A NEW ANTIBIOTIC, 1-(*p*-HYDROXYPHENYL)-2,3-DIISOCYANO-4-(*p*-METHOXYPHENYL)-BUTA-1,3-DIENE. II

ELUCIDATION OF THE STRUCTURE

(STUDIES ON ANTIVIRAL AND ANTITUMOR ANTIBIOTICS. III)

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In our screening for antiviral antibiotics using the agar diffusion-plaque inhibition method, a new antibiotic was obtained in crystalline form from the filter cake of the fermented broth of *Dichotomomyces albus* SAITO. The structure of the antibiotic was determined to be 1-(*p*-hydroxyphenyl)-2,3-diisocyano-4-(*p*-methoxyphenyl)-buta-1,3-diene from the interpretation of the ultraviolet absorption, infrared absorption, nuclear magnetic resonance and mass spectra.

A new antiviral antibiotic was found in the filter cake of the fermented broth of *Dichotomomyces albus* SAITO by utilizing the paper disc-agar diffusion-plaque inhibition method[1]. The antibiotic effectively inhibits plaque formation of Newcastle disease virus strain Miyadera (NDV) infected on primary chick embryo fibroblast cell monolayer (CEF) and the growth of some pathogenic bacteria, such as *Shigella flexneri* and *Staphylococcus aureus*[2].

The structure of the antibiotic was studied by examining the ultraviolet (UV), infrared absorption (IR), mass and nuclear magnetic resonance (NMR) spectra of the antibiotic. It is remarkable that the antibiotic has an isocyano grouping in the molecule. The structure proposed is 1-(*p*-hydroxyphenyl)-2,3-diisocyano-4-(*p*-methoxyphenyl)-buta-1,3-diene on the basis of the spectral evidences and from IR comparison with known antibiotics with isocyano groupings, xanthocillin X (XX) and its dimethylated derivative[3].

Determination of the Molecular Weight

The molecular weight of the antibiotic was determined by mass spectroscopy. At an early stage of this study, no molecular ion peak could be found in the mass spectrum due to the low volatility of the antibiotic. Increasing the temperature of the sample heater of the direct inlet system resulted in the appearance of the parent peak. At 190°C in the sample heater and under the pressure of 10^{-4} mmHg, the molecular ion, m/e 302, became the base peak.

Determination of the Molecular Formula

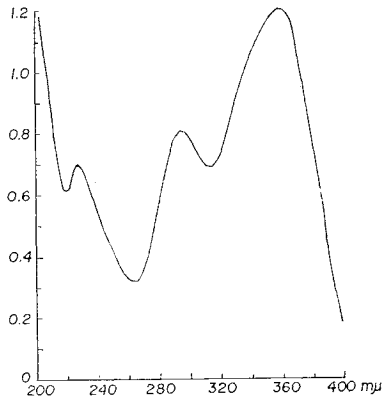
The molecular formula, $C_{19}H_{14}N_2O_2$, was assigned for the antibiotic on the basis of the molecular weight, 302, and microelementary analyses.

Calcd. for $C_{19}H_{14}N_2O_2$: C 75.50, H 4.63, N 9.27.
Found: C 75.55, H 4.45, N 9.30.

Ultraviolet Absorption Spectrum

Ultraviolet absorption spectrum was recorded by a Cary Model 11 M in methanol. The molar absorptivity of the antibiotic was λ_{max} 236 mμ (14,200), λ_{max} 295 mμ (17,200) and λ_{max} 360 mμ (30,000), as shown in Fig. 1. The high absorptivity suggested the presence of highly conjugated system in the molecule, consistent with the observation that the molecular ion is the most abundant in the mass spectrum under certain conditions.

Fig. 1. Ultraviolet absorption spectrum



Infrared Absorption Spectrum

The antibiotic showed a strong characteristic band at 2,100 cm^{-1} in the IR spectrum, as shown

Fig. 2. Infrared absorption spectrum (KBr disc)

