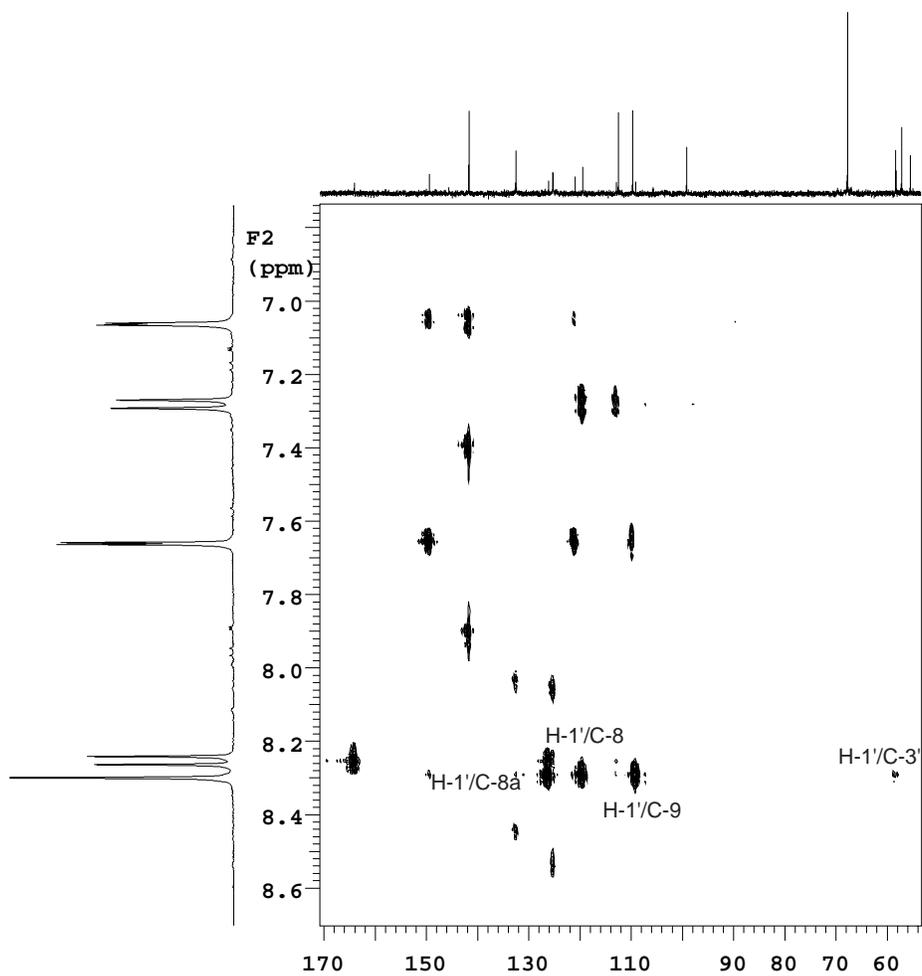
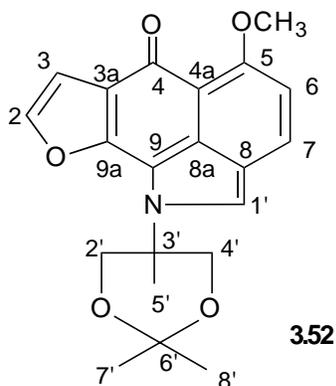


Figure 14. Expanded HMBC Spectrum of **3.52**

The imine structure was excluded by its mass spectrum, which indicated its composition to be $C_{21}H_{21}NO_5$. This composition also agreed with its ^{13}C NMR spectrum, which showed the presence of only one carbonyl carbon and not two as in the expected product. All these data implied that one of carbonyl groups was replaced with C-N group as in **3.52**.

Based on all these data, compound **3.52** was determined to have the structure shown.



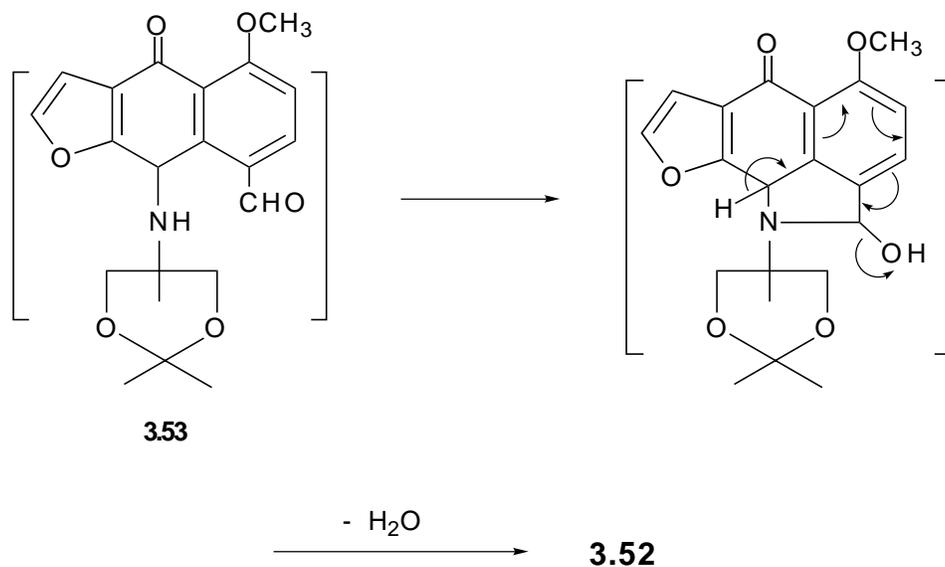
Complete NMR data for **3.52** are shown in Table 8.

Table 8. NMR data for Compound **3.52**

position	δ_C^b	δ_H^c	HMBC
2	141.6	7.66(1H, d, J=2.0)	C-3, C-3a, C-9a
3	109.7	7.06(1H, d, J=2.0)	C-2, C-9a
4	176.6		
5	164.0		
6	112.5	7.28(1H, d, J=8.8)	C-8, C-4a
7	132.5	8.25(1H, d, J=8.8)	C-1', C-5, C-8a
8	119.4		
9	109.1		
3a	120.9		
4a	123.3		
8a	126.1		
9a	149.4		
OCH ₃	57.1	4.12(3H, s)	C-5
1'	125.2	8.30(1H, s)	C-8, C-9, C-8a, C-3'
2', 4'	67.7	a. 4.69(1H, d, J=13.1) b. 4.45(1H, d, J=13.1)	C-3', C-6' C-3', C-6'
3'	58.3		
5'	21.4	1.76(3H, s)	C-2', C-3', C-4'
6'	99.1		
7'	19.4	1.58(3H, s)	C-6', C-8'
8'	28.6	1.25(3H, s)	C-6', C-7'

