⁂Studies on the Antioxidants: XI. Oxidation Products of Concomitantly Used Butylated Hydroxyanisole and Butylated Hydroxytoluene

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ABSTRACT

The effects of antioxidants used concomitantly were studied for their contribution to the autoxidation process and for the functional mechanism of the antioxidating process. In this study, an equal mixture of butylated hydroxyanisole (BHA) and butylated hydroxytoluene (BHT) in benzene was irradiated with ultraviolet rays and the chemical structures and antioxidant activities of the resulting oxides were derived. The chemical changes of the substrates and oxides were followed quantitatively by gas chromatography. The resulting oxide was confirmed to be 3,3′,5′-tri-*tert*-butyl-5-methoxy-2,4′-dihydroxydiphenyl methane, which was a dehydrogenated dimer of BHA and BHT. The stability study on this compound by AOM showed its strong activity as an antioxidant on lard and soybean oil.

INTRODUCTION

In our previous studies (1-3), we have proven that an antioxidant, butylated hydroxyanisole (BHA), generated free radical groups during the antioxidation processes, resulting in oxidation products with active antioxidative activities and contributing to the antioxidative activity of the substrate. There are many papers on the oxidation products of butylated hydroxytoluene (BHT) from the same standpoint (4-7).

Generally, several antioxidants have been used as mixtures of one another. The fates of the antioxidant molecules in the mixtures have important factors concerned with synergism (8-11). However, these factors are not yet satisfactorily clarified at present. We therefore studied a mixture of BHA and BHT as the antioxidant mixture from the same standpoint.

This paper will report the isolation of the oxidation products which resulted from ultraviolet (UV) irradiation on the benzene solution of equal molarity BHA and BHT mixture. The oxidation product was identified as 3,3′,5′-tri-*tert*-butyl-5-methoxy-2,4′-dihydroxy diphenyl methane. Moreover, this oxidation product still retained the antioxidative activity. The quantitative analyses of the oxidation products were followed up during the degradation process.

EXPERIMENTAL PROCEDURES

Materials

The BHA used was 2-*tert*-butyl-4-methoxyphenol (mp 64 C) which was obtained by the repeated recrystallization of Sustane (Nikki Universal Co., Tokyo) from petroleum ether.

The BHT used was obtained by the repeated crystallization of a commercial product of BHT (mp 69-71 C) (Nikki Universal Co., Tokyo) from ethanol.

2,2′-Dihydroxy-5,5′-dimethoxy-3,3′-di-*tert*-butyldiphenyl (2a) and 2′,3-di-*tert*-butyl-2-hydroxy-4′,5-dimethoxy-diphenyl ether (2b) were isolated by silica gel column chromatography from the irradiated BHA-benzene solution (1) (mp 2a, 224.5 C; 2b, 78-79 C).

1,2-bis(3,5-Di-*tert*-butyl-4-hydroxyphenyl) ethane (BE) and 3,5,3′,5′-tetra-*tert*-butylstylbenquinone (SQ) were isolated from the oxidation products of BHT-benzene solution by silica gel column chromatography with potassium ferricyanide according to the Cook et al. method (5) (mp 169-170 C).

Lard, methyl oleate and soybean oil were the same products used in our previous paper (12).

Assay Procedure

Thin layer chromatographic (TLC) examination of the products was made on 25-mm silica gel coated plates. These were, in most cases, developed in hexane/toluene solution (1:1) sprayed with 1% 2,6-dichloroquinone-4-chloroimide ethanol solution.

Gas chromatography (GLC) was carried out under the following conditions with hydrogen flame detector, Model G-80, Yanagimoto Co.: condition 1. Column: 1% Silicone SE-52 on Gaschrom-Q; column temperature: heated from 150 to 270 C at 6 C/min, injector temperature: 250 C; carrier gas: N_2, 10 ml/min; condition 2. Column: 5% silicone OV-17 on Chromosorb W AW; column temperature: 250 C; carrier gas: N_2, 12.5 ml/min.

Column chromatography was carried out using a column with 3.5 mm id and packed with 35 g of 100-mesh Silicagel (Kanto Kagaku Co., Tokyo). The column was eluted by benzene/hexane, 1:1.