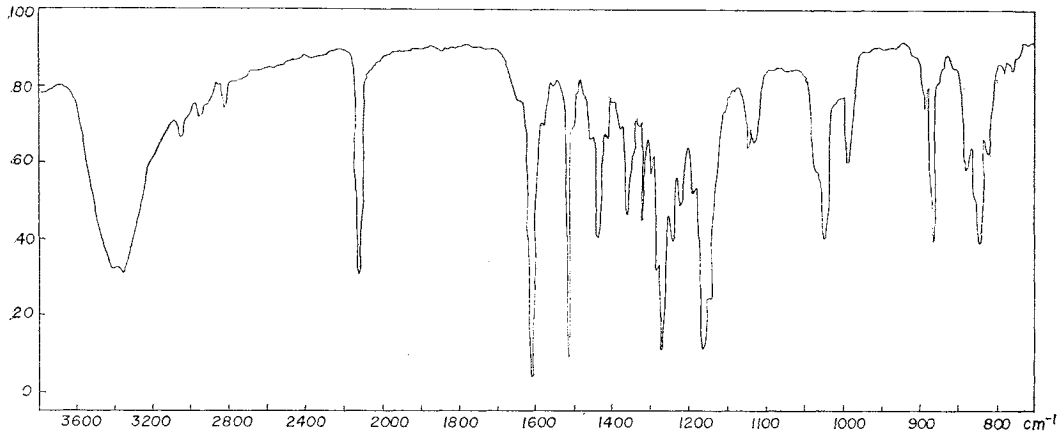
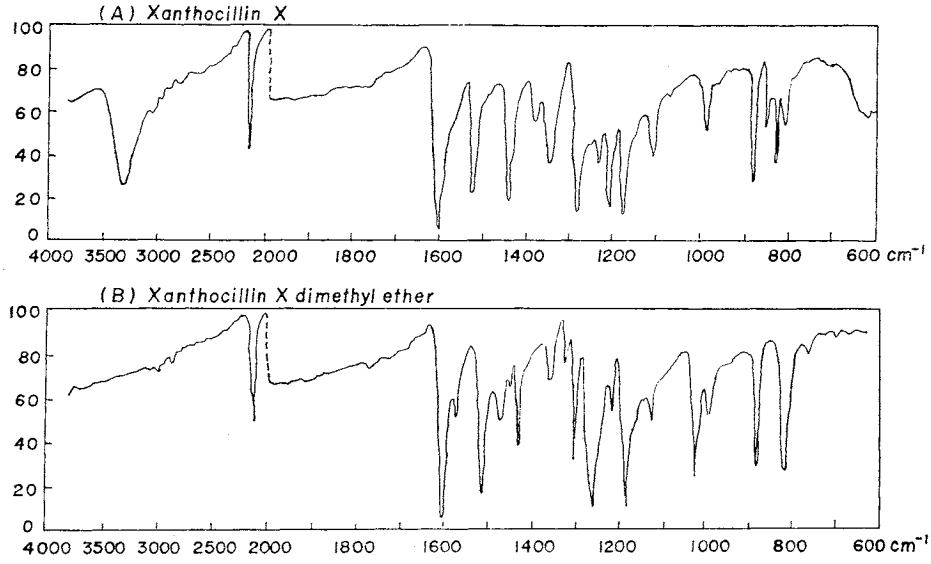


Fig. 3. IR Spectra of xanthocillin X (A) and xanthocillin X dimethyl ether (B)

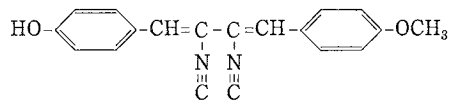


in Fig. 2. The band in this region is attributable to the absorption of the groupings of either X≡Y or X=Y=Z types[4]. The presence of unstable alkyne or allene groupings was improbable because the antibiotic is stable for several months at room temperature, and at 100°C for an hour at neutral pH. The presence of either cyano or isocyano groupings was indirectly suggested from the absence of absorption bands due to other groups containing nitrogen. The cyano group ordinarily shows a medium absorption band in the region between 2,260~2,240 cm^{-1} and when the group is attached to a highly conjugated carbon atom, the band shifts at most to 2,240~2,222 cm^{-1} [5]. Isocyanides display strong absorption in the 2,185~2,105 cm^{-1} region resulting from C-N stretching. Therefore, the strong band at 2,100 cm^{-1} was assigned to isocyanide.

The presence of phenyl grouping was evident from the bands at 3,020, 1,607, 1,512, 960 and 859 cm^{-1}. The presence of phenolic hydroxyl grouping was also indicated by the bands at 3,380 and 1,156 cm^{-1}.

Xanthocillin X (XX) is an only antibiotic with an isocyano grouping in the molecule among the fungal metabolites[3]. XX was found by ROTHE as an antimicrobial agent from the filter cake of the fermented broth of *Penicillium notatum*, and was determined to be 1,4-(*p*-hydroxyphenyl)-2,3-diisocyano-buta-1,3-diene by HAGEDORN and TÖNJES[3]. When the IR of our antibiotic was compared with those of XX and its dimethylated derivative (Fig. 3), many similarities were observed, although the molecular formulas are clearly different; our antibiotic is $C_{19}H_{14}N_2O_2$; XX, $C_{18}H_{12}N_2O_2$; its dimethylated derivative, $C_{20}H_{16}N_2O_2$. The presence of a methoxyl grouping attached to the phenyl ring was suggsted by bands at 2,830 and 1,270 cm^{-1} from the comparison of IR of the antibiotic obtained with that of the dimethylated derivative.

