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Full Length Research Paper

Biotransformation of n – phenyl cyclohexenylimide and 3, 5 – dinitrobenzoic acid

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N - phenyl cyclohexenylimide and 3,5 - dinitrobenzoic acid were subjected to biotransformation on maize fermenting system. These compounds were left for two weeks in the fermenting system and later extracted with a mixture of n - hexane, diethyl ether and ethanol. The TLC of the biotransformed products were carried out. The structures proposed for the isolated biotransformed products were based on FT - IR and NMR spectral data. One of the biotransformed products arose from the reduction of N - phenyl cyclohexenyl imide while decarboxylation of 3,5-dinitrobenzoic acid resulted in 1,3-dinitrobenzene.

Key words: Biotransformation, N – phenyl cyclohexenyl imide, 3,5-dinitrobenzoic acid, fermenting system.

INTRODUCTION

Biotransformation is the process of changing organic compounds into other forms by the use of micro-organisms. A lot of micro-organisms such as bacteria, algae, fungi etc have been used to effect biotransformation of many organic compounds (David et al., 1977). Pasteur in 1858, reported the use of the fungus *Penicillium glaucum* to obtain L -ammonium tartrate from DL - ammonium tartrate by selective destruction of the D - enantiomer. A lot of enzymes are found in micro-organisms which enable them to achieve highly selective metabolic changes in the structure of foreign organic compounds. Another method of maximizing diversity from a single natural product is the use of biological systems in the form of whole cells or isolated enzymes to modify the molecule. The process involves incubation of the isolated natural product with one or several microbial cultures, allowing the enzymes of each organism to act on the compound to produce modified forms (Sarker et al., 2006). The enzymes in these micro-organisms affect different kinds of chemical changes such as epoxidation, reduction, oxidation, dealkylation and hydroxylation. Two things are sought after in biotransformation studies of organic compounds. One is the production of less toxic compound from parent compound, while the other is the production of potentially useful metabolite (Hanson, 1995). The advantages of biotransformation lie in the fact that many organisms are able to carry out numerous site-specific and stereospecific reactions that are very challenging to a chemist.

The disadvantages of biotransformation is that it is not very predictive except where purified enzymes are used. Groups of organisms could be built up that carry out various classes of reaction and the general modifications a given compound undergoes could be known, but such empirical rules are not absolute or predictive (Sarker et al., 2006).

This study was undertaken to subject N – phenyl cyclohexenyl imide and 3,5 - dinitrobenzoic acid to bio-transformation using micro organisms present in fermenting maize, isolate the biotransformed constituents from the solution of the fermenting system and elucidate the structures of the biotransformed components using spectroscopic / analytical techniques. Studies regarding the molecular structure – biological activity relationship of maleimide indicates that their microbial activity could be related to the presence of the double bond in the imidic ring or the nitrogen atom next to the benzene ring. This could lead to electronic interactions between maleimides and microbial cells (Cechinel and Yunes, 1998, Dantas et al, 2000).

Therefore, the significance of the study is to carry out biochemical transformation of the two compounds and identify/isolate new constituents with potential biological properties.

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Lima et al. (1999) found prominent antimicrobial activity in imidic compounds which were effective to inhibit the growth of *Escherichia coli*, *Staphylococcus aureus*, *Candida albicans*, *Microsporum canis* and *Penicillium*. It has also been proven that benzoic acid inhibits the growth of mould and yeast (Warth, 1991).

EXPERIMENTAL

Materials and Reagents

The materials / reagents used in the experiment include fermenting maize, N-phenyl cyclohexenyl imide and 3, 5 - dinitrobenzoic acid. The following solvents were also used in the course of the experiment: n- hexane, ethanol, diethyl ether and dichloromethane which were all redistilled before use.

Collection and processing of samples

Some quantities of maize were purchased at the local market within Ilorin metropolis. The maize was soaked in a plastic container for two days. The fermenting maize grains were washed and grinded at the mill on the third day. 30 g each of this was weighed and put into two different containers and were labeled MZ-1 and MZ-2. The control experiment sample was labeled CMZ. 16 mg each of N - phenyl cyclohexenyl imide (Cpd -1) and 3, 5 -dinitrobenzoic acid (Cpd -2) were weighed and introduced into the fermenting maize system. The system was shaken properly for 1 h to form a homogeneous mixture. The containers were covered and left for two weeks.

