NEW BIOSYNTHETIC ANTHRACYCLINES RELATED TO BARMINOMYCINS INCORPORATING BARBITURATES IN THEIR MOIETY

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Three new anthracyclines, FCE 21424 (**2**), FCE 24366 (**3**) and FCE 24367 (**4**), were isolated from culture broths of *Streptomyces peucetius* and its mutant strains after addition of sodium barbiturates during the fermentation. Structural assignment, achieved through spectroscopic and degradative studies, that the new anthracyclines had a common barminomycin-like structure incorporating different barbiturate moieties.

The new anthracyclines were found to display outstanding cytotoxicity and remarkable potency "*in vivo*" against P388 ascitic leukemia.

In our search for new biosynthetic anthracyclines *Streptomyces peucetius*[1] the daunorubicin (**1**) producing microorganism and some of its mutants were cultured in the presence of barbituric acid and analogs with the aim of shifting the production pattern of known anthracyclines and/or further broadening their intrinsic biosynthetic versatility. Under these conditions culture broths of *S. peucetius* and its mutants, such as *S. peucetius* var. *caesius*[2], *S. peucetius* var. *carneus*[3] and *S. peucetius* var. *carminatus*[4], showed an increase of cytotoxic and antibacterial activity.

This paper describes the production, isolation, structural characterization and preliminary biological activity data of three new barminomycin-like anthracyclines, FCE 21424 (**2**), FCE 24366 (**3**) and FCE 24367 (**4**), incorporating barbituric acids in their structure and endowed with remarkable cytotoxicity and potency against experimental tumors[5,6].

Fermentation

S. peucetius (strain ATCC 29050)[1], *S. peucetius* var. *caesius* (strain ATCC 27952)[2], *S. peucetius* var. *carneus* (strain ATCC 21354)[3] and *S. peucetius* var. *carminatus* (strain ATCC 31502)[4] were cultured under aerobic conditions at 28°C in a production medium consisting of glucose 6%, dried yeast 3%, NaCl 0.2%, KH_2PO_4 0.1%, $CaCO_3$ 0.2%, $MgSO_4 \cdot 7H_2O$ 0.01%, $FeSO_4 \cdot 7H_2O$ 0.001%, $ZnSO_4 \cdot 7H_2O$ 0.001% and $CuSO_4 \cdot 5H_2O$ 0.001% (pH 6.7 before sterilization). After 48 hours of fermentation an addition of sodium barbiturate (5%, w/v, aqueous suspension) was made in order to reach a 4 g/liter concentration, and the fermentation was continued for a further 120 hours period.

Isolation

Early analytical work was performed on the organic phase obtained upon sonication (1 minute, 25°C, pH 7.5) of broth samples diluted with two volumes of a $CHCl_3$-MeOH mixture 9:1. Bioactive anthracyclines in the organic phase were monitored by bioautography with *Micrococcus luteus* (strain ATCC 9341) after chromatography on thin silica gel plates (Merck F254) developed with $CHCl_3$ - MeOH - toluene

(7 : 3 : 3). Major active anthracyclines, designated FCE 21424 (**2**), FCE 24366 (**3**) and FCE 24367 (**4**), were found to occur at Rf values of 0.55, 0.65 and 0.60 upon addition of the sodium salts of barbituric acid, its 1,3-*N*,*N*-dimethyl- and 2-thio analog respectively.

The broth (4 liters) of *S. peucetius* cultured in the presence of sodium barbiturate was filtered. Red pigments were extracted from mycelium with acetone and from filtered broth (pH 7.5) with ethyl acetate. Combined organic extracts were subjected to silica gel column chromatography using a mixture of $CHCl_3$ - MeOH - toluene (7 : 3 : 3) as eluant. Concentration of selected fractions gave pure FCE 21424 (**2**, 50 mg). Compound **2** was also isolated in comparable yields from cultured broths of *S. peucetius* mutant strains.

Following the same procedure, FCE 24366 (**3**) and FCE 24367 (**4**) were obtained in yields comparable to that of **2** from cultured broths of both *S. peucetius* and its mutant strains upon addition of sodium 1,3-*N*,*N*-dimethylbarbiturate and sodium 2-thiobarbiturate respectively.

