## NOTES

# THE ANTIBIOTIC XK-41 COMPLEX

## II. STRUCTURAL IDENTIFICATION

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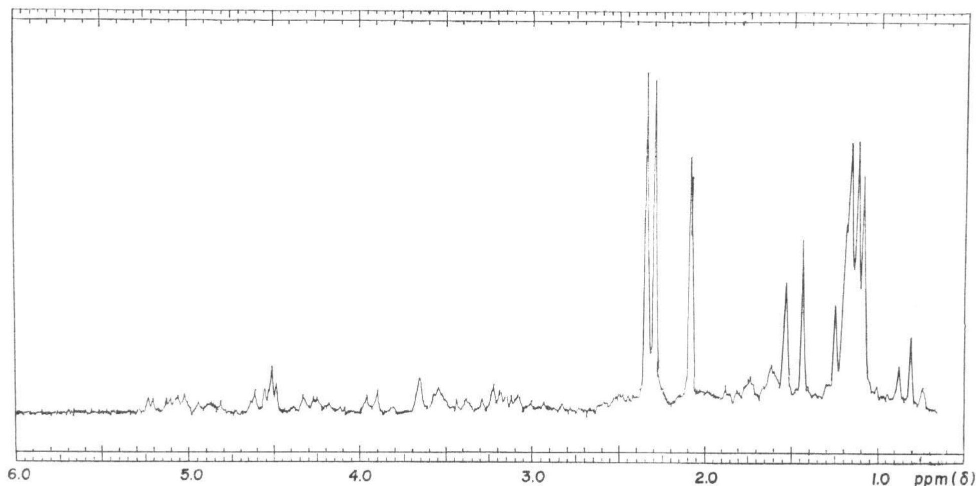
The antibiotic XK-41 complex, isolated from a *Micromonospora* species, consists of five major components which have been produced and isolated as previously detailed.[1] We wish now to report the identity of these compounds determined solely from physical methods involving primarily NMR and mass spectra. NMR has been extensively employed in these laboratories for the identification of macrolide antibiotics arising both from synthetic[2,3] and biosynthetic[4~7] sources. Similarily electron impact (EIMS)[8,9] and chemical ionization mass spectrometry (CIMS)[10,11,12] using isobutane as the reagent gas have been shown to be especially useful in the structural analysis and characterization of macrolide antibiotics. The XK-41 antibiotics have been shown to be megalomicin A, B, $C_1$ $C_2$ and 4″-propionylmegalomicins A; the latter antibiotic has not been previously isolated from natural sources.

## Results

The NMR spectrum of XK-41-$A_2$ (Fig. 1) is typical of members of this complex and permits assignment of these compounds to a specific antibiotic class. The high field region between 0.5~1.6 ppm contains a significantly large number of resonances arising from *C*-methyl groups. The extent of branching that these methyl groups indicate is typical of the propionate derived 14-membered ring macrolide family of antibiotics. Previously recognized important members of this class of antibiotics are the erythromycins, oleandomycin and lankamycin.† Consistent with this observation are the numerous single proton multiplets which appear downfield of

Fig. 1. NMR spectrum of XK-41-$A_2$




† For a recent review of macrolide antibiotics, see ref. 13, 14.

2.0 ppm and the absence of vinyl or aromatic resonances below 5.0 ppm. No *O*-methyl resonance is observed in the 3.0 ppm region of the spectra indicating that the common neutral sugars cladinose, oleandrose, arcanose or chalcose are not present but rather mycarose may be a component of this compound. Mycarose has previously been encountered in erythromycin C and more recently in megalomicin A.[15] Two singlets at 2.29 and 2.34 ppm, attributable to dimethylamino resonances, are further suggestive of the megalomicin antibiotics which contain a second basic 6-deoxy sugar in addition to the one generally found in the other macrolides. Two other prominent peaks at 2.07 and 2.08 ppm are characteristic of *O*-acetyl groups and indicate that XK-41-$A_2$ is likely a di-*O*-acetylmegalomicin. This assignment was confirmed by mass spectra which also located the position of substitution of the *O*-acetyl groups.