Nuclear magnetic resonance (NMR) spectrum was measured by a Hitachi spectrometer Model R-20A, 60 Mc. The solvents used were DMSO and $CDCl_3$. The chemical shift was expressed as ppm using tetramethylsilane (Me_4Si) as the internal standard. Mass spectrum was measured by a Hitachi mass spectrometer Model RMU-7L.

UV Irradiation and Oxidation Products Isolation

BHA (1.8 g) and BHT (2.2 g) were dissolved in ca. 50 ml benzene and the solution was placed into a glass dish12 cm in diameter and 2 cm deep. The solution was UV-irradiated for 15 days in a UV irradiation box. The distance between the light source and the samples was set at 30 cm. Evaporated benzene was supplemented intermittently.

The BHA/BHT-benzene photooxidized solution was distilled under reduced pressure to remove benzene and applied to silica gel column chromatography, then eluted by benzene/hexane (1:1). The reaction process was examined by TLC and GLC.

All fractions, including the oxidation products, were placed into the same batch and after removing the solvent, the products were isolated and purified by column chromatography. White crystal (AT-1) was obtained after recrystallization from petroleum ether.

Acetylated AT-1 was produced when 1 ml pyridine and 1 ml acetic anhydride were added to 50 ml AT-1 and allowed to react over a boiling water bath for 1 hr. Then the reaction mixture was extracted with ethyl ether/chloroform (4:1) and rinsed with 10% HCl, 5% Na_2CO_3

solution and water in that sequence. It was dried over anhydrous Na_2SO_4 and the solvent was then eliminated. The products were purified by column chromatography.

Assay of BHA, BHT and Oxidation Products in Benzene Solution under UV Irradiation

Preparation of the calibration curves. The 0.25% dichloromethane solutions of BHA and BHT and 0.05% dichloromethane solutions of 2a, 2b, BE, SQ and AT-1 were prepared individually. Of these solutions, exactly 1.0 ml, 2.0 ml, 3.0 ml and 5.0 ml were transferred into each 10-ml mess flask. Exactly 1 ml of 1% 2-methyl-6-*tert*-butylphenyl and 0.2% trioctyl amine dichloromethane solution was added to each solution as the internal standard and dichloromethane was added to bring the level up to 10 ml. The calibration curves of these solutions were prepared as under GLC condition 2.

Reaction condition. Benzene solution (20 ml) of 0.18 g BHA and 0.02 g BHT were placed in a glass dish 7 cm in id and 2 cm deep and UV-irradiated continuously. The distance between the light source and the samples was set at 30 cm. The temperature of the equipment was maintained at 25 ± 3 C. The sample vol was adjusted at exactly 20 ml with benzene every 24 hr. The 0.1-ml sample solution each of BHA, BHT and the oxidant products was assayed by GLC as follows: a mixture of 0.1 ml irradiated solution, 0.2 ml solution of the internal standard and 1.7 ml dichloromethane was used as the samples for GLC analysis. The sample solutions were assayed as under GLC condition 2. The losses by sampling were corrected for all samples.

Determination of antioxidative activity. The peroxide values (POV) were measured according to the Wheeler (13) procedure. Active oxygen method (AOM) was applied. The oil sample (20 ml) was placed in oxidation tubes (25" x 20"). The temperature was kept at 97.8 C (± 0.2 C) in oil bath. Clean air was passed at a rate of 2.33 ml/sec. The apparatus can use 10 tubes at once; it is designed so that air passes into the test tubes constantly through carefully selected capillaries. The POV of the samples were taken at constant intervals.

The antioxidative activity as POV was determined by setting the test tubes with 20 ml-methyl oleate solutions of 3.6 mg BHA (1 mM), 4.4 mg BHT (1 mM) and a mixture of 1.8 mg BHA and 2.2 mg BHT (each 0.5 mM) and also with 20 ml methyl oleate as the control. The samples were flushed by air under heated conditions and were taken at constant intervals. The stability of the test oil is expressed by the starting time to the time it takes the oil to reach POV 100 m equiv/kg. The antioxidative activities on lard or soybean oil as the base oil were compared according to the same procedure.