Physico-chemical Properties

Anthracyclines FCE 21424, FCE 24366 and FCE 24367 were obtained as fine red needles after crystallization from ethyl acetate. The compounds are soluble in acetone, dioxane, *N*,*N*-dimethylformamide, *N*,*N*-dimethyl sulfoxide, slightly soluble in chloroform, dichloromethane, ethyl acetate and lower alcohols, but practically insoluble in *n*-hexane and water. The novel anthracyclines are unstable in acidic media. Selected physico-chemical properties are listed in Table 1.

Structure Assignment

Daunorubicin (**1**) and 7-deoxydaunomycinone, both identified by direct comparison of their IR, UV, ^{1}H NMR and MS[7], were obtained respectively after mild acid hydrolysis (dioxane - 0.1 N aqueous acetic acid (1 : 1) at 85°C for 2 hours) and after hydrogenolysis (dioxane with 5% palladium charcoal - barium sulfate at 25°C for 1 hour) from **2**, **3** and **4**.

The sugar moiety obtained upon hydrogenolysis of **2** was hydrolyzed (0.1 N acetic acid at 85°C for 2 hours) to give a mixture of hydrolysis products among which daunosamine[7] and barbituric acid were isolated and identified by direct comparison with authentic samples. After treatment with 2,4-dinitrophenylhydrazine, the 2,4-dinitrophenylhydrazone of crotonaldehyde was also obtained and identified by direct comparison (TLC and MS) with an authentic sample.

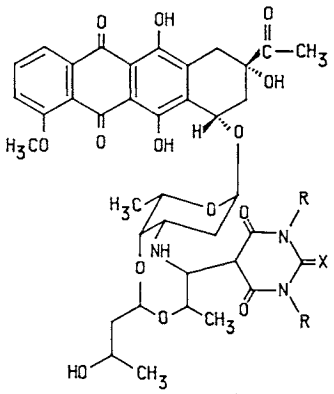
In addition to daunosamine and crotonaldehyde (as its 2,4-dinitrophenylhydrazone), 1,3-*N*,*N*-dimethyl-

Table 1. Physico-chemical properties of new anthracyclines.

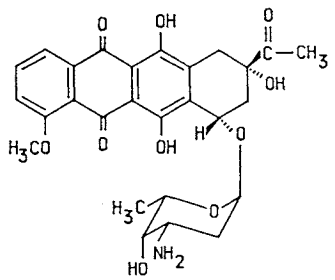
Properties	FCE 21424 (**2**)	FCE 24366 (**3**)	FCE 24367 (**4**)
MP (°C, dec)	205～208	195～196	210～211
$[\alpha]_D^{23}$ (*c* 0.1, MeOH)	+340°	ND	ND
UV and VIS λ_{max}^{MeOH} nm ($E_{1\,cm}^{1\%}$)	236 (350), 255 (395), 293 (67), 480 (148), 496 (150), 534 (107)	234 (500), 254 (425), 290 (110), 480 (144), 496 (146), 530 (85)	234 (450), 254 (385), 290 (90), 480 (145), 496 (145), 530 (80)
IR (KBr) cm^{-1}	3430, 1715, 1600, 1450, 1420, 1380, 1230, 1225, 1120, 1070, 1040, 990	3430, 1720, 1680, 1590, 1450, 1380, 1360, 1290, 1230, 1215, 1120, 1090, 1060, 1040, 995	3440, 1715, 1615, 1595, 1520, 1450, 1420, 1380, 1360, 1290, 1240, 1215, 1190, 1120, 1030, 990
MW (FAB-MS) *m/z* (MH^+)	782	810	798
Molecular formula	$C_{38}H_{43}N_3O_{15}$	$C_{40}H_{47}N_3O_{15}$	$C_{38}H_{43}N_3O_{14}S$

ND: Not determined.

Fig. 1. Structures of the new anthracyclines.



FCE 21424 (**2**) R=H X=O
FCE 24366 (**3**) R=CH_3 X=O
FCE 24367 (**4**) R=H X=S



Daunorubicin (**1**)

Table 2. ^{13}C NMR chemical shifts of **3**.

