(9.3, 624 - HOAc), 425 (1.0, a_1b_2 + H), 365 (1.9, 425 - HOAc), 201 (32.5, R_1Ac), 200 (100, R_2Ac), 169 (13.3), 157 (6.5).

Pseudoerythromycin A 6,9:9,12-spiroketal 2,4″,13-triacetate: crystals from acetone–water after column chromatographic purification, 49% yield; mp 110–116 °C; IR (KBr) 3400, 3000–2700, 1720 (ester, lactone), 1440, 1365, 1230, 1160, 895 cm^{-1}; MS, *m/e* (relative intensity), 841 (0.8, M), 799 (3.3, M - CH_2CO), 741 (5.7, g + H), 740 (5.2, g), 641 (0.2, b_1 + H), 624 (0.3, b_2), 560 (5.7, h + H), 201 (44.6, R_1Ac), 200 (100, R_2Ac), 181 (78.6), 169 (10.7), 157 (5.0), 123 (47.2).

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Registry No. 1, 114-07-8; 1 (triacetate), 31357-45-6; 2, 527-75-3; 3, 33396-29-1; 3 (triacetate), 105882-74-4; 4, 33275-72-8; 5, 23893-13-2; 5 (triacetate), 23893-10-9; 6, 105900-46-7; 6 (triacetate), 105882-69-7; 7, 105882-69-7; 7 (triacetate), 105882-75-5; 8, 105900-47-8; 9, 105882-70-0; 9 (triacetate), 105882-76-6; 10, 105882-71-1; 11, 105882-72-2.

Elsamicins A and B, New Antitumor Antibiotics Related to Chartreusin. 2. Structures of Elsamicins A and B

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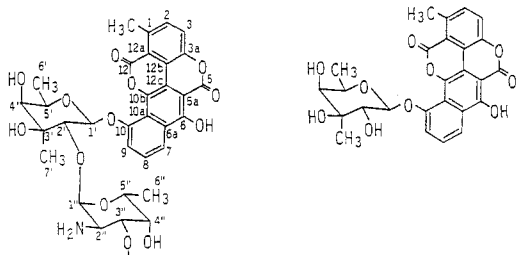
The structures of new antitumor antibiotics elsamicins A (**1a**) and B (**1b**), produced by an unidentified actinomycete strain J907-21, have been established by a combination of chemical degradation, spectral analysis, and X-ray diffraction. They are structurally similar to chartreusin containing chartarin as an aglycon, but differ to each other in sugar moieties. Elsamicin A possesses two novel sugars, 2-amino-2,6-dideoxy-3-*O*-methyl-D-galactose and 6-deoxy-3-*C*-methyl-D-galactose. The presence of the amino sugar makes elsamicin A remarkably water-soluble and more bioactive than chartreusin. Elsamicin B differs from elsamicin A in that it lacks the amino sugar moiety.

Results and Discussion

The search for anticancer drugs, especially drugs with novel chemotypes, has been accelerated by the demands of modern cancer chemotherapy. In our continuing screening for antitumor antibiotics in the fermentation broths using mouse leukemia P388, we have isolated two new antibiotics, elsamicins A (**1a**) and B (**1b**) from an



1a

1b

unidentified actinomycete strain No. J907-21 (ATCC-39417) collected in El Salvador.[1] Both antibiotic components showed antibacterial activity against gram-positive bacteria and anaerobic organisms. **1a**, the predominant component, produced a strong effect in prolonging the life span of mice with leukemia P388, leukemia L1210, and melanoma B16, while **1b** was devoid of antitumor activity.

In this report we present structural studies which show that **1a** and **1b** are similar to chartreusin[2] (**2**). They have the same aglycon, chartarin, but differ in the sugar moieties. **2** was extensively studied because of its promising activity in experimental tumor models.[3] However, it did not proceed to clinical study primarily because of its poor water solubility and rapid bile excretion. Since **1a** contains a new amino sugar (elsaminose) and a neutral sugar (elsarose), it is soluble in water, especially under acidic conditions, and exhibits different pharmacokinetics from **2**.

1a and **1b** were isolated from the fermentation broth of strain J907-21 by 1-BuOH extraction followed by column chromatography on nonionic porous polymer resin and silica gel.[1] **1a** and **1b** were obtained as yellow rods from $CHCl_3$–MeOH. **1a** crystals contain 1 equiv of MeOH as solvate: $C_{33}H_{35}NO_{13}$–CH_3OH, mp 225–226 °C, $[\alpha]^{26}_D$ +124° (*c* 0.5, pyridine); **1b**, $C_{26}H_{22}O_{10}$, mp 271–272 °C dec., $[\alpha]^{26}_D$ −8° (*c* 0.5, pyridine). The UV spectra of **1a** and **1b** exhibited absorption maxima at 236, 266, 398, and 422 nm in neutral and acidic solution and at 240, 268, and 436 nm in alkaline solution, suggesting a chartarin-like chromophore.[4] Comparison of the ^{13}C NMR spectra with that

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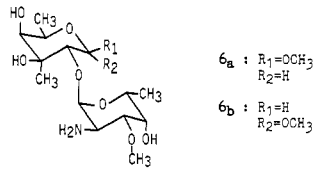
Table I. ^{13}C NMR Data of Elsamicins A and B, Disaccharide (6b), and Chartreusin

