A NEW ANTIBIOTIC XK-90

II. THE STRUCTURE OF XK-90

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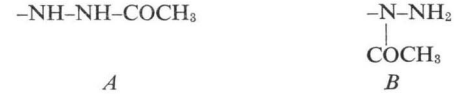
The new antibiotic, XK-90, produced by *Streptomyces* sp. is active against Gram-positive and Gram-negative bacteria. The structure has been determined as N-acetyl-N′-(3-formyl-4-hydroxyphenyl)hydrazine (**1**) and is the second example of a naturally occurring antibiotic having the phenylhydrazine skeleton.

XK-90 was found in the culture broth of *Streptomyces* sp., the taxonomy of which, and isolation of the antibiotic were recently reported by TAKASAWA *et al.*[1] Although its activity is not strong, XK-90 is an antibiotic classified as a hydrazo compound, the occurrence of which is very rare[2] and it offers additional information on the structure-activity relationship among this class of compounds having biological activity. In the present paper structure determination of the antibiotic is reported.

XK-90 (**1**), $C_9H_{10}N_2O_3$, m.p. 128~129°C, shows hydroxyl (3300 cm^{-1}), amide (1660 cm^{-1}), and aromatic (1600 and 1513 cm^{-1}) groups in its ir spectrum (KBr). The nmr spectrum of XK-90 exhibited signals at δ 10.62 (1H, s), 9.83 (1H, s), 6.87~7.18 (3H, m), and a sharp singlet at δ 2.09 accompanied with a sharp but weaker singlet at δ 2.16* (total integration of the both peaks corresponds to 3H) at 60°C assignable to a hydrogen bonding phenolic OH, CHO, aromatic ring protons and N-$COCH_3$, respectively. In addition to these, two broad signals were observed at δ 6.06 and 5.75.

Treatment of XK-90 with dimethyl sulfate gave the trimethyl derivative (**2**), the nmr of which showed three sharp singlets at δ 3.89, 3.09, and 2.90 attributable to a methoxy and two N-methyl groups, respectively. The signals at δ 10.62, 6.06, and 5.75 observed in the nmr of XK-90, were not observed as expected. At this point, it was deduced that XK-90 has three active protons and is a derivative of hydroxybenzaldehyde, and the remaining substituent (N_2H_2-$COCH_3$) must be either one of partial structures *A* and *B*. The problem of this selection was solved by mass spectra. The mass spectrum



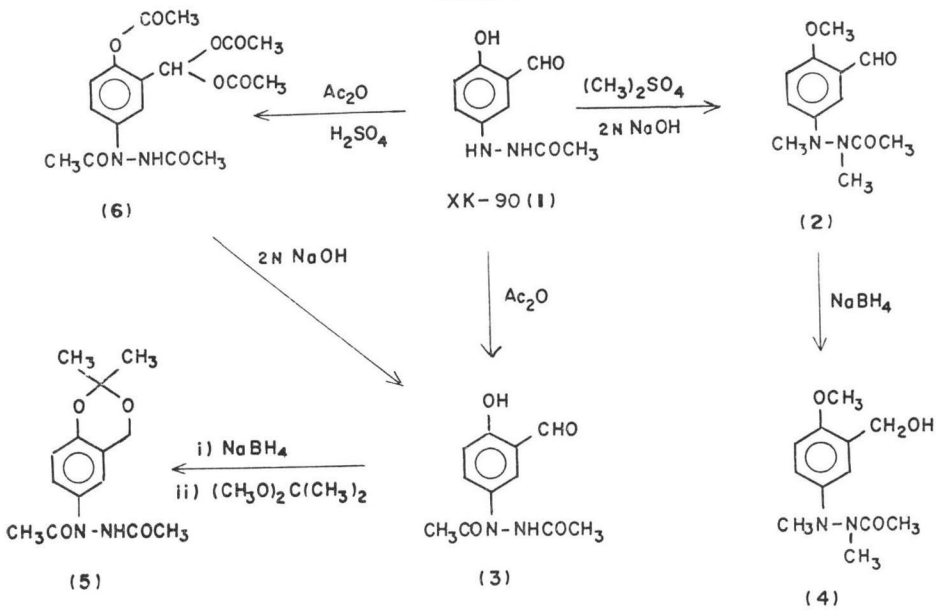
A *B*

of XK-90 showed intense fragments at *m/e* 152, 136, and 108. Fragmentation of this compound was analyzed with the aid of high resolution mass measurements as shown in scheme II**.