Carbon	[a]	[b]	Carbon	[a]	[b]
Aglycone moiety			Daunosamine moiety		
1	118.8	118.3	1′	99.5	99.3
2	136.0	135.7	2′	29.0	29.8
3	119.5	119.8	3′	49.3	49.3
4	160.6	160.9	4′	74.6	75.1
4a	119.8	120.6	5′	64.4	64.3
5	186.3	(186.4)[c]	6′	16.6	16.5
5a	110.6	111.3	Acetal moiety		
6	155.9	156.2	1″	106.1	107.4
6a	(135.1)[c]	(135.4)[c]	2″	44.6	43.8
7	70.9	70.0	3″	62.6	62.8
8	36.2	35.0	4″	[23.9][c]	[23.6][c]
9	75.0	75.0	5″	73.6	75.1
10	31.4	33.2	6″	60.2	65.1
10a	(135.5)[c]	(134.4)[c]	7″	21.0	21.1
11	154.2	155.6	Barbiturate moiety		
11a	110.6	111.3	CH_3-N-1‴	26.7	27.2
12	186.3	(187.0)[c]	CH_3-N-3‴	27.1	27.5
12a	(134.5)[c]	(133.6)[c]	2‴	152.4	152.5
13	211.6	212.0	4‴	162.6	164.2
14	[23.8][c]	[24.2][c]	6‴	163.3	165.5
4-OCH_3	56.4	56.6	5‴	76.5[d]	58.4[d]

[a] In DMSO-d_6 at 50 MHz. Assignments based on 2D-heteronuclear correlation experiment.
[b] In $CDCl_3$ at 50 MHz.
[c] Assignments can be exchanged within the same parenthesis.
[d] Singlet in off resonance experiments.

and 2-thio-barbituric acid were identified among the hydrolysis products of the sugar moiety of **3** and **4** respectively. Among the acidic hydrolysis products of carminomycins II and III[8], rubeomycins B and B_1[9], crotonaldehyde was previously identified and considered as a degradation product in acidic medium of 3-hydroxybutanal, the common constituent of the acetal moiety of known baumycin-like anthracyclines[10,11]. Data from chemical degradation indicated for the new anthracyclines **2**~**4** a common baumycin-like structure incorporating different barbituric acids in their structure, as shown in Fig. 1.

In fact consideration of ^{13}C and ^{1}H NMR data (shown in Tables 2 and 3) suggested the presence of the acetal chain found in baumycins A_1, A_2, B_1, B_2[10,11] and in related rubeomycins A, A_1[9], but with a difference at C-6″, which was now identified as the methine carbon at δ 60.3 (**3**, DMSO). Its corresponding hydrogen was a doublet in the range 4.3~4.5 ppm with a coupling constant of about 11 Hz with the vicinal 5″-H. This characteristic value suggested the presence of a 8-membered ring, as depicted in structures **2**~**4**, considering also that the chemical shift of C-3′ of the daunosamine moiety was indicative of a substitution on the nitrogen.

The site of attachment of the barbiturate ring was at C-5‴, since only strictly similar products (**2**~**4**) were obtained with different barbiturates, and the signal of the three carbonyl carbons of the ring were found in the expected range[12~15]. For compounds **2**~**4**, 5‴-H was detected in DMSO in the 7~8 ppm

Table 3. ^{1}H NMR chemical shifts[a] for compounds **2**~**4**.

