MAGGIEMYCIN AND ANHYDROMAGGIEMYCIN: TWO NOVEL ANTHRACYCLINONE ANTITUMOR ANTIBIOTICS

ISOLATION, STRUCTURES, PARTIAL SYNTHESIS AND BIOLOGICAL PROPERTIES†

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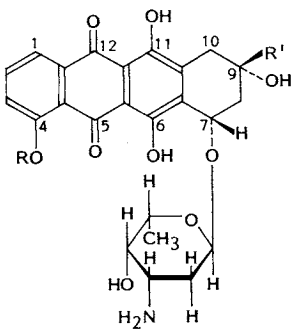
Two new anthracyclinone antitumor antibiotics, maggiemycin (**6**, NSC-D344012) and anhydromaggiemycin (**8**) have been isolated from a culture of an unspeciated *Streptomyces* (ATCC No. 39235). Bioautography against *Bacillus subtilis* was used for the preliminary detection of these anthracyclinones. Structures were proposed based on their UV-visible, IR, ^{1}H NMR, ^{13}C NMR spectra, electron impact (EI) and high-resolution EI-MS and confirmed by partial synthesis and a direct correlation with ε-rhodomycinone. Both the anthracyclinones are active against KB, P388 and L1210 murine tumor cell lines; however, anhydromaggiemycin was more active than maggiemycin. A number of related anthracyclinones have also been prepared and their biological activity has been determined. The structure-activity relationship of these new anthracyclinones is also discussed.

Anthracycline antibiotics,[1~5] in particular daunorubicin (**1**), doxorubicin (**2**) and carminomycin (**3**), have invoked considerable interest because of their impact on cancer chemotherapy. Most of the biologically active anthracyclines are glycosides with one or more sugar residues attached to the aglycone part. No biological activity for any anthracycline aglycone has been reported in the literature. ARCAMONE[5] in his book on doxorubicin states: "... In the anthracycline antibiotics, the presence of the amino sugar residue is an important structural requirement for bioactivity, as a biological action due to the isolated aglycone moiety has never been recorded..."

In this paper, we report on the fermentation, isolation, structures, partial synthesis and biological properties of two novel anthracyclinones which have been found active *in vitro* against KB, P388 and L1210 murine cell lines.



Daunorubicin (**1**)	R=CH_3	R'=$COCH_3$
Doxorubicin (**2**)	R=CH_3	R'=$COCH_2OH$
Carminomycin I (**3**)	R=H	R'=$COCH_3$

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Materials and Methods

General

MP's were determined on a Kofler micro hot stage apparatus and are uncorrected. UV-visible (UV-VIS) spectra were taken on a GCA/McPherson UV-VIS spectrophotometer, Model EU-700-32. IR spectra (KBr pellet) were run by Mr. N. RISSER on a Perkin-Elmer spectrophotometer, Model 180. ^{1}H NMR spectra were determined by Dr. D. WILBUR on a Varian XL-100 spectrometer equipped with a Nicolet Instrument Corp. TT 100 Fourier transform accessory. ^{13}C NMR spectra were run on a Jeol FX 60 spectrometer by Ms. M. KLINE. Electron impact (EI)-MS were run by Mr. S. HUANG on a Finnigan 3300 GC/MS with a 6000 MS data system at 70 eV (solid probe). High resolution (HR)EI-MS were measured by Dr. G. MCCLUSKY on a Vg Micromass ZAB-2F mass spectrometer, equipped with a combination EI, chemical ionization (CI), field ionization (FI), field desorption (FD) ion source and VG 2035 data system. Analytical (0.25 mm) TLC were carried out on E. Merck precoated silica gel plates with fluorescent indicator. Following solvent systems were developed and used in TLC: A) Chloroform (100%); B) chloroform - ethyl acetate (80 : 20); C) chloroform - acetone (80 : 20); D) chloroform - methanol (80 : 20); E) chloroform - acetic acid (95 : 5); F) chloroform - acetone - acetic acid (90 : 8 : 2); G) chloroform - hexane - acetic acid (50 : 50 : 5); H) ether - petroleum ether (1 : 1); I) chloroform - benzene - methanol (6 : 3 : 1); J) chloroform - benzene - methanol (7 : 3 : 2); K) benzene - ethyl acetate - methanol - formic acid - water (5 : 5 : 1.5 : 1 : 0.3); L) chloroform - hexane - methanol (5 : 5 : 1); and M) chloroform - heptane - methanol - acetic acid (40 : 40 : 18 : 2). Two types of E. Merck silica gel (TLC grade Silica gel 60 H and column grade 70~230 mesh ASTM) were used for column chrmatography. HPLC was performed using Waters Associates Model 6000A solvent delivery system, a U6K septumless injector (Waters Associates) and a Schoeffel SF 770 variable wavelength UV detector. The detector was set at 254 nm and 0.04 absorbance unit full scale. The separations were carried out on a C_{18} μBondapak column (3.9 mm × 30 cm, Waters Associates) and methanol - pH 2.0 water (with phosphoric acid) (65 : 35) as the solvent at a flow rate of 2 ml/minute.[6]

Cytotoxicity Determinations

The cytotoxicity against the KB, P388 and L1210 cell lines was determined under contracted accessory testing services provided through the National Cancer Institute, U.S.A.[7,8]

Derivation of Variant Strain PD J566-A21

All natural variant strains were derived from a culture of an unspeciated *Streptomyces* designated PD J566.[9] Strain V1 was isolated as the predominant colony with morphological type of PD J566. Strain A21 (ATCC No. 39235) was isolated as a natural variant of strain V1, recognizable as a blue colony among the normal red colonies of strain V1.[10] Strains were maintained as spore suspensions in 10% glycerol, 0.5% Tween 80 at a temperature of −196°C.