Solvent extractions of the samples

The fermenting mixture was extracted using a mixture of diethyl ether, n -hexane and ethanol (1:1:1). The mixture was decanted and filtered. The filtrate was concentrated on a water bath to obtain the crude biotransformed compound (Sarker et al., 2006).

Thin Layer Chromatography (TLC) test

The crude biotransformed extracts were spotted on TLC pre - coated plates (Silica gel G_{F254}, 0.25 mm Merck .W. Germany) (Abdul and Quian, 1986) using the solvent system, n-hexane / diethyl ether (1:1) plus 3 drops of ethanol. They were developed and thereafter, the plates were dried and chromatographic spots were observed under the UV lamp.

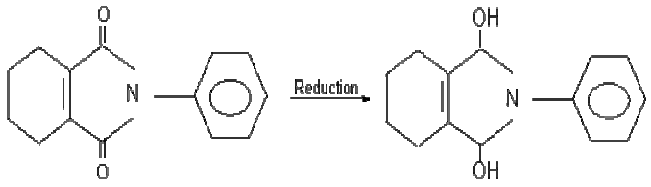
Spectroscopic measurements

IR spectra were recorded as a film on potassium bromide disks and nujol mull using a NICOLET AVERTER 330 FT - IR (Thermal Electron Corporation Spectrum Instrument). Proton and Carbon - 13 NMR spectra were recorded on MERCURY - 200 BB SPECTROMETER, operating at a basic frequency of 200 MHz for ^{1}H and 75 MHz for ^{13}C.

RESULTS AND DISCUSSION

Thin Layer Chromatography (TLC)

The results obtained from the chromatograms of the crude biotransformed extracts are as follows: Cpd-1 and the identified biotransformation product in MZ, showed fluorescence at different wavelengths. The R_f value of the biotransformation product (0.5) was higher than that of Cpd-1 (0.2). It therefore appears that Cpd-1 had undergone biotransformation in MZ. Cpd-1 showed fluorescence at 254 nm while the biotransformation product showed at 366 nm under the UV lamp. Equally interesting is the observation that Cpd-2 and the biotransformation product in MZ were observed at the same R_f (0.2). Furthermore the spot observed for MZ



Equation 1. Biotransformation of Cpd-1.

disappeared in the chromatogram of MZ-2 and instead, a new spot with a much lower R_f (0.3) was observed. Whether this spot arose from the MZ spot or Cpd-2 was unclear.

Infrared spectroscopic analysis

The identity of the functional groups present in the organic compounds and biotransformed products were established using FT-IR spectroscopy. Clearly, the IR revealed that biotransformation affected the carbonyl functional group of Cpd-1 leading to the formation of an hydroxyl group. Comparing the IR spectra of Cpd -1 and MZ -1, it would be seen that there was a strong - OH absorption in the spectrum of MZ -1. This implies that reduction of Cpd -1 had occurred. It could be inferred from the intensity of the - OH absorption that the reduction affected the two carboxyl functional groups. However, in addition to the hydroxyl stretching, the carbonyl stretching frequency was also present in form of a small shoulder at about υ1700 cm^{-1} (Figure 6).

The implication of this is that probably only one of the two carboxyl functional groups had been converted to the hydroxyl functional group. The ambiguity was later resolved after the ^{1}H – NMR was taken. A biosynthetic scheme was proposed for the biotransformation as shown in Equation 1.

There is support for biotransformation of Cpd -2 on MZ because, the -OH stretching was very weak. This indicates that the hydroxyl stretching intensity which was strong in Cpd -2 had been affected in MZ -2. The intensity of the C=O absorption was considerably weaker than was registered for Cpd-2. This makes one to suspect that

Table 1. ^{1}H-NMR spectrum of MZ-1.

Proton number	Chemical shift (δ)	Multiplets
5 H	7.5 - 7.3	m
1 H	5.35 - 5.30	D
2 H	4.8	S
1 H	3.6 - 3.4	S

