Tridec-1-ene-3,5,7,9,11-pentayne, an Ovicidal Substance from Xanthium canadense

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Recently, the screening for insect development inhibitory activity of methanol extracts of higher plants by our novel "Drosophila test" led to the discovery of an ovicidal substance, cis-DME from Solidago altissima L.1) The screening test revealed that the methanol extracts of roots of Xanthium canadense Mill. also inhibit hatching of the fly eggs. It was expected that the roots of X. canadense also contain polyacetylenic compounds with an ovicidal activity similar to that of cis-dehydromatricaria ester (DME), because both plants belong to the same family, Compositae. We have examined the methanol extract of roots of X. canadense for ovicidal activity and isolated a C13 ene-pentayne substance as the active principle.

In this paper we wish to report the isolation and the ovicidal activity of this polyacetylene.

The methanol extract of the roots was concentrated in vacuo, diluted with water and reextracted with benzene. The organic extract was adsorbed to a silica gel (Merck, 0.2—0.5 mm) column and successively eluted with 0 %, 5 %, 10 %, 25 %, 50 % and 100 % ethyl acetate in n-hexane. The active fraction eluted with 10% ethyl acetate in n-hexane was then subjected to dry column chromatography over silica gel (Merck 60 PF254) with n-hexane as an eluant. The slightly yellow colored eluate which showed one spot detected by UV lamp on its thin-layer chromatogram (silica gel) and one peak on its gas chromatogram (1 % OV-1 on Shimalite W) was obtained. Evaporation of the solvent under reduced pressure at room temperature gave the yellowish crystals which instantly turned black and insoluble in any solvents and so the melting point of them could not be measured by a conventional method. Accordingly, this substance had to be handled in a nitrogen atmosphere and stored as a dilute solution in a refrigerator, and the solvent had to be removed in vacuo below 5°C just before use in spectroscopic measurements and bioassays.

This substance (M+: m/e 162, base peak) had acetylenic bands at near 2200 cm⁻¹ and vinyl bands in the position of vinyl acetylenes, at 930, 962 and 1863 cm⁻¹ in its IR spectrum. The UV spectrum of this substance, \( \lambda_{\text{max}} (\text{nm}) (e): 252.5 (2100), 257 (2500), 265 (4000), 271 (2900), 286.5 (3300), 308 (52), 328.6 (86), 351 (140), \) showed the characteristic of an ene-yne conjugated system and resembled in its pattern that of the naturally occurring ene-pentayne, so called the "4100 pigment," which is widely distributed in Compositae plants.2,4) In its PMR spectrum this substance showed only one methyl signal (2.0 ppm, singlet), well consistent in chemical shift with the signal of the methyl group attached to the conjugated pentayne system (2.05 ppm, singlet).3) The signals of three olefinic protons (5.8 ~ 6.0 ppm) also indicated the presence of a vinyl group. In addition, the catalytic hydrogenation of this substance gave a hydrocarbon which was identified as n-tridecane by GC-MS. Accordingly, this substance was concluded to be tridec-1-ene-3, 5, 7, 9, 11-pentayne (C₁₃ ene-pentayne).

We tested the ovicidal activity of the C₁₃ ene-pentayne to the fruit-fly, Drosophila melanogaster and the house-fly, Musca domestica using the paper disk method described previously.3) Because of its extreme instability mentioned above, the C₁₃ ene-pentayne had decomposed considerably before the placement of eggs and exhibited much less activity if the paper disk soaked with it had been fully dried up. Therefore, 20 µl of an appropriate concentration of the hexane solution was applied to a paper disk (φ 1.0 cm) with a microsyringe, and ten eggs were placed to the disk immediately after evaporation of the solvent.

The results are shown in Fig. 1. The C₁₃ ene-pentayne exhibited 50 % inhibition of hatching of both flies at a dose of 0.8 ~ 1.0 µg/cm², while cis-DME at a dose of 5 µg/cm². The C₁₃ ene-pentayne had higher ovicidal potency than cis-DME and the decomposition

![FIG. 1. Effect of the C₁₃ ene-pentayne on Hatching of Flies (paper disk method).](image-url)

-○-, the fruit-fly, Drosophila melanogaster; ○—○, the house-fly, Musca domestica.

1) Search for Insect Development Inhibitors in Plants. Part II. See Reference 1.)
products of the C_{18} ene-pentayne seemed to be less active. From these data it is suggested that not the ester but the acetylenic groups contribute to the ovicidal activity, and its activity increases with an increase in the number of conjugated triple bonds in carbon chain of polyacetylene.

Many polyacetylenic compounds were isolated from higher plants,\(^1\) and their physiological activities to plants,\(^2\) animals,\(^3\)\(^4\) and microorganisms\(^5\)\(^6\)\(^7\)\(^8\)\(^9\) were reported. The activity against insects, however, remained to be studied extensively.

Recently, Kogiso et al. reported that 3-cis, 11-trans and 3-trans, 11-trans-trideca-1, 3, 11-triene-5, 7, 9-triyne isolated from the flowers of *Carthamus tinctorious* L. has the nematicidal activity.\(^10\) It is noticeable that the nematicidal triene-triyne has the same C_{18} carbon skeleton as our ovicidal substance, the C_{18} ene-pentayne.

It may be interesting to examine the ovicidal activity of different kinds of polyacetylenes and elucidate the relationship between the molecular structure and the activity.

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**REFERENCES**