Discovery and Development of Anthracycline Antitumour Antibiotics

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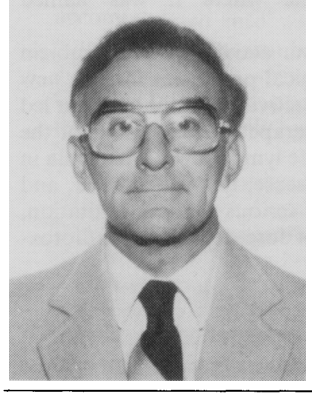
1 Introduction

The clinical treatment of neoplastic diseases relies on the complementary procedures of surgery, radiation treatment, immunotherapy, and chemotherapy. The latter technique has matured from its earliest applications of mustard alkylating agents in the 1940s to a vigorous and increasingly rationally-based discipline which is contributing significantly to the management of human malignancies.[1] Initially, as a result of the introduction of activity-based screens, the main contribution of the organic chemist was in isolation and structure elucidation. As the field of chemotherapy matured and several promising natural anticancer agents were identified[1] the interests of organic chemists turned towards total synthesis of such natural products.

However, a more urgent need soon arose from the common experience of clinically limiting toxicities of most anticancer drugs, *i.e.* the necessity to develop less toxic clinical drug candidates.[1] Thus the role of the synthetic organic chemist or medicinal chemist turned towards analogue development during this, still largely empirical, phase of chemotherapy. Hand-in-hand with this considerable synthetic effort, which uncovered several promising clinical leads, biochemical pharmacology, or study of the mechanisms of action of clinical anticancer agents,[1] afforded deeper insight into drug metabolism and mode of action. More recently, therefore, the field of synthetic organic chemistry, which has become increasingly sophisticated in the interim and has been complemented by the methods of microbial chemistry, has been faced with new synthetic challenges, occasioned by the identification of hitherto unrecognized cellular targets for anticancer drugs, such as topoisomerases, and helicases.

The armamentarium of the oncologist currently includes about 40—50 clinically useful chemical agents.[1] The paradigm of cytotoxic anticancer agents is doxorubicin, an anthracycline, which is still amongst the most widely prescribed and effective of anticancer agents.[1b] The present review attempts to summarize the discovery and isolation of anthracyclines, the elucidation of their structure, stereochemistry, and absolute configuration, and synthetic efforts towards improvement of therapeutic efficacy.

J. William Lown was born in Blyth, Northumbria and received the Ph.D. and D.I.C. degrees in organic chemistry at Imperial College in 1959. Following postdoctoral work with Professor R. U. Lemieux at the University of Alberta, Canada, he joined the faculty of that institution where, since 1974, he has been Professor of Organic Chemistry. Professor Lown was Alberta Heritage Foundation for Medical Research Professor-in-Residence from 1984—85 and was appointed McCalla Research Professor in 1988. Dr. Lown has published over 280 refereed research articles mainly in the field of cancer chemotherapy. He was awarded the Paul Ehrlich Prize for 1992 by the Association de Recherches Scientifique Paul Neumann for his work in chemotherapy and was elected Fellow of the International Union Against Cancer in 1992. Professor Lown serves as Associate Editor of Cancer Research *and is on the editorial boards of* Anti-Cancer Drug Research, Free Radical Biology and Medicine, J. Cellular Pharmacology, *and* Heterocyclic Communications.



2 Discovery and Isolation of the First Anthracyclines

2.1 Structure Determination and Conventions

The first anthracycline, whose structure was elucidated, β-rhodomycin II (1), was isolated from *Streptomyces purpurascens* by Brockmann and Bauer in 1950.[2] Subsequently, in the late 1950s, a red compound isolated from *Streptomyces* sp. from a soil sample collected in India was shown by Arcamone and his colleagues at Farmitalia to be rhodomycin B (2), a component of the rhodomycin complex studied earlier by the German workers (Figure 1). Although the earliest discovered anthracyclines displayed potent antibacterial activity in culture, their high toxicity in mice precluded their further development as antitumour agents. Two principal classes of microbial products were investigated by Brockmann and co-workers.[2] The first group which included the rhodomycinones, the isorhodomycinones, the rhodomycins, and the isorhodomycins, was isolated from *Streptomyces purpurascens*, while the second group, together with the pyrromycinones and the glycoside pyrromycin, were isolated from a related strain. The corresponding deoxyaglycones (subsequently shown to be important metabolites of anthracyclines) were also isolated and characterized.

These early studies served to identify some of the structural features and different substitution patterns characteristic of the aglycones of many anthracyclines subsequently identified.

Another important structural feature established by the early workers was the stereochemistry of the sugar moieties rhodosamine (3a), 2-deoxy-L-fucose (3b), and rhodinose (3d) contained in the first group of anthracyclines investigated. A significant observation was that the isolation of 7-deoxy compounds such as ζ-rhodomycinone, ζ-pyrromycinone, and ζ-isorhodomycinone indicated that reductive elimination of the C-7 oxygen could take place during anthracycline biosynthesis.[2] This subsequently proved to be an important metabolic pathway of the anthracyclines.

2.2 Biogenetic Considerations

The biosynthesis of two different anthracyclinones, ε-pyrromycinone and daunomycinone, were found to start with a propionate unit.[3] Acetate units are added to complete the polyketide intermediate, which is later transformed and developed into the anthracyclines.[3] This hypothesis, subsequently confirmed by ^{14}C labelling experiments, was used to account for the ethyl substituent in ε-pyrromycinone. Such biogenetic schemes including the use of ^{13}C labelling could then be used to rationalize the formation of a number of anthracyclines, including aklavinone, ε-rhodomycinone, ε-pyrromycinone, and daunorubicin, and to anticipate the formation of other structures.

The biosynthesis of anthracycline antibiotics has been studied genetically using various blocked mutants of anthracycline-producing *Streptomyces* and by ^{13}C NMR analysis.[3] The results of these studies show that there are two biosynthetic pathways in the formation of the polyketide: daunomycinone, pyrromycinone, rhodomycinones, and aklavinone are derived from nine

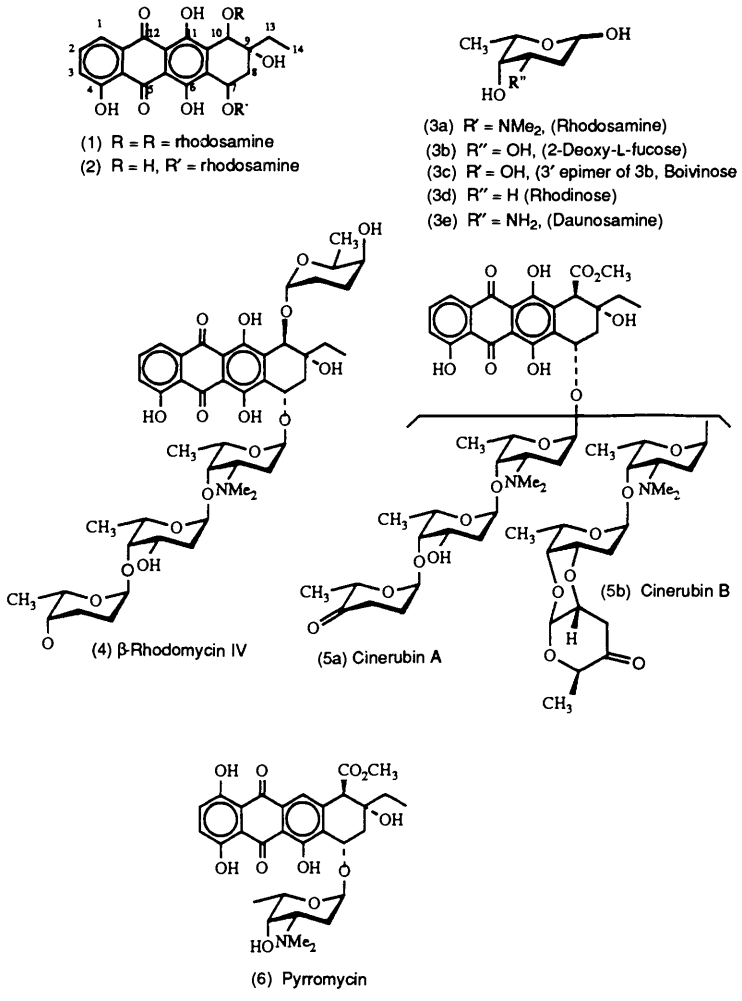


Figure 1 Representative anthracycline structures and numbering system in the chromophore.

acetate units and one unit of propionate; steffimycinone and nogalarol are built from ten acetate units.

