SYNTHESES OF 9-SUBSTITUTED JOSAMYCIN, 13-SUBSTITUTED ISOJOSAMYCIN AND THEIR TETRAHYDRO DERIVATIVES

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Derivatives of josamycin and isojosamycin modified at C-9 and C-13 have been prepared by reaction sequences involving treatment of josamycin with alcohols, phenol or 1-methyl-1*H*-tetrazol-5-ylthiol in acidic media. Several tetrahydro derivatives of josamycin and isojosamycin have also been prepared by reaction sequences involving catalytic hydrogenation. From the ^{1}H NMR studies, it was found that the conformation of the macro-lactone portion of 13-*O*-methylisojosamycin dimethylacetal, a key intermediate, is flexible and changeable with variation of the solvent.

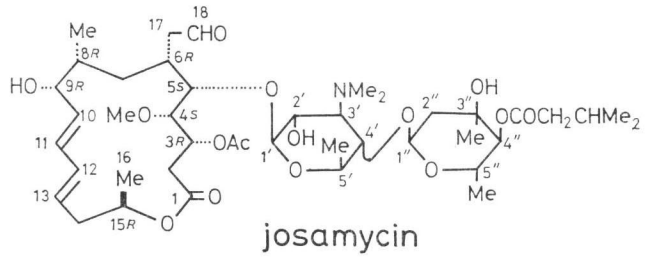
Josamycin[1] (leucomycin A_3[2]) is a macrolide antibiotic which has widely been used clinically and is produced by *Streptomyces narbonensis* var. *josamyceticus*. In attempts to improve the clinical characteristics, we have undertaken to prepare 9-*O*-alkyl derivatives of josamycin and 13-*O*-alkyl derivatives of isojosamycin. Josamycin is known to be converted, in aqueous acidic media, to isojosamycin (isoleucomycin A_3[3]), which has a hydroxyl group at C-13 produced by rearrangement[4] of the conjugated dienol system of josamycin. We found that the desired *O*-alkyl groups could be introduced into josamycin and isojosamycin by treatment of josamycin with alcohols in acidic media. The reactions of josamycin with phenol or 1-methyl-1*H*-tetrazol-5-ylthiol are also described.

Syntheses

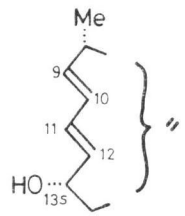
Treatment of josamycin in methanol containing *p*-toluenesulfonic acid (0°C, 12 hours) gave a mixture of 9-*O*-methyljosamycin dimethylacetal (**1**) and 13-*O*-methylisojosamycin dimethylacetal (**2**) in a ratio of *ca.* 1:1 in a moderate total yield (66%). The above reaction conditions were the best among the conditions tested in terms of the yield, and superior to the yields of josamycin and isojosamycin obtained by the treatment of josamycin in an acidic aqueous medium. As by-products, methyl 4-*O*-isovaleryl-α- and -β-mycarosides[5,6] (**3** and **4**), josamycin dimethylacetal[7] (**5**), and several demycarosyl derivatives of josamycin and isojosamycin were obtained. Separation of **1** and **2** was difficult on account of their similar mobilities but was accomplished by repeated column chromatography to give pure **1** and **2** in low yields.

Similar treatment of josamycin with ethanol, propanol, or butanol gave mixtures of the corresponding 9-*O*-alkyljosamycin dialkylacetal [**10**(ethyl), **24**(propyl), **33**(butyl)] and 13-*O*-alkylisojosamycin dialkylacetal [**11**(ethyl), **25**(propyl), **34**(butyl)] in 47% (for ethyl compound), 28% (propyl) and 14% (butyl) yield, respectively. In each case, the formation of josamycin dialkylacetal [**14**(ethyl), **28**(propyl),

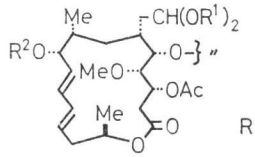
Chart 1.

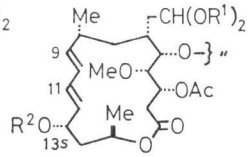


josamycin

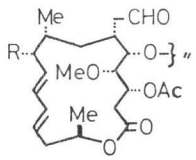


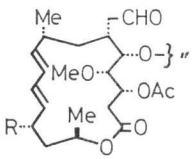
isojosamycin

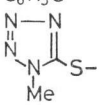


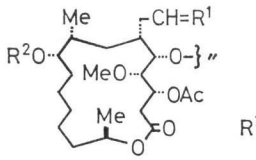


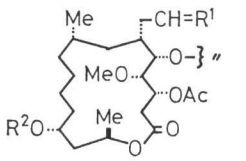
R^1	R^2		
Me	Me	1	2
Me	H	5	
Et	Et	10	11
Et	H	14	
Pr	Pr	24	25
Pr	H	28	
Bu	Bu	33	34
Bu	H	35	



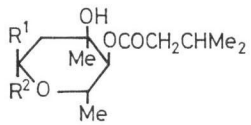


R		
EtO	20	21
i-PrO	40	41
t-BuO	42	43
$NCCH_2CH_2O$	44	45
C_6H_5O	46	47
	48	49

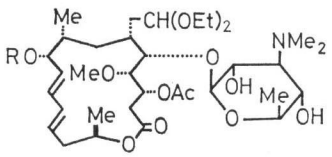




R^1	R^2		
$(MeO)_2$	Me	6	7
O	Me	8	9
$(EtO)_2$	Et	18	19
O	Et	22	23
$(PrO)_2$	Pr	29	30
O	Pr	31	32
$(BuO)_2$	Bu	36	37
O	Bu	38	39



R^1	R^2	
MeO	H	3
H	MeO	4
EtO	H	12
H	EtO	13
PrO	H	26
H	PrO	27



R	
Et	15
H	17

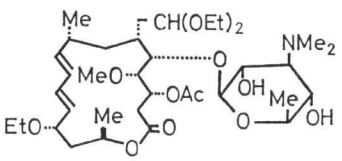


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Table 1. Physicochemical properties of products.

