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COUMARIN CONSTITUENTS OF THE CHINESE TALLOW TREE (*SAPIUM SEBIFERUM*)

PEIQUAN YANG[1] and A. DOUGLAS KINGHORN*

Program for Collaborative Research in the Pharmaceutical Sciences and Department of Medicinal Chemistry and Pharmacognosy, College of Pharmacy, Health Sciences Center, University of Illinois at Chicago, Chicago, Illinois 60612

The Chinese tallow tree [*Sapium sebiferum* (L.) Roxb.], which is native to semitropical areas in the People's Republic of China, has become naturalized in the southeastern United States (1,2). This tree is of considerable economic importance; for example, the seed tallow has applications in soap and candle making, while the seed kernel oil is used as an illuminant and as a drying oil (1). The tree grows very rapidly and has therefore been investigated for its woody biomass potential (2,3). In traditional Chinese medicine, the bark of the stems and roots of *S. sebiferum* (Chinese name "Wu-Jui") is used as a diuretic and a cathartic, as well as for the treatment of certain schistosomiasis infections (4).

In the present report, we wish to describe the isolation of three coumarin constituents of the bark and roots of the Chinese tallow tree, which were obtained through droplet counter-current chromatography. While no coumarins have been isolated before from this species, previous investigators have shown the presence of phloroacetophenone 2,4-dimethyl ether (5), xanthoxylin (6), and moretenone, moretenol, daucosterol, and a xanthoxylin glycoside (7) in the roots of *S. sebiferum*. Prior phytochemical studies on the bark of this species have afforded sebiferic, aleuritolic, and sebiferenic acids (8,9), 3-epimoretenol (10), and 3,4-di-*O*-methylellagic acid (11), as well as a flavanone-*O*-glycoside with antimicrobial properties (12).

Two of the three coumarins obtained in this study were readily identified by direct comparison with authentic samples. Thus, 6,7,8-trimethoxycoumarin was identified as a *S. sebiferum* bark constituent, and scopoletin was obtained from a root extract. The third such constituent was isolated from the roots of the Chinese tallow tree as an oil. It exhibited a molecular weight of 266 daltons, and demonstrated spectroscopic characteristics typical of a simple coumarin. In its ^{1}H-nmr spectrum, four methoxy resonances were observed at δ 3.902, 3.971, 3.980, and 4.045 ppm, and it was apparent that the α-pyrone ring was unsubstituted (13), due to the presence of doublets at δ 7.947 ppm (H-4) and δ 6.299 ppm (H-3). On the basis of the interpretation of these data, this isolate was identified as 5,6,7,8-tetramethoxycoumarin. The ^{1}H-nmr characteristics of this compound were found to be closely comparable to those published for synthetic 5,6,7,8-tetramethoxycoumarin (14). To date, 5,6,7,8-tetramethoxycoumarin has been identified as a constituent of only one plant, namely, *Artemisia tridentata* ssp. *vaseyana* (big sagebush) (15,16), as a result of which this compound was accorded the trivial name, artelin. However, this assignment was made more than a decade ago using only 0.5 mg of isolate (17). Direct comparison of the original spectral data of artelin with those of our isolate from *S. sebiferum* indicated several differences. In particular, the ^{1}H-nmr chemical shift of H-3 at δ 6.80 and the mass spectral base peak at *m/z* 105 of artelin were not

[1]A Visiting Scholar at the World Health Organization Collaborating Centre for Traditional Medicine, College of Pharmacy, University of Illinois at Chicago, on leave from Sichuan Medical College, Chengdu, Sichuan, People's Republic of China.

evident in the spectral data of our sample. Therefore, we must conclude that the previous identification of 5,6,7,8-tetramethoxycoumarin from *A. tridentata* ssp. *vaseyana* is erroneous, and that this compound has been obtained in the present investigation for the first time as a natural product.