Several anthraquinones such as aklanonic acid have been isolated from *Streptomyces* sp. 21MET43717 and blocked mutants of *S. galibeus* MA144-M1 as intermediates at an early stage in the biosynthesis of anthracyclines. Treatment of *S. griseus* with MNNG (methyl-*N'*-nitro-*N*-nitroguanidine) provided the blocked mutants OIP7 and IP3 which are incapable of producing daunorubicin. These mutants incorporate the total radioactivity of (*U*-^{14}C) alkanonic acid into ε-rhodomycinone, daunomycinone, 7-deoxy-ε-rhodomycinone, and IP/II (γ-hydroxy-bisanhydro-ε-rhodomycinone). The closure of the decaketide chain derived from acetate and/or propionate units subsequently forms aklavinone *via* a series of polyketide intermediates; 1,8-dihydroxyanthraquinones, aklavinones, and aklanonic acid. Aklavinone is then converted into rhodomycinones and pyrromycinones by oxidation, and glycosidation leads to the corresponding biologically active glycosides (Scheme 1).

In the biosynthetic pathway to daunorubicin and doxorubicin it is probable that daunomycinone (15) is synthesized from aklavinone (12) *via* ε-rhodomycinone (13) because all daunorubicin products of *Streptomyces* can produce ε-rhodomycinone concomitantly in the culture broth, and their blocked mutants capable of accumulating ε-rhodomycinone and its glycosides or of accumulating aklavinone and 7-deoxyaklavinone have been isolated.[3] In fact it has been confirmed that daunorubicin can be produced by a daunorubicin non-producing mutant of *S. coeruleorubidus* in the fermentation medium to which either ε-rhodomycinone or aklavinone was added.

In this manner the biosynthetic sequence of anthracyclines was deduced from microbial modification and glycosidation of various anthracyclinones using a variety of blocked mutants derived from *S. galilaeus*, *S. coeruleorubidus*, *S. purpurascens*, and *S. peucetius*.

3 Daunorubicin

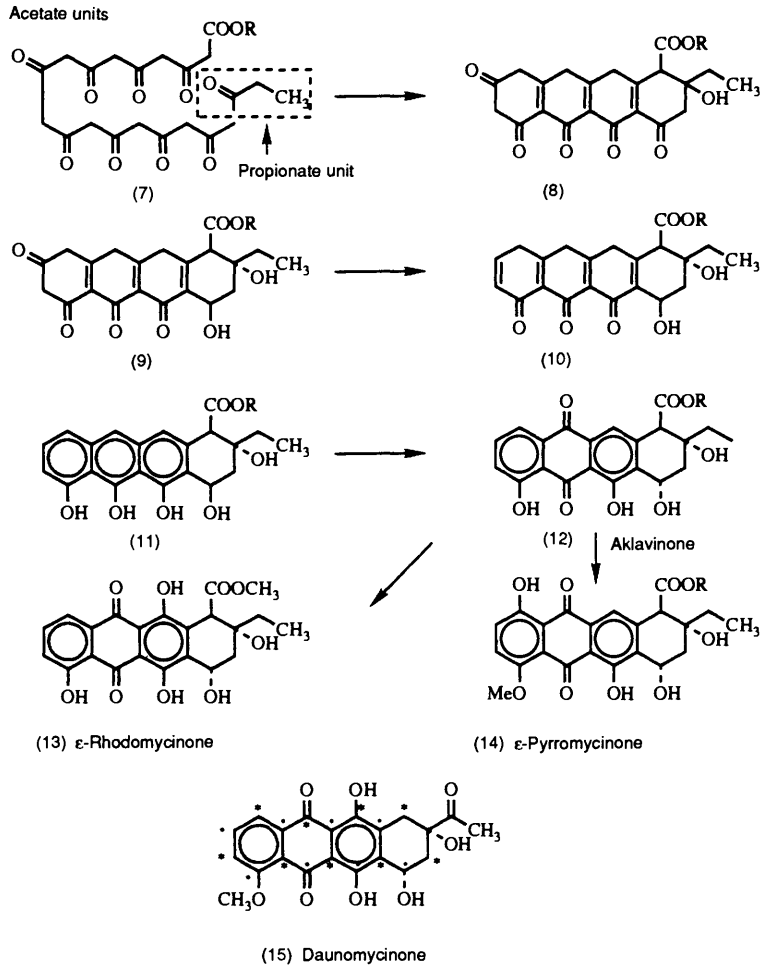
3.1 Isolation, Degradative Studies, and Properties

An important advance in anthracyclines was the discovery and isolation of daunorubicin in the early 1960s, since it was the first antibiotic of this class to show activity against acute leukaemia in man. The antibiotic was discovered independently in the laboratories of Farmitalia, where it was named daunomycin[4a] and, in those of Rhône-Poulenc where it was named rubidomycin.[4b]

The first report on the antitumour activity of daunorubicin indicated exceptional pharmacological properties for this new antibiotic. The marked anticancer activity of daunorubicin led to clinical trials where the main therapeutic indications of the drug are leukaemias, especially acute lymphocytic leukaemia in children but also in Hodgkins's disease, lymphosarcoma, and reticular cell sarcoma. The most serious clinical limitation, which was recognized early, is that of dose-dependent cardiotoxicity (*vide infra*).[1b]

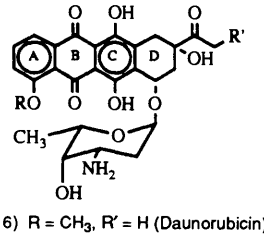
3.2 Stereochemistry and Absolute Configuration

The positions of the substituents and the stereochemistry of the aglycone were then established by a series of chemical degrada-

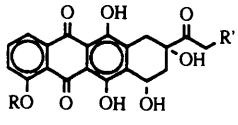


Scheme 1 Hypothetical biosynthetic pathway of anthracyclines and distribution of ^{13}C enrichment when [1-^{13}C] acetate (•) or [2-^{13}C] acetate (*) were fed to cultures of *S. peucetius*.

tions. The aminosugar component daunosamine (3e) proved to be 3-amino-2,3,6-trideoxy-L-*lyxo*-hexopyranose, a compound not previously found from natural sources. The absolute configuration was determined by comparison of the molar rotations of (3e) with those of known 2,6-dideoxyhexapyranoses and their methyl glycosides, and the result was confirmed by ^{1}HNMR spectroscopy. Thus daunosamine has structure (3e). Thus the structure of daunorubicin is represented by (16) and was subsequently confirmed by *X*-ray crystallographic analysis of the *N*-bromoacetyl derivative of daunorubicin.



(16) R = CH_3, R′ = H (Daunorubicin)
(17) R = CH_3, R′ = OH (Doxorubicin)
(18) R = R′ = H (Carminomycin)



(15) R = CH_3, R′ = H (Daunomycinone)
(19) R = CH_3, R′ = OH (Adriamycinone)
(20) R′ = R′ = H (Carminomycinone)

4 Doxorubicin (Adriamycin)

4.1 Isolation and Anticancer Activity

Doxorubicin (17) was isolated from the cultures of one of the varieties derived from *Streptomyces peucetius* strain, *i.e.*, *S. Peucetius* var. *caesius*. This agent generated immediate excitement because of its outstanding antitumour properties which were established by Di Marco *et al.*[6]