Compound (ratio)	Nature*	$[\alpha]_D^{15}$ (c 1, $CHCl_3$)	Rf**	Molecular formula	Analysis: Found (Calcd.)		
					C	H	N
1+**2** (1: 1)	a.p.	−41°	0.42 (0.56)	$C_{45}H_{77}NO_{16}$	60.98 (60.86)	8.71 (8.74)	1.42 (1.58)
1	a.p.	−40°	0.42	$C_{45}H_{77}NO_{16}$	60.99	8.61	1.52
2	a.p.	−42°	0.42	$C_{45}H_{77}NO_{16}$	61.03	8.66	1.41
5	a.p.	−44°	0.26 (0.28)	$C_{44}H_{75}NO_{16}$	60.21 (60.46)	8.48 (8.65)	1.67 (1.60)
6	84~85°C	−52°	0.42	$C_{45}H_{81}NO_{16}$	60.45 (60.59)	8.88 (9.15)	1.48 (1.57)
7	a.p.	−60°	0.42	$C_{45}H_{81}NO_{16}$	60.33	8.88	1.56
8	a.p.	−70°	0.40 (0.35)	$C_{43}H_{75}NO_{15}$	61.17 (61.04)	8.78 (8.94)	1.52 (1.66)
9	a.p.	−68°	0.40 (0.35)	$C_{43}H_{75}NO_{15}$	60.96	8.83	1.63
10+**11** (1: 1)	a.p.	−60°	0.46	$C_{48}H_{83}NO_{16}$	62.28 (61.98)	9.03 (8.99)	1.49 (1.51)
14	a.p.	−45°	0.29	$C_{46}H_{79}NO_{16}$	60.95 (61.24)	8.69 (8.83)	1.61 (1.55)
18+**19** (1: 1)	a.p.	−55°	0.46	$C_{48}H_{87}NO_{16}$	61.55 (61.71)	9.10 (9.39)	1.33 (1.50)
20	a.p.	−59°	0.44	$C_{44}H_{73}NO_{15}$	61.89 (61.73)	8.65 (8.60)	1.50 (1.64)
21	a.p.	−54°	0.40	$C_{44}H_{73}NO_{15}$	61.58	8.45	1.45
22+**23** (1: 1)	a.p.	−40°	0.42	$C_{44}H_{77}NO_{15}$	61.19 (61.44)	8.82 (9.02)	1.49 (1.63)
24+**25** (1: 1)	a.p.	−60°	0.49, 0.51	$C_{51}H_{89}NO_{16}$	62.96 (63.00)	8.92 (9.22)	1.32 (1.44)
28	a.p.	−48°	0.32	$C_{48}H_{83}NO_{16}$	62.06 (61.98)	8.94 (8.99)	1.39 (1.51)
29+**30** (1: 1)	a.p.	−57°	0.49, 0.51	$C_{51}H_{93}NO_{16}$	61.71 (62.74)	9.34 (9.60)	1.40 (1.43)
31+**32** (1: 1)	a.p.	−63°	0.45	$C_{45}H_{79}NO_{15}$	62.07 (61.83)	9.09 (9.11)	1.38 (1.60)
33+**34** (0.8: 1)	a.p.	−59°	0.56, 0.58	$C_{54}H_{95}NO_{16}$	63.77 (63.94)	9.20 (9.44)	1.27 (1.38)
35	a.p.	−47°	0.35	$C_{50}H_{87}NO_{16}$	62.40 (62.67)	8.96 (9.15)	1.52 (1.46)
36+**37** (0.8: 1)	a.p.	−51°	0.56, 0.58	$C_{54}H_{99}NO_{16}$	63.94 (63.69)	9.53 (9.80)	1.33 (1.38)
38+**39** (0.8: 1)	a.p.	−78°	0.47	$C_{46}H_{81}NO_{15}$	62.35 (62.21)	9.12 (9.19)	1.88 (1.58)
40+**41** (3: 1)	a.p.	−57°	0.35, 0.38	$C_{45}H_{75}NO_{15}$	62.37 (62.12)	8.72 (8.69)	1.53 (1.61)
42+**43** (1.5: 1)	a.p.	−63°	0.37, 0.41	$C_{46}H_{77}NO_{15}$	62.21 (62.49)	8.67 (8.78)	1.55 (1.58)
42	a.p.	−60°	0.41	$C_{46}H_{77}NO_{15}$	61.89	8.52	1.68
44+**45** (1: 1)	a.p.	−56°	0.40, 0.44	$C_{45}H_{72}N_2O_{15}$	61.08 (61.34)	8.00 (8.24)	2.98 (3.18)
44	a.p.	−61°	0.44	$C_{45}H_{72}N_2O_{15}$	61.11	8.20	3.00
46	a.p.	−66°	0.47	$C_{47}H_{78}NO_{15}$	63.21 (63.28)	8.01 (8.25)	1.38 (1.57)
47	a.p.	−64°	0.32	$C_{47}H_{73}NO_{15}$	63.40	8.10	1.48
48	152~155°C	−57°	0.39	$C_{44}H_{71}N_5O_{14}S$	57.34 (57.06)	7.84 (7.73)	7.68 (7.56)
						S 3.61 (3.46)	
49	a.p.	−32°	0.35	$C_{44}H_{71}N_5O_{14}S$	56.80	7.45	7.50
						S 3.26	

* Melting points or amorphous powder (a.p.).

** The value obtained on TLC with benzene - acetone (3: 1). The values in parentheses are for benzene - methanol (10: 1).

Table 2. Minimal inhibitory

Test organism	JM*	4HJM*	8	9	20	21	22+23	31+32
			4H9Me*	4H13Me*	9Et	13Et	4H9Et 4H13Et	4H9Pr 4H13Pr
Staph. aureus 193	12.5	12.5	12.5	25	12.5	25	12.5	25
〃 〃 EMf**	3.12	3.12	3.12	3.12	6.25	6.25	6.25	6.25
〃 FDA 209P	0.78	1.56	1.56	1.56	3.12	6.25	0.78	3.12
〃 Smith	0.78	0.78	0.78	1.56	1.56	3.12	0.78	3.12
〃 MS 8800	>100	>100	>100	>100	>100	>100	>100	>100
Micrococcus flavus FDA 16	<0.2	<0.2	0.39	0.39	0.78	0.78	<0.2	1.56
Sarcina lutea PCI 1001	<0.2	<0.2	0.39	<0.2	0.39	0.39	<0.2	0.78
B. subtilis NRRL B-558	0.78	0.78	1.56	1.56	1.56	3.12	1.56	3.12
Corynebact. bovis 1810	0.2	0.78	0.78	0.78	0.78	1.56	0.2	1.56
E. coli NIHJ	>100	>100	>100	>100	>100	>100	>100	>100
〃 K-12	>100	>100	>100	>100	>100	>100	>100	>100
K. pneumoniae PCI 602	50	100	100	50	>100	>100	>100	>100
Sh. dysenteriae JS 11910	6.25	12.5	25	25	50	100	100	100
Sal. enteritidis 1891	12.5	25	50	50	50	100	100	100
P. aeruginosa A3	>100	>100	>100	>100	>100	>100	>100	>100

* Abbreviations: JM, josamycin; 4HJM, tetrahydrojosamycin[8]; 4H is for the tetrahydro compounds;
** Erythromycin-resistant strain.

35(butyl)] was observed in the early stage of reaction, followed by the formation of the desired 9-*O*- and 13-*O*-alkyl derivatives together with some alcoholyzed products formed by cleavage of the mycarosyl bonding. These were alkyl 4-*O*-isovaleryl-α- and -β-mycarosides (**12** and **13** for ethyl, **26** and **27** for propyl), de(mycarosyl)josamycin dialkylacetal (**17** for ethyl), 9-*O*-alkyl-de(mycarosyl)josamycin dialkylacetal (**15** for ethyl), and 13-*O*-alkyl-de(mycarosyl)isojosamycin dialkylacetal (**16** for ethyl).

Treatment of josamycin with isopropanol, *tert*-butanol, 2-cyanoethanol or phenol in a similar manner gave no dialkyl- or diphenylacetal derivative, giving only monosubstituted derivatives, namely, 9-*O*-isopropyl- (**40**), 9-*O*-(*tert*-butyl)- (**42**), 9-*O*-(2-cyanoethyl)- (**44**), and 9-*O*-phenyljosamycin (**46**), together with the corresponding 13-*O*-alkylisomers, that is 13-*O*-isopropyl- (**41**), 13-*O*-(*tert*-butyl)- (**43**), 13-*O*-(2-cyanoethyl)- (**45**), and 13-*O*-phenylisojosamycin (**47**), in yields of 8% (for the mixture of **40** and **41**), 9.9% (for the mixture of **42** and **43**), 52% (for the mixture of **44** and **45**), 9% (**46**) and 42% (**47**). No formation of the expected diacetal derivative, and low yields of the desired *O*-alkyl derivatives except for the cyanoethyl derivatives may be ascribed to the bulkiness of the alkyl groups introduced. In the case of cyanoethylation, formation of the corresponding dialkyl-acetals may be expected, but none of them were isolated.

