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BIOGENESIS OF THIOPHENE AND SELENOPHENE DERIVATIVES IN *LIATRIS SP.* AND *TAGETES ERECTA*

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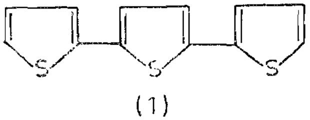
Abstract

A number of selenophene derivatives were found to be present in the ethereal extracts of the roots of *Tagetes* and *Liatris*. Studies with radioactive selenium showed that inorganic selenium but not selenomethionine could be incorporated into selenophenes

Introduction

At least six thiophene derivatives have been isolated from the ethereal extracts of the roots of *Tagetes erecta*, two of these compounds have been identified as terthienyl (1) and 5-(but-3-en-l-ynyl)-2-2-bithienyl (2) by comparison with authentic samples (Bohlman et al, 1962, 1963, 1963a Bohlman & Herbst, 1962 Bohlman & Kleine, 1963, Bohlman et al. 1964). Bohlman and coworkers have also reported that thiophene derivatives as well as other sulphur containing compounds like thioethers and sulphoxides invaribly occur alongwith polyacetylenes. It was therefore concluded (Bohlman, 1967) that thiophenes are synthesised in nature by a reaction equivalent to the addition of hydrogen sulphide to polyacetylenes. Schulte et al, (1965) have indicated that cysteine and sulphate are used as a source of sulphur in the biosynthesis of thiophenes. The present studies were undertaken to isolate the thiophene derivatives and their selenium analogues (selenophenes) when plants were fed wth 75Se-L-selenomethionine and inorganic 75Se i.e. 75Se-sodium selenite and 75se-sodium selenate to *Tagetes* and *Liatris*. The synthesis of authentic samples of selenium analogues of thiophenes has been described elsewhere (Hasanin et al. 1965).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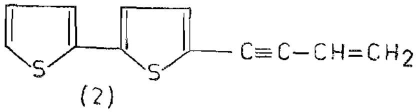
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Materials and Methods

Radioactive 75se-L-selenomethionine was obtained from the Radio-chemical centre Amersham, England. It was fed to the plants by two different methods.

Method A: 1 ml ^{75}Se-L-selenomethionine (0.26 mc) was diluted to 90 ml with distilled water. One month old plants of the two species were selected the roots were cleaned and the plants were placed separately in 100 ml glass cylinders containing the above radioactive material. The plants were allowed to grow in the laboratory under diffused light for 48 hours. The plants were then removed from the cylinders, the roots were washed with water and then disintegrated in distilled water (50 ml) with a Waring blender for 10 minutes. The solution was extracted with ether and washed three times with ether. The ethereal layer was separated dried over sodium sulphate and then evaporated. The residue was chromatographed on silica gel plates with chloroform: benzene (9 : 1) and ethyl acetate: chloroform: methanol (2 : 2 : 1), using authentic samples of terthienyl and 5-(but-3-en-l-ynyl)-2-2'-bithienyl.

Method B: In his method it was not necessary to remove the plants from the pots. A white cotton thread was inserted with a small needle through the stem of the plant about 2 cm above the roots. The two ends of the thread were then dipped into a small beaker containing 0.26 mc. radioactive ^{75}Se-L-selenomethionine solution. This solution was taken up by the plant in about 15 hours. A few drops of water were added twice to wash the beaker as completely as possible. During this period the plants were exposed to ordinary daylight. After 48 hours the plants were removed from the pots, the roots were washed with water and then harvested in the manner described under method A.

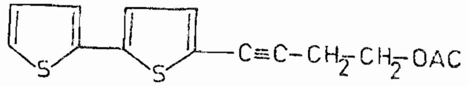
^{75}Se-sodium selenite and ^{75}Se-sodium selenate were used as inorganic sources of radioactive selenium. Before carrying out experiments with radioactive selenium, the toxicity of this element to plants was also studied. For this purpose five week old 3" high plants were selected and fed hydroponically with sodium selenite and sodium selenate solutions of varying concentrations. Plants fed with sodium selenite with concentration of selenium as high as 10 mEq were found to grow in normal manner while sodium selenate was found to be toxic and a concentration of 10 mEq selenium caused drying of leaves. After a week the plants were removed, dried and roots and leaves were cut into small pieces and allowed to stand on ether for two days. The ethereal layer was dried over sodium sulphate and solvent was evaporated. Thin layer chromatography on silica gel plates in pentane showed the presence of thiophenes in the roots but not in the leaves.

Three, five week old *Tagetes erecta* plants were fed hydroponically with 0.57mg Se (0.34 mc.) in 1 ml water, transferred to a test tube containing the plant. The tube was filled wih 'Phostrogen' solution. Two control plants were also grown similarly. Loss of water due to evaporation was made up by the addition of distilled water.

The plants were removed after a week and the roots were washed, dried on filter paper and then with hot air, cut into small pieces and soaked with ether for three days. The ethereal layer was separated dried over sodium sulphate and evaporated to dryness. TLC. on silica gel plates using n-pentane as solvent and autoradiography was then performed. Uv. spectra of the compounds obtained from thin layer plates were recorded on a unicam spectrophotometer.