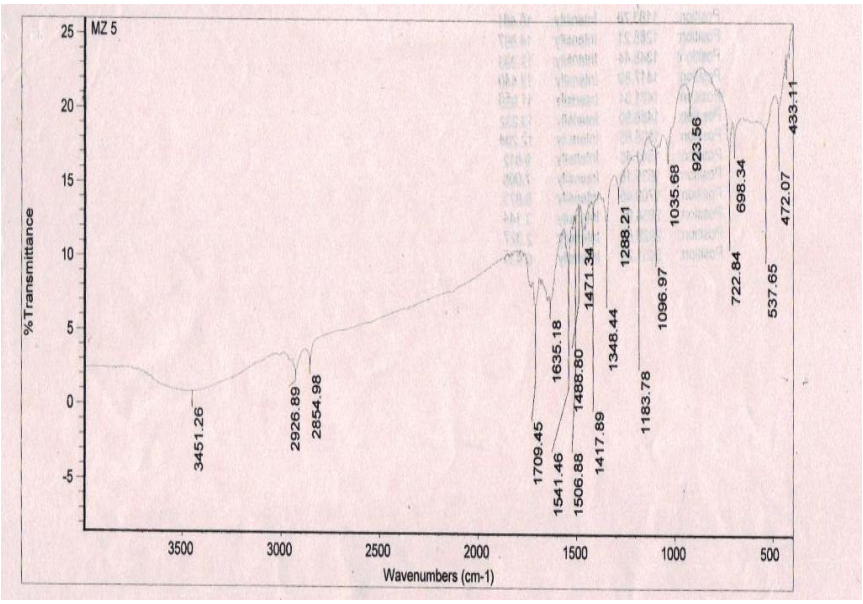


Figure 1. IR Spectrum of MZ – 2.

that Cpd-2 had been decarboxylated in MZ. Such decarboxylation process involving a carboxylic group directly attached to the aromatic ring is uncommon. Decarboxylation is more frequently encountered with β - keto carboxylic acid and amino acids. There is no evidence from spectroscopic point of view that the nitro groups were in any way affected as these absorptions could still be seen in the spectrum of MZ -2 (Figure 1).

Evidence for a 1,3-disubstituted aromatic ring was obtained from the finger print region of the IR spectrum (772 - 694 cm^{-1}). The two absorptions at 722 and 698 cm^{-1} are prominent (Figure 1). The NMR spectral data of MZ-1 (Table 1) that is the biotransformation product of Cpd -1 (N-Phenyl cyclohexenyl imide) lent support to the conclusions arrived at from the IR spectral data.

A multiplet resonance at δ 7.5 - 7.3 ppm confirms the aromatic ring. The doublet at δ 5.35 - 5.30 ppm as well as the quartet of doublet at δ 4.3 - 4.1 ppm were attributed to the compound of the substrate (MZ) which was extracted along with the biotransformed product. The long range interaction of the allylic proton with hydroxyl functional group may be responsible for the chemical shift to δ 4.8 ppm. The allylic protons are registered at δ 2.4 ppm. The diethyl ether solvent signal at δ1.35 - 1.20 ppm and δ 3.8 - 3.6 ppm were recognized. The hydroxyl group in the biotransformed product was represented by the broad singlet registered at δ 3.6 - 3.4 ppm. The methylene protons are represented by the signals at δ 1.8 ppm (Figure 3). The signals ascribed to the main compound extracted from the substrate along with the biotransformed product were reconfirmed by a separate NMR spectrum of a control sample (Figures 4 and 7).

A comparison of the NMR data as stated above with literature (Lie et al., 1995) shows that the compound extracted along with the biotransformation product is a lipid. This is not unusual, since the substrate (MZ) itself contains significant amount of lipid. The ^{13}C - NMR spectra (Figure 5) showed the expected absorptions. The carbonyl absorption which showed at δ 176 ppm was attributed to the carbonyl functional group in the unbiotransformed starting material. This implies that the conversion was incomplete.

The sp^2 hybridized carbon of MZ-1 showed resonance at δ 141 ppm, aromatic/olefinic carbons at δ 129 -126 ppm, sp^3 hybridized carbons at δ 58 - 18 ppm, -CH_2 of aliphatic group at δ29 ppm. The ^{13}C- NMR data of the

