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# ADDITIONAL BIOLOGICALLY ACTIVE CONSTITUENTS OF THE CHINESE TALLOW TREE (*SAPIUM SEBIFERUM*)

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The Chinese tallow tree [*Sapium sebiferum* (L.) Roxb., Euphorbiaceae], a species native to the People's Republic of China now naturalized in the southeastern United States, is of considerable interest as an economic plant (1,2). Following our previous isolation of the new natural product, 5,6,7,8-tetramethoxycoumarin, and other coumarins from the bark and roots of *S. sebiferum* (3), we now wish to report the identification of six constituents of the leaves of this species, several of which are of known biological activity. Other workers have isolated ellagic acid, friedelin, sitosterol, and three phorbol esters from this plant part (4,5).

In the present investigation, a $CHCl_3$-soluble fraction of an MeOH extract of the leaves of the Chinese tallow tree was found to display significant in vitro activity against the P-388 lymphocytic leukemia test system when assessed using standard protocols (6). Phorbol esters, which have previously been shown to possess activity in this test system (7), were shown by application of a skin-irritancy test (8) to be absent from the plant sample studied in this investigation. Cytotoxic activity was traced by activity-guided fractionation to the *S. sebiferum* constituent, gallic acid, which exhibited an $ED_{50}$ of 0.7 μg/ml in the P-388 cell culture system. This compound has previously been found to show inhibitory activity against KB (9,10) and HeLa (11) cells but to be inactive when tested in vivo in the P-388 lymphocytic leukemia system over the dose range 50–200 mg/kg (9). Other compounds obtained in this study were astragalin, (−)-loliolide, kaempferol, quercetin, and β-sitosterol glycoside, none of which was found to be significantly cytotoxic to P-388 cells. However, astragalin is reported to have in vivo P-388 activity (12), and (−)-loliolide is a potent ant-repellent (13).

## EXPERIMENTAL

PLANT MATERIAL.—Leaf samples of *S. sebiferum* were collected from authenticated tree specimens cultivated at the University of Houston Coastal Center near Hitchcock, Texas.

EXTRACTION AND FRACTIONATION.—The dried plant material (2.15 kg) was exhaustively extracted with MeOH, and the dried residue taken up into $Me_2CO$. The $Me_2CO$-soluble extract was partitioned between petroleum ether (bp 60–80°) and MeOH-$H_2O$ (20:3), with the polar layer then extracted sequentially with $CHCl_3$, $Me_2CO$, and a second quantity of $CHCl_3$. The second $CHCl_3$-soluble residue was found to exhibit an $ED_{50}$ of 3.8 μg/ml when tested against cultured P-388 cells and was chromatographed over Si gel to afford gallic acid (0.04 g, 0.0019% w/w) as a cytotoxic constituent, with the inactive flavonoids, kaempferol (0.8 g, 0.037% w/w) and quercetin (0.9 g, 0.042% w/w), being obtained as major constituents from adjacent fractions. Astragalin (0.02 g, 0.0093% w/w) was obtained by fractionation of the initial $Me_2CO$ extract, and (−)-loliolide (0.009 g, 0.00042% w/w) and β-sitosterol glycoside (1.07 g, 0.05% w/w) were obtained by fractionation of the initial $CHCl_3$ extract. With the exception of gallic acid, none of these compounds was significantly cytotoxic against P-388

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cells. All of the isolates were identified by physical and spectral data comparison (mp, $[\alpha]_D$, uv, ir, $^1H$ nmr, $^{13}C$ nmr, ms) and/or direct comparison with authentic samples. Full details are available from the senior author.

BIOLOGICAL ACTIVITY OF THE ISOLATES.—When tested against P-388 lymphocytic leukemia cells according to standard protocols (6), gallic acid, kaempferol, quercetin, astragalin, (−)-loliolide, and β-sitosterol glycoside exhibited $ED_{50}$ values, respectively, of 0.7, 6.7, 40, 8.2, 15, and 53 μg/ml. An isolate is considered active in this system if it shows an $ED_{50}$ of ≤4.0 μg/ml (6).

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