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ANTITUMOR AGENTS FROM BOHEMIC ACID COMPLEX, VI.[1] SCHAUNARDIMYCIN

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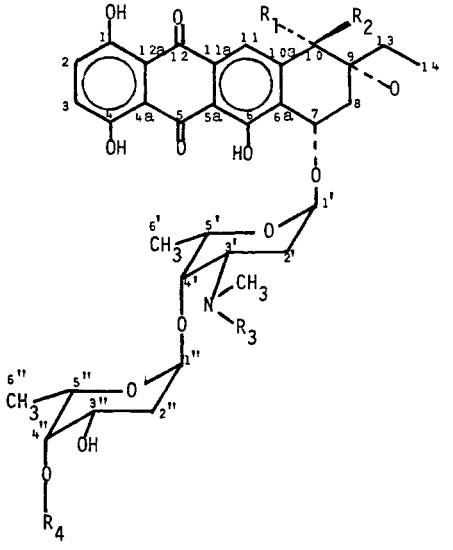
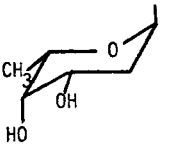
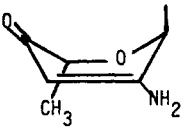
Previously, we have reported the isolation and structure determination of six novel anthracycline antitumor antibiotics from the bohemic acid complex **1-6** (Figure 1) (1-3). We now report the isolation and structure determination of schaunardimycin **7**.[2]

During the course of large scale fermentation for the production of marcellomycin (**1**), one fermentation produced moderate amounts of a new antibiotic which eluted closely behind **1** on silica gel chromatography. The presence of this new component greatly complicated large-scale chromatography using CH_2Cl_2-MeOH-NH_3 based systems on silica gel as described earlier (2). On a preparative scale, considerable tailing of





α-2-deoxy-L-fucosyl (DF)



α-rednosyl (R)

	R^1	R^2	R^3	R^4	
1	H	CO_2CH_3	CH_3	DF	Marcellomycin
2	H	CO_2CH_3	H	DF	Alcindoromycin
3	CO_2CH_3	H	CH_3	DF	Mimimycin
4	H	CO_2CH_3	CH_3	R	Rudolphomycin
5	H	CO_2CH_3	CH_3	H	Musettamycin
6	CO_2CH_3	H	CH_3	H	Collinemycin
7	H	CO_2CH_3	H	H	Schaunardimycin

FIGURE 1. Structures of the Anthracyclines from Bohemic Acid Complex.

7 into **1** was observed. We attribute this to the greater basicity of **7** due to the secondary amine function.

In our earlier work, the separation of alcindoromycin (**2**) from the complex had been achieved using Sephadex LH-20 eluted with toluene-MeOH. When

[1]For Part V, see Doyle *et al.* (1).

[2]It is the practice in this laboratory to name certain cultures after operas. The culture that produces bohemic acid complex (C36145, ATCC31127) was named after La Boheme. Novel components of the anthracycline complex have been given trivial names based on characters in the libretto of La Boheme.

separation of **7** from **1** was attempted using this system, only partial resolution was observed. Substitution of $CHCl_3$ for toluene-MeOH led to resolution of two major components of the mixture as well as several minor ones. Essentially, pure **7** was obtained from the second major fraction. Application of the method on a preparative scale gave pure marcellomycin (**1**) needed for clinical trials.

The structure of schaunardimycin was determined by nmr studies. The cmr chemical shifts for **7** were very similar to those of musettamycin except for those arising from the carbon atoms in the vicinity of the nitrogen atom on the amino sugar. Strong upfield shifts were observed for C-3′ (61.8 ppm to 54.8 ppm) and the *N*-methyl groups (42.7 ppm to 33.2 ppm) with lesser downfield shifts for C-2′ (29.2 ppm to 31.8 ppm) and C-4′ (73.6 ppm to 77.7 ppm). The pmr spectrum was very similar to that of musettamycin except that the *N*-methyl signal integrated for only three protons and had moved downfield from 2.22 to 2.35.

Biologically, schaunardimycin appears to have about a tenth of the potency of musettamycin and around one-twentieth that of marcellomycin (cf. Table 1).

EXPERIMENTAL

MATERIALS AND EQUIPMENT.—Sephadex LH-20 was purchased from Pharmacia Fine Chemicals. Lab-Crest columns with Solv-Seal type joints for low dead volume connections (Fischer and Porter, Lab-Crest Scientific Division) were used for the final chromatographic run (1). Earlier work in a six-inch column utilized homemade set-ups in our pilot plant area. Nmr spectra were run on a Varian XL-100 nmr spectrometer.