Fermentation

Fermentations were initiated by transferring 0.05 to 0.5 ml of a spore suspension to a baffled shake flask containing 1/5 of the nominal flask volume of seed medium (Medium S). Medium S contained (w/v): Corn starch 1.5%, Pharmamedia (Traders Protein, Fort Worth, Texas) 0.5%, Nutrisoy defatted soy flour (Archer-Daniels Midland Co., Decatur, Illinois) 0.5%, Red Star autolyzed yeast (Universal Foods Corp., Milwaukee, Wisconsin) 0.1%, sodium chloride 0.25% and calcium carbonate 0.5%.

Seed cultures were incubated for 3 days at 28~30°C on a 2.5-cm stroke rotary shaker at 250 rpm. Seed cultures were then used to inoculate (at a ratio of 10%) baffled shake flasks containing 1/5 of the nominal flask volume of production medium (Medium P). Medium P contained (w/v): Glucose monohydrate 5%, herring meal (The Mearl Corp., Eastport, Maine) 1.2%, Red Star autolyzed yeast 0.5%, Nutrisoy 1.25%, sodium chloride 0.33%, calcium carbonate 1.0% and Prochem 51 (Prochemco, Inc., Lake Station, IN) 0.2%. Production cultures were incubated for 7 days at 28~30°C with rotary shaking at 250 rpm (2.5-cm stroke).

Large-scale fermentations were carried out for 7 days at 30°C with an aeration rate of 5 liters/minute and an agitation rate of 500 rpm in 14-liter Microferms (New Brunswick Scientific Co.) containing 10 liters of medium P, but with 2% Prochem 51.

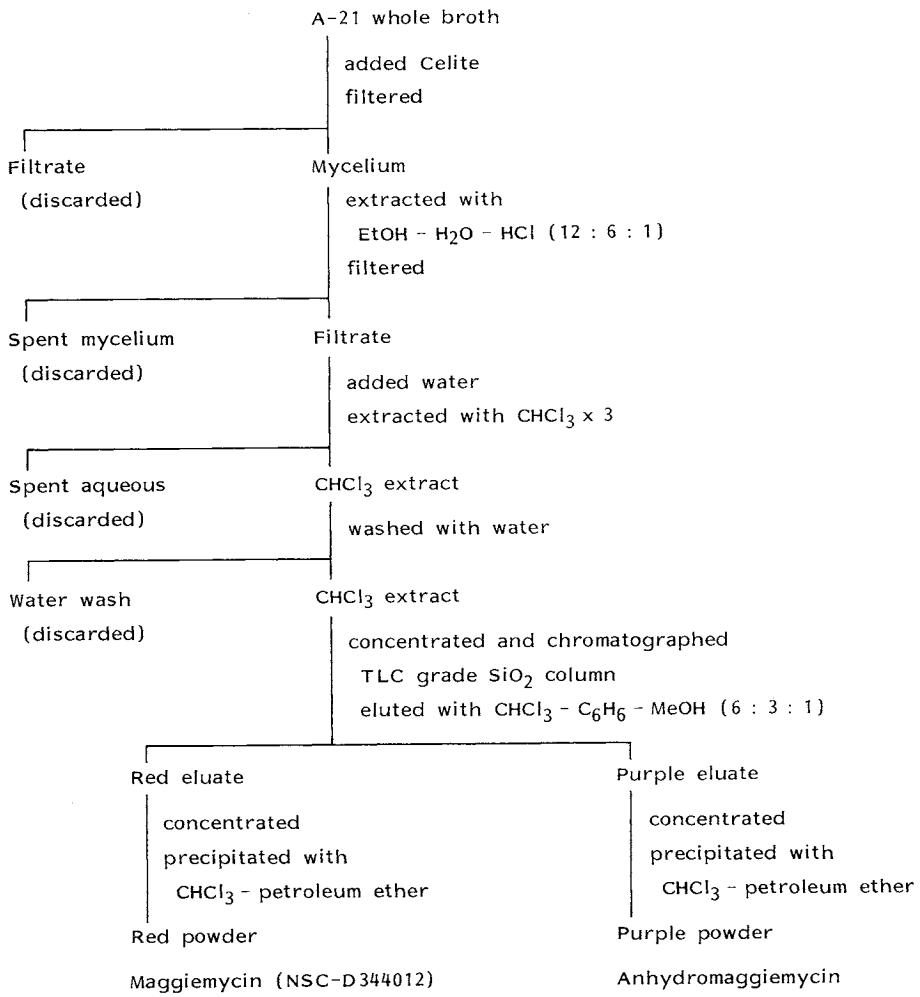
Isolation of Maggiemycin and Anhydromaggiemycin

At the end of the fermentation, cultures were supplemented 8 g/liter of Celite (an inert filter aid; Johns-Manville Co.) and the aqueous phase was removed by filtration through Whatman No. 1 filter paper and discarded. The mycelial filter cake was extracted with 1/3 of the original culture volume of a mixture of ethanol - water - conc hydrochloric acid (12 : 6 : 1). The extract was recovered by filtration through Whatman No. 1 filter paper and the filter cake was re-extracted twice as above. The 3 extract filtrates were pooled and 2 liters aliquots were diluted with 2 liters of tap water, then extracted 3 times with chloroform volumes of 200 ml, 100 ml and a final 100 ml. The chloroform phases were recovered, pooled, and concentrated by evaporation *in vacuo* at 40°C to produce the crude compound.

