CHEMICAL MODIFICATION OF STEFFIMYCIN B

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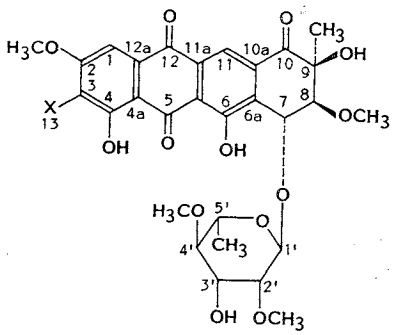
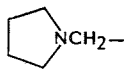
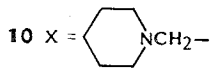
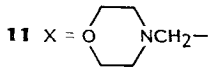
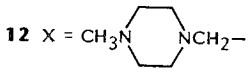
Fifteen 3-substituted analogues of steffimycin B (**1**) have been synthesized and their activity against P388 murine leukemia has been determined. Three of these were substantially more active than the parent compound.

Steffimycin and steffimycin B (**1**) were isolated some years ago,[1,2] and their structures were established by WILEY *et al.*[3] and ARARA.[4] These compounds are anthracycline antibiotics, but they are unusual in this family since they do not contain an amino sugar moiety. The steffimycins have only borderline antitumor activity against P388 murine leukemia while almost all of the anthracyclines containing nitrogen are active in this assay. Experiments in our laboratory had yielded 3-iodo-**1** as an unexpected product. These results were interpreted to confirm that the presence of a hydroxyl group and a methoxyl group *ortho* to the 3 position in **1** activated that position to electrophilic substitution. This suggested ways of introducing other halogens, nitrogen-containing substituents, and other substituents at that position. Such analogues (especially the nitrogen-containing ones) might have enhanced anti-leukemic activity relative to **1**. It was found that such substitution occurred, and the 3-substituent analogues described in this paper were prepared.

Fig. 1.



1 X = H
2 X = $HOCH_2$—
3 X = OHC—
4 X = HON=CH—
5 X = CH_3ON=CH—
6 X = C_6H_5NHN=CH—
7 X = $(CH_3)_2NCH_2$—
8 X = $(n\text{-}C_4H_9)_2NCH_2$—
9 X = 

10 X = 
11 X = 
12 X = 
13 X = F
14 X = Cl
15 X = Br
16 X = I

Chemical

Nitrogen-containing analogues of **1** were prepared in two series, carbonyl (**4**~**6**) and basic amino derivatives (**7**~**12**). It was considered that a formyl group, which could then be converted to nitrogen-containing substituents, could be introduced into position 3 by direct introduction, but this did not prove to be the case necessitating an indirect route. This route consisted of treating **1** with CH_2O under basic conditions to prepare **2** which was then oxidized to **3**. In both cases, but especially the latter, yields were poor. Oxidation was not achieved by the usual benzylic hydroxyl oxidation procedures but re-

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Table 1. ^{13}C Chemical shift assignments of **1**~**6** and **13**~**16**.

Position No.	**1**	**2**	**3**	**4**	**5**	**6**	**13**	**14**	**15**	**16**
1	109.67	104.19	102.86	102.89	103.14	102.41	105.65	104.17	104.14	103.28
2	168.16	164.94	167.57	163.48	164.91	162.20	154.90	162.46	163.66	166.01
3	108.22	122.33	118.72	118.56	119.95	119.56	137.98	110.88	107.19	84.72
4	166.28	162.49	166.49	162.01	162.58	161.89	152.02	162.31	162.32	163.44
4a	111.50	110.48	112.18	111.51	112.16	104.15	112.08	116.49	110.90	110.02
5	191.03	190.81	188.98	189.11	189.52	189.01	191.59	190.97	190.90	190.30
5a	119.92	118.35	117.42	116.69	117.15	116.57	118.63	118.30	118.32	117.91
6	162.86	162.12	162.51	161.77	162.41	161.44	162.36	159.93	161.12	162.12
6a	134.38	133.84	132.57	132.54	132.92	133.04	129.52	132.44	133.10	132.87
7	73.13	71.96	72.00	71.75	72.11	77.45	71.95	71.68	71.96	71.89
8	87.55	85.78	85.77	85.53	86.36	86.04	85.70	85.64	85.66	85.53
9	77.71	76.71	76.74	76.31	76.75	76.77	76.80	76.70	76.75	76.53
10	200.04	198.99	198.98	198.73	199.09	199.29	198.98	199.90	198.90	198.73
10a	136.97	135.71	139.74	135.09	135.79	134.82	136.02	135.96	135.99	135.81
11	116.94	117.45	114.89	112.73	112.57	113.29	117.94	117.75	117.80	113.49
11a	136.09	134.31	135.63	135.09	135.46	133.25	133.88	133.98	134.00	134.71
12	181.41	179.92	180.17	180.09	180.37	180.91	179.66	179.78	179.99	179.76
12a	134.71	134.19	134.46	133.48	134.04	133.13	133.24	133.01	133.55	133.87
13		53.23	190.54	143.74	147.77	142.80				
1′	102.36	100.82	100.78	100.56	100.79	100.79	100.72	100.68	100.71	100.59
2′	82.53	80.60	80.54	80.86	80.60	80.67	80.55	80.47	80.50	80.36
3′	71.87	71.24	71.30	70.66	71.32	71.23	71.36	71.25	71.31	71.11
4′	83.98	83.31	83.34	82.89	83.99	83.37	83.37	83.29	83.33	83.16
5′	70.35	69.13	69.09	66.75	69.08	69.07	69.60	69.08	69.10	68.91
9-CH_3	24.88	22.93	22.85	22.43	22.96	23.04	22.93	22.84	22.91	22.75
5′-CH_3	17.98	17.98	17.96	17.49	17.98	17.94	18.04	17.92	17.79	17.79
2′-OCH_3	61.57	60.90	60.97	60.53	60.36	60.92	61.08	60.99	60.96	60.70
8-OCH_3	61.34	60.03	60.04	59.56	60.04	60.11	60.10	60.00	60.03	59.86
4′-OCH_3	60.14	58.93	58.91	58.51	58.38	58.84	58.98	58.94	58.92	58.77
2-OCH_3	58.01	56.75	57.32	56.52	56.75	56.67	57.21	57.31	57.42	57.36
3-$CHNOCH_3$					62.70					
1″						159.10				
2″						112.46				
3″						129.57				
4″						121.46				
5″						129.57				
6″						112.46				

