methazine solubility was dependent on the sulfonamide content in the system (Table III). At low sulfamethazine concentrations (0.1 and 0.2 g %), citric acid did not significantly increase the lowered sulfamethazine solubility because of the saturation of the surface by the adsorbed benzoic acid. At higher sulfamethazine concentrations (4 and 10 g %), citric acid increased the equilibrium solubility by virtue of its effect on lowering the pH.

In conclusion, the effect of electrolytes such as sodium citrate on adsorption should be interpreted not only on the basis of changing the dielectric constant of the system (5) but also on the possible effect of the electrolyte on the system pH. This may alter the ionization of electrolytic adsorbates and the charge characteristics of the adsorbent.

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Isolation of Myricadiol, Myricitrin, Taraxerol, and Taraxerone from *Myrica cerifera* L. Root Bark

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Abstract □ Isolation and identification of three triterpenes, myricadiol, taraxerol, and taraxerone, and a flavonoid glycoside, myricitrin, from the root bark of *Myrica cerifera* L. are reported.

Keyphrases □ Myricadiol—isolation and identification from *Myrica cerifera* root bark □ Myricitrin—isolation and identification from *Myrica cerifera* root bark □ Taraxerol—isolation and identification from *Myrica cerifera* root bark □ Taraxerone—isolation and identification from *Myrica cerifera* root bark □ *Myrica cerifera* L.—isolation and identification of three triterpenes and one flavonoid glycoside

"Bayberry bark" (myrtle wax or southern bayberry), the dried root bark of *Myrica cerifera* L. (Myricaceae), is known to exhibit astringent, emetic, and antipyretic activities when administered orally (1, 2). In medical practice, it has been used externally as a stimulant for indolent ulcers (1). Recently, the aqueous extracts and the tannin fractions of *Acacia villosa, Krameria ixina,* and *K. triandra* were prepared, bioassayed, and found to be tumorigenic in rats (3, 4). In a continuation of these studies, the authors isolated tannins of *M. cerifera* L. root bark and leaves and are currently examining them for carcinogenicity. The present paper describes the identification of four crystalline nontannin compounds isolated during the fractionation of tannins from the root bark of *M. cerifera.*

EXPERIMENTAL

Isolation of Triterpenes—The powdered root bark[1] of *M. cerifera* (1 kg) was extracted with 3 liters of petroleum ether (bp 38–47°) in a continuous extractor for 24 hr. The defatted bark powder was then similarly extracted with benzene (3 liters) for 24 hr. The benzene extract was concentrated (~25 ml) and chromatographed on a column (2.5 cm i.d. × 30 cm) of neutral alumina[2] (40 g). The following sequence of solvents with increasing polarity was used for elution: petroleum ether (1 liter), petroleum ether–benzene (2:1 mixture, 1 liter; 1:1 mixture, 1 liter), benzene (1 liter), and benzene–chloroform (2:1 mixture, 1.5 liters).

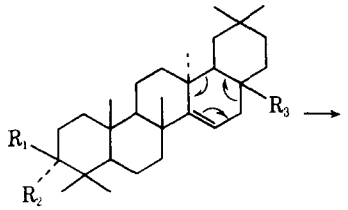
Taraxerone (I)—The petroleum ether–benzene (2:1) eluant of the alumina column was evaporated to dryness, and the residue was crystallized from petroleum ether–benzene to yield 280 mg of taraxerone, mp 238–239° [lit. (5) mp 242–244°]; mass spectrum: m/e 424 (M^+), 409 ($M - CH_3$), and 300 ($M - C_9H_{16}$). The IR and mass spectra of the isolated compound were identical to those of reference taraxerone. The melting point of the reference compound remained unchanged when mixed with the plant isolate.

Taraxerol (II)—Removal of the solvents from the petroleum ether–benzene (1:1) eluant of the alumina column and crystallization of the resulting residue from methanol yielded 141 mg of taraxerol, mp 282–283° [lit. (5) mp 282–283°]; mass spectrum: m/e 426 (M^+), 411 ($M - CH_3$), and 302 ($M - C_9H_{16}$). It formed a monoacetate derivative (IV), mp 302° [lit. (5) mp 304–305°]; mass spectrum: m/e 468 (M^+), 453 ($M - CH_3$), 408 ($M - HOCOCH_3$), and 344 ($M - C_9H_{16}$). The IR and mass spectra of both the isolated alcohol and its acetate derivative were identical to those of reference taraxerol and its acetate, respectively. The melting points of both reference compounds were unaltered when admixed with the respective plant products from *M. cerifera.*

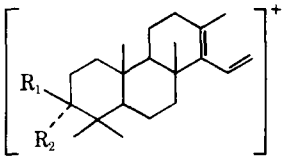
Myricadiol (III)—The benzene–chloroform (2:1) eluant of the alumina column deposited a semicrystalline precipitate when left overnight at room temperature. The precipitate was crystallized from hot alcohol (95%) to afford 450 mg of myricadiol, mp 267–268° [lit. (6) mp 273–274°]; mass spectrum: m/e 442 (M^+), 427 ($M - CH_3$), and 302 ($M - C_9H_{16}O$). Acetylation of the isolated compound with acetic anhydride–pyridine gave a diacetate (V), mp 254° [lit. (6) mp 256.5°]; mass spectrum: m/e 526 (M^+), 511 ($M - CH_3$), 466 ($M - HOCOCH_3$), and 344 ($M - C_{11}H_{18}O_2$). The IR

[1] *The plant material* used was collected in South Carolina and provided by Dr. J. F. Morton, Morton Collectanea, University of Miami, Coral Gables, Fla.

[2] Brockmann activity I, 80–200 mesh, Fisher Scientific Co.



I: $R_1, R_2 = O, R_3 = CH_3$
II: $R_1 = OH, R_2 = H, R_3 = CH_3$
III: $R_1 = OH, R_2 = H, R_3 = CH_2OH$
IV: $R_1 = OCOCH_3, R_2 = H, R_3 = CH_3$
V: $R_1 = OCOCH_3, R_2 = H, R_3 = CH_2OCOCH_3$



a

m/e 300, $R_1 = R_2 = O$
m/e 302, $R_1 = OH, R_2 = H$
m/e 344, $R_1 = OCOCH_3, R_2 = H$

Scheme I

and mass spectra of both the isolated diol and its diacetate derivative were identical to those of reference myricadiol and its diacetate, respectively. The melting points of both reference compounds remained unchanged when mixed with the respective plant isolates.

Isolation of Myricitrin—Following benzene extraction of the defatted bark powder, the marc was extracted with 95% alcohol (3 liters). The alcohol extract was concentrated (~150 ml) and, upon cooling, deposited a crystalline precipitate. The precipitate was recrystallized from alcohol to furnish 2.5 g of myricitrin, mp 