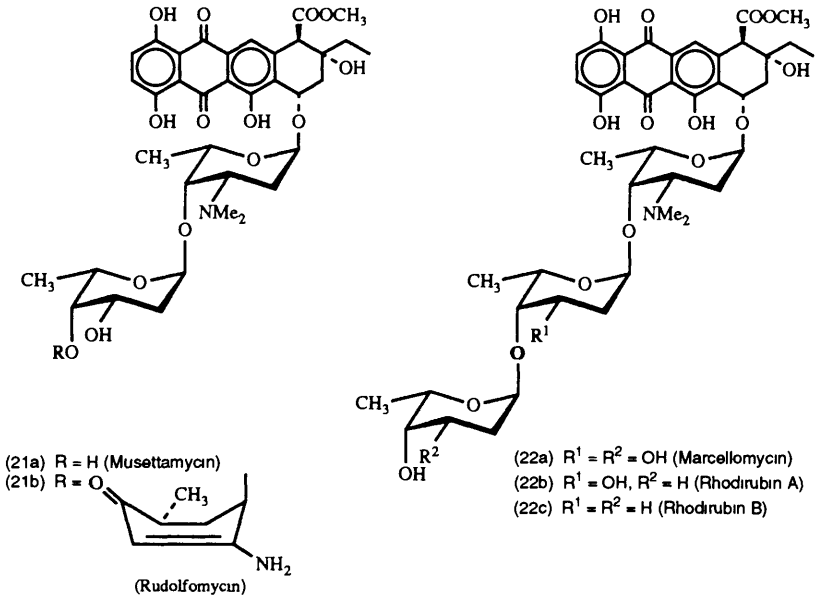
Doxorubicin exhibited the same kind of marked inhibitory effect on tumour growth as daunorubicin but was generally more potent.[6] Clinical trials confirmed the early promise of this valuable agent. The principal clinical limitation however remains, as in the case of daunorubicin, the risk of cardiotoxicity that ranges from a delayed and insidious cardiomyopathy to irreversible heart failure (*vide infra*).[1b]

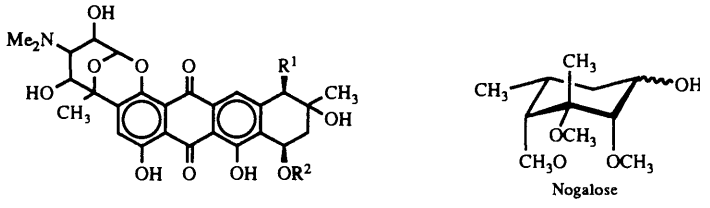
5 Classification of Anthracyclines and Nomenclature

5.1 Classification on the Basis of Structure

The β-rhodomycins I, II, III, and IV and the related pyrromycins have been mentioned in connection with the pioneering structural elucidation in Section 2.[2] The cinerubins A and B are related structurally and were isolated from strains of *Streptomyces*. Workers at the Bristol-Myers Company reported the isolation of new anthracyclines from the bohemic acid complex including marcellomycin (22a), musettamycin (21a), rudolfomycin (21b), and the rhodirubins A and B (22b and c), which were shown by chemical transformations to be related to the pyrromycins.[7a]

In 1975 investigations at the Sanraku-Ocean Co. and the Institute of Microbial Chemistry in Tokyo led to the isolation and identification of the anthracyclines aclacinomycins A and B.[7b] These agents bear the same trisaccharides as cinerubins A and B respectively (5a, 5b), but the aglycone was novel and it is designated aklavinone (12). A related antibiotic was found to correspond to the aclacinomycins but lacking the two terminal





(23) $R^1 = CO_2CH_3$, R^2 = Nogalose (Nogalamycin)
(24) $R^1 = CO_2CH_3$, $R^2 = CH_3$ (7-*O*-Methylepinogalorol)
(25) R^1 = H, R^2 = Nogalose (Nogalamycin))
(26) R^1 = H, $R^2 = CH_3$ (7-*O*-Methylepinogalorol)

sugar residues and therefore is equivalent to a 1-deoxypyrromycin structure

Wiley and his co-workers at the Upjohn company isolated a group of anthracyclines [(23)—(26)] which differed substantially in the chromophore moiety [8] The nogalamycins attracted the attention of synthetic chemists both because of their novel structural features and because certain examples exhibited superior antitumour activity The lead compound nogalamycin (23) was chemically modified to prepare the 'noga' and 'nogala' series of analogues Each of these compounds exists as two possible stereoisomers, namely the *con* and *dis* configurations with respect to C-7, which are not interconvertible The structure (7*R*)-*O*-methylnogarol (menogaril) (26) shows superior anticancer activity and entered Phase I/II clinical trials

The structures of additional representative compounds belonging to other groups of anthracyclines are also given (Figure 2) and include baumycin A1 and A2 (27a and b), steffimycins A and B (28a and b), isoquinocycline A (29), elloramycin (30), and barminomycin I (31)

5.2 Classification on the Basis of Mechanism of Action

It soon became evident that one of the significant modes of action of many anthracyclines was connected with their ability to bind intercalatively to double helical DNA (Figures 3 and 4) An obvious further classification followed into those anthracyclines (including daunorubicin, doxorubicin, and carminomycin) which bind to DNA and those agents which do not (principally semi-synthetic derivatives such as the AD series, including AD32 and AD41) The discovery of inhibition of topoisomerase II action by certain non-intercalative anthracyclines,[9] which has important implications in both mode of action and drug resistance, emphasizes the reality of this classification and presents new synthetic challenges (*vide infra*)

More extensive and detailed examination of the biochemical pharmacology of anthracyclines revealed additional mechanistically related classes based on their ability to inhibit nucleolar RNA synthesis Two classes of anthracyclines are defined, based upon their selectivities for the inhibition of nucleolar precursor ribosomal RNA (NO-RNA) synthesis Class I anthracyclines are nucleolar non-selective, inhibiting both DNA and NO-RNA synthesis at approximately equivalent concentrations Class II anthracyclines are nucleolar selective, inhibiting NO-RNA synthesis at concentrations 200—1300-fold lower than those required to inhibit DNA synthesis

These two classes of agents may be further subdivided When comparing the affinity constants for binding to DNA, the Class I anthracyclines can be subdivided into carminomycin (low K_{app}, apparent binding constants) *versus* pyrromycin and doxorubicin (intermediate and high K_{app} respectively) In addition, the Class II anthracyclines marcellomycin, rudolfomycin, musettamycin, and aclacinomycin are distinct from 10-decarbomethoxy marcellomycin, 10-descarbomethoxy rudolfomycin, collinemycin, and mimimycin, based on DNA binding characteristics and biological activities

6 Chemical Reaction and Transformations of Anthracyclines

6.1 Reactions within the Aglycone

Acylation of the alcoholic and phenolic hydroxyl groups occurs readily, except for the tertiary hydroxyl at position 9 The phenolic hydroxyl groups are methylated by dimethyl sulfate or methyl iodide and mild base such as potassium carbonate In contrast, methylation of the aliphatic hydroxyl group at position 7 requires more vigorous conditions Demethylation of anthracycline *O*-methyl ethers may be effected with either a

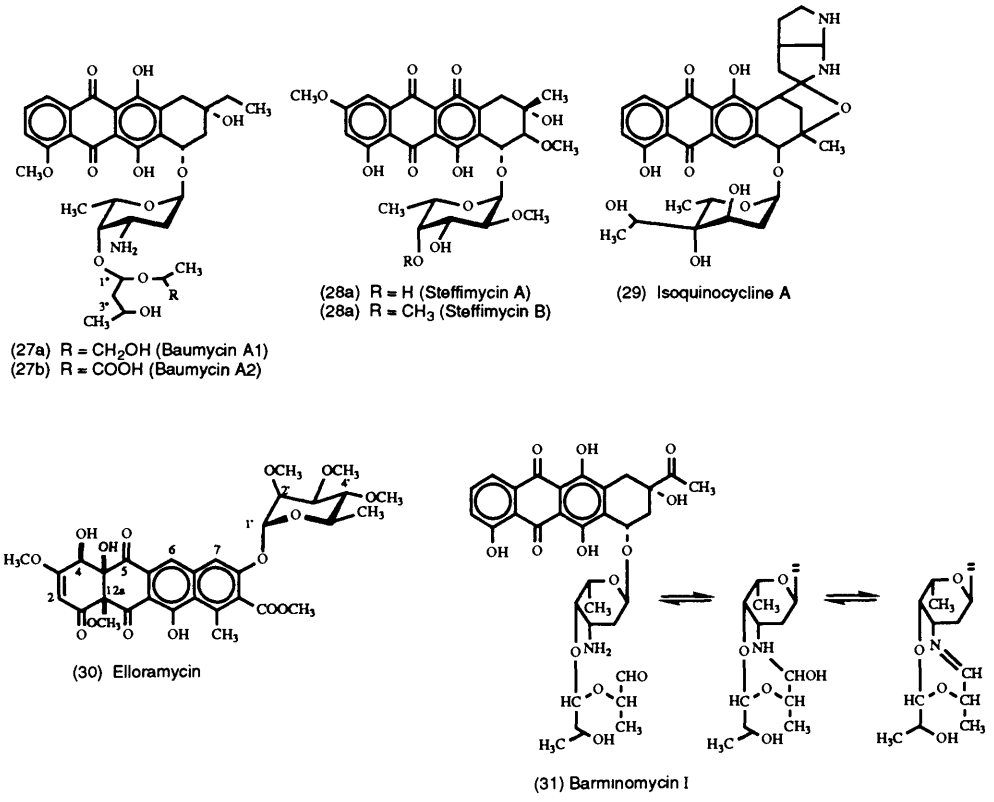


Figure 2 Representative alternative anthracycline structures.

