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MEDICINAL PLANTS OF EAST TENNESSEE. CHEMICAL CONSTITUENTS OF *CHRYSANTHEMUM LEUCANTHEMUM* L.

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ABSTRACT

The methylene chloride extract of the medicinal herb *Chrysanthemum leucanthemum* has yielded three crystalline compounds identified as acacetin (5,7-dihydroxy-4'-methoxyflavone), scopoletin (7-hydroxy-6-methoxycoumarin), and 2,6-dimethoxy-p- benzoquinone.

INTRODUCTION

Exploration of the plant kingdom for chemical compounds of medicinal value has been an important activity of man since prehistoric times. Herbalism and native folk medicine have been and continue to be sources of useful therapy. Indeed, in the nineteenth century, when organic chemists took up the study, this valuable medical information began to pass into the hands of scientists. Since that time, the constitution and activity of many well-known vegetable drugs—for example quinine, atropine, morphine, strychnine, tetrahydrocannabinol, cocaine, and others—have become known. Yet thousands of plant species remain unexplored despite the scrutiny of natural products chemists throughout the world.

As a part of a continuing study of the medicinal plants of East Tennessee (Waddell et al., 1979), we have examined the constituents of *Chrysanthemum leucanthemum* (family Compositae), a plant reported to have been used to restore color after jaundice, for wounds, asthma and consumption, and as an insect powder (Coon, 1963). Furthermore, the estrongenic and toxic effects of this plant on experimental animals have recently been studied (Bergeron and Goulet, 1980).

METHODS

Plant material was collected in May 1972 on highway 153 near Chickamauga Dam, Chattanooga, Tennessee, and identified by Dr. G. S. Van Horn, Department of Biology, UTC. A voucher specimen, CHRL-CD-572-TGW, is on file. This plant material was sun-dried prior to long term storage. Column chromatography was done on Baker Analyzed Reagent silica gel, 40-140 mesh. Eastman chromatogram sheets (Kodak 13179 silica gel) were used for thin-layer chromatography. The TLC solvent system was 20% acetone in chloroform and chromatograms were visualized by UV light and iodine. UV spectra were taken on a Hitachi 100-80 Computerized Spectrophotometer. Melting points were recorded with a Thomas-Hoover apparatus and are corrected.

Extraction of *Chrysanthemum leucanthemum.*

An 1810 g portion of the dried, aerial parts of *C. leucanthemum* was ground in a Wiley mill (model 4) fitted with a 2 mm screen. The powdered plant material was extracted for three days using a Soxhlet apparatus and the extract was evaported to dryness leaving a dark-green tar. This extract was dissolved in 150 ml of hot ethanol whereupon 375 ml of hot water was added. The resulting mixture was stirred for 20 minutes at 75 degrees C and refrigerated. After four days, the mixture was filtered through Celite and the yellow filtrate was extracted with six 150 ml portions of chloroform. The combined chloroform extract was dried over anhydrous magnesium sulfate, filtered, and evaporated to dryness using a rotary evaporator to give 5.0 g of a dark, amber oil.

This final extract was chromatographed on a column of silica gel (2.5 x 45 cm) according to the following scheme:

Fraction No.	*Fraction volume*	*Eluted with*
—	300 ml	25% chloroform in hexane
—	300 ml	50% chloroform in hexane
1-3	20 ml each	50% chloroform in hexane
4-20	20 ml each	75% chloroform in hexane
21-42	20 ml each	100% chloroform

Fractions to be combined were identified by TLC. Com-

bined fractions 26-36 were rechromatographed on a silica gel column (1.3 x 50 cm) eluting with chloroform. From this second chromatography, fractions 6-22 yielded 1 mg of crystalline *1*. Numbers 24-26 deposited 11 mg of *2*, and 35-42 gave 2 mg of *3*.

2,6-dimethoxy-p-benzoquinone (*1*): λ max (methanol) 282 nm; mass spectrum (m/e)-168(47% of base peak), 140(8%), 138(15%), 125(12%), 97(12%), 80(30%), 69(100%). Compound *1* was identified by direct comparison (TLC, UV,MS) with an authentic sample (Aldrich Chemical Co.).

7-hydroxy-6-methoxycoumarin (*2*): λmax(methanol) 342 nm, 293, 250sh, 227; mass spectrum(m/e)-192(base peak), 177(59%), 164 (24%), 149(46%), 121(17%). Compound *2* was identified by direct comparison (TLC,UV,MS, mixture mp) with an authentic sample (Fluka Chemical Corp.).

5,7-dihydroxy-4′-methoxyflavone (*3*): λ(methanol) 332 nm, 300sh, 270; (NaOMe, methanol) 351, 295sh, 276; (NaOAc, methanol) 368, 291sh, 277; (NaOAc, H_3BO_3, methanol) 335, 295sh, 267; ($AlCl_3$, methanol) 379, 344, 302, 277, 259sh; ($AlCl_3$, HCl, methanol) 379, 340, 301, 278, 257sh; mass spectrum(m/e)-284(base peak), 241 (15%), 152(8%), 132(21%). Compound *3* was identified by comparison of detailed UV and mass spectra with published data. Melting point of *3* = 262-5 deg, lit. value 261-3 deg (Geissman, 1962)

Results and Discussion

The methylene chloride extract of the aerial parts of *C. leucanthemum,* upon further fractionation and extensive silica gel chromatography, yielded three crystalline compounds, discussed below in order of their elution from the column.

