BIO-HYDROLYSIS OF 5-(4-ACETOXY-1-BUTINYL)-2, 2^1-BITHIENYL ISOLATED FROM *TAGETES ERECTA*

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ABSTRACT

Four naturally occurring acetylenicthiophenes: 5-(3-buten-1-nyl)-2,2^1-bithienyl; alpha-terthienyl; 5-(4-acetoxy-1-butinyl)-2,2^1-bithienyl; and 5-(4-hydroxy-1-butinyl)-2,2^1-bithienyl, isolated from the root- extract of *Tagetes erecta* (African Marigold) were subjected to bio-hydrolysis and antibacterial activity studies**.** The extraction was achieved through soaking of 250g dried root sample in petroleum ether and diethyl ether (7:3%) solvent system for three days. Separation was carried out using silica gel self-coated glass plates. The thiophenes were identified through colour reactions with vanillin spray reagents and UV spectral characteristics compared with literature. Isolated compounds were separately incorporated into cassava pulp and fermented for two days before analysis. One of the isolated compounds, 5-(4-acetoxy-1-butinyl)-2,2^1-bithienyl, was hydrolysed on fermentation for two days in cassava substrate. The isolated thiophenes showed no antimicrobial inhibition against the tested microorganisms. It is hoped that this study might contribute to wider knowledge and scope of application of natural thiophenes and their biotransformation products.

Key words: Acetylenicthiophenes, bio-hydrolysis, fermentation, microflora, *Tagetes erecta*

INTRODUCTION

Natural acetylenicthiophenes are well known group of natural products among the plant family, *Asteraceae* [1]. Thiophenes have been obtained from several species of *Tagetes*, including *Tagetes erecta*. They equally occur in *Dyssodia* and *Porophyllum* and many other tribes of the *Asteraceae*, thus representing natural products with wide distribution [2]. The biogenesis of natural thiophenes had been traced to natural acetylenes as precursors [3].

These natural thiophenes which are secondary metabolites, possess two to three thiophene rings linked usually at alpha carbons. Isolation, structural studies and applications of natural thiophenes have been widely reported in literature. For example, the phototoxic properties of natural thiophenes toward the larvae of mosquito, *Aedesintrudens*, has been demonstrated [4].

Alpha-terthienyl was found to be exceptionally phototoxic. Also herbicidal efficacy of alpha-terthienyl has been established [5]. Thiophene derivatives are used for the preparation of other products which are of wider applications such as antimicrobial, antiviral (HIV-1) protease inhibitor, insecticidal and anticancer activities [6]. Although, a lot have been reported on the isolation, characterization and applications of natural thiophenes, reports on the biodegradation or biotransformation are rare in literature. However, biotransformation of 5-(4-hydroxyl-1-butinyl)-2, 2^1-bithienyl was reported by Sutfeld and Towers [7].

Because of the broad spectrum of relatively similar compounds, with multiple functionality, found in *Targetes*, this plant seems suitable for studies of microbial transformation. Studies of microbial transformations reported in literature often involved purified strains of microorganisms. It is however known that microorganisms are present as microflora in some food systems where they perform important roles in fermentation. Thus, microflora of fermenting cassava substrate has been selected for the present study.

The microflora responsible for cassava fermentation is of two types: heterolactic and homolactic bacteria [8]. The heterolactic bacteria, *Leuconostocmesenteroides* are predominant during the early stage of cassava fermentation, and these are subsequently replaced by homolactic bacteria, *Lactobacillus plantarum*, due to inability of *Leuconostocmesenteroides* to tolerate an increase in acidity associated with natural cassava fermentation [9].

Thus, the aim of this research was to investigate possible structural modifications of acetylenicthiophenes found in *Tagetes erecta* using micro-organisms. This was borne out of the inherent advantages of employing micro-organisms to effect synthesis hitherto difficult to achieve in normal laboratory synthesis. Some of these advantages include the fact that reactions can often be carried out under the mild conditions of pH and temperature. Also, non-activated postions in a molecule can be functionalised as demonstrated in the microbiological oxidation of steriods [10].

MATERIALS AND METHODS

Materials

The *Tagetes erecta* seeds used were obtained from the University of Ilorin Botanical gardens. Chemicals and solvents used included petroleum ether, diethyl ether, sulphuric acid, methanol,

acetic acid and vanillin. Other materials used were filter paper (whatman, England), silica gel, precoated silica gel (60F254, 0.2MM), Thin Layer Chromatography (TLC) plates, aluminium foil and petri dishes. Other chemicals were obtained from standard suppliers of laboratory chemicals and were of analytical grade.

