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Biotransformation of Acetylenic Thiophenes Isolated from *Tagetes patula* (African Marigold)

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Abstract: The biotransformation of four naturally occurring acetylenic thiophenes; 5-(3-buten-l-nyl)-2, 2[1]-bithienyl (I), alpha-terthienyl (II); 5-(4-acetoxy-1-butinyl)-2, 2[1]-bithienyl (III); and 5-(4-hydroxy-1-butinyl)-2, 2[1]-bithienyl (IV) isolated from the root of *Tagetes Patula nana* (African marigold) by the microflora in fermenting cassava substrate was investigated. One of the thiophenes, 5-(4-acetoxy-1-butinyl)-2, 2[1]-bithienyl (III) was successfully hydrolised after 7 days of fermentation.

Key words: Biotransformation, tagetes patula, acetylenic thiopheness, microflora, fermentation, cassava root

INTRODUCTION

Natural acetylenic thiophenes are well known group of natural products among the Asteraceae (Bohlmaun, 1973). Thiophenes have been obtained from several species of Tagetes among which is Tagetes Patula (African marigold). They equally occur in Dyssodia and porophyllum (Downum and Tower, 1983).

These natural thiophenes which are secondary metabolites possess one to three thiophene rings linked usually at the alpha carbons. Isolation, structural studies and applications of natural thiophenes toward the lavae of mosquito had been demostrated (Arnason, 1986). Alpha-Tertheinyl was found to be exceptionally phototoxic. Also herbicidal efficacy of alpha-terthenyl has been established (Lambert *et al*., 1991). Thiophene derivatives are used for preparation of other products which are of wider applications.

Although, a lot have been reported on the isolation, characterization and applications of natural thiophenes, not much has been reported on the biodegradation or transformation. However, biotransformation of 5-(4-acetoxy-1-butinyl)-2, 2[1]-bithienyl by a purified enzyme preparation from tagetes patula to 5-(4-hydroxyl-1-butinyl)-2, 2[1]-bithienyl have been reported (Sutfeld and Towers, 1982).

Because of the broad spectrum of relatively similar compounds with multiple functionality found in the Tagetes, this plant seems suitable for studies of microbiological transformation. With this objective in view, the present study on the biotransformation of thiophenes indigenous to Tagetes Patula was undertaken.

Studies of microbiological transformations reported in the literature employed purified strains of micro-organisms. It is however known, that micro-organisms are present as microflora in some food systems. Thus, microflora of fermenting cassava substrate have been selected for the present study.

Possible products of the biotransformation of thiophenes isolated from Tagetes Patula.

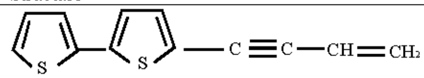
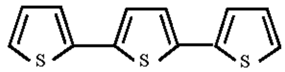
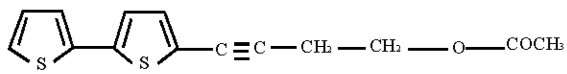
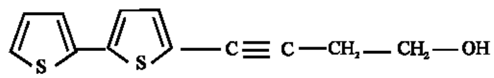
The microflora responsible for cassava fermentation are of two types; heterolactic and homolactic bacteria (Girand *et al*., 1993). The heterolactic bacteria-Leuconostic mesenteroides are predominant during the early stage of cassava fermentation and these are subsequently replaced by homolactic bacteria-Lactobacillus plantarums, due to the inability of leuconostic mesenteroids to tolerate an increase in acidity associated with natural cassava fermentation (Oyewole and Odunfa, 1990). In this research, we investigated the ability of these microflora to transform or degrade natural thiophenes indigenous to Tagete Patula. It is hoped that this study might contribute to wider knowledge and scope of application of natural thiophenes and their biotransformation products.

MATERIALS AND METHODS

The Tagetes Patula seeds used where obtained locally, Chemicals and solvents used include 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) TLC plates, aluminium foil and petri dishes. All other chemicals were obtained from standard suppliers of laboratory chemicals and were of analytical grade.

Extraction: The roots of 70 days old Tagetes patula were dried and chopped into small sizes using scissors 250.0 g

Table 1: Structures and names of four thiophene derivatives isolated from Tagetes Patula

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

of the root was weighed and extracted by soaking in petroleum ether and diethyl ether (1:1$^{v}/_{v}$) and allowed to stand for three days. The extract was then concentrated using rotary evaporator.

Thin layer chromatographic analysis: Portions of the sample extract were applied to precoated silica gel, 60F254, 0.2mm thin layer chromatographic (tlc) plates. The plates were developed in petroleum ether and diethyl ether (7:3 $^{v}/_{v}$) 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 (5 g of vanillin in 9 mL of methanol, 0.5 mL of H_2SO_4 and 3 drops of acetic acid) and was gently heated on a hot plate.