In ppm (δ) obtained from $CDCl_3$ solutions except for **1** and **6** which were in CD_3SOCD_3 and $CDCl_3$-CD_3OD, respectively. Trimethylsilane was the internal reference.

quired pyridinium chlorochromate. Compound **3** was then converted to its carbonyl derivatives by the usual procedures. Selectivity was not a problem as the carbonyl at C-10 reacts very sluggishly with the usual carbonyl reagents. That **2** and compounds derived from it have a substituent at C-3 is shown by their ^1H NMR spectra. In the ^1H NMR spectrum of **1** chemical shifts of δ 6.75 and δ 7.08 present as doublets are assigned to 3-H and 1-H. In compounds **2**~**5** the resonance for the higher field proton has disappeared. A singlet at δ 7.36~7.42 is present and must arise from 1-H. Since the 3-H proton is no longer present the substituents must be at that position. Substitution at C-3 in the other two series of compounds rests on the same argument.

The Mannich reaction was used to introduce dialkylaminomethyl groups into **1** at C-3. Compounds **7**~**12** were prepared in this way. Yields were poor in all cases and purification was quite difficult.

Table 2. ^{13}C Chemical shift assignments of **7**~**12**.

Position No.	7	8	9	10	11	12
1	112.49	112.75	112.24	112.73	111.10	110.52
2	163.45	166.07	165.39	165.81	165.13	163.50
3	103.02	101.94	102.3	101.85	103.55	102.06
4	162.26	163.59	163.71	163.32	163.57	162.79
4a	115.62	115.19	116.51	116.10	117.09	115.79
5	189.84	189.94	189.13	188.56	190.21	188.64
5a	118.64	116.59	119.39	119.61	118.81	117.96
6	162.04	162.3	162.45	162.37	162.61	161.23
6a	132.56	133.18	133.15	133.07	133.08	132.01
7	71.72	72.16	72.15	72.14	72.10	71.10
8	85.58	85.89	86.00	85.99	85.90	84.91
9	76.42	76.71	76.72	76.68	76.72	75.67
10	198.82	197.28	199.29	199.32	199.04	198.07
10a	135.12	135.76	135.12	135.17	135.49	134.23
11	116.87	116.59	117.07	116.26	118.62	117.02
11a	133.34	134.79	134.94	134.62	134.44	133.56
12	180.33	181.58	181.28	181.74	180.40	179.62
12a	132.84	135.58	133.67	133.48	133.78	132.71
13	50.44	49.00	49.02	53.52	50.19	49.52
1′	100.42	100.65	100.78	100.69	100.86	99.86
2′	80.34	80.56	80.71	80.67	80.73	79.83
3′	70.94	71.28	71.78	71.21	71.29	70.09
4′	83.08	83.97	83.38	83.33	83.36	82.70
5′	68.72	68.83	69.01	68.92	69.13	68.10
9-CH_3	22.72	22.98	23.05	23.00	23.04	22.05
5′-CH_3	17.69	17.94	17.97	17.94	18.00	16.92
2′-OCH_3	60.60	60.91	60.84	60.86	60.84	59.79
8-OCH_3	59.67	59.94	59.99	59.91	60.04	58.94
4′-OCH_3	58.59	58.84	58.84	58.82	58.89	57.84
2-OCH_3	56.55	56.43	56.43	56.30	56.57	55.42
1″	44.02	53.29	53.75	53.82	53.42	53.65
2″		27.06	23.60	25.18	66.85	51.67
3″		20.46		23.43		
4″		13.79				
CH_3N						44.72

In ppm (δ) obtained from $CDCl_3$ solutions containing trimethylsilane as internal reference. Carbon atoms in the R_2n groups are given the same number in both R's.

A number of other procedures for purifying these compounds other than those mentioned in the experimental were tried. None were superior to those actually used, and most were inferior.

Halogenation of **1** to give **13**~**16** was done by a variety of procedures differing in each case. Fluorination was done with CF_3OF, chlorination with $(CH_3)_3COCl$, bromination with elemental Br_2, and iodination by a periodic acid procedure similar to that of SUZUKI.[5]

Poor analytical values were obtained for a number of these compounds. Melt solvate assay results were interpreted to indicate solvent occlusion. However, high resolution mass spectra indicated quite clearly the molecular formulas proposed.

Biological

All of the compounds prepared except **6** retained the Gram-positive antibacterial activity of **1**.

The halogenated compounds and those compounds derived from 3-formyl **1** were inactive against P388 murine leukemia. However, there was considerable enhancement of the *in vivo* anti-leukemic activity in the case of three of the Mannich reaction products (Table 3). While **1** has, at most, borderline activity in this assay, compounds **9**, **10** and **12** showed substantial activity in the range of many of the anthracyclines containing amino sugars. For example the % T/C of daunorubicin has been reported to be 175.[6,7]

Table 3. Anti-leukemic activity of compounds **1**~**16**.

Compounds	Dose (mg/kg/day)	T/C (%)
1	400	110
2	50	118
3	50	125
4	100	122
5	100	105
6	100	110
7	25	120
8	25	120
9	25	160
10	25	156
11	50	130
12	50	160
13	400	105
14	400	115
15	400	100
16	400	100

P388 leukemia cells (10^6) were injected intraperitoneally on day 0, and drug was injected on days 1, 5 and 9.