The crude compound (~600 mg) was dissolved in chloroform and loaded onto a TLC grade silica gel column (6.5 × 29 cm). The column was eluted with chloroform (100%, 100 × 10 ml, discarded); chloroform - acetic acid (99 : 1, 146 × 10 ml, discarded, 21 × 10 ml, pure red compound, 14 × 10 ml, mixture); chloroform - acetic acid (98 : 2, 13 × 10 ml, pure purple compound) and chloroform - acetic acid (95 : 5, 90 × 10 ml, mixture).

The purple compound moved faster in TLC using chloroform or chloroform - acetic acid as the solvents, but on column the red compound eluted first. Every third fraction was checked by TLC and the pure red and purple fractions were pooled separately, concentrated and precipitated from chloroform - petroleum

Scheme 1. Isolation and purification method for maggiemycin (NSC-D344012) and anhydromaggiemycin from A-21 whole broth.



ether. The precipitates were washed with petroleum ether (3×10 ml) and dried under high vacuum to produce a red and a purple powder (Scheme 1). The red powder (198.8 mg) designated as maggiemycin (NSC-D344012) was active against *Bacillus subtilis* and *Staphylococcus aureus* and had ED_{50} 2.7 μg/ml against KB cell lines and ED_{50} 4.4 μg/ml against P388 cell lines. Maggiemycin had the first mp 190~195°C at which time needles begin to form which then melt at 250°C; $[\alpha]_D^{21}$ +4.6° (*c* 0.1, dioxane); Rf 0.82 (solvent C), 0.69 (solvent I), 0.57 (solvent L); UV λ_{max}^{MeOH} nm (ε) 220 (sh, 21,176), 240 (36,435), 285 (10,364), 502 (14,023) (Fig. 1); IR (KBr) cm^{-1} 3525, 3450, 2975, 1725, 1660, 1625, 1610, 1475, 1460, 1440, 1410, 1380, 1375, 1340, 1310, 1290, 1200, 1160, 1110, 1075, 1055, 1025, 990, 950,

Fig. 1. UV-VIS spectrum of maggiemycin.

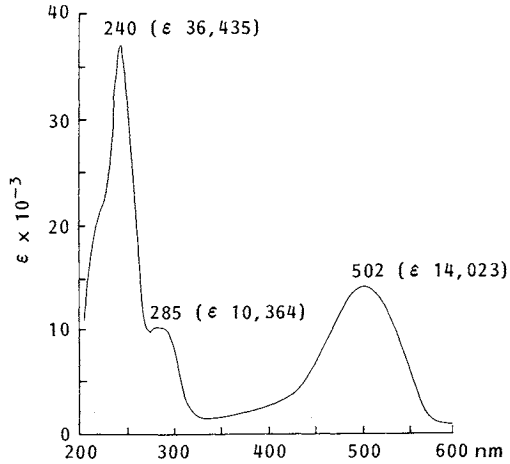


Fig. 2. IR spectrum of maggiemycin (KBr).

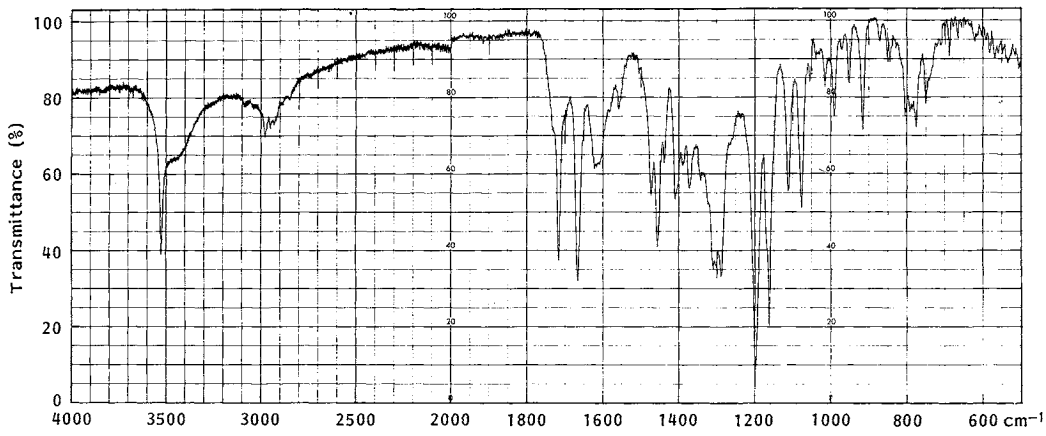


Fig. 3. ^1H NMR spectrum (100 MHz) of maggiemycin in $CDCl_3$.

Shaded peaks disappear on D_2O shake.