	3		**2**	**4**
	$CDCl_3$	DMSO-d_6[b]	DMSO-d_6	DMSO-d_6
Aglycone moiety				
1-H	7.96 (8.0)	7.86	7.89	7.88
2-H	7.74 (8.0, 8.0)	7.62	7.64	7.62
3-H	7.34 (8.0)	7.86	7.89	7.88
4-OCH_3	4.02	3.94	3.96	3.95
7-H	5.14	4.88	4.76	4.89
8-H	2.1~2.3	2.09	1.7~2.1	2.20
9-OH	4.11	5.46	5.69	5.68
10-H	3.17, 2.81 (18.8)	2.95	3.06, 2.82 (18.2)	3.03, 2.84 (18.0)
13-CH_3	2.39	2.22	2.19	2.20
6-OH	13.94	13.97	14.04	ND
11-OH	13.15	13.18	13.25	ND
Daunosamine moiety				
1′-H	5.52	5.20	5.22	5.22
2′-H	1.8~2.1	1.7~1.9	1.7~2.1	1.6~1.9
3′-H	4.05	3.35	[c]	3.40
4′-H	4.88	4.74	4.76	4.70
5′-H	4.25 (6.4)	4.14 (6.6)	4.02 (6.7)	4.08 (6.5)
6′-CH_3	1.32 (6.4)	1.15 (6.6)	1.13 (6.7)	1.14 (6.5)
Acetal moiety				
1″-H	4.94	4.80	4.9~5.1	4.77
2″-H	1.8~2.1	1.7~1.9	1.7~2.1	1.6~1.9
3″-H	3.65	3.75	3.78	3.75
3″-OH	ND	4.54 (4.8)	4.55 (4.8)	4.56 (5.0)
4″-CH_3	1.25 (6.0)	1.08 (6.7)	1.08 (6.1)	1.06 (6.5)
5″-H	5.40	5.06 (4.8, 11.0)	4.9~5.1	4.95
6″-H	4.57 (10.7)	4.47 (11.0)	4.34 (11.0)	4.31 (10.7)
7″-CH_3	1.16 (5.7)	0.96 (4.8)	0.97 (5.7)	0.99 (5.0)
Barbiturate moiety				
N-1‴, N-3‴	3.11	2.97	9.32	
Substitution	2.88	2.97	9.12	10.4~10.7
5‴-H[d]	ND	8.38	7.64	7.80
		7.45	7.50	7.60

[a] Spectra recorded at 200 MHz. Coupling constants in parenthesis.
[b] Assignments in DMSO based on 2D-homocorrelation experiment.
[c] Overlap by H_2O from solvent.
[d] Seen as enolic form; each signal integrates for 0.5 H.
ND: Not determined.

region as two broad signals, each integrating for half-hydrogen (Table 3). This indicates the predominance of the enolic form of the barbiturate ring in the polar solvent as expected[16]. In $CDCl_3$ the triketo form must exist in rapid equilibrium with the enolic form, since 5‴-H is never detected and the corresponding C-5‴ is dramatically shielded (δ 58.4, **3**) with respect to its position in DMSO (δ 76.4), and appeared as a singlet in off-resonance experiments in both solvents. A study[17] on the keto-enol tautomerism in barbituric acid derivatives proved that in the 5-unsubstituted-*N*-monoalkyl (or *N*,*N*-dialkyl)-barbituric acids only the 2-thio derivatives show extensive tautomerization toward a predominant enol form. All other derivatives are present exclusively as the triketo form in polar and apolar solvents alike. It is likely that substitution at position 5 of the barbiturate ring changes significantly the position of the equilibrium. In fact our ^{13}C NMR findings for **3** clearly support the presence of a tautomeric equilibrium, shifted toward the enol form in DMSO and toward the triketo form in $CDCl_3$, as seen from the relative shieldings of C-5‴.

Moreover, since the change in electron density at C-5‴ in changing solvent polarity was directly reflected in the shielding of C-6″ (δ 60.2 in DMSO, δ 65.1 in $CDCl_3$, **3**), this carbon was firmly established as the site of attachment of the acetal chain to the barbiturate ring. The difference in chemical shifts observed in **3** (DMSO, $CDCl_3$) between substituents at N-1‴ and N-3‴ and between carbons 4‴ and 6‴ (Table 2) is not due to chirality at C-5‴, since the planar enolic form is always present, if not the predominant one. In fact magnetic non-equivalence in the presence of a chiral substituent at C-5 of a barbiturate ring seems to be generalized only when the triketo form is the only one present [15], and was observed only in the pure neutral species for *C*-glycosyl barbiturates[18]. In our case the symmetry induced by the enolic form is overcome by the strong asymmetry of the environment.

FD-MS of compounds **2**~**4** did not show the molecular ion owing to its thermal lability; for all compounds the ion with the highest mass was at *m*/*z* 653, corresponding to the loss of the barbiturate moiety from the molecular ion, and probably having a cyclic imine structure as already shown for barminomycins I and II[19]. The value of the molecular weight was obtained by FAB-MS. The data are reported in Table 4. The most intense peak for all compounds was at *m*/*z* 321, as already seen in the

Table 4. Positive ion FAB-MS data of compounds **2**~**4** (*m*/*z* (relative intensity)).