Experimental

3-Hydroxymethylsteffimycin B (2)

A solution of 11.0 g (18.7 mmol) of **1** in 250 ml of 25% $(CH_3)_3N$ solution was stirred at room temp while adding dropwise 250 ml of 37% CH_2O solution. The reaction mixture was heated to 80°C for 4 hours, cooled to room temp and stirred for 16 hours. The reaction mixture was evaporated *in vacuo* to a volume of about 300 ml. After the addition of 500 ml of H_2O, the solution was adjusted to pH 6.0 with 1 N HCl and extracted with five 250-ml portions of CH_2Cl_2 - CH_3OH (9 : 1). The combined extracts were evaporated *in vacuo* to an oily residue which was dissolved in a small amount of CH_2Cl_2 - CH_3OH (9 : 1) to which was added 500 ml of Skellysolve B. The supernatant was decanted, and the residue was dried *in vacuo*. The residue was then chromatographed on 550 g of silica gel using first 3 liters of CH_2Cl_2, then 2.5 liters of CH_2Cl_2 - CH_3OH (95 : 5) before color appeared after which one hundred and fifty-five 20-ml fractions were collected. On the basis of TLC in $CHCl_3$ - CH_3OH (9 : 1; Rf 0.40) fractions 1~14 and fractions 15~45 were pooled. After evaporation of the pools *in vacuo* there was obtained 7.51 and 4.19 g, respectively, with the second pool being pure **2**. The first pool residue was chromatographed on 375 g of silica gel eluting with CH_2Cl_2 - CH_3OH (95 : 5) and collecting two hundred 6-ml fractions after color appeared. Fractions 73~150 were combined as **2** on the basis of TLC as above and evaporated *in vacuo*, yield 1.56 g. The total yield of pure **2** was 5.75 g (49%).

Five hundred mg of the second lot of **2** was dissolved in 5 ml of CH_2Cl_2. Four ml of cyclohexane was added slowly to the boiling solution. After the mixture had stood at room temp for 24 hours it was filtered to give 381 mg; mp 235~238°C; $[\alpha]_D^{25}$ +9.6° (*c* 0.237, $CHCl_3$); UV $\lambda_{max}^{CH_3OH}$ nm (ε) 216 (sh, 16,685), 233 (19,900), 280 (18,240), 439 (11,370); IR ν_{max} (Nujol) cm^{-1} 3440, 1750, 1660, 1595, 1550, 1425, 1365, 1310, 1250, 1080, 1045, 1010, 905, 860, 820, 760, 750; ^1H NMR ($CDCl_3$) δ 1.42 (3H, d), 1.52 (3H, s), 3.08 (1H, t), 3.57 (11H, s), 3.78 (2H, m), 4.03 (3H, s), 4.80 (2H, s), 5.19 (1H, d), 5.62 (1H, br s) 7.42 (1H, s), 8.31 (1H, s), 12.30 (1H, s), 12.90 (1H, s); fast atom bombardment mass spectrum (FAB-MS) *m/z* 618; calcd 618.

Anal Calcd for $C_{30}H_{34}O_{14}$: C 58.25, H 5.54.
Found: C 57.83, H 5.55.

3-Formylsteffimycin B (3)

A solution of 1.04 g (4.8 mmol) of finely ground pyridinium chlorochromate in 100 ml of spectroscopic grade CH_2Cl_2 was stirred, and a solution of 1.92 g (3.1 mmol) of **2** in 200 ml of spectroscopic grade CH_2Cl_2 was added. The solution was stirred at room temp for 4 hours and evaporated to dryness *in vacuo*. The residue was dissolved in 200 ml of CH_2Cl_2 - CH_3OH (9 : 1), and the solution was washed

with two 100-ml portions of 0.1 N HCl and 100 ml of H_2O. Evaporation *in vacuo* gave 1.77 g. This was chromatographed on 177 g of silica gel eluting with 800 ml of CH_2Cl_2, 900 ml of CH_2Cl_2 - CH_3OH (95 : 5), 850 ml of CH_2Cl_2 - CH_3OH (9 : 1) and collecting three hundred and fifty 5-ml fractions. On the basis of TLC in CH_2Cl_2 - CH_3OH - H_2O (78 : 20 : 2; Rf 0.55) fractions 231~420 were combined and evaporated *in vacuo*, weight 666 mg. The residue was dissolved in a mixture of 145 ml of CH_3OH - H_2O (13 : 16) and acidified to pH 2 with 1.0 N HCl. The CH_3OH was removed by evaporation, and the aqueous portion was extracted with three 50-ml portions of $CHCl_3$. Evaporation *in vacuo* of the combined extracts gave 482 mg whose TLC in the above system showed that it was mostly **3**.

One hundred and nine mg was subjected to countercurrent distribution in a 335-ml Ito Coil planetary centrifuge using the two phases of $CH_3C_6H_5$ - $CHCl_3$ - CH_3OH - H_2O (4 : 1 : 4 : 1) with the upper phase being used as the stationary phase and collecting sixty 10-ml fractions. Combination of fractions 32~37 on the basis of TLC in the above system and evaporation *in vacuo* gave 25 mg homogeneous by TLC; mp 265~267°C (dec); $[\alpha]_D^{25}$ +83° (*c* 0.248, $CHCl_3$); UV $\lambda_{max}^{CH_3OH}$ nm (ε) 232 (24,115), 279 (19,620), 441 (10,445); IR ν_{max} (Nujol) cm^{-1} 3460, 1710, 1695, 1690, 1644, 1595, 1475, 1375, 1320, 1240, 1200, 1185, 1090, 1010, 860, 765, 750; ^1H NMR ($CDCl_3$) δ 1.31 (3H, d), 1.45 (3H, s), 2.98 (1H, t), 3.48 (11H, s), 3.67 (1H, s), 4.06 (3H, s), 5.23 (1H, d), 5.55 (1H, br s), 7.42 (1H, s), 8.28 (1H, s), 10.4 (1H, s), 13.16 (2H, s); FAB-MS *m/z* 616; calcd 616.

