# Photocycloaddition Reaction of 8-Methoxypsoralen and 5, 7-Dimethoxycoumarin with Maleimide

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$C_4$-Photocycloaddition of 8-methoxypsoralen (8-MOP) and 5,7-dimethoxycoumarin (DMC) to maleimide was studied in order to elucidate the mechanism of the photobiological activities of these molecules. The photoreaction was carried out in chloroform solution and frozen aqueous solution state. The major product was isolated and characterized by spectroscopic methods. The photoadduct between 8-MOP and maleimide was shown to be an 1:1 $C_4$-cycloadduct through the photocycloaddition of 4',5'-furyl double bond of 8-MOP to maleimide. The stereochemistry of cyclobutane ring of this photoadduct is consistent with the *anti* configuration. The photoadduct between DMC and maleimide was shown to be an 1:1 $C_4$-cycloadduct through the photocycloaddition of 3,4-pyrone double bond of DMC to maleimide.

## Introduction

Psoralens are known to photoreact with pyrimidine bases in DNA under irradiation with near UV light. Various physiological actions such as skin erythema on human and quinea pig skin, mutagenic and lethal effects in bacteria, inactivation of DNA viruses, inhibition of tumor-transmitting capacity of various tumor cells, and disorders in the development of sea urchin eggs fertilized with sperm have been attributed to this photoreaction.[1,2] Psoralens plus UVA light (PUVA) have been used in the treatment of skin diseases such as psoriasis and vitiligo[3] for a long time.

The binding of these furocoumarins to nucleic acids has recently been utilized for structure determination of certain DNA and RNA molecules.[4-9] Much work has been done on the correlation between the skin-photosensitizing ability of various furocoumarins and their molecular structure.[10-17] Among other things, these studies have shown that both the 4',5'-furyl- and 3,4-pyrone-functional groups are required for biological activity. It has also been postulated that the most likely mechanism for skin-photosensitization involves the formation of interstrand cross-links in DNA through the photocycloaddition of the 4', 5'- and 3,4-double bonds of the furocoumarin molecules.[18,19] This idea is supported by the evidence that only psoralens with bifunctional groups are capable of producing lethal and mutagenic effects when applied to bacterial cultures.[10,20] Although most monofunctional coumarin derivatives are inactive,[14,15] 5,7-dimethoxycoumarin (DMC) has been shown to be highly active photobiologically.[21]

Maleimides are heterocyclic compounds having a conjugated double bond with two carbonyl functional groups. It has been shown that maleimides have a strong quenching effect on the triplet state of the furocoumarins although the cycloadducts are formed with low quantum yields. When the equilibrium constants of molecular complex formation in the ground state and rate of initial formation of fluorescent substances between the furocoumarins and maleimides by the long wavelength UV light are measured, the values for the DNA and those of maleimides are identical with each other. From these experimental data, maleimides appear to be a better model compound for DNA than thymine and cytosine *in vitro*.[22,23]

For these reasons, we undertook the study of the photocycloaddition reaction of 8-methoxypsoralen (8-MOP) and DMC to maleimide as an important model for the photoreaction of 8-MOP and DMC with pyrimidine bases and nucleic acids.

## Materials and Methods

*Materials.* Extra pure chloroform, dioxane and ethyl acetate (Wako Chemical Co. or Cica Chemical Co.) were used as received or after distillation. Maleimide was synthesized according to the method reported by Tawney *et al.*[24] The purified maleimide can be recrystallized from ethyl acetate or chloroform. The compound was further purified by sublimation if necessary. 8-Methoxypsoralen (8-MOP) and 5,7-dimethoxycoumarin (DMC) were obtained from Sigma and Aldrich Chemical Co. respectively. DMC was recrystallized from ethanol or used as received. 8-MOP was used without further purification.

*Irradiation Apparatus.* Irradiations were carried out in a Rayonet Photochemical Reactor (The Southern New England Ultraviolet Company) Model RPR-208 or RPR-100 equipped with 350 nm fluorescent lamps. Two modules of Model RPR-208 were stacked together and arranged in a horizontal positon, allowing the photolysis of frozen aqueous solution state samples.