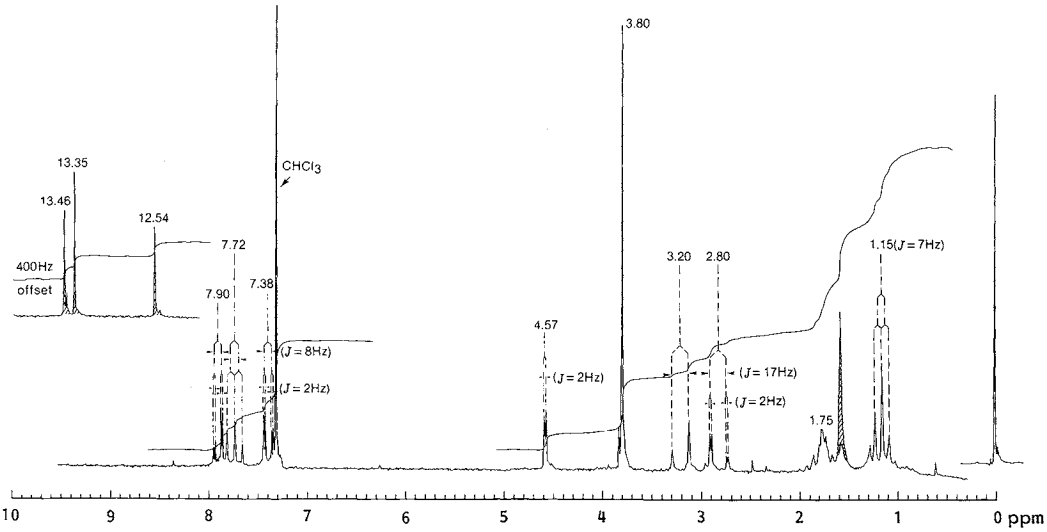


Fig. 4. ^{1}H decoupled ^{13}C NMR spectrum (FX-60, DMSO-d_6) of maggiemycin.

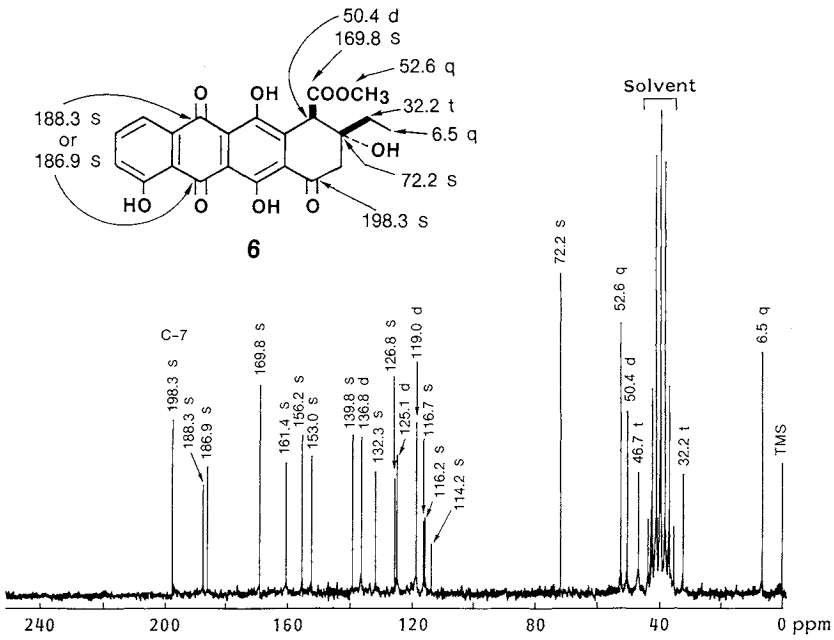
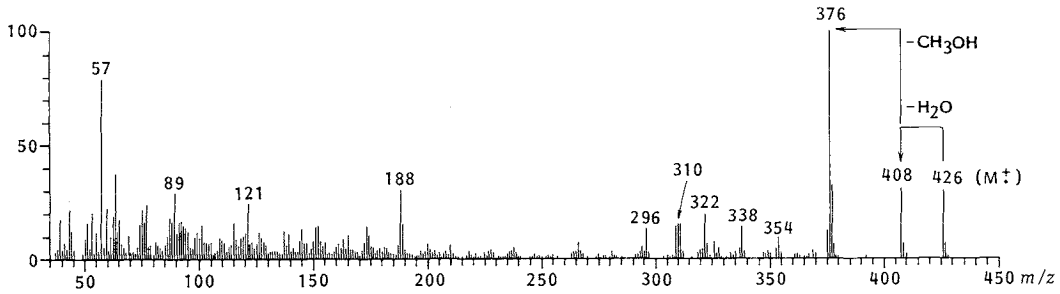


Fig. 5. EI-MS of maggiemycin.



920, 800, 775, 750 (Fig. 2); ^{1}H NMR (100 MHz, $CDCl_3$) Fig. 3 and Table 2; ^{13}C NMR (FX-60, DMSO-d_6) Fig. 4; EI-MS (70 eV) m/z 426 (M^+), 408 ($M-H_2O$), 376 ($M-H_2O-CH_3OH$) (Fig. 5); *Anal* Calcd for $C_{22}H_{18}O_9$: MW 426.0944. Found: MW 426.0947 (HREI-MS).

The purple powder (128 mg) was designated as anhydromaggiemycin and had mp 252~256°C; Rf 0.29 (solvent C), 0.62 (solvent I), 0.31 (solvent L); UV λ_{max}^{MeOH} nm (ε) 205 (18,976), 260 (32,154), 320 (5,712), 510 (10,025) 547 (17,311), 590 (17,450); IR (KBr) cm^{-1} 3420, 2970, 2950, 1720, 1600, 1568, 1515, 1465 (sh), 1445, 1435, 1375, 1280, 1240, 1190, 1150, 1120, 1088, 1050, 1020, 985, 972, 890, 850, 828, 810, 765, 695, 650; ^{1}H NMR (100 MHz, $CDCl_3$) Table 2; EI-MS (70 eV) m/z 408 (M^+), 377, (100%), 375, 361, 348, 347, 333, 320, 319, 305, 291, 263, 247, 246, 227, 221, 219, 218, 217, 205, 203, 202, 201, 200, 199, 191, 190, 189, 188, 187, 182, 181, 179, 178, 177, 176, 175, 174, 173, 172, 166, 165, 163, 160, 152, 151, 150, 147, 146, 145, 138, 137, 129, 125, 124, 123, 122, 121, 120, 119, 118, 117, 116, 115, 109, 105, 104, 103, 101, 100, 99, 96, 95, 94, 91, 89, 88, 87, 83, 82, 81, 77, 76, 75, 69, 63, 58, 57, 55 amu; *Anal* Calcd for $C_{22}H_{16}O_8$: MW 408.0835. Found: MW 408.0840 (HREI-MS).

