tion of 1% prednisolone in CMP, which is used in dentistry as a pulp-capping agent, would have a shelf-life of 2 to 5 years.

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Keyphrases

Prednisolone—stability in CMP
Degradation—prednisolone
Blue tetrazolium reaction—assay
IR spectrophotometry—analysis

Aster pilosus (*Compositae*) I. Isolation of Hyperoside (Quercetin-3-β-D-mono-galactoside) from the Leaves

By NORMAN R. FARNSWORTH, HILDEBERT WAGNER, LUDWIG HÖRHAMMER, and HANNS-PETER HÖRHAMMER

Aster pilosus **leaves were investigated and found to contain several flavonoids, as evidenced by thin-layer chromatography. Purification of a crude ethanol extract, followed by polyamide column chromatography, resulted in the isolation of one of the flavone constituents, which was found to be identical with hyperoside (quercetin-3-β-D-mono-galactoside).**

ALTHOUGH SEVERAL HUNDRED SPECIES of the genus *Aster* have been botanically described, relatively little is known of the phytochemical constituents of this group of plants. *Aster tataricus* roots have yielded the triterpenes friedelin, epifriedelanol, and shionone, in addition to astersaponin (hederagenin glucoside) (1–6) and the flavone quercetin (7). Quercetin, carotenoids, and galactose have also been detected in the pollen of this species (8). The latter three compounds have similarly been detected in the pollen grains of *Aster yomena* and *A. ageratoides* var. *ovatus* (8). *Aster tripolium* roots have yielded the polyacetylene compound 2-*trans*:8-*trans*-matricarianol (9) and *A. spinosus*, *cis*-lachnophyllum ester (10). Also, 2-*cis*: 8-*cis* matricaria ester has been isolated from the roots of *A. mongolicus*, *A. lautureanus*, and *A. novae angliae* (9), and angelic acid ester from *A. novae belgii* (11). Delphinidin diglycosides have been detected in the flowers of *A. amellus* var. *riverslea* and *A. sinensis* (12), cyanidin-3, 5-dimonoside in the leaves of *A. ericoides* (13), and callistephin (pelargonidin-3-glucoside), as well as chrysanthemin (cyanidin-3-glucoside), have been isolated from *A. chinensis* (14). A number of volatile oil constituents has also been identified in *Aster indicus* by means of gas chromatography (15).

Several *Aster* species have been used as folkloric remedies in the treatment of skin diseases (*A. novae angliae*) (16), rectal disorders, angina, eye inflammation, and stomach acidity (*A. amellus*, *A. tripolium*) (16), syphilis, bone caries, and coughs. (*A. bakerianus*) (17), intestinal parasites, and abdominal pain (*A. erigeroides*, *A. filifolius*) (17), and headache (*A. muricatus*) (17). In addition, various species have been recorded as being toxic to livestock, having strong emetic and purgative activity, producing depression, and having antiseptic properties (16, 17).

On the experimental side, *A. pilosus* var. *demotus* (18), *A. scaber* (19), *A. tataricus* (19), and *A. japonicus* (20) have been reported to elicit antitumor activity, whereas *A. divaricatus* extracts have shown strong inhibitory action against *Mycobacterium tuberculosis* (21). Defatted ethanol extracts of several native *Aster* species have been shown to elicit varying degrees of central system depression and/or autonomic activity in mice (22) and *A. divaricatus* is inhibitory for the pseudorabies virus in tissue culture tests (22).

No definitve phytochemical studies have been reported in the literature for *Aster pilosus* and biological activity reports on this species are limited (22–24).

On the basis of a lack of phytochemical information on *A. pilosus*, and because of interesting folkloric uses recorded for this genus of plants, a preliminary chemical study of the leaves was initiated.

EXPERIMENTAL

Plant Material—The plant material used in this investigation was the dried leaves of *Aster pilosus* Willd. (*Compositae*), collected during September 1966 at Pittsburgh, Pa. and authenticated by Dr. L. K. Henry, Carnegie Museum, Pittsburgh, Pa.

Received December 7, 1967, from the Institut für Pharmazeutische Arzneimittellehre der Universität München, Munich, Germany, and the Department of Pharmacognosy, School of Pharmacy, University of Pittsburgh, Pittsburgh, PA 15213

Accepted for publication February 6, 1968.

This investigation was supported, in part, by a research grant-in-aid from Eli Lilly and Company, Indianapolis, Indiana 46206. One of the authors (N.R.F.) is grateful to the Alexander von Humboldt Stiftung for a research fellowship which made this work possible.

