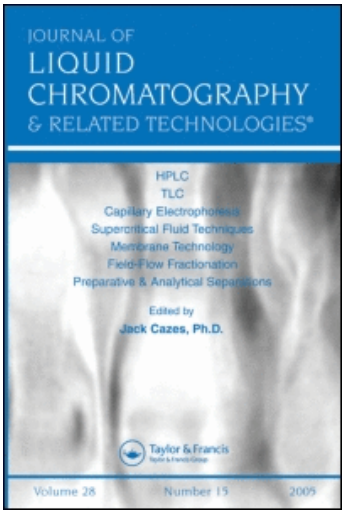


Journal of Liquid Chromatography & Related Technologies

Publication details, including instructions for authors and subscription information:
http://www.informaworld.com/smpp/title~content=t713597273

Structural Investigation of the Antibiotic Sporaviridin. XV. Preparative-Scale Preparation of Sporaviridin Components by HSCCC

Ken-Ichi Harada[a]; Ikumi Kimura[a]; Akiko Yoshikawa[a]; Makoto Suzuki[a]; Hiroyuki Nakazawa[b]; Shigehiko Hattori[c]; Kyoichi Komori[c]; Yoichiro Ito[d]

[a] Faculty of Pharmacy, Meijo University, Tempaku, Nagoya, Japan [b] The Institute of Public Health, Minato, Tokyo, Japan [c] Shimadzu Corporation Nakagyo, Kyoto, Japan [d] Laboratory of Technical Development, National Heart, Lung, and Blood Institute, Bethesda, Maryland

To cite this Article Harada, Ken-Ichi , Kimura, Ikumi , Yoshikawa, Akiko , Suzuki, Makoto , Nakazawa, Hiroyuki , Hattori, Shigehiko , Komori, Kyoichi and Ito, Yoichiro(1990) 'Structural Investigation of the Antibiotic Sporaviridin. XV. Preparative-Scale Preparation of Sporaviridin Components by HSCCC', Journal of Liquid Chromatography & Related Technologies, 13: 12, 2373 — 2388

To link to this Article: DOI: 10.1080/01483919008049039

URL: http://dx.doi.org/10.1080/01483919008049039

JOURNAL OF LIQUID CHROMATOGRAPHY, 13(12), 2373-2388 (1990)

STRUCTURAL INVESTIGATION OF THE ANTIBIOTIC SPORAVIRIDIN. XV. PREPARATIVE-SCALE PREPARATION OF SPORAVIRIDIN COMPONENTS BY HSCCC

KEN-ICHI HARADA[1], IKUMI KIMURA[1], AKIKO YOSHIKAWA[1], MAKOTO SUZUKI[1] HIROYUKI NAKAZAWA[2], SHIGEHIKO HATTORI[3], KYOICHI KOMORI[3], AND YOICHIRO ITO[4]

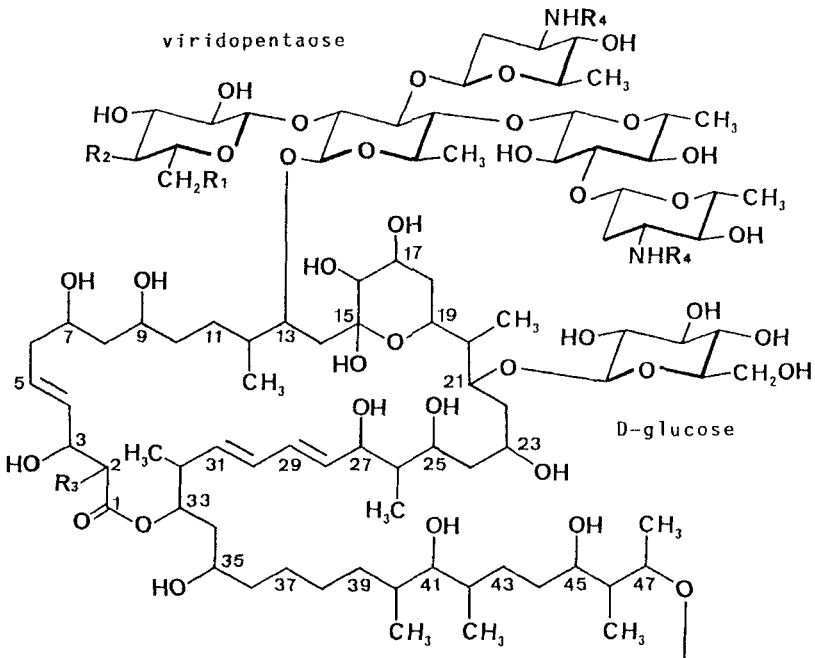
[1]*Faculty of Pharmacy, Meijo University*
Tempaku, Nagoya 468, Japan
[2]*The Institute of Public Health*
Minato, Tokyo 108, Japan
[3]*Shimadzu Corporation*
Nakagyo, Kyoto 604, Japan
[4]*Laboratory of Technical Development*
National Heart, Lung, and Blood Institute
Bethesda, Maryland 20892