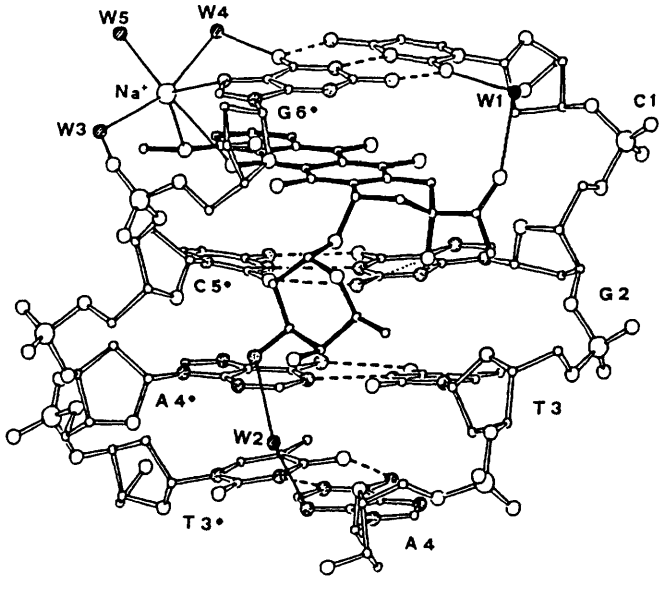


Figure 3 Daunorubicin-d(CpGpTpApCpG) complex showing intermolecular interactions.

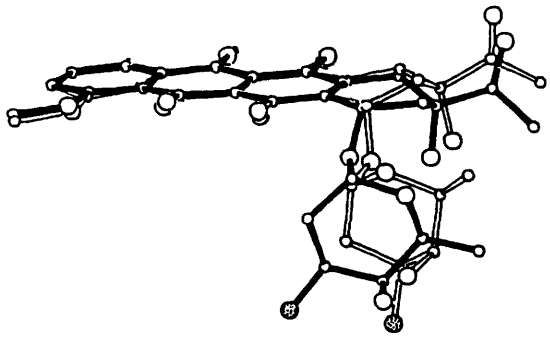


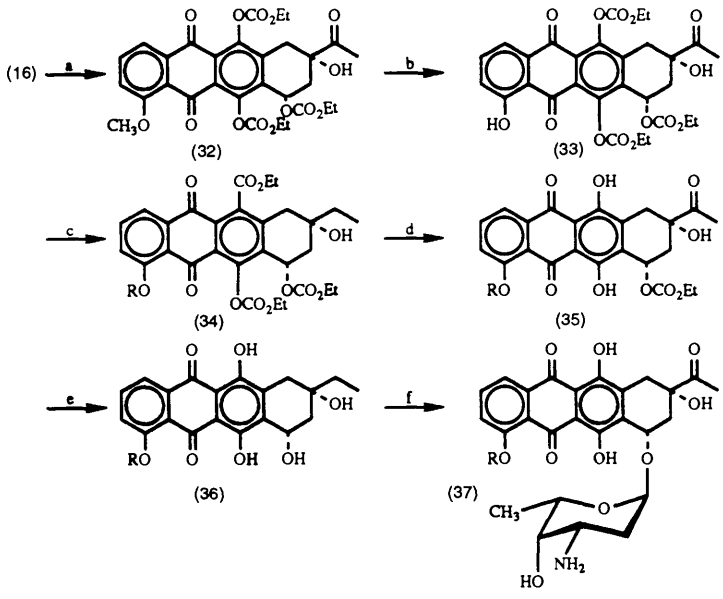
Figure 4 The conformation of daunorubicin in the complex with d(CpGpTpApCpG) (filled bonds) compared with the conformation of daunorubicin crystallized by itself (open bonds).

Lewis acid such as aluminium chloride in benzene, or under milder conditions with sodium thiocresolate.[4a]

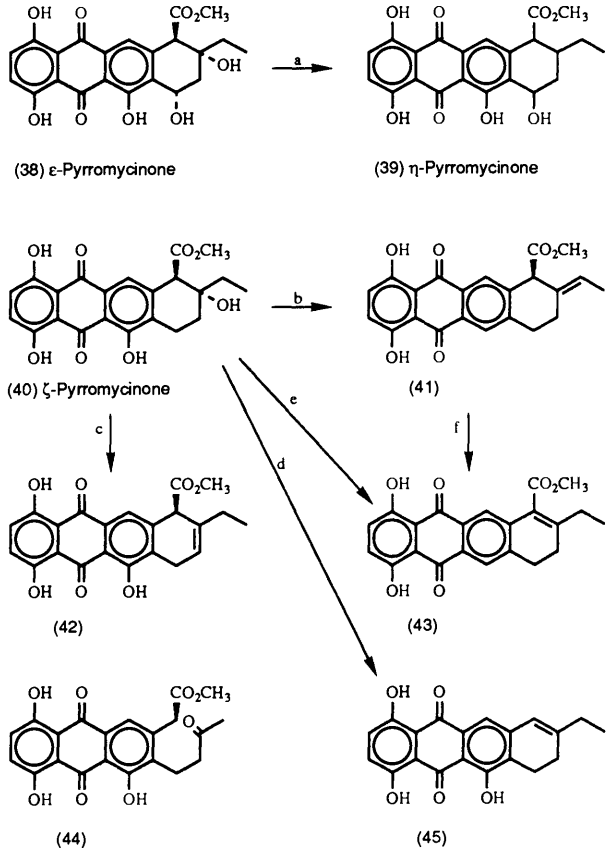
Side-chain bromination at the 14 carbon of daunomycinone occurs readily and the bromine may be readily displaced with the sodium or potassium salts of carboxylic acids[10] to give, for example, the valuable 14-octanoate series; or 14-ethers may be obtained by solvolysis in the presence of sodium triflate. A useful intermediate (Scheme 2) derived from the aglycone is the selectively protected triethoxycarbonyl derivative prepared with ethoxycarbonyl chloride and pyridine to give, for example, compound (32). This could be selectively dealkylated with aluminium bromide to (33). Ethoxylation, followed by treatment with morpholine, permitted selective removal of the phenolic ethoxycarbonyl protection. Removal of the remaining ethoxycarbonyl group at the 7-hydroxyl position may be effected with sodium hydroxide, and glycosidation affords (37).

Dehydration of the aliphatic ring of the chromophore occurs relatively readily under acidic conditions because the hydroxyl groups present are either tertiary or benzylic. Thus bis-hydration of 7,9-diols results in aromatization of ring D. The 7-deoxy aglycones (40) cannot aromatize in ring D by dehydration, and instead one or other olefinic product is formed depending on the dehydrating agent used. Heating of (40) at 230 °C gives (43) together with the ring-opened compound (44), (Scheme 3).

Side-chain oxidative degradation of the aglycone in 13-dihydro-*N*-trifluoroacetyl daunorubicin (46) may be effected by sodium metaperiodate, (Scheme 4) and affords the ketone (47) which, after reduction to the alcohol, may be used for the preparation of alternative derivatives. The 13-carbonyl group of both daunorubicin and doxorubicin is reduced to the alcohol by potassium borohydride. Conversion of the acetyl side chain of daunorubicin into the corresponding 9-ethyl group may be effected by reduction of the 13-thioketal with Raney nickel.



Scheme 2 Reaction conditions: (a), EtOCOCl, pyridine; (b), $AlBr_3$; (c), RI, Ag_2O; (d), morpholine; (e), OH^-; (f), daunosaminyl chloride, CF_3SO_3Ag, MeOH, OH^-.