Anal Calcd for $C_{30}H_{32}O_{14}$: C 58.43, H 5.24.
Found: C 58.26, H 5.52.

3-Formylsteffimycin B Oxime (**4**)

A mixture of 200 mg (0.32 mmol) of **3**, 26.3 mg (0.38 mmol) of $NH_2OH \cdot HCl$, 45 mg of Na_2CO_3, 50 ml of EtOH, and 50 ml of H_2O was stirred for 18 hours. The resulting solution was poured into 100 ml of H_2O, and the EtOH was removed by evaporation *in vacuo*. The aqueous was adjusted to pH 7.5 with 0.1 N HCl and extracted with five 50-ml portions of $CHCl_3$. The $CHCl_3$ extracts were combined and evaporated to dryness *in vacuo*, weight 181 mg. A portion of this (62 mg) was chromatographed on 19 g of silica gel eluting with $CHCl_3$ - CH_3OH (95 : 5) to give 40 mg. This was dissolved in 10-ml of $CHCl_3$ and washed with three 10-ml portions of H_2O. The $CHCl_3$ solution was evaporated *in vacuo* to give 14 mg; mp 170~183°C (dec); Rf 0.12 (TLC in $CHCl_3$ - CH_3OH (9 : 1)); $[\alpha]_D^{25}$ +128° (*c* 0.236, $CHCl_3$); UV $\lambda_{max}^{CH_3OH}$ nm (ε) 233 (20,790), 291 (16,815), 440 (7,470); IR ν_{max} (Nujol) cm^{-1} 3350, 1700, 1665, 1610, 1565, 1450, 1375, 1090, 1020, 750; ^1H NMR ($CDCl_3$) δ 1.40 (3H, d), 1.52 (3H, s), 3.10 (1H, t), 3.57 (11H, s), 3.74 (1H, d), 4.08 (3H, s), 5.19 (1H, d), 5.61 (1H, br s), 7.42 (1H, s), 8.25 (1H, s), 8.53 (1H, s); FAB-MS *m/z* 631.1896 (calcd for $C_{30}H_{33}NO_{14}$, 631.1981).

Anal Calcd for $C_{30}H_{33}NO_{14}$: C 57.05, H 5.27, N 2.22.
Found: C 55.28, H 5.16, N 2.26.

3-Formylsteffimycin B Methoxime (**5**)

A mixture of 200 mg (0.32 mmol) of **3**, 36 mg (0.43 mmol) of $CH_3ONH_2 \cdot HCl$, 212 mg of Na_2CO_3 and 100 ml of EtOH - H_2O (1 : 1) was stirred at room temp for 18 hours. The reaction mixture was poured into 100 ml of H_2O, and the solution was adjusted to pH 4.25 with 1 N HCl. Extraction with four 25-ml fraction portions of $CHCl_3$ and evaporation *in vacuo* gave 181 mg of product which was chromatographed on 36 g of silica gel eluting with $CHCl_3$ - CH_3OH (95 : 5) and collecting 4.5 ml fractions. Fractions 15~24 were combined on the basis of TLC in $CHCl_3$ - CH_3OH (9 : 1; Rf 0.44) and evaporated *in vacuo*. The residue was crystallized from 25 ml of EtOH to give 54 mg homogeneous by TLC; mp 108~122°C; $[\alpha]_D^{25}$ +13° (*c* 0.625, $CHCl_3$); UV $\lambda_{max}^{CH_3OH}$ nm (ε) 233 (23,860), 295 (29,410), 439 (12,900); IR ν_{max} (Nujol) cm^{-1} 3480, 1705, 1670, 1610, 1570, 1460, 1370, 1255, 1190, 1030, 760; ^1H NMR ($CDCl_3$) δ 1.41 (3H, d), 1.51 (3H, s), 3.05 (1H, t), 3.58 (11H, s), 3.69 (1H, s), 4.04 (3H, s), 5.16 (1H, br s), 5.56 (1H, br s), 7.36 (1H, s), 8.29 (1H, s), 8.45 (1H, s); FAB-MS *m/z* 645.2070 (calcd for $C_{31}H_{35}NO_{14}$, 645.2057).

Anal Calcd for $C_{31}H_{35}NO_{14}$: C 57.67, H 5.46, N 2.17.
Found: C 57.20, H 5.59, N 2.13.

3-Formylsteffimycin B Phenylhydrazone (**6**)

A solution of 1 ml of phenylhydrazine in 20 ml of CH_3OH was added to a solution of 200 mg

(0.32 mmol) of **3** in 20 ml of CH_3OH. One drop of CH_3COOH was added, and the solution was boiled for 12 minutes and poured into 50 ml of H_2O. The mixture was extracted with four 50-ml portions of $CHCl_3$. The combined extracts were evaporated *in vacuo* to give 240 mg which was chromatographed on 48 g of silica gel eluting with $CHCl_3$ - CH_3OH (95 : 5) and collecting one hundred and twenty 4-ml fractions. On the basis of TLC in $CHCl_3$ - CH_3OH (9 : 1; Rf 0.61) fractions 31~37 were combined and evaporated *in vacuo* giving 99 mg; mp 187~194°C; UV $\lambda_{max}^{CH_3OH}$ nm (ε) 202 (32,335), 234 (32,405), 263 (25,025), 282 (22,840), 384 (14,825), 436 (12,000); IR ν_{max} (Nujol) cm^{-1} 3440, 3375, 1700, 1660, 1600, 1540, 1455, 1375, 1320, 1250, 1215, 1090, 1025, 750; ^1H NMR ($CDCl_3$ - CD_3OD) δ 1.38 (3H, d), 1.49 (3H, s), 3.12 (1H, t), 3.62 (3H, s), 3.65 (6H, s), 3.75 (1H, d), 4.00 (2H, s), 5.18 (1H, d), 5.53 (1H, br s), 6.8~7.5 (6H, m), 8.23 (1H, s); FAB-MS *m/z* 706.2351 (calcd for $C_{36}H_{38}N_2O_{13}$, 706.2374).