The authors wish to acknowledge the technical assistance of Mr O. Seligmann and Misses M. Seitz and E. Sedlaczek.

Voucher specimens representative of the collection have been deposited in the herbaria of Carnegie Museum and the Department of Pharmacognosy, University of Pittsburgh. The plant material was air-dried and milled to a coarse powder prior to extraction.

Preparation of Crude Flavonoid Extract—The dried leaves (2.56 Kg.) were continuously extracted in a Lloyd extractor with skellysolve B (*n*-hexane) for 72 hr. and the resulting extract was concentrated to remove all traces of solvent by means of a flash evaporator *in vacuo* at 40°. The green, oily residue weighed 37.1 Gm. (Fraction *A*). Next, the skellysolve-B exhausted plant material was continuously extracted in the Lloyd extractor for 72 hr. with methanol, and the methanol extract was concentrated to dryness to yield 435 Gm. of a dark-green powder (Fraction *B*).

Thin-layer chromatography of Fraction *B* on Kieselgel G, and polyamide plates, as well as paper chromatographic examination, revealed the presence of several flavonoid components, as evidenced by color reactions of resolved components after treatment of the chromatograms with lead subacetate solution, and viewing under ultraviolet light (25). At least three major and two minor flavonoids could be detected in this manner.

A 210-Gm. batch of Fraction *B* was mixed with 3 L. of distilled water and heated on a steam bath for several hours until the extract was concentrated to about 1 L. The mixture was refrigerated overnight, during which time a considerable amount of nonflavonoid material precipitated from solution, which was removed by centrifugation. The clear supernatant was again concentrated on a steam bath to about 0.5 L., followed by refrigeration overnight. Very little precipitation occurred following this treatment, and the extract was again clarified by centrifugation to yield a clear brown solution. Thin-layer chromatography of this extract revealed the presence of chlorophyll, thus it was diluted with an equal volume of water and again concentrated to about 0.5 L. and refrigerated. This treatment resulted in an additional precipitation of nonflavonoid pigments, which were removed by centrifugation.

This clarified extract was next extracted six times in a separator with separate 200-ml. portions of ethyl acetate, the combined extracts being taken to dryness *in vacuo* to yield 14.37 Gm. of Fraction *B-1*. The aqueous phase was then extracted with six separate 200-ml. portions of ethyl acetate–methanol (95:5). Removal of solvent *in vacuo* yielded 3.01 Gm. of Fraction *B-2*.

Isolation of Hyperoside—A glass chromatographic column (5.5 × 55 cm.) was filled with a methanol slurry of fine particle size polyamide, prepared as previously described (26), to a packed height of 48 cm. in the column. Fraction *B-2* (3.1 Gm.) was dissolved in methanol, mixed with a small amount of polyamide, and allowed to air dry. The mixture was then placed on the top of the prepared column and methanol was continuously added, with 25-ml. fractions being collected and monitored by means of TLC on Kieselgel G plates, using methanol as the developing solvent. In this manner, a total of 95 fractions was collected and combined as follows on the basis of TLC data: 1–11, 12–21, 22–27, 28–39, 40–46, and 47–95. Combined fractions 47–95 were taken to dryness, and crystallized from methanol to yield 0.017 Gm. of pale yellow crystals. After drying the crystals *in vacuo* at 100° for 24 hr., they exhibited an m.p. of 226–229° dec. Thin-layer chromatography of the isolate on Kieselgel G plates, using a solvent system of ethyl acetate–methanol–water (100:16.5:13.5) revealed a single spot at R_f 0.38 after treatment of the chromatogram with lead subacetate solution, and viewing under ultraviolet light (366 mμ). Further, TLC of the isolate on polyamide, using methanol as the developing solvent, revealed a single spot at R_f 0.28 under the same conditions. An infrared absorption spectrum (KBr) was superimposable with those of reference samples of hyperoside (quercetin-3-β-D-mono-galactoside) derived from natural sources, and synthesized (Fig. 1). Thin-layer chromatography of the isolate in the two systems previously described, showed it to have an R_f value identical with the two samples of reference hyperoside.

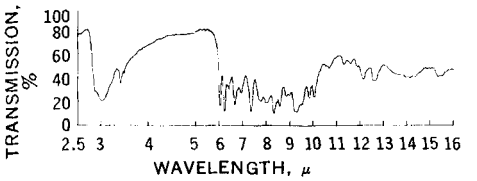

Fig. 1—Infrared absorption spectrum of hyperoside (KBr).