* In the nmr of XK-90 and its derivatives, almost all of the signals due to N-$COCH_3$ were observed as rather broad peaks at room temperature, but they became sharper at elevated temperature. However, these signals still remained as two peaks except in the case of **2**. This kind of phenomenon is often encountered in N-acetylhydrazines, which is explained as being due to hindered rotation around C-N bond of the amide unit[3].

** Electron induced fragmentation studies of hydrazines are very limited in their number and only a few reports on alkyl and phenylhydrazines have been published[4,5]. In this regard, XK-90 and its derivatives are good model compounds of acylated hydrazines for mass spectrometric studies.

Scheme I



The mass spectrum of **2** could be interpreted in the same manner. If XK-90 has the partial structure *B*, the mass of **2** is expected to exhibit fragment ions at *m/e* 150(h) and 122(i) instead of *m/e* 164(e) and 136(f), respectively.

The monoacetate (**3**) gave a well-resolved nmr spectrum in the aromatic region, where it was clearly shown that three aromatic protons were located on the 1, 2, and 4-positions (see Table 1). On the other hand, sodium borohydride reduction of **3**, followed by treatment with 2,2-dimethoxypropane, gave an isopropylidene derivative (**5**). This means that the hydroxyl group is located in the position *ortho* to the aldehyde group in the aromatic ring in XK-90.

Table 1. Chemical shifts and coupling constants of aromatic protons

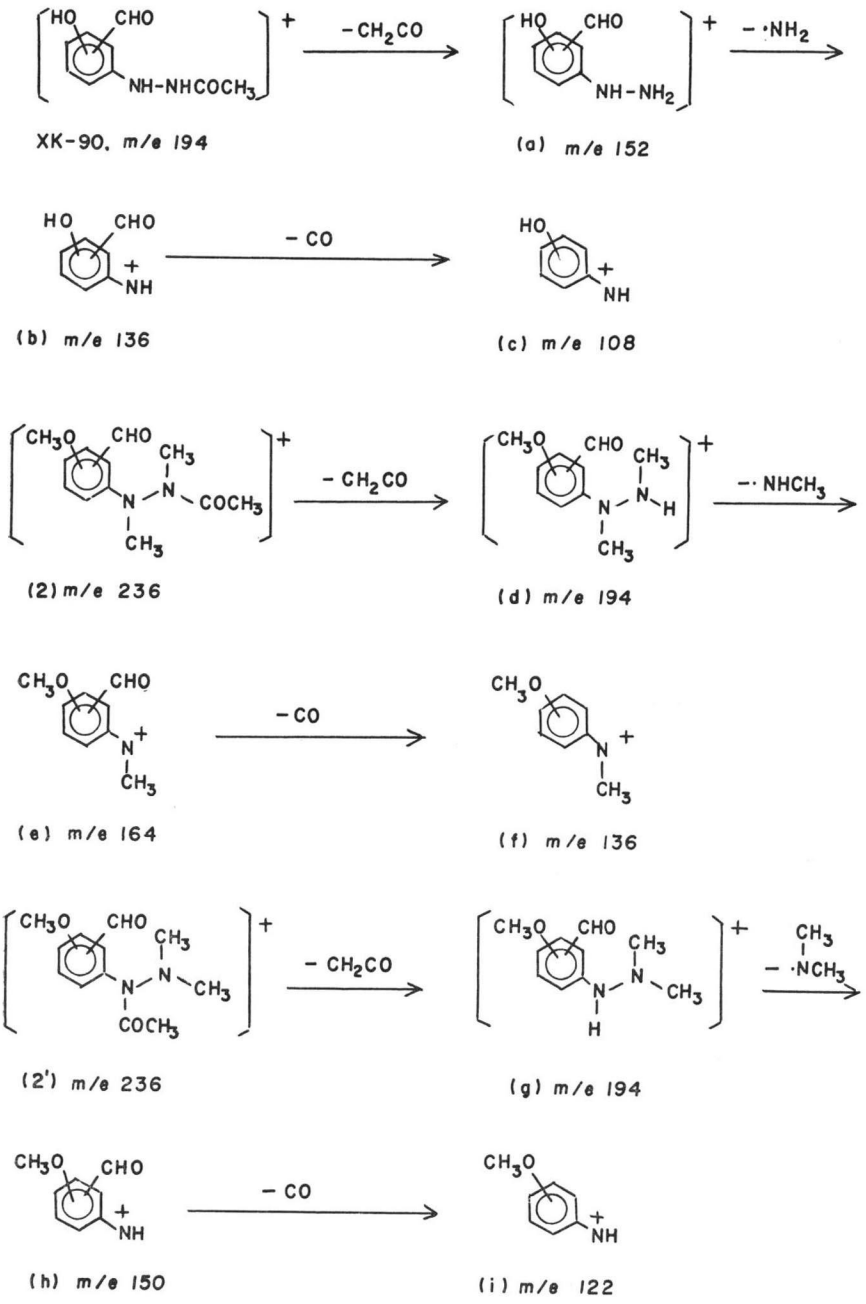
Compounds	H_A	H_B	H_C
2	7.15(s)	6.93(s)	6.93(s)
3	7.67(d, 2.9)	7.50(dd, 8.8, 2.9)	6.96(d, 8.8)
4	6.65(d, 3.3)	6.56(dd, 3.3, 8.8)	6.81(d, 8.8)
5	7.08(br. s)	7.03~7.20(br. d)	6.77(d, 8.5)