ε-Rhodomycinone (**4**)

ε-Rhodomycinone used here was isolated from the daunorubicin fermentation broth and had mp 215~218°C; $[\alpha]_D^{21}$ +232.5° (*c* 0.1, dioxane); $[\alpha]_D^{21}$ +104.5° (*c* 0.1, $CHCl_3$); (literature[9] mp 220~222°C,

$[\alpha]_D$ +80° (*c* 0.5, $CHCl_3$)); Rf 0.03 (solvent: Chloroform-heptane-methanol, 5:5:1), 0.66 (solvent: Chloroform-methanol-formic acid, 80:20:2); UV λ_{max}^{MeOH} nm (ε) 526 (9,153), 491 (13,197), 471 (10,927), 284 (7,485), 250 (23,734), 231 (38,280), 205 (16,213); ^{1}H NMR ($CDCl_3$) Table 2; ^{13}C NMR ($CDCl_3$) δ 6.8, 32.7, 34.5, 51.6, 52.4, 62.7, 71.4, 111.4, 111.5, 116.0, 119.7, 124.9, 133.4, 135.0, 137.1, 137.5, 155.9, 157.0, 162.7, 171.2, 186.0, 190.0; EI-MS (70 eV) *m/z* 428 (M^+, 100%), 410, 392, 368, 360, 351, 339, 333, 323, 322, 321, 294, 293, 292, 268, 265, 249, 239, 237, 221, 212, 211, 203, 202, 189, 181, 175, 174, 173, 165, 163, 152, 146, 145, 139, 128, 127, 121 amu.

Preparation of Maggiemycin from ε-Rhodomycinone

A stirred solution of ε-rhodomycinone (1 g) in acetone (100 ml) was cooled to 0°C and 4 ml of Jones reagent (prepared from 26.7 g Cr_2O_3 and 23 ml conc H_2SO_4, diluted to 100 ml with water) was added to it, dropwise. The reaction mixture was stirred at 0°C for 30 minutes and at *ca.* 22°C for 16 hours. Excess of the reagent was quenched by 2-propanol, and the mixture was diluted with water. The reaction product was extracted with chloroform (3×200 ml) and the combined chloroform extract was washed with water (2×200 ml) and brine (2×200 ml), and was dried over sodium sulfate. TLC in solvent systems C, I and L indicated the oxidation had occurred and the sample was almost pure. Sodium sulfate was removed by filtration and the filtrate was concentrated under vacuum. The residue was passed through a small column of silica gel and the eluate was concentrated and precipitated from chloroform-petroleum ether to produce 687 mg (yield 68%) of red powder, mp 191~195°C; mixture mp with authentic sample 190~195°C; $[\alpha]_D^{21}$ +2.3° (*c* 0.1, dioxane). TLC, IR, ^{1}H NMR and EI-MS were identical to the authentic sample.

Preparation of Anhydromaggiemycin from Synthetic Maggiemycin

A stirred solution of maggiemycin (110 mg) and *p*-toluenesulfonic acid (50 mg) in benzene (200 ml) was refluxed using a Dean stark trap to remove water formed in the reaction mixture. The course of the reaction was followed by TLC. After 4 hours the reaction mixture was cooled, diluted with water and the benzene and aqueous layers were separated. The benzene layer was washed with water (3×100 ml) and dried (Na_2SO_4). Removal of solvent and precipitation of the residue from chloroform-petroleum ether produced 84.9 mg (84% yield) of a purple powder: MP 250~254°C. TLC, IR, ^{1}H NMR and EI-MS of this product were identical to the authentic natural anhydromaggiemycin.

Daunomycinone (**5**)

Daunomycinone used in this work was prepared from daunorubicin by mild acid hydrolysis using the reported procedure.[11] The purified product had: MP 210~211°C; $[\alpha]_D^{25}$ +105.0° (*c* 0.1, $CHCl_3$); $[\alpha]_D^{21}$ +175.4° (*c* 0.11, dioxane) (literature[11] mp 213~214°C, $[\alpha]_D$ +193° (*c* 0.1, dioxane)).

Preparation of 7-Oxodaunomycinone (NSC-D344013)

7-Oxodaunomycinone was prepared from 500 mg of daunomycinone and 2 ml of Jones reagent by stirring at 0°C for 24 hours and then working up the product in a manner described for partial synthesis of maggiemycin. The residue was purified over silica gel column using chloroform-hexane-methanol (5:5:1) as the eluent. The pure fractions were combined based on TLC and precipitated from chloroform-petroleum ether to produce a red powder which showed activity against KB, P388 and L1210 cell lines with ED_{50} of 5.6, 3.4 and 1.9 μg/ml, respectively. It had mp 125~127°C; Rf 0.41 (solvent C), 0.35 (solvent L) and 0.89 (solvent: Chloroform-methanol-formic acid, 80:20:2); $[\alpha]_D^{21}$ +3.7° (*c* 0.1, $CHCl_3$); $[\alpha]_D^{21}$ +1.5° (*c* 0.1, dioxane); UV λ_{max}^{MeOH} nm (ε) 550 (sh, 6,463), 515 (9,909), 493 (10,468), 275 (sh, 13,243), 250 (23,357), 237 (25,929), 224 (sh, 20,154); *Anal* Calcd for $C_{21}H_{16}O_8$: MW 396. Found: MW 396 (EI-MS, 70 eV).

