Phytochemistry, Vol. 22, No. 2, pp. 594–595, 1983.
Printed in Great Britain.

0031-9422/83/020594-02$03.00/0

PHOTOTOXIC SUBSTANCES FROM *FLAVERIA TRINERVIS AND SIMIRA SALVADORENSIS*

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(*Received* 28 *June* 1982)

Key Word Index—*Flaveria trinervis*; Compositae; *Simira salvadorensis*; Rubiaceae; phototoxins; α-terthienyl; harman.

Abstract—Phototoxic secondary metabolites are reported from *Flaveria trinervis* and *Simira salvadorensis*. A sensitive test for phototoxicity to *Saccharomyces cereviceae* was used to identify activity in plant material, crude extracts and purified compounds. This method led to the isolation and identification of α-terthienyl from tops of *F. trinervis* and harman from bark of *S. salvadorensis*.

INTRODUCTION

Although photosensitizing secondary substances have been known as agents of photodermatitis in humans and range animals, it was not until recently that their role as protective agents in plants has been suggested. The phototoxic activity of certain secondary substances to insects [1], fungi [2] and even other plants [3] suggests that light-mediated toxicity confers certain evolutionary advantages on the plants which possess them. In addition to realizing the importance of light in plant–pest relations, the number of known photosensitizers has been greatly increased by the identification of polyacetylenes [4], furanoquinoline alkaloids [5] and β-carboline alkaloids [6] as phototoxic substances. The photosensitizing properties of these compounds was perhaps overlooked in the past because many of them absorb at wavelengths in the near-UV range of sunlight and are colorless. Another reason for overlooking these compounds has been the lack of a sensitive screening method. An efficient method devised by Daniels [7], has now been successfully used to identify phototoxic members of the Asteraceae [4], and was more recently used to screen selected plants of several families collected in Belize and Venezuela. [Arnason, T., Reyes, I., Lambert, J. D. H. and Towers, G. H. N., unpublished results]. Two strongly phototoxic plants, *Simira salvadorensis* and *Flaveria trivernis* were identified in these trials. The present study reports the isolation and identification of the phototoxic substances of these plants.

RESULTS AND DISCUSSION

Simira salvadorensis is identified by local Maya farmers in Belize because a slash mark on the white bark turns red a few minutes after injury. Ethanol extracts of the bark are a brilliant fluorescent red, which suggested that an extended quinone, such as the red fluorescent and highly phototoxic hypericin of *Hypericum* spp., might be present. The extract was, in fact, highly phototoxic to yeast (Table 1), but the assumption that the major phototoxic material is an extended quinone proved to be erroneous as chromatography of the extract, on Si gel, yielded a colorless compound with bright blue fluorescence and a higher MW non-fluorescent red compound. The former was phototoxic, but the latter was inactive as were the other major fractions. The phototoxic compound was identified as harman and its phototoxicity (Table 1) was comparable to that reported previously [6].

Flaveria trinervis was among the most phototoxic plants (Table 1) identified in the Venezuelan survey. A polyacetylene was suspected as the phototoxic agent and in the extraction procedure, the petrol fraction was found to be phototoxic while the plant residue and aqueous phase were inactive. Chromatography on Si gel of the non-polar fraction yielded a blue fluorescent compound, which was found to be highly phototoxic (Table 1), and was subsequently identified as α-terthienyl. The isolated compound was comparable in its activity to authentic α-terthienyl [4].

Harman has been previously reported from another species of *Simira*, *S. rubra* [8].

Bohlmann *et al.* [9] have reported the occurrence of α-terthienyl and other polyacetylenes in the roots of *F. trinervata* and *F. repanda*, both of which are probably synonyms of *F. trinervis*. However, the present report of α-terthienyl in the tops of the plant is the first record of polyacetylenes, or their derivatives, from aerial portions of any plant in the genus and is important if light absorption is considered.

The high degree of phototoxicity of above-ground portions of both *Flaveria trinervis* and *Simira salvadorensis* suggested that these two species may be well protected from some pests by the photosensitizing effects of their secondary compounds. In addition, this study indicates how the use of biological activity testing can be used to assist in the elucidation of secondary chemistry.