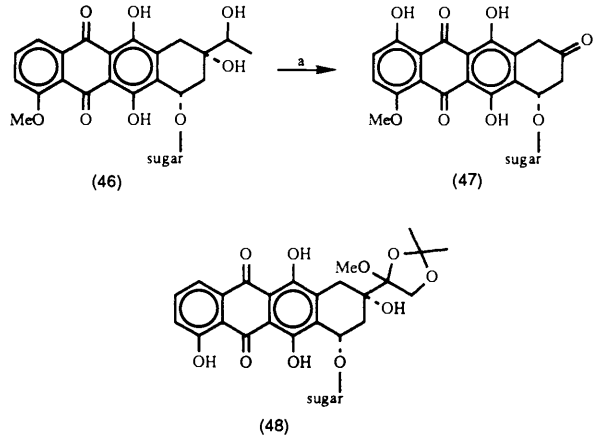


Scheme 3 Reaction conditions: (a), *p*-TSA, or HCl; (b), p-TSA; (c), P_2O_5; (d), HBr; (e) 230 °C; (f) pyridine, Et_3N.

However the 7-hydroxyl group is also subject to some hydrogenolysis under these conditions. Alternatively sodium cyanoborohydride reduction of the 13-*p*-toluenesufonylhydrazone of daunomycinone affords the 7,13-dideoxy derivative.

6.2 Reactions within the Aminosugar Moiety

Methylation of the amino group in the daunosamine moiety of anthracyclines gives either the *N,N*-dimethyl derivative or the quaternary ammonium salt, depending on the reaction conditions. Selective methylation of the 6- and 11-phenolic hydroxyl group of daunorubicin may be effected after protection of the amino group of the sugar moiety as its trifluoroacetyl derivative. Similarly *N,N*-dibenzyl derivatives of daunorubicin and doxorubicin proved to be valuable, both in terms of their antitumour activity and in metabolic studies.[11]

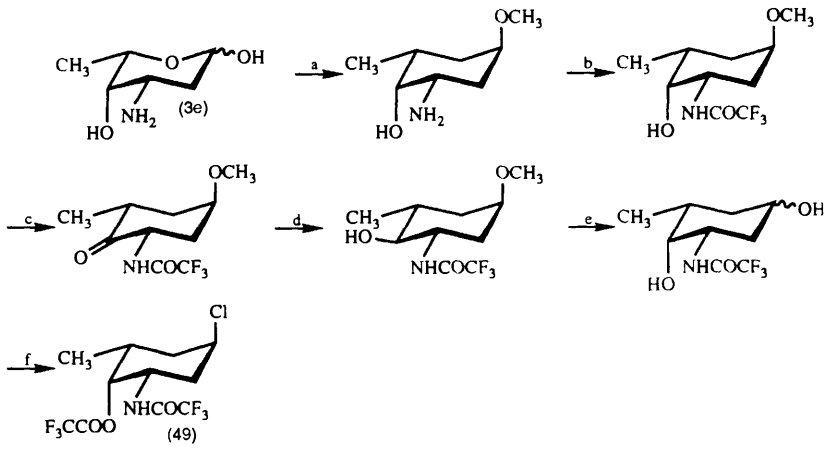


Scheme 4 Reaction conditions: (a), $NaIO_4$.

Acylation of the amino group in the daunosamine moiety is readily accomplished. The *N*-trifluoroacetyl derivates (AD series) are especially noteworthy because of their expression of antitumour activity in spite of lacking the ability to bind intercalatively to DNA.[12] Representative chemical transformations of daunosamine (3e) leading to an acosamine derivative (49) are shown in Scheme 5. Additional amino sugar derivatives are discussed in Section 8.

6.3 Reactions of the Glycosides

Once the clinical potential of doxorubicin was recognized, a chemical transformation of intact glycosides that became of immediate interest was the interconversion of daunorubicin into doxorubicin. This could be effected (Scheme 6) by trifluoroacetylation of daunorubicin, followed by *O*-acyl exchange, to the *N*-trifluoroacetyl derivative, which on photohalogenation gave (51).[13] Replacement of the iodo substitute with acetate gave (52), and the latter was converted into *N*-trifluoroacetyl doxorubicin (53) upon treatment with a weak base. Hydrolysis of (54) followed by acid treatment afforded doxorubicin (17). Subse-



Scheme 5 Representative chemical transformations of daunosamine leading to *N,O*-ditrifluoroacetyl-α-acosaminyl chloride. Reaction conditions: (a), MeOH/H^+; (b), 1, $(CF_3CO)_2O$; 2, MeOH; (c) RuO_2/KIO_4; (d), $NaBH_4$; (e) $AcOH/H_2O$; (f), $(CF_3CO)_2O$, HCl/Et_2O.

quently a more direct conversion of daunorubicin into doxorubicin was developed by electrophilic bromination of (50) at C-14 followed by replacement of the bromine by hydroxyl with mild base treatment.[13]

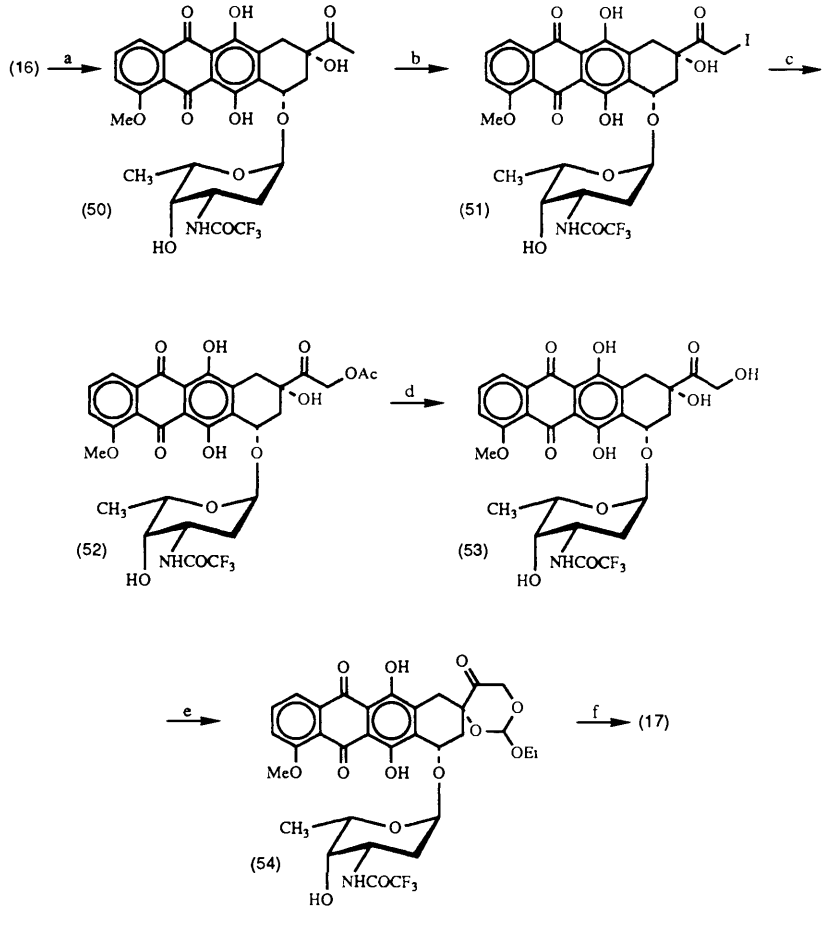
The reverse transformation, of doxorubicin into daunorubicin, was used for the synthesis of [14-^{14}C]daunorubicin by Arcamone and co-workers.[13] [14-^{14}C]Daunorubicin was converted into [14-^{14}C] doxorubicin *via* the 14-bromo derivative as described previously.[13]

Scheme 6 Reaction conditions: (a) $(CF_3CO)_2O$, MeOH; (b), I_2, CaO, THF; (c), NaOAc; (d), Na_2CO_3; (e), OH^-; (f) H^+.

6.4 Metabolism

The two main and characteristic routes of metabolic transformation of the anthracyclines in animals and in man are the reduction of the side chain carbonyl group to a secondary alcohol and reductive deglycosidation with formation of 7-deoxyaglycones.[14] The enzyme catalysing the first reaction is a cytoplasmic aldoketo reductase, native to all tissues (although it has been referred to as daunomycin reductase). It is also capable of reducing daunomycinone, and other anthracycline aglycones, to the 13-dihydro compound (46).[14] These conversions are stimulated by NADPH and do not require oxygen.