Isolation of 9-*O*-ethyljosamycin (**20**) and 13-*O*-ethylisojosamycin (**21**) from the reaction mixture obtained by the procedure described was difficult on account of the low yield of the desired derivatives. However, when josamycin was treated in acetonitrile in the presence of trifluoroacetic acid for rather long reaction period of time (1 month), **20** and **21** were formed in detectable amounts, and they were separated, after laborious column chromatography, in low yields.

concentration (mcg/ml).

38+39	40+41	42	42+43	44	44+45	46	47	48	49
4H9Bu 4H13Bu	9*i*Pr 13*i*Pr	9*t*Bu	9*t*Bu 13*t*Bu			9Ph	13Ph		
12.5	12.5	12.5	25	12.5	12.5	25	12.5	6.25	6.25
6.25	3.12	6.25	6.25	6.25	6.25	12.5	12.5	1.56	1.56
6.25	3.12	1.56	3.12	0.78	1.56	6.25	3.12	0.78	0.78
3.12	1.56	3.12	3.12	0.78	1.56	3.12	0.78	0.39	0.39
>100	>100	>100	>100	>100	>100	>100	>100	>100	>100
1.56	0.78	1.56	1.56	0.2	0.78	0.39	<0.2	<0.2	0.39
<0.2	0.78	0.39	0.78	0.39	0.39	0.39	0.39	<0.2	0.2
6.25	3.12	3.12	3.12	0.78	1.56	3.12	0.78	0.39	0.39
1.56	0.78	0.39	0.78	0.39	0.78	0.78	0.39	0.2	0.2
>100	>100	>100	>100	>100	>100	>100	>100	>100	>100
>100	>100	>100	>100	>100	>100	>100	>100	>100	>100
>100	>100	>100	>100	>100	100	>100	>100	50	25
>100	>100	>100	>100	50	50	>100	>100	12.5	12.5
>100	>100	>100	>100	50	50	>100	>100	6.25	6.25
>100	>100	>100	>100	>100	>100	>100	>100	>100	>100

9 Alkyl and 13 Alkyl is for the 9-*O*-alkylated and 13-*O*-alkylated compounds, respectively.

Since acetalation of the aldehyde group at C-18 gave rise to compounds having no antibacterial activity, the acetal derivatives described above were deacetalated after catalytic hydrogenation of the double bonds, the alkoxyl groups at C-9 or C-13 being fixed in that positions by the latter reaction. The tetrahydro acetals [**6** and **7** (Me derivatives), **18** and **19**(Et), **29** and **30**(Pr), **36** and **37**(Bu)] were deacetalated to give 9-*O*-alkyl-10,11,12,13-tetrahydrojosamycin [**8**(Me), **22**(Et), **31**(Pr), **38**(Bu)] and 13-*O*-alkyl-9,10,11,12-tetrahydroisojosamycin [**9**(Me), **23**(Et), **32**(Pr), **39**(Bu)].

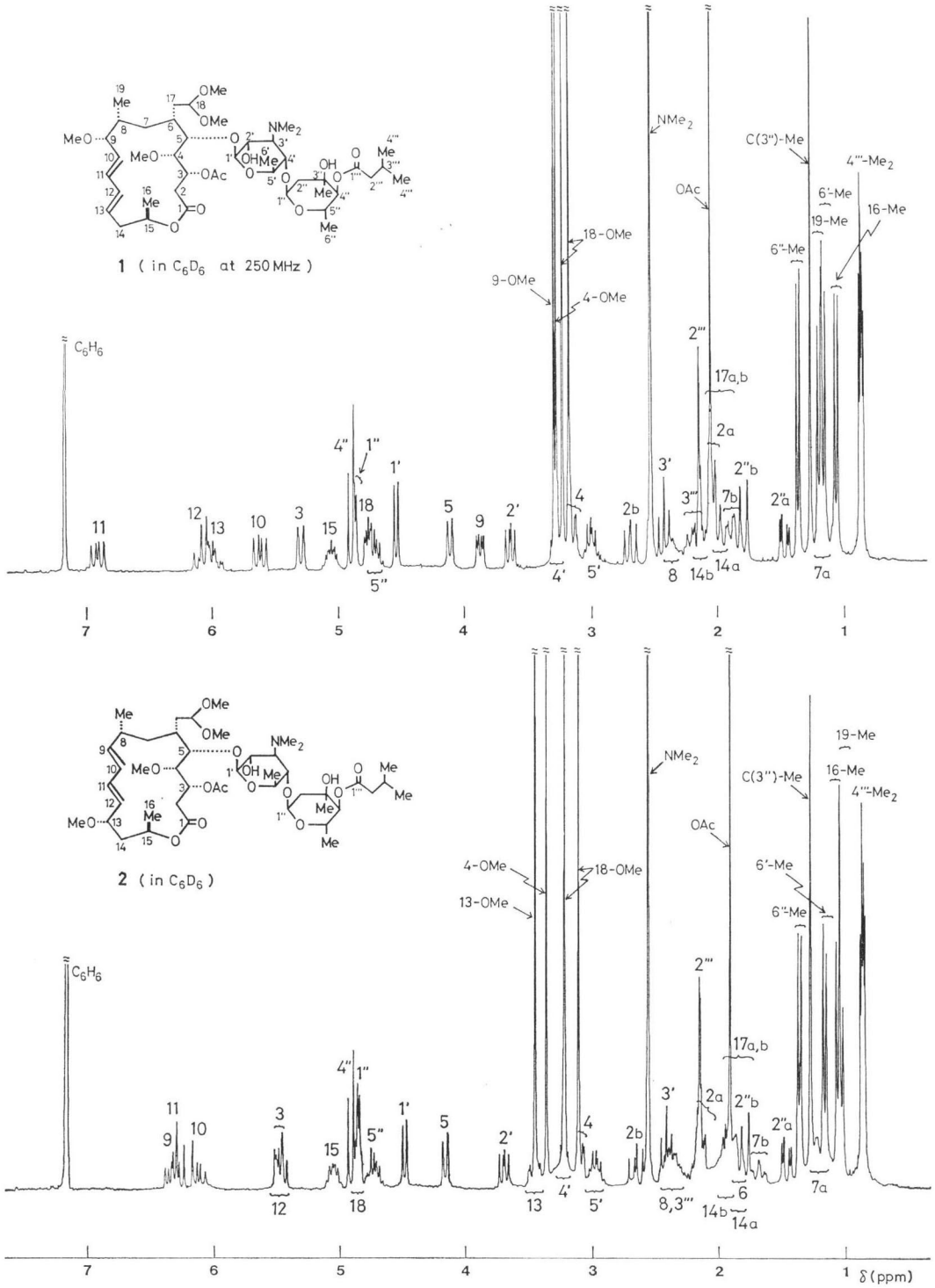
In another experiment, josamycin was treated with 1-methyl-1*H*-tetrazol-5-ylthiol, which has often been used in the preparation of β-lactam antibiotics[8], to give 9-(1-methyl-1*H*-tetrazol-5-ylthio)josamycin (**48**) and 13-(1-methyl-1*H*-tetrazol-5-ylthio)isojosamycin (**49**) in moderate yields, respectively, without formation of thioacetals.

Physicochemical properties of the above mentioned prepared are shown in Table 1, and the antibacterial spectra of 9-*O*-alkyljosamycins, 9-*O*-alkyl-tetrahydrojosamycins, 13-*O*-alkylisojosamycins, 13-*O*-alkyltetrahydroisojosamycins, **48** and **49** are shown in Table 2 with those of josamycin and tetrahydrojosamycin[9,10]. No substantial difference was observed in the antibacterial spectra.