Extraction

The roots of 70 days old *Tagetes erecta* plants were dried and chopped into small sizes using scissors. Approximately 250.0g of the root was weighed and extracted by soaking in petroleum ether and diethyl ether (1:1%) solvent system. Portions of the sample extract were applied to pre-coated silica gel, 60 F254, 0.2mm TLC plates. The plates were developed in petroleum ether and diethyl ether (7:3) solvent system. Spots were viewed at both 254 and 366nm under an ultraviolet lamp and the spots seen were marked with pencil. The developed thin layer chromatographic plate was then sprayed with freshly prepared vanillin reagent (5g of vanillin in 9mL of methanol, 0.5mL of H_2SO_4 and 3 drops of acetic acid) and was gently heated on a hotplate.

The remaining crude extract was applied to a silica gel self-coated glass plates. After development, various zones on the plates were carefully scraped into separate flasks and diluted with petroleum ether and diethyl ether (1:1%). The isolated components were further purified by repeated TLC. Components were identified by their colour reactions with vanillin and the UV_{max} obtained from UV spectrophotometer [2].

Preparation of cassava substrate

Cassava tubers were peeled by hand using a knife. They were washed clean and grated by using a hand grater**.** About 0.2g each of thefour thiophene derivatives isolated were separately incorporated into cassava pulp and analysed. Samples for analysis were taken daily up to the 7^{th} day. Each sample was eluted with petroleum ether and diethyl (1:1%), concentrated and subjected to TLC analysis. Spots were viewed at both 254 and 366nm under an ultraviolet lamp. Thereafter, the plates were sprayed with freshly prepared vanillin reagent.

pH determination

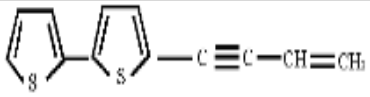
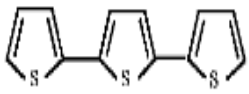
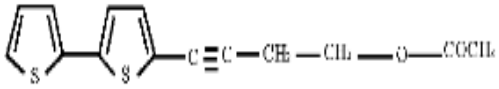
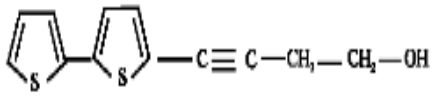
Half of each fermented samples was homogenized in distilled water for 1 min and the pH was determined using a standardized pH meter (Jeneway 3505 pH meter, UK).

RESULTS AND DISCUSSION

Thiophenes analysis and biotransformation study

Four compounds were isolated from the dried root of *Tagetes erecta*. The isolated compounds were defined by their colour reactions with vanillin reagent and UV spectral characteristics. The four components are shown in Table 1.

Table 1: Names and structures of four thiophene derivatives isolated from T*agetes erecta*

Compound	Structure	Name
I		5-(3-buten-1-nyl)-2,2-bithienyl
II		Alpha-terthienyl
III		5-(4-acetoxy-1-butinyl)-2,2'bithienyl
IV		5-(4-hydroxy-1-butinyl)-2,2'-bithienyl

On the7th day of fermentation, the pH of the cassava substrate incorporated with thiophene derivatives dropped to 2.8 from an initial value of 6.6 for all the derivatives (Fig. 1). A similar drop in pH from 6.0 to 2.6 for the control, i.e., the cassava without thiophenes, was also observed during the same period of fermentation. The rapid drop in pH during the first 3 days and the gradual drop thereafter followed the usual pattern observed during cassava fermentation. It therefore implies that thiophenes incorporation into cassava substrates did not affect the fermentation process and that the activities of the fermentation microflara were not inhibited by the thiophenyl compounds.

