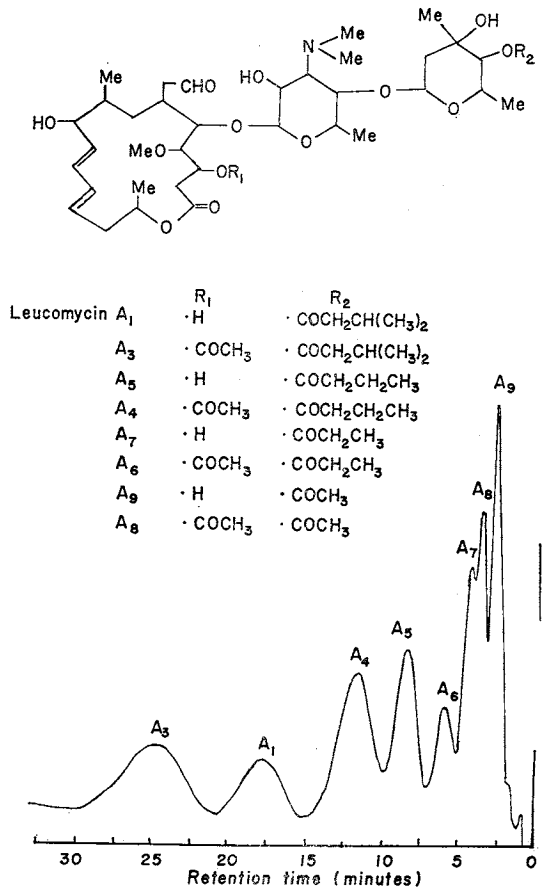
FAST LIQUID CHROMATOGRAPHY OF MACROLIDE ANTIBIOTICS

Sir;

Sixteen-membered ring macrolide antibiotics containing multi-components have been discovered one after another in recent years. In addition to carbomycins, spiramycins, and leucomycins,[1] complexes such as maridomcyins,[2] YL-704,[3] SF-837,[4] and espinomycins,[5] *etc.* have been isolated. Structure determination has become rapidly attainable by the application of mass spectrometry. Structural difference among these macrolide antibiotics lies in the acyl group at the 3-position of the chromophoric lactone moiety and the acyl group at the 4-position of mycarose. Different combinations of these groups give rise to the numerous macrolides that have been discovered and those yet to be discovered. For the separation and detection of these antibiotics with similar chemical structure, paper chromatography[6] and thin-layer chromatography[1] have hitherto been employed but they proved to be insufficient. For example, it was necessary to use the combination of thin-layer chromatography using alumina, developing with ethyl acetate and that using silica gel with acetone-benzene, for detection of each component of the leucomycins produced by *Streptomyces kitasatoensis*.

We have examined the analyses of macrolide antibiotics, centering around leucomycins using fast liquid chromatography, with satisfactory results which will be dealt with in this paper. The sample used was a leucomycin complex from *S. kitasatoensis* No. 23-1, which produces leucomcyins A_1 through A_9. Those eight factors whose structures have been determined were analyzed on a JASCO Associates Analytical Model FLC-150 using both chemically bonded column (normal phase and reverse phase) and adsorption columns and a variety of carriers. The eight components of the antibiotic were completely separated and detected, as shown in Fig. 1, by the use of the chemically bonded column (reverse phase), JASCO PACK SV-02-500®, with a mixture of methanol-M/15 acetate buffer (pH 4.9)-acetonitrile (35 : 60 : 5, v/v/v) as a carrier. Assignment of each peak in Fig. 1 was made with reference to the retention time obtained with authentic samples. Changes in the composition ratio of the carrier solvents and in the pH of the buffer resulted in separatory ability and in changes of retention time. For example, increase in the quantity of acetonitrile resulted, in general, in shorter retention times and a change in pH from 4.9 to 4.5, reduced the separation of the initially eluted components, especially A_7 and A_8. In order to establish a good separation of structurally similar components of larger molecules like leucomycin, it is necessary to reduce the amount of the sample applied, to choose a detector with high sensitivity and to be able to select the wave length agreeing with the intrinsic absorption of the chromphor

Fig. 1. Fast liquid chromatogram of leucomycin complex



Instrument: JASCO FLC-150. Detector: JASCO UVIDEC-2 (232.5 nm) spectrophtometer. Separation mode: Partition. Column: JASCOPACK SV-02-500. Column temparature: Ambient. Column pressure: 65 kg/cm².

Mobile Phase: MeoH-M/15 acetate buffer (pH 4.9)-acetonitrile (35 : 60 : 5). Flow rate: 1.0 ml/min.

Table 1. Retention time of macrolide antibiotics in fast liquid chromatography.

Macrolide antibiotics		Retention time (min.)
Leucomycin	A_1	17.5
〃	A_3	25.8
〃	A_5	8.0
〃	A_4	11.5
〃	A_7	4.0
〃	A_6	6.0
〃	A_9	2.5
〃	A_8	3.5
〃	U	2.0
〃	V	1.5
Spiramycin	I	2.5
〃	III	5.1
Tylosin		3.5
Magnamycin	A	18.5
〃	B	28.0
Erythromycin	A	3.0
〃	B	2.4

of the compounds. In the present study, a spectrophotometer equipped with a detector,* capable of selecting the wavelength in the range of 200~850 nm was used. The curve shown in Fig. 1 was obtained by selecting the wavelength of the UV absorption of leucomycin at 232.5 nm. The use of 253.7 nm, the wavelength generally applied, gave poor detection, showing only five peaks, even under the same concentration of the sample injected. From a comparison of the retention time of the leucomycin complex, it was found that components A_3, A_4, A_6, and A_8, having an acetyl group in the 3-position of the lactone, showed larger retention time and better separation than components A_1, A_5, A_7, and A_9, having a hydroxyl group in the same position.

The results of fast liquid chromatography carried out on other macrolides under the same conditions as shown in Fig. 1 are given by retention time in Table 1, which also gives the results of leucomycins U and V, the trace components of leucomycin. These components were completely separated and detected.

As stated above, it has become clear that structurally similar components of macrolide antibiotics containing many such components can be separated accurately and detected by fast liquid chromatography using a minute quantity (0.1~0.2 μg) of the sample. This technique is considered to be a great contribution towards the future discovery and identification of new components in macrolide antibiotics. It will also be possible to obtain structural informations at the same time by varying the wavelength, and this fast liquid chromatography may in future be used not only for macrolides but also for the analyses of natural organic compounds.

* We used the UVIDECK-2 spectrophotometer manufactured for trial which was kindly supplied by JASCO Co., Ltd.

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