Structural Studies

The structure of **1** was determined by comparison of the ^{1}H NMR spectra of **1** and josamycin dimethylacetal[7] (**5**) measured in chloroform-*d* ($CDCl_3$) at 250 MHz. Major differences between the spectra were the appearance of the C(9)OMe group (δ 3.58) in **1**, and the change in the shift values of

Fig. 1.


1 (in C_6D_6 at 250 MHz)
2 (in C_6D_6)
C_6H_6
NMe2
OAc
C(3")-Me
4"'-Me2
6'-Me
19-Me
16-Me
6"-Me
18-OMe
9-OMe
4-OMe
13-OMe
17a,b
2a
2"b
2"a
7b
7a
14b
14a
δ (ppm)

H-9 (**5**: δ 4.20, **1**: δ 3.58); other signals appeared at similar shifts with similar patterns in both compounds with respect to the corresponding protons. The spectrum of **1** in benzene-d_6 (C_6D_6) is shown in Fig. 1. All shift-values and coupling constants of **1** (Table 3) were certified by decoupling. No noticeable differences in J values of the corresponding protons were observed in $CDCl_3$ and C_6D_6, indicating that the conformations of **1** in both solvents are substantially the same. The shift-values of the corresponding protons were, however, quite different, for some protons.

The structure of **2** was next studied. Double irradiation starting from H-15 (the proton was clearly distinct from other protons by the indication of its δ-value and the splitting pattern) to H-8 through H-13 revealed the sequence of HC(15)Me–C(14)HH–HC(13)OMe–HC(12)=C(11)H–HC(10)=C(9)H–HC(8)Me–C (see Experimental and Table 3). This result indicates that the first double bond (at C-12) appears at the 3rd carbon from C-15 and, therefore, **2** has the same structure with that of isojosamycin with respect to the macro-lactone ring. Therefore, the absolute configuration at C-13 should be *S*.

In order to clarify the conformation of the macro-lactone ring of **2**, J values of **2** in $CDCl_3$ or C_6D_6 were measured. The results are shown in Table 3 and Fig. 1. It is worthwhile to compare the above results with those obtained by X-ray crystallography of de(mycarosyl)isojosamycin reported by HIRAMATSU *et al.*[11,12], because the structures of the macro-lactone rings of both the compounds are considered to be substantially the same. The dihedral angles (cited in the 13th column of Table 3) relating to vicinal hydrogens (H_2–C_B–C_A–H_1) of de(mycarosyl)isojosamycin are calculated from the reported[11,12] dihedral angles created by C_1–C_A and C_2–C_B in C_2–$\overset{H}{\underset{|}{C}}_B$–$\overset{H}{\underset{|}{C}}_A$–$C_1$ system of the compound, with an assumption that the hydrogens attached at each carbon are bonded without deviation from the ideal positions of tetrahedral geometry. The $^3J_{H,H}$ values shown in the last column of the Table are claculated from the modified Karplus equation based on the calculated angles (on the 13th column). For the $^3J_{H,H}$ of the C–C chain between C(6)–C(8), the equation of ABRAHAM[13,14] ($J=12.4\cdot\cos^2\phi$ for $0\leqq\phi\leqq90°$, and $J=14.3\cdot\cos^2\phi$ for $90°\leqq\phi\leqq180°$) is adopted, and for the other $^3J_{H,H}$ of the saturated bondings, the equation ($J=14.4\cdot\cos^2\phi$ for $0\leqq\phi\leqq90°$, and $J=11\cdot\cos^2\phi$ for $90°\leqq\phi\leqq180°$) is adopted, the latter equation being made from the original Karplus equation[15,16] by adding the assumption that $^3J_{H,H}$ is 3.6 Hz for dihedral angle of 60°, 0 Hz for 90°, and 11 Hz for 180°, the J values being accepted[17] in sugar chemistry. Comparison of the calculated J values (on the last column) with those of **2** obtained in C_6D_6 (on the 11th column) showed reasonable agreement with respect to the corresponding values. The J values of **2** measured in $CDCl_3$ (on the 10th column), however, showed discrepancy with the values relating to H-14 (Table 3). The results indicate that the conformation of the macro-lactone ring of the crystalline de(mycarosyl)isojosamycin and that of compound **2** dissolved in C_6D_6 resemble each other, and that the conformation in the range of C(13)–C(15) is suggested to be flexible and changeable depending on the solvent used.

On the flexibility in the range of C-13 to C-15, we want to add another observation obtained by changing the solvent ratio of $CDCl_3$ and C_6D_6. In neat $CDCl_3$, H-15 appeared at δ 4.99 as a comparatively sharp multiplet (sextet?), the assignment being made by the irradiation at δ 1.22 [C(15)-Me], whereupon H-15 became double doublets [apparant triplet; $J\simeq6$ and $\simeq7$ Hz ($=J_{14a,15}$ and $J_{14b,15}$)*] by the disappearance of the couplings with the C(15)-methyl protons. In C_6D_6, however, H-15 ap-

* It seems that, in $CDCl_3$, dihedral angles formed between C_{12}-C_{13} and C_{14}-C_{15} ($+78°$), and C_{13}-C_{14} and C_{15}-O ($-84°$) reported[11,12] in the crystalline state, change to $\sim+40°$ and $\sim-50°$, respectively.

Table 3. ^{1}H NMR data of **1** and **2**.

	1					**2**					H, H*1	angle(°)*1	J(Hz)*2
	in $CDCl_3$ (δ)	in C_6D_6 (δ)	$J_{H,H}$	in $CDCl_3$ J(Hz)	in C_6D_6 J(Hz)	in $CDCl_3$ (δ)	in C_6D_6 (δ)	$J_{H,H}$	in $CDCl_3$ J(Hz)	in C_6D_6 J(Hz)			
H–2a	2.26 d	2.03 d	2a,2b	13.5	13	2.21 d	2.14 dd	2a, 2b	15	15.5			
			2a, 3	≃1	≃1			2a, 3	≃0	≃1.5	2a, 3	57	4.3
–2b	2.73 dd	2.69 dd	2b, 3	11	11	2.72 dd	2.65 dd	2b, 3	11	12	2b, 3	177	11
–3	5.08	5.29 d(br)	3, 4		≃1.5	5.22 d(br)	5.47 d(br)	3, 4	≃1.5	≃2.5	3, 4	50	5.9
–4	3.25	3.14 dd				~3.2	3.08 dd(?)						
–5	3.89 d	4.11 d	4, 5	9.5	9	3.87 d	4.15 d	4, 5	9.5	9.5	4, 5	175	10.9
			5, 6	≃0.5	≃0.5			5, 6	≃0.5	≃0.5	5, 6	61	3.4
–6	~1.63					~1.7	~1.9						
–7a	0.90 br t?	~1.2				1.19	1.22 ?						
–7b	1.47 dt	~1.87 dt?	7a, 7b	≃12.5	≃12.5	1.42 dt	1.68 dt	7a, 7b	≃12.5				
			6, 7a	≃12.5				6, 7a	≃12.5	≃12.5	6, 7a	156	11.9
			6, 7b					6, 7b			6, 7b	84	0.2
			7a, 8	≃2				7a, 8			7a, 8	179	14.3
			7b, 8	3				7b, 8	2.5	3.3	7b, 8	61	3.4
–8	~2.15	~2.35				~2.3	~2.35						
–9	3.58 dd	3.87 dd	8, 9		4	6.23 dd	6.33 dd	8, 9	6.5	6.8			
			9, 10	9.5	9.5			9, 10	15.5	15.5			
–10	5.47 dd	5.62 dd	10, 11	15	15	6.10 dd	6.12 dd	10, 11	9.5	9.5			
–11	6.54 dd	6.91 dd	11, 12	10.5	9.5	6.22 dd	6.27 dd	11, 12	15	15			
–12	6.09 dd	6.08 dd	12, 13	15	15	5.37 dd	5.46 dd	12, 13	9	9.5			
–13	5.74 ddd	6.00 ddd				~3.6	3.44 (dt?)						
–14a	~2.09	1.98	13, 14a	10.5	{7.5	1.89 t	~1.86	13, 14a	≃7	3.3	13, 14a	78	0.6
–14b	~2.16	~2.1	13, 14b	3.8	{4		~2	13, 14b	≃7	≃9.5	13, 14b	162	9.9
			14a, 15	3	4			14a, 15	{≃6	≃0	14a, 15	84	0.2
			14b, 15	10	10.5			14b, 15	{≃7	9.5	14b, 15	156	9.2
–15	5.03 m	5.05 m				4.99 m(sextet)	5.04 m						
–17a, –17b		≃2.0~2.2	17a, 18		{3.8,	1.55~1.85	1.83~2.0						
			17b, 18		{7.5								
–18	~4.5	4.78 dd				~4.5	~4.85						