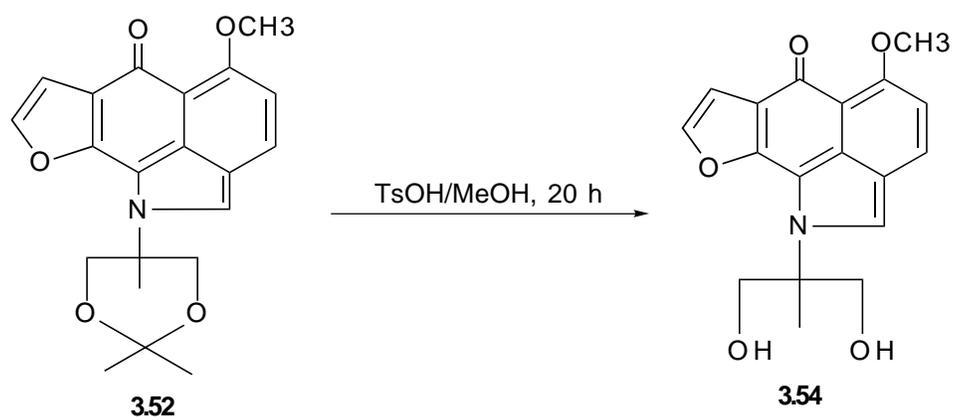
^a Date recorded in CD₃OD at 400MHz (¹H) and 100 MHz (¹³C). ^b Assignments were determined by HETCOR and HMBC experiments. ^cMultiplicities and coupling constants (in Hz) are listed.

The formation of **3.52** can be explained by an intramolecular amination reaction. After reductive amination giving the secondary amine **3.53**, an intramolecular amination followed by dehydration could occur to yield the stable enamine **3.52**, as shown in Scheme 9.

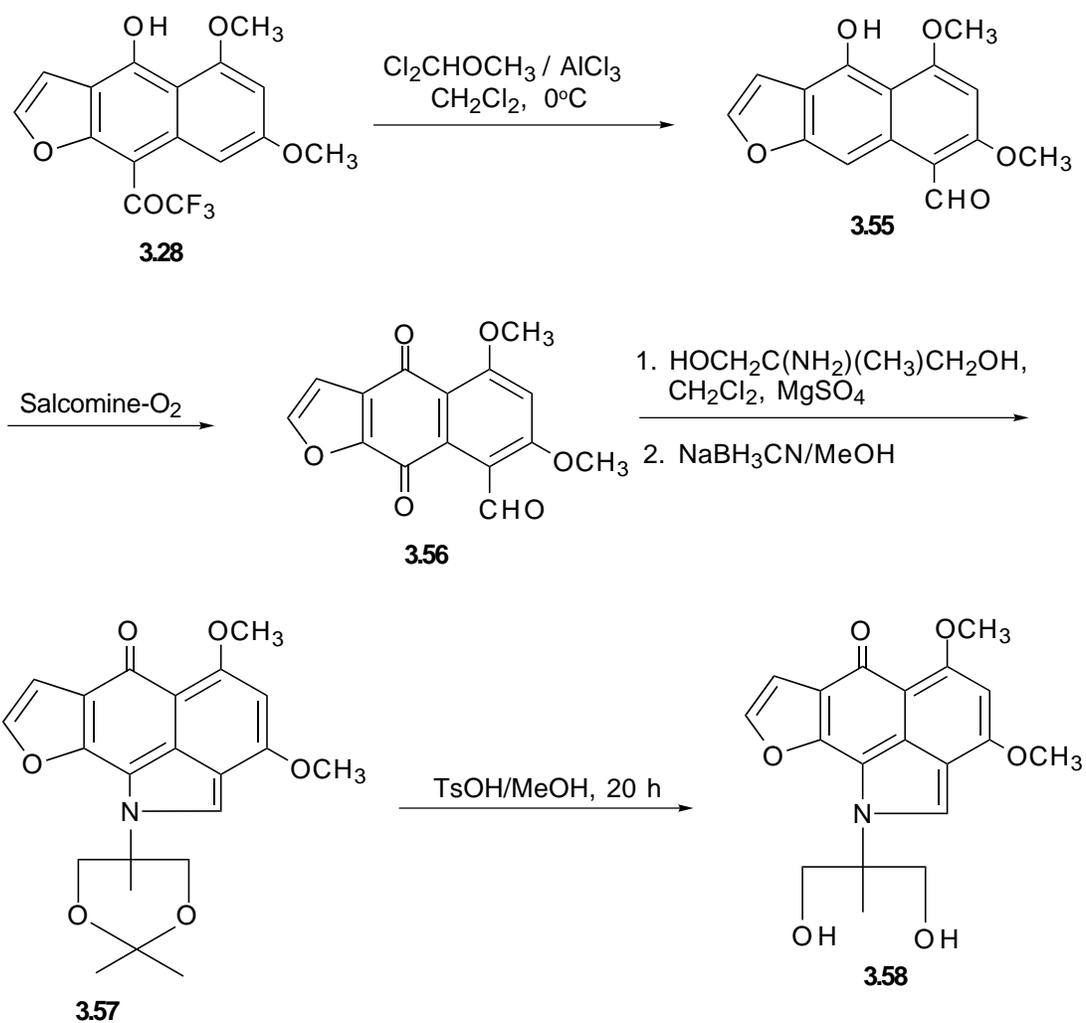


Scheme 9. Possible Mechanism for Formation of **3.52**

Hydrolysis of **3.52** with *p*-toluenesulfonic acid in methanol overnight gave compound **3.54** in 80% yield.



Application of the same reaction sequence to quinone **3.28** gave the corresponding product **3.58** (Scheme 7).



Scheme 10. Synthetic Scheme for Compound **3.58**

3.2.3.3 Summary and Biological Activity of Compounds **3.54** and **3.58**

The attempted syntheses of furanonaphthoquinones with a hydroxyamino side chain on the C-ring (benzene ring) were not successful. The desired target compound **3.42**

underwent intramolecular amination to give enamine **3.52**. Similarly amination of **3.56**, which possesses one more methoxy group at the 7 position, afforded the related product **3.58**.

Compounds **3.54** and **3.58** did not show any activity in the yeast bioassay, or in a cytotoxicity assay. This evidence tends to suggest that the furanonaphthoquinone structure is essential for the observed bioactivity of this class of compounds.