RESULTS AND DISCUSSION

Formation of Oxidation Products

A benzene solution of BHA and BHT (equal mol) was UV-irradiated. The progress of the reaction was examined by TLC. Figure 1 shows the gas chromatogram and thin layer chromatogram on the samples irradiated for 15 hr. The retention times of BHT and BHA on GLC were 4.1 min. (A) and 4.5 min (B), respectively. Two of the oxidation products of BHA, e.g., 2',3-di-*tert*-butyl-2-hydroxy-4',5-dimethoxy diphenyl ether (2b) and 2,2'-dihydroxy-5,5'-dimethoxy-3,3'-di-*tert*-butyl diphenyl (2a) have retention times at 18 min (D) and 19 min (E), respectively. The retention times of BHT oxidation products, e.g., 1,2-bis-(3,5-di-*tert*-butyl-4-hydroxyphenyl) ethane (BE) and 3,5,3',5'-tetra-*tert*-butyl stylbenquinone (SQ) were 23 min (G) and 29 min (H), respectively, and that of 3,5-di-*tert*-butyl-4-hydroxybenzaldehyde (T-ald) has a peak at 8 min (C). The new peak with the retention time at 21 min was assumed to be one of the phenol compounds because it developed color with 2,6-dichloroquinone-4-chloroimide solution on TLC.

Isolation and Purification of Oxidation Products

The reaction solution was processed by column chromatography in order to isolate a compound which has a peak retention time at 21 min on GLC. The fractions which were eluted after SQ came out of the column were purified again by column chromatography. The white crystal was obtained by recrystallization with petroleum ether. This compound had a single peak on GLC with retention time 21 min and has a single spot on the TLC plate with Rf value 0.3 on TLC. Therefore, it is assumed to be the F oxidation product of Figure 1 (called AT-1).

Chemical Structure of Oxidation Product

The AT-1 isolated was a white crystal with mp 106-107 C. It was soluble in ethanol, hexane, chloroform and other solvents, but was insoluble in water.

The mass spectrometry (MS) showed the molecular ion peak at 398 m/e. The molecular formula was agreed upon as $C_{26}H_{38}O_3$ as derived from the elemental analysis.

Figure 2 shows the NMR spectrum. Several signals were observed in the chart: 3 signals can be attributed to *tert*-butyl groups (1.32 ppm, 27 H), 1 to a methoxyl group (3.70 ppm, 3 H), and 1 to a methylene group (3.87 ppm, 2 H). Two peaks at 4.40 ppm (1 H) and 5.08 ppm (1 H)

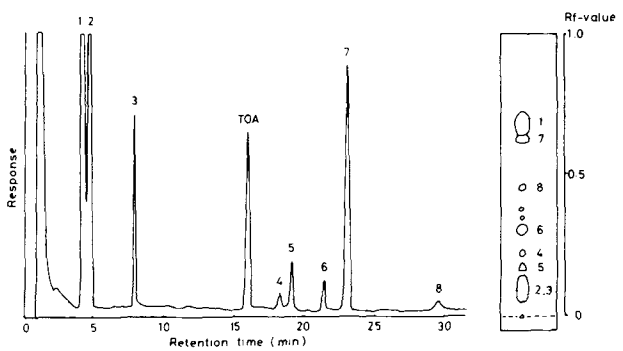


FIG. 1. Gas chromatogram and thin layer chromatogram of oxidation products of BHA-BHT mixture in benzene under irradiation with UV light. A: BHT; B: BHA; C: T-ald; D: 2b; E: 2a; F: AT-1; G: BE; H: SQ.

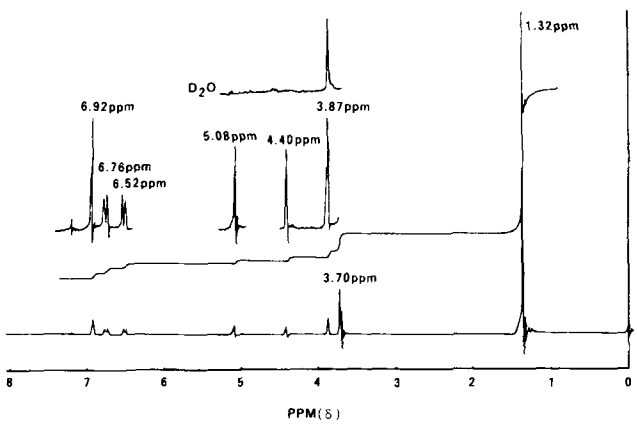


FIG. 2. Nuclear magnetic resonance spectrum (60 MHz) of AT-1.