The EIMS of XK-41-$A_2$ (Fig. 2) showed a small but observable molecular ion at *m/e* 960 which by high resolution mass measurement gave a molecular formula of $C_{48}H_{84}N_2O_{17}$ (calc. *m/e* 960.5770; meas. *m/e* 960.5783). The CIMS revealed a relatively intense protonated molecular ion at *m/e* 961. In both cases the molecular weights are consistent with the formulation of this compound as a di-*O*-acetylmegalomicin A.

Prior experience has shown that significant fragmentation peaks in the high mass region

Fig. 2. EIMS and CIMS of XK-41-$A_2$

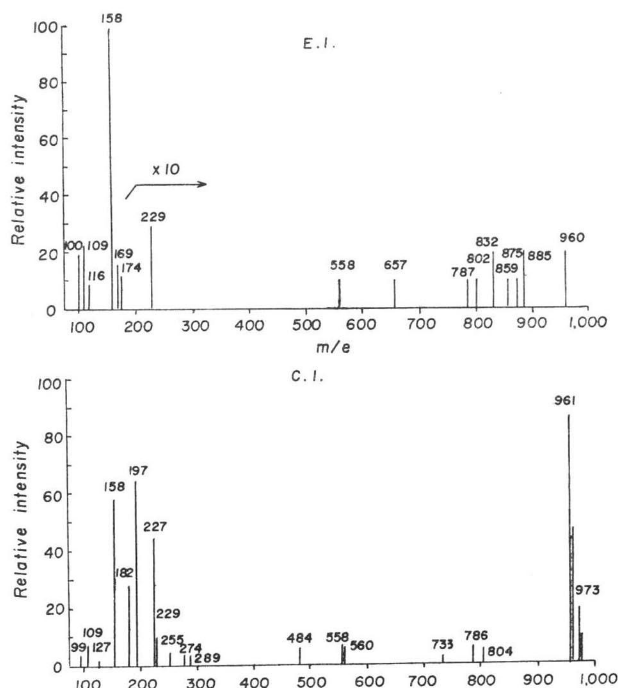
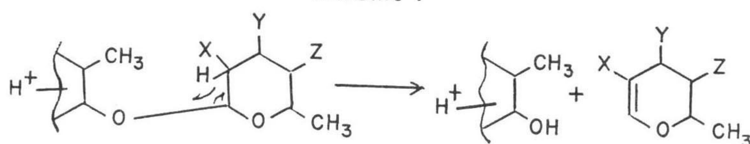



Table 1. EIMS Mass spectral fragments

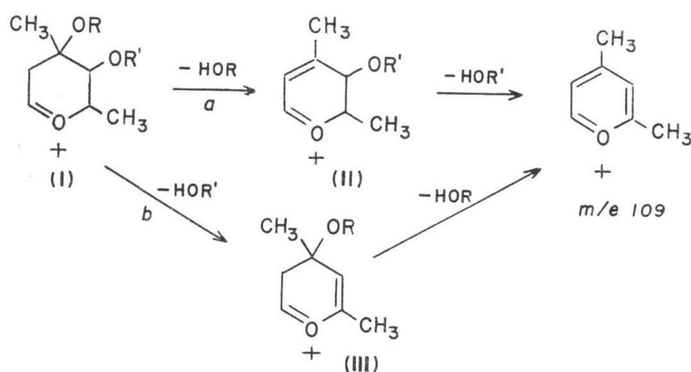
| | XK-41-$A_1$ | XK-41-$A_2$ | XK-41-$B_1$ | XK-41-$B_2$ | XK-41-C |
|---|---|---|---|---|---|
| $M^{+\cdot}$ | 974 | 960 | 918 | 932 | 876 |
| I | 243 | 229 | 187 | 201 | 145 |
| II | 169 | 169 | 169 | 183 | 127 |
| III | 183 | 169 | 127 | 127 | 127 |
| IV | 816 | 802 | 760 | 774 | 718 |