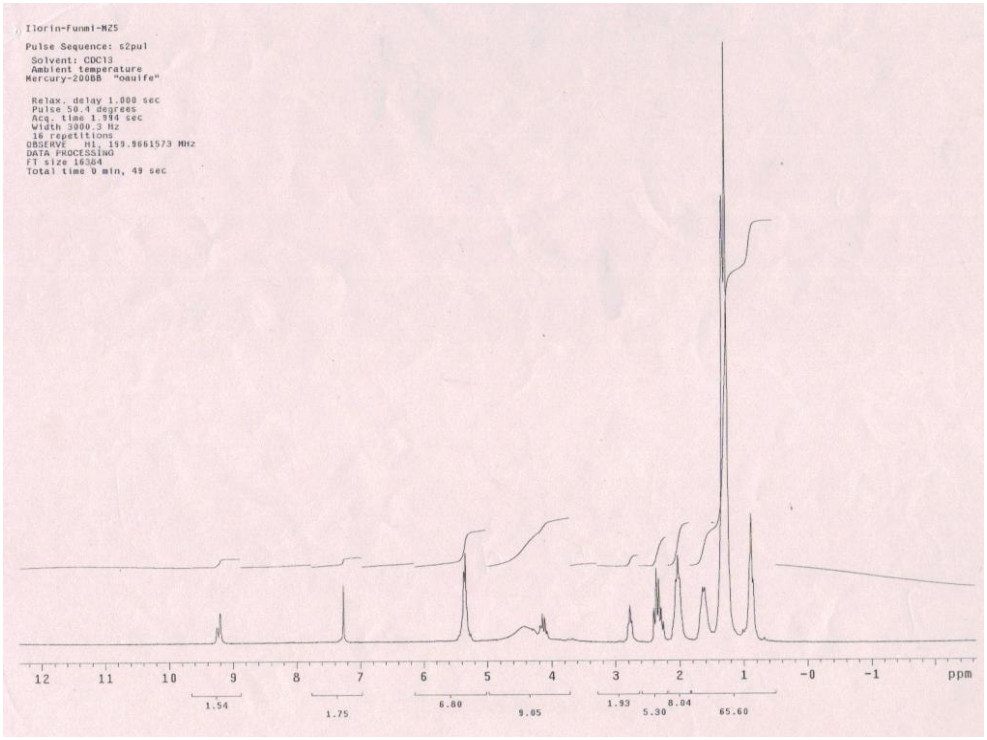


Figure 2. ^{1}H NMR Spectrum of MZ – 2.