*Spectroscopic measurements.* Infrared spectra were recorded on a Perkin-Elmer Model 267 spectrophotometer using potassium bromide pellets. Ultraviolet-visible spectra were recorded on a Cary 17 spectrophotometer. NMR spectra were measured on a Varian T-60A and EM-360 spectrometer in deuterated chloroform or perdeuterated pyridine against

tetramethylsilane internal standard. Fluorescence spectra were recorded on an Aminco-Bowman spectrofluorimeter with Aminco XY-recorder.

*Irradiation of 8-Methoxypsoralen and Maleimide.* 8-MOP (300 mg) and 907mg of maleimide (molar ratio 1.5: 10) were dissolved in 300 m*l* chloroform. The solution was deoxygenated by bubbling dry nitrogen through the solution for 2 h and irradiated in a preparative photochemical reactor equipped with UV lamps (RPR-208, RUL-3500 Å lamps). The irradiation time was about 100 h.

*Analysis and Isolation of 8-MOP-maleimide Photoadduct.* Irradiated samples were analyzed by silica gel TLC utilizing a dichloromethane-acetone (90:10 v/v) mixture as a developing solvent and visualized by Mineral light (Model UVS-11; 250 nm and 350 nm). The major product was isolated by preparative TLC.

*Irradiation of 5,7-Dimethoxycoumarin and Maleimide. Chloroform solution state irradiation.* DMC (150 mg) and 471 mg of maleimide (molar ratio 1.5:10) were dissolved in 300 m*l* chloroform. The solution was deoxygenated by bubbling dry nitrogen through the solution for 2 h and irradiated in a preparative photochemical reactor equipped with UV lamps. The irradiation time was about 50 h.

*Frozen Aqueous Solution State Irradiation:* DMC and maleimide (molar ratio 1.5:10) were dissolved in dioxane-water (1:9 v/v). The solution was poured into a Petri dish and frozen in a refrigerator. The thickness of the sample was less than 7 mm. The Petri dishes containing the frozen solution were placed at 15 cm distance from the RUL-3500 Å lamps arranged in a horizontal position and irradiated for 50 h. The temperature of reaction chamber was maintained below 0°C during the photolysis in order to keep the samples from melting.

*Analysis and Isolation of DMC-Maleimide Photoadduct.* Irradiated samples were analyzed by silica gel TLC utilizing a dichloromethane-acetone-cyclohexane (90:55:45 v/v) mixture as a developing solvent and visualized by Mineral light. The major product was isolated by column chromatography.

## Results and Discussion

*Photoreaction of 8-MOP with Maleimide.* 8-MOP did not photoreact with maleimide in frozen aqueous solution state and in nondeoxygenated chloroform solution. 8-MOP photoreacted with maleimide in chloroform solution only when solution is deoxygenated. This indicates that the formation of photocycloadduct between 8-MOP and maleimide is obtained through a triplet state of 8-MOP which is efficiently quenched by oxygen.

Poppe and Grossweiner (1975) found that 8-MOP is a good photodynamic sensitizer in aqueous solution. Singlet oxygen ($^1O_2$) generated by triplet energy transfer is the principal reactive intermediate in the inactivation of lysozyme. The earlier studies also found that most of the excited states of psoralen and coumarin undergo nonradiative decay ($\Phi_f$= 0.019, $\Phi_p$=0.13 for psoralen, $\Phi_f$=0.009, $\Phi=_p$0.055 for coumarin, respectively). The reason of the very rapid nonradiative decay of 8-MOP is that an $^3(n,\pi^*)$ state lies at only slightly lower energy level than the $^1(\pi,\pi^*)$ state, thus facilitating the intersystem crossing to the triplet manifold.[16] This is in agreement with the observation that the formation of photocycloadduct between 8-MOP and maleimide is quenched by oxygen. Therefore, it is very probable that the photocycloaddition reaction of 8-MOP and maleimide undergoes through an excited triplet state.

*Characterization of 8-MOP-maleimide Photoadduct.* The photoproducts of 8-MOP-maleimide were analyzed by silica gel TLC. The major product was isolated by preparative TLC and recrystallized in methanol. The structure of the 8-MOP-maleimide photoadduct was characterized by the spectroscopic methods as described below.