EXPERIMENTAL

GENERAL EXPERIMENTAL PROCEDURES.—Melting points were measured on a Kofler apparatus and are uncorrected. The uv and ir spectra were obtained, respectively, on a DB-G grating spectrophotometer and on a Nicolet MX-1 FT-IR interferometer. ^{1}H-nmr spectra were recorded in $CDCl_3$, using TMS as internal standard, employing a Nicolet NT-360 instrument (360 MHz) or a Varian T-60A instrument, with a Nicolet TT-7 Fourier Transorm attachment (60 MHz). Low-resolution mass spectra were measured using a Varian MAT-112S double-focusing spectrometer, operating at 70 eV. Droplet counter-current chromatography (dccc) was performed at room temperature using a Model A instrument (Tokyo Rikakikai, Tokyo, Japan), that was equipped with a 10-ml sample chamber. Ascending development was employed for dccc, at a pressure of 2-4 kg/cm^2, and fractions (120 drops each) were collected into an automatic fraction collector. Preparative tlc was conducted on silica gel GHLF (Analtech, Inc., Newark, Delaware), with 250 μm thick layers, using cyclohexane-Et_2O-EtOAc (1:1:1, solvent 1) and $CHCl_3$-Et_2O (19:1, solvent 2) as developing solvents.

PLANT MATERIAL.—Separate samples of the roots and stem bark of *S. sebiferum*, collected in January 1983, were supplied by Dr. H.W. Scheld. These were collected from authenticated specimens cultivated at the University of Houston Coastal Center, near Hitchcock, Texas.

EXTRACTION AND FRACTIONATION.—Dried, chipped *S. sebiferum* roots (50 g) were macerated with three changes of MeOH at room temperature, and the residue obtained on solvent was exhaustively extracted with Me_2CO. After filtering and drying, the Me_2CO-soluble residue was partitioned between MeOH-H_2O (17:3, 100 ml) and hexane (3×100 ml). The polar layer was adjusted to a 1:1 v/v MeOH-H_2O ratio by the addition of 70 ml H_2O and was extracted with 3×100 ml CH_2Cl_2. The dried CH_2Cl_2 residue was fractionated by dccc, using a saturated mixture of hexane-Et_2O-*n*PrOH-EtOH-H_2O (7:16:6:10:8) as solvent, with the upper phase used as mobile phase. The sample (0.5 g) was dissolved in equal (5 ml) quantities of upper and lower phases prior ro introduction to the dccc apparatus. Fractions 78-136 from the dccc separation were purified by preparative tlc in solvent 2 (Rf 0.43) to afford 5.2 mg of 5,6,7,8-tetramethoxycoumarin. Fractions 137-280 from this preliminary separation afforded 130 mg of scopoletin, after repeated preparative tlc in solvent 2 (Rf 0.17).

This extraction and fractionation procedure was repeated with 50 g of *S. sebiferum* bark flakes. Dccc fractions 151-180 of the bark were purified by sequential preparative tlc in solvent 2 (Rf 0.36) and solvent 1 (Rf 0.33) to provide 5.5 mg of 6,7,8-trimethoxycoumarin.

5,6,7,8-TETRAMETHOXYCOUMARIN.—This root constituent (5.2 mg, 0.01% w/w), obtained as a yellow oil, exhibited the following data: uv λ max 346 nm (log ε 3.42); ir ν max (AgCl) 1734, 1598, 1470, 1449, 1418, 1393, 1356, 1145, 1068, and 1045 cm^{-1}; ^{1}H nmr (360 MHz, $CDCl_3$) δ 3.902, 3.971, 3.980, 4.045 (3H each, s, 4×-OCH_3), 6.299 (1H, d, J=9.9 Hz, H-3), and 7.947 (1H, d, J=9.9 Hz, H-4); ms *m/z* 266 (M^+, 100%), 251 (86), 223 (45), 208 (26), 193 (12), 180 (11), 165 (14), 109 (22), and 94 (12). The ^{1}H-nmr data obtained for this isolate compare very closely with those obtained for a synthetic sample of 5,6,7,8-tetramethoxycoumarin (14).

SCOPOLETIN.—This crystalline isolate, obtained from *S. sebiferum* roots (130 mg, 0.26% w/w), mp 195-197°, exhibited uv, ir, ^{1}H-nmr, and ms data consistent with literature values (13,18). Identity was confirmed by direct comparison (mmp, ms, tlc) with an authentic sample.

6,7,8-TRIMETHOXYCOUMARIN.—This isolate from the bark of *S. sebiferum* (5.5 mg, 0.011% w/w) exhibited a mp of 103-105°. On the basis of the comparison of its physical and spectroscopic data with those of the three known simple coumarins which have three methoxy groups in the aromatic ring, namely, 6,7,8- (19,20), 5,6,7- (20,21), and 5,7,8- (22,23) trimethoxycoumarins, this isolate was identified as 6,7,8-trimethoxycoumarin. Identity was confirmed by direct comparison (mmp, ^{1}H nmr, ms, tlc) with an authentic sample.

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