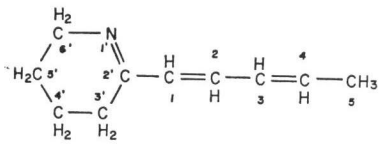
# 1-[2-(3, 4, 5, 6-TETRAHYDROPYRIDYL)]-1, 3-PENTADIENE AN N-METHYLTRANSFERASE INHIBITOR PRODUCED BY ACTINOMYCETES

Sir:

In our systematic screening studies of specific enzyme inhibitors[1], we found a new fermentation product, 1-[2-(3, 4, 5, 6-tetrahydropyridyl)]-1, 3-pentadiene (THPP), that inhibited the N-methyltransferase of rabbit lung[2] (Fig. 1). The isolation of THPP from culture broth of actinomyces strain MD736-C6 as well as the structure and activity of THPP are presented.

The inhibition of N-methyltransferase was assayed by the method of AXELROD[2] modified as follows: The reaction mixture, 250 $\mu$l, contained 25 $\mu$moles of sodium phosphate buffer (pH 8.5), 0.25 $\mu$mole of tryptamine, 0.025 $\mu$mole of S-adenosyl-L-methionine-methyl-[$^3$H] (1 $\mu$Ci/$\mu$mole) and 50 $\mu$l of N-methyltransferase solution prepared from rabbit lung. After incubation at 37°C for 60 minutes, 500 $\mu$l of 0.5 M borate buffer (pH 10.0) was added and the [$^3$H]-methylated product was extracted into 4 ml of toluene-isoamylalcohol (97:3). The radioactivity in a 2-ml aliquot was determined in BRAY'S solution by a liquid scintillation counter. THPP at $8.9\times10^{-5}$ M produced 50% inhibition of the reaction.

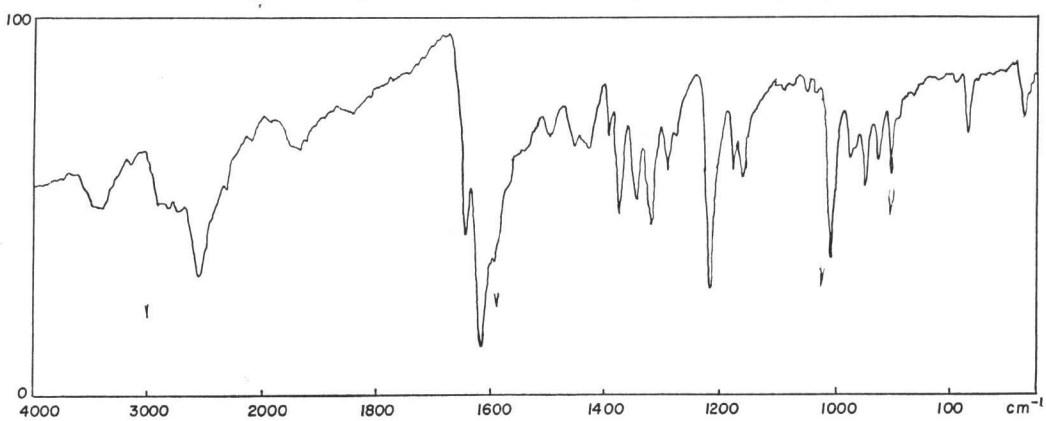
Fig. 1. The structute of THPP



Strain MD736-C6 was shake-cultured at 27°C on a reciprocal shaker (130 rpm) in a medium (125 ml in each SAKAGUCHI flask) containing 2.0% glucose, 2.0% starch, 2.0% soy bean meal ("Soya Meal" Nishin Seiyu Co., Ltd.), 0.5% yeast extract, 0.25% NaCl, 0.32% $CaCO_3$, 0.0005% $CuSO_4\cdot5H_2O$, 0.0005% $MnCl_2\cdot4H_2O$ and 0.005% $ZnSO_4\cdot7H_2O$, pH 7.4. Production of THPP was shown to reach the maximum after 72~96 hours in shake culture fermentations. In a aerated jar fermentation at 27°C, concentration of THPP reached the maximum after 48 hours.