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 eluted with petroleum ether and diethyl ether (1:1$^{v}/_{v}$). The isolated components were further purified by repeated PTLC. Components were identified by their colour reactions with vanillin and the UV max. obtained from UV spectrophotometer.

Preparation of cassava substrate: Cassava tubers were peeled by hand using a knife. They were washed clean and grated by using a hard grater. 0.2 g each of four thiophene derivatives isolated were separately incorporated into cassava pulp and analysed.

Sample for analysis were taken after 24, 72 h and so on. Each sample was eluted with petroleum ether and diethyl ether (1:1$^{v}/_{v}$), concentrated and subjected to Tlc using precoated silica gel 60 F254, 0.2 mm Tlc plates. Spots were viewed at both 254 and 366nm under an ultraviolet lamp. Thereafter, the plates were sprayed with freshly prepared vanillin reagent.

Table 2: Spectra data of thiophenes isolated from Tagetes Patula

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

pH determination: For 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.

RESULTS AND DISCUSSION

Thiophenes analysis and biotransformation study: Four compounds were isolated from the dried root of Tagetes Patula. The isolated compounds were identified by their colour reactions with vanillin reagent and uv spectral characteristics. The 4 compounds identified are shown in Table 1.

On the 7th day of fermentation, the PH of the cassava substrate incorporated with the thiophene derivatives dropped to 2.8 from an initial PH value of 6.6 for all the derivatives. A similar drop in PH from 6.0-2.6 for the control, i.e., the cassava without thiophenes was also observed after the same period of fermentation. The rapid drop in PH during the first 3 days and the gradual drop thereafter follow the usual pattern observed during cassava fermentation. It therefore implies that thiophenes incorporation into the cassava substrates did not affect the fermentation process and that the activities of the fermentation microflora were not inhibited by the thiophenyl compounds.

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

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

Compound	Colour with vanillin before fermentation	Colour with vanillin after fermentation	Rf values in pet-ether:ether (7.3) befor 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

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 366 nm). Also, their Rf values in petroleum ether and ether ($7:3^{v}/_{v}$) solvent system remained the same as previously observed during the tlc analysis of the crude extract. But cpd III shared colour reaction typical of IV, with vanillin spray reagent. Also, the Rf value was typical of cpd IV (Table 2 and 3).

CONCLUSION

The aim of this research was to investigate possible structural modifications of acetylenic thiophenes (I-IV) found in Tagetes patula using micro-organism. This was born out of the inherent advantages in employing micro-organisms to effect synthesis hitherto difficult to achieve in normal laboratory synthesis. Some of these advantages include the fact hat, reactions can often be carried out under mild conditions of PH and temperature and non-activated positions in a molecule can be functionlized as demonstrated in the microbiological oxidation of stereoids at carbon (Peterson and Nurry, 1952).

The microflora of fermented cassava substrate chosen for this experiment was able to transform or degrade compound III, i.e. 5-(4-acetoxy-1-butinyl)-2, 2^{1}-bithienyl to compound IV, 5-(4-hydroxy-1-butinyl)-2,2^{1}-bithienyl out of the four thiophnyl compounds that were investigated after 7 days of fermentation. This may imply that the biotransfirmation 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 micro flora brings about rapid biotransformation. Trials with other systems containing specific micro-organism shall for future research work in this area be worthwhile.

REFERENCES

Arnason, T., 1986. Phototoxicity of naturally occurring and synthetic thiophenes and acetylenes analogous to mosquito Larvae; Phytochemistry, 25: 1609-1611.

Bohlmaun, F., 1973. Naturally occurring acetylenes. Academic Press, Inc. London.

Downum, K.R. and G.H.N. Tower, 1983. Analysis of thiophenes in the Tageteae (Asteraceae) by HPLC, J. Nat. Prod., 46: 98-103.

Girand, E., L. Gosselin and N. Rainbault, 1993. Production of a Lactobacillus plantarum starter with Linamase and amylase activities for cassava fermentation; J. Sci. Food Agric., 62: 77-82.

Lambert, J.D.H., G. Camphbell, J.T. Anason and W. Najaki, 1991. Herbicidal properties of alpha-terthienyl, a naturally occurring phototoxins. Gan. J. Plant Sci., 71: 215-218.

Oyewole, O.B. and S.A. Odunfa, 1990. characterization and distribution of lactic acid bacteria in cassava fermentation during fufu production; J. Applied Bacteriol., 68: 145-152.

Peterson, D.H. and H.C. Nurray, 1952. Microbiological oxidation of steroids at carbon-11; Journal of American Chemistry Society.

Sutfeld, R. and G.H.N. Towers, 1982. 5-C4-acetoxy-1-butinyl)-2,21-bithiophene; acetate asterase from Tagetes Patula. Phytochemistry, 21: 277-279.