FCE 21424 (**2**):	782 (2, MH^+), 598 (2), 398 (4), 382 (8), 364 (9), 363 (21), 339 (14), 337 (12), 321 (100), 218 (13), 200 (27), 167 (55), 147 (19), 130 (9), 113 (14)
FCE 24366 (**3**):	810 (1, MH^+), 598 (3), 398 (3), 382 (6), 364 (7), 363 (18), 339 (13), 337 (10), 321 (100), 218 (11), 200 (37), 195 (70), 147 (17), 130 (9), 113 (17)
FCE 24367 (**4**):	798 (3, MH^+), 598 (2), 398 (4), 382 (8), 364 (8), 363 (17), 339 (13), 337 (12), 321 (100), 218 (33), 200 (86), 183 (91), 147 (17), 130 (25), 113 (46)

Table 5. Antibacterial activity.

Test organism	MIC (μg/ml)			
	Daunorubicin	FCE 21424	FCE 24366	FCE 24367
Staphylococcus aureus FDA 209P	6.25	12.5	0.78	6.25
S. aureus Smith	25	12.5	0.78	6.25
Micrococcus luteus ATCC 9341	1.56	1.56	0.19	0.78
Streptococcus pyogenes ATCC 12384	3.12	6.25	3.12	6.25
S. faecium ATCC 8043	>100	>100	12.5	100
Klebsiella pneumoniae ATCC 10031	>100	50	0.78	25
Escherichia coli 02636	6.25	12.5	1.56	12.5
Mycobacterium phlei ATCC 354	25	25	6.25	12.5

Table 6. Effect of FCE 21424 (**2**), FCE 24366 (**3**), FCE 24367 (**4**), doxorubicin and daunorubicin on the growth of HeLa, P388/S and P388/R cell cultures.

Compound	IC_{50} (ng/ml)		
	HeLa[a]	P388/S[b]	P388/R[b]
FCE 21424 (**2**)	0.23	0.07	2.5
FCE 24366 (**3**)	0.09	0.035	1.2
FCE 24367 (**4**)	0.7	0.003	3.8
Doxorubicin	18	9.2	350
Daunorubicin (**1**)	19	9.5	750

[a] Cells are exposed to the drugs for 24 hours then plated.
[b] Cells are exposed to the drugs for 48 hours then plated.

Table 7. Antitumor activity of the new anthracyclines against P388 ascitic leukemia in mice[a].

Compound	Dose (mg/kg)[b]	T/C (%)[c]	Toxic deaths[d]
FCE 21424 (**2**)	0.06	140	0/10
	0.12	145	0/10
	0.25	145	0/10
	0.50	160	0/10
FCE 24366 (**3**)	0.012	130	0/10
	0.025	135	0/10
	0.05	150	0/10
	0.1	165	1/10
FCE 24367 (**4**)	0.3	145	0/10
	0.45	150	1/10
	0.68	155	1/10
Daunorubicin (**1**)	2.9	160	0/10
	4.4	150	7/10

[a] Mice where treated ip on day 1 after tumor cell inoculation.
[b] Administered in a Tween 80 - water (1 : 9) suspension.
[c] Median survival time expressed as percentage of untreated controls.
[d] Evaluated on the basis of macroscopic autoptic fundings.

FAB-MS of anthracyclines[20] but very intense ions, deriving from the barbiturate moiety, were also present at *m/z* 167, 195 and 183 for **2**, **3** and **4**, respectively.

Other fragments derive from daunomycinone (*m/z* 398, 382, 364, 363, 339, 337), from daunosamine (*m/z* 147, 130, 113) and from the daunomycin moiety bearing the C-1″–C-4″ acetal chain (*m/z* 598), or from the latter bound to daunosamine (*m/z* 218 accompanied by its monoanhydrous derivative at *m/z* 200).

Biological Activity

The antimicrobial activity of the new anthracyclines was compared to that of daunorubicin using the standard tube dilution procedure. The results are shown in Table 5. The cytotoxic activity of anthracyclines **2**, **3**, **4**, daunorubicin (**1**) and doxorubicin was compared *in vitro* on cultured HeLa cells and on two different murine P388 leukemia cell lines, one sensitive (P388/S) and one resistant (P388/R) to doxorubicin. As shown in Table 6 the new anthracyclines **2**, **3** and **4** were found to be cytotoxic for HeLa and P388/S cell lines at dosage levels several times lower than those of daunorubicin and doxorubicin, being also active against P388/R cell line which is cross-resistant to most anthracyclines.