3.3 EXPERIMENTAL

General Experimental Procedures. Mp's were determined on a Kofler hot-stage apparatus and are uncorrected. The ^1H - and ^{13}C -NMR spectra were recorded on a Varian Unity 400 spectrometer at 400 and 100.57 MHz, respectively. ^1H - ^1H COSY, ^1H - ^{13}C HETCOR, HMQC and HMBC NMR experiments were performed on the same spectrometer, using standard Varian pulse sequences. Mass spectra were taken on a VG 7070 E-HF instrument. Chromatography was performed using Si gel Merck G60(230-400 mesh), preparative TLC with Si gel GF254 plates (Analtech, 500 mm, 20x20 cm), and reversed-phase preparative TLC with Whatman PLKC18F linear K reversed-phase (500 mm, 20x20 cm) plates.

2-Acetoxy-2,3-dihydro-naphtho[2,3-*b*]furan-4,9-dione (3.17) and 2-acetoxy-2,3-dihydro-naphtho[1,2-*b*]furan-4,5-dione (3.18). To a solution of 2-hydroxy-1,4-naphthoquinone (**3.16**) (1.0 g, 5.8 mmol) and vinyl acetate (5.34 mL, 58 mmol) in acetonitrile (20 mL) at 0°C was added ceric ammonium nitrate (3.0 g, 5.5 mmol) with stirring. The mixture was stirred at 0°C for 1 hour. Then the reaction solution was diluted with water and extracted with ethyl acetate 3 times. The combined extract was washed with water, and dried over sodium sulfate. Evaporation followed by repeated column chromatography on silica gel with chloroform-methanol afforded pure compounds **3.17** and **3.18**. Data for **3.17**: orange crystals (364 mg, 24.3%), mp 189-191°C; ^1H NMR (CDCl_3) δ 2.15 (3H, s), 3.11 (1H, dd, $J = 17.2, 2.8$), 3.39 (1H, dd, $J = 17.2, 7.6$), 7.01 (1H, dd, $J = 7.6, 2.8$), 7.60 (1H, m), 7.66 (2H, m), 8.08 (1H, m); ^{13}C NMR (CDCl_3) δ 20.8, 32.6, 99.4, 113.8, 124.8, 126.9, 129.8, 130.6, 132.2, 134.8, 168.0, 169.0, 175.2, 180.3; EIMS m/z 258 (M^+), 216, 198, 188, 170, 159. Data for **3.18**: yellow crystals (526 mg, 35.2%), mp 187-189°C; ^1H NMR (CDCl_3) δ 2.12 (3H, s), 3.17 (1H, dd, $J =$

18.4, 2.4), 3.45 (1H, dd, $J = 18.4, 7.6$), 6.99 (1H, dd, $J = 7.6, 2.4$), 7.72 (2H, m), 8.09 (2H, m); ^{13}C NMR (CDCl_3) δ 20.8, 33.6, 98.3, 123.3, 126.2, 126.5, 131.5, 132.7, 133.3, 134.2, 158.3, 168.7, 176.7, 181.7; EIMS m/z 258 (M^+), 217, 199, 189.

Naphtho[2,3-b]furan-4,9-dione (3.15). To a solution of **3.18** (200 mg, 0.77 mmol) in THF (5 mL) lithium bis (trimethylsilyl) amide (1.6 mL of a 1.0 mol dm^{-3} solution in THF, 1.6 mmol) was added at -78°C with stirring. After 15 minutes the reaction solution was diluted with water and extracted with ethyl acetate three times. The ethyl acetate was collected, washed with water, and dried over sodium sulfate. Evaporation and column chromatography on silica gel with hexanes-ethyl acetate gave compound **3.15** as a yellow solid (50 mg, 64.7%). mp $225\text{-}226^\circ\text{C}$ (lit⁸⁷ $225\text{-}225.5^\circ\text{C}$); ^1H NMR (CDCl_3) δ 7.01 (1H, d, $J = 1.6$), 7.76 (2H, m), 7.78 (1H, d, $J = 1.6$), 8.22 (2H, m); ^{13}C NMR (CDCl_3) δ 108.6, 126.9, 127.1, 130.5, 132.5, 133.2, 133.8, 133.9, 148.6, 152.7, 173.6, 180.5; EIMS m/z 198 (M^+), 170, 142, 114.

General procedure for the preparation of hydroxymethylfuroic acids 3.22 and 3.23. To diisopropylamine (7mL, 50 mmol) at -10°C under argon was added *n*-butyllithium (20 mL of a 2.5 mol dm^{-3} solution in hexanes, 50 mmol) with stirring. After 15 minutes the resulting solution was diluted with dry THF (40 mL), and cooled to -78°C . A solution of 3-furoic acid (2.8 g, 25 mmol) in dry THF (20 mL) was added. The solution was stirred at -78°C for 30 minutes and a solution of either 3-methoxybenzaldehyde or 3, 5-dimethoxybenzaldehyde (25 mmol) in THF (20 mL) was added. After 15 minutes the solution was allowed to reach ambient temperature over *ca.* 15 minutes. The resulting solution was diluted with water and washed with ethyl acetate. The aqueous portion was acidified with hydrochloride acid and extracted with ethyl

acetate 3 times. The combined extracts were washed with water, dried over sodium sulfate, and evaporated to yield the crude product. Column chromatography of this product on silica gel with chloroform-methanol afforded a fraction as an oil which was crystallized from chloroform-hexanes giving pure compounds.

2-[(3-Methoxyphenyl)hydroxymethyl]-3-furoic acid (3.22): white solid (5.06 g, 82%), mp 85-87°C (lit⁷¹80°C); ¹H NMR (CD₃OD)δ 3.73 (3H, s), 6.13 (1H, s), 6.63 (1H, d, *J* = 2.0), 6.74 (1H, ddd, *J* = 0.8, 2.8, 8.0), 6.92 (2H, m), 7.17 (1H, t, *J* = 8.0), 7.23 (1H, d, *J* = 2.0); ¹³C NMR (CD₃OD)δ 55.1, 69.1, 111.4, 111.8, 113.2, 114.5, 118.5, 129.4, 141.4, 142.3, 159.5, 161.5, 166.5; EIMS *m/z* 248 (M^{•+}), 230, 201, 187, 135, 123, 115, 77.

2-[(3,5-Dimethoxyphenyl)hydroxymethyl]-3-furoic acid (3.23): white solid (5.28 g, 76%), mp 132-133°C (lit⁷⁰136°C); ¹H NMR (CD₃OD)δ 3.63 (6H, s), 6.07 (1H, s), 6.22 (1H, dd, *J* = 2.4), 6.47 (2H, d, *J* = 2.4), 6.54 (1H, d, *J* = 2.0), 7.18 (1H, d, *J* = 2.0); ¹³C NMR (CD₃OD)δ 55.7, 68.7, 100.4, 105.3, 112.0, 115.7, 143.2, 144.9, 162.1, 162.3, 166.8; EIMS *m/z* 278 (M^{•+}), 260, 231, 161, 139, 123, 95.