Table 3. (Continued)

	1					2					H, H*1	angle(°)*1	J(Hz)*2
	in $CDCl_3$ (δ)	in C_6D_6 (δ)	$J_{H,H}$	in $CDCl_3$ J(Hz)	in C_6D_6 J(Hz)	in $CDCl_3$ (δ)	in C_6D_6 (δ)	$J_{H,H}$	in $CDCl_3$ J(Hz)	in C_6D_6 J(Hz)			
3-O-Ac	2.12 s	2.05 s				2.10 s	1.91 s						
4-OMe	3.28 s	(3.27 s)				3.31 s	3.36 s						
C(8)-Me	0.98 d	1.19 d		7	7	1.08 d	1.04 d		7	7			
9-OMe	3.55 s	(3.29 s)*3											
13-OMe						3.58	3.44 s						
C(15)-Me	1.26 d	1.05 d		7	7	1.22 d	1.06 d			6			
C(18) $(OMe)_2$	3.20 s	(3.17 s)				3.21 s,	3.10 s,						
	3.22 s	(3.22 s)				3.27 s	3.31 s						
H–1′	4.52 d	4.55 d	1′, 2′	7.5	7.5	4.44 d	4.49 d	1′, 2′	7.5	7.5			
–2′	~3.57	3.64 dd	2′, 3′		10	~3.57	3.69 dd	2′, 3′		10			
–3′	~2.45	2.42 t	3′, 4′		10	~2.5	2.4	3′, 4′		10			
–4′	3.2~3.4	~3.2	4′, 5′		9	3.25~3.35	~3.2	4′, 5′		9			
–5′		3.0 m	5′, 6′				2.69 dq	5′, 6′	7.5				
C(5′)-Me	1.29 d	1.15 d			7	1.29 d	1.15 d						
NMe_2	2.51 s	2.53 s				2.51 s	2.56 s						
–1″	5.08 d	4.87 d	1″, 2″a	3.5	3.5	5.09 d	4.85 d	1″, 2″a	3.5	3.5			
			1″, 2″b	≃0	≃0			1″, 2″b	≃0	≃0			
–2″a	1.84 dd	1.47 dd	2″a,2″b	14	14.5	1.85 dd	1.47 dd	2″a,2″b	14	14.5			
–2″b	2.02 d	1.79 d				2.01 d	1.79 d						
C(3″)-Me	1.12 s	1.26 s				1.13 s	1.28 s						
–4″	4.63 d	4.90 d	4″, 5″	10	10	4.63 d	4.91 d	4″, 5″	10	10			
–5″	~4.5	4.71 dq				~4.5	4.73 dq	5″, Me		6			
C(5″)-Me	1.14 d	1.36 d	5″, Me		7	1.15 d	1.36 d		7.5				
–2‴		~2.15				2.3 d	~2.16						
–3‴	~2.2	~2.18				~2.1 (?)	~2.35						
C(3‴)-Me_2	0.95 d	0.85 d		7	7	0.98 d	0.86 d		7	7			
	0.98 d	0.87 d		7	7		0.87 d			7			

*1 Dihedral angle formed, for example, by H–C_{2a} and C_3–H in a H–C_{2a}–C_3–H system (the H's involved are cited in the column of H, H).

*2 J values calculated by the angles cited in the left column.

*3 Tentatively assigned.

peared as an unresolved multiplet (δ 5.04). On irradiation at C(15)-Me, the multiplet became a sharp doublet indicating that $J_{14a,15}$ and $J_{14b,15}$ should be 9.5 and $\simeq$0 Hz. It may be interesting to find the mixture-point of $CDCl_3$-C_6D_6, at which the above change in $J_{14,15}$ value will occur. This was found to be at the mixture between 10: 1 ~ 5: 1 of $CDCl_3$ - C_6D_6. In the former mixture (10: 1), H-15 (δ 5.04) appeared in the same pattern as observed in chloroform-*d*, but in the latter mixture, H-15 appeared as an unresolved multiplet (δ 4.98) similar to that observed in C_6D_6. Gradual increase in the ratio of C_6D_6 gave no further change in the pattern of H-15. On the signals of H-13, similar change in *J* values were also observed.

Experimental

General

Thin-layer chromatography (TLC) was carried out on precoated silica gel HPTLC 60 F_{254} (E. Merck, Darmstadt) with benzene - acetone (3: 1), unless otherwise stated, with detection by spraying with sulfuric acid, followed by slight heating. Column chromatography was performed on Kieselgel 60, 230 ~ 400 mesh (E. Merck), or on Kieselgel 60 prepacked column for LC, size B (E. Merck) (Column A) unless otherwise stated. ^{1}H NMR spectra were recorded at 90 MHz with a Varian EM-390 spectrometer or at 250 MHz with a Bruker WM 250 spectrometer.

A Mixture of 9-*O*-Methyljosamycin Dimethylacetal (**1**) and 13-*O*-Methylisojosamycin Dimethylacetal (**2**)

A solution of josamycin (258 mg as base) and anhydrous *p*-toluenesulfonic acid (80 mg) in dry methanol (2.6 ml) was kept in an ice-bath for 12 hours. Triethylamine (63 mg) was added and the solution was concentrated *in vacuo*. The residue was dissolved in chloroform (15 ml) and the solution was washed with saturated aqueous sodium hydrogen carbonate, water, dried over sodium sulfate, and evaporated. The residue showed, on TLC, spots of Rf 0.91 (trace) and 0.73 (trace) [methyl 4-*O*-isovaleryl-α- and -β-mycarosides (**3** and **4**), respectively], 0.42 (major, **1** and **2**), 0.26 (trace, josamycin dimethylacetal (**5**)], 0.2 (trace, josamycin) and 0.1 (minor, 9-*O*-methyldemycarosyljosamycin dimethylacetal and 13-*O*-methyldemycarosylisojosamycin dimethylacetal[4]?). Separation by column chromatography gave a mixture of **1** and **2**, 183 mg (66%).

Treatment of josamycin in a similar manner changing only the temperature (20°C, 12 hours) gave, after column chromatography, **3** and **4** in better yields than those obtained in the former experiment to give thick syrups (25 and 50%, respectively).