ABSTRACT

Preparative-scale separation of the six sporaviridin components, very complicated basic glycoside antibiotics produced by Streptosporangium viridogriseum, was carried out by high speed countercurrent chromatography (HSCCC). The following two points, selection of proper solvent system and stationary phase retention, were carefully examined. Many two-phase solvent systems were searched, so that a solvent system, n-butanol:diethylether:water =10:4:12, was selected and other operating conditions were also optimized. The HSCCC was performed under the optimized conditions and gave satisfactory results.

INTRODUCTION

Sporaviridins (abbreviated as SVD) are basic and water-soluble antibiotics, and were first isolated in 1963 (1). They are active against gram-positive bacteria, acid-fast bacteria and trichophyton. Since the intact antibiotics are very unstable under basic conditions, the isolation and structural determination were carried out using, more stable derivatives, N-acetylsporaviridins (N-Ac-SVD) and recently their structures have been proposed as shown in Fig. 1 (2). They comprise the six components and each has a 34-membered lactone and seven monosaccharide units, a pentasaccharide (viridopentaose) and two monosaccharides. Because the structural differences among them are derived from those of methyl or ethyl group at C-2 and three viridopentaoses A, B and C (3) at C-13, the six components (A_1 - C_2) of N-Ac-SVD exist. But, these N-acetylated derivatives show no biological activity and it was required to separate and purify the intact components. In order to obtain each component a preparative HPLC was optimized in consideration of their properties. The mobile phase containing aqueous ammonium chloride depressed the unfavorable chemical behavior and gave considerably good separation (Fig. 2). The six components were successfully isolated and each component isolated had expectedly the microbial activity (4). However, it is laborious to do preparative HPLC works and indeed it took many runs to obtain about 10 mg of each component from the complex mixture. We found an additional



SVD-A_1: R_1=H, R_2=OH, R_3=C_2H_5, R_4=H
SVD-A_2: R_1=H, R_2=OH, R_3=CH_3, R_4=H
SVD-B_1: R_1=H, R_2=NH_2, R_3=C_2H_5, R_4=H
SVD-B_2: R_1=H, R_2=NH_2, R_3=CH_3, R_4=H
SVD-C_1: R_1=OH, R_2=OH, R_3=C_2H_5, R_4=H
SVD-C_2: R_1=OH, R_2=OH, R_3=CH_3, R_4=H

N-Ac-SVD-A_1: R_1=H, R_2=OH, R_3=C_2H_5, R_4=Ac
N-Ac-SVD-A_2: R_1=H, R_2=OH, R_3=CH_3, R_4=Ac
N-Ac-SVD-B_1: R_1=H, R_2=NHAc, R_3=C_2H_5, R_4=Ac
N-Ac-SVD-B_2: R_1=H, R_2=NHAc, R_3=CH_3, R_4=Ac
N-Ac-SVD-C_1: R_1=OH, R_2=OH, R_3=C_2H_5, R_4=Ac
N-Ac-SVD-C_2: R_1=OH, R_2=OH, R_3=CH_3, R_4=Ac