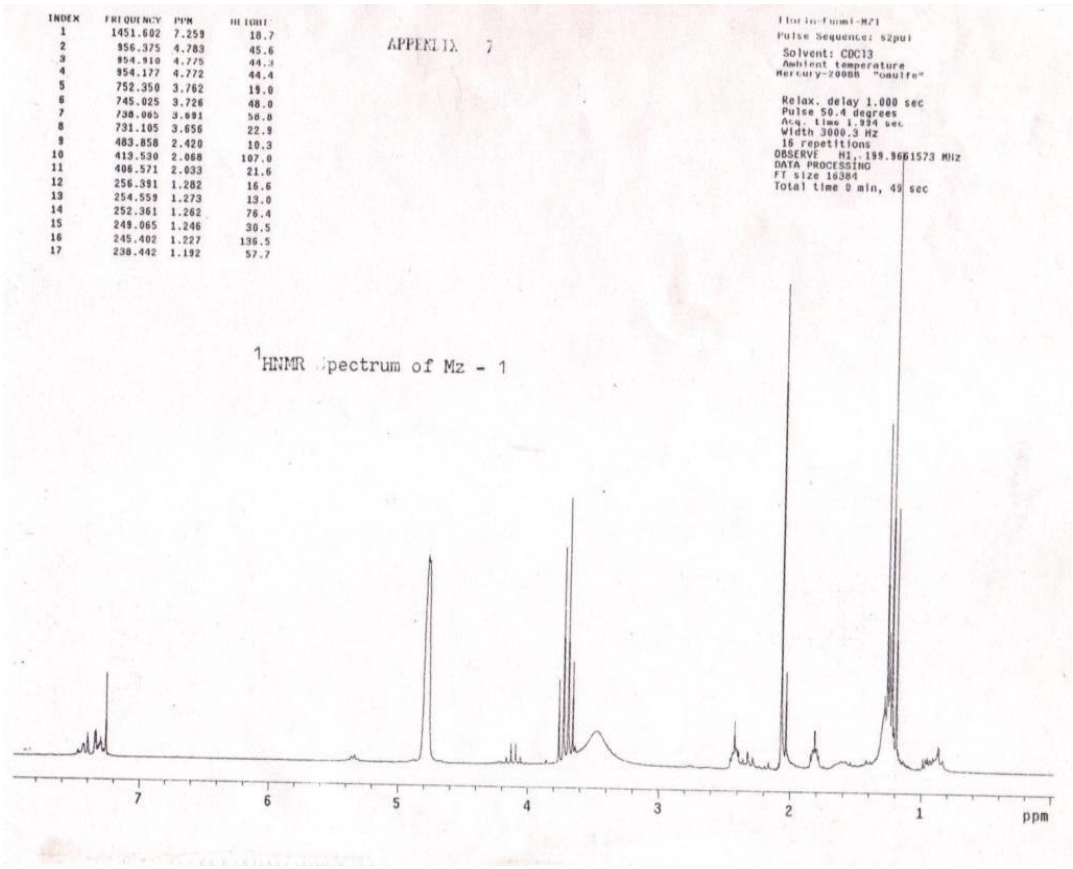


Figure 3. ^{1}H - NMR Spectrum of MZ – 1.

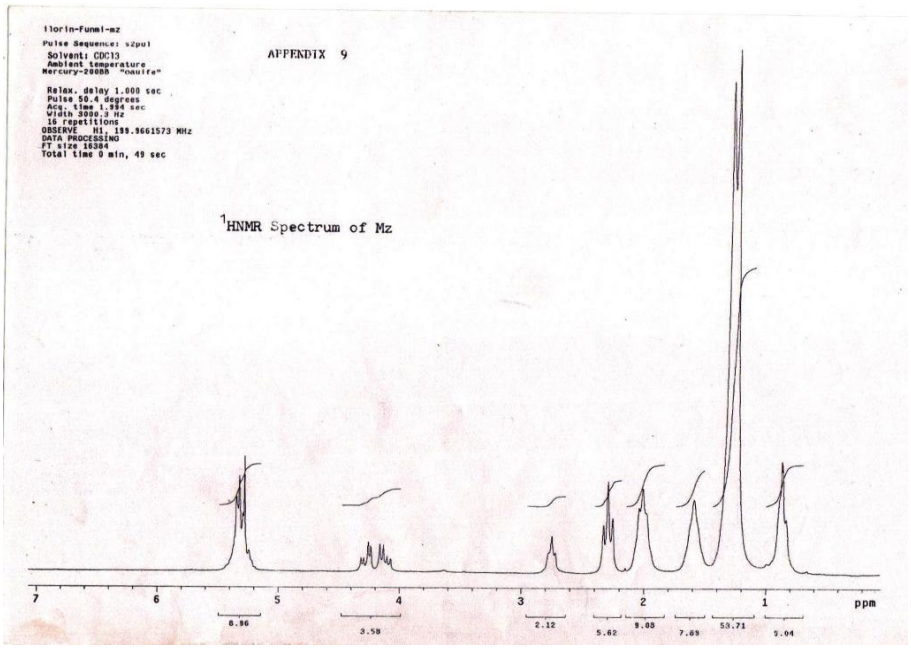


Figure 4. ^{1}H - NMR Spectrum of MZ.