The UV absorption spectrum of the photoadduct is nearly superimposable with that of DMC. The $\lambda_{max}$ of 8-MOP is observed at 300 nm and the $\lambda_{max}$ of DMC is at 324 nm. The $\lambda_{max}$ of photoadduct appears at 324 nm. The $\lambda_{max}$ and shape of the UV absorption spectra indicate that the 4',5'-furyl double bond of 8-MOP is saturated. The photoadduct was easily photosplit into 8-MOP and maleimide on 254 nm UV light irradiation for 5 min. This suggests that the photoadduct is composed of 8-MOP and maleimide formed by $2\pi+2\pi$ cycloaddition of 4',5'-furyl double bond of 8-MOP to C=C double bond of maleimide.

The infrared spectra do not show any shift of the carbonyl stretching band of 8-MOP at 1710 $cm^{-1}$ which is the same with that of DMC. This intact carbonyl stretching band indicates that 3, 4-pyrone double bond of 8-MOP is not affected on photoreaction. New signals at 3450, 3200 $cm^{-1}$ ($\nu_{NH}$), and 1582 $cm^{-1}$ (amide II band) are observed. A new carbonyl stretching band is also shown at 1770 cm.$^{-1}$ This is due to the antisymmetric carbonyl strectching band of succinimide structure which is formed by the saturation of the double bond of maleimide on photoreaction. A characteristic cyclobutane ring deformation band appeared at 830 $cm^{-1}$ which is not observed in 8-MOP or in maleimide spectra. This also suggests that the photoadduct is a $C_4$-photocycloaddition product of 8-MOP and maleimide formed through a 2+2 addition of the 4',5'-furyl double bond of 8-MOP to the double bond of maleimide.

The fluorescence spectra show a small blue shift of $\lambda^{fl}_{max}$ from 465 nm in 8-MOP to 450 nm in the photoadduct due to the saturation of 4', 5'-furyl double bond of 8-MOP. The $\lambda^{fl}_{max}$ of the photoadduct is nearly the same as that of 4', 5'-dihydropsoralen.

The proton nuclear magnetic resonance spectra of the photoadduct taken in chloroform-d with a drop of trifluoroacetic acid (Figure 1) do not show the olefinic proton peaks of 4', 5'-furyl double bond which were observed at 6.85 ppm and 7.82 ppm in 8-MOP. This also indicates the loss of the 4',5'-furyl double bond of 8-MOP through a 2+2 photocycloaddition with maleimide. It is therefore clear that the first quantum of near UV light absorbed by the 8-MOP causes the formation of $C_4$-monoadduct between 4',5'-furyl double bond of 8-MOP and 5, 6-double bond of a pyrimidine base in DNA and the second quantum has to be absorbed by a 4',5'-monoadduct rather than a 3, 4-monoadduct to form a