Preparation of Anhydro-7-oxodaunomycinone

Anhydro-7-oxodaunomycinone was prepared from 80 mg of 7-oxodaunomycinone following the previously described method for anhydromaggiemycin to produce 50.3 mg of the pure product: MP 275~279°C; UV λ_{max} ($MeOH-CHCl_3$, 1:1) nm (ε) 569.5 (34,621), 529 (31,958), 495 (16,409), 465.5 (sh, 6,357), 378 (3,093), 275 (sh, 42,955), 260 (52,748); IR (KBr) cm^{-1} 3450 (weak), 3000 (weak), 1690, 1585, 1508, 1460, 1445, 1435, 1360, 1320, 1270, 1240, 1215, 1190, 1178, 1120, 1055, 1035, 885, 835, 810, 768,

730, 700, 585, 480; EI-MS (70 eV) *m/z* 378 (M^+, 100%), 360, 345, 335, 317, 307, 292, 277, 264, 249, 236, 208, 182, 163, 152, 104, 87, 76, 63, 57 amu; *Anal* Calcd for $C_{21}H_{14}O_7$: MW 378.0739. Found: MW 378.0738 (HREI-MS).

Results

It was found that a natural variant strain PD J566-A21, recognized by its formation of blue colonies on nutrient agar and obtained through natural variants V-1 and V1-1 from parent strain PD J566, produced fast-moving antimicrobial and antitumor antibiotics when cultured in a suitable medium (discussed in Materials and Methods section). The bioautography of this fermentation extract repeatedly showed antimicrobial activity in the region where there was no activity in the earlier daunorubicin fermentation broths. It was of interest, therefore, to isolate these fast-moving biologically active components and determine their structures and biological activity. The isolation of these components from the whole broth is shown in Scheme 1.

The A-21 whole broth was filtered, after addition of 8 g/liter of Celite. The mycelium containing the biologically active material was then extracted with 1/3 volume of a mixture of ethanol - water - hydrochloric acid (12 : 6 : 1) and filtered. An equal volume of water was added to the filtrate and was extracted three times with chloroform. The combined chloroform extract was washed with water and concentrated under vacuum to afford the crude compound.

Various solvent systems were developed for the isolation of pure compounds on TLC. It was found that when the TLC plate was developed first in chloroform and then chloroform - acetic acid (95 : 5), the purple spot moved far ahead of the red spot. The crude compound was therefore chromatographed on a TLC grade silica gel column eluting with chloroform (100%), chloroform - acetic acid (99 : 1), chloroform - acetic acid (98 : 2), and chloroform - acetic acid (95 : 5). Based on the TLC, various fractions were combined to produce a red and a purple eluate. These were separately concentrated and precipitated with chloroform - petroleum ether to yield red and purple powder. The red powder has been designated as maggiemycin (NSC-D344012) and the purple powder as anhydromaggiemycin. The physico-chemical properties of maggiemycin and anhydromaggiemycin are summarized in Table 1.

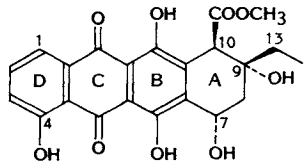
Structures of Maggiemycin and Anhydromaggiemycin

From the Rf, UV, molecular weights, elemental composition (Table 1) and biogenetic consideration

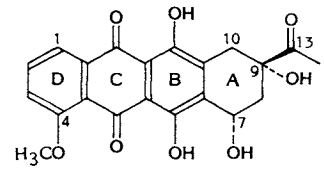
Table 1. Properties of maggiemycin and anhydromaggiemycin.

	Maggiemycin (NSC-D344012)	Anhydromaggiemycin
Producing organism	*Streptomyces* sp. (ATCC 39235)	*Streptomyces* sp. (ATCC 39235)
Appearance	Red powder	Purple powder
MP (°C)	190 ~ 195	252 ~ 256
$[\alpha]_D^{21}$	+4.6° (Dioxane)	
UV λ_{max}^{MeOH} nm (ε)	240 (36,435), 285 (10,364), 502 (14,023)	262 (31,515), 325 (sh, 5,372), 515 (sh, 10,922), 550 (16,832), 590 (16,921)
MW	426.0947 (Δm = 0.3 mmu)	408.0840 (Δm = 0.5 mmu)
Molecular formula	$C_{22}H_{18}O_9$	$C_{22}H_{16}O_8$
Rf[a] S1	0.69	0.62
S2	0.57	0.31
S3	0.82	0.29

[a] Using analytical TLC silica gel plates. Solvent system S1: Chloroform - benzene - methanol (6 : 3 : 1), S2: chloroform - hexane - methanol (5 : 5 : 1), S3: chloroform - acetone (80 : 20).

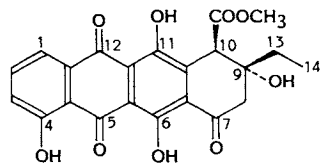


ε-Rhodomycinone (**4**)
$C_{22}H_{20}O_9$, 428



Daunomycinone (**5**)
$C_{21}H_{18}O_8$, 398

Fig. 6. Possible structures for maggiemycin.



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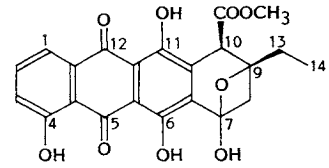

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of maggiemycin and anhydromaggiemycin, it was clear that these two antibiotics fall into the anthracycline group of antibiotics and are probably anthracyclinones of the ε-rhodomycinone (**4**) or daunomycinone (**5**) type. The ^{1}H NMR spectrum of maggiemycin (Fig. 3) was reminiscent of the ε-rhodomycinone group of antibiotics (methyl triplet centered at δ 1.15 (J=7 Hz) and methoxy singlet at δ 3.80), suggesting that it did not belong to the daunomycinone type.

