

## COMPOUNDS OF BENZOIC ACID IN HEMIPARASITIC SCROPHULARIACEAE

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### SUMMARY

It is demonstrated that ethanolic extracts from a number of hemiparasitic Scrophulariaceae species contain a compound with an absorption maximum at 230 nm, which is absent in non-parasitic members of the family.

The peak at 230 nm could be attributed to benzoic acid. It occurs in *Rhinanthus serotinus* in free form, as an ester with aucubin, and bound to an unknown compound.

### INTRODUCTION

In the course of a study on the interactions between host and parasite (HOFSTRA & KLAREN 1973) it was found that the absorption spectrum of ethanolic extracts from dried material of the hemiparasite *Rhinanthus serotinus* (Scrophulariaceae) shows a conspicuous peak at 230 nm which was not found in extracts from several host plant species.

The absorption maximum at 230 nm was also found in ethanolic extracts from other hemiparasitic Scrophulariaceae, viz. *Rhinanthus minor*, *Melampyrum pratense*, *Melampyrum arvense*, *Odontites verna*, *Parentucellia viscosa*, and *Bellardia trixago*. In the extract from *Pedicularis palustris* an absorption maximum is found at 240 nm. In *Euphrasia* spec., however, a peak at these wavelengths could not be detected.

Extracts from the non-parasitic Scrophulariaceae: *Scrophularia nodosa*, *Penstemon* spec., *Antirrhinum majus*, *Linaria vulgaris*, *Digitalis purpurea*, *Mimulus luteus* and *Veronica chamaedrys* did not show a maximum in this range of the ultraviolet spectrum (fig. 1).

## 2. EXPERIMENTS AND RESULTS

2.1. To investigate if the absorption peak at 230 nm bears any relation to aucubin or other iridoid glucosides, which frequently occur in Scrophulariaceae (KOOIMAN 1970), aucubin from *Rhinanthus serotinus* leaves was partly purified according to TRIM & HILL (1952) by adsorption on activated charcoal from an aqueous extract and subsequent elution with ethanol. The unknown compound and aucubin coincided in the obtained extract.

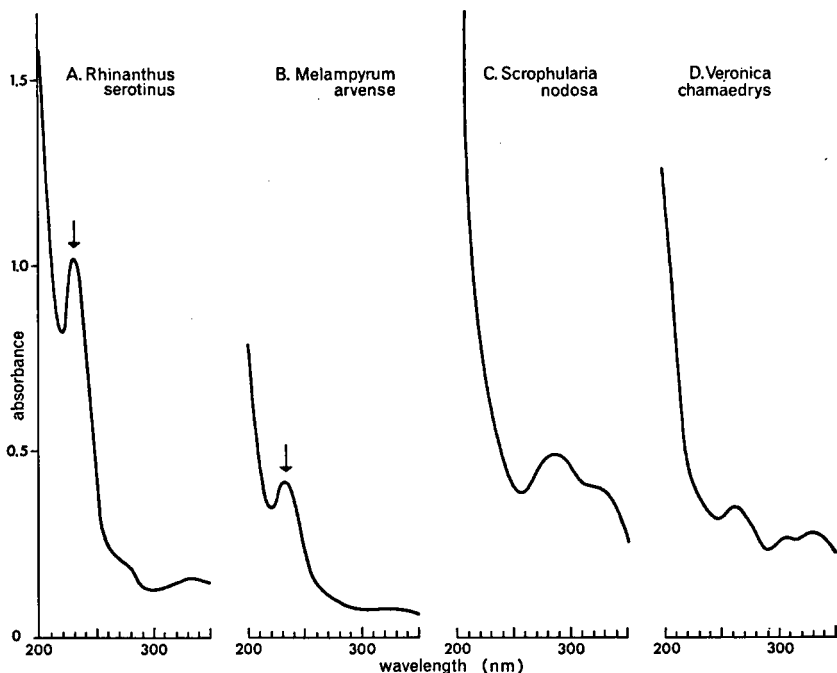


Fig. 1. Absorption spectra of ethanolic extracts from hemiparasitic (A, B) and non-parasitic (C, D) Scrophulariaceae.

2.2. In the isolation procedure of aucubin according to BRAECKE (1923) the peak at 230 nm was detectable up to the last but one crystallization. In the final step, however, crystals were obtained which showed an absorption maximum at 255 nm in ethanolic solution.

2.3. That the peak at 230 nm is not caused by aucubin also follows from its absence in other aucubin-containing species. Moreover, the E<sub>230</sub> in extracts from the leaves of *Rhinanthus serotinus* is much higher than in extracts from the roots (where it does not appear as a peak), whereas the aucubin content in the leaves is lower than in the roots.

Aucubin contents were determined colorimetrically at 570 nm as described by BRODA et al. (1969) after heating at 65°C of an extract with 0.5% p-dimethylaminobenzaldehyde in ethanol and concentrated hydrochloric acid (1:1).

2.4. In order to separate the unknown compound and aucubin an ethanolic extract from dried *Rhinanthus serotinus* leaves was examined by paper chromatography in n-butanol-ethanol-water (40:11:19 v/v) by the method of KOOIMAN (1970). The chromatogram was divided into 20 strips, which were eluted in ethanol. The UV spectra of the eluates were determined in a Perkin-Elmer Double Beam Spectrophotometer. The fractions were tested for aucubin with p-dimethylaminobenzaldehyde reagent (pDAB).

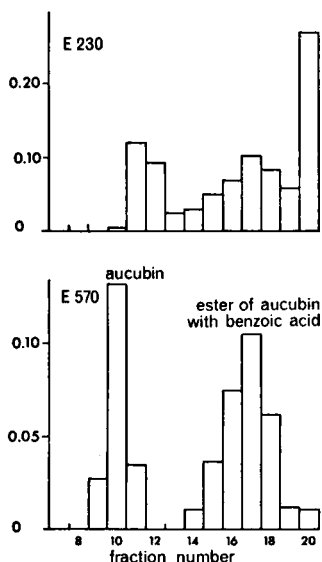


Fig. 2. Distribution of the E230 peak and aucubin (E570 in pDAB-test) after chromatography of an ethanolic extract from dried *Rhinanthus serotinus* leaves.

Fig. 2 shows that the E230 is caused by three compounds. The one with a maximum in fraction 17 ( $R_F$  0.82) appears to correspond with an ester of aucubin. This ester was demonstrated by FIKENSCHER et al. (1969) in leaves of *Rhinanthus glaber* (= *Rhinanthus serotinus*) and proved to be the ester of aucubin and benzoic acid. Drying of plant material causes partial saponification of the ester (FIKENSCHER et al. 1969).

Benzoic acid has an absorption maximum at 230 nm. The maximum in fraction 20 is probably due to free benzoic acid.

The compound with a maximum at 230 nm in fraction 11 ( $R_F$  0.51) could be separated from aucubin ( $R_F$  0.46) by repeated chromatography. During this treatment it lost some benzoic acid (which appeared again in fraction 20). This compound has not yet been identified.

The spectrophotometric data suggest that benzoic acid, in free or bound form (or in both), occurs more frequently in hemiparasitic Scrophulariaceae than hitherto was known. Although the occurrence of benzoic acid and hemiparasitism in this group may be a mere coincidence, based on taxonomic relationship, the possibility that benzoic acid has a specific function in the hemiparasites should not be excluded.

#### ACKNOWLEDGEMENT

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