of CIMS result from a four-centered cleavage of the sugars from the macrolide ring (Scheme I),[10~12] while simple cleavages at the glycosidic linkages are also exhibited in the EIMS.[8~9] These peaks are confirmed by oxonium ions at correspondingly lower mass. Further, the substitution pattern of the neutral sugar is suggested by the relative intensities of the ions which result from loss of the oxygen substituents at C-3″ and C-4″ (Scheme II). The preferred pathway, *a*, yields the more intense fragment, unless R′ is the more labile substituent. In the case of XK-41-$A_2$, loss of one basic sugar (rhodosamine or desosamine, both have identical formulas) results in a peak at *m/e* 802 with the accompanying oxonium ion at *m/e* 158 as the base peak in the spectrum. Loss of the neutral sugar results in a peak at *m/e* 731 with a corresponding fragment peak at *m/e* 229. The empirical formulas of these peaks determined by high resolution measurement requires that both acetyl groups are substituted on the neutral sugar of XK-41-$A_2$. Confirmation of this

Scheme I




Scheme II




Scheme III

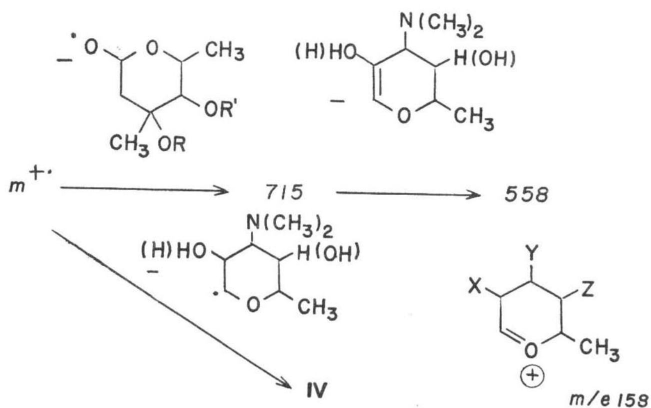
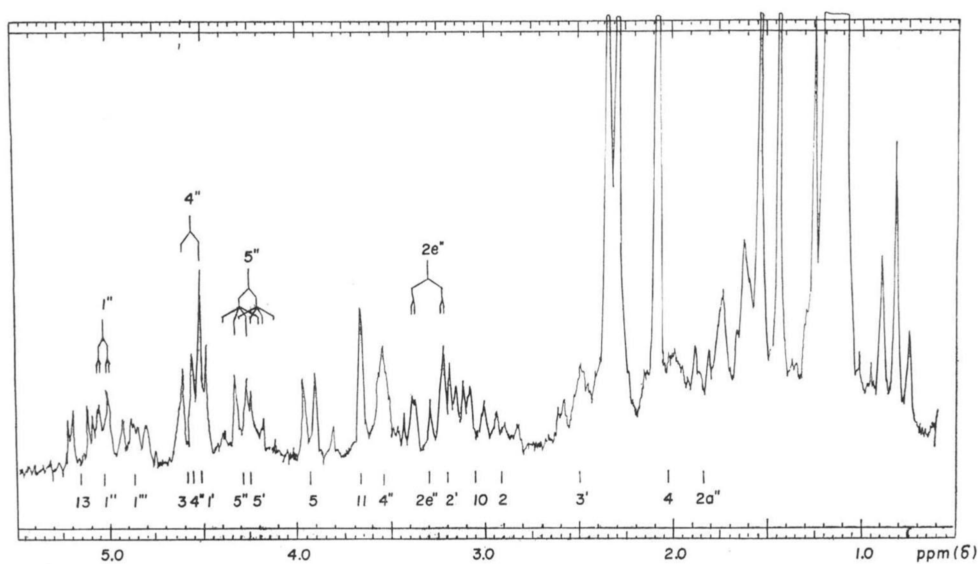