Fig. 1. Structures of sporaviridins and N-acetylsproaviridins

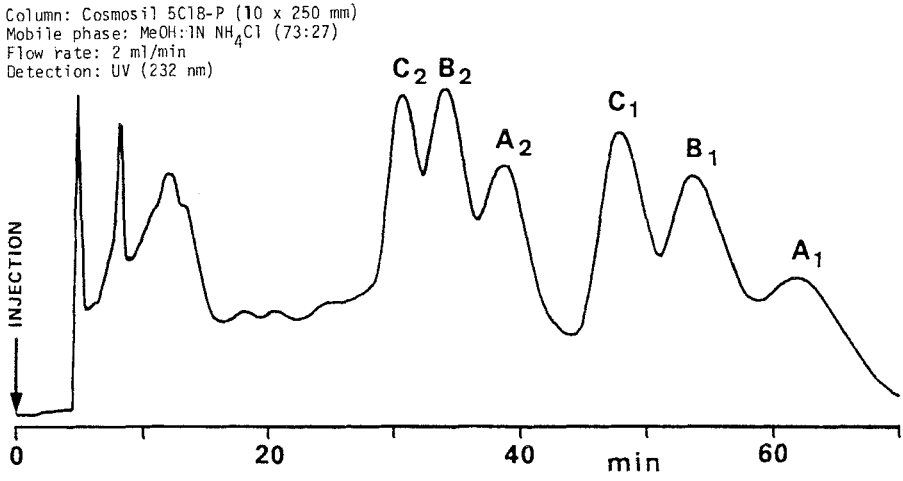


Fig. 2. High performance liquid chromatogram of the intact sporaviridins under preparative HPLC condiitions

inconvenience that it is difficult to remove ammonium chloride from the desired fraction.

In the present study we tried to apply high speed countercurrent chromatography (HSCCC) instead of HPLC to the preparative-scale separation for the intact free bases of SVD.

EXPERIMENTAL

The apparatus used was a Shimadzu prototype of the coil planet centrifuge for HSCCC with 160 m length of 1.6 mm (id) teflon tubing around a 6.5 cm diameter column holder making multiple coiled layers. The total capacity was 325 ml and the column was rotated at about 800 rpm. Solvents were delivered with

a Shimadzu LC-6A pump. After filling the stationary phase, followed by sample injection, mobile phase was eluted at 3 ml/min. The detection was performed using UV (232 nm) with a Shimadzu SPD-6A. Eluate was collected in a Pharmacia FRAC-100 fraction collector and fractions were analyzed by the following HPLC and TLC. The HPLC conditions were: pump; Shimadzu LC-5A, detector; Shimadzu SPD-2A, column; Cosmosil 5C18 (Nacalai Tesque, 4.6 x 150 mm), mobile phase; methanol:1N ammonium chloride (76:24), detection; UV (232 nm) and the TLC conditions were: plate; RP-18 $F_{254}S$ and RP-18W$F_{254}S$ (Merck), mobile phase; methanol:1N ammonium chloride (80:20), detection; 1% ceric sulfate-10% sulfuric acid. Sporaviridins were kindly supplied by Tanabe Seiyaku Ltd (Osaka, Japan).

RESULTS AND DISCUSSION

Our objective in this study was to separate efficiently the six components of the intact SVD by HSCCC. We focused on the following two points to attain the objective, selection of proper solvent system and stationary phase retention.

Selection of proper two-phase solvent system

Precise separation by HSCCC is mainly dependent upon the selection of a proper two-phase solvent system. To select the suitable solvent system, settling time of the two-phase solvent system used, and partition coefficients of the individual components and solubility of the SVD complex were carefully

considered. A two-phase solvent system with less than 30 sec of settling time is desirable for high retention of stationary phase. The SVD complex is only soluble in polar solvents such as water, methanol and n-butanol and in fact it was extracted with n-butanol (1). Additionally a mobile phase, chloroform:methanol:7% ammonium hydroxide, was used for separation on normal phase TLC. Therefore, two solvent systems, chloroform:water and n-butanol:water were mainly examined.