THPP was extracted from the cultured broth by the following procedure: 20 liters of the filtrate was adjusted to pH 7.0 with 6 N HCl and passed through a column (1 liter) of Amberlite IRC-50 ($Na^+$-form); after the column was washed with 5 liters of distilled water, THPP was eluted with 3 liters of 1 N HCl; the active eluate was neutralized with 6 N NaOH and adsorbed on a carbon column (200 ml); the column was washed with 2 liters of distilled water and thereafter THPP was eluted with 50% aqueous acetone; the active fractions were collected and evaporated *in vacuo*, yielding 7 g pale brown powder. The powder thus obtained was applied to a silica-gel column chromatography (300 ml) developed with *n*-butanol. The active fractions were combined and concentrated to dryness, and the yellowish residue was dissolved in a minimal volume of distilled water. After adjust-

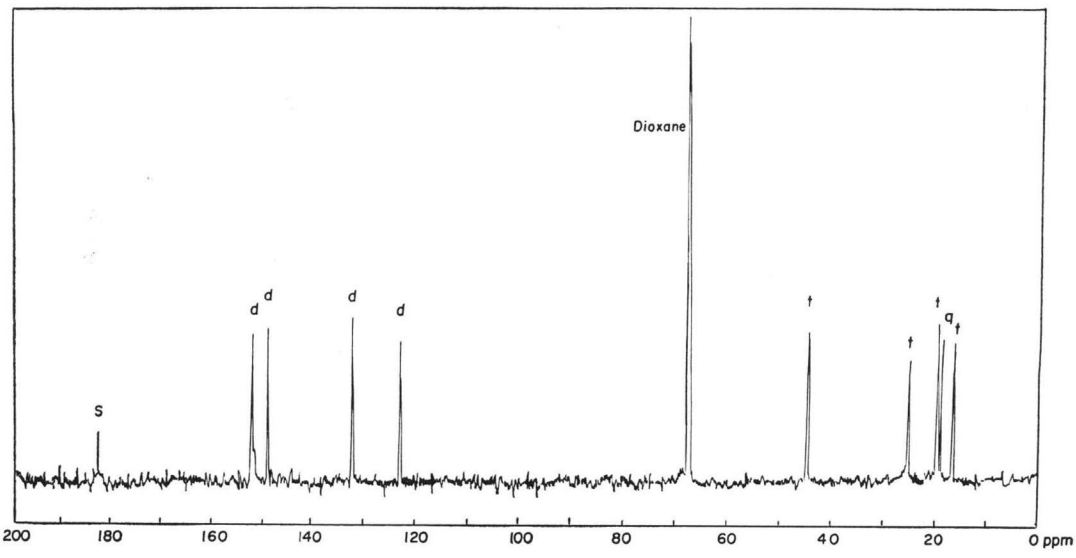
Fig. 2. IR spectrum of THPP monohydrochloride (KBr)

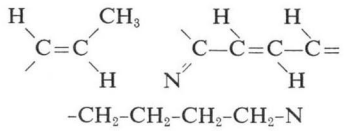


ing to pH 7.0 with 1 N NaOH, the solution was subjected to CM-Sephadex C-25 ($Na^+$-form) column chromatography (200 ml), eluting with 0.2 M NaCl. To remove sodium chloride, THPP was adsorbed from the active eluate on a carbon column, and eluted with 50% aqueous acetone. Concentrating the eluate, THPP monohydrochloride (620 mg) was obtained as colorless crystals analyzing as follows: found: C, 64.12; H, 8.65; N, 7.92; Cl, 18.26: calcd. for $C_{10}H_{15}N \cdot HCl$: C, 64.68: H, 8.68; N, 7.54; Cl, 19.09; m.p. 192°C (dec.); $[\alpha]_D^{25}=0$ (*c* 1, $H_2O$); pKá 9.9 (tit. eq. 169±8 as monohydrochloride). The IR spectrum of THPP monohydrochloride is shown in Fig. 2. The molecular formula was further confirmed by high resolution mass spectrometry ($M^+$, *m/e* 149.1189, calcd. for $C_{10}H_{15}N$, *m/e* 149.1203). The compound gives positive RYDON-SMITH and potassium permanganate reactions, and a yellow color in ninhydrin reaction on thin-layer chromatography (Silica gel G). THPP gives a UV maximum at 303 nm (ε 33,000) in 0.01 N HCl, and the maximum shifts to 267 nm in 0.01 N NaOH. In cyclohexane, it gives three maxima at 253 nm (ε 28,200), 261 nm (ε 34,200) and 272 nm (ε 24,500), indicating the presence of a triene group. On catalytic hydrogenation in propanol with $PtO_2$ under atmospheric pressure, THPP absorbs three moles of hydrogen, and gives a hexahydroderivative which is crystallized as the monohydrochloride, m.p. 142°C. This hexahydroderivative produced the molecular ion with *m/e* 155.1670 (calcd. for $C_{10}H_{21}N$: *m/e* 155.1672). The hexahydroderivative was converted to the mono-N-acetate by treatment with acetic anhydride in pyridine and blue color with nitroprusside (sodium)-acetaldehyde. THPP itself gives neither the acetyl product nor the blue color in the nitroprusside reaction.