Results

^{75}Se-L-selenomethionine when fed to *Tagetes erecta* by either of the two methods described did not result in the formation of selenophenes containing radioactive material. The substances moving with the authentic samples of terselenyl and and 5-(but-3-en-l-ynyl)-2″-biselenyl were found to be devoid of any radioactivity. T. l. c. of the ethereal extracts of the roots of *Tagetes erecta* fed on ^{75}Se-sodium selenite showed radioactivity in compounds chromatographyically identical to terthienyl (1), 5-(but-3-en-l-ynyl)-2″ bithienyl (2), 5-(4-acetoxybut-l-ynyl)- 2, 2″-bithenyl (3) and 2-Acetyl-3-hydroxyl-5-(prop-l-ynyl)-thiophene (4).



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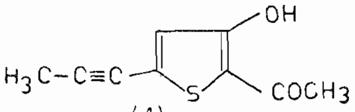


(4)

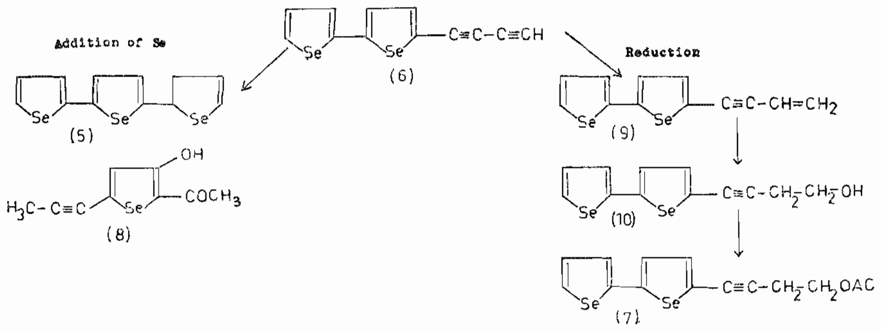
Selenophene derivatives isolated from the roots of *Liatris* fed on organic ^{75}Se did not possess radioactivity. The roots of the plants fed on ^{75}Se-sodium selenite, however showed the incorporation of radioactive selenium in a compound chromatographically and spectrosccpically identical to compound (4).

Discussion

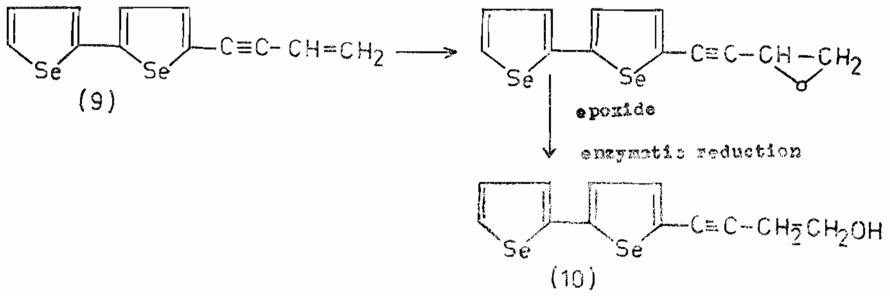
Previous investigations have indicated that thiophenes are produced by the plants by the addition of sulphur to polyacetylenes. (Bohlman & Herbst, 1962, Bohlman & Kleine, 1963; Bohlman, 1967; Schulte 1965). Experiments with 35S have however, shown that methionine and cysteine but not hydrogen sulphide can be incorporated into thiophenes.

The present studies were undertaken with a view to isolate selenophene derivatives from the roots of *Tagetes erecta* and *Liatris* when these plants were fed on organic and inorganic sources of selenium. Selenium shows remarkable resemblence in its chemical behaviour to sulphur, the isolation of radioactive selenophene derivatives could, therefore, lead to the possible explanation for the mechanism of biosynthesis of thiophenes. Laggett and Epstein (1965) have shown selenate and sulphate anions to possess identical affinities for organic compounds. According to Trelease (1945) selenium could replace sulphur in a protein molecule. Assuming that selenium analogues of thiophenes behave spectroscopically and chromatographically identical to their respective thiophenes, it could be expected that they are produced by the plants in a similar manner. However unlike sulphur, selenium in organic form could not be incorporated into selenophenes. Selenophene derivatives having selenium could, however, be isolated from the ethereal extracts of the roots of both plants when they were supplied with inorganic source of selenium. Radioactive selenium was found to be incorporated into terselenyl (5), 5-(but-3-en-l-ynyl)-2, 2'-biselenyl (6), 5-(4-acetoxybut-l-ynyl)-2, 2'-biselenyl (7) and 2-Acetyl-3-hydroxyl-5-(prop-l-ynyl)-selenophene (8) which are selenium analogues of compounds (1), (2), (3) and (4). respectively.

Terselenyl (5) may be formed from either (6) or (7) according to the following route. The scheme also suggests a possible relationship between the compounds that have been isolated from *Liatris* and *Tagetes.*



Conversion of compound (9) to (10) appears to involve an unusual anti-Markownikoff addition of water on the double bond. The addition of water could, however, possibly take place via an epoxide intermediate followed by preferential enzymatic reduction as shown below:



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