Isolation and Identification of Emodin (1,3,8-Tri-hydroxy-6-methyl Anthraquinone) from *Rumex hymenosepalus*, Family Polygonaceae

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Abstract □ The anthraquinone pigment, emodin, has been isolated and its presence positively verified from a nonpolar extract of the dried, ground tubers of *Rumex hymenosepalus*. Isolation was accomplished by preparative TLC on silica gel layers. Identification was made by visible, IR, NMR spectroscopy and TLC, with use of commercial samples of emodin.

Keyphrases □ Emodin isolation—*Rumex hymenosepalus* □ TLC—separation, identity □ IR spectrophotometry—identity □ Visible spectrophotometry—identity □ NMR spectroscopy—identity

The isolation of chrysophanic acid and physcion was reported recently (1). The authors reported that although the possible presence of emodin had been previously reported (2), no trace of it could be found in their investigation (1).

In a previous investigation, the author isolated and identified a potential antitumor fraction from extracts of the tubers of *Rumex hymenosepalus* (3, 4). During this investigation, nonpolar extracts showed the presence of several anthraquinone pigments.

EXPERIMENTAL

Materials and Methods—The tubers used in this experiment were obtained from the University of Arizona Agricultural Experimental Station, Mesa, Arizona by Norris Gilbert. Positive identification was made by Richard Barr, Research Associate at the University of Arizona Natural Products Laboratories, College of Pharmacy, Tucson, Arizona. IR spectra were made on a spectrophotometer (Perkin-Elmer Infracord) by KBr pellets. Visible absorption spectra were measured in ethanol, using a spectrophotometer (Beckman BD). NMR spectra were determined on a high resolution spectrophotometer (Varian A-60), using acetone and $CDCl_3$, with tetramethylsilane as internal reference. TLC was performed on Silica Gel G plates, and preparative TLC was performed on plates (20 × 20 cm.) coated with 1-mm. thick layer of Silica Gel G which was activated at 130° for 1 hr.

Preparation of Extract—A tannin-free extact was obtained by the following extraction procedure. An ether extract was obtained from 600 g. of the ground, dried tubers. The ether extract was washed with water and separated. The ether phase was evaporated *in vacuo*, resulting in an amorphous residue. This material was taken up in chloroform and subsequently washed several times with water. The chloroform phase was separated from the aqueous phase and the chloroform evaporated *in vacuo*. Five grams of amorphous, yellow-orange residue was obtained. This residue was subjected to exploratory TLC with chloroform as the solvent and ceric sulfate (2%) as the spray reagent. The exploratory TLC revealed the presence of several separate materials in the nonpolar extract. Two distinct bright orange spots appeared at R_f 0.3 and 0.75. The spot at R_f 0.75 was subsequently identified as consisting of the pigments, chrysophanic acid, and physcion, previously reported. (1) The spot at R_f 0.3 fluoresced orange when examined under UV light; and when sprayed with 10% sodium hydroxide, a cherry-red color was noted which indicated the presence of emodin (5).

Isolation of Emodin—The residue obtained from the nonpolar extract was subjected to preparative TLC with chloroform as the eluent. Twenty plates were prepared according to Stahl (6) and the silica gel was spread 1 mm. thick by a Desaga applicator (Brinkmann) (7). The bands which developed at R_f 0.3 were eluted with ether and chloroform. The resulting orange-yellow crystals were recrystallized several times with chloroform to give a crystalline isolate with a melting point of 255° identical to that of a reference sample of emodin (K & K Labs, Plainview, N. Y.). TLC of the isolate and reference emodin in several systems [chloroform; benzene–methyl formate–formic acid (7:24:1); benzene–glacial acetic acid (60:30)] showed identical R_f values. An IR spectrum of the isolate was superimposable with that of reference emodin. Carbonyl absorption peaks at 1,625 and 1,677 $cm.^{-1}$ showed only one carbonyl is chelated. Visible absorption spectra of isolate and reference emodin, in ethanol, (maximum absorbance 435 mμ) were also superimposable. NMR spectra of isolate and reference sample of emodin were identical. Peaks were present at δ:1.25 singlet, 7.02 (broad, 3 cyc./sec.), 7.49 (broad, 3 cyc./sec.), 7.19 (doublet, J 2.5 cyc./sec.). 6.57 (doublet, J 3 cyc./sec.).

The possible presence of emodin was reported in 1878 (2). More recent investigations of *Rumex hymenosepalus* reported no trace of it could be found (1). An extensive literature search failed to show the confirmation of the presence of emodin in this species. This investigation has isolated and identified emodin in the ground, dried tubers. This was carried out by preparative TLC on silica gel layers, IR, visible, NMR spectroscopy, and TLC with reference sample of emodin.

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ACKNOWLEDGMENTS AND ADDRESSES

Received November 15, 1968, from the *Pharmacy Department, University of Arizona, Tucson, AZ*

Accepted for publication February 20, 1969.

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