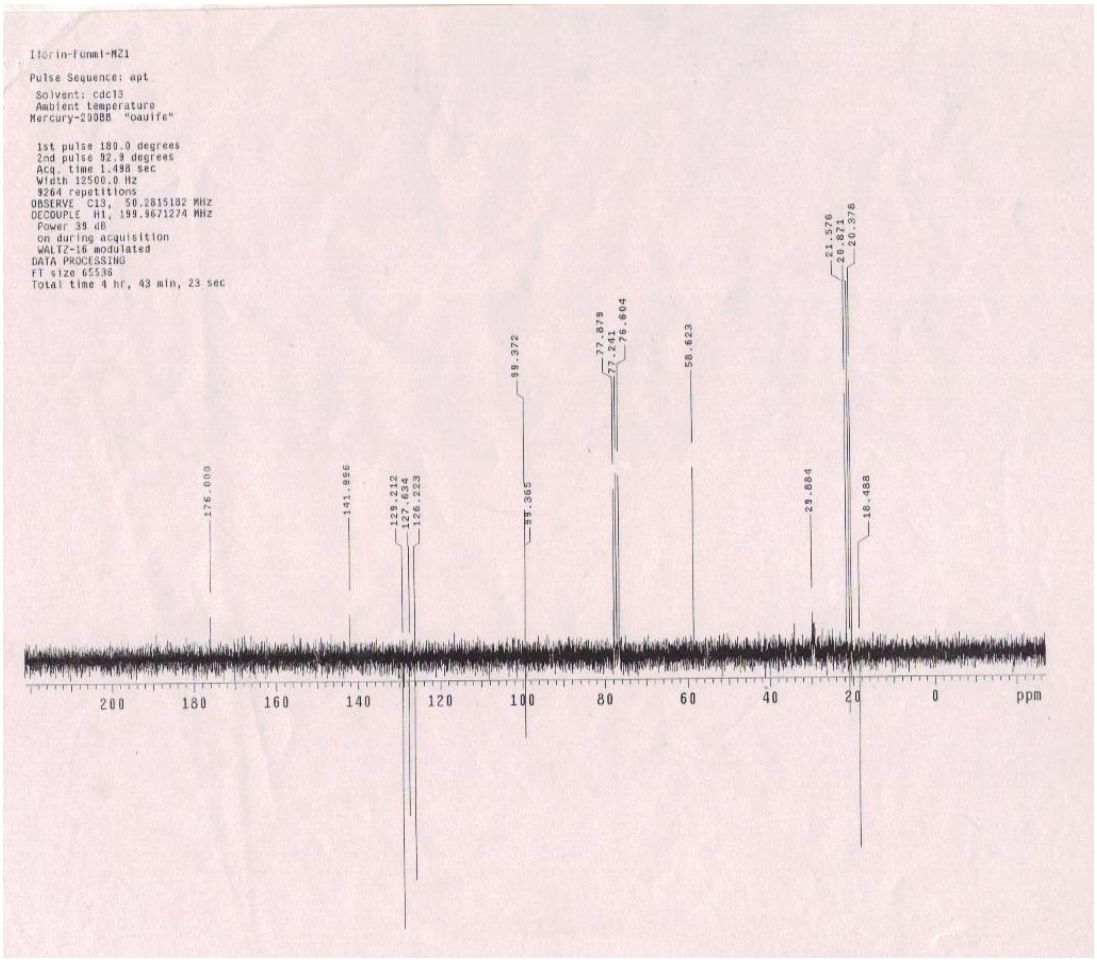


Figure 5. ^{13}C NMR Spectrum of MZ – 1.

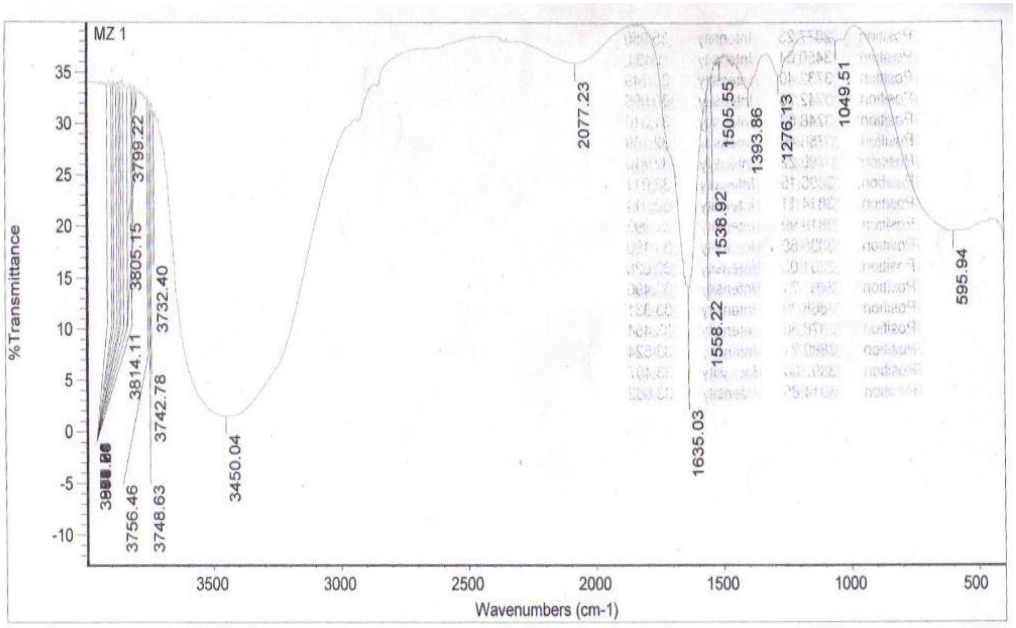


Figure 6. IR Spectrum of MZ – 1.