Partition coefficient (K value) was estimated by the following simple test tube experiment. The SVD complex was partitioned with a two-phase solvent system which had been already equilibrated and the resulting upper and lower phases were analyzed by HPLC. Total and each partition coefficients were determined by dividing the corresponding peak area of the upper phase by that of the lower phase. Table 1 shows the total and individual partition coefficients of the SVD components with chloroform:ethanol:water and chloroform:ethanol:methanol:water systems together with their settling times. The total partition coefficient can predict approximate elution volume and the separation among the six components depends on the degree of dispersion of the six values. Ideally, these values should be dispersed around K=1. In order to understand well and quickly we devised to express these results using the figure as shown in Fig. 3. It is ready to predict that any chloroform-containing solvent systems cannot separate completely all components but probably it would clearly separate between SVD-C_1 and C_2. In fact, we were

Table 1. Partition coefficients of SVD components with chloroform solvent systems

solvent system	s.t.	total	C_2	B_2	A_2	C_1	B_1	A_1
C:E:M:W								
5:2:2:4	17	1.49	2.89	1.54	1.06	2.03	1.12	1.10
5:2:3:4	21	1.32	2.38	1.40	1.08	1.53	0.97	1.01
5:3:3:4	34	0.73	1.41	0.77	0.64	1.05	0.67	0.71
5:4:3:4	60	0.84	0.69	1.14	0.89	0.94	0.69	0.67
5:3:3:3	51	1.16	1.85	1.19	1.03	1.29	1.09	0.84
C:E:W								
7:13:8	110	0.33	0.38	0.34	0.29	0.34	0.33	0.32
5:2:3	18	4.22	14.00	4.25	3.41	5.66	2.99	2.63
5:3:3	20	0.57	1.80	0.57	0.46	0.77	0.35	0.28
5:4:3	26	0.80	1.43	0.68	0.65	1.06	0.55	0.61
5:3:4	16	1.25	2.93	1.33	1.04	1.91	0.73	0.66
5:4:2	24	0.69	1.53	0.72	0.56	0.86	0.43	0.33
5:4:1	127	0.46	0.91	0.40	0.37	0.58	0.33	0.32
4:3:3	23	0.71	1.92	0.60	0.55	0.98	0.42	0.37
3:3:3	32	0.39	0.86	0.36	0.31	0.47	0.26	0.29

C:chloroform E:ethanol M:methanol W:water s.t.:settling time(sec)

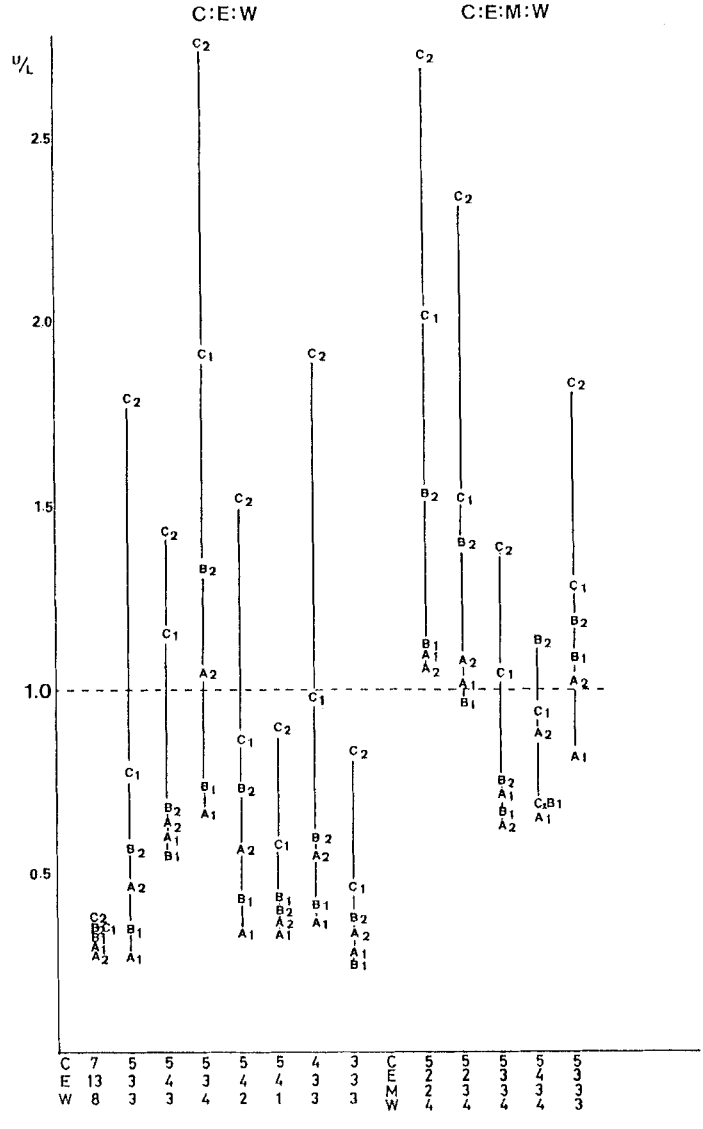


Fig. 3. Partition coefficients of SVD componenets with chloroform solvent system

not able to attain our purpose using any chloroform solvent systems.