Anal Calcd for $C_{36}H_{38}N_2O_{13}$: C 61.18, H 5.42, N 3.96.
Found: C 59.45, H 5.36, N 3.30.

3-Dimethylaminomethylsteffimycin B (**7**)

A solution of 11 g (17.8 mmol) of **1** in 250 ml of 26% $(CH_3)_2NH$ was stirred while adding dropwise 250 ml of 37% CH_2O solution. The reaction mixture was stirred at 80~85°C for 22 hours. Three hundred ml of H_2O was added, and the solution was adjusted to pH 6.2 with conc HCl. The solution was extracted with five 250-ml portions of $CHCl_3$. The combined extracts were evaporated *in vacuo* to a weight of about 7 g which was chromatographed on 300 g of silica gel eluting with $CHCl_3$ - CH_3OH (95 : 5). On the basis of TLC in $CHCl_3$ - CH_3OH - H_2O (78 : 20 : 2; Rf 0.43) those fractions containing **7** were combined and evaporated *in vacuo*, yield 527 mg; mp 134~137°C; $[\alpha]_D^{25}$ +119° (*c* 0.72, $CHCl_3$); UV $\lambda_{max}^{CH_3OH}$ nm (ε) 216 (sh, 27,930), 229 (28,380), 266 (23,800), 278 (23,235), 441 (9,805); IR ν_{max} (Nujol) cm^{-1} 3410, 1700, 1670, 1605, 1435, 1375, 1320, 1285, 1250, 1185, 1110, 1020, 915, 750; ^1H NMR ($CDCl_3$) δ 1.41 (3H, d), 1.52 (3H, s), 2.58 (6H, s), 3.14 (1H, t), 3.61 (11H, s), 3.82~3.90 (3H, d), 4.07 (3H, s), 5.25 (1H, br s), 5.86 (1H, br s), 7.34 (1H, s), 8.32 (1H, s); FAB-MS *m/z* 645.2413 (calcd for $C_{32}H_{39}NO_{13}$, 645.2421).

Anal Calcd for $C_{32}H_{39}NO_{13}$: C 59.53, H 6.09, N 2.17.
Found: C 57.90, H 6.29, N 1.74.

3-Di-*n*-butylaminomethylsteffimycin B (**8**)

A solution of 2 g (3.4 mmol) of **1** in 30 ml of $(n\text{-}C_4H_9)_2NH$ was stirred while adding dropwise 30 ml of 37% CH_2O. Fifty ml of EtOH was added, and the solution was heated at 75~80°C for 68 hours. The reaction mixture was evaporated *in vacuo* to a black oil. This was mixed with 100 ml of ether, and 500 ml of Skellysolve B was added. The supernatant was decanted, and the residue was dried *in vacuo*, weight 2.38 g. The residue was chromatographed on 224 g of silica gel eluting successively with 120 ml of $CHCl_3$ and 2 liters of $CHCl_3$ - CH_3OH (95 : 5). Those fractions containing **8** as determined by TLC in $CHCl_3$ - CH_3OH (9 : 1; Rf 0.58) were combined and evaporated *in vacuo* to give 226 mg; mp 119~125°C; UV $\lambda_{max}^{CH_3OH}$ nm (ε) 271 (18,040), 441 (17,160), 510 (sh, 3,775); IR ν_{max} (Nujol) cm^{-1} 3410, 1705, 1665, 1610, 1460, 1375, 1315, 1250, 1195, 1090, 1030, 760; ^1H NMR ($CDCl_3$) δ 0.97 (6H, t), 1.39 (3H, d), 1.51 (3H, s), 1.15~1.88 (4H, m), 2.70 (4H, m), 3.03 (1H, t), 3.58 (11H, s), 3.68 (2H, m), 4.02 (3H, s), 5.18 (1H, d), 5.62 (1H, br s), 7.39 (1H, s), 8.34 (1H, s); FAB-MS *m/z* 729.3356 (calcd for $C_{38}H_{51}NO_{13}$, 729.3360).

Anal Calcd for $C_{38}H_{51}NO_{13}$: C 62.54, H 7.04, N 1.92.
Found: C 61.47, H 7.14, N 1.76.

3-Pyrrolidinomethylsteffimycin B (**9**)

A solution of 2 g (3.4 mmol) of **1** in 34 ml of pyrrolidine was stirred while adding 34 ml of 37% CH_2O solution dropwise. After the solution had been stirred at room temp for 48 hours, it was poured into 500 ml of H_2O, and the pH was adjusted to 7.35 with conc HCl. Extraction with five 200 ml portions of $CHCl_3$ and evaporation of the combined extracts *in vacuo* gave 2.82 g. This material was chromatographed on 140 g of silica gel eluting with 200 ml of $CHCl_3$, 1 liter of $CHCl_3$ - CH_3OH (98 : 2), and 2.2 liters of $CHCl_3$ - CH_3OH (95 : 5). On the basis of TLC in $CHCl_3$ - CH_3OH - H_2O (78 : 20 : 2; Rf 0.47) those fractions containing **9** were combined and evaporated *in vacuo*, yield 755 mg;

mp >250°C (dec); UV $\lambda_{max}^{CH_3OH}$ nm (ε) 215 (sh, 21,570), 229 (21,910), 272 (18,655), 444 (6,105), 510 (sh, 3,725); IR ν_{max} (Nujol) cm^{-1} 3390, 1700, 1660, 1605, 1450, 1370, 1315, 1245, 1185, 1085, 1020, 750; ^1H NMR ($CDCl_3$) δ 1.39 (3H, d), 1.52 (3H, s), 1.93 (4H, s), 2.85 (4H, s), 3.06 (1H, t), 3.56 (8H, s), 3.59 (3H, s), 3.70 (2H, m), 4.01 (3H, s), 4.05 (1H, s), 5.20 (1H, d), 5.66 (1H, br s), 7.39 (1H, s), 8.32 (1H, s); FAB-MS *m/z* 671.2595 (calcd for $C_{34}H_{41}NO_{13}$, 671.2578).