General procedure for the preparation of benzylfuroic acids 3.24 and 3.25:

To a suspension of sodium iodide (17.9 g, 120 mmol) in dry acetonitrile (35 mL) chlorotrimethylsilane (17.1 mL, 120 mmol) was added with stirring followed by a solution of either **3.22** or **3.23** (20 mmol) in dry acetonitrile (20 mL) and the mixture was stirred at room temperature for 15 minutes. The mixture was diluted with water and extracted with ethyl acetate 3 times. The combined extracts were washed with aqueous sodium thiosulphate solution, water, and dried over sodium sulfate. Evaporation afforded the crude product which was re-crystallized in chloroform-hexanes to give the pure compounds.

⁸⁷ Mathieson, J. W., Thomson, R. H. *J. Chem. Soc. (C)*, **1971**, 153

2-(3-Methoxybenzyl)-3-furoic acid (3.24): white crystals (4.0 g, 85%), mp 70-72°C (lit⁷¹80°C); ¹H NMR (CDCl₃) δ 3.79 (3H, s), 4.37 (2H, s), 6.72 (1H, d, *J* = 2.0), 6.79 (1H, ddd, *J* = 0.8, 2.4, 8.0), 6.86 (1H, dd, *J* = 0.8, 2.4), 6.89 (1H, dd, *J* = 0.8, 8.0), 7.23 (1H, dd, *J* = 8.0), 7.29 (1H, d, *J* = 2.0); ¹³C NMR (CDCl₃)δ 33.6, 55.1, 110.9, 112.0, 113.2, 114.6, 121.2, 129.5, 138.5, 141.4, 159.7, 161.8, 169.6; EIMS *m/z* 232 (M^{•+}), 214, 199, 171, 115.

2-(3,5-Dimethoxybenzyl)-3-furoic acid (3.25): white crystals (4.19 g, 80%), mp 110°C (lit⁷⁰112°C); ¹H NMR (CDCl₃) δ 3.77 (6H, s), 4.31 (2H, s), 6.34 (1H, dd, *J* = 2.2), 6.45 (2H, d, *J* = 2.2), 6.71 (1H, d, *J* = 2.0), 7.29 (1H, d, *J* = 2.0); ¹³C NMR (CDCl₃) δ 34.3, 55.6, 99.4, 107.7, 112.0, 114.0, 141.1, 142.6, 161.8, 162.3, 167.0; ; EIMS *m/z* 262 (M^{•+}), 244, 182, 158, 115, 77, 63.

4-Hydroxy-5-methoxy-9-trifluoroacetylnaphtho[2,3-b]furan (3.26). To a stirred solution of **3.24** (3.99g, 17.2 mmol) in dry dichloromethane (50 mL) was added trifluoroacetic anhydride (3.67 mL, 25 mmol) at 0°C. After stirring at this temperature for 4 hours, the solution was diluted with water and extracted with dichloromethane. The organic phase was collected, washed with water and dried over sodium sulfate. Evaporation and repeated column chromatography on silica gel with chloroform and hexanes-ethyl acetate gave **3.26** as yellow crystals (1.73g, 32.4%) and a mixture of **3.26** and **3.27** (0.7g). Data for **3.26**: mp 128°C (lit⁷¹127-128°C); ¹H NMR (CDCl₃) δ 4.12 (3H, s), 6.82 (1H, d, *J* = 7.8), 7.02 (1H, d, *J* = 2.3), 7.46 (1H, dd, *J* = 7.8, 9.0), 7.63 (1H, d, *J* = 2.3), 8.33 (1H, d, *J* = 9.0), 10.80 (1H, s); ¹³C NMR (CDCl₃) δ 56.5, 102.4, 103.4, 104.0, 111.0, 114.2, 115.1, 118.7, 128.9, 134.3, 144.8, 155.6, 156.9, 157.1, 180.5; EIMS *m/z* 310 (M^{•+}), 241, 226, 198, 170.

4-Hydroxy-5,7-dimethoxy-9-trifluoroacetylnaphtho[2,3-b]furan (3.28). To a stirred solution of **3.26** (610 mg, 2.3 mmol) in dry dichloromethane (10 mL) was added trifluoroacetic anhydride (0.35 mL, 2.3 mmol) at 0°C. After stirring at this temperature for 2 hours, the solution was diluted with water and extracted with dichloromethane. The organic phase was collected, washed with water and dried over sodium sulfate. Evaporation and repeated column chromatography on silica gel with chloroform and hexanes-ethyl acetate gave **3.28** as yellow crystals (500 mg, 64%). mp 169°C (lit⁷⁰ 168°C); ¹H NMR (CDCl₃) δ 3.95 (3H, s), 4.11 (3H, s), 6.52 (1H, d, *J* = 2.0), 7.00 (1H, d, *J* = 2.0), 7.59 (1H, d, *J* = 2.0), 8.09 (1H, d, *J* = 2.0); ¹⁹F NMR (CDCl₃)δ -75.27; ¹³C NMR (CDCl₃)δ 55.5, 56.5, 97.2, 97.4, 101.2, 104.0, 106.7, 107.6, 109.9, 112.7, 115.3, 118.5, 121.8, 136.4, 143.8, 144.1, 149.0, 156.5, 158.1, 158.3, 161.2, 179.5; EIMS *m/z* 340 (M^{•+}), 271, 256, 228, 200, 157, 69.

General procedure for the preparation of furonaphthoquinones 3.19 and 3.20. Hydrogen peroxide (2.0 mmol, 30% w/w in water) was added to a stirred solution of either **3.26** or **3.28** (0.15 mmol), sodium hydroxide (20 mg, 0.5 mmol) and sodium carbonate (20 mg, 0.2 mmol) in distilled water (4 mL), and the mixture was stirred at room temperature for 4 hours. The solution was acidified, and then extracted with ethyl acetate 3 times. The combined extracts was washed with water and dried over sodium sulfate. Evaporation and column chromatography on silica gel with hexanes-ethyl acetate afforded pure compounds.

5-Methoxy-4,9-dihydronaphtho[2,3-b]furan-4,9-dione (3.19): yellow needles (26.8 mg, 69.4%), mp 183-184°C (lit⁷¹ 190-191°C); ¹H NMR (CDCl₃)δ 4.03 (3H, s), 6.96 (1H, d, *J* = 2.0), 7.33 (1H, dd, *J* = 0.8, 8.0), 7.69 (1H, dd, *J* = 8.0, 8.0), 7.33 (1H, d, *J* = 2.0), 7.90 (1H, d, *J* = 0.8, 8.0). ¹³C NMR (CDCl₃)δ 56.6, 109.0, 118.6, 119.9, 132.1,

134.8, 134.9, 148.7, 151.0, 160.5, 173.2, 180.1; EIMS m/z 228 (M^+), 199, 170, 154, 142, 114.