Fig. 4. Structure of xanthocillin X methyl ether

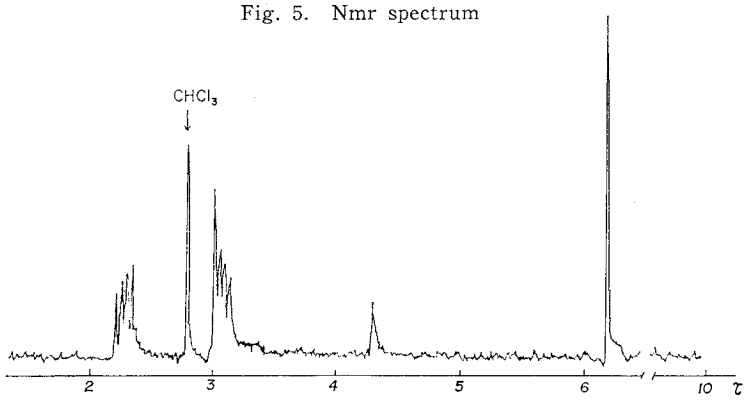


Therefore, it was considered that the most probable structure would be 1-(*p*-hydroxyphenyl)-2,3-diisocyano-4-(*p*-methoxyphenyl)-buta-1,3-diene (Fig. 4).

Nuclear Magnetic Resonance Spectrum (NMR)

The proposed structure was confirmed by NMR and mass spectra. Fig. 5 shows the NMR spectrum recorded by a Japan Electron Optics Model JNM 4H-100 in deuterochloroform using tetramethylsilane as an internal standard. A singlet at τ 6.18

Fig. 5. Nmr spectrum

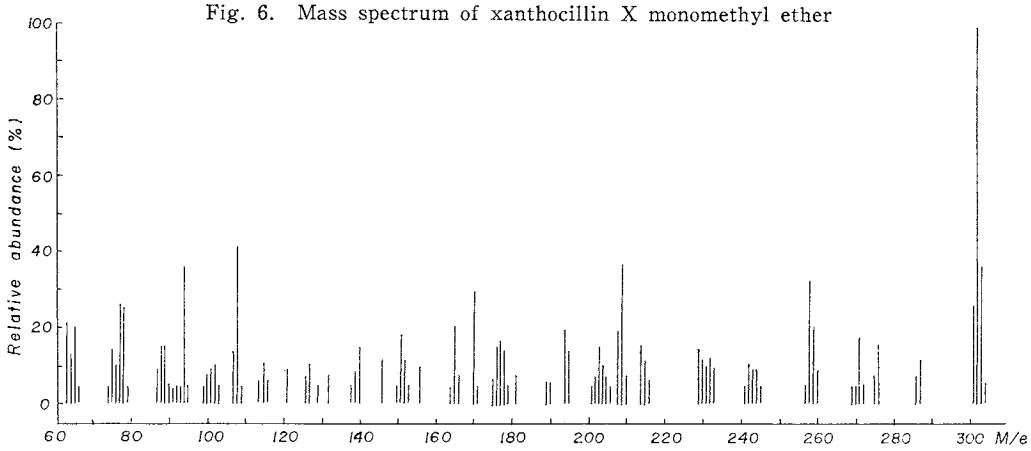


corresponds to 3 protons and clearly indicated the presence of a methoxyl group attached to phenyl[6]. A singlet at τ 4.16 corresponding to one proton disappeared on addition of deuterium oxide. The signal shifted to τ 2.96 when the spectrum was recorded in deuteroacetone. Thus, the signal was assigned to a phenolic hydroxyl group. Signals of the protons attached to the rings and double bonds were involved in two quartets at τ 3.08 (6 H) and τ 2.29 (4 H) coupled to each other with a J=5 cps. This is one of the rare instances that the protons attached to olefinic bonds coupled with those attached to a phenyl ring. The positions of the substituents at the rings were determined to be *para* rather than *meta* or *ortho* from the chemical shifts because three signals with more complicated forms would appear in the spectrum if the positions were *meta* or *ortho*. The proposed structure is in good accordance with the evidence from the NMR spectrum.

Mass Spectrum

The parent peak (m/e, 302), is the base peak which suggested a highly conjugated structure of the antibiotic (Fig. 6). The fragment ions formed by eliminating methyl (m/e, 287), methoxyl (m/e, 271) and isocyano (m/e, 276) groupings from the molecular ion were present in the spectrum. Strong rearrangement peaks at m/e 108 and 94 might be anisole and phenol ions, respectively, indicating the presence of these moieties in the molecule. Thus, the assigned structure was consistent with the fragmentation pattern in the mass spectrum.

Fig. 6. Mass spectrum of xanthocillin X monomethyl ether



Conclusion

All evidence supported the assigned structure, 1-(*p*-hydroxyphenyl)-2,3-diisocyano-4-(*p*-methoxyphenyl)-buta-1,3-diene.

Acknowledgements

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