position no.	1a[d]	1b[d]	6b[e]	2[d]
1	138.5, s	137.7, s		138.4, s
2	133.4, d	134.1, d		133.0, d
3	120.6, d	121.7, d		120.6, d
3a	145.4, s	146.1, s		146.0, s
5	163.3, s	163.5, s		163.6, s
5a	95.9, s	96.0, s		96.6, s
6	155.7, s	156.2, s		155.4, s
6a	126.0, s	126.1, s		126.0, s
7	116.6, d	117.5, d		116.3, d
8	128.2, d	129.3, d		128.3, d
9	115.8, d	116.4, d		114.3, d
10	152.4, s	154.5 s		154.0, s
10a	118.0, s	117.8, s		117.8, s
10b	138.7, s	139.6, s		138.5, s
12	158.5, s	158.9, s		158.3, s
12a	116.9, s	117.0, s		116.8, s
12b	118.7, s	118.7, s		119.3, s
12c	107.8, s	108.0, s		108.1, s
1-CH_3	21.7, q	22.2, q		21.6, q
1′	96.7, d	102.3, d	104.1, d	99.6, d
2′	80.0, d	73.9, d	79.9[a], d	79.8, d
3′	73.2, s	73.9, s	74.7, s	72.0, s
4′	76.0,[a] d	76.7, d	77.3,[a] d	71.2, d
5′	69.3, d	70.2, d	70.4, d	70.2, d
3′-CH_3	19.6, q	20.0, q	18.9, q	
5′-CH_3	16.8[b], q	18.1, q	16.6,[c] q	16.5,[a] q
1′-OCH_3			58.1, q	
1″	96.4, d		100.5, d	99.3, d
2″	49.7, d		50.3, d	67.4, d
3″	75.9,[a] d		80.7,[a] d	77.9, d
4″	66.8, d		67.8,[b] d	66.9, d
5″	65.3, d		67.5,[b] d	65.7, d
3″-OCH_3	55.1, q		56.6, q	55.8, q
5″-CH_3	16.5,[b] q		16.3[c], q	16.4,[a] q

[a-c] Assignment may be interchanged. [d] In Me_2SO-d_6. [e] In D_2O.

of **2** (Table I) revealed that both **1a** and **1b** contained all 18 sp^2 carbons and one aromatic methyl group assignable to the chartarin (**3**) nucleus. In addition to these carbon signals, the spectrum of **1a** displayed three C-CH_3, one O-CH_3, two anomeric carbons (δ 96.4 and 96.7) and eight sugar ring carbons (δ 49.7–80.0) which indicated a disaccharide moiety. One of the sugar carbons appeared to be substituted by an amino function since its resonance at δ 49.7 was at a significantly higher field than the other, oxygen bearing carbons. These ^{13}C NMR data further indicated two 6-deoxyhexoses having one C-CH_3, one O-CH_3, and one amino group. Upon acetylation in anhydrous MeOH and in pyridine, **1a** afforded a mono-*N*-acetate (**4**) and a tetra-*N*,*O*-acetate (**5**), respectively. The ^1H NMR spectra of **4** and **5** (Table II) showed two doulet methyls at around δ 1.2–1.5, one singlet methyl (δ 1.32 or 1.48), one OCH_3 (δ 3.25 or 3.29), and one *N*-acetyl group (δ 0.80). That **1b** lacked the amino sugar moiety of **1a** was clearly demonstrated in the ^{13}C NMR. The mass spectrum of **4** indicated a molecular ion at *m/z* 695 and strong fragment ions at *m/z* 334 (chartarin, base peak), *m/z* 362 (*N*-acetyl disaccharide), and *m/z* 202 (*N*-acetyl sugar). The molecular ion of **5** appeared at *m/z* 821 along with fragment ions at *m/z* 376 (monoacetylchartarin) and *m/z* 334 (chartarin, base peak), *m/z* 446 (triacetyl disaccharide), and *m/z* 244 (diacetyl sugar). Acid hydrolysis of **1a** with 0.4 N methanolic hydrogen chloride yielded a yellow crystalline product (**3**) and an anomeric mixture of a ninhydrin-positive disaccharide (**6**). **3** (*m/z* 334, $C_{19}H_{10}O_6$)



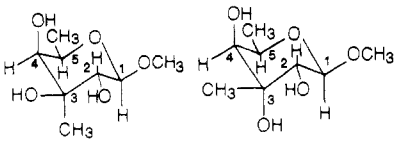
was identified as chartarin[4] by direct comparison with an authentic sample prepared from **2**. The sugar fragment **6** was separated by a weakly acidic ion exchange resin to α- (**6a**, minor) and β-methyl glycoside (**6b**, major). Molecular formulae of **6a** and **6b** were established as $C_{15}H_{29}NO_8$ on the basis of EI-mass ($M^+ + 1$, *m/z* 352) and ^{13}C NMR. In their ^1H NMR spectra (Table II), the anomeric protons of **6a** appeared at δ 4.90 (d, J = 4.3 Hz) and 4.98 (d, J = 3.5 Hz), while those of **6b** at δ 4.50 (d, J

Table II. ^1H NMR Data of Elsamicin Derivatives and the Hydrolysis Products

position no.	4[b]	5[c]	3[d]	6a[e]	13a[c]
2	7.1–7.4	7.78 (d, 8.9)[a]	7.74 (d, 8.8)[a]		
3	7.1–7.4	7.53 (d, 8.9)[a]	7.57 (d, 8.8)[a]		
6-OH	11.54 (s)		11.44 (s)		
7	8.03 (d, 8.5)	7.95 (d, 8.5)	7.90 (dd, 8.4, 2.2)		
8	7.50 (t, 8.5)	7.68 (t, 8.5)	7.58 (t, 8.4)		
9	7.1–7.4	7.40 (br d)	7.26 (dd, 8.4, 2.2)		
10-OH			10.45 (s)		
1-CH_3	2.54 (s)	2.61 (s)	2.80 (s)		
$OCOCH_3$		2.95 (s)			
1′	5.36 (d, 8.2)	5.42 (d, 8.2)		4.85 (d, 4.5)	4.74 (d, 4.2)
2′	3.3–4.5	4.25 (br d)		3.78 (d, 4.5)	3.81 (d, 4.2)
3′-CH_3	1.32 (s)	1.48 (s)		1.26 (s)	1.44 (s)
4′	3.3–4.5	5.02 (s)		3.42 (s)	4.89 (s)
5′	3.3–4.5	4.13 (br s)		4.12 (q, 6.7)	4.12 (q, 6.5)
5′-CH_3	1.2–1.4 (d)	1.21 (d, 6.4)		1.15 (d, 6.7)	1.15 (d, 6.5)
3′-OH		1.86 (br s)			1.86 (br s)
1″	5.75 (d, 3.8)	5.90 (d, 3.2)		5.12 (d, 3.5)	4.96 (d, 4.0)
2″	3.3–4.5	4.38–4.42		3.48 (dd, 3.5, 11.0)	4.41 (ddd, 4.0, 10.4, 8.8)
3″	3.3–4.5	3.40 (br s)		3.74 (dd, 3.0, 11.0)	3.47 (dd, 3.0, 10.4)
4″	3.3–4.5	5.40 (s)		4.10 (d, 3.0)	5.38 (d, 3.0)
5″	3.3–4.5	4.38–4.42		4.27 (q, 6.7)	4.36 (q, 6.4)
3″-OCH_3	3.29 (s)	3.25 (s)		3.31 (s)[a]	3.35 (s)[a]
5″-CH_3	1.2–1.5 (d)	1.21 (d, 6.4)		1.15 (d, 6.7)	1.14 (d, 6.4)
2′-NHAc		5.40–5.42			5.84 (d, 8.8)
$NHCOCH_3$	0.80 (s)	0.78 (br s)			2.03 (s)
$OCOCH_3$		2.16, 2.25			2.18, 1.19
OCH_3				3.37 (s)[a]	3.40 (s)[a]