As mentioned earlier sporaviridin complex was extracted with n-butanol from the fermentation broth. Indeed, when SVD complex was partitioned with n-butanol and water, it passed almost completely into the upper phase (Table 2). This result indicate that the solubility of the n-butanol phase must be decreased and a non-polar solvent such as n-hexane and diethylether was added to n-butanol and water solvent system as the modifier. The optimization for obtaining the suitable solvent system was done by the following way. Initially, volumes of n-butanol and water were fixed to be 10 ml and that of diethylether was varied, so that a solvent system, n-butanol:diethylether:water=10:4:10, was selected. Next, volumes of n-butanol and diethylether were fixed and that of water was changed from 11 to 15 ml. In the case of 10:4:12, the almost equally dispersed partition coefficient among the six components were obtained as shown in Fig. 4. This solvent system is most suitable for the separation of the six components at present.

Other operating conditions

Satisfactory retention of the stationary phase throughout the separation by HSCCC should be always considered. As mentioned above a two-phase solvent system with a shorter settling time is more advantageous. Other important factors such as elution speed of mobile phase, revolution speed of column, operating temperature

Table 2. Partition coefficients of SVD components with n-butanol solvent systems

solvent system	s.t.	U[ml]	L[ml]	total	C_2	B_2	A_2	C_1	B_1	A_1
n-Bu:W										
10:10	11	10.8	9.2	5.66	2.96	6.41	6.65	4.87	8.81	9.09
10:15	12	10.2	14.8	4.94	2.35	5.33	6.30	4.24	7.45	9.61
10:20	14	9.8	20.2	6.13	2.77	7.03	7.19	5.17	10.62	12.57
10:25	15	9.6	25.4	4.57	2.01	5.15	6.43	3.80	7.17	8.59
n-Bu:DE:W										
10: 1:10	13	11.6	9.4	3.61	1.69	3.60	4.55	3.11	5.03	7.13
10: 2:10	12	12.8	9.2	3.34	1.58	3.45	4.08	2.86	4.92	5.71
10: 3:10	10	13.8	9.2	2.21	0.96	2.17	2.78	1.84	3.34	4.19
10: 4:10	10	14.9	9.1	1.29	0.50	1.12	1.59	1.04	1.85	2.91

10: 5:10	10	15.9	9.1	0.94	0.38	0.78	1.11	0.74	1.25	2.12
10: 6:10	10	17.0	9.0	0.98	0.39	0.90	1.19	0.81	1.39	1.70
10: 7:10	10	18.0	9.0	0.80	0.24	0.63	1.10	0.59	1.08	1.82
10: 8:10	11	18.9	9.1	0.68	0.44	0.51	0.78	0.59	0.95	1.37
10: 9:10	11	20.1	8.9	0.60	0.21	0.49	0.78	0.46	0.78	1.31
10:10:10	10	21.0	9.0	0.41	0.12	0.35	0.45	0.29	0.63	0.96
10: 4:11	13	14.5	10.5	0.95	0.31	0.89	1.24	0.70	1.50	2.00
10: 4:12	15	14.5	11.5	1.13	0.38	1.09	1.41	0.80	1.85	2.32
10: 4:13	15	14.6	12.4	1.09	0.37	1.05	1.51	0.73	1.58	2.10
10: 4:14	15	14.5	13.5	0.88	0.29	1.09	1.17	0.57	1.22	1.74
10: 4:15	14	14.5	14.5	1.32	0.49	1.22	1.84	1.01	1.77	2.55

n-Bu:n-butanol DE:diethylether W:water s.t.:settling time(sec)