5,7-Dimethoxy-4,9-dihydronaphtho[2,3-b]furan-4,9-dione (3.20): yellow needles (35 mg, 92%), mp 238°C C (lit⁷⁰242-243°C); ¹H NMR (CDCl₃)δ 3.97 (3H, s), 3.98 (3H, s), 6.73 (1H, d, $J = 2.5$), 6.94 (1H, d, $J = 1.8$), 7.41 (1H, d, $J = 2.5$), 7.71 (1H, d, $J = 1.8$);). ¹³C NMR (CDCl₃)δ 56.0, 56.5, 104.2, 104.5, 109.2, 114.5, 132.4, 136.9, 148.7, 151.2, 162.7, 164.7, 173.0, 179.1; EIMS m/z 258 (M^+), 229, 157, 129, 100, 63.

7-Methoxy-4,9-dihydronaphtho[2,3-b]furan-4,9-dione (3.29). Hydrogen peroxide (0.2 mL, 2.0 mmol, 30% w/w in water) was added to a stirred solution of the mixture of **3.26** and **3.27** (70 mg), sodium hydroxide (20 mg, 0.5 mmol) and sodium carbonate (20 mg, 0.2 mmol), and the mixture was stirred at room temperature for 4 hours. The solution was acidified, and then extracted with ethyl acetate 3 times. The combined extracts were washed with water and dried over sodium sulfate. Evaporation and column chromatography on silica gel with hexanes-ethyl acetate afforded **3.19** (35 mg, 68%) and **3.29** (10 mg, 19%). Data for **3.29**: yellow crystals, mp 177-179°C; ¹H NMR (CDCl₃)δ 3.97 (3H, s), 6.98 (1H, d, $J = 2.0$), 7.19 (1H, dd, $J = 2.4, 8.4$), 7.68 (1H, d, $J = 2.4$), 7.76 (1H, d, $J = 2.0$), 8.14 (1H, d, $J = 8.4$); ¹³C NMR (CDCl₃)δ 55.9, 108.5, 108.7, 111.1, 119.5, 126.4, 129.4, 130.8, 134.6, 148.7, 164.3, 173.9, 179.9; EIMS m/z 228 (M^+), 199, 143, 115, 101, 75, 63.

3-Phenyliodonio-1,2,4-trioxo-1,2,3,4-tetrahydronaphthalenide (3.31). To a stirred solution of 2-hydroxy-1,4-naphthoquinone (2.06 g, 11.8 mmol) in chloroform 40 mL was added a solution of (diacetoxyiodo)benzene (3.8g) dissolved in chloroform (25 mL) at 0°C. The mixture was stirred at this temperature for 1 hour and allowed to be stirred for 4 more hours at room temperature. Filtration gave an orange precipitate which

was washed with chloroform and dried to afford compound **3.31** (3.9 g, 88%) as orange crystals, mp >320°C; ¹H NMR (DMSO)δ 7.38 (2H, m), 7.50 (1H, m), 7.70 (1H, m), 7.79 (1H, m), 7.84 (2H, m), 7.95 (1H, m), 8.03 (1H, m); ¹³C NMR (DMSO) δ 103.0, 113.9, 126.8, 126.9, 130.6, 130.8, 131.1, 132.2, 133.1, 133.4, 134.6, 169.9, 174.8, 181.0; FABMS *m/z* 377 (M+1⁺); EIMS *m/z* 204, 172, 104, 76.

2-Hydroxymethyl-4,9-dihydronaphtho[2,3-b]furan-4,9-dione (3.32). To a solution of **3.31** (1.0 g, 2.7 mmol) in pyridine (40 mL) was added propargyl alcohol (1.6 mL, 27 mmol) and cuprous oxide (1.0 g) at 80°C. The mixture solution was stirred at this temperature for 2 hours. The solution was filtered and diluted with 10% aq. HCl. The acidic solution was extracted with ethyl acetate for 3 times. The combined organic phase was washed with water and dried over sodium sulfate. Evaporation and repeated column chromatography on silica gel with chloroform-acetone gave compound **3.32** as orange solid (150 mg, 25%). mp 202-203°C; ¹H NMR (CDCl₃)δ 4.69 (2H, s), 6.87 (1H, s), 7.78 (2H, m), 8.17 (2H, m); ¹³C NMR (CDCl₃)δ 56.1, 104.7, 126.3, 126.4, 131.0, 132.0, 132.6, 133.5, 133.6, 151.6, 162.4, 173.2, 180.3; EIMS *m/z* 228 (M⁺), 212, 183, 172, 113, 104, 76.

2-Formyl-4,9-dihydronaphtho[2,3-b]furan-4,9-dione (3.33). To a solution of **3.32** (90 mg, 0.39 mmol) in dichloromethane (5 mL) was added pyridinium chlorochromat (0.3 g). The mixture was stirred for 20 hours at room temperature. Then the solution was diluted with water and extracted with ethyl acetate 3 times. The combined extract was washed with water and dried over sodium sulfate. Evaporation and column chromatography on silica gel with chloroform-acetone afforded **3.33** as a yellow solid (63 mg, 70%). mp 183-186°C; ¹H NMR (CDCl₃)δ 7.67 (1H, s), 7.81 (2H, m), 8.24 (2H, m), 9.96 (1H, s); ¹³C NMR (CDCl₃)δ 114.4, 127.3, 127.4, 130.4, 132.5, 133.1, 134.4, 134.6,

153.8, 154.8, 173.9, 179.1, 179.5; HREIMS m/z 226.0266 ($M^{\bullet+}$) (calcd for $C_{13}H_6O_4$, 226.0265); EIMS m/z 226 ($M^{\bullet+}$), 197, 169, 141, 114.

General procedure for the preparation of 3.30 and its analogs. To a solution of **3.33** (10 mg, 0.044 mmol) in dichloromethane (3 mL) was added 2-amino-2-methyl-1,3-propanediol, or 2-amino-2-methylpropanol, or 2-amino-2-ethyl-1,3-propanediol, or 2-amino-2-hydroxymethyl-1,3-propanediol, or 3-amino-2,3-propanediol (0.88 mmol) and magnesium sulfate (100 mg). The mixture was stirred for 24 hours. The solution was filtered and evaporated to dry. The residue was dissolved in methanol (2 mL). The resulting solution was adjusted to pH 6-7 by acetic acid and was added sodium cyanoborohydride (14 mg, 0.22 mmol). The mixture was stirred for 30 minutes. The solution was diluted with water and extracted with ethyl acetate 3 times. The ethyl acetate extracts were collected and dried over sodium sulfate. Evaporation and purification by preparative TLC eluting with chloroform-methanol afforded pure compounds.