[a] Assignments may be interchanged. [b] 80 MHz, in $CDCl_3$. [c] 360 MHz, in $CDCl_3$. [d] 80 MHz, in Me_2SO-d_6. [e] 200 MHz, in D_2O.

Table III. ^{1}H NMR Data of Methyl β-Elsaroside and Methyl β-D-Virenoside (CD_3OD)



position no.	9b	12b
1	4.20 (d, 7.8)	4.40 (d, 7.8)
2	3.53 (d, 7.8)	3.29 (d, 7.8)
3		
4	3.40	3.40
5	3.83 (q, 6.2)	4.12 (q, 6.2)
3-CH_3	1.22 (s)	1.34 (s)
5-CH_3	1.30 (d, 6.2)	1.21 (d, 6.2)
1-OCH_3	3.50 (s)	3.48 (s)

= 8.0 Hz) and 5.09 (d, J = 3.5 Hz). **6a** and **6b** resisted further acid hydrolysis and underwent extensive degradation under conditions designed to cleave the glycoside linkage. **6b** was, thus, converted to mono-*N*-acetyl derivative (**7b**, $M^+ + 1$, m/z 394) which was hydrolyzed in 4.5 N methanolic hydrogen chloride. Silica gel purification of the products afforded four sugar fragments, α- and β-methyl glycosides of an *N*-acetylaminodideoxymono-*O*-methylhexose (**8a** and **8b**, methyl elsaminoside, $C_{10}H_{19}NO_5$, $M^+ + 1$, m/z 234 and M^+ – MeOH, m/z 201) and α- and β-methyl glycosides of a monodeoxy-C-methylhexose (**9a** and **9b**, methyl elsaroside, $C_8H_{16}O_5$, $M^+ - OCH_3$, m/z 161). $Ba(OH)_2$ treatment of **8a** yielded free aminoglycoside (**10a**, $M^+ - OCH_3$, m/z 160) whose ^{1}H NMR showed no $COCH_3$ and a substantial upfield shift (by 1.18 ppm) of the C_2 ring proton in comparison to that of **8a**. The ring protons which were analyzed in first order ($J_{1_\beta-2}$ = 3.8 Hz, J_{2-3} = 10.5 Hz, J_{3-4} = 3.0 Hz, J_{4-5} = 1.0 Hz, J_{5-6} = 6.4 Hz), indicated **10a** to be a methyl 2-amino-2,6-dideoxygalactoside. The position of the OCH_3 was determined to be C-3 on the basis of the fact that O-acetylation of **8a** (**11a**, $M^+ + 1$: m/z 276) resulted in the low-field shift of the C_4-ring proton by 1.32 ppm in the ^{1}H NMR. The above NMR data coupled with the optical rotational value (**10a**: $[\alpha]^{23}_D$ +106°) strongly assigned that elsaminose (**10**) was 2-amino-2,6-dideoxy-3-*O*-methyl-D-galactose. The ^{1}H NMR of **9a** and **9b** clearly indicated the absence of a ring proton at C-3. The observed coupling constants of **9a** ($J_{1_\beta-2}$ = 4.5 Hz, J_{4-5} < 1.0 Hz and J_{5-6} = 6.7 Hz) and **9b** ($J_{1_\alpha-2}$ = 7.8 Hz, J_{4-5} < 1.0 Hz and J_{5-6} = 6.5 Hz) and the splitting patterns suggested the 6-deoxy-3-*C*-methylgalactose or 6-deoxy-3-*C*-methylgulose (virenose[5]) structures for elsarose. An authentic sample of methyl β-D-virenoside (**12b**)[6] was differentiated from **9b** by TLC and ^{1}H NMR. In their ^{1}H NMR (Table III), the H_1 and H_5 protons of **12b** were observed at considerably lower field (H_1 δ 4.40 and H_5 δ 4.12) than those of **9b** (H_1 δ 4.20 and H_5 δ 3.83), indicating that C_3-OH of **12b** was in an axial and that of **9b** in an equatorial orientation. The optical rotation values of **9a** (+152°) and **9b** (–33°) and $\Delta[M]_{CuAm}$ value[7] observed for **9a** (–1310°) allowed us to assign the D configuration to this sugar.

The structure of disaccharide **6** and the nature of its linkage in **1a** were established by the following spectral data. The mass spectra of **6a** and **6b** exhibited strong

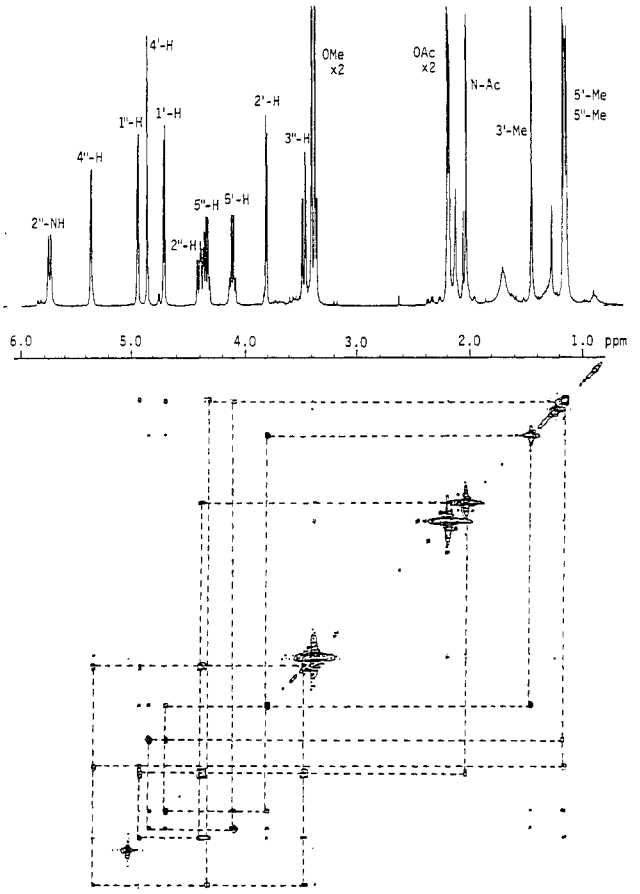


Figure 1. ^{1}H–^{1}H shift correlated spectroscopy (COSY) of **13a**.