The pmr spectrum of THPP (Fig. 1) monohydrochloride in $D_2O$ is as follows: $\delta$ 2.32 (3H, C-5 methyl, d, $J_{4,5}$ 7 Hz), $\delta$ 2.20~2.45 (4H, C-5′ methylene and C-4′ methylene, m), $\delta$ 3.25~3.50 (2H, C-3′ methylene, m), $\delta$ 3.95 ~4.20 (2H, C-6′ methylene, m), $\delta$ 6.60~7.20 (3H, C-1 methine, C-3 methine & C-4 methine), $\delta$ 7.78 (1H, C-2 methine, dd, $J_{1,2}$ 15 Hz, $J_{2,3}$ 9Hz). The value of coupling constant indicates the *trans* configuration of protons at C-1 and C-2. NOE was observed between methyl at 2.36 ppm and methine at 6.8~6.9 ppm, and it is a valid proof of a *trans* configuration of protons at C-3 and C-4. Besides these data, double resonance experiments indicated the presence of the following partial structures;

Fig. 3. $^{13}C$-NMR spectrum of THPP monohydrochloride
The symbols s, d, t and q mean singlet, doublet, triplet and quartet under off-resonance CW decoupling.





The structure of THPP was conclusively determined by $^{13}C$ NMR studies, as shown in Fig. 3. This spectrum indicates that there are four olefinic (122.8, 132.3, 149.3 and 152.3 ppm respectively) and one quaternary carbons (182.4 ppm) in the low field, and five carbon atoms (16.4, 18.4, 19.3, 25.0 and 44.5 ppm) in the high field. One of the latter (18.4 ppm) could be assigned to the methyl group by the off-resonance technique. From the facts mentioned above, the structure of THPP was determined as shown in Fig. 1. This structure was also supported by high resolution mass spectrometry of the hexahydroderivative: *m/e* 84.0842 (base peak, $C_5H_{10}N$ from piperidine) and 71.0858 (11.4%, $C_5H_{11}$ from pentyl group chain).

The inhibition of N-methyltransferase by THPP was studied. The enzyme was purified 20-fold by first centrifuging rabbit lung at $105,000\times g$. The supernatant was then fractionated with ammonium sulfate, followed by gel filtration on Sephadex G-150[2]. THPP showed competitive-type inhibition with both tryptamine and S-adenosyl-L-methionine in LINEWEAVER-BURK plots. The inhibition constants, Ki values, of THPP against tryptamine and S-adenosyl-L-methionine were $2.4\times10^{-5}$ M and $3.0\times10^{-5}$ M, respectively. Km values for tryptamine and S-adenosyl-L-methionine with this enzyme were $9.1\times10^{-4}$ M and $2.3\times10^{-5}$ M, respectively. Though THPP competed with S-adenosyl-L-methionine, THPP at 100 $\mu$g/ml ($5.4\times10^{-4}$ M) did not inhibit either catechol-O-methyltransferase[3] or S-adenosyl-L-methionine decarboxylase[4]. THPP showed neither antibacterial, nor antifungal activities at 200 $\mu$g/ml. Toxicity of THPP to mice was observed after intravenous injection: $LD_{50}$, 5.9 mg/kg.

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