Table 2. Ring proton chemical shifts of the components of the XK-41 complex and erythromycin C

| | XK-41-$A_1$ | XK-41-$A_2$ | XK-41-$B_1$ | XK-41-$B_2$ | XK-41-C | Ery. C |
|---|---|---|---|---|---|---|
| H-2 | 2.91 | 2.92 | 2.98 | 2.97 | ~3.0 | ~3.0 |
| H-3 | 4.57 | 4.56 | 4.72 | 4.71 | 4.74 | 4.14 |
| H-5 | 3.93 | 3.93 | 3.90 | 3.90 | 3.89 | 3.51 |
| H-11 | 3.65 | 3.66 | 3.66 | 3.66 | 3.65 | 3.84 |
| H-13 | 5.15 | 5.16 | 5.17 | 5.16 | 5.18 | 5.06 |
| H-1′ | 4.53 | 4.52 | 4.45 | 4.46 | 4.34 | 4.37 |
| H-2′ | 3.20 | 3.20 | 3.18 | 3.17 | 3.18 | ~3.2 |
| H-1″ | 5.03 | 5.03 | 5.22 | 5.22 | 5·14 | 5.01 |
| H-2e″ | 3.30 | 3.30 | ~2.2 | ~2.2 | 2.21 | 2.21 |
| H-4″ | 4.57 | 4.55 | 4.57 | 4.57 | 2.92 | 2.96 |
| H-5″ | ~ 4.3 | 4.26 | ~4.3 | 4.24 | 3.95 | 3.85 |
| H-1‴ | 4.86 | 4.87 | 4.88 | 4.88 | 4.89 | — |
| H-4‴ | 3.54 | 3.54 | 3.55 | 3.55 | 3.54 | — |
| H-5‴ | 4.29 | 4.29 | 4.30 | ~4.3 | 4.30 | — |
| 6-$CH_3$ | 1.54 | 1.53 | 1.55 | 1.54 | 1.60 | 1.50 |
| 3″-$CH_3$ | 1.43 | 1.44 | ~1.2 | ~1.2 | ~1.2 | ~1.2 |
| $N(CH_3)_2$ | 2.29 | 2.29 | 2.28 | 2.26 | 2.26 | 2.27 |
| $N(CH_3)_2$ | 2.35 | 2.34 | 2.34 | 2.33 | 2.33 | — |
| OAc | — | 2.07 | 2.07 | — | — | — |
| OAc | 2.08 | 2.08 | — | — | — | — |

Fig. 3. Assignments made of ring proton resonances



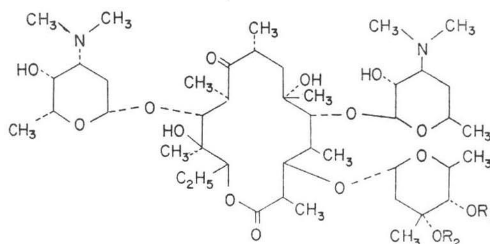
substitution was given by the observation of ions arising from successive losses of the neutral species acetic acid at *m/e* 169 and 109. Therefore XK-41-$A_2$ is formulated as 3″, 4″-di-*O*-acetylmegalomicin A also designated as megalomicin $C_1$. Additional prominent fragments in the EIMS of this compound and their probable origin are collected in Table 1.

Analysis of the aglycone and sugar ring proton resonances in the NMR of XK-41-$A_2$ (Table 2) also assign the position of acylation. The assignments made of ring proton resonances are shown in Fig. 3. Unprimed numbers refer to protons at the indicated position on the aglycone ring, primed numbers for those on the desosamine ring, double primed numbers for mycarose and triple primed for rhodosamine. Most significant is the downfield shift of H-4″ (4.55 ppm) compared to its position in erythromycin C (2.96 ppm) indicating that the 4″-hydroxyl group is a site of acylation. The unusual chemical shift of H-2e″ at 3.30 ppm compared to its position in erythromycin C (2.21 ppm) is indicative of acylation of the vicinal tertiary hydroxyl at the 3″-position. This unusual chemical shift effect is also seen in the spectrum of 3″, 4″, 5, 11-tetra-*O*-acetyl-3-*O*-α-L-mycarosyl erythronolide B (3.36 ppm) compared to the unacetylated monoglycoside (2.14 ppm). Also diagnostic of acylation of the 3″-hydroxyl group is the singlet methyl resonance at 1.44 ppm attributable to the 3″-methyl group. This singlet is shifted to higher field (~1.2 ppm) in the spectrum of erythromycin C and other macrolides which are not 3″-acyl derivatives.