fragment ions at m/z 319 (M^+ – MeOH) and 160 (oxonium ion of elsaminose) in addition to their protonated molecular ion at m/z 352 indicating the elsaminosylelsarose sequence of the disaccharides. N,O-Acetylation of **6a** (**13a**) resulted in a low-field shift (0.9–1.3 ppm) of three protons ($H_{4'}$, $H_{2''}$, and $H_{4''}$) but no shift of $H_{2'}$ (δ 3.8, d, J = 4.2 Hz) in the ^{1}H NMR (Table II). Unambiguous assignments for all protons were provided by ^{1}H–^{1}H shift correlated spectroscopy of **13a** (COSY, Figure 1) which strongly supported the assigned structures of elsaminose and elsarose. In the COSY spectrum, the anomeric proton of elsaminose ($H_{1''}$) resonated at δ 4.96 as a doublet with J = 3.5 Hz, indicating the α-linkage of the sugar. The COSY spectrum also revealed the presence of a long-range W-type coupling between $C_{2''}$-H and $C_{3''}$-CH_3 which can be explained by the axial configuration of both $C_{2''}$-H and $C_{3''}$-CH_3. The β-pyranoside linkage of **6** to chartarin was assigned by the magnitude of the coupling of the anomeric proton ($H_{1'}$ δ 5.3–5.4, d, J = 8.2 Hz, Table II). **1a** exhibited UV spectra superimposable to those of chartreusin at various pH's, and the IR spectra of **1a** and chartreusin in chloroform solution showed the same pattern of carbonyl absorptions ($\nu_{C=O}$, 1690 and 1725 cm^{-1}) indicating that **6** was linked to the C_{10}-OH of chartarin in the same way as chartreusin. Furthermore, the phenolic proton of **1a** was observed at δ 11.44 in the ^{1}H NMR, which indicated a hydrogen bond between the hydroxyl (C_6-OH) and C_5 carbonyl.[8] As discussed previously, **1b** is apparently an analogue of **1a** lacking the amino sugar moiety.

It is noteworthy that the $C_{2''}$-*N*-acetyl group of elsaminose resonated at unusually high field (δ 0.80) in **4** and

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(6) The authentic sample of methyl 6-deoxy-3-*C*-methyl-β-D-gulopyranoside (methyl β-virenoside) was kindly supplied by Dr. J. Yoshimura.

(7) Umezawa, S.; Tsuchiya, T.; Tatsuta, K. *Bull. Chem. Soc. Jpn.* **1966**, *39*, 1235.

(8) The ^{1}H NMR of chartarin (**3**) exhibited two phenolic protons at 10.45 and 11.44 ppm. The latter proton was observed in the ^{1}H NMR of **1a** and **1b**.

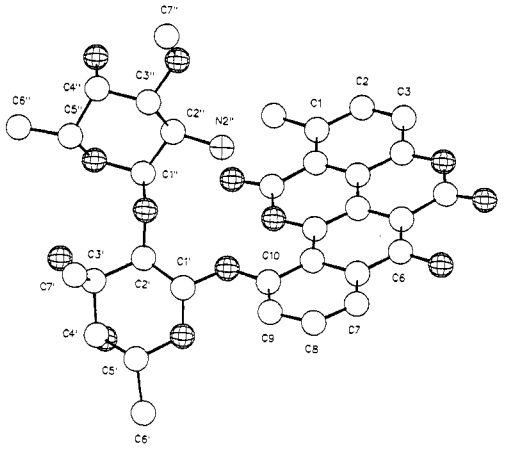

Figure 2. Computer-generated perspective drawing of the final X-ray model of elsamicin A. Hydrogens are omitted for clarity.

5. This was most plausibly interpreted as having the acetyl group over the chartarin ring and in the deshielding cone. This was also found to be the case with **2**. In the ^{1}H NMR of pentaacetylchartreusin (**14**), one of the acetyl groups was observed at δ 0.75 which was assigned to the acetyl on $C_{2''}$-hydroxyl of D-digitalose. The X-ray crystallographic analysis described below fully supported this analysis as well as the general structure of **1a**.

A computer generated perspective drawing of the final X-ray model of elsamicin A is displayed in Figure 2. The asymmetric unit in the X-ray determination consisted of two independent molecules. Since they had the same structure, configuration, and conformation, only one is illustrated. The X-ray experiment determined only the relative configuration, and the absolute configuration was set by the D sugars. The chartarin portion of elsamicin A is planar and the best plane of the disaccharide portion is twisted approximately 40° away from the aromatic plane. As suggested by the NMR studies on the *N*-acetyl derivative, the $N_{2''}$ substituent is pointing toward the aromatic portion. If there were no change in conformation, the *N*-acetyl methyl would be directly over the aromatic rings. Both sugars are in the chair conformation with most substituents equatorial.

Experimental Section

Thin layer chromatography (TLC) was performed on precoated silica gel plates (Kiesel gel 60F_{254}, Merck, layer thickness 0.25 mm). The IR spectra were determined on a JASCO IRA-1 spectrometer in KBr pellet or chloroform solution and the UV spectra on a Shimazu UV-200 spectrometer in methanol solution. The ^{1}H NMR and ^{13}C NMR spectra were recorded on a Varian Model FT80A, a Varian XL-200, or a Bruker WM-360 spectrometer operated in Fourier transform mode using tetramethylsilane as the internal standard. The ordinary mass spectra were obtained with a Hitachi RMU-6MG mass spectrometer modified with an in-beam/electron impact system and high-resolution spectra on a Hitachi-M80 mass spectrometer. Optical rotations were determined with a JASCO model DIP-140.