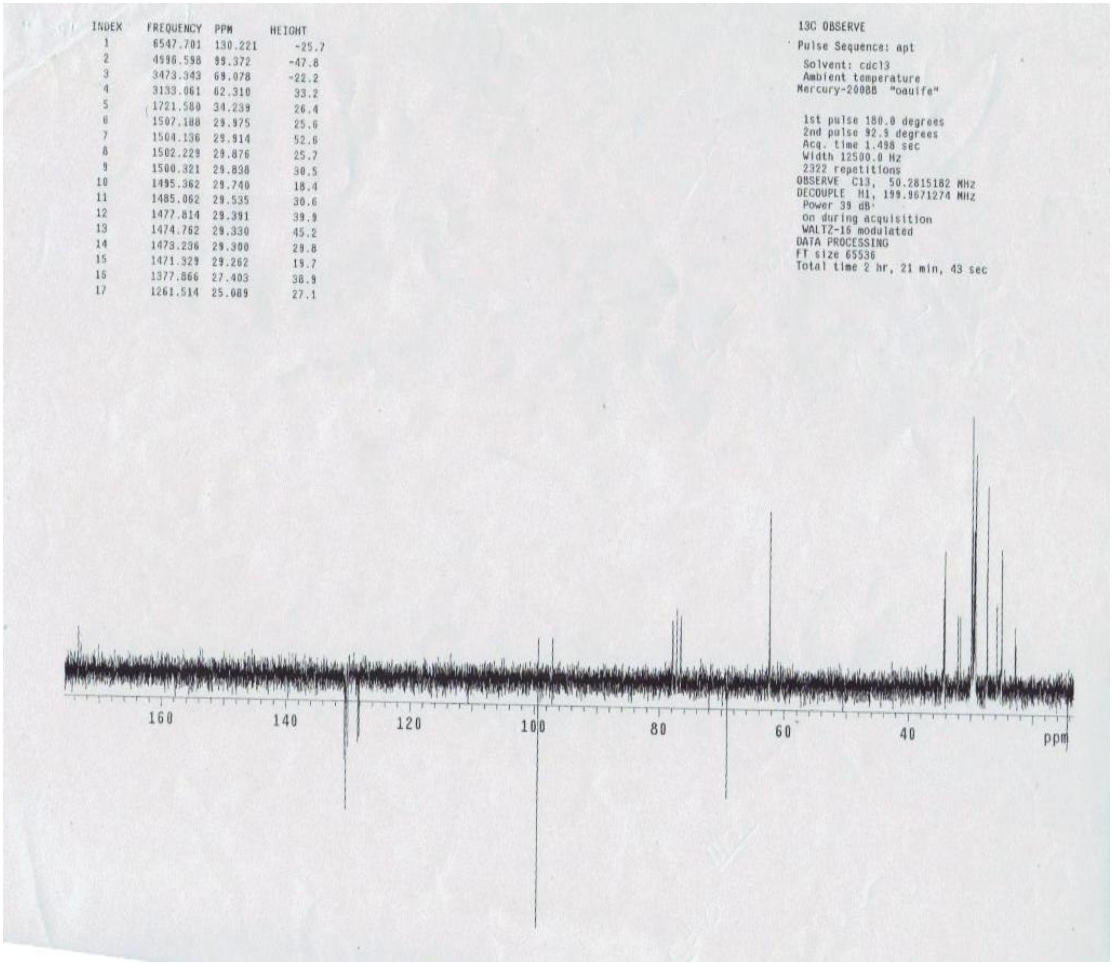
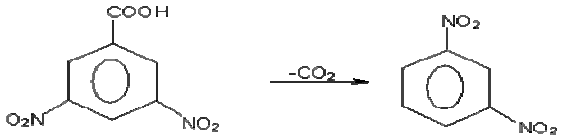


Figure 7. 13C NMR Spectrum of MZ.

substrate, MZ itself is as follows: C=O absorption of ester at δ 173ppm, -CH of olefinic group at δ 129ppm, $-CH_2$ bonded to oxygen at δ 77 ppm and $-CH_2$ of aliphatic group at δ29 ppm.

NMR Spectroscopic analysis of MZ-2.

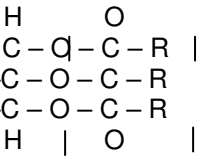
The ^{1}H-NMR of MZ-2 (Figure 2) shows clearly that a major component of the substrate was extracted with the biotransformed compound whose main signal at δ 9.25 ppm is attributed to the aromatic protons. These signals appeared as two small broad singlets. This problem was resolved by extracting the main component of the substrate that is a control sample after two weeks. The ^{1}H – NMR spectrum of this component enabled us to eliminate the signals that belong to the substrate



Equation 2. The formation of 1,3 – dinitrobenzene.

component only.