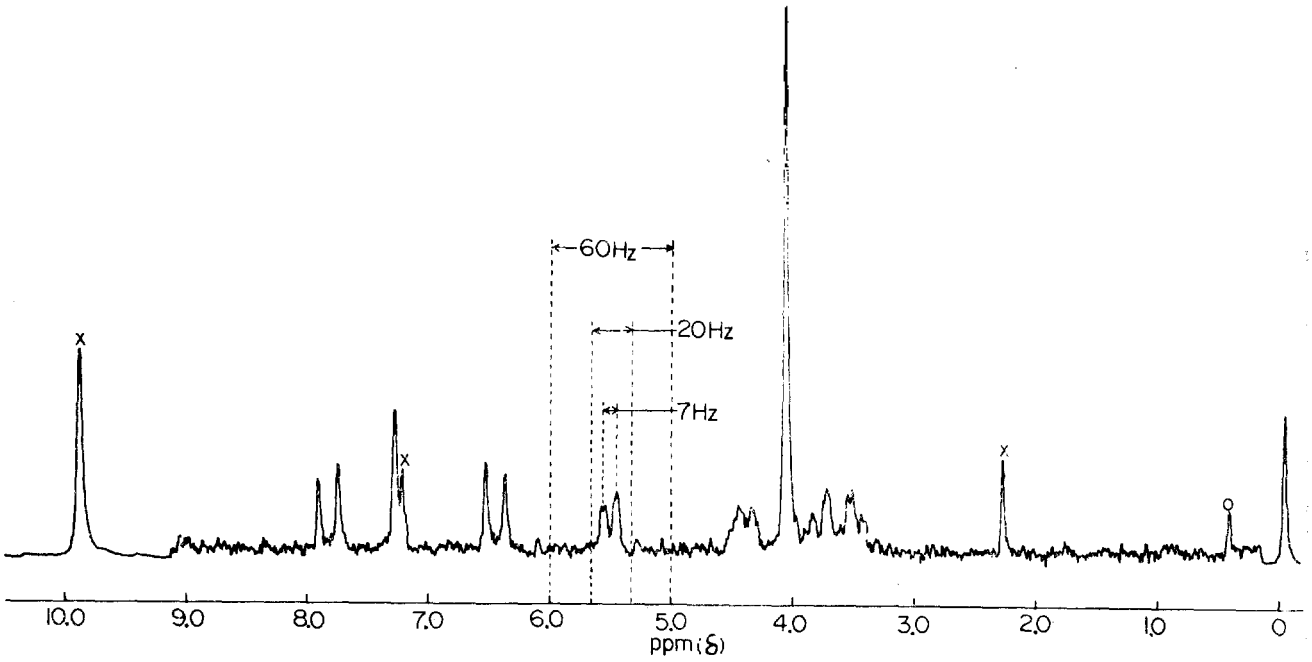
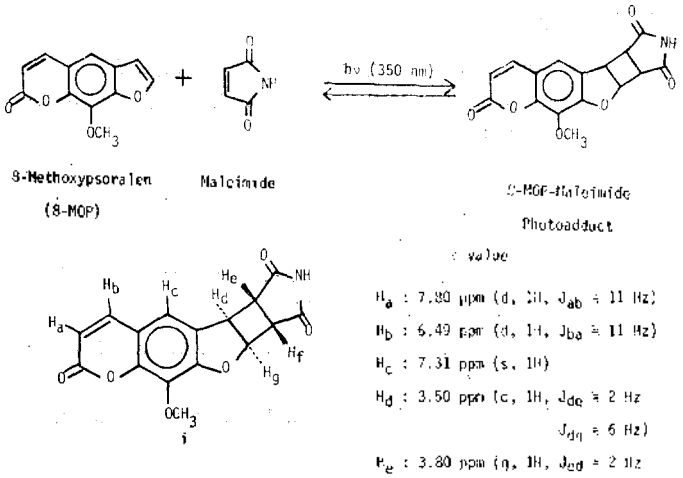



**Figure 1.** $^1$H NMR spectrum of 8-MOP–maleimide photoadduct (chloroform-d/$CF_3COOH$). X: solvent peak o: impurity.

cross-link in DNA. This is in contrast with the higher reactivity of the pyrone double bond of 8-MOP in the triplet excited state.

To distinguish whether the stereochemical configuration of cyclobutane ring in the photoadduct is *syn* or *anti*, coupling constants and chemical shifts of protons in the cyclobutane ring are very important.

It is predicted that if vicinal protons are coupled at *syn* position (dihedral angle $\phi=0°$), the coupling constant is about 8.0 Hz, and about 2.2 Hz at *anti* position ($\phi=109.5°$). These coupling constants are estimated by the relationship between the dihedral angle and vicinal coupling constants, first proposed by Karplus.[25]




New signals between 3.45 ppm and 5.60 ppm are observed in a spectral region typical for cyclobutane ring protons.[26,27] The vicinal coupling constants in the cyclobutane ring of 8-MOP-maleimide photoadduct at *syn* position are observed as 6 Hz (for 4',5'-protons of 8-MOP) and 7 Hz (for maleimide protons), respectively. The vicinal coupling constants at *anti* position are observed as about 2.0 Hz. This coupling constants between vicinal protons explain that the cyclobutane ring of 8-MOP-maleimide photoadduct has *anti* configuration.

*Characterization of DMC-maleimide Photoadduct.* The UV absorption spectra of the photoadduct are nearly superimposable with those of a DMC dimer and of a 1:1 DMC-thymine adduct; thus, the loss of conjugation in DMC-maleimide photoadduct cause a blue shift of the $\lambda_{max}$ from 324 nm to 276 nm. The photoadduct was easily photosplit into DMC and maleimide on 254 nm UV light irradiation for one minute. This suggests that the photoadduct is composed of DMC and maleimide, probably a $C_4$-cycloadduct.