The reductive scission of the glycosidic bond is catalysed by a reductive hydrolase, the action of which appears to be rare since there are no other examples of enzyme catalysis of this otherwise chemically facile reaction. Rat liver microsomal preparations were found to convert both daunorubicin and daunorubicinol into their aglycones.[14] The scission of the glycosides by both



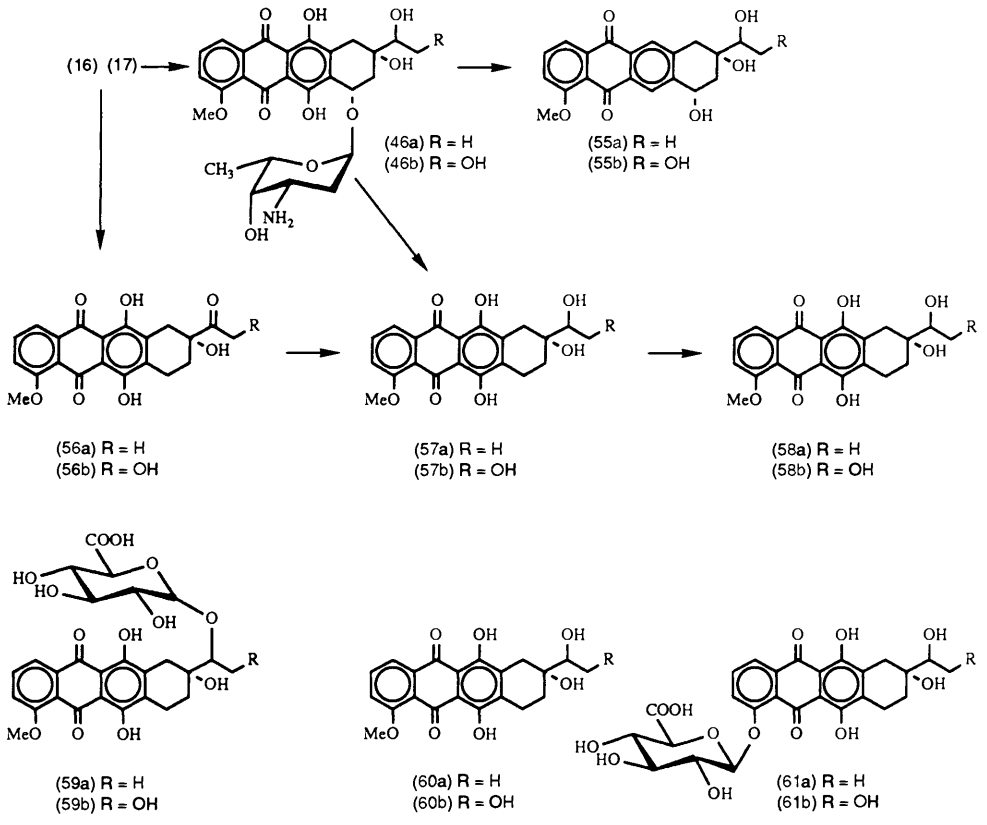


Figure 5 Principal metabolites of the anthracyclines

routes is of immediate pharmacological and clinical concern because, while the aglycone and its 7-deoxy counterpart do not appear to contribute to the cytotoxic/anticancer activity, they appear to contribute to general side-effect toxicities Although the liver was the most effective organ in glycoside cleavage reactions, lung, kidney, brain, skeletal muscle, and heart also showed significant activities [14]

The principal metabolites of the anthracyclines are also subject to conjugation [14] The following aglycone-derived metabolites have been identified from the urine of patients following treatment with daunorubicin (Figure 5) 13-dihydrodaunomycinine, 7-deoxydaunomycinone, 7-deoxy-13-dihydrodaunomycinine (55a), 4-methyl-7-deoxy-13-dihydrodaunomycinone (58a), 4-demethyl-7-deoxy-13-dihydrodaunomycinone-4-*O*-sulfate (60a), 4-demethyl-7-deoxy-13-dihydrodaunomycinone-4-*O*-β-D-glucuronide (61a), and 7-deoxy-13-dihydrodaunomycinone-13-*O*-β-D-glucuronide (59a) [14]

7 Overview of Anthracycline Development

7.1 Synthetic Strategy

The recognition of the anticancer activity of daunorubicin and particularly the clinical potential of doxorubicin began to attract the attention of synthetic organic chemists in the early 1970s The pioneering work in this field is due to Wong and co-workers who reported the synthesis of the racemic aglycone derivative 4-demethoxy-7-*O*-methyldaunomycinone [15] The synthetic strategy was essentially A + CD → ABCD Subsequently Wong and co-workers reported the total synthesis of racemic daunomycinone following essentially the same strategy Some of the synthetic approaches were adopted and adapted in the Farmitalia laboratories where, for example, resolution of a key racemic substituted tetralin intermediate opened a route to a number of different daunomycinone analogues obtained in optically pure form Following this method both enantiomeric forms of 4-demethoxydaunomycinone were prepared

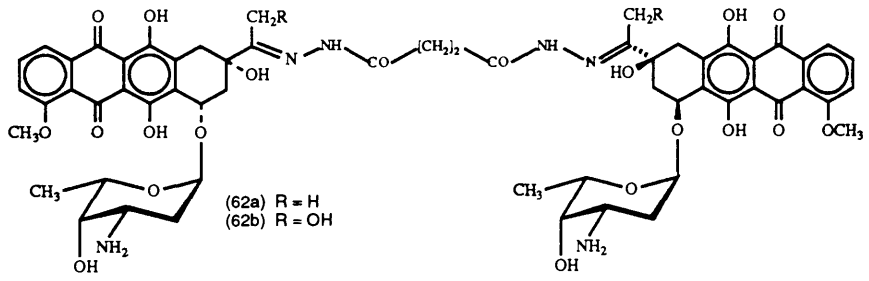
Major synthetic questions that were subsequently addressed, in an effort which is international in scope, included regioselectivity in the construction of the chromophore, stereoselectivity at positions 7 and 9, stereocontrolled synthesis of L-daunosamine, and efficient glycosidic coupling procedures

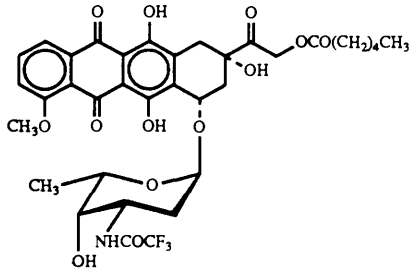
7.2 Microbial Transformations

Another valuable and complementary route to new and potentially useful anthracycline structures is by the action of microbial cultures, and particularly by the use of blocked mutants [3] Since 1981 about 20 new analogues of daunorubicin, doxorubicin, and carminomycin have been reported [3] The baumycin-producing *Streptomyces* D788,[16] which is different from the well-known baumycin-producing strains of *S coeruleorubidus* and *S peucetius* is transformed by treatment with MNNG to a mutant which lacks the ability to form 4′-*O*-substitution products Fermentation using this mutant, which cannot produce baumycins, allows the accumulation of daunorubicin and concomitantly produces a new water-soluble anthracycline D788-1, identified as 10-carboxyl-13-deoxocarminomycin [16] Further mutation of strain GI-1 with MNNG provided a doubly blocked mutant strain RPM-5 which produces an intensely potent minor component called oxaunomycin,[16] in addition to 13-deoxocarminomycin, 13-dihydrocarminomycin, and 10-decarboxy-13-deoxycarminomycin The structure of oxaunomycin is 7-*O*-(α-L-daunosaminyl)-β-rhodomycinone

Two strains of *Actinomadura roseoviolacea* produce the new anthracyclines *N*-formyl-13-dihydrocarminomycin[16] and akrobomycin (9,10-anhydro-13-deoxocarminomycin)[16] respectively A blocked mutant MnW1 of the carminomycin-producer *Actinomadura roseoviolacea* can convert anthracyclinones into the corresponding glycosides, *e g* conversion of ε-rhodomycinone into carminomycins and bio-inactive 4-*O*-(β-D-glucopyranosyl)-ε-rhodomycinone, ε-pyrromycinone to the novel 1-hydroxy-11-deoxycarminomycin II [16]

In conjunction with biosynthetic studies of anthracycline oligosaccharides, the microbial glycosidation of biologically inactive anthracyclinones is aimed at preparing new anthracyclines with better therapeutic indices An aclacinomycin negative mutant strain KE303 derived from *S galilaeus* MA144-M1 has the property of glycosidating the C-7 or C-10 position of various

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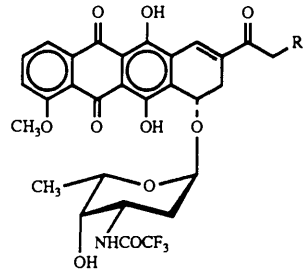
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aglycones, *e.g.* 2-hydroxaklavinone, β-rhodomycinone, β-isorhodomycinone, α-2-rhodomycinone, and α-citromycinone with mono, di, or trisaccharides.[16] 2-Hydroxyaclacinomycins A and B, betacalamycins A, M, N, S, and T,[16] CG10, CG11, and CG12 are produced by adding the corresponding aglycone to a culture of the blocked mutant KE303.