The structures of the remaining members of the XK-41 complex (Table 3) were determined in a totally analogous manner. In each case high resolution EIMS provided a molecular ion and empirical formula with characteristic fragment ions (Table 1 and Scheme III) which determined the identity of the acyl groups and indicated that they were substituted on the neutral sugar. The presence of a *m/e* 588 peak in each compound is noteworthy since this establishes that an identical aglycone ring is present in all members of the complex. Analysis of the ring proton NMR spectra (Table 2) with particular emphasis on the chemical shifts of H-2e″ and H-4″ determined the specific site of *mono*- or *di*-acylation. It should be noted that in the case of XK-41-$A_1$, the data do not unambiguously determine the specific site of substitution of the individual acyl groups.

After completion of this work, a patent[16] and series of papers[17,18] have appeared from investigators at the Schering Corporation detailing their independent determination of the structures of the megalomicin complex. In these papers they reported the structures of three additional members of the complex in addition to the previously described megalomicin A.[15] The structures of four components of the XK-41 complex are identi-

Table 3. Structures of the components of the XK-41 complex




| Component | Empirical formula | $R_1$ | $R_2$ | Designation |
|---|---|---|---|---|
| XK-41-$A_1$ | $C_{49}H_{36}N_2O_{17}$ | $CH_3CH_2CO$- | $CH_3CO$- | Megalomicin $C_2$ |
| XK-41-$A_2$ | $C_{48}H_{84}N_2O_{17}$ | $CH_3CO$- | $CH_3CO$- | Megalomicin $C_1$ |
| XK-41-$B_1$ | $C_{46}H_{32}N_2O_{16}$ | $CH_3CO$- | H- | Megalomicin B |
| XK-41-$B_2$ | $C_{47}H_{34}N_2O_{16}$ | $CH_3CH_2CO$ | H- | — |
| XK-41-C | $C_{44}H_{30}N_2O_{15}$ | H- | H- | Megalomicin A |

cal to those given for the components of the megalomicin complex as noted in Table 3. Thin-layer comparisons of the XK-41 and megalomicin complexes corroborate the identities indicated. In addition, the spectral and chemical characteristics of the megalomicins are mirrored by the corresponding XK-41 complex component (*cf*. experimental section). It appears that XK-41-$B_2$ has not yet been identified in the natural megalomicin complex; however, 4″-*O*-propionylmegalomicin A of synthetic origin is described.

## Experimental

General: NMR spectra were determined in $CDCl_3$ solution at 55°C on a Varian Associates HA-100 spectrometer. Assignments of specific protons were made by analogy with erythromycin compounds[2~7] and are generally supported by spin decoupling experiments. The EIMS were run on an A.E.I. MS-902 by the direct insertion technique at source temperatures between 170°C and 190°C above ambient. The CIMS were obtained using an A.E.I. MS-9 equipped with a SRIC CIS-2 chemical ionization source and isobutane as the reagent gas.

XK-41-$A_1$ (Megalomicin $C_2$): White amorphous crystals; m.p. 147.4~149°C (reported[17] 147~150°C). Anal. Calcd. for $C_{49}H_{86}N_2O_{17}$: C 60.35, H 8.89 and N 2.87. Found: C 59.93, H 8.86 and N 2.20. A maximum absorption in the UV spectrum in methanol was 279 nm, at which $E^{1\%}_{1cm}$ was 0.52. $[\alpha]^{20}_D-101.9°$(*c* 1, ethanol) (reported[17] −102°). The infrared spectrum showed peaks at the following wavelengths in $cm^{-1}$: 700, 810, 840, 870, 900, 910, 960, 1000, 1016, 1030, 1075, 1085, 1120, 1170, 1245, 1320, 1350, 1380, 1465, 1630, 1690, 1740, 2800, 2830, 2838, 2900, 3000, 3480.