PRELIMINARY LARGE-SCALE FRACTIONATION OF CRUDE MATERIALS ON SEPHADEX LH-20.—Dry Sephadex LH-20, 13 kg, was swollen over an 18-h period in 40 liters of toluene-MeOH (8:2 v/v). The excess solvent was then decanted and 20 liters of toluene-MeOH (9:1 v/v) added followed by reequilibration. The latter process was repeated twice. The slurry was then loaded into a six-inch diameter glass column, equipped with a fritted glass disc and needle valve take-off at the bottom, to a height of 48 in.

Post-marcellomycin fractions from Prep 500 runs rich in schaunardimycin were pooled to give a starting solid, 57 g, which was dissolved in 600 ml of the mobile phase. Filtration showed very little insoluble matter.

The sample was applied to the column with rinsing to a total of about one liter of charge solution and elution begun at about 50 ml/min. The first orange band began to elute after 8 liters of void volume. The major colored band eluted after another 3.75 liters. At this point, fractions of 200-300 ml were taken and pooled according to hplc analyses (3). Solids were recovered from these fractions by evaporation of solvents *in vacuo*. Results are given in Table 2.

FINAL SEPHADEX LH-20 FRACTIONATION OF A SCHAUNARDIMYCIN-RICH FRACTION.—Sephadex LH-20 (1 kg) was swollen in excess $CHCl_3$ and packed into a 110×5 (ID) cm Fischer-Porter glass column equipped at the top with a short extender. After passing solvent through the column until the bed was settled, the extender was carefully detached to remove excess packing and a frit and cap placed on the top to give an effective bed height of 110 cm. Schaunardimycin-rich solids, 2.5 g from the pooled fractions 33-37 of the previous experiment, were dissolved in about 10 ml $CHCl_3$ and applied to the top of the column. The latter was then developed at a flow rate of 3-5 ml/min with $CHCl_3$. After the void volume, of 700 ml, fractions were collected every 4 min and combined according to absorbance peaks as determined by a Brinkmann PC/600 colorimeter at 420 nm. Results are given in Table 3.

Essentially pure shaunardimycin was obtained (as a dark red amorphous solid, 296 mg) by concentration and drying at ~22° *in vacuo* of fraction 11: ir ν max (KBr) 3460, 2975, 2940, 1736, 1600, 1452, 1320, 1296, 1220, 1162, 1118, 1010, 988, 908, and 730 cm^{-1}; uv λ max (MeOH) 234 nm (ϵ 4.23×10^4), 257 (ϵ 2.02×10^4), 287-294 (ϵ 7.26×10^3), 481 (ϵ 1.20×10^4, shoulder), 492 (ϵ 1.31×10^4), 510 (ϵ 9.66×10^3, shoulder), and 523-525 (ϵ 8.00×10^3); 360 MHz, pmr ($CDCl_3$) δ 1.09 (t, 3H, C-14 methyl protons), 1.24-1.30 (8H, c-6′ and C-6″ methyls, C-13, CH_2), 1.52 (broad qt, 1H), 1.67-1.93 (m, 5H), 2.06 (broad m, 1H), 2.08 (broad m, 1H), 2.30 (broad s, 1H), 2.35 (broad s, 3H, N-CH_3), 2.53 (broad d of d, 1H), 2.64 (broad d, 1H), 3.65-3.72 (partly obscured, 2H), 3.71 (s, 3H, $COOCH_3$), 4.06-4.12 (m, partly obscured, 2H), 4.12 (s, 1H, C-10H), 4.19 (m, 1H), 4.45 (broad, s, 1H), 4.96 (broad s, 1H, C-1″H), 5.23 (broad s, 1H, C-7H), 5.44 (broad

[3]System: CH_2Cl_2-CH_3OH-NH_4OH 96:4:0.5 (lower phase) on μ-Porosil.

TABLE 1. Effect of Schaunardimycin on L1210 Leukemia[a] and Reference to Musettamycin and Marcellomycin