On the basis of their splitting patterns, differentiation between the aromatic ring protons could be made independently of their chemical shifts. The biggest chemical shift change (0.50 ppm to higher field) was observed on the isolated proton(H_A) when the aldehyde group of **2** was reduced to the hydroxymethyl group (**4**), whereby H_B and H_C moved to higher field by 0.37 and 0.12 ppm, respectively. The same kind of chemical shift changes were observed between compounds 3 and 5 (Table 1). These findings led a conclusion that the isolated proton is located in the position *ortho* to the aldehyde group and the full structure of XK-90, therefore, has been determined as N-acetyl-N'-(3-formyl-4-hydroxyphenyl)hydrazine (**1**). So far we know, XK-90 is the second example of a naturally occurring antibiotic having the phenylhydrazine skeleton[6].

Experimental

"Working-up" refers to extracting the reaction mixture with ethyl acetate followed by drying the extracts over Na_2SO_4, filtering and evaporating to afford the products. For tlc, precoated silica gel

Scheme II



F-254 plates (Merck) were used with ethyl acetate as the solvent unless otherwise stated. Nmr spectra were recorded on a Nihondenshi JNM-PS-100 spectrometer operating at 100 MHz in the FOURIER transform mode in deuteriochloroform with tetramethylsilane as an internal standard. Sweep width of 2 KHz with 4,096 data points were used throughout to give chemical shifts with an accuracy of ± 0.49 Hz (± 0.05 ppm). A pulse width of 11.5 μsec, "tilt angle" of 65°, and repetition time of 5 seconds were used. Ms spectra were measured with a Nihondenshi JMS-01SG-2 spectrometer operating at 75 eV and ir spectra with a Shimazu IR-27G spectrometer.

XK-90 (1)

For the isolation and characterization of XK-90, see reference 1. Yellowish needles from AcOEt, m.p. 128~129°C. Ms; *m/e* 194.0788 ($C_9H_{10}N_2O_3$)=M^+, 152.0610 ($C_7H_8N_2O_2$), 136.0401 ($C_7H_6NO_2$) and 108.0529 (C_6H_6NO). Ir (KBr); 3300, 1660, 1600, 1513, 1480, 1270, 1170, 826, 775, and 743 cm^{-1}. Nmr (at room temperature); δ 2.09 and 2.16 (each s, total 3H, N-$COCH_3$*).

Anal. Calcd. for $C_9H_{10}N_2O_3$: C, 55.66; H, 5.19; N, 14.43%
Found: C, 55.64; H, 5.19; N, 14.15%

N,N',O-Trimethyl XK-90 (2)

To a solution of 11 mg of XK-90 in 0.5 ml of dimethyl sulfate was added 1.0 ml of 2 N NaOH aqueous solution periodically for 6 hours at room temperature. After standing overnight at −20°C, working-up of the reaction mixture gave an oily residue. Standing the product after purification with tlc resulted in yellowish crystals (3.0 mg). Ir ($CHCl_3$); 2940, 1658, 1497, 1385, 1178, and 1025 cm^{-1}. Nmr (at room temperature); δ 2.13 (s, 3H, N-$COCH_3$), 2.90 and 3.09 (each s, 3H, 2×NCH_3), 3.89 (s, 3H, OCH_3), and 10.41 (s, 1H, CHO). Ms; *m/e* 236 (M^+), 194, 193 (base peak), 164, 163 (high resolution measurements showed the compositions of these fragments to be $C_{12}H_{16}N_2O_3$, $C_{10}H_{14}N_2O_2$, $C_{10}H_{13}N_2O_2$, $C_9H_{10}NO_2$, and $C_9H_9NO_2$, respectively), 150, 149, 136, 135, 121, and 120.

