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ULTRAVIOLET-MEDIATED ANTIBIOTIC ACTIVITY OF THIOPHENE COMPOUNDS OF *TAGETES*

G. F. Q. CHAN,* G. H. NEIL TOWERS† and J. C. MITCHELL‡

*Faculty of Pharmaceutical Sciences, †Department of Botany, and ‡Division of Dermatology, The University of British Columbia, Vancouver, Canada

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Key Word Index—*Tagetes patula*; Compositae; phototoxicity; thiophenes; 5-(3-buten-1-ynyl)-2,2′-bithienyl; α-terthienyl.

Abstract—Two intensely mauve UV fluorescent compounds isolated from *Tagetes* root were found to be phototoxic to *Candida albicans*. By chromatography on alumina followed by gel filtration on Sephadex LH-20, the compounds were identified as 5-(3-buten-1-ynyl)-2,2′-bithienyl and α-terthienyl.

INTRODUCTION

Linearly annulated furanocoumarins, psoralens, which are mainly found in plants belonging to the Umbelliferae, Rutaceae, Moraceae and Leguminosae can elicit a phototoxic reaction and melanin formation when the skin is irradiated with long UV light (320–360 nm) [1,2].

Achillea millefolium, a member of the Compositae, was reported [3] to evoke photodermatitis but the evidence is inconclusive. Daniels [4] in a microbiological assay of plant materials for phototoxicity, reported that the achene of marigold, also of the Compositae, produced phototoxicity, viz. a clear zone of inhibition of growth of *Candida albicans* after long UV irradiation. As furanocoumarins have not been reported from plants of the Compositae, we undertook a chemical investigation of the compound(s) responsible for phototoxicity in marigold (*Tagetes patula*). Recently, we have observed that a number of other species in the Compositae are phototoxic to *Candida* [5].

Thiophene derivatives have been isolated from species of *Tagetes* including *T. erecta*, *T. minuta* and *T. patula*. From the petals of *T. erecta* an intense blue-fluorescing compound was isolated and identified as α-terthienyl [6]. Subsequently *Tagetes* root was found to be a superior source of α-terthienyl [7]. This compound possesses nematocidal properties, as does a second compound isolated from *Tagetes* root, namely 5-(3-buten-1-ynyl)-2,2′- bithienyl [8]. Since then, many other thiophene derivatives have been isolated from this genus [9,10].

We have now isolated two intensely mauve fluorescent compounds with phototoxic activity from *Tagetes patula* root and identified them as 5-(3-buten-1-ynyl)-2,2′-bithienyl and α-terthienyl.

RESULTS AND DISCUSSION

Chromatography of the marigold root extract on alumina produced 13 bands on the column, four of which were fluorescent. After extrusion of the alumina, three bright mauve fluorescent bands were eluted and each subjected to LH-20 chromatography. Assay for phototoxicity was carried out with LH-20 fractions. One of the fractions yielded a pale yellow oil identified as 5-(3-buten-1-ynyl)-2,2′-bithienyl, identified by UV, IR, NMR and mass spectrum [7,9,10]. A second fraction yielded approximately 45 mg of a pale yellow ppt, which was recrystallized from $CHCl_3$–MeOH as long yellow needles and identified as α-terthienyl by mp, UV, IR, NMR and mass spectrum [7,10].

It is not known whether these thiophene compounds, which are phototoxic towards *C. albicans*, also exhibit phototoxicity for human skin.

At present, a study with α-terthienyl regarding this aspect is underway. *Tagetes minuta*, a common weed in subtropical America and south eastern Africa, is a source of irritant as well as allergic contact dermatitis [11]. Photoaddition of psoralens with the pyrimidines of DNA to form a cyclobutane derivative is responsible for epidermal cell damage observed in cutaneous phototoxic reactions [12]. It is not known whether the thiophene ring of our compounds undergoes a similar reaction with DNA of *C. albicans* to yield a lethal photoadduct or whether the mode of action differs.