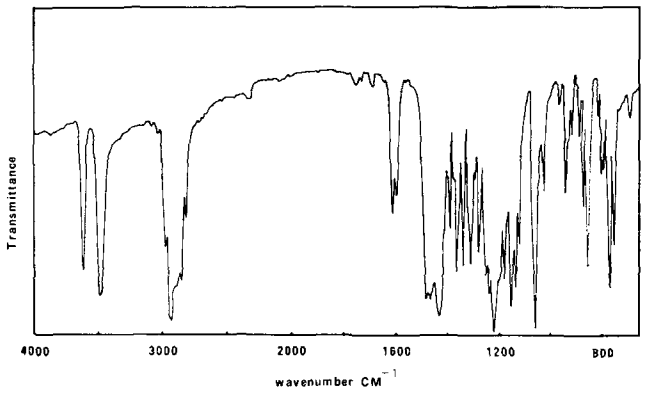
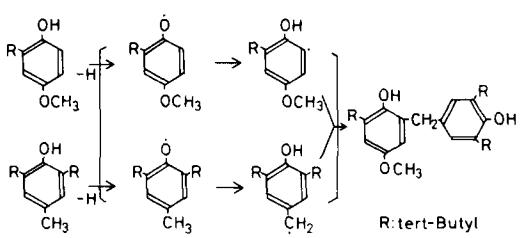


FIG. 3. Infrared spectrum of AT-1.

disappeared after treatment with deuterium, so they were assumed to be 2 OH groups. These assumptions were made from the absorption in IR spectrum (Fig. 3) at 3,480 cm^{-1} and 3,610 cm^{-1} which may have resulted from the stretching vibration of hydroxyl groups. The acetylation product AT-1 was considered to be the monoacetylated compound having OH groups because it showed the molecular ion peak at 440 m/e in the mass spectrum, but lost a peak at 4.40 ppm (1 H) in the NMR spectrum, then developed a new peak at 3.64 ppm (3 H); a peak of 3,480 cm^{-1} disappeared between 2 peaks at 3,480 cm^{-1} and 3,610 cm^{-1} in IR spectrum, and developed a new peak at 1,760 cm^{-1} which resulted from the ester. The presence of 2 OH groups is supported by these data. In the nuclear proton field, the doublets were present which showed m-coupling at 6.52 ppm (1 H, J = 3 Hz) and 6.76 ppm (1 H, J = 3 Hz). These doublets were formed by the hydrogens at positions 3 and 5 of BHA, and a singlet at 6.92 ppm (2 H) resulted from the hydrogens of positions 3 and 5 of BHT.

AT-1 was identified as 3,3′,5′-tri-*tert*-butyl-5-methoxy-2,4′-dihydroxydiphenylmethane as shown in Scheme I



SCHEME I. Formation of AT-1.

in reviewing these data just mentioned. It was considered to be one of the dimerization products of the free radicals produced by the dehydrogenation of the methyl group of BHT and nuclear hydrogen of position 6 of BHA when we were considering the molecular structure of AT-1. This shows that the antioxidant was dehydrogenated by some oxidation factors to generate free radicals which eventually dimerized with the same species of the free radicals or with the different species of free radicals to form the oxidation product. An interesting suggestion is that the complex fate of antioxidant molecules exists during the autoxidation processes.

Assay of BHA, BHT and Oxidation Products by GLC

The simultaneous assay procedures for the substrates and oxidation products in a solvent mixture of BHA and BHT irradiated with UV light were examined. The 5 samples of oxidation products; 2a, 2b, BE, SQ and AT-1 were assayed. The oxidation products were isolated well and identified by SE-52 column chromatography under GLC condition 1 whereas BHA and BHT were not so satisfactorily isolated. Seven sample compounds were isolated well using an OV-17 column and raising the temperature from 120 to 290 C under GLC condition 2. The calibration curves of BHA and BHT and those of the oxidation products were prepared in a range between 0-1.25 mg/ml and 0-0.25 mg/ml, respectively. After the peaks for BHA and BHT appeared, the oxidation products were assayed by a 10-fold increase in detection sensitivity.