The infrared spectra show a shift of the carbonyl stretching band from 1710 $cm^{-1}$ in DMC to 1750 $cm^{-1}$ in the photoadduct. The same shift is observed in the DMC dimer, $C_4$-cycloadduct of DMC to tetramethylethylene, and $C_4$-cycloadduct of DMC to thymine. The infrared spectral data are consistent with the saturation of 3,4-pyrone double bond of DMC on photolysis. This spectra also show other strong carbonyl stretching band of maleimide at 1710 $cm^{-1}$, indicating the loss of double bond of maleimide in the photoadduct. Some new peaks appear at 3450, 3200 $cm^{-1}$ ($\nu_{NH}$), 1590 $cm^{-1}$ (amide II band) and 1243 $cm^{-1}$ (C-N stretching) in the IR spectra of the photoadduct. A characteristic cyclobutane ring deformation band appeared at 850 $cm^{-1}$ which is not observed in DMC or maleimide spectra. This suggests that the photoadduct is a $C_4$-photocycloaddition product of DMC and maleimide.

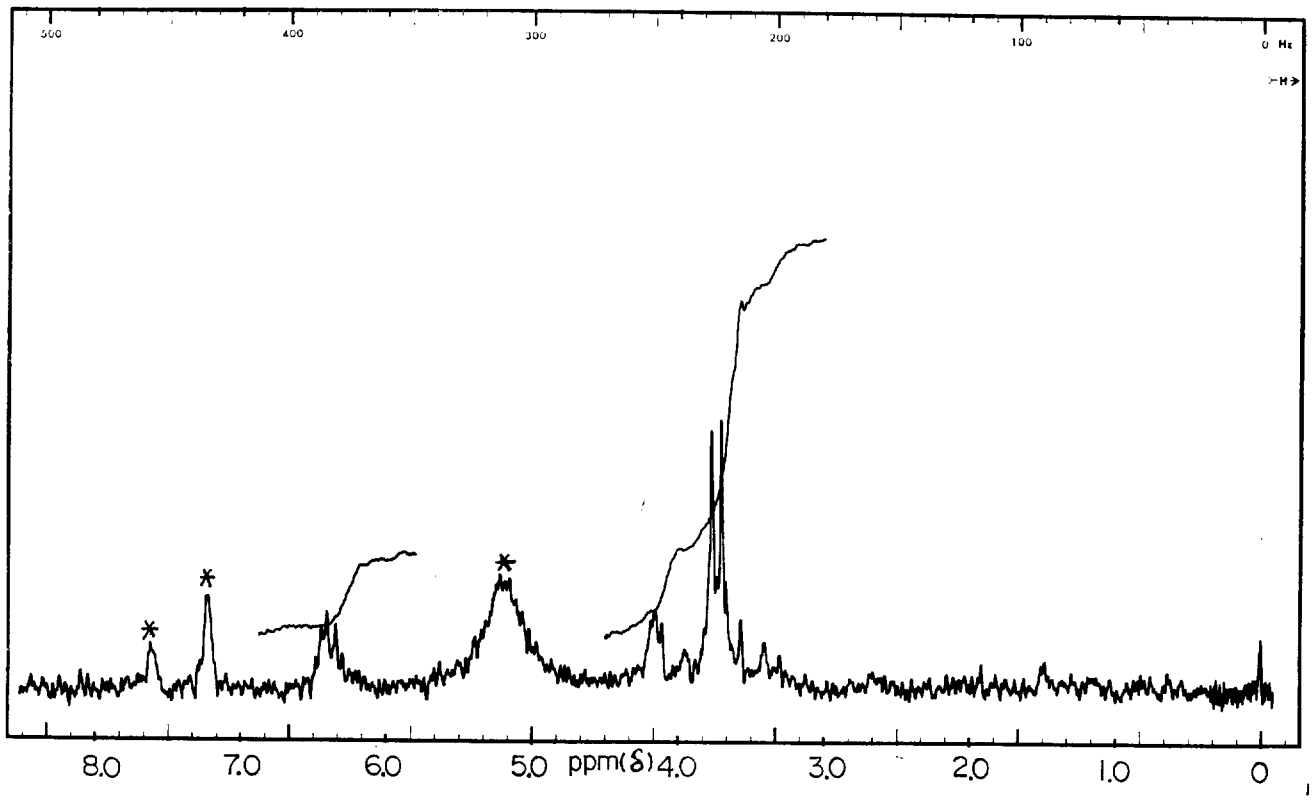
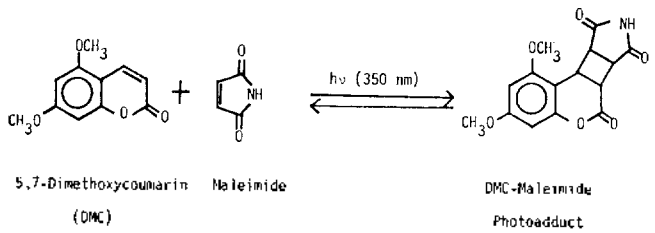