3: $[\alpha]_D^{15}$ −137° (*c* 2, chloroform) [Ref.[5,6] −135° (*c* 1.33, chloroform)]. ^{1}H NMR (in $CDCl_3$ at 250 MHz): δ 0.98 (6H d, C(3′)-Me_2), 1.11 (3H s, C(3)-Me), 1.18 (3H d, C(5)-Me), 1.88 (1H dd, $J_{1,2ax}$ 4 Hz, $J_{2,2}$ 15 Hz, H-2_{ax}), 2.01 (1H dd, $J_{1,2eq}\simeq$1 Hz, H-2_{eq}), 2.15 (1H m, H-3′), 2.295 (1H d, *J* 6 Hz) and 2.300 (2H, d, *J* 8 Hz, COCH_2CH), 3.39 (3H s, OMe), 4.00 (1H dq, H-5), 4.65 (1H d, $J_{4,5}$ 10 Hz, H-4), 4.80 (1H slightly broadened d, H-1). Irradiation of C(3′)-Me_2 collapsed the multiplet of H-3′ to an apparent triplet (*J* 7 Hz).

4: $[\alpha]_D^{15}$ +14° (*c* 3, chloroform) [Ref.[5,6] +13.5° (*c* 1.33, chloroform)]. ^{1}H NMR (in $CDCl_3$ at 250 MHz): δ 0.99 (6H d), 1.15 (3H s), 1.18 (3H d), 1.62 (1H ddd, $J_{1,2ax}$ 10 Hz, $J_{2,2}$ 15 Hz, $J_{2ax,3\text{-}OH}$ 2.5 Hz, H-2_{ax}; became dd on deuteration), 1.82 (1H d, disappeared on deuteration, HO-3), 2.02 (1H dd, $J_{1,2eq}$ 2.5 Hz, H-2_{eq}), 2.15 (1H m), 2.275 (1H d, *J* 6 Hz), 2.280 (1H d, *J* 8 Hz), 2.49 (OMe), 3.85 (H-5), 4.63 (H-4), 4.73 (1H dd, H-1).

To obtain josamycin dimethylacetal (**5**) in better yield, josamycin was likewise treated for 5 hours in an ice-bath, whereupon **5** was isolated, after column chromatography, in 25% yield, $[\alpha]_D^{21}$ −66° (*c* 1, ethanol) [Ref.[7] −64.0° (*c* 1.0, ethanol)].

^{1}H NMR (in $CDCl_3$ at 250 MHz): δ 2.12 (3H s, Ac), 2.51 (6H s, NMe_2), 3.21 and 3.28 [each 3H s, CH(O*Me*)$_2$], 3.55 (3H s, C(4)-OMe), 4.20 (1H dd, H-9), 5.65 (1H dd, H-10), 5.74 (1H ddd, H-13), 6.07 (1H dd, H-12), 6.53 (1H dd, H-11). $J_{8,9}$ 4.5, $J_{9,10}$ 10, $J_{10,11}$ 16, $J_{11,12}$ 11, $J_{12,13}$ 15 Hz; $J_{13,14a}$, $J_{13,14b}$, 3.5 and 11 Hz.

Separation of **1** and **2**

A mixture (1.76 g) of **1** and **2** was chromatographed on a column of silica gel with benzene - hexane (1: 1)→benzene - hexane - acetone (4: 4: 3) (gradually changed). From the earlier and the later fractions, a mixture of **1** and **2** which was rich in **1** (0.54 g) and a mixture of **1** and **2** (0.98 g) were obtained, respectively. The former and the latter mixtures were, separately, chromatographed twice more on "Column A" with benzene - hexane - acetone (2: 2: 1) to afford pure **1** (183 mg) and pure **2** (310 mg).

^{1}H NMR of **2** (in $CDCl_3$ at 250 MHz): Irradiation at δ 4.99 (H-15) caused the doublet of C(15)Me and the triplet of H-14 to collapse to a singlet and a doublet (J 7.5 Hz), respectively. Irradiation at δ 1.88 (H-14, H-2''a) caused the multiplet (sextet) of H-15 and the doublet of H-1'' to collapse to a quartet (J 7.5 Hz) and a singlet, respectively. Irradiation at δ 3.56 (H-13, 2', MeO-13) caused the triplet of H-14, quartet of H-12, and the doublet of H-1' to collapse to a doublet (J 7.5 Hz), a doublet (J 15 Hz), and a singlet, respectively. Irradiation of H-12 caused the quartet of H-11 to collapse to a doublet. Irradiation at δ 6.22 (H-11, 9 and partially 10) caused the quartet of H-12 to collapse to a doublet (J 9 Hz). Irradiation at δ 2.3 (H-8 and $COCH_2$-) caused the doublet of C(8)Me; the quartet of H-9, and the double triplets of H-7b to collapse to a singlet, a doublet (J 15 Hz), and a triplet ($J\simeq$ 12.5 Hz), respectively. Irradiation at δ 1.2 (H-7a) caused the double triplets of H-7b to collapse to a broadened doublet. Irradiation of H-7b caused the signals between δ 1.6~1.7 to change and the doublet of H-5 to sharpen. Irradiation at 1.6~1.7 (H-6, 17a, 17b) caused the double triplets of H-7b to collapse to a doublet. Irradiation of H-5 caused the signals at δ~3.2 to change. Irradiation of H-4 collapsed the doublet of H-5 to a singlet, and the slightly broadened doublet of H-3 to a sharp doublet. Irradiation of H-3 collapsed the double doublets of H-2b to a doublet (J 15 Hz), but no change was observed for the doublet of H-2a by the irradiation. By this irradiation, a small doublet ($J_{3,4}\simeq$1.5 Hz) at δ 3.18 became a singlet. Irradiation of H-2b collapsed the doublet of H-2a and that of H-3 to a singlet, respectively. Irradiation at δ 4.48 (H-18, 1', 5'') caused the multiplet at δ 1.55~1.85 to change. By this irradiation the doublet of H-4'' and that of C(5'')-Me also collapsed to a singlet, respectively.

^{1}H NMR of **2** (in 10: 1, $CDCl_3$ - C_6D_6 at 250 MHz): Irradiation at δ 5.04 (H-15) caused the triplet of H-14 (δ 1.94, $J_{13,14}$ 7 Hz) to collapse to a doublet.

^{1}H NMR of **2** (in 1: 1, $CDCl_3$ - C_6D_6 at 250 MHz): Signals of H-13 appeared at δ 3.48 as double triplets ($J_{12,13}=J_{13,14b}\simeq$9.5 Hz, $J_{13,14a}\simeq$3.3 Hz) without overlapping with other signals. Irradiation at δ 1.83 (H-14a) collapsed the double triplets of H-13 to double doublets.

A Mixture of 9-*O*-Ethyljosamycin Diethylacetal (**10**) and 13-*O*-Ethylisojosamycin Diethylacetal (**11**)

A solution of josamycin (2.19 g) and anhydrous *p*-toluenesulfonic acid (685 mg) in dry ethanol (22 ml) was kept in an ice-bath. After 1 hour, on checking by TLC, josamycin (Rf 0.2) almost disappeared and josamycin diethylacetal (**14**) was the major product. After 24 hours, the reaction mixture showed, on TLC, spots of Rf 0.92 and 0.75 [ethyl 4-*O*-isovaleryl-α- and -β-mycarosides (**12** and **13**), respectively], 0.46 (major, **10** and **11**), 0.29 (**14**), 0.12 [a mixture of 9-*O*-ethyl-de(mycarosyl)josamycin diethylacetal (**15**) and 13-*O*-ethyl-de(mycarosyl)isojosamycin diethylacetal (**16**)] and 0.04 [de(mycarosyl)-josamycin diethylacetal (**17**)]. After addition of triethylamine (0.7 ml), the reaction mixture was worked up as described for **1** and **2** and the crude product was subjected to column chromatography (silica gel was charged with benzene) with benzene - ethanol (20: 1→6: 1, gradually changed, finally with 3: 1).