Mono-*N*-acetylelsamicin A (4). Elsamicin A (**1a**, 50 mg) was stirred with acetic anhydride (0.5 mL) in anhydrous methanol (5 mL) for 3 h at room temperature. The reaction mixture was evaporated to a residue which was chromatographed on silica gel by using $CHCl_3$–MeOH (95:5). The UV-absorbing fractions were pooled and concentrated to give mono-*N*-acetylelsamicin A (**4**, 42 mg): $[\alpha]^{23}_D$ −29° (*c* 0.5, MeOH); MS, *m/z* 695 (M^+), 494 (M^+ − elsaminose), 362 (M^+ − chartarin), 334 (base peak, chartarin); IR (KBr) 3400, 1720, 1690, 1380, 1250, 1070, 1040 cm^{-1}; ^{1}H NMR (Table II); ^{13}C NMR ($CDCl_3$) δ 169.0, 164.4, 158.0, 156.7, 152.5, 146.0, 140.2, 138.3, 132.9, 127.4, 126.5, 120.6, 118.9, 118.4, 118.3 (2C), 117.2, 108.6, 100.6, 98.3, 97.8, 96.4, 81.5, 78.9, 75.8, 73.4, 70.0, 66.9, 56.7, 47.8, 22.4, 21.9, 17.3, 16.8, 16.4.

***N,O*-Tetraacetylelsamicin A (5).** Elsamicin A (**1a**, 50 mg) was treated with acetic anhydride (5 mL) in the presence of pyridine (5 mL) for 3 days at room temperature. The reaction mixture was evaporated to dryness and the residue purified on silica gel chromatography using $CHCl_3$–MeOH (98:2) to give *N,O*-tetraacetylelsamicin A (**5**, 38 mg): IR (KBr) 3400, 1780, 1740, 1670, 1380, 1250, 1070, 1040 cm^{-1}; MS, *m/z* 821 (M^+), 779 (M^+ − CH_2=C=O), 446 (M^+ − chartarin), 376 (*O*-acetylchartarin), 334 (chartarin), 244 (elsaminose); ^{1}H NMR (Table II); ^{13}C NMR ($CDCl_3$) δ 170.9 (×2), 169.1, 169.0, 158.5, 156.5, 152.9, 146.8, 145.9, 144.1, 139.7, 133.5, 130.5, 128.3, 120.1, 118.7, 118.3 (2 C), 117.6, 117.3, 111.4, 108.5, 100.9, 98.5, 81.9, 76.8, 76.3, 72.4, 69.4, 68.6, 66.3, 57.0, 48.4, 22.4, 22.1, 21.1, 20.9 (2 C), 20.1, 16.8, 16.5.

Acid Methanolysis of 1a: Chartarin (3) and Disaccharides (6a and 6b). A solution of elsamicin A (**1a**, 1.5 g) in 0.4 N methanolic hydrogen chloride (300 mL) was refluxed for 1 h at 80 °C. After cooling, yellow crystals deposited in the reaction solution were collected by filtration and dried in vacuo. Chartarin (**3**, 723 mg): IR (KBr) 3420, 3210, 1690, 1610, 1580, 1505 cm^{-1}; MS, *m/z* 334 (M^+); ^{1}H NMR (Table II).

The filtrate was neutralized with Amberlite IR-45 resin (OH^-) and concentrated in vacuo to afford a pale-yellow syrup (**6**, 919 mg, an anomeric mixture of disaccharide **6a** and **6b**). The R_f values on TLC developed with 1-BuOH–AcOH–H_2O (63:10:27) were 0.22 for **6a** and 0.16 for **6b**. **6** (600 mg) was charged on an Amberlite CG-50 column (NH_4^+, 800 mL) which was developed with H_2O. Each fraction (8 mL) was monitored by ninhydrin reagent on a SiO_2 plate. The first ninhydrin-positive fractions (no. 101–131) were pooled, evaporated to a small volume, and subjected to a column of Sephadex LH-20 packed in 50% MeOH. Elution was carried out with the same solvent and the ninhydrin-positive fractions were concentrated in vacuo to yield the α-methyl glycoside form (**6a**, 229 mg) as a white amorphous powder: mp 79–82 °C; $[\alpha]^{27}_D$ +211° (*c* 1, H_2O); MS, *m/z* 352 (M^+ + 1), 160; high-resolution EIMS, *m/z* 352.1968 (calcd for $C_{15}H_{30}NO_8$ 352.1972); ^{1}H NMR (Table II); ^{13}C NMR (D_2O) δ 99.5, 98.1, 80.7, 77.5, 77.2, 73.0, 68.0, 67.5, 66.2, 56.8, 55.6, 50.2, 20.3, 16.5, 16.3.

Anal. Calcd for $C_{15}H_{29}NO_8 \cdot {}^1/_4H_2O$: C, 50.62; H, 8.35; N, 3.93. Found: C, 50.52; H, 8.30; N, 3.85.

The second ninhydrin-positive fractions (no. 148–210) of the Amberlite CG-50 chromatography were combined and purified by using Sephadex LH-20 chromatography to afford homogeneous β-methyl glycoside (**6b**, 402 mg): mp 80–83 °C; $[\alpha]^{27}_D$ +116° (*c* 1, H_2O); MS, *m/z* 352 (M^+ + 1), 160; IR (KBr) 3400, 2950, 1620, 1140, 1070, 1040, 1000, 870 cm^{-1}; ^{1}H NMR (200 MHz, D_2O + DCl) δ 1.16 (6 H, d, *J* = 6.7 Hz), 1.19 (3 H, s), 3.38 (3 H, s), 3.45 (3 H, s), 3.3–3.5 (3 H, m), 3.51 (1 H, dd, *J* = 3.0 and 11.0 Hz), 3.92 (1 H, q, *J* = 6.7 Hz), 4.05 (1 H, d, *J* = 3.0 Hz), 4.32 (1 H, q, *J* = 6.7 Hz), 4.46 (1 H, d, *J* = 8.0 Hz), 5.17 (1 H, d, *J* = 3.5 Hz); ^{13}C NMR (Table I).

Anal. Calcd for $C_{15}H_{29}NO_8 \cdot {}^1/_2H_2O$: C, 49.99; H, 8.39; N, 3.89. Found: C, 50.15; H, 8.31; N, 3.73.