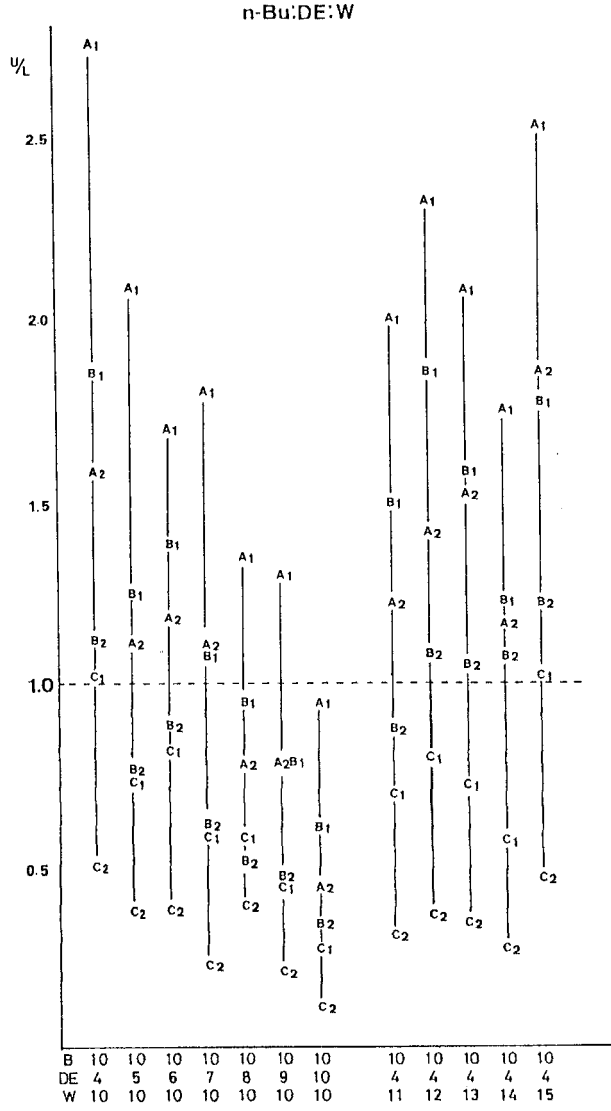


Fig. 4. Partition coefficients of SVD compnents with n-butanol solvent systems

and how to load sample were also examined (5) and the following conditions were optimized: solvent system; n-butanol:diethylether:water=10:4:12 (settling time; 15 sec), partition coefficient: total U/L; 1.13, C_2; 0.38, C_1; 0.80, B_2; 1.09, A_2; 1.41, B_1; A_1; 2.32. stationary phase: upper phase. mobile phase: lower phase. flow rate; 3 ml/min. elution: head to tail. injection: sandwich injection (sample solution is injected after filling stationary phase and then mobile phase is eluted). revolutional speed: 800 rpm. operation temperature: 37-40 C .

Separation of the SVD components by HSCCC

We have successfully isolated the six components of sporaviridins by the HPLC (4), but it was quite laborious and required the repeated operation as stated previously.

The preparative separation of the six components by HSCCC was performed under the optimized conditions. In this experiment the retention of the stationary phase was 75.4%, and the total elution time and elution volume were 3.5 hr and 500 ml, respectively. The six components were eluted in the order of their partition coefficients, SVD-C_2, C_1, B_2, A_2, B_1 and A_1. The purified components, A_1 (1.3 mg), A_2 (0.6 mg), B_1 (0.7 mg), B_2 (0.5 mg), C_1 (1.1 mg) and C_2 (1.4 mg) were obtained from 15 mg of the complex mixture and their high performance liquid chromatograms are shown in Fig. 5. Two or three peaks around the retention time of 3 min correspond to the pseudoaglycones produced by the cleavage of the glycosidic linkage at C-13 of the aglycones (6). The ratio of the