**Figure 2.** $^1$H NMR spectrum of DMC–maleimide photoadduct (pyridine-$d_5$) ×; solvent peak.




The proton nuclear magnetic resonance spectra of the photoadduct taken in pyridine-$d_5$ (Figure 2) do not show the 3, 4-pyrone double bond protons which were observed at 6.09 ppm and 7.90 ppm in DMC. This also indicates the loss of the 3,4-pyrone double bond of DMC through 2+2 photocycloaddition. New signals between 3.30 ppm and 4.16 ppm are observed in a spectral region typical for cyclobutane ring protons. The remainder of the spectra is consistent with the $C_4$-cycloadduct structure of DMC and maleimide.

The results suggest that 8-MOP-mleimide 4,'5'-monoa-ddu ct can undergo $C_4$-photocycloaddition reaction with maleimide on further irradiation with long wavelength UV light

## Conclusion

When 8-MOP and maleimide mixture (molar ratio 1.5: 10) in chloroform is irradiated with 350 nm UV light, a $C_4$-cycloadduct is formed as a major product. The adduct is formed through $2\pi+2\pi$ addition reaction betweenm aleimide C=C double bond and 4', 5'-furyl double bond of 8-MOP instead of expected 3,4-pyrone double bond. DMC and maleimide mixture yielded a $C_4$-photocycloadduct as a major photolysis product through $2\pi+2\pi$ addition reaction between pyrone double bond of DMC and maleimide double bond. The results suggest that 8-MOP initially form 4',5'-cycloadducts with pyrimidine bases in DNA on irradiation with long wavelength UV light and these monoadducts undergo photocycloaddition reaction on the pyrone double bond with another molecule of pyrimidine bases resulting in cross-links in DNA on further irradiation.

*Acknowledgements.* This investigation was supported by PHS Grant Number 5 ROI CA 21729, awarded by the National Cancer Institute, DHHS, U. S. A.

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# Machanism on the Formation of Bis-9, 9'-thioxanthenylmethane from the Reaction of Thioxanthylium Ion with Dimethylmercury(I)

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9-Methylenethioxanthene(6) was synthesized and for the first time good mp and spectral data were taken. Reaction of (6) with thioxanthylium ion (1) in acetonitrile led to a carbenium addition adduct (8) which then was either attacked by a variety of nucleophiles subsequently added or underwent deprotonation reaction to give an olefin (13). From these reactions. was obtained bis-9,9'-thioxanthenylmethane (2). These results indicate clearly that (2) can be formed via (8) by accepting hydride. Isolation of (2) and (6) from the reaction of (1) with 9-methylthioxanthylium ion (18) also supports the involvement of (8) in the reaction of (1) with dimethylmercury. However, addition of thioxanthene radical (4) to (6) has not baen ruled out.

## Introduction

Thioxanthylium ion (1) prepared from the oxidation of thioxanthene by thianthrene cation radical perchlorate has been used to synthesize 9-arylthioxanthene and 9-thioxanthyl -phosphonium salts.[1] The products are believed to be formed by the nucleophilic attack of aromatics bearing an electron donating group and phosphines, respectively.

In contrast, the reactions of (1) with organomercurials were explained by an electron-transfer from organomercurials to (1), followed by a radical combination reaction between thioxanthene radical (4) and alkyl or aryl radical originated from organomercurials.[2] Therefore, from the reaction with diphenyl- and dibenzylmercury under $N_2$ atmosphere were