XK-41-$A_2$ (Megalomicin $C_1$): White amorphous crystals: m.p. 239~241°C (reported[17] 243~246°C). Anal. Cald. for $C_{48}H_{84}N_2O_{17}$: C 59.98, H. 8.81 and N. 2.91. Found: C 60.37, H. 8.43 and N 2.34. A maximum absorption in the UV spectrum was 278 nm, at which $E^{1\%}_{1cm}$ was 0.96. $[\alpha]^{20}_D-107.8°$ (*c* 1, ethanol) (reported[17] −102°). The infrared spectrum showed peaks at the following wavelengths in $cm^{-1}$: 700, 840, 865, 900, 910, 960, 990, 1000, 1040, 1050, 1075, 1085, 1110, 1120, 1165, 1230, 1245, 1325, 1350, 1380, 1400, 1460, 1630, 1695, 1740, 2800, 2810, 2819, 2830, 2835, 3500.

XK-41-$B_1$ (Megalomicin B): White needle crystals; m.p. 141°C with foaming and completely at 150°C (reported[17] 135~140°C). Anal. Calcd. for $C_{46}H_{82}N_2O_{16}$: C 60.11, H 8.99 and N 3.05. Found C 58.91, H. 8.93 and N 2.65. A maximum absorption in the UV spectrum was 261~265 nm in methanol, at which $E^{1\%}_{1cm}$ was 1.0. $[\alpha]^{20}_D-89.5°$ (*c* 1, ethanol) (reported[17] −92°). The infrared spectrum showed peaks at the following wavelengths in $cm^{-1}$: 700, 780, 840, 865, 900, 920, 930, 970, 995, 1005, 1040, 1075, 1118, 1140, 1165, 1245, 1270, 1345, 1380, 1395, 1465, 1635, 1715, 1740, 2800, 2810, 2819, 2830, 2835, 3500.

XK-41-$B_2$ (4″-*O*-propionylmegalomicin A): White needle crystals; m.p. 212~212.5°C. Anal. Cald. for $C_{47}H_{84}N_2O_{16}$: C 60.49, H 9.07 and N 3.00. Found: C 58.97, H 10.53 and N 2.98. The UV absorption