Mono-*N*-acetyl-6b (7b). **6b** (370 mg) was treated with acetic anhydride (3 mL) in anhydrous methanol (30 mL) for 5 h at room temperature. The solution was concentrated to dryness to give the mono-*N*-acetyl derivative (**7b**, 469 mg): $[\alpha]^{23}_D$ +114° (*c* 0.5, MeOH); MS, *m/z* 394 (M^+ + 1), 362 (M^+ − OCH_3); IR (KBr) 3500, 3340, 1650, 1530, 1040 cm^{-1}; ^{1}H NMR (D_2O) δ 1.25 (3 H, d, *J* = 6.7 Hz), 1.28 (3 H, d, *J* = 6.7 Hz), 1.32 (3 H, s), 2.10 (3 H, s), 3.44 (3 H, s), 3.50 (3 H, s), 3.64 (1 H, dd, *J* = 3.0 and 11.0 Hz), 3.44–4.30 (m), 4.37 (1 H, q), 4.43 (1 H, d, *J* = 8.0 Hz), 5.07 (1 H, d, *J* = 3.5 Hz).

***N,O*-Triacetyl-6a (13a).** **6a** (12 mg) was dissolved in acetic anhydride (0.1 mL) and pyridine (1 mL) and the mixture stirred overnight at room temperature. The solution was evaporated in vacuo to a sticky residue which was applied on the top of a silica gel column (20 mL). Upon developing with $CHCl_3$–MeOH (20:1), the anthrone-positve eluate was combined and concentrated to afford *N,O*-triacetyl-**6a** (**13a**, 10 mg): mS, *m/z* 478 (M^+ + 1); IR (KBr) 3450, 1740, 1680, 1540, 1240, 1050 cm^{-1}; ^{1}H NMR (Table II).

Acid Methanolysis of 7b: Methyl *N*-Acetylelsaminosides (8a and 8b) and Methyl Elsarosides (9a and 9b). **7b** (450 mg) was hydrolyzed with 4.5 N methanolic hydrogen chloride (40 mL) under reflux for 40 min. After being neutralized by Amberlite

IR-45 resin (OH^-), the reaction solution was concentrated and subjected to a silica gel column (450 mL) which had been prewashed and packed by the lower phase of $CHCl_3$–MeOH–28% NH_4OH (6:1:1) mixture. The column was developed with the same solvent and eluate collected in 12-mL fractions. Upon monitoring by anthrone reagent, the first anthrone-positive fractions (no. 45–55) were pooled and concentrated in vacuo to give **8a** (140 mg). Subsequent elution followed by evaporation of the appropriate eluate fractions yielded **8b** (22 mg, no. 84–92), **9a** (80 mg, no. 109–128), and **9b** (84 mg, no. 136–165) in the order of elution. R_f values on TLC developed with the lower phase of $CHCl_3$–MeOH–28% NH_4OH (2:1:1) were **8a**, 0.57; **8b**, 0.49; **9a**, 0.31; and **9b**, 0.28. Methyl elsarosides (**9a** and **9b**) were clearly differentiated from methyl β-D-virenoside by the TLC (R_f 0.35). Methyl *N*-acetyl-α-elsaminoside (**8a**): MS, m/z 234 ($M^+ + 1$); IR 3500, 3310, 1650, 1560, 1060 cm^{-1}; ^1H NMR (D_2O) δ 1.28 (3 H, d, J = 6.3 Hz, 5″-CH_3), 2.05 (3 H, s, 2″-$NCOCH_3$), 3.37 (3 H, s, 1″-OCH_3), 3.39 (3 H, s, 3″-OCH_3), 3.59 (1 H, dd, J = 3.0 and 10.5 Hz, 3″-H), 4.05 (1 H, q, J = 6.3 Hz, 5″-H), 4.10 (1 H, br d, J = 3.0 Hz, 4″-H), 4.15 (1 H, dd, J = 3.8 and 10.5 Hz, 2″-H), 4.79 (1 H, d, J = 3.8 Hz, 1″-H). Methyl *N*-acetyl-β-elsaminoside (**8b**): ^1H NMR (D_2O) δ 1.32 (3 H, d, J = 6.3 Hz, 5″-CH_3), 2.05 (3 H, s, 2″-$NCOCH_3$), 3.39 (3 H, s, 3″-OCH_3), 3.41 (1 H, dd, J = 8.3 and 3.6 Hz, 3″-H), 3.48 (3 H, s, 1″-OCH_3), 3.76 (1 H, q, J = 6.3 Hz, 5″-H), 3.85 (1 H, t, J = 8.3 Hz, 2″-H), 4.02 (1 H, dd, J = 3.6 and 1.2 Hz, 4″-H), 4.38 (1 H, d, J = 8.3 Hz, 1″-H). Methyl α-elsaroside (**9a**): colorless syrup; $[\alpha]^{25}_D$ +152° (*c* 0.5, $CHCl_3$); $[\alpha]^{22}_{435}$ +244° (*c* 0.25, H_2O), $[\alpha]^{22}_{435}$ −438° (*c* 0.25, CuAm), $\Delta[M]_{CuAm}$ = (−438−224) × 1.92 = −1309°; MS, m/z 161 ($M^+ - OCH_3$); high-resolution EIMS, m/z 161.0813 (calcd for $C_7H_{13}O_4$ 161.0814); ^1H NMR ($CDCl_3$) δ 1.32 (3 H, d, J = 6.9 Hz, 5′-CH_3), 1.37 (3 H, s, 3′-CH_3), 2.88 (3 H, br, OH), 3.45 (4 H, s, 1′-OCH_3 and 4′-H), 3.91 (1 H, d, J = 4.5 Hz, 2′-H), 4.05 (1 H, q, J = 6.9 Hz, 5′-H), 4.76 (1 H, d, J = 4.5 Hz, 1′-H); ^{13}C NMR (D_2O) δ 100.4 (d), 77.0 (d), 73.7 (s), 71.1 (d), 66.2 (d), 56.0 (q), 20.0 (q), 16.7 (q). Anal. Calcd for $C_8H_{16}O_5 \cdot {}^1/_2H_2O$: C, 47.75; H, 8.52. Found: C, 47.44; H, 8.47.