To further characterize the isolate, a small amount (2.0 mg.) in 5.0 ml. of methanol was hydrolyzed using 5.0 ml. of 10% hydrochloric acid under reflux for 2 hr. The hydrolysate was cooled and extracted several times with ether to remove the aglycone. Thin-layer chromatography of the concentrated ethereal extract on Kieselgel G plates, using a solvent system of benzene–pyridine–formic acid (36:9:5), showed the aglycone to have an R_f value of 0.25, and identical with that of reference quercetin, in addition to R_f values of 0.36 for the ethereal extract and reference quercetin after TLC on Kieselgel G, using a solvent system of toluene–ethyl formate–formic acid (5:4:1).

Identification of the sugar moiety was achieved by neutralizing the ether-extracted hydrolysate (*vide supra*) using silver oxide, concentrating the aqueous phase to about 0.5 ml., and spotting the extract on Schleicher and Schull No. 2043 b chromatography paper, followed by ascending development using solvent systems of isopropanol–pyridine–water–acetic acid (8:8:4:1), as well as *n*-butanol–pyridine–water (6:4:3), each for 18 hr. The aqueous hydrolysate and D-galactose both exhibited R_{GA} values of 1.0 following treatment of the dried chromatograms with benzidine spray reagent and heating at 105° for 15 min. These data are consistent with an identification of the isolate from *Aster pilosus* leaves as the flavone glycoside hyperoside (quercetin-3-β-D-mono-galactoside).

SUMMARY

An investigation of the leaves of *Aster pilosus* Willd. (*Compositae*) has resulted in the isolation of a flavone glycoside, which has been identified as hyperoside (quercetin-3-β-D-mono-galactoside). This flavone has been previously isolated from several higher plants (7), but this is the first report of its presence in any *Aster* species. The only flavonoid isolated from this genus previously is the flavone

aglycone quercetin, from *A. tataricus* roots and pollen (7, 8), and the pollen of *A. ageratoides* var. *ovatus* (8), as well as *A. yomena* (8).

The identity of additional flavonoids present in this plant will be reported at a later date.

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Keyphrases

Aster pilosus leaves
Quercetin-3-β-D-mono-galactoside—isolated
TLC—separation, identity
Column chromatography—separation
IR spectrophotometry—structure

Bacterial Contamination in Some Nonsterile Antibiotic Drugs

By MACK WHITE, FRANCES W. BOWMAN, and AMIEL KIRSHBAUM

Methods have been developed for the determination of microbial contamination in some nonsterile antibiotic preparations. Microorganism counts have been performed on both antibacterial and antifungal antibiotic powders. The highest counts were found in samples from the antifungal antibiotic.

In 1964, an extensive investigation into microbiological contamination of medical preparations was conducted in Sweden (1). A report of these findings was submitted to the Royal Swedish Medical Board, including a proposal for an upper limit of 100 bacteria per gram in nonsterile medicinals.

The *Antibiotic Regulations* of the Food and Drug Administration (FDA), the USP, and the NF require that antibiotics for injection be sterile, and they specify sterility tests for these preparations. None of these compendia, however, require sterility for oral or topical antibiotics, except for surgical powders and some ophthalmic and otic solutions. Tolerances are not specified in the official compendia for microbial contamination of nonsterile drugs except for one topical antibiotic preparation; the *Antibiotic Regulations* limits the contamination of this preparation to not more than 10 microorganisms per Gm. (2). No other oral or topical antibiotics undergo routine microbial examination as part of the antibiotic certification program.

Received January 10, 1968, from Division of Antibiotics and Insulin Certification, Food and Drug Administration, Washington, DC 20204
Accepted for publication February 9, 1968.

Recently, several batches of Agent F, an antifungal antibiotic, were found to be grossly contaminated with microorganisms, Although sterility is not required for the powder or dosage forms of this antifungal antibiotic, gross contamination would indicate a possible violation of the good manufacturing practices required by FDA (3). This possibility led to a study of the problem and to the development of suitable methods for the determination of microbial contamination in nonsterile antibiotic preparations.

EXPERIMENTAL

Materials—Prepare the following nutrient agar: peptone, 6.0 Gm.; yeast extract, 3.0 Gm.; beef extract, 1.5 Gm.; agar, 15.0 Gm.; and enough distilled water to make 1000.0 ml. The pH should be 6.5 to 6.6 after sterilization.

Prepare peptone water by dissolving 1 Gm. of peptic digest of animal tissue, USP or equivalent, in sufficient distilled water to make 1,000 ml. Dispense 99-ml. portions into flasks and sterilize in an autoclave at 121° for 20 min. The final pH should be 7.1 ± 0.1.

Method I—Powder—Place 1.0 Gm. of powder into 99 ml. of sterile peptone water. From this sus-