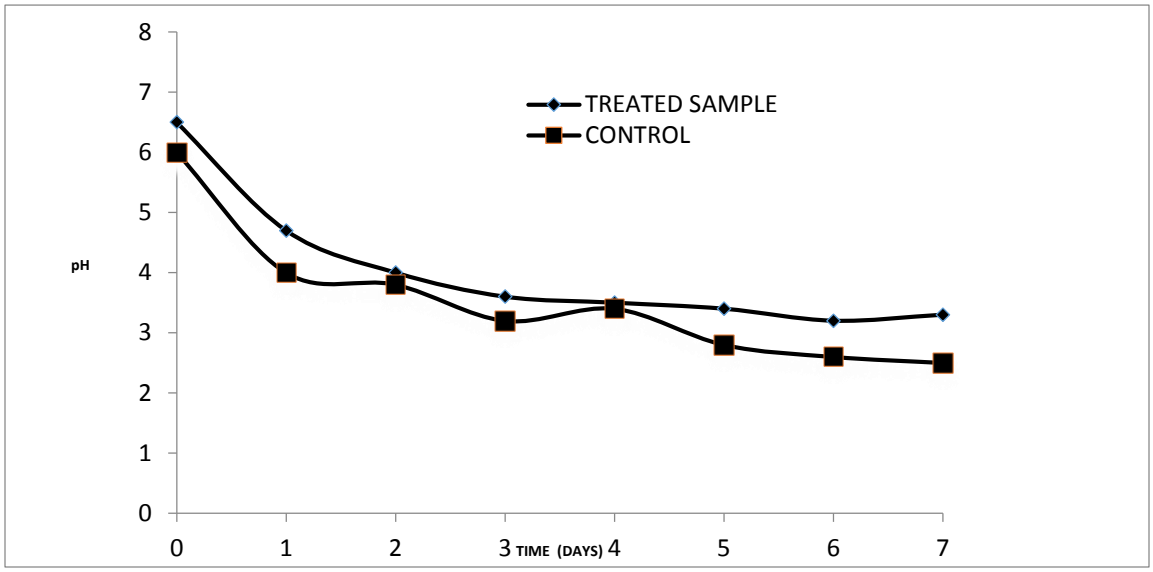


Figure 1: pH changes of the fermented cassava substrate containing thiophenesand the control

Furthermore, the TLC and colour reaction analysis of the cassava substrates incorporated with thiophenyl compounds showed that only compound III was affected by the fermentation process (Table 2).

Table 2: Spectra data of thiophenes isolated from *TagetesPatula*

	Fluorescence in UV light		
	UV max. (nm)		
Compound	254nm	366nm	(Main maximal)
I	Brown	Blue	338
II	Brown	Yellowish	350
III	Brown	Blue	324
IV	Brown	-	334

All the compounds apart from III observed on the chromatogram retained their original characteristic colour with vanillin spray reagent and with respect to fluorescence under the UV lamp (254 and 366nm). Also, their Rf values in petroleum ether and ether (7:3%) solvent system remained the same as previously observed during the TLC analysis of the crude extract. But III

shared colour reaction typical of IV with vanillin spray reagent. Also, the Rf value was typical of IV (Tables 2 and 3).

Table 3: Colour reactions of thiophenes (I-IV) before and after fermentation

Compound	Colour with Vanillin before fermentation	Colour with Vanillin before fermentation	Rf values in Pet-ether:ether (7:3) before fermentation	After fermentation
I	Greenish-blue	Greenish-blue	0.84	0.85
II	Greenish-yellow	Greenish-yellow	0.82	0.83
III	Blue	Purple	0.65	0.34
IV	Purple	Purple	0.33	0.35

CONCLUSION

The microflora of fermented cassava substrate chosen for this experiment was able to transform or degrade 5-(4-acetoxy-1-butinyl)-2,2^1-bithienyl to 5-(4-hydroxy-1-butinyl)-2,2^1-bithienyl out of the four thiophenyl compounds that were investigated after 7 days of fermentation. This may imply that the biotransformation of the remaining natural thiophenes requires definite (stereo) selectivity which perhaps was not available in the medium/substrate chosen. Added to this may be that specific strain rather than microflora brings about rapid biotransformations. Trials with other systems containing specific micro-organism shall for future research work in this srea be worthwhile.

REFERENCES

1. Bohlmaun, F. (1973). Naturally occurring acetylenes. Academic press, inc. London. 155-180
2. Downum, K.R. & Tower, G.H.N. (1983). Analysis of thiophenes in Tagateae (Asteraceae) by HPLC, J. Nat. Prod., 46: 98-103.
3. Jente, R., Olatunji. G. A, & Basold, F. (1981). Formation of Natural thiophene derivatives from acetylenes by *Tagetes patula*. Phytochem. 20(9), 2169-2175.
4. Amason, T. (1986). Phototoxicity of naturally occurring andsynthetic thiophenes and acetylenes analogous to mosquito Larvae:Phytochem., 25, 1609-1611.
5. Lambert, J. D.H., Camohbell, G., Anason, J.T. & Najaki, W. (1991). Herbicidal properties of alpha-terthienyl, a naturally occurring photo toxins. Can. J. Plant Sci., 71, 215-218.
6. Ibrahim, S. R.M., Abdallah, H.M., El-Halawany, A.M. & Mohamad, G. A. (2016). Naturally

occurringthiophene: Isolation, purification, structural elucidation, and evaluation of bioactivities. Phytochem Rev., 15,197-220.

7. Sutfeld, R. & Towers, G.H.N. (1982). 5-(4-acetoxy-1-butinyl)-2,2[1]-bithiophene; acetate asterase from Tagetes Patula. Phytochem. 21, 227-279.
8. Girand, E., Gosselin, L. & Rainbault, N. (1993). Production of a *Lactobacillus plantarum* starter with Linamase and amylase activities for cassava fermentation; J. Sci. Food Agric., 62, 77-82.
9. Oyewole, O.B. & Odunfa, S.A. (1990). Characterization and distribution of lactic acid bacteria in cassava fermentation during fufu production; J. Applied Bacteriol., 68, 145-152.
10. Peterson, D.H. &Nurray, H.C. (1952). Microbiological oxidation of steroids at carbon-11; J. Amer. Chem. Soc. 1870-1871.