12: 65 mg (9%), thick syrup, $[\alpha]_D^{15}-123°$ (c 1, chloroform). ^{1}H NMR (in $CDCl_3$ at 250 MHz): δ 3.47 and 3.78 (each 1H dq, J 7 and 10 Hz, CH_2CH_2O–), 4.05 (1H m, H-5), 4.66 (1H d, $J_{4,5}$ 10 Hz, H-4), 4.92 (1H slightly broadened d, H-1).

Anal. Calcd. for $C_{14}H_{26}O_5$: C, 61.29; H, 9.55.
Found: C, 61.35; H, 9.41.

13: 200 mg (28%), m.p. 35.5~37°C, $[\alpha]_D^{15}+15°$ (c 1, chloroform). ^{1}H NMR (in $CDCl_3$ at 250 MHz): δ 3.55 and 3.94 (CH_3CH_2O–), 4.84 (1H dd, J 2.5 and 9.5 Hz, H-1).

Anal. Calcd. for $C_{14}H_{23}O_5$: C, 61.29; H, 9.55.
Found: C, 61.23; H, 9.38.

A mixture of **10** and **11**, 1.16 g (47%). ^{1}H NMR (in $CDCl_3$ at 250 MHz): δ 2.07 (s, Ac for **11**), 2.11 (s, Ac for **10**), 2.50 (s, NMe_2 for **11**), 2.51 (s, NMe_2 for **10**), 3.55 (3H s, C(4)-OMe).

14: 250 mg (11%). ^{1}H NMR (in $CDCl_3$ at 250 MHz): δ 2.10 (3H s, Ac), 2.50 (6H s, NMe_2), 3.55 (3H s, C(4)-OMe), 4.23 (1H dd, H-9). The signal pattern for the olefin protons was almost the same with that of **5**.

A mixture of **15** and **16**, 457 mg (25%), amorphous powder, $[\alpha]_D^{15}$ −19° (*c* 1, chloroform). ^{1}H NMR (in $CDCl_3$ at 250 MHz): δ 2.08 (s, Ac for **16**), 2.12 (s, Ac for **15**), 2.56 (6H s, NMe_2), 3.55 (3H s, MeO-4).

Anal. Calcd. for $C_{36}H_{63}NO_{12}$: C, 61.60; H, 9.05; N, 2.10.
Found: C, 61.31; H, 8.79; N, 1.90.

17: 161 mg (9%), amorphous powder $[\alpha]_D^{15}$ −6° (*c* 1, chloroform). ^{1}H NMR ($CDCl_3$): δ 2.10 (Ac), 2.55 (NMe_2), 3.55 (C(4)-OMe), 4.24 (dd, H-9), 4.52 (1H dd, $J_{17a,18}$, $J_{17b,18}$ 3.8 and 7.5 Hz, H-18), 4.57 (1H d, $J_{1',2'}$ 7.5 Hz, H-1'), 4.97~5.1 (2H, H-3, -15), 5.67 (1H dd, H-10), 5.73 (1H ddd, H-13), 6.06 (1H dd, H-12), 6.49 (1H dd, H-11).

Anal. Calcd. for $C_{34}H_{59}NO_{12}$: C, 60.60; H, 8.83; N, 2.08.
Found: C, 60.74; H, 8.80; N, 2.17.

A Mixture of 9-*O*-Propyljosamycin Dipropylacetal (**24**) and 13-*O*-Propylisojosamycin Dipropylacetal (**25**)

A mixture of josamycin (478 mg) and anhydrous *p*-toluenesulfonic acid (150 mg) in dry propanol (8 ml) was kept in an ice-bath for 24 hours. After usual work-up, products were isolated.

Propyl 4-*O*-isovaleryl-α-mycaroside (**26**), 12 mg (7%), thick syrup, $[\alpha]_D^{15}$ −119° (*c* 1, chloroform). ^{1}H NMR (in $CDCl_3$ at 250 MHz): δ 3.36 and 3.68 (each 1H dt, *J* 7 and 10 Hz, $CH_3CH_2CH_2O$-), 4.91 (1H d, H-1).

Anal. Calcd. for $C_{15}H_{28}O_5$: C, 62.47; H, 9.79.
Found: C, 62.42; H, 9.58.

Propyl 4-*O*-isovaleryl-β-mycaroside (**27**), 54 mg (32%), thick syrup, $[\alpha]_D^{15}$ +15° (*c* 1, chloroform). ^{1}H NMR (in $CDCl_3$ at 250 MHz): δ 3.41 and 3.82 (each 1H dt, *J* 7 and 10 Hz, $CH_3CH_2CH_2O$-), 4.82 (1H dd, H-1).

Anal. Found: C, 62.27; H, 9.52.

A mixture of **24** and **25**, 149 mg (28%). ^{1}H NMR (in $CDCl_3$ at 250 MHz): 2.07 (s, Ac for **25**), 2.11 (s, Ac for **24**), 2.50 (s, NMe_2 for **25**), 2.51 (s, NMe_2 for **24**), 3.56 (3H s, C(4)-OMe).

Josamycin dipropylacetal (**28**), 65 mg (12%). ^{1}H NMR (in $CDCl_3$ at 250 MHz): δ 2.10 (3H s, Ac), 2.51 (6H s, NMe_2), 3.56 (3H s, C(4)-OMe).

A Mixture of 9-*O*-Butyljosamycin Dibutylacetal (**33**) and 13-*O*-Butylisojosamycin Dibutylacetal (**34**)

Josamycin (441 mg) was similarly treated in *n*-butanol (4.4 ml) containing *p*-toluenesulfonic acid (140 mg) in an ice-bath for 24 hours in a manner as described above to give a mixture of **33** and **34**, 75 mg (14%) and josamycin dibutylacetal (**35**), 61 mg (12%). ^{1}H NMR (in $CDCl_3$ at 250 MHz) of the mixture of **33** and **34**: δ 2.07 (Ac for **34**), 2.10 (Ac for **33**).

9-*O*-Ethyljosamycin (**20**) and 13-*O*-Ethylisojosamycin (**21**)

A mixture of josamycin (1.93 g), dry ethanol (1.56 ml) and trifluoroacetic acid (0.27 ml) in dry acetonitrile (20 ml) was allowed to stand at room temperature for 30 days. The reaction mixture showed, on TLC, 7 spots of 0.46 (a mixture of **10** and **11**), 0.44 (**20**), 0.40 (**21**), 0.29 (**14**), 0.2 (josamycin), 0.12 and 0.04. Usual work-up as described above gave a crude mixture, which was chromatographed on a column of silica gel with benzene→benzene - acetone (3: 1) (gradually changed) to give a pure mixture of **20** and **21**. Chromatography of the mixture by "Column A" with benzene - acetone (4: 1) gave **21**, 34 mg (1.7%) and the fractions containing **20** mainly. The latter was again chromatographed on "Column A" with benzene - hexane - acetone (2: 1: 1) and the fractions containing **20** was further chromatographed on Sephadex LH-20 (developed with methanol) to give pure **20**, 34 mg (1.7%).

^{1}H NMR ($CDCl_3$). **20**: δ 2.13 (3H s, Ac), 2.56 (6H s, NMe_2), 3.57 (3H s, C(4)-OMe), 9.72 (1H s,

CHO). Olefin protons assignable to H-10, 11, 12 and 13 gave almost the same δ and J values with those of **1**.

