Note

PRODUCTION OF 2-METHYL-4[3*H*]-QUINAZOLINONE, AN INHIBITOR OF POLY(ADP-RIBOSE) SYNTHETASE, BY BACTERIUM

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(Received for publication July 23, 1990)

While screening the culture filtrate of microorganisms for specific inhibitors against various kinds of enzymes, we have succeeded in isolation of a variety of new compounds which specifically inhibit the target enzymes.[1~3] Among such compounds was included benadrostin, an inhibitor of poly(ADP-ribose) synthetase.[4,5] In this paper, we report the discovery of another inhibitor of this enzyme, 2-methyl-4[3*H*]quinazolinone from the culture broth of *Bacillus cereus* BMH225-mF1. Although the compound has already known structure,[6,7] it seems to be worth while to report the first isolation from a microbial source and its characterization because of its potential usefulness.

Poly(ADP-ribose) synthetase used was partially purified from calf thymus according to the method of SHIZUTA *et al.*[8] The activity was measured as reported previously,[4] to determine a concentration of the inhibitor required for 50% inhibition (IC_{50}). The inhibitor was produced by shaking culture of *B. cereus* BMH225-mF1 in a medium containing glucose 2.0%, Prorich (Ajinomoto Co., Inc.) 2.0%, sodium glutamate 0.5%, $CaCO_3$ 0.5% (adjusted to pH 7.4 with 5 N NaOH before sterilization). The maximum production was attained after 72 hours cultivation at 27°C. The culture broth was centrifuged at 10,000 rpm for 10 minutes and the supernate (25 liters) was adsorbed on a Diaion HP-20 column (3 liters, 9.5 × 42 cm), which was washed with water and eluted with 50% aqueous $(CH_3)_2CO$. The active eluate was concentrated under reduced pressure to give a brownish powder (14.5 g, IC_{50} 64 μg/ml). A solution of this powder in $CHCl_3$ was appried to a $CHCl_3$-filled column of silica gel (300 ml, 5.2 × 14 cm), which was eluted with $CHCl_3$ - MeOH (5 : 1) to gave a light brownish active powder (2.31 g, IC_{50} 29 μg/ml). The powder in MeOH was subjected to a Sephadex LH-20 column (1.5 liters, 4.4 × 100 cm) chromatography developed with MeOH to give a slightly yellowish active powder (69.0 mg, 0.39 μg/ml). The powder in MeOH was subjected to silica gel TLC developed with $CHCl_3$ - MeOH (10 : 1). The MeOH extract of the Rf 0.37 fraction was concentrated to dryness. It was dissolved in MeOH and applied to Sephadex LH-20 column (45 ml, 1.5 × 25 cm) chromatography eluted with MeOH to give a colorless active powder. The inhibitor was purified by repeated crystallization from $CHCl_3$ to yield colorless needles (19.9 mg, IC_{50} 0.17 μg/ml, mp 232~234°C).

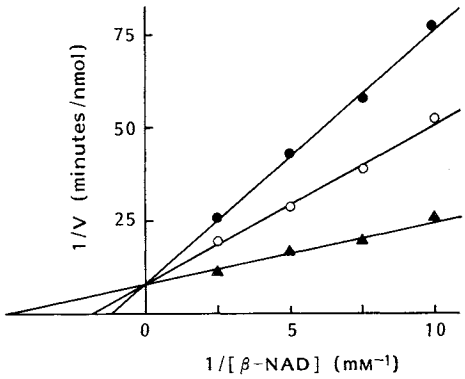
The inhibitor is soluble in MeOH, EtOH, $(CH_3)_2CO$, EtOAc, $CHCl_3$ and benzene, but insoluble in H_2O and *n*-hexane. It gives positive color reaction with potassium permanganate reagent. On silica gel TLC, it gave a single spot at Rf 0.37 ($CHCl_3$ - MeOH, 10 : 1). The elemental analysis was as follows; Calcd for $C_9H_8N_2O$: C 67.49, H 5.03, N 17.49. Found: C 67.49, H 5.22, N 17.35. EI-MS (*m/z*) 160 (M^+). UV λ_{max}^{MeOH} nm (log ε) 225 (4.61), 263 (4.11), 303 (3.85), 314 (3.77) in MeOH, and 232 (4.59), 269 (4.01), 291 (3.86), 302 (3.76) in 0.01 N HCl - MeOH, and 229 (4.58), 280 (4.16), 309 (3.96) in 0.01 N NaOH - MeOH; IR (KBr) cm^{-1} 3160, 3040, 2910, 1690, 1670, 1610, 1470, 1420, 1380, 1345, 1295, 1250, 1140, 880, 780; ^1H NMR (400 MHz, $CDCl_3$) δ 2.60 (3H, s), 7.47 (1H, dt, J=8.3 and 1.6 Hz), 7.68 (1H, br d, J=8.3 Hz), 7.77 (1H, dt, J=8.3 and 1.6 Hz), 8.29 (1H, dd, J=8.3 and 1.6 Hz), 12.05 (1H, br s); ^{13}C NMR (100 MHz, $CDCl_3$) δ 22.0 (q), 120.2 (s), 126.1 (d), 126.3 (d), 127.0 (d), 134.8 (d), 149.4 (s), 153.1 (s), 164.1 (s). These data suggested has 2-methyl-4[3*H*]-quinazolinone as the structure of the inhibitor.

Table 1. Inhibitory effect of 2-methyl-4[3*H*]quinazolinone and other inhibitors on poly(ADP-ribose) synthetase.

	IC_{50} (μM)
2-Methyl-4[3*H*]quinazolinone	1.1
Benadrostin	35
Benzamide	8.3
Nicotinamide	74

Fig. 1. Lineweaver-Burk plot of inhibition of poly-(ADP-ribose) synthetase by 2-methyl-4[3*H*]quinazolinone.

● I=0.04 μg/ml, ○ I=0.02 μg/ml, ▲ 0 μg/ml.



This was supported by the long range selective proton decoupling experiment.

The inhibitory activity of various inhibitors against poly(ADP-ribose) synthetase are shown in Table 1. 2-Methyl-4[3*H*]quinazolinone is the strongest among the inhibitors tested. Inhibition of the inhibitor against poly(ADP-ribose) synthetase is competitive with the substrate (Fig. 1). The *Ki* and *Km* values are 1.1×10^{-6} M and 3.1×10^{-4} M, respectively. Mice tolerated an ip injection of 250 mg/kg of the inhibitor to indicate its low acute toxicity.

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