Monoacetate of XK-90 (3)

(1) XK-90, 15 mg, was treated with 3 ml of Ac_2O at room temperature overnight. After evaporation of the solvent, the residue was purified by preparative tlc (developed with benzene - acetone, 1: 1) to give 18 mg of colorless oil which crystalized gradually during standing. Ir ($CHCl_3$); 3240, 3000, 2850, 1660, 1585, 1482, 1370, 1280, and 600 cm^{-1}.

Nmr (at 60°C); δ 2.04 and 2.02 (each s, 3H, 2×N-$COCH_3$), 8.04 (br. s, 1H, NH), 9.82 (s, 1H, CHO), and 10.97 (s, 1H, phenolic OH). Ms; *m/e* 236 (M^+), 194 (base peak), 152, 151, and 136.

(2) XK-90 tetraacetate (6), 18 mg, was treated with 2 N NaOH aqueous solution at room temperature for 5 minutes. The reaction mixture was adjusted to pH 4 with AcOH. Working-up of the extract in the usual way gave 9 mg of an oily product, which was identified with the monoacetate (3) by comparison of its Rf value on tlc and nmr with those of 3 obtained in the procedure (1).

Reduction of 2 to 4

A small amount of 2 was treated with excess $NaBH_4$ in iso-PrOH for 5 hours at room temperature. After evaporation of the solvent, the residue was extracted with MeOH and then purified by tlc. Nmr (at room temperature); δ 2.00 and 2.15 (each s, total 3H, N-$COCH_3$), 2.90 and 3.05 (each s, 3H, 2×N-CH_3), 3.81 (s, 3H, OCH_3), 4.64 (s, 2H, benzyl CH_2), a signal due to OH was not assigned clearly because of its overlapping of the signals owing to a small amount of impurities (δ 1.25~2.15). Ms; *m/e* 238 (M^+), 196, 195 (base peak), 166, and 165.

Isopropylidene derivative (5)

Monoacetate (3), 25 mg, was treated with 8 mg of $NaBH_4$ in 1 ml of THF for 2.2 hours, then the reaction mixture was adjusted to pH 3 by addition of 0.2 N hydrochloric acid and saturated with NaCl. After working-up, the crude product was dissolved in 1 ml of 2,2-dimethoxypropane and was stirred in the presence of 1 mg of *p*-TsOH·H_2O at room temperature for 1.5 hours. Working -up of the reaction mixture gave 10 mg of an oily product, which was purified by preparative tlc. Ir ($CDCl_3$); 3260, 3000, 1665, 1495, 1376, 1270~1200, 1138, 1115, 960, and 765 cm^{-1}. Nmr (at 60°C); δ 1.53 (s, 6H, isopropylidene), 2.00 (s, 6H, 2×N-$COCH_3$), 4.79 (s, 2H, benzyl CH_2) and 7.74 (br. s, 1H, NH). Ms; *m/e* 278 (M^+), 236, 220, 178 (a high resolution measurement of these fragment ions referred to $C_{14}H_{18}N_2O_4$, $C_{13}H_{18}NO_3$, $C_{11}H_{12}N_2O_3$, and $C_9H_{10}N_2O_2$, respectively), 86 and 84 (base peak).

Tetraacetate of XK-90 (6)

A solution of 15 mg of XK-90, 25 μl of H_2SO_4 in 1 ml of Ac_2O was allowed to stand for 1 day at room temperature. The reaction mixture was diluted with ice-water, and neutralized with $NaHCO_3$. Working-up of the resulting solution gave 19 mg of oil. Ir ($CHCl_3$); 3280, 3000, 1761, 1715~1670, 1495, 1375, 1250~1180, 1012, and 605 cm^{-1}. Nmr (at room temperature); δ 1.98 (s, 3H, N-$COCH_3$), 2.11 (s, 9H, 2×$OCOCH_3$ and N-$CHCH_3$), 2.33 (s, 3H, ph-$OCOCH_3$), 7.09 (d, 9.0, 1H, ph-H), 7.12 (br. dd,

1H, ph-H), 7.72 (d, 2.2, 1H, ph-H), and 8.4~9.3 (broad peak, 1H, NH). Ms; *m/e* 380 (M^+), 338, 320, 278, 236, 194 (base peak), 152, and 43.

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