According to Lie et al. (1995), these signals belong to a glycerol ester of the type:



$R = CH_2 – CH_2 – (CH_2)_{14} – CH_3$

Apparently, the acid alkyl rest are saturated as no signal could be found between δ 5 -6 ppm. The following signals which were observed in the spectrum of MZ-2 (Figure 2) were all attributed to the substrate main compound MZ; δ 5.5 - 5.2, δ 4.20 - 4.05, δ 2.8 - 2.7, δ 2.10 - 1.95, δ 1.4 - 1.2 and δ 1.0 - 0.8 ppm. Having eliminated the signals belonging to MZ in the ^{1}H -NMR of MZ-2, the signal belonging to a 1, 3-disubstituted aromatic remained and these are the signals at δ 9.25 ppm (two broad singlets).

The ^{1}H –NMR signals belonging to the substrate were later confirmed by running a separate NMR for the substrate control sample alone (Figure 4 and 7). The observed signals are documented below: δ 5.4 - 5.2, δ 4.3 - 4.1, δ 2.4 - 2.2, δ 1.7 - 1.5, δ 1.4 - 1.2 and δ 1.0 -0.8 ppm all of which agree with those identified in MZ-5.

Therefore, it is correct to assume decarboxylation resulting in the formation of 1, 3 - dinitrobenzene as shown in Equation 2. Although preparation of 1,3–dinitrobenzene does not present any synthetic problem, the decarboxylation from the aromatic nucleus is not common as encountered for instance in amino acids, α,α dicarboxylic acids or ß - keto esters.

CONCLUSION

N-phenyl cyclohexenyl imide underwent reduction in maize; the two carbonyl groups of the compound were biotransformed into hydroxyl groups. 3,5-dinitrobenzoic acid was decarboxylated in maize to 1,3-dinitro benzene. According to Omemu et al. (2007), the following yeast strains present in fermenting maize; *Saccharomyces cerevisiae, Candida Knisei, Candida tropicalis, Geotrichum candidum, Geotrichum fermentas* and *Rhodotorula graminis* exhibit lipase, esterase and amylase activities. In enzyme classification, lipases hydrolyze lipids (triglycerides). These enzymes are hydrolases which are therefore responsible for the biotransformation of Cpd -1. The proposed structures of the biotransformed products, the identification of the enzymes responsible for the biotransformation and the mechanism of the biotransformations would be the subject of future investigation.

REFERENCES

Abdul H, Quian X (1986). Transformation of Steroids by algae. J. Nat. Products 49: 244-248.

Cechinel FV, Yunes R (1998). Estrategias para a obtencao e compostos meicinals, conceitos nso bre moifica cao Quim Nova 21: 99–105.

Dantas ZMR, Lima EO, Vasconcelos FPA, Cechinel FV (2000). Suspceptibilidade in vitroe especies de Candida a maleimidas naftalimiase succinimidas. Rev. Bras. Farm 81: 31-35.

David PJ, Wiese DR, Rosazza JP (1977). Microbial transformation of natural antitumor in formation of N – (2) – Nor – D tertradine: J. Natural Products 40(3): 239 – 243.

Hanson J (1995). An introduction to biotransformations in Organic Chemistry. Oxford University Press. 2 – 68, ISBN 0-71674-541-0.

Lie K, Jie MSF, Lam CC (1995). ^{1}H Nuclear magnetic resonance spectroscopic studies of saturated, acetylenic and ethyl triacylglycerols. Chem. Phys. Lipids, 77-155.

Lima EO, Quziroz EF, Andricopulo A, Yunes RA, Correa R, Cechinel FV (1999). Evaluation of antifungal activity of N – aryl – maleimides and N – phenyl alkyl – 3,4–dichloro maleimides. Biol. Soc. Chim. Quim. 44: 185-189.

Omemu AM, Oyewole OB, Bankole MO (2007). Significance of yeasts in the fermentation of maize for Ogi production. J. Food Microbiol., 24(6): 571 – 576.

Sarker SD, Zahid L, Alexander IG (2006). Natural Products Isolation. Humana Press. pp. 486 – 488.

Warth AD (1991). Mechanism of action of benzoic acid on *Zygosaccharomyces bailii*: Effects on glycolytic metabolite levels, energy production and intracellular pH. Appl. Environ. Microbiol., 24: 114-117.