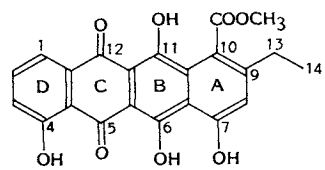
A comparison of the ^{1}H NMR data of ε-rhodomycinone with maggiemycin and anhydromaggiemycin is shown in Table 2. The three aromatic protons C-1, C-2 amd C-3 of ε-rhodomycinone appear as doublet of doublets (dd) centered at δ 7.37 (J=8 and 2 Hz), triplet centered at δ 7.76 (J=8 Hz) and another pair of doublets (dd) centered at δ 7.83 (J=8 and 2 Hz). The C-7 proton appears as a broad signal at δ 5.44 (goes to a triplet (J=4 Hz) on D_2O shake), and the C-10 proton appears at δ 4.36. The two methylene groups C-13 and C-8 appear at δ 1.76 (m, J=7 Hz) and 2.36 (d, J=4 Hz). The methyl groups appear at δ 1.23 (t, J=7 Hz) and 3.81. The phenolic hydroxyls appear at δ 12.14, 12.97 and 13.50.

A comparison of these data with maggiemycin ^{1}H NMR (Fig. 3 and Table 2) clearly reveal that there is no proton at C-7 in the maggiemycin structure. Because of this change, the C-8 methylene protons appear at δ 2.80 (1H, dd, J=17 and 2 Hz) and 3.20 (1H, d, J=17 Hz). The coupling between one of the C-8 protons and the C-10 proton was confirmed by spin decoupling studies. This suggests that ε-rhodomycinone and maggiemycin are different only in the substitution pattern in ring A. The probable structures for maggiemycin could then be written as **6** or **7**.

The ^{13}C NMR spectrum of maggiemycin (Fig. 4) was instrumental in distinguishing between these two structures. From structure **7** one would expect a signal in the region δ 100 for the C-7 carbon, whereas structure **6** would show C-7 carbon around δ 200. A signal at δ 198.3 rules out the structure **7** and clearly suggests structure **6** for maggiemycin. The assignment of other important signals is indicated in Fig. 4.

Further comparison of the ^{1}H NMR spectra of ε-rhodomycinone (**4**) and maggiemycin (**6**) with the ^{1}H NMR of anhydromaggiemycin (Table 2) clearly shows the absence of C-7 and C-10 protons and also the methylene grouping at C-8 in anhydromaggiemycin. The most characteristic peak in the spectrum

Fig. 7. Structure of anhydromaggiemycin (**8**).



was a new singlet in the aromatic region at δ 7.22 which was not present in the other two compounds. Based on the elemental composition (Table 1) and the above ^{1}H NMR data, the only possible structure for anhydromaggiemycin could be written as **8**.

Partial Synthesis of Maggiemycin (**6**) and Anhydromaggiemycin (**8**)

The structure maggiemycin (**6**) and anhydromaggiemycin (**8**) were confirmed by their partial synthesis from, and thus correlation with ε-rhodomycinone.

ε-Rhodomycinone, a by-product of daunorubicin fermentations, was first isolated by extraction and column chromatography over silica gel and identified by comparison of $[\alpha]_D$, IR, ^{1}H NMR, ^{13}C NMR spectra and EI-MS with an authentic sample and was used for the synthesis.

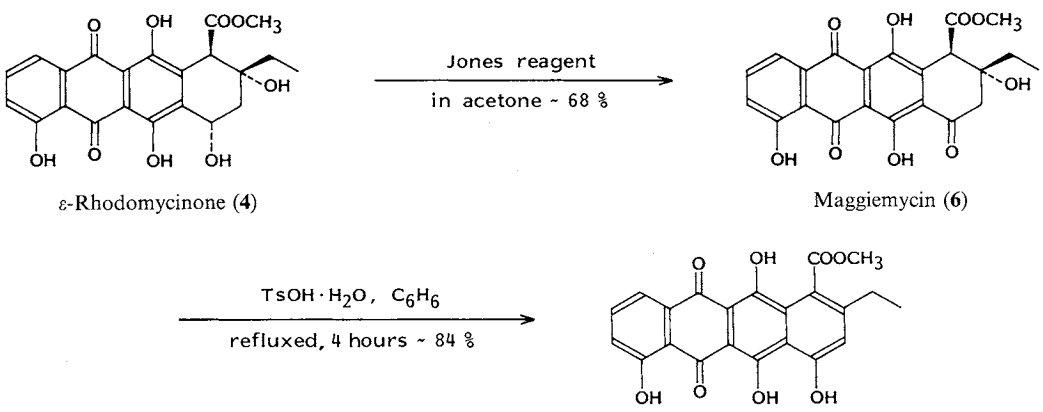
Treatment of ε-rhodomycinone with Jones reagent in acetone produced maggiemycin in a yield of 68%. The product was isolated by extraction with chloroform followed by silica gel chromatography. The synthetic material had mp, TLC, UV, IR, ^{1}H NMR and EI-MS identical to that of natural maggiemycin. The optical rotation of the synthetic material ($[\alpha]_D^{21}$ +2.3°) was also in agreement with the rotation of the

Table 2. Comparison of ^{1}H NMR chemical shifts of maggiemycin, anhydromaggiemycin and ε-rhodomycinone.