Material	Treatment Schedule[b]	Dose, IP (mg/kg/inj)	MST[c] (Days)	Effect MST[c] (% T/C[d])	AWG[e] (g) Day 5	Survivors Day 5 (30)
Schaunard-	d.1	25.6	8.0	133	−1.4	6/6
imycin		12.8	8.0	133	+0.7	6/6
		6.4	7.0	117	+2.5	6/6
		3.2	7.0	117	+3.2	6/6
		1.6	6.5	108	+2.7	6/6
		0.8	6.0	100	+2.8	6/6
		0.4	6.0	100	+2.8	6/6
		0.2	6.0	100	+2.5	6/6
	qd 1↦5	6.4	8.0	133	+1.6	6/6
		3.2	8.0	133	+2.8	6/6
		1.6	7.5	125	+3.0	6/6
		0.8	7.0	117	+3.5	6/6
		0.4	7.0	117	+2.6	6/6
		0.2	7.0	117	+2.6	6/6
Musettamycin . . .	d.1	25.6	TOX[f]	TOX[f]	TOX[f]	2/6
		12.8	10.0	167	−2.3	6/6
		6.4	9.5	158	+0.3	6/6
		3.2	8.0	133	+0.8	6/6
		1.6	8.0	133	+0.7	6/6
		0.8	7.0	117	+2.6	6/6
		0.4	6.5	108	+2.7	6/6
		0.2	6.0	100	+2.7	6/6
	qd 1↦5	6.4	6.0	100	−1.9	5/6
		3.2	10.0	167	−1.2	6/6
		1.6	9.5	158	+0.3	6/6
		0.8	8.0	133	+1.4	6/6
		0.4	7.0	117	+2.3	6/6
		0.2	6.5	108	+2.8	6/6
Marcellomycin . .	d.1	12.8	TOX[f]	TOX[f]	TOX[f]	1/6
		6.4	9.5	158	−1.9	4/6
		3.2	8.5	142	−1.7	6/6
		1.6	8.5	142	−0.5	6/6
		0.8	8.0	133	+1.4	6/6
		0.4	7.0	117	+1.8	6/6
		0.2	6.5	108	+2.8	6/6
		0.1	6.0	100	+2.7	6/6
Control		Saline	6.0	—	+2.4	10/10

[a]Host: CDF_1 ♀ mice. Tumor inoculum: 10^6 ascites cells implanted ip.
[b]d.1=single dose on day one; qd 1↦5=single dose given daily, days 1-5.
[c]MST=median survival time.
[d]% T/C=(MST treated/MST control) x 100; % T/C ·125 considered significant antitumor activity.
[e]AWG=average weight gain.
[f]<4/6 mice alive on Day 5.

s, 1H, C-1′H), 7.29 (s, 2H, C-2H and C-3H), and 7.70 (s, 1H, C-11H); cmr ($CDCl_3$, 25.2 MHz) δ 190.7 (c-5), 185.8 (C-12), 171.3 (CO_2CH_3), 162.3 (C-6), 158.5 (C-4), 157.9 (C-1), 142.5 (C-10a), 132.9 (C-11a), 131.4 (C-6a), 130.1 (C-2), 129.7 (C-3), 120.5 (C-11), 114.9 (C-5a), 112.4 (C-4a), 112.2 (C-12a), 102.0 (C-1′), 100.4 (C-1″), 77.7 (C-4′), 71.7 (C-9), 71.2

TABLE 2. First Sephadex LH-20 Chromatography (Toluene-MeOH)

Fractions	Volume	Weight	Nature
1	8000 ml	—	void volume
2-10	3750 ml	—	non-active pigments-discarded
11-21	2650 ml	12.3 g	mainly marcellomycin
22-29	1900 ml	39.7 g	marcellomycin+schaunardimycin
30-33	800 ml	2.0 g	mainly schaunardimycin
33-37	~6000 ml	5.3 g	mainly schaunardimycin

TABLE 3. Final Sephadex LH-20 Chromatography ($CHCl_3$)

Combined Fraction No.	Volume	Weight	Nature
1	20 ml	—	no weight, discarded
2-7	1490 ml	253 mg	frontal peak+2-3 minor peaks
8	60 ml	518 mg	marcellomycin+earlier eluting peaks
9	185 ml	187 mg	marcellomycin+earlier eluting peaks
10	225 ml	732 mg	marcellomycin+schaunardimycin
11	80 ml	296 mg	schaunardimycin
12	610 ml	416 mg	schaunardimycin+late peak[a]
13	1475 ml	61 mg	column wash, mixture
14	[b]	115 mg	mixture

[a]Presumably mimimycin.
[b]A color band at the top of the column was stripped off with MeOH.

(C-7), 71.0 (C-4″), 68.3 (C-5′), 66.9 (C-5″), 65.7 (C-3″), 57.2 (C-10), 54.8 (C-3′), 52.5 ($COOCH_3$), 34.0 (C-8), 33.2 ($NHCH_3$), 33.0 (C-13), 32.2 (C-2″), 31.8 (C-2′), 17.7 (C-6′), 16.9 (C-6″), 6.7 (C-14).

Anal. calcd for $C_{35}H_{43}NO_{14}$: C, 59.90; H, 6.18; N, 2.00. Found: C, 59.66; H, 6.50; N, 1.80.

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