Figure 4 shows the quantitative change of the substrates and oxidation products when an equivalent solution of BHA and BHT was UV-irradiated. The amounts of BHA and BHT gradually decreased. This indicates that substrates themselves were oxidized in proportion to the irradiation time. About 73% of BHA remained after a 120-hr irradiation and ca. 65% remained after the maximal 240-hr irradiation. Generally, the amount of the substrate was reduced gradually. But BHT remained at ca. 20% after the 240-hr irradation. BHT was more significantly degraded by oxidation than BHA.

Most of the oxidation product was BE, which was derived from BHT. The amount of BE reached was a maximum at 0.06 mmol (12% of the amount of BHT added) after 144 hr of irradiation, but it reduced thereafter and reached ca. 0.052 mmol after the maximal 240-hr irradiation. SQ was increasingly formed with increased time. The maximal amount of SQ was 0.01 mmol. It was therefore speculated that BE oxidized to SQ. The amounts of 2a and 2b increased linearly with a slight gradient and reached 0.028 mmol (corresponding to 5.6% of BHA added) and 0.005 mmol (corresponding to 1.0% of BHA added) in 240 hr, respectively. The amount of AT-1 formed reached 0.003 mmol after 144 hr of irradiation and stayed at the constant level with negligible fluctuation.

Antioxidative Activity of AT-1

Kurechi (2) and Harano (7) have studied the antioxidative activity of the oxidation products, i.e., 2a, 2b BE and

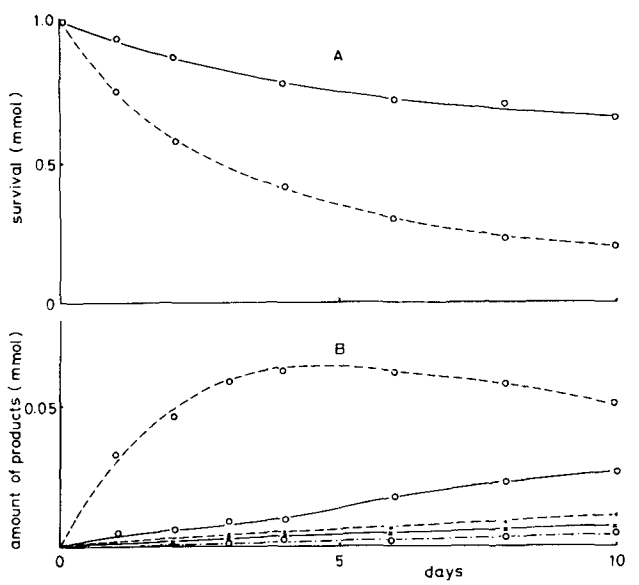


FIG. 4. Change of residual BHA and BHT(A) and their oxidation products (B) with time under irradation of UV light in benzene. 1.0 mmol BHA and BHT were added to 20 ml benzene. (A) o—o BHA, o– –o BHT; (B) o–·–o AT-1, o—o 2a, x—x 2b, o– –o BE, •—• SQ.

SQ. Every oxidation product except SQ was reported to have antioxidative activity. The antioxidative activity of AT-1 on methyl oleate, lard and soybean oil as the base oil was studied. Figures 5-7 show the effects of AT-1 in these base oils by POV curves when they were evaluated by AOM. Table I shows the antioxidative ratio (A.R.) calculated from the relative ratios of the induction period over that of BHT.

TABLE I

Antioxidative Effect of BHA, BHT, Their Mixture and AT-1

	Methyl oleate A.R.[a]	Lard A.R.	Soybean oil A.R.
BHT	1.00	1.00	1.00
BHA	1.42	1.53	0.02
BHA,BHT mix	1.66	1.67	0.55
AT-1	1.03	1.66	1.47

[a]Antioxidative ratio.

As shown in Figure 5 (POV curve), AT-1 and BHT behaved similarly when methyl oleate was used as the base oil. The induction period was extended by ca. 28 hr. It was proven that they retain the antioxidative activity, and the order, compared by the oxidation preventive ratios, was: mixture of BHA/BHT > BHA > AT-1 ≒ BHT.

AT-1 developed significant antioxidative activity when lard was used as the base oil as proven from the POV curve (Fig. 6). The antioxidative activity of AT-1 was almost the same as that of the BHA/BHT mixture. The induction period was extended at ca. 56 hr; oxidation preventive ratio was 1.66.