The microbial reduction of daunorubicin to daunorubicinol first reported in 1975 can also be effected with strains of *Streptomyces lavendulae*, *S. roseochromogenes*, *Corynebacterium simplex*, and *Bacterium cycloxydans*.[16] These strains are also capable of converting carminomycin into 13-dehydrocarminomycin.[16] *Corynebacterium equi* has been used for preparative scale selective side-chain reduction of *N*-acetyldaunorubicin to *N*-acetyl-13-dihydrodaunorubicin.[16h]

8 Clinical Experience of Doxorubicin Leading to Analogue Development

The initial observation of the outstanding antitumour activity of doxorubicin[1] directly stimulated the studies on the synthesis of anthracycline aglycones and total synthesis of the natural antibiotics. Early clinical studies revealed toxicities however, especially the risk of cardiotoxicity.[4] This led directly to analogue development, and therefore to a systematic exploration of anthracycline chemistry in the hope that such toxic effects could be minimized or eliminated.

8.1 Side-chain Modification

Obvious functional sites for derivatization in the anthracyclines were at C-13 and C-14. This gave rise to products from reduction at C-13, derivatization of the C-13 carbonyl 14-esters and thioesters, 14-amino derivatives and compounds derived from oxidative degradation of the doxorubicin side chain and 14-ethers of doxorubicin. Reduction of the C-13 carbonyl group may be effected microbially (*vide supra*) or chemically with potassium borohydride. The regioselectivity of the reaction is ensured by the rapid air oxidation of the hydroquinone.

13-Deoxo analogues of the anthracyclines (of interest because of the presence of the C-9 ethyl group in many promising natural anthracyclines) were produced by sodium cyanoborohydride reduction, daunomycinone or daunorubicin tosylhydrazone. Conventional functionalization of the carbonyl group occurs unremarkably to afford semicarbazones, thiosemicarbazones, oximes, and substituted hydrazones. A variation of the latter was to prepare linked anthracycline bishydrazones (62) by reaction with the corresponding dihydrazide in methanol.

Esters of doxorubicin were obtained by reaction of 13-bromodoxorubicin with the sodium salts of carboxylic acids. Variants of this type of derivative include double esters and thioester structures. Side chain fatty acid derivatives proved especially valuable for uptake and cellular distribution studies. Of particular interest in this regard was the *N*-trifluoroacetyldoxorubicin 14-valerate (63).

The early recognition that intercalative binding of anthracyclines to doubled helical DNA was an important component of the cytotoxic action of anthracyclines led to the consideration of net charges on the molecule. Therefore amine functions were introduced at C-14 by reaction of 14-bromodaunorubicin with amines.[18] Periodate oxidation of 13-dihydroxyrubicin *N*-trifluoroacetate followed by reduction of the resulting C-9 formyl compound to a C-9 hydroxymethyl derivative and de-*N*-acylation gave C-9-hydroxymethyldaunorubicin. Reaction of the hydroxyaldehyde with diazomethane affords both *N*-trifluoroacetyldaunorubicin and an epoxide.

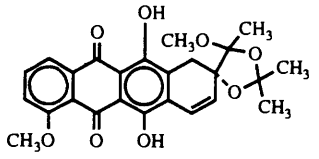
Many of the derivatives obtained by functionalization at C-9 and C-10 are obtained from the 8,10-anhydro-*N*-trifluoroacetyldaunorubicin which is obtained by treating daunorubicin hydrochloride with trifluoracetic anhydride in the presence of collidine at 0 °C.[19] Catalytic hydrogenation of (64) in the presence of $Pd/BaSO_4$, followed by base treatment gives a 9-deoxydaunorubicin.

The presence of a methoxy substituent at C-8 in the steffimycins stimulated interest in developing routes to alternatively C-8 substituted structures. Protection of the C-9-OH and C-13 carbonyl in daunomycinone by treatment with dimethoxypropane and *p*-toluenesulfonic acid permits selective C-7—C-8 dehydration to give (65).[20] Epoxidation of (65) proceeds normally to give (66). Opening of the oxirane ring with methanol and mild acid gives the 7-methoxy-8-hydroxy derivative. Methylation with methyl iodide and sodium hydride in tetrahydrofuran and deprotection with trifluoroacetic acid gives the new 8-substituted aglycone (67).[20]

8.2 Chromophore Modification

The recognition of the intercalative capacity of the anthracyclines and its bearing on their biological properties naturally focused attention on the chromophore.

Development of effective routes, both total synthetic and semisynthetic, to 4-demethoxydaunorubicins produced especially potent analogues.[21] These developments focused attention on ring A of the chromophore and routes to 1,4- and 2,3-disubstituted anthracyclines were pursued. Additional stu-



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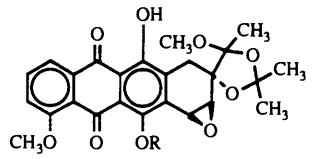

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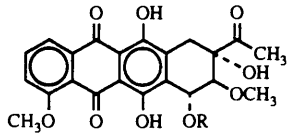

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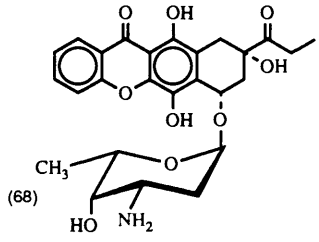

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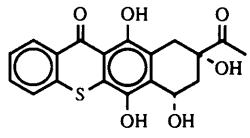

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dies along these lines led to 5-*O*-methyl and 11-*O*-methyl derivatives.

In addition to the above chemical modifications of the chromophore, mechanistic considerations also influenced the direction of synthetic studies. The recognition of the generation of free radical species following microsomal or chemical activation of the quinone moiety of anthracyclines raised the question of the contribution of this pathway to either the cytotoxicity or cardiotoxicity.[22] Chromophore-modified glycosides were prepared by total synthesis in which the bioreducible quinone group was replaced by γ-pyrone (68) or γ-thiapyrone (69) moeities,[22] of which (68) proved to be cytotoxic.

8.3 Aminosugar Modification

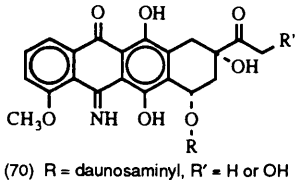
The first synthesis of L-daunosamine was reported by Goodman *et al.* starting from L-rhamnose.[23] An alternative procedure was developed by Horton and Weckerle starting from methyl-α-D-mannopyranoside.[24] The synthesis of D-daunosamine and D,L-daunosamine have also been described.[25]

Systematic exploration of the L-arabino analogues of these glycosides led to the new clinical candidate drugs 4′-epidoxorubicin and 4′-epidaunorubicin.[26] Additional sugar modifications are the 4-deoxy analogues, 4′-methyl derivatives, and 4′-*C*-methylated analogues.[26] Configurational analogues of doxorubicin and daunorubicin that have been synthesized and evaluated and include: (i) those belonging to the L-series but possessing the same configuration at C-1′-(α-glycosides), *i.e.* the L-*ribo* and L-*xylo* analogues; (ii) those belonging to the L-series but showing inverted configuration at C-1′-(β-glycosides); and (iii) those belonging to the D-series both in the α and β anomeric forms.[1,26]

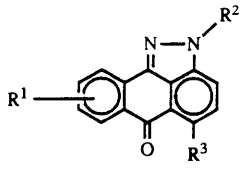
8.4 Serendipitous Modification

Results from the systematic modification of anthracyclines have been complemented by a number of fortuitous discoveries that have influenced the synthetic strategy of analogue development. The first involves the treatment of daunorubicin with methanolic ammonia during certain manipulations that had the unexpected result of producing a stable 5-imino derivative (70).[27] The imino group is presumably introduced selectively because of the additional stabilization afforded by the hydrogen bonds to the flanking oxygen functions.