In preliminary *in vivo* experiments against P388 ascitic leukemia in mice, reported in Table 7, the new anthracyclines were found as active as daunorubicin at dosage levels several times lower.

Discussion

Previous studies described the effect of barbituric acids on anthracycline biosynthesis by *Streptomyces coeruleorubidus*, *S. peucetius* and *Streptomyces galilaeus*[21,22] and on rifamycin biosynthesis by *Streptomyces mediterranei*[23] (later renamed *Nocardia mediterranei*); however to our knowledge no example of 5-unsubstituted barbiturate incorporation into microbial metabolites has been reported. Recently barminomycins I and II[19] and SN-07 chromophore[24] have been described and found to differ from other baumycin-like anthracyclines by the presence of a CHO group at C-5″ in the acetal moiety. These compounds have been considered to be in equilibrium with cyclic carbinolamine and imine forms. These findings and the present work allow us to consider the new anthracyclines **2**~**4** as deriving from the reaction of a

biosynthetic intermediate, such as 4-*O*-methylbarminomycin, in the cyclic carbinolamine form with a 5-unsubstituted barbituric acid, both present in the cultural media. In conclusion the present report suggests that in addition to microbial fermentation and/or biotransformation, hybrid biosynthesis is also capable of discovering new classes of anthracyclines endowed with unusual structural features and biological activity.

Experimental

TLC was performed on precoated glass plates (0.25 mm) of Silica gel 60F-254 (Merck, Darmstadt, FRG). MP's, obtained with a SMP-20 apparatus (Büchi), are uncorrected. Optical rotations were measured with a Jasco-DIP 140 polarimeter in 10 cm tube. IR spectra were recorded with a Perkin-Elmer Model 457 spectrophotometer. UV/VIS spectra were run on a Spectracomp 601 (C. Erba Strumentazione) spectrophotometer.

NMR spectra were recorded on a XL-200 Varian spectrometer operating at 200 MHz for proton and at 50 MHz for carbon observations.

Chemical shifts are expressed in δ (ppm) from zero TMS. All proton assignments were checked by suitable homonuclear decoupling experiments. Assignments for **3** in DMSO-d_6 are based on 2D-homonuclear correlation and 2D-heteronuclear ^{13}C-^{1}H experiments run at 35°C (Fig. 3).

FD-MS were recorded on a Varian Mat 311-A mass spectrometer equipped with a combined field ionization (FI)/FD/EI ion source using benzonitrile activated emitters. The voltage difference between the field emitter anode and the cathode was about 9 kV. The emitter heating current was in the range 16~30 mA and the source temperature was 200°C. FAB-MS were recorded on a VG Analytical 70-70 EQ mass spectrometer fitted with its own standard FAB ion source employing xenon atoms of 8 keV kinetic energy. The samples were dissolved in a mixture of glycerol and thioglycerol. Normalization of the FAB spectra has been carried out from the value of 100 *m/z*.

Isolation

The whole broth (4 liters) from a fermentation of *S. peucetius* (strain ATCC 29050) obtained after addition of sodium barbiturate was filtered at pH 7.5 using diatomaceous earth (3% w/v) as filter aid. The wet filter cake was extracted with three 3-liter portions of acetone. The acetone extracts were concentrated to 1 liter and extracted with two 1-liter portions of ethyl acetate at pH 7.5. The filtered broth was extracted by the same procedure and all the ethyl acetate extracts were combined, dried over anhydrous sodium sulfate and concentrated to a small volume (50 ml). By addition of *n*-hexane (250 ml) FCE 21424 was obtained as a crude brown purple powder (0.1 g). The crude material, dissolved in a $CHCl_3$-MeOH-toluene (7:3:3) mixture, was chromatographed on a silica gel dry column using the above mentioned mixture as eluant. Selected fractions picked by TLC, were concentrated to a small volume, and a 5-fold volume of *n*-hexane was added, gave pure FCE 21424 (0.05 g) that after crystallization from ethyl acetate was obtained as fine red needles: MP 205~208°C (dec); $[\alpha]_D^{23}$ +340° (*c* 0.1, MeOH); UV and IR spectra

Fig. 2. FAB-MS of compound **4**.