Figure 7 shows the POV curves in a system where soybean oil was used as the base oil and where AT-1 again developed antioxidative activity. It extended the induction period for ca. 22 hr; oxidation preventive ratio was 1.31. In soybean oil, AT-1 has the largest antioxidative activity, and sequentially BHT, a mixture of BHA/BHT and BHA had activities ranging in this order from greatest to least.

The antioxidative activity of AT-1 was studied using 3 kinds of base oil; AT-1 retained its antioxidative activity in all 3. AT-1 was clarified by AOM to stabilize any base oil; however, its activity depends on the variety of base oil, e.g., its activity on methyl oleate was almost the same as that on BHT and nearly the lowest. But its antioxidative activity on lard and soybean oil were the highest and was almost the same or more as a BHA/BHT mixture. Reviewing these results, when antioxidants were used concomitantly, an oxidation product AT-1 which was formed from 2 different kinds of antioxidants, and other oxidation products which were formed by one kind of antioxidant, were found to contribute to prevention of oxidation in oil.

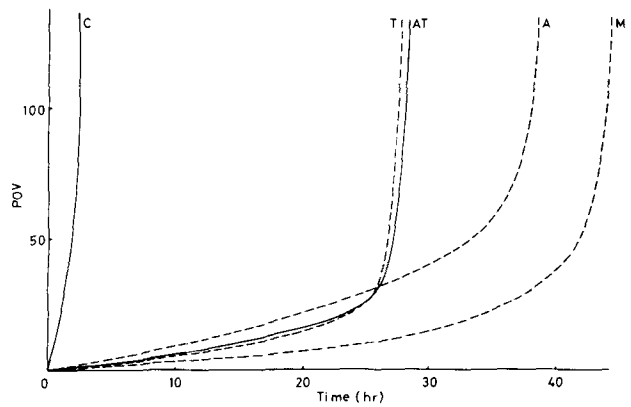


FIG. 5. Antioxidative effect of BHA, BHT, their mixture and AT-1 on methyl oleate. C: control; A: BHA (1 mM); T: BHT (1 mM); M: BHA (0.5 mM) + BHT (0.5 mM); AT: AT-1 (0.5 mM).

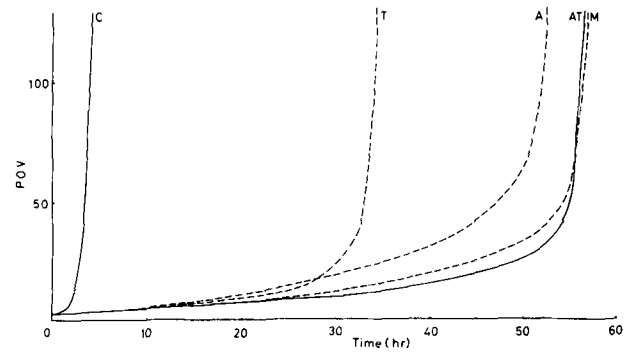


FIG. 6. Antioxidative effect of BHA, BHT, their mixture and AT-1 on lard. C: control; A: BHA (1 mM); T: BHT (1 mM); M: BHA (0.5 mM) + BHT (0.5 mM); AT: AT-1 (0.5 mM).

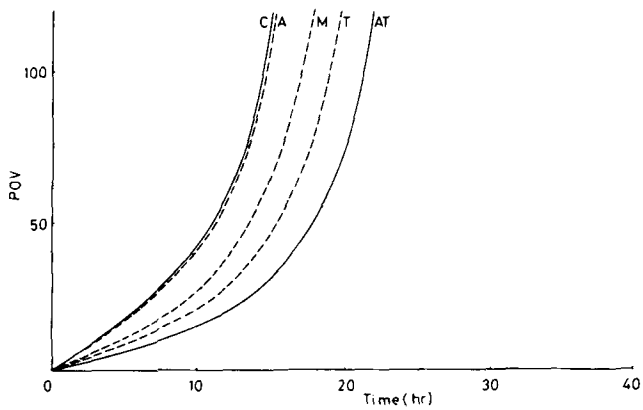


FIG. 7. Antioxidative effect of BHA, BHT, their mixture and AT-1 on soybean oil. C: control; A: BHA (1 mM); T: BHT (1 mM); M: BHA (0.5 mM) + BHT (0.5 mM); AT: AT-1 (0.5 mM).

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[Received July 2, 1979]