2-Methyl-2-[2'-(4',9'-dihydronaphtho[2',3'-b]furan-4',9'-dionylmethyl)

amino]-1,3-propanediol (3.30): yellow solid (8.6 mg, 61%), mp 193-195°C; 1H NMR (DMSO) δ 0.92 (3H, s), 2.08 (1H, br s), 3.27 (4H, d, $J = 5.5$), 3.88 (2H, s), 4.47 (2H, dd, $J = 5.5$), 6.91 (1H, s), 7.85 (1H, m), 8.07 (2H, m); ^{13}C NMR (DMSO) δ 18.8, 39.4, 57.5, 64.9, 79.6, 105.1, 126.7, 126.8, 131.5, 132.6, 133.1, 134.4, 134.7, 151.6, 164.8, 172.9, 180.8; HRFABMS m/z 316.1182 ($M+1^+$) (calcd for $C_{17}H_{18}NO_5$, 316.1185); EIMS m/z 226 ($M^+ - C_4H_9O_2$), 197, 169, 141, 114, 113, 76.

2-Methyl-2-[2'-(4',9'-dihydronaphtho[2',3'-b]furan-4',9'-dionylmethyl)

amino]propanol (3.34): yellow solid (12.6 mg, 96%), mp 187-189°C; 1H NMR (CD_3OD) δ 1.13 (6H, s), 3.42 (2H, s), 3.93 (2H, s), 6.91 (1H, s), 7.81 (2H, m), 8.16 (2H, m); ^{13}C NMR (DMSO) δ 24.0, 39.6, 54.2, 68.6, 105.1, 126.7, 126.9, 131.4, 132.6, 133.1,

134.4, 134.7, 151.6, 164.9, 173.0, 180.9; HRFABMS m/z 300.1244 ($M+1^+$) (calcd for $C_{17}H_{17}NO_4$, 300.1234).

2-Hydroxymethyl-2-[2'-(4',9'-dihydronaphtho[2',3'-b]furan-4',9'-dionyl methyl)amino]-1,3-propanediol (3.35): yellow solid (13.7 mg, 94%), mp > 320°C; 1H NMR (DMSO) δ 3.38 (6H, d, $J = 5.0$), 3.99 (2H, s), 4.38 (3H, t, $J = 5.0$), 6.93 (1H, s), 7.84 (2H, m), 8.07 (2H, m); ^{13}C NMR (DMSO) δ 60.0, 61.3, 104.7, 126.3, 126.4, 131.1, 132.2, 132.6, 134.0, 134.3, 151.2, 164.3, 172.5, 180.5; HRFABMS m/z 332.1143 ($M+1^+$) (calcd for $C_{17}H_{17}NO_6$, 332.1134).

2-Eethyl-2-[2'-(4',9'-dihydronaphtho[2',3'-b]furan-4',9'-dionylmethyl) amino]-1,3-propanediol (3.36): yellow solid (12.9 mg, 89%), mp > 320°C; 1H NMR (CD_3OD) δ 0.93 (3H, t, $J = 7.5$), 1.50 (2H, q, $J = 7.5$), 3.53 (4H, d, $J = 4.1$), 3.95 (2H, s), 6.91 (1H, s), 7.81 (2H, m), 8.17 (2H, m); ^{13}C NMR (DMSO) δ 7.1, 22.3, 48.6, 58.9, 62.1, 104.7, 126.3, 126.4, 131.0, 132.2, 132.7, 134.0, 134.3, 151.2, 164.2, 172.5, 180.5; HRFABMS m/z 330.1354 ($M+1^+$) (calcd for $C_{18}H_{19}NO_5$, 330.1341).

3-[2'-(4',9'-dihydronaphtho[2',3'-b]furan-4',9'-dionylmethyl)amino]-1,2-propanediol (3.37): yellow solid (12.6 mg, 95%), mp 148-150°C; 1H NMR (CD_3OD) δ 2.65 (1H, dd, $J = 8.0, 12.0$), 2.80 (1H, dd, $J = 4.0, 12.0$), 3.51 (2H, d, $J = 5.0$), 3.75 (1H, m), 3.99 (2H, s), 6.92(1H, m), 7.82(2H, m), 8.15(2H, m); ^{13}C NMR (DMSO) δ 45.8, 52.0, 64.3, 70.6, 105.2, 126.3, 126.5, 131.0, 132.2, 132.7, 134.0, 134.3, 151.4, 163.3, 172.6, 180.5; HRFABMS m/z 302.1034 ($M+1^+$) (calcd for $C_{16}H_{15}NO_5$, 302.1028).

8-Formyl-4-hydroxy-5-methoxynaphtho[2,3-b]furan (3.44). To a solution of **3.26** (1.4 g, 4.5 mmol) in dichloromethane (50 mL) was added α,α -dichloromethyl methyl ether (4.15 mL, 45 mmol) and aluminum chloride (0.65 g, 6.7 mmol) at 0°C. The mixture was stirred for 30 minutes. Then the solution was diluted with water and extracted with

ethyl acetate for 3 times. The ethyl acetate was collected and extracted with 5% aq. sodium hydroxide solution for 3 times. The combined basic solution was acidified with hydrochloric acid and extracted with ethyl acetate 3 times. The combined extracts were washed with water and dried over sodium sulfate. Evaporation and column chromatography on silica gel with chloroform followed by preparative TLC eluting with hexanes-ethyl acetate gave pure compound **3.44** as a yellow solid (275 mg, 25.3%). mp 158-161°C; ¹H NMR (CDCl₃)δ 4.17 (3H, s), 6.74 (1H, d, *J* = 8.0), 7.01 (1H, d, *J* = 2.4), 7.63 (1H, d, *J* = 2.4), 7.75 (1H, d, *J* = 8.0), 9.11 (1H, s), 9.69 (1H, s), 10.11 (1H, s); ¹³C NMR (CDCl₃)δ 56.5, 97.8, 100.4, 104.0, 109.9, 115.7, 125.8, 130.7, 140.0, 145.4, 148.5, 157.7, 162.4, 192.0; HREIMS *m/z* 242.0579 (M^{•+}) (calcd for C₁₄H₁₀O₄, 242.0579); EIMS *m/z* 242 (M^{•+}), 214, 199, 171, 115, 87.