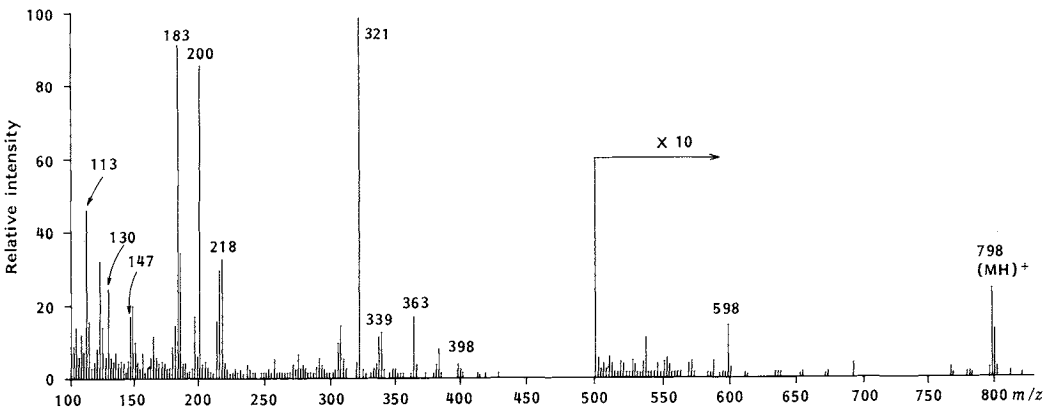
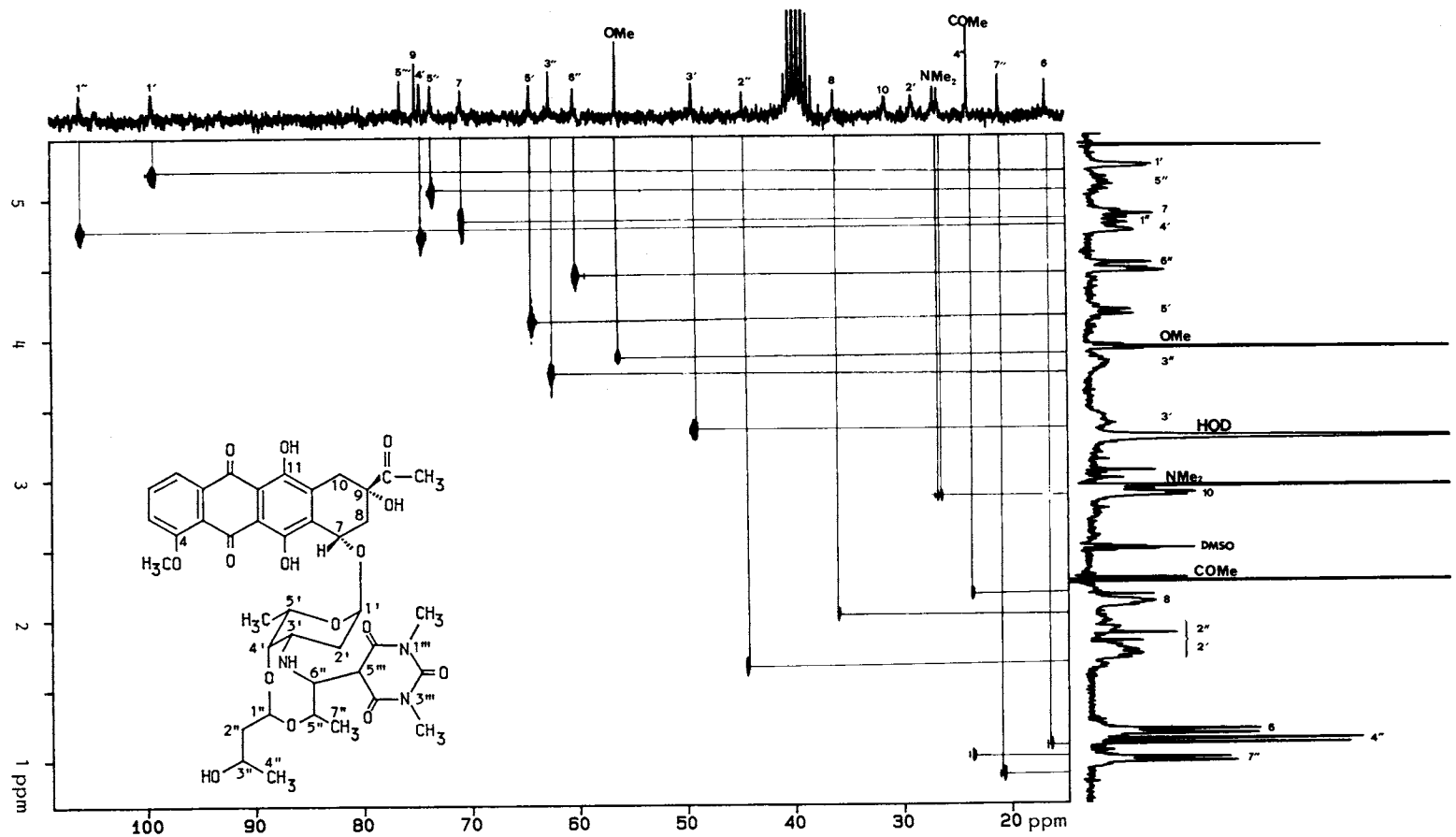