Methyl β-elsaroside (**9b**): colorless syrup; $[\alpha]^{25}_D$ −33° (*c* 0.5, $CHCl_3$); $\Delta[M]_{CuAm}$ = −1119°; MS, m/z 161 ($M^+ - OCH_3$); ^1H NMR ($CDCl_3$) δ 1.32 (3 H, s, 3′-CH_3), 1.37 (3 H, d, J = 6.0 Hz, 5′-CH_3), 3.42 (1 H, br s, 4′-H), 3.47 (3 H, br, OH), 3.60 (3 H, s, 1′-OCH_3), 3.69 (1 H, d, J = 7.8 Hz, 2′-H), 3.83 (1 H, q, J = 6.0 Hz, 5′-H), 4.22 (1 H, d, J = 7.8 Hz, 1′-H); ^{13}C NMR (D_2O), δ 103.5 (d), 76.9 (d), 74.9 (s), 73.7 (d), 70.6 (d), 58.0 (q), 18.6 (q), 16.7 (q).

Alkaline Hydrolysis of 8a: Methyl α-Elsaminoside (10a). **8a** (50 mg) was hydrolyzed with barium hydroxide saturated water (20 mL) under reflux for 3 days. The solution was neutralized with dry ice, and precipitated $BaCO_3$ was removed by filtration. The filtrate was concentrated in vacuo to a small volume and applied on a solumn of Amberlite CG-50 (NH_4^+, 100 mL). The column was washed with H_2O and then developed with 0.2 N NH_4OH. The ninhydrin-positive fractions were combined and concentrated to afford methyl α-elsaminoside as a pale yellow syrup (**10a**, 25 mg): $[\alpha]^{23}_D$ +106° (*c* 0.2, MeOH); MS, m/z 160 ($M^+ - OCH_3$); ^1H NMR (D_2O) δ 1.26 (3 H, d, J = 6.4 Hz, 5″-CH_3), 2.97 (1 H, dd, J = 3.8 and 10.5 Hz, 2″-H), 3.41 (3 H, s, OCH_3), 3.43 (3 H, s, OCH_3), 3.45 (1 H, dd, J = 3.0 and 10.5 Hz, 3″-H), 4.00 (1 H, q, J = 6.4 Hz, 5″-H), 4.02 (1 H, br d, J = 3.0 Hz, 4″-H), 4.72 (1 H, d, J = 3.8 Hz, 1″-H); ^{13}C NMR (D_2O), δ 100.7 (d), 80.7 (d), 67.5 (d), 67.1 (d), 56.7 (q), 56.1 (q), 50.0 (d), 16.4 (q).

O-Acetylation of 8a (11a). **8a** (20 mg) was acetylated by stirring with acetic anhydride (0.3 mL) and pyridine (1 mL) overnight at room temperature. The reaction mixture was evaporated to dryness and the residue was purified by silica gel chromatography developing with $CHCl_3$–MeOH (50:1). Anthrone positive eluate was concentrated to give a white solid (22 mg) which was crystallized from a mixture of *n*-hexane and benzene to afford colorless needles of **11a** (12 mg); mp 163–164 °C (lit.[9] 147–148 °C); $[\alpha]^{23}_D$ +154° (*c* 0.3, $CHCl_3$) (lit. $[\alpha]_D$ +104.2 (*c* 0.92, $CHCl_3$)); MS, m/z 276 ($M^+ + 1$); IR (KBr) 3320, 1730, 1650, 1550, 1240, 1060 cm^{-1}; ^1H NMR ($CDCl_3 + D_2O$), δ 1.18 (3 H, d, J = 6.4 Hz, 5″-CH_3), 2.02 (3 H, s, 2″-$NHCOCH_3$), 2.18 (3 H, s, 4″-$OCOCH_3$), 3.33 (3 H, s, OCH_3), 3.36 (3 H, s, OCH_3), 3.43 (1 H, dd, J = 3.2 and 10.5 Hz, 3″-H), 3.98 (1 H, q, J = 6.4 Hz, 5″-H), 4.37 (1 H, dd, J = 3.8 and 10.5 Hz, 2″-H), 4.76 (1 H, d, J = 3.8 Hz, 1″-H), 5.34 (1 H, br d, J = 3.2 Hz, 4″-H).

Anal. Calcd for $C_{12}H_{21}NO_6$: C, 52.35; H, 7.69; N, 5.09. Found: C, 52.47; H, 7.80; N, 4.91.

Acid Methanolysis of Elsamicin B (1b): Chartarin (3) and Methyl Elsaroside (9a and 9b). A solution of elsamicin B (**1b**, 2 mg) in 0.4 N methanolic hydrogen chloride (2 mL) was heated under reflux for 1 h. Yellow crystals which precipitated in the solution were filtered and dried in vacuo (0.5 mg). The crystals were identical with chartarin by TLC and IR. Neutral sugars in the filtrate were identified as a mixture of **9a** and **9b** by TLC (R_f, 0.31 and 0.28) developed with the lower phase of $CHCl_3$–MeOH–28% NH_4OH (2:1:1).

Pentaacetylchartreusin (14). Chartreusin (**2**) was isolated from the fermentation broth of a *Streptomyces* strain, No. H470-503, and was identified with the authentic sample by spectral analysis. **2** (50 mg) was stirred with acetic anhydride (5 mL) in pyridine (5 mL) for 2 days. The mixture was concentrated in vacuo and the residue subjected to silica gel chromatography developed with $CHCl_3$–MeOH (100:1). Concentration of the UV-absorbing eluate afforded pentaacetylchartreusin as a yellow solid (**14**, 46 mg): IR (KBr) 1780, 1740, 1360, 1230, 1065 cm^{-1}; ^1H NMR ($CDCl_3$) δ 0.75 (3 H, s), 1.22 (3 H, d, J = 6.4 Hz), 1.29 (3 H, d, J = 6.4 Hz), 2.08 (3 H, s), 2.10 (3 H, s), 2.23 (3 H, s), 2.61 (3 H, s), 2.94 (3 H, s), 3.22 (3 H, s), 3.53 (1 H, dd, J = 3.2 and 10.5 Hz), 3.9–4.2 (2 H, br q), 4.52 (1 H, dd, J = 8.5 and 10.2 Hz), 4.89 (1 H, dd, J = 3.2 and 10.5), 5.2–5.4 (3 H, m), 5.44 (1 H, d, J = 8.2 Hz), 6.12 (1 H, d, J = 3.6 Hz), 7.3–8.0 (5 H, m).