Carbons	Chemical shift[a]		
	Maggiemycin (**6**)	Anhydromaggiemycin (**8**)	ε-Rhodomycinone (**4**)
CH_3	1.15 t (J=7 Hz), 3.80 s	1.30 t (J=7 Hz), 4.03 s	1.23 t (J=7 Hz), 3.81 s
CH_2	1.75 m, 2.80 dd (1H, J=17, 2 Hz), 3.20 d (1H, J=17 Hz)	2.72 q (J=7 Hz)	1.76 m (J=7 Hz), 2.36 d (J=4 Hz)
>CH	4.57 d (J=2 Hz)		4.36 s, 5.44 t (J=4 Hz)
=CH	7.38 dd (J=8, 2 Hz), 7.72 t (J=8 Hz), 7.90 dd (J=8, 2 Hz)	7.22 s, 7.28 dd (J=8, 2 Hz), 7.73 t (J=8 Hz), 8.06 dd (J=8, 2 Hz)	7.37 dd (J=8, 2 Hz), 7.76 t (J=8 Hz), 7.83 dd (J=8, 2 Hz)

[a] δ in ppm relative to TMS as internal reference (0.00). J in Hz.

Scheme 2. Partial synthesis of maggiemycin and anhydromaggiemycin from ε-rhodomycinone.



ε-Rhodomycinone (**4**)

Maggiemycin (**6**)

Anhydromaggiemycin (**8**)

Table 3. *In vitro* cytotoxicity of various anthracyclinones against established cell lines.

Anthracyclinones	ED_{50} (μg/ml)[a]		
	KB	L1210	P388
Maggiemycin (**6**)	2.70	5.20	4.40
Anhydromaggiemycin (**8**)	0.51	0.26	0.62
ε-Rhodomycinone (**4**)		30.0	26.0
7-Oxodaunomycinone	5.6	1.9	3.4
Anhydro-7-oxodaunomycinone		16.0	47.0
Daunomycinone (**5**)	>100	8.9	21.6

[a] The dose level of pure compounds at which 50% inhibition of growth *in vitro* is noted vs. untreated controls. Any compound having $ED_{50}<4.0\,\mu g/ml$ is called active and is of interest for further development. Compounds having $ED_{50}>4.0\,\mu g/ml$ are called inactive.

natural product ($[\alpha]_D^{21}$ +4.6°), thus establishing the stereochemistry at C-9 and C-10.

When maggiemycin was refluxed with *p*-toluenesulfonic acid in benzene for 4 hours, anhydromaggiemycin was obtained in a yield of ~84% which was identical to the natural product in all physico-chemical properties. The preparation of these compounds is summarized in Scheme 2.

Structure-activity Relationship

Maggiemycin and anhydromaggiemycin were active against *B. subtilis* and *S. aureus* and showed *in vitro* antitumor activity against KB, P388 and L1210 cell lines. We next decided to check and compare the *in vitro* antitumor activity of these newly isolated anthracyclinones with some of the known anthracyclinones. A literature search revealed that no antitumor activity has been reported for any of the known anthracyclinones. In order to make a suitable comparison, 7-oxodaunomycinone and anhydro-7-oxodaunomycinone, the corresponding analogues of **6** and **8** from daunomycinone series, were synthesized from daunomycinone (**5**) by the methods discussed earlier. These were all tested for *in vitro* activity against one or all of the murine tumor cell lines KB, L1210 and P388. The results are summarized in Table 3. In the ε-rhodomycinone series, ε-rhodomycinone was inactive, whereas maggiemycin and anhydromaggiemycin were active, the latter being more active. In the daunomycinone series, daunomycinone and anhydro-7-oxodaunomycinone were inactive, whereas 7-oxodaunomycinone was active.

Discussion

In the course of our studies with the large-scale production of daunorubicin (**1**, NSC-82151) by fermentation,[12~14] constant efforts were directed to increase the daunorubicin titer by changing medium composition and by isolating higher producing mutants. As a result of the strain development work, a number of mutants were isolated that differ from the parent strain (*Streptomyces* sp. PD J566) in anthracycline production.[15] One mutant was found to produce novel anthracyclinones maggiemycin and anhydromaggiemycin. To our knowledge, these are the first anthracycline aglycones to demonstrate biological activity; they are active against *B. subtilis*, *S. aureus*, and cell lines KB, L1210 and P388.

Some structure activity relationships for anthracyclinones can be suggested from the cytotoxicity data in Table 3. If ring A is saturated, then activity is enhanced by the presence of carbonyl at C-7. Enhancement ranged from 5-fold (daunomycinone series, L1210 cells) to over 18-fold (daunomycinone series, KB cells). In the compounds with ring A saturated, the daunorubicin-related compounds are slightly more active than the ε-rhodomycinone-related compounds; thus, a carbonyl at C-13 may be important. If ring A is aromatic, then the C-10 carbomethoxy greatly enhances activity compared to the C-13 carbonyl (Table 3). Replacement of the C-7 hydroxyl of anhydromaggiemycin with hydrogen eliminates cytotoxicity (R.

PANDEY and M. TOUSSAINT; unpublished observations), so the C-7 hydroxyl is essential for activity. The C-10 carbomethoxy has also been shown to be important for the cytotoxic activity of the anthracycline glycosides marcellomycin and rudolfomycin.[16] These are considered class II anthracyclines, since they inhibit RNA synthesis at concentrations 10-fold lower than those required to inhibit DNA synthesis.[17] It would be interesting to test whether anhydromaggiemycin falls into class I or class II on this basis. Removal of the C-10 carbomethoxy from nogalamycin had only a slight effect on cytotoxicity.[18]

Over 400 anthracyclines have been or are being tested *in vivo*, but to our knowledge none of them have aglycones similar to those reported here. Since the aglycones reported here are cytotoxic, we believe that suitable modifications such as attachment of an amino sugar to a position other than C-7, may lead to more effective anthracyclines.

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