Anal Calcd for $C_{34}H_{41}NO_{13}$: C 60.80, H 6.15, N 2.09.
Found: C 60.13, H 6.31, N 1.99.

3-Piperidinomethylsteffimycin B (**10**)

A solution of 2 g (3.4 mmol) of **1** in 55 ml of piperidine was stirred while adding dropwise 30 ml of 37% CH_2O solution. The solution was stirred at 90~95°C for 3 hours, cooled to room temp and evaporated *in vacuo* to about 50 ml. The residue was dissolved in 100 ml of ether, and 500 ml of Skellysolve B was added. The supernatant was decanted, and the residue was partitioned between 200 ml of $CHCl_3$ and 200 ml of H_2O. The $CHCl_3$ layer was evaporated *in vacuo*, and the residue was chromatographed on 115 g of silica gel by ascending dry column chromatography eluting with 1.62 liters of $CHCl_3$ then $CHCl_3$ - CH_3OH (9 : 1) obtaining 5.1 g of oily material. This was chromatographed on 50 g of silica gel eluting with EtOAc, then EtOAc - CH_3OH (95 : 5), and $CHCl_3$ - CH_3OH (9 : 1) collecting thirty-two 10-ml fractions, 103 fractions, and 95 fractions, respectively. On the basis of TLC in $CHCl_3$ - CH_3OH (9 : 1; Rf 0.35) fractions 147~185 were combined and evaporated *in vacuo*, yield 435 mg. Fractions 186~228 were combined and evaporated *in vacuo*, yield 176 mg. The latter was purified further by preparative TLC in $CHCl_3$ - CH_3OH (95 : 5), yield 130 mg. The two products were combined and chromatographed on 60 g of silica gel eluting with $CHCl_3$ - CH_3OH - H_2O (85 : 14 : 1) collecting fifty 4-ml fractions. Fractions 26~40 were combined on the basis of TLC and evaporated *in vacuo*, yield 120 mg; mp 157~172°C (dec); $[\alpha]_D^{25}$ +171° (*c* 0.076, $CHCl_3$); UV $\lambda_{max}^{CH_3OH}$ nm (ε) 229 (25,895), 272 (21,030), 444 (9,130); IR ν_{max} (Nujol) cm^{-1} 3430, 1705, 1665, 1615, 1325, 1290, 1255, 1095, 1030, 755; ^1H NMR ($CDCl_3$) δ 1.37 (3H, d), 1.53 (3H, s), 1.70~2.3 (6H, m), 3.0~3.25 (5H, m), 3.57, 3.61 (9H, 2×s), 3.74 (2H, m), 4.01 (3H, s), 4.68 (1H, d), 5.23 (1H, d), 5.71 (1H, br s), 7.23 (1H, s), 8.27 (1H, s); FAB-MS *m/z* 685.2723 (calcd for $C_{35}H_{43}NO_{13}$, 685.2734).

Anal Calcd for $C_{35}H_{43}NO_{13}$: C 61.30, H 6.32, N 2.04.
Found: C 59.81, H 6.32, N 1.96.

3-Morpholinomethylsteffimycin B (**11**)

A solution of 2 g (3.4 mmol) of **1** in 34 ml of morpholine was stirred while adding 34 ml of 37% CH_2O solution dropwise. The solution was stirred and heated at 80~85°C for 23 hours. The reaction mixture was evaporated *in vacuo* to a brown oil which was chromatographed on 100 g of silica gel by dry column ascending chromatography eluting with $C_2H_5COCH_3$ - CH_3COCH_3 - H_2O (70 : 20 : 11) and collecting 10-ml fractions. Fractions 5~34 were combined and evaporated *in vacuo*. The resultant residue was dissolved in 100 ml of CH_2Cl_2 to which was added 900 ml of Skellysolve B. The supernatant was decanted, and the residue (2.34 g) was chromatographed on 115 g of silica gel eluting with $CHCl_3$ - CH_3COCH_3 - Skellysolve B (16 : 3 : 4), $CHCl_3$ - CH_3OH (95 : 5), and $CHCl_3$ - CH_3OH (9 : 1) using 1.47 liters, 750 ml, and 200 ml, respectively. Those fractions containing **11** were combined on the basis of TLC in $CHCl_3$ - CH_3OH (9 : 1; Rf 0.46) and evaporated *in vacuo* to give 1.72 g. Two hundred mg was purified by preparative TLC in the above $CHCl_3$ - CH_3OH - Skellysolve B system, yield 99 mg; mp 155~165°C (dec); UV $\lambda_{max}^{CH_3OH}$ nm (ε) 216 (24,835), 231 (25,659), 279 (22,568), 442 (12,283); IR ν_{max} (Nujol) cm^{-1} 3400, 1700, 1665, 1600, 1450, 1370, 1315, 1280, 1250, 1090, 1010, 860, 745; ^1H NMR ($CDCl_3$) δ 1.40 (3H, d), 1.51 (3H, s), 2.62 (4H, m), 3.25 (m), 3.56 (11H, s), 3.75 (5H, m), 4.03 (3H, s), 5.15 (1H, d), 5.58 (1H, br s), 7.42 (1H, s), 8.32 (1H, s); FAB-MS *m/z* 687.2521 (calcd for $C_{34}H_{41}NO_{14}$, 687.2527).

Anal Calcd for $C_{34}H_{41}NO_{14}$: C 59.38, H 6.01, N 2.04.
Found: C 58.74, H 6.23, N 1.98.

