ISOLATION OF KAEMPFERITRIN FROM *JUSTICIA SPICIGERA*

K. L. EULER and M. ALAM*

Department of Medicinal Chemistry and Pharmacognosy, College of Pharmacy, University of Houston, Houston, Texas 77004

A number of plants have the common name firecracker plant due to their narrow orange flowers. One of these, *Justicia spicigera* Schlectend. [*Jacobinia spicigera* (Schlectend.) L. H. Bailey] (Acanthaceae), is a shrub which is indigenous to Mexico and south to Columbia. It is cultivated and used in Texas as a medicinal herb called *muicle*.

The leaves of the plant have been used medicinally as a stimulant and antidysenteric (1) and also to treat scabies (2). A plant substance termed "Mexican Blue" has been used for whitening clothes (3). An early investigation into the plant indicated the presence of a colorless compound mohintline which turned green, then blue, upon exposure to air (4). An additional, but previously unreported, use of the plant is to treat a variety of gastrointestinal disorders, including nausea, stomachache, cramps and diarrhea.

EXPERIMENTAL[1]

PLANT MATERIAL.—The plant material examined in the present study was cultivated and collected in Edinburg, Texas, and a voucher specimen is on deposit in our department.

EXTRACTION.—Air dried and milled leaves (113 g) were exhaustively extracted with ethanol in a Soxhlet extractor, and the ethanol extract was evaporated to dryness to yield an oily residue (19 g). The residue was partitioned between equal volumes of hexane and water. The aqueous layer was separated and reextracted twice with hexane, followed by chloroform, and finally with methylethyl ketone (MEK). The MEK extract (4.7 g) was evaporated to dryness.

CHROMATOGRAPHY.—A 1.3 g sample of the dried MEK extract was chromatographed on a silica gel 60 (E. Merck mesh 70–230) column (1″ x 30″) which was eluted with 15 percent methanol in chloroform. Fractions of 15 ml volume were collected. Rechromatography of the residue (0.50 g) from the desired fractions of the first column was performed on a smaller column (0.5 x 20″) packed with silica gel 60 (70–230 mesh) and eluted with 15 percent methanol in chloroform. Recrystallization of the crystalline precipitate with methanol gave yellow needles—Compound I (0.3 g) mp 220–221°; ir, νmax (KBr) 3400, 1660, 1600 cm^{-1}; uv, λmax (MeOH) 350, 265, 244 nm; ^{1}H-nmr, (DMSO-d_6) δ 0.81 (3H, d, J=5.1 Hz) and 1.13 (3H, d, J=5.4 Hz) (6H, rhamnose-CH_3), 645 (1H, d, J=2.2 Hz, 6-H), 6.71 (1H, d, J=2.2 Hz, 8-H), 6.93 (2H, d, J=8.7 Hz, 2',6'-H), 7.78 (2H, d, J=8.7 Hz, 3',5'-H), 7.84 (1H, broad s, 4'-OH) 12.59 (1H, s, 5-OH).

PREPARATION OF THE ACETATE.—The acetate derivative was prepared by refluxing 50 mg of I in 5 ml of pyridine-acetic anhydride (2:1 v/v) for 2 hours. The ^{1}H-nmr spectrum gave 8 singlets, each integrating for 3H; δ 2.31 and 2.32; 1.94, 1.97, 1.98, 2.04, 2.09 and 2.14, indicating the presence of 2 aromatic OH's and 6 alkyl linked OH's in I.

HYDROLYSIS OF I. Compound I (5 mg) was hydrolyzed by refluxing with methanol (5 ml) and 0.2 N H_2SO_4 (5 ml) for 40 min. The methanol was removed by evaporation, and the reaction mixture was extracted with chloroform. The aqueous layer was neutralized with $Ca(OH)_2$, filtered and lyophilized. The TMS-ether derivative of the residue on gc gave a retention time which matched exactly with TMS-rhamnose. The chloroform layer, upon evaporation and recrystallization with methanol, gave yellow crystals, mp 279°; $C_{15}H_{10}O_6$ (Calc. 286.04774; found 286.04767); ms, (rel. intensities) m/z 286 (49.5%, M^+), 258 (17%, M^+-CO), 153 [48%, M^+-$C_8H_5O_2$, fragment A (5)], 121 [29%, M^+-$C_8H_5O_4$, fragment B (5)], 93 (26%, M^+-$C_9H_5O_5$); uv, λmax (MeOH) 367, 322sh, 266, 255 nm.

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[1]Melting points were determined on a Thomas Hoover Capillary Melting Point Apparatus and are uncorrected. The uv spectra were recorded on a Perkin-Elmer Hitachi 200 spectrophotometer. The ir spectrum was recorded on a Perkin-Elmer 283 infrared spectrophotometer with polystyrene calibration at 1601 cm^{-1}. The ^{1}H-nmr spectra were recorded on a Varian XL-100 spectrometer. Mass spectra were recorded on a Hewlett-Packard 5930A spectrometer equipped with a 5933A data system. A Varian Aerograph model 2400 gas chromatograph equipped with a hydrogen flame ionization detector was used for gc determinations. The glass column (⅛″ x 6′) was packed with 2.5% SE-30 on gas chrom Q (80–100 mesh). The column temperature was 130°, the detector and injection port temperature were 250°, and the carrier gas (N_2) flow rate was 30 ml/min.

DISCUSSION

The aglycone of hydrolyzed I was identified as kaempferol by its mp, mw, ms fragmentation pattern (5), ^{1}H-nmr spectrum (6) and the uv spectrum in methanol and in various reagents [eg, NaOMe, $AlCl_3$, $AlCl_3$/HCl (7)]. On the basis of the ^{1}H-nmr spectrum of the acetate derivative and the gc retention time for the TMS-ether of the aqueous portion of the hydrolyzed glycoside, the glycoside was determined to contain two rhamnose units.

On the basis of the ^{1}H-nmr and uv spectra and the mp, I of *J. spicigera* was thus identified as kaempferol-3,7-bisrhamnoside (kaempferitrin), which was first isolated from *Indigo arrecta* (8) then from the leaves of *Trischosantes cucumeroides* and *T. japaniea* (9) and also from a number of other plants.

A trace amount of a kaempferol trirhamnoside was also isolated from the initial column chromatography. The ^{1}H-nmr spectrum of the isolated glycoside showed the presence of three rhamnose units. After hydrolysis the aglycone was identified as kaempferol and the sugar as rhamnose by gas chromatography of the TMS-ether. No other flavonoid (free or glycoside) was present in either hexane, chloroform, MEK, or aqueous fractions.

ACKNOWLEDGMENTS

The authors wish to thank Mrs. Mary Lou Hinojosa and Ms. Eloise Moreno for their help in the collection of plant material. This work was supported in part by a grant (E-745) from The Robert A. Welch Foundation, Houston, Texas.

Received 10 November 1980

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