Single-Crystal X-ray Diffraction Analysis of Elsamicin A (1a). Suitable crystals of **1a** were grown from $CHCl_3$/MeOH solutions by slow evaporation. Preliminary X-ray diffraction photographs displayed only triclinic symmetry. Accurate lattice constants of a = 9.580 (1), b = 9.1065 (9), and c = 22.052 (3) Å and α = 102.498 (8)°, β = 107.236 (8)°, and γ = 65.104 (8)° were determined from a least-squares fit of 15 diffractometer-measured 2θ values. Assuming a plausible density of 1.37 g/mL combined with the known optical activity of elsamicin A, required space group P_1 with two molecules of composition $C_{33}H_{35}O_{13}N$–CH_3OH forming the asymmetric unit (unit cell). All unique diffraction maxima with 2θ > 114° were collected using graphite monochromated Cu Kᾱ radiation (1.54178 Å) and variable speed, 1° ω-scans. Of the 4961 reflections collected in this way, 4280 (86%) were judged observed and used in all subsequent calculation.[10] The structure was phased with some difficulty using the MULTAN series of programs. The initial phasing model was extended with weighted Fourier refinements until all of the non-hydrogen atoms were present. Hydrogens were located by difference syntheses following partial refinement. Block-diagonal least-squares refinements with anisotropic non-hydrogen atoms and isotropic hydrogens have converged to a standard crystallographic residual 0.0563 for the observed data. Additional crystallographic details are available.[11]

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(11) Crystallographic parameters have been deposited with the Cambridge Crystallographic Data File, University Chemical Laboratory, Lensfield Road, Cambridge, CB2 1EW, England, and are available from them. Please give a complete literature citation when ordering.

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Registry No. **1a**, 97068-30-9; **1b**, 97068-31-0; **2**, 6377-18-0; **3**, 34170-23-5; **4**, 106544-47-2; **5**, 106544-48-3; **6a**, 106544-49-4; **6b**, 106544-50-7; **7b**, 106544-51-8; **8a**, 106544-53-0; **8b**, 106544-54-1; **9a**, 106622-98-4; **9b**, 106622-99-5; **10a**, 106544-55-2; **11a**, 53958-79-5; **13a**, 106544-52-9; **14**, 106544-56-3.

Supplementary Material Available: Tables of fractional coordinates and thermal parametes, bond distances, bond angles, and torsion angles for **1a** (16 pages). Ordering information is given on any current masthead page.

Aqueous Cycloadditions Using Glyco-Organic Substrates. 1. Stereochemical Course of the Reaction[1]

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New water-soluble *trans*-butadienyl ethers were synthesized by using free glucose as the hydrophilic part. Aqueous cycloaddition with a variety of dienophiles showed rate and stereoselectivity enhancement in comparison with similar peracetylated dienes in organic solvents. After cycloaddition and eventually functional group manipulations, the sugar moiety was smoothly removed by enzymatic hydrolysis to give highly functionalized chiral cyclohexane derivatives. In this way, as a model, the new (1*S*,2*S*)-2-(hydroxymethyl)-2-methylcyclohexanol was prepared.

Introduction

In view of the widespread occurrence in nature of highly functionalized six-atom rings, (4 + 2) cycloaddition reactions continue to provide inventive strategies for natural product elaboration. Their successes are due to the perfect cis stereospecificity, the good regioselectivity, and the fairly good yields which are usually obtained. However, for preparative purposes, the normal reaction requires activated dienes (with electron-donating substituents) and activated dienophiles (with electron-withdrawing substituents) to proceed at temperatures compatible with the thermal stability of more and more complex targets. Therefore, intensive efforts have been made concerning new modes of activations of both partners,[2] new catalysts,[3] as well as theoretical studies to elucidate mechanistic aspects. In this connection, there are extensive studies of the influence of solvents on the kinetic and stereochemical outcome of cycloaddition. Several correlations of rate and/or endoselectivity with solvent parameters[4-6] have been published, but none included water, so that the prevailing opinion seems to be, more or less, that the influence of the solvent—independent of the system investigated—was relatively small.[4,7] In contrast, in 1980, Breslow's group[8] reported in a pioneering paper remarkable rate and stereoselectivity enhancements when the cycloadditions were conducted in water in comparison with organic solvents. It was also pointed out that such reactions could be achieved on a preparative scale without loss of stereoselectivity, even if one of the reactants was only sparingly soluble in water. Subsequently, Grieco et al.[9] reported utilization of dienes attached to a carboxylate group in preparative aqueous cycloaddition.

Although water has been considered as a possible solvent in Diels–Alder reactions ever since their discovery,[10] its poor solvent properties for dienes made it look unpromising. Consequently, in numerous papers on the subject water is absent from the list of solvents under investigation, undoubtedly a fact which discouraged its utilization. Since Breslow's paper, the effects of water have been much debated.[11] Undoubtedly, they cannot be explained only by the polarity of water, as the reaction of cyclopentadiene with methyl acrylate gave less endo adduct in formamide (ϵ 109) and *N*-methylacetamide (ϵ 133) than in water[12] (ϵ 80). Moreover, the lack of endo selectivity in water in the case of the polar dienophile PhSOCH=CHCO$_2$H[13] suggested a relation to the hydrophobic effect. This has been confirmed and clearly demonstrated using solutions of "structure-breaking" or "structure-making" salts in the case of the reaction of cyclopentadiene with methyl vinyl ketone.[8] When two nonpolar molecules are dissolved in water, they tend to aggregate. This entropy-driven association is well-known and is of importance in biological chemistry.[14] So, the rate enhancement could be the result of an entropy-favorable process. The question now arises whether the stereoselectivity could have the same origin. In 1974, Dack[15] stressed the importance of considering the activation volume $\Delta V^{\ddagger}$, in relation with the solubility parameter δ of the solvent for chemical reactivity. Water, with the highest known solubility parameter (δ 23 cal$^{1/2}$ mL$^{-3/2}$), then appeared as a solvent of choice for a reaction with negative activation volume between two nonpolar

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