21: δ 2.09 (3H s, Ac), 2.55 (6H s, NMe_2), 3.60 (3H s, C(4)-OMe), 9.72 (1H s, CHO). Olefin protons assignable to H-9, 10, 11 and 12 gave almost the same δ and J values with those of **2**.

A Mixture of 9-*O*-Isopropyljosamycin (**40**) and 13-*O*-Isopropylisojosamycin (**41**)

A solution of josamycin (620 mg) and *p*-toluenesulfonic acid (190 mg) in dry isopropanol (6 ml) was kept at room temperature for 7 hours, then triethylamine (150 mg) was added to stop the reaction. On checking by TLC, the solution showed spots of Rf 0.35 and 0.38 ($\sim$3: 1 in strength) (**40** and **41**, major), 0.2 (josamycin). Several other spots were also observed. Silica gel column chromatography with benzene - acetone (4: 1) gave a mixture of **40** and **41**, 52 mg (8%).

^{1}H NMR ($CDCl_3$): δ 2.27 (s, Ac for **41**) and 2.31 (s, Ac for **40**) (3H in total), 2.55 (6H s, NMe_2), 3.59 (s, C(4)-OMe for **40**) and 3.61 (s, C(4)-OMe for **41**), 9.77 (1H s, CHO). The signal pattern ascribed to olefin protons resembled that of **1** more than that of **2**.

9-*O*-(*t*-Butyl)josamycin (**42**) and 13-*O*-(*t*-Butyl)isojosamycin (**43**)

A solution of josamycin (1.07 g) in dry acetonitrile (10 ml) containing *t*-butanol (2.3 ml) and trifluoroacetic acid (0.18 ml) was kept at room temperature for 75 hours. The reaction mixture contained **42** (Rf 0.41), **43** (Rf 0.37), josamycin (Rf 0.2) and other minor products. Separation by column chromatography gave pure **42**, 17.5 mg (1.5%) and a mixture of **42** and **43**, 46 mg (8.4%).

^{1}H NMR ($CDCl_3$) of **42**: 2.30 (3H s, Ac), 2.56 (6H s, NMe_2), 3.59 (3H s, C(4)-OMe), 9.76 (1H s, CHO).

9-*O*-(2-Cyanoethyl)josamycin (**44**) and 13-*O*-(2-Cyanoethyl)isojosamycin (**45**)

To a solution of josamycin (1.52 g) in dry acetonitrile (15 ml) was added 2-cyanoethanol (1.25 ml) and trifluoroacetic acid (410 mg), and the mixture was kept at room temperature for 2.5 hours. Usual work-up gave a mixture of **44** and **45**, 831 mg (52%). A part of the mixture was subjected to chromatography on "Column A" to give pure **44** in a low yield.

^{1}H NMR of **44** ($CDCl_3$): δ 2.33 (Ac), 2.57 (NMe_2), 3.60 (C(4)-OMe), 9.77 (CHO).

9-*O*-Phenyljosamycin (**46**) and 13-*O*-Phenylisojosamycin (**47**)

A solution of josamycin (2.05 g) in dry acetonitrile (20 ml) containing phenol (4.7 ml) and trifluoroacetic acid (0.19 ml) was kept at room temperature for 30 hours. The solution showed, after addition of triethylamine, 4 spots of Rf 0.44 (**46**, minor), 0.32 (**47**, major), 0.2 (josamycin, slight) and 0.04. Separation by column chromatography with benzene - acetone (4: 1) gave **46**, 197 mg (9%) and **47**, 933 mg (42%).

^{1}H NMR ($CDCl_3$), **46**: δ 2.34 (Ac), 2.58 (NMe_2), 3.60 (C(4)-OMe), 9.77 (CHO). **47**: 2.28 (Ac), 2.58 (NMe_2), 3.65 (C(4)-OMe), 9.77 (CHO).

9-(1-Methyl-1*H*-tetrazol-5-ylthio)josamycin (**48**) and 13-(1-Methyl-1*H*-tetrazol-5-ylthio)isojosamycin (**49**)

To a solution of josamycin (244 mg) in acetonitrile (2.4 ml) was added 1-methyl-1*H*-tetrazol-5-ylthiol (58 mg) and trifluoroacetic acid (62 mg), and the mixture was kept at room temperature for 20 hours. The solution, after addition of triethylamine (0.11 ml), showed, on TLC, spots of 0.39 (**48**), 0.35 (**49**) and 0.2 (josamycin). Silica gel column chromatography with benzene - acetone (4: 1) gave **48**, 62.5 mg (15%) and **49**, 84 mg (33%).

^{1}H NMR ($CDCl_3$), **48**: δ 2.32 (Ac), 2.55 (NMe_2), 3.59 (C(4)-OMe), 3.94 (NMe), 9.82 (CHO). **49**: 2.23 (Ac), 2.55 (NMe_2), 3.61 (C(4)-OMe), 3.94 (NMe), 9.72 (CHO).

General Procedure for Hydrogenation of Diene Compounds (**1**, **2**, **10**, **11**, **24**, **25**, **33** and **34**)

Starting diene compound (*ca.* 0.5 mmole) dissolved in methanol [10 ml (in the cases of **1** and **2**)] or ethanol (10 ml, for the other compounds) was hydrogenated under the atmospheric pressure of hydrogen in the presence of palladium black for 1 hour. The products showed, on TLC, the same Rf values with those of the corresponding starting materials, respectively, but, unlike the starting materials, the spots can not be detected under the UV light [Super-light LS-D1 (2537 Å), Nikko Sekiei Works, Japan]. The reaction mixture was filtered and concentrated to give the corresponding 9-*O*-

alkyltetrahydrojosamycin dialkylacetal (**6**, **18**, **29**, **36**) and 13-*O*-alkyltetrahydroisojosamycin dialkylacetal (**7**, **19**, **30** and **37**). They were obtained quantitatively in all cases. In their ^{1}H NMR spectra, signals for olefin protons had disappeared.

General Procedure for Deacetalation of 9-*O*-Alkyltetrahydrojosamycin Dialkylacetals (**6**, **18**, **29** and **36**) and 13-*O*-Alkyltetrahydroisojosamycin Dialkylacetals (**7**, **19**, **30** and **37**).

To a solution of starting tetrahydroacetal (*ca.* 0.5 mmole) dissolved in acetonitrile (10 ml) was added 0.05 M hydrochloric acid (15 ml) under cooling and the solution was kept at 20°C for 18 hours (for **6** and **7**), 24 hours (for **18** and **19**), 50 hours (for **29** and **30**) or 120 hours (for **36** and **37**). The reaction mixture showed, on checking by TLC with benzene - acetone (3: 1) or benzene - methanol (10: 1) (for **6** and **7**), major spots at the Rf values slightly smaller than those of the corresponding starting materials. Some other slight spots ascribable to by-products were also observed. The reaction periods mentioned above were determined in order to give the biggest spots, on TLC, of the deacetalation products, respectively. After working up in a usual manner, the crude products obtained were purified by column chromatography with benzene - acetone (5: 1) or 4: 1 (for **6** and **7**). The yields of deacetalation products were as follows: **8** (40%), **9** (40%), a mixture of **22** and **23** (80%), a mixture of **31** and **32** (49%) and a mixture of **38** and **39** (38%); the above fluctuation in yields being, in part, ascribable to the ease or difficulty of the purification by column chromatography. In their ^{1}H NMR spectra, aldehyde protons appeared at $\delta \simeq 9.77$ as 1H singlets in all cases.

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