EXPERIMENTAL

Plant material. Simira salvadorensis (Standl.) Standl. (Syn. *Sickingia salvadorensis* Standl.) is a small tree of the semi-evergreen tropical forest zones of Central America and our specimens were collected from a mixed tropical hardwood association known locally as high bush at Indian Church, Belize during a 3 year agro-forestry study. Bark chips were placed in

Table 1. Phototoxicity of plant parts and extracts to *S. cereviceae*

Test material	Zone of inhibition (mm) Light*	Dark
Simira salvadorensis		
bark extract (1 g/ml)	3	0
Flaveria trinervis		
leaf	5	0
root	5	0
stem	5	0
achene	5	0
disk flower	0	0
ray flower	0	0
Harman (from *Simira*) (50 μg/disk)	4	0
α-Terthienyl (from *Flaveria*) (10 μg/disk)	10	0

* Light treated plates were exposed to 4 hr near-UV from a bank of four Westinghouse BLB tubes (F 20T12). Intensity was 5 W/m^2.

EtOH and transported to Canada for analysis. Identification was made by J. D. Dweyer of the Missouri Botanical Gardens where voucher specimens have been deposited. *Flaveria trinervis* (Spreng) C. Mohr was collected at Rubio, Tachira, Venezuela, in dry the premontaine forest zone (Bosque seco premontano) at *ca* 600 m elevation. Verification of identification was made by V. M. Badillo of l'Instituto de Botanica Agricola, Mascaray, and a voucher specimen has been deposited in the herbarium.

Phototoxic assays. Crushed plant material, or 8 mm filter paper disks treated with plant extracts, were placed on Sabouraud agar plates previously treated with a lawn of *Saccharomyces cerevisiae* [NRC (Canada) culture No. XY222-1A α-WT]. Plates were prepared in duplicate and one was treated under near-UV Westinghouse BLB lamps (F20T12, 5 W/m^2) or, in some cases, full spectrum solar simulating lamps (Durotest Vita lite 48112, 400 W/m^2). The other plate was maintained in darkness. After 24 hr post-treatment incubation at 37°, zones of inhibition were measured.

Isolation and identification of terthienyl. Leaves and flowers of *Flaveria trinervis*, collected in EtOH were ground in a Waring blender and the suspension filtered. The extract was diluted with an equal vol. of H_2O and extracted × 3 with petrol (bp 30–60°). After reduction in vol. the petrol extract was passed through a column of Si gel (80–200 mesh) developed with petrol. A blue fluorescing fraction was the only one detected in the first 1000 ml collected. The fractions were reduced in vol. and further chromatographed on thin-layer Si gel plates with $CHCl_3$–hexane (1:1) or Et_2O–petrol (1:9), using authentic α-terthienyl and bithienylvinylacetylene as reference compounds. The *Flaveria* extract had a major component with identical UV and fluorescent characteristics to an authentic sample of α-terthienyl and a trace component corresponding to the bithienyl. The major component isolated had identical UV characteristics (λ_{max} 350 and 250 nm) and mass spectrum (*m/z* 248) to authentic α-terthienyl. The concn of α-terthienyl in the fresh plant material was estimated to be 60 mg/kg.

Isolation and identification of harman. Ca 300 g of pieces of *Simira salvadorensis* bark and EtOH (300 ml) were mixed in a Waring blender for several min. The resulting mesh was filtered and the vol. of the filtrate was reduced to 50 ml. C_6H_6 (10 ml) was added and the vol. was then decreased to 10 ml. This resulted in a H_2O-free soln which was chromatographed on a 60 × 2.5 cm Si gel (60–200 mesh) column. Elution with CH_2Cl_2–MeOH (19:1) gave two products which have yet to be identified. On TLC the third substance eluted (*ca* 30 mg) showed a bright blue fluorescence when exposed to UV. Crystallization from CH_2Cl_2–hexane gave a pure compound, mp 230–235° (dec.); IR ν_{max}^{KBr} cm^{-1}: 1625, 1604, 1567, 1504; UV λ_{max}^{EtOH} nm: 230, 236, 246, 288, 335, 348; δ(CDCl) 2.78 (3H, *s*), 7.20–7.60 (3H, aromatic protons), 7.84 and 8.36 (1H and 1H, *d*, $J = 3$ Hz), 8.10 (1H, $J = 4$ Hz), 9.0 (1H, *br*); *m/z* 182 (100), 181 (33), 155 (10), 154 (20). This data compares well with previously reported properties for harman [10]. The isolated compound was found to be identical in all respects with an authentic sample of 1-methyl-β-carboline or harman.

Acknowledgement—This work was supported by NSERC and an Agriculture Canada grant (EMR) to one of us (T.A.).

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