Compound *1* gave a molecular ion at m/e 168 in a mass spectrum(MS) which was identical to a published spectrum of 2,6-dimethoxy-p-benzoquinone (Mass Spectrometry/Data Center, 1974). A direct comparison (TLC,UV) of *1* with authentic 2,6-dimethoxy-p-benzoquinone confirmed their identity. 2,6-Dimethoxy-p-benzoquinone has not previously been reported to occur in *Chrysanthemum* species.

Compound *2* gave a molecular ion at m/e 192 in its mass spectrum. Other fragment ions at m/e 177, 164, 149, and 121 were indicative of a coumarin structure containing one hydroxy and one methoxy group (Barnes and Occolowitz, 1964). A direct comparison (TLC, UV, mixture mp) of compound *2* with authentic scopoletin(7-hydroxy-6-methoxycoumarin) established their identity and the structure of *2*. Scopoletin is a poisonous compound which has previously been isolated from *C. leucanthemum* (Dargaeva and Brutko, 1977) and *C. segetum* (Oksuz and Wagner, 1982). A wide range of pharmacological properties has been described for a variety of coumarins (Joshi et al., 1980) and the occurrence of scopoletin in *C. leucanthemum* may contribute to this plant's medicinal activities.

Compound *3* displayed UV and mass spectra which were identical to the detailed published spectra (Mabry et al., 1970) (Mass Spectral Data Base, 1978) of acacetin (5,7-dihydroxy-4′-methoxyflavone). In addition, the melting point of *3,* 262-5 deg, matched closely the literature value of 261-3 (Geissman, 1962). Acacetin has been previously isolated from *Artemesia* species (family Compositae) (Rodriquez et al., 1972) and, as a 7-0-glycoside derivative, from *Chrysanthemum indicum* (Chatterjee et al., 1981). Although the biological properties of acacetin itself are not known, flavones have been reported to display mutagenic (Bjeldanes and Chang, 1977) and insect-repelling activity (Dreyer and Jones, 1981).

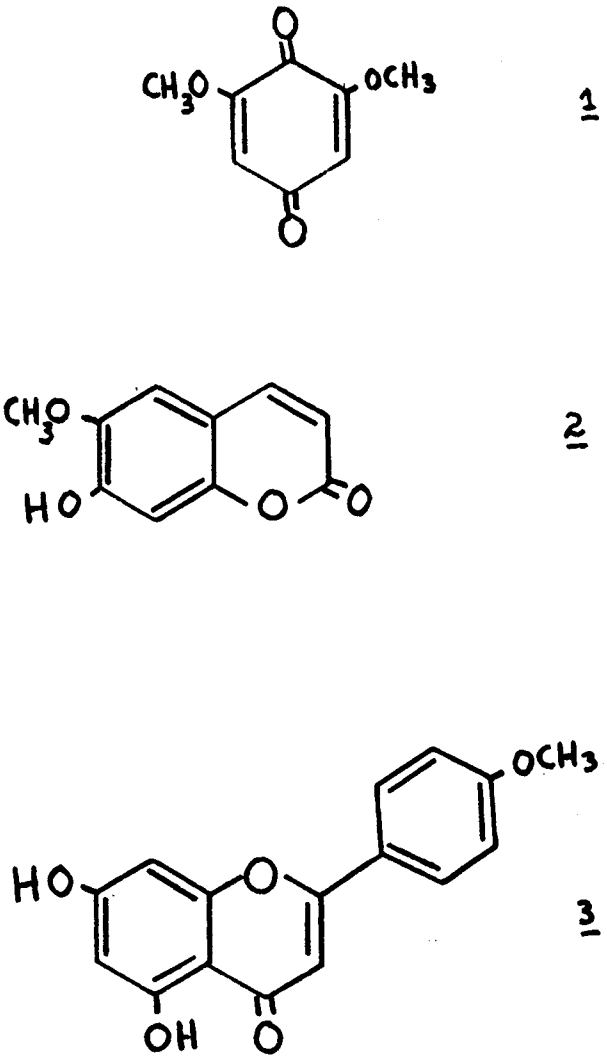

FIG. 1. *Compounds 1, 2, and 3 which were investigated in this study.*

The work-up procedure described in the Methods section has been shown to concentrate sesquiterpene lactones (Geissman, 1966), a class of natural products common in many *Chrysanthemum* species (Bloszuk et al., 1978). In the present study, the final extract lacked the characteristic gamma-lactone infrared absorption indicating the absence of significant quantities of sesquiterpene lactones in *C. leucanthemum.*

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