Fig. 3. 2D-Heterocorrelation map for compound **3** (aliphatic region).



are reported in Table 1.

Anal Calcd for $C_{38}H_{43}N_3O_{15}\cdot 3\frac{1}{2}H_2O$: C 54.02, H 5.97, N 4.97.
Found: C 54.18, H 5.94, N 4.95.

FCE 21424 (**2**) was also isolated in comparable yields from cultured broths of *S. peucetius* mutant strains.

Following the same procedure FCE 24366 (**3**) and FCE 24367 (**4**) were isolated in yield comparable to that of **2** from cultured broths of both *S. peucetius* and its mutants upon addition of sodium 1,3-*N*,*N*-dimethylbarbiturate and sodium 2-thiobarbiturate respectively. Physico-chemical properties of compounds **3** and **4** are reported in Table 1.

Hydrolysis

A solution of FCE 21424 (0.1 g) in dioxan (2 ml) and 0.1 N aqueous acetic acid (2 ml) was heated under stirring for 2 hours at 85°C. The reaction mixture, diluted with water (10 ml) was adjusted to pH 8.5 (0.1 N NaOH) and extracted with $CHCl_3$. The extract, washed with water and dried on anhydrous sodium sulfate, was concentrated to a small volume. Addition of methanolic 0.5 N HCl gave a red crystalline compound (0.05 g): MP 186~187°C (dec), identical in mp, TLC, IR, UV, NMR and MS with daunorubicin·HCl (**1**).

Following the same procedure **1** was obtained by acid hydrolysis of both FCE 24366 (**3**) and FCE 24367 (**4**).

Hydrogenolysis

A solution of FCE 21424 (0.2 g) in dioxan (20 ml) was hydrogenated in the presence of 5% palladium charcoal-barium sulfate (1 g) for 1 hour at 25°C. The reaction mixture was filtered, diluted with water (20 ml) and extracted with $CHCl_3$. The organic phase after concentration to dryness gave a red crystalline residue (0.06 g), mp 228~230°C, identified by mp, TLC, IR, UV, NMR and MS as 7-deoxydaunomycinone.

The almost colorless aqueous phase, containing the sugar moiety, was treated with N aqueous acetic acid (5 ml), then heated at 85°C for 2 hours under stirring. Daunosamine and barbituric acid in the reaction mixture were identified by direct comparison with authentic samples on TLC (PrOH-EtOAc-H_2O-NH_4OH, 7:1:3:1). When an aliquot of the reaction mixture was treated with a concentrated solution of 2,4-dinitrophenylhydrazine in 2 N HCl, and stirred for 2 hours at 25°C, a yellow-red precipitate was obtained and identified as crotonaldehyde 2,4-dinitrophenylhydrazone by comparison (TLC and MS) with an authentic sample.

Following hydrogenolysis and mild acid hydrolysis, FCE 24366 (**3**) and FCE 24367 (**4**) gave the same degradation products but different barbituric acids, namely 1,3-*N*,*N*-dimethyl- and 2-thio-barbituric acid, respectively.

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