3-(4-Methylpiperazinomethyl)steffimycin B (**12**)

A solution of 2 g (3.4 mmol) of **1** in 30 ml of *N*-methylpiperazine was stirred while adding 30 ml of 37% CH_2O solution dropwise. The solution was heated at 80~82°C for 4 hours then stirred at

room temp for 18 hours. The reaction mixture was partitioned between 1.5 liters each of the two phases of $CHCl_3$ - CH_3OH - H_2O (1 : 1 : 1). The lower phase was removed and evaporated *in vacuo* to an oil. The upper phase was adjusted to pH 7.2 with conc HCl extracted with four 400-ml portions of $CHCl_3$. The combined extracts were evaporated *in vacuo*, and the residues were combined and dissolved in 50 ml of ether to which was added 200 ml of Skellysolve B. Filtration gave 2.0 g which was chromatographed on 125 g of silica gel using $CHCl_3$ - CH_3OH (95 : 5) for 775 ml then $CHCl_3$ - CH_3OH (9 : 1) for 775 ml. On the basis of TLC in $CHCl_3$ - CH_3OH - H_2O (78 : 20 : 1; Rf 0.42) the fractions containing **12** were combined and evaporated *in vacuo*, yield 464 mg; mp 133~139°C; $[\alpha]_D^{25}$ +29° (*c* 0.3798, $CHCl_3$); UV $\lambda_{max}^{CH_3OH}$ nm (ε) 215 (18,305), 231 (19,390), 279 (17,605), 440 (10,220); IR ν_{max} (Nujol) cm^{-1} 3375, 1695, 1650, 1590, 1440, 1360, 1305, 1275, 1240, 1080, 1015, 740; ^1H NMR ($CDCl_3$) δ 1.38 (3H, d), 1.52 (3H, s), 2.27 (3H, s), 2.35~2.77 (8H, m), 3.04 (1H, t), 3.53 (3H, s), 3.57 (6H, s), 3.73 (2H, s), 3.98 (3H, s), 5.20 (1H, d), 5.63 (1H, br s), 7.40 (1H, s), 8.34 (1H, s); FAB-MS *m/z* 700.2844 (calcd for $C_{35}H_{44}N_2O_{13}$, 700.2843).

Anal Calcd for $C_{35}H_{44}N_2O_{13}$: C 59.99, H 6.33, N 4.80.
Found: C 57.06, H 6.15, N 4.23.

3-Fluorosteffimycin B (**13**)

A solution of 5.0 g (8.5 mmol) of **1** in 125 ml of $CHCl_3$ was stirred while bubbling CF_3OF through for 2 minutes followed by refluxing for 18 hours. The solution was shaken with 125 ml of H_2O, the $CHCl_3$ layer was removed, and the aqueous layer was extracted with two 75-ml portions of $CHCl_3$. The $CHCl_3$ layers were combined and evaporated *in vacuo*, yield 5.14 g. This solid was purified by chromatographing five times on silica gel using 532 g, 65.5 g, 318 g, 326 g and 203 g of silica gel successively. Fractions containing **13** were combined in each case on the basis of TLC in $CH_3C_6H_5$ - $CH_3COCH_2CH(CH_3)_2$ - CH_3OH (65 : 25 : 10; Rf 0.23). The solvents used were the above in the first and third chromatographies, the same solvent in the ratio 74 : 25 : 1 in the second, and the same solvent in the ratio 70 : 25 : 5 in the last two, yield 661 mg; mp 218~227°C; UV λ_{max}^{EtOH} nm (ε) 214 (25,600), 232 (29,200), 278 (26,260), 434 (8,750), 535 (4,350); IR ν_{max} (Nujol) cm^{-1} 2955, 2855, 1715, 1635, 1435, 1420, 1390, 1260, 1190, 1165, 1115, 1075, 1035; ^1H NMR ($CDCl_3$) δ 1.33 (3H, d), 1.45 (3H, s), 3.01 (1H, t), 3.49 (3H, s), 3.52 (6H, d), 3.68 (1H, d), 4.02 (3H, s), 5.12 (1H, d), 5.53 (1H, br s), 7.46 (1H, s), 8.30 (1H, s), 11.69 (1H, s), 12.69 (1H, s); FAB-MS *m/z* 606; calcd 606.

Anal Calcd for $C_{29}H_{31}FO_{13}$: C 57.43, H 5.15.
Found: C 56.02, H 5.19.

3-Chlorosteffimycin B (**14**)

A solution of 5.0 g (8.5 mmol) of **1** in 125 ml of $CHCl_3$ was stirred while adding 1.4 g (13 mmol) of $(CH_3)_3COCl$. The solution was refluxed for 18 hours, and 125 ml of H_2O was added. The $CHCl_3$ layer was removed, and the aqueous layer was extracted with two 75-ml portions of $CHCl_3$. The combined extracts were evaporated *in vacuo*, yield 6.41 g. This material was chromatographed on 500 g of silica gel eluting with $CH_3C_6H_5$ - CH_2Cl_2 - CH_3OH (70 : 25 : 5) and collecting 20-ml fractions. Fractions 233~275 were combined on the basis of TLC in the above system in the ratio 65 : 20 : 10 (Rf 0.25) and evaporated *in vacuo*, weight 1.47 g. Chromatography was repeated on 25.5 g of silica gel using the TLC solvent system, yield 1.32 g; mp 268~273°C; UV λ_{max}^{EtOH} nm (ε) 220 (28,000), 233 (29,730), 282 (27,760), 437 (9,900), 545 (4,450); IR ν_{max} (Nujol) cm^{-1} 2955, 2920, 2855, 1710, 1625, 1465, 1405, 1380, 1210, 1190, 1115, 1100, 1050, 1005; ^1H NMR ($CDCl_3$) δ 1.33 (3H, d), 1.44 (3H, s), 3.02 (1H, t), 3.50 (3H, s), 3.52 (6H, s), 3.60 (1H, d), 4.01 (3H, s), 5.49 (1H, d), 5.95 (1H, br s), 7.35 (1H, s), 8.24 (1H, s); FAB-MS *m/z* 622; calcd 622.