EXPERIMENTAL

Extraction of plant material. 560 g of clean *Tagetes patula* roots were cut up and extracted 4× in a Waring blender with a total of 8 l. of hot EtOH. After removal of solid material, the ethanolic extract was evaporated *in vacuo* and residue dissolved in 2 l. dry Et_2O. The dried Et_2O soln. was evaporated to dryness. A total of 500 ml of petrol (60–80°) was added in several stages to the brown residue until extracts yielded no further fluorescence on addition of petrol. The combined petrol soln was used for column chromatography.

Isolation of fluorescent compounds. The petrol soln of the plant extract was applied to a 2 × 20 cm column containing previously activated alumina with 10% Celite in petrol (60–80°). The column was developed with 50 ml petrol. Of the 13 bands observed on the column, 4 were fluorescent in long UV light: (1) a 1·5 cm mauve band 2·1 cm from the top of the column, (2) a 1·0 cm bright mauve band 6·3 cm down the column, (3) a 4·5 cm mauve fluorescent band adjacent to the 1·0 cm band, and followed by (4) another 4·5 cm bright mauve fluorescent band.

After extrusion of the column, the 1 cm bright mauve band was no longer discernible as a separate band from the adjacent 4·5 cm mauve band, and was thereafter treated as one section from the column. The three sectioned fluorescent zones were eluted exhaustively with dry Et_2O. Each Et_2O soln was evaporated *in vacuo*.

The Et_2O eluate of the bottom 4·5 cm bright mauve band yielded a golden oil, which was taken up in 5 ml MeOH and applied to a 2·5 × 32 cm Sephadex LH-20 column in MeOH. The column was eluted with 450 ml MeOH and 5 ml fractions were collected. Measurement of absorption of each fraction at 350 nm and fluorescence under long UV light were used to monitor column eluates. Pooled fractions of each of the 3 peaks and a trailing blue fluorescence were separately evaporated *in vacuo*.

The Et_2O eluate of the combined 1 cm bright mauve and 4·5 cm mauve band from the sectioned alumina column was chromatographed in a similar manner on LH-20. In this case, gel filtration yielded mainly 2 peaks plus some trailing blue fluorescence.

Candida albicans (UBC 54) assay [4]. Several small wells were cut in the Sabouraud dextrose agar plates equal in number to the tests to be carried out. The wells were spaced uniformly around the plates so that zones of inhibition would not overlap. Approximately 3 ml suspension of *C. albicans* was prepared from a 24 hr slant. The plates were each streaked with sterile cotton swabs in one direction and cross-wise in the other direction. About 1 μl of each sample solution was added to two separate wells on one plate. A control of the solvent itself was also run. Test plates were incubated at room temp. for at least 24 hr under illumination provided by 2 Blacklite F 15T8-BL fluorescent tubes (320–390 nm). Each assay was run on duplicate plates also, along with a control plate, which was placed under the fluorescent lamps but covered with dark plastic to remove UV radiation. A clear zone of inhibition of growth around the sample wells after UV irradiation indicated phototoxicity.

5-(3-buten-1-ynyl)-2,2'-bithienyl. λ_{max} (MeOH) 254, 346 nm, γ_{max} (thin film) cm^{-1}: 2205 (–C≡C–), 1602, 973 (–C=C–), 848 (2-thienyl). NMR (60 Hz, $CDCl_3$, TMS): δ 7·09 (5H, *m*, bithienyl), 5·80 (3H, *m*, C=C), M^+ 216.

α-terthienyl. Mp (uncorr.) 91–92° (lit. 92–93° [7]), λ_{max} (MeOH) 253, 353 nm, γ_{max} (KBr) cm^{-1}: 840 (2-thienyl), 806 (thiophene-2,5-diyl), 698 (2-thienyl). NMR δ 7·10 (*m*, bithienyl), M^+ 248.

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