Fig. 4. UV absorption spectrum of XK-41-$B_2$

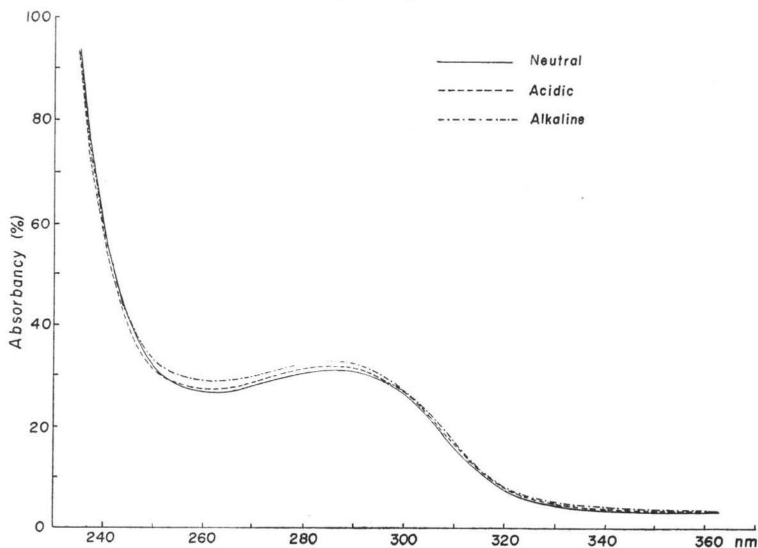


Fig. 5. IR absorption spectrum of XK-41-$B_2$

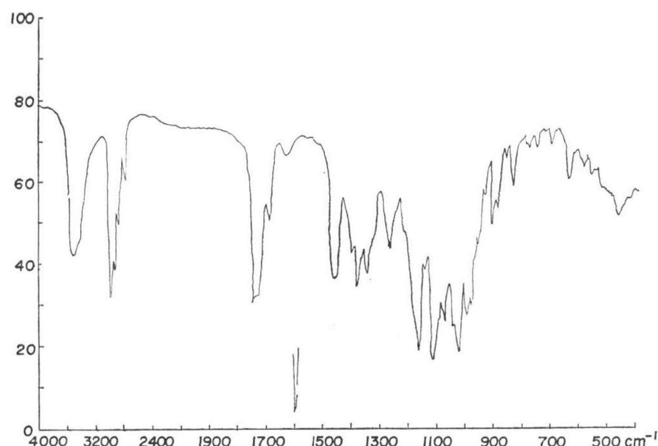



Table 4. Ascending paper chromatography of XK-41-$B_2$

| Developer | Rf value | Developing period (hour) |
|---|---|---|
| 20% (w/v) Ammonium chloride | 0.60 | 3 |
| Water-saturated *n*-butanol | 0.85 | 15 |
| *n*-Butanol - acetic acid - water (3:1:1) | 0.73 | 15 |
| Water-saturated ethyl acetate | 0.00 | |
| Water-satured *n*-butanol containing 2% (w/v) of *p*-toluene sulfonic acid and 2% (v/v) of piperidine | 0.87 | 15 |
| 5% (w/v) Ammonium chloride | 0.70 | 3 |
| Water-saturated methyl isobutyl ketone | 0.75 | 3 |
| Water-saturated methyl isobutyl ketone containing 1% (w/v) of *p*-toluene sulfonic acid | 0.87 | 3 |
| Acetone·water (1:1) | 0.80~0.90 | 3 |
| Methanol - *n*-butanol - water (1:4:2) containing 21% (w/v) of methyl orange | 0.89 | 15 |

spectrum, as illustrated in Fig. 4, shows a maximum at 286 nm in methanol, at which $E^{1\%}_{1cm}$ was 0.62, $[\alpha]^{20}_{D}-92.3°$ ($c$ 1, ethanol). The infrared spectrum, as shown in Fig. 5, showed peaks at the following wavelengths in $cm^{-1}$: 700, 750, 780, 840, 860, 890, 910, 935, 955, 980, 1000, 1030, 1070, 1115, 1140, 1170, 1270, 1345, 1380, 1460, 1630, 1690, 1740, 2800, 2880, 2980, 3500.

The Rf values of XK-41-$B_2$ obtained as a result of paper chromatography using various solvent systems are shown in Tables 4 and 5. XK-41-$B_2$ is distinct in Rf values from four known megalomicin components as evident from Table 5.

Table 5. Thin-layer chromatography of XK-41-$B_2$ and megalomicins

| Components | Rf values |
|---|---|
| XK-41-$A_1$ | 0.85 |
| XK-41-$A_2$ | 0.80 |
| XK-41-$B_1$ | 0.45 |
| XK-41-$B_2$ | 0.55 |
| XK-41C | 0.20 |

An alumina sheet (type E produced by Merck & Co.) activated at 120°C for 2 hours was used. The development was carried out at room temprature for 3 hours using a solvent system comprising 9 parts by volume of ethylacetate and one part by volume of methanol as a developer.

## Summary

The structures of five components of the macrolide antibiotic XK-41 complex have

been determined by NMR and mass spectrometry. Four components have been shown to be identical to the megalomicins A, B, $C_1$ and $C_2$. XK-41-$B_2$ has been found to be 4″-*O*-propionylmegalomicin A which has not been previously isolated from natural sources.

Acknowledgements

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