8-Formyl-5-methoxy-4,9-dihydronaphtho[2,3-b]furan-4,9-dione (3.45). To a solution of **3.44** (90 mg, 0.37 mmol) in acetonitrile (10 ml) was added salcomine (20 mg). The mixture was stirred for 40 minutes while oxygen was bubbled through. The solution was filtered and evaporated. Column chromatography of the residue on silica gel with chloroform afforded pure compound **3.45** as a yellow solid (55 mg, 58%). mp 199°C; ¹H NMR (CDCl₃)δ 4.09(3H, s), 6.98 (1H, d, *J* = 2.0), 7.40 (1H, d, *J* = 8.8), 7.78 (1H, d, *J* = 2.0), 8.04 (1H, d, *J* = 8.8), 10.64 (1H, s); ¹³C NMR (CDCl₃)δ 56.9, 109.0, 118.1, 120.6, 131.8, 132.1, 135.1, 135.2, 149.4, 150.6, 163.3, 174.5, 179.1, 191.6; HREIMS *m/z* 256.0372 (M^{•+}) (calcd for C₁₄H₈O₅, 256.0372); EIMS *m/z* 256 (M^{•+}), 241, 149, 115, 91, 83, 69, 57.

The formation of 3.46. To a solution of **3.45** (14 mg, 0.055 mmol) in dichloromethane (3 mL) was added 2-amino-2-methyl-1,3-propanediol (115 mg, 1.1 mmol) and magnesium sulfate (100 mg). The mixture was stirred for 24 hours. The

solution was filtered and evaporated to dry. The residue was dissolved in methanol (2 mL). The resulting solution was adjusted to pH 6-7 by acetic acid and sodium cyanoborohydride (17 mg, 0.28 mmol) added. The mixture was stirred for 30 minutes. The solution was diluted with water and extracted with ethyl acetate 3 times. The ethyl acetate extracts were collected and dried over sodium sulfate. Evaporation and purification by preparative TLC eluting with chloroform-methanol afforded pure compound **3.46** as a yellow solid (9.4 mg, 50%); ^1H NMR: see Table; ^{13}C NMR: see Table; HRFABMS m/z 342.0975 ($\text{M}+1^+$) (calcd for $\text{C}_{18}\text{H}_{17}\text{NO}_6$, 342.0978); CIMS m/z 342 ($\text{M}+1^+$), 309, 254, 212, 140.

Protection of 2-amino-2-methyl-1,3-propanediol. To a solution of 2-amino-2-methyl-1,3-propanediol (4.0 g, 38 mmol) in *N,N*-dimethylformamide (25 mL) was added benzyl chloroformate (5.72 mL, 38 mmol). After the mixture was stirred for 4 hours, 2,2-dimethoxypropane (10 mL) and pyridinium *p*-toluenesulfonate (300 mg) were added to this solution, which was allowed to stir for another 20 hours. Then the solution was diluted with water and extracted with ethyl acetate 3 times. The combined extracts were washed with water and dried over sodium sulfate. Evaporation gave crude product which was crystallized with hexanes-ethyl acetate to afford pure product **3.50** as white crystals (8.0 g, 75%). mp 109°C; ^1H NMR (CDCl_3) δ 1.28 (3H, s), 1.42 (3H, s), 1.43 (3H, s), 3.66 (2H, d, $J = 12.0$), 3.90 (2H, d, $J = 12.0$), 5.08 (2H, s), 7.31-7.37 (5H, m); ^{13}C NMR (CDCl_3) δ 18.8, 19.2, 27.8, 49.3, 66.3, 67.1, 98.2, 128.0, 128.1, 128.5, 136.5, 155.3; EIMS m/z 279 (M^+), 264, 248, 221.

Hydrogenolysis of 3.50. To a solution of **3.50** (1.92 g, 6.9 mmol) in absolute methanol (30 mL) was added palladium on activated carbon (10%, 200 mg). The mixture was stirred for 2 hours under hydrogen, and the solution filtered, and evaporated under

vacuum to give pure product **3.51** as a colorless liquid (0.98 g, 98%). ^1H NMR (CD_3COCD_3) δ 0.96 (3H, s), 1.32 (3H, s), 1.34 (3H, s), 1.97 (2H, br s), 3.40 (2H, d, $J = 12.0$), 3.60 (2H, d, $J = 12.0$); ^{13}C NMR (CD_3COCD_3) δ 20.8, 20.9, 25.6, 46.6, 70.1, 97.4; CIMS m/z 146 ($\text{M}+1^+$), 130, 88, 73, 57.

The formation of 3.52. To a solution of **3.45** (10 mg, 0.039 mmol) in dichloromethane (3 mL) was added **3.51** (180 mg, 1.2 mmol) and magnesium sulfate (100 mg). The mixture was stirred for 20 hours. The solution was filtered, diluted with water and extracted with ethyl acetate. The combined extracts were washed with water and dried over sodium sulfate. After removal of the solvent by evaporation, the residue was dissolved in methanol (2 mL). The resulting solution was adjusted to pH 6-7 by acetic acid and sodium cyanoborohydride (12 mg, 0.19 mmol) added. The mixture was stirred for 30 minutes. The solution was diluted with water and extracted with ethyl acetate 3 times. The ethyl acetate extracts were collected and dried over sodium sulfate. Evaporation and purification by preparative TLC eluting with chloroform-methanol afforded pure compound **3.52** as a pink solid (6.8 mg, 46%). ^1H NMR: see Table; ^{13}C NMR: see Table; HREIMS m/z 367.1420 (M^+) (calcd for $\text{C}_{21}\text{H}_{21}\text{NO}_5$, 367.1420); EIMS m/z 367 (M^+), 255, 239, 210, 152, 71, 59.

Hydrolysis of 3.52. To a solution of **3.52** (4.5 mg, 0.012 mmol) in the mixture of methanol and water (95:5, 2 mL) was added *p*-toluenesulfonic acid (10 mg). The mixture was stirred for 20 hours at room temperature. The solution was then diluted with water and extracted with ethyl acetate 3 times. The combined extracts were washed with water and dried over sodium sulfate. Evaporation and purification by preparative TLC eluting with chloroform-methanol afforded pure compound **3.54** as a pink solid (3.2 mg, 80%). ^1H NMR (CD_3OD) δ 1.91 (3H, s), 4.17 (3H, s), 4.18 (2H, d, $J = 12.0$), 4.40 (2H, d, $J =$

12.0), 7.16 (1H, d, $J = 2.0$), 7.32 (1H, d, $J = 8.8$), 7.65 (1H, d, $J = 2.0$), 8.29 (1H, s), 8.34 (1H, d, $J = 8.8$); ^{13}C NMR (CD_3OD) δ 22.6, 55.7, 64.7, 67.0, 108.0, 108.8, 110.0, 110.8, 118.3, 119.7, 125.2, 127.9, 133.5, 140.6, 149.0, 165.2, 179.2; HRFABMS m/z 328.1200 ($\text{M}+1^+$) (calcd for $\text{C}_{18}\text{H}_{18}\text{NO}_5$, 328.1185).