Anal Calcd for $C_{29}H_{31}ClO_{13}$: C 55.91, H 5.02, Cl 5.69.
Found: C 55.89, H 4.98, Cl 5.21.

3-Bromosteffimycin B (**15**)

A solution of 5.0 g (8.5 mmol) of **1** in 50 ml of anhydrous pyridine was stirred while adding dropwise 0.460 ml (8.92 mmol) of Br_2. The solution was stirred at room temp for 24 hours, and 500 ml of 3 N HCl was added. The resulting mixture was extracted with three 250 ml portions of $CHCl_3$ - CH_3OH (9 : 1). The combined extracts were evaporated *in vacuo* to give 5.45 g of residue. This was

chromatographed on 500 g of silica gel eluting with $CH_3C_6H_5$ - CH_2Cl_2 - CH_3OH (70 : 25 : 5) and collecting 20-ml fractions. Fractions 76~152 were combined on the basis of TLC in $CH_3C_6H_5$ - $CH_3COCH_2CH(CH_3)_2$ - CH_3OH (65 : 25 : 10; Rf 0.27) and evaporated *in vacuo*, yield 3.93 g. A portion of this was recrystallized from EtOH; mp 275°C (dec); UV λ_{max}^{EtOH} nm (ε) 220 (sh, 28,020), 231 (29,400), 283 (28,170), 440 (10,330), 545 (4,440); IR ν_{max} (Nujol) cm^{-1} 2955, 2855, 1715, 1625, 1465, 1405, 1385, 1210, 1190, 1170, 1115, 1090, 1040; ^1H NMR ($CDCl_3$) δ 1.41 (3H, d), 1.53 (3H, s), 3.08 (1H, t), 3.54 (3H, s), 3.55 (6H, s), 3.77 (1H, s), 4.14 (3H, s), 5.19 (1H, d), 5.61 (1H, br s), 7.49 (1H, s), 8.40 (1H, s), 12.54 (1H, s), 12.80 (1H, s); FAB-MS *m/z* 666/668; calcd 666/668.

Anal Calcd for $C_{29}H_{31}BrO_{13}$: C 52.18, H 4.68, Br 11.97.
Found: C 51.78, H 4.70, Br 12.24.

3-Iodosteffimycin B (**16**)

A mixture of 5 g (8.5 mmol) of **1** and 1 liter of H_2O was added to a solution of 19.4 g (85.0 mmol) of H_5IO_6 in 1 liter of H_2O with stirring. The mixture was stirred and refluxed for 72 hours, cooled to room temp and extracted with four 500-ml portions of $CHCl_3$ - CH_3OH (9 : 1). The combined extracts were filtered, and the filtrate was evaporated *in vacuo*, yield 7.51 g. This material was chromatographed on 610 g of silica gel eluting with $CH_3C_6H_5$ - $CH_3COCH_2CH(CH_3)_2$ - CH_3OH (70 : 25 : 5) and collecting 20-ml fractions. Those fractions containing **16** (90~225) as determined by TLC in the above solvent system in the ratio of 65 : 25 : 10 (Rf 0.30) were combined and evaporated *in vacuo* to give 5.06 g; mp 247~253°C; UV λ_{max}^{EtOH} nm (ε) 231 (28,680), 246 (22,710), 287 (26,400), 441 (11,250), 550 (3,410); IR ν_{max} (Nujol) cm^{-1} 3490, 1710, 1675, 1615, 1575, 1315, 1280, 1240, 1135, 1090, 1025, 755; ^1H NMR ($CDCl_3$) δ 1.53 (3H, d), 1.57 (3H, s), 3.08 (1H, t), 3.50 (9H, s), 3.68 (1H, s), 4.14 (3H, s), 5.19 (1H, d), 5.60 (1H, d), 7.40 (1H, s), 8.40 (1H, s), 12.89 (1H, s), 13.20 (1H, s); FAB-MS *m/z* 714; calcd 714.

Anal Calcd for $C_{29}H_{31}IO_{13}$: C 48.75, H 4.37, I 17.76.
Found: C 48.57, H 4.57, I 16.03.

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References

1) BERGY, M. E. & F. REUSSER: A new antibacterial agent (U-20,661) isolated from a streptomycete strain. Experientia 23: 254~257, 1967
2) BRODASKY, T. F. & F. REUSSER: Steffimycin B, a new member of the steffimycin family: Isolation and characterization. J. Antibiotics 27: 809~813, 1974
3) KELLY, R. C.; I. SCHLETTER, J. M. KOERT, F. A. MAC KELLAR & P. F. WILEY: Structures of steffimycin and steffimycin B. J. Org. Chem. 42: 3591~3596, 1977
4) ARARA, S. K.: Molecular structure, stereochemistry and interactions of steffimycin B, a DNA-binding anthracycline antibiotic. J. Biomol. Struct. Dyn. 3: 377~385, 1985
5) SUZUKI, H.: Direct iodination of polyalkyl-benzenes. *In* Organic Synthesis. Vol. 51. *Ed.*, R. E. BENSON, pp. 94~95, John Wiley & Sons, Inc., New York, 1971
6) ARCAMONE, F.; G. CASSINELLI & S. PERCY: Recent developments in the chemistry of doxarubicin-related anthracycline glycosides. *In* Anthracycline Antibiotics. *Ed.*, H. S. EL KHADEM, p. 71, Academic Press, New York, 1982
7) OKI, L.: Microbial transformations of anthracycline antibiotics and development of new anthracyclines. *In* Anthracycline Antibiotics. *Ed.*, H. S. EL KHADEM, p. 86, Acedemic Press, New York, 1982