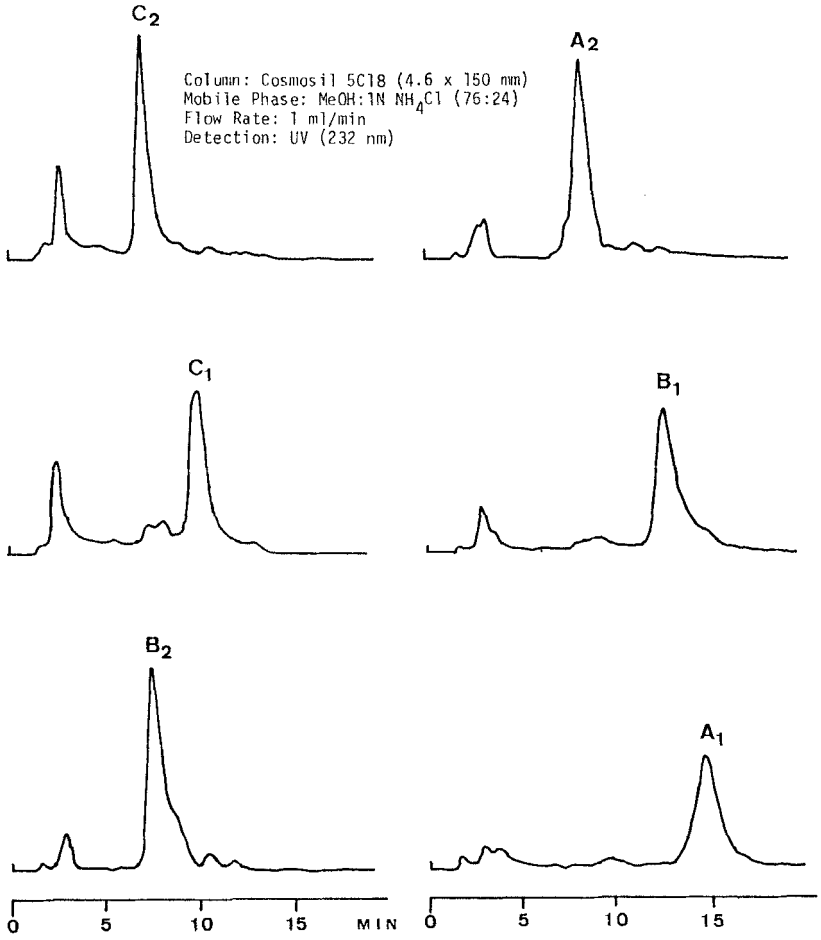


Fig. 5. High performance liquid chromatograms of the purified components of SVD

purified components to the starting SVD complex was about 40%. In the case of 100 mg of the complex mixture the similar results were obtained.

Additionally, an interesting difference was found in the elution behavior between HSCCC and HPLC of SVD. In the case of the HSCCC their order was C_2, C_1, B_2, A_2, B_1 and A_1, whereas C_2, B_2, A_2, C_1, B_1 and A_1, were eluted in order in the HPLC. Although both are characterized to be reversed phase separation mode, the substituent R_1 operated mainly on the elution order in the HSCCC, but the substituent R_3 affected considerably the elution in the HPLC (Fig. 1).

In conclusion we used HSCCC to separate efficiently the intact SVD which are very complicated glycoside antibiotics and are composed of six closely related components. Many two-phase solvent systems were examined, so that a solvent system, n-butanol:diethylether:water=10:4:12, was selected and other operating conditions were also optimized. Finally the HSCCC was carried out under the optimized conditions and gave satisfactory results. From these results, we made sure that HSCCC is powerful and potential for preparative-scale separation. This separation method is being applied to other complicated natural products.

REFERENCES

1. T. Okuda, Y. Ito, T. Yamaguchi, T. Furumai, M. Suzuki and M. Tsuruoka, J. Antibiotics, Ser A, 19, 85 (1966).

2. I. Kimura, Y. Ota, R. Kimura, T. Ito, Y. Yamada, Y. Kimura, Y. Sato, H. Watanabe, Y. Mori, K.-I. Harada, M. Suzuki and T. Iwashita, Tetrahedron Lett., 23, 1921 (1987).

3. K.-I. Harada, S. Ito, M. Suzuki and T. Iwashita, Chem. Pharm. Bull., 31, 3829 (1983).

4. K.-I. Harada, I. Kimura, T. Sakazaki and M. Suzuki, J. Antibiotics, to be submitted.

5. Y. Ito, CRC Critical Review in Anal. Chem., 17 (Issue 1), 65 (1986).

6. I. Kimura, K. Yamamoto, K.-I. Harada and M. Suzuki, Tetrahedron Lett., 28, 1917 (1987).