8-Formyl-4-hydroxy-5,7-methoxynaphtho[2,3-b]furan (3.55). To a solution of **3.26** (1.2 g, 3.5 mmol) in dichloromethane (50 mL) was added α,α -dichloromethyl methyl ether (3.22 mL, 35 mmol) and aluminum chloride (0.5 g, 5.2 mmol) at 0°C . The mixture was stirred for 30 minutes. The solution was then diluted with water and extracted with dichloromethane for 3 times. The dichloromethane was collected and extracted with 5% aq. sodium hydroxide solution for 3 times. The combined basic solution was acidified with hydrochloric acid and extracted with ethyl acetate 3 times. The combined extracts were washed with water and dried over sodium sulfate. Evaporation and repeated column chromatography on silica gel with chloroform gave pure compound **3.55** as a yellow solid (306 mg, 32%). mp 230°C ; ^1H NMR (CDCl_3) δ 4.01 (3H, s), 4.15 (3H, s), 6.40 (1H, s), 6.94 (1H, d, $J = 2.0$), 7.56 (1H, d, $J = 2.0$), 9.23 (1H, s), 9.47 (1H, s), 10.71 (1H, s); ^{13}C NMR (CDCl_3) δ 56.3, 56.4, 89.2, 97.4, 103.7, 105.5, 110.3, 113.7, 131.6, 144.5, 148.2, 158.7, 164.5, 166.4, 189.9; HREIMS m/z 272.0685 ($\text{M}^{\bullet+}$) (calcd for $\text{C}_{15}\text{H}_{12}\text{O}_5$, 272.0685).

8-Formyl-5-methoxy-4,9-dihydronaphtho[2,3-b]furan-4,9-dione (3.56). To a solution of **3.55** (90 mg, 0.33 mmol) in dichloromethane (10 mL) was added salcomine (20 mg). The mixture was stirred for 40 minutes while oxygen was bubbled through. The solution was filtered and evaporated. Column chromatography of the residue on silica gel with chloroform afforded pure compound **3.56** as a yellow solid (51 mg, 54%). mp 235°C ; ^1H NMR (CDCl_3) δ 3.95(3H, s), 4.07 (3H, s), 6.75 (1H, s), 6.96 (1H, d, $J = 2.0$),

7.75 (1H, d, $J = 2.0$), 10.32 (1H, s); ^{13}C NMR (CDCl_3) δ 56.4, 56.7, 100.1, 109.2, 113.8, 124.2, 132.7, 135.4, 149.5, 150.2, 161.7, 163.9, 173.7, 179.2, 191.3; HRCIMS m/z 287.0541 ($\text{M}+1^+$) (calcd for $\text{C}_{15}\text{H}_{11}\text{O}_6$, 287.0556).

The formation of 3.57. To a solution of **3.56** (8 mg, 0.028 mmol) in dichloromethane (3 mL) was added **3.51** (120 mg, 0.83 mmol) and magnesium sulfate (100 mg). The mixture was stirred for 20 hours. The solution was filtered, diluted with water and extracted with ethyl acetate. The combined extracts were washed with water and dried over sodium sulfate. After removal of the solvent by evaporation, the residue was dissolved in methanol (2 mL). The resulting solution was adjusted to pH 6-7 by acetic acid and sodium cyanoborohydride (9 mg, 0.14 mmol) added. The mixture was stirred for 30 minutes. The solution was diluted with water and extracted with ethyl acetate 3 times. The ethyl acetate extracts were collected and dried over sodium sulfate. Evaporation and purification by preparative TLC eluting with chloroform-methanol afforded pure compound **3.57** (6.3 mg, 57%) as a yellow solid. ^1H NMR (CD_3OD) δ 1.26 (3H, s), 1.58 (3H, s), 1.70 (3H, s), 4.19 (3H, s), 4.20 (3H, s), 4.38 (2H, d, $J = 12.8$), 4.64 (2H, d, $J = 12.8$), 6.66 (1H, s), 7.19 (1H, d, $J = 2.0$), 7.65 (1H, d, $J = 2.0$), 8.37 (1H, s); ^{13}C NMR (CD_3OD) δ 19.1, 21.3, 28.9, 57.2, 57.3, 58.8, 68.1, 92.5, 99.9, 107.6, 109.6, 110.4, 111.1, 122.0, 126.3, 127.2, 142.4, 149.6, 166.1, 171.0, 176.0; EIMS m/z 397 (M^+), 269, 240.

Hydrolysis of 3.57. To a solution of **3.57** (4.0 mg, 0.010 mmol) in a mixture of methanol and water (95:5, 2 mL) was added *p*-toluenesulfonic acid (10 mg). The mixture was stirred for 20 hours at room temperature. Then the solution was diluted with water and extracted with ethyl acetate 3 times. The combined extracts were washed with water and dried over sodium sulfate. Evaporation and purification by preparative TLC eluting with chloroform-methanol gave pure compound **3.58** as a yellow solid (2.5 mg, 70%). ^1H

NMR (DMSO) δ 1.72 (3H, s), 3.95 (2H, d, $J = 11.2$), 4.06 (3H, s), 4.12 (3H, s), 4.13 (2H, d, $J = 11.2$), 6.60 (1H, s), 7.13 (1H, d, $J = 2.0$), 7.63 (1H, d, $J = 2.0$), 8.18 (1H, s); ^{13}C NMR (DMSO) δ 19.8, 56.5, 56.6, 64.1, 66.4, 91.5, 106.3, 108.6, 108.8, 109.4, 119.9, 121.8, 125.8, 140.9, 147.7, 161.1, 166.0, 173.8; HRFABMS m/z 358.1297 ($\text{M}+1^+$) (calcd for $\text{C}_{19}\text{H}_{20}\text{NO}_6$, 358.1291).

IV. CONCLUSIONS

In the preceding chapters two approaches to the search for new antitumor agents have been described.

One approach, the isolation of bioactive compounds from plant material, was discussed in chapter 2. Directed by mechanism-based yeast bioassay a sesquiterpenoid was isolated which showed selective DNA damaging activity and cytotoxicity.

Another approach, the synthesis of potential antitumor compounds, using known cytotoxic natural products as model compounds, was covered in chapter 3. A series of furanonaphthoquinone derivatives with hydroxyamino side chain had been synthesized, which showed cytotoxicity against H4IIE rat hepatoma cell. The bioassay data also showed that these compounds act by a different mechanism of action than their parent furanonaphthoquinone derivatives.

IV. APPENDIX

^1H and ^{13}C NMR Spectra of Selected Compounds