The 5-imino derivatives of daunorubicin and doxorubicin



(70) R = daunosaminyl, R′ = H or OH



(71)

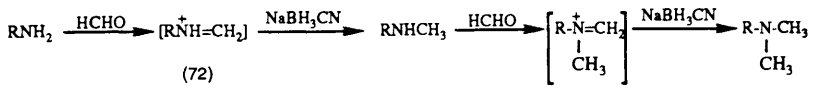
proved to be quite potent, and perhaps more significantly, less cardiotoxic.[27] This serendipitous finding led directly to the synthetic rationale for producing the promising anthrapyrazoles (71) and, retrospectively, in interpretations of the reactivity and toxicity properties of such agents as mitoxantrone.

Another valuable observation emerged from Acton's group during their development of a general method for *N*-alkylation of daunorubicin and doxorubicin.[28] This method is based on the *N*,*N*-dimethylation of amines that uses formaldehyde as a source of the methyl groups, sodium cyanoborohydride as reducing agent, and aqueous acetonitrile as the solvent (Scheme 7). This selectivity under mild conditions is convenient for work with daunorubicin and doxorubicin because concomitant reduction of the 13-ketone to the 13-dihydro derivative could be minimized as a side reaction.[29] Similar use of sodium cyanoborohydride has recently afforded the intensely potent *N*-(5,5-diacetoxypent-l-yl)doxorubicin.[30]

The morpholino anthracyclines are a special subclass of *N*-alkyl derivatives from doxorubicin and daunorubicin. Use of glutaraldehyde gave piperidino derivatives incorporating the amino-N in a new ring. As logical variants additionally incorporating a ring-O, the morpholino analogues were synthesized by reductive alkylation with 2,2′-oxybisacetaldehyde. Neutral by-products of this synthesis unexpectedly included an exceptionally potent derivative which proved to be the α-cyanomorpholine derivative (73).[29]

Formation of these α-cyano compounds can be explained by a mechanism of the reductive alkylation involving iminium intermediates in two stages. Iminium ions are excellent alkylating species and at either of the two intermediate stages can evidently capture CN^-, which is present as an impurity in the $NaBH_3CN$ reagent or as a product of its consumption (Scheme 8).

Not only are the cyanomorpholinoanthracyclines exceptionally potent as anticancer agents but they also appear to function quite differently from conventional anthracyclines in inducing, for example, DNA interstrand cross-links.[29]



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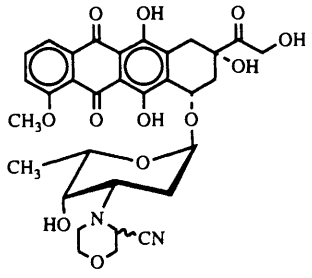

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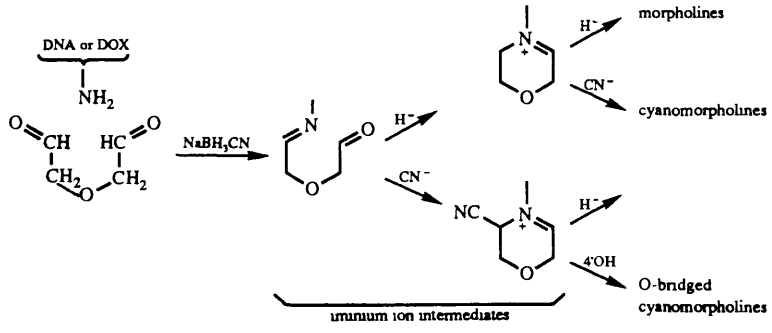
Scheme 7



Scheme 8 Intermediates in the formation of morpholinoanthracylines.

9 Synthetic Challenges in Response to Mechanistic Findings

The anthracyclines are unique amongst clinical anticancer agents in terms of the breadth and depth of prolonged enquiry into all aspects of their properties. Among the most challenging problems has been the elucidation of their mode of action. The consensus view is that the anthracyclines exert several parallel cytotoxic mechanisms,[22] which in turn pose new synthetic challenges for the organic chemist. Some of these challenges have been met, but with qualified success. Thus while an adequate biochemical basis has yet to be established for the cardiotoxicity of the anthracyclines, the pragmatic view was taken that it might be connected to redox cycling of the quinone moiety.[22a] This interpretation was offered *post facto* for the apparently lower cardiac toxicity of 5-iminodaunorubicin[27] and mitoxantrone,[31] and was used to justify the design and synthesis of the promising anthrapyrazoles[32] and related structures.

A new challenge to synthetic chemists has arisen from the discovery of DNA topoisomerase II and helicases as an intracellular target of anthracyclines.[9] The DNA intercalating analogues of doxorubicin and daunorubicin as well as certain of the non-intercalating *N*-acylanthracyclines are apparently topoisomerase II-targeted drugs.[9] Metabolic activation of 14-acyl analogues of *N*-acylanthracyclines by ubiquitous non-specific esterases is a prerequisite for the drug interaction with the enzyme–DNA complexes. The mapping analysis of DNA cleavage mediated by topoisomerase II has shown a similar pattern among various anthracyclines.[9] Molecular pharmacological evidence suggests that anthracyclines interfere with the breakage–reunion reaction of topo-II by (i) stabilizing the complex between the enzyme and DNA, (ii) changing its non-cleavable state into a cleavable one, thus (iii) triggering a sequence of events leading to cell death.[9] Drugs with *N*-trifluoroacetyl or *N*-pentafluoropropionyl substitution of the sugar moiety are substantially more effective than analogues with *N*,*N*-dibenzyl or *N*-acetyl substitution. Since some of the compounds known to inhibit topo-II DNA complexes are poor DNA binders, drug intercalation may not be a precondition of a drug-induced inhibition of topo-II. Doxorubicin and several analogues may bind to the enzyme–DNA adducts and form a ternary complex. Such a proposed complex implies that a topo-II molecule has a domain which represents a binding site for the drug. The domain may have become accessible following formation of the non-cleavable complex between topo-II and DNA. Subsequent binding of anthracyclines to topo-II inhibits the enzyme function and stabilizes the cleavable complex.[9] The removal of bulky side chains of AD32 or AD143, which is a precondition of the drug interaction with topo-II, may reduce steric hindrance and facilitate the formation of drug–topo-II DNA complexes.[9]

These studies suggest strategies for development of new anthracycline analogues. These would include identification of various substituents of anthracyclines responsible for topo-II inhibition. This, in turn, could lead to new analogues which, unlike doxorubicin, stabilize the topo-II DNA complex with high efficiency, and which may translate into higher anticancer potency.

Additional synthetic challenges arise from the extremely serious problem of resistance to chemotherapy in the management of cancer patients. There are indications that resistance to topo-II mediated anthracyclines may be related to low levels of topo-II in quiescent human leukaemic and cancer cells.[9] Systematic exploration of the structural requirements in anthracyclines for efficient topo-II inhibition may therefore also contribute to the resistance problem.[33] Such challenges arising directly from a more sophisticated view of anthracycline action will require the organic chemist to work more closely with enzymologists and molecular pharmacologists in the future.

10 Prospects

Synthetic organic chemistry has contributed significantly in all phases of anthracycline development. These contributions include isolation and structure determination, establishment of absolute configuration, organization into different structural classes, exploration of the characteristic chemical properties and

reactions of anthracyclines, the development of procedures for total synthesis of steadily increasing sophistication and control, and finally, in response to clinical challenges, the generation of anthracycline analogues on a massive scale for biological testing

During the long effort extending over 25 years or more the role of the organic chemist has changed The major challenge is no longer in the area of total synthesis of natural antibiotics Increasingly effective microbial transformations can generate new candidates for biological screens Analogue development in response to clinical observations of severe toxicity of existing anthracyclines encouraged exploration of the basic chemical properties of these agents Well over 2000 analogues have been synthesized to date in this largely pragmatic and semi-empirical development programme The immediate problem of cardiotoxicity has eased somewhat as a result of the introduction and co-administration of ICRF-187 Newer and greater challenges have emerged for the organic chemist from recent mechanistic studies These include compounds synthesized especially to address questions related to topoisomerase II or helicase[33] mediated cell effects and the possibly related questions of drug resistance [32] Additional synthetic challenges are posed by the need to develop improved cell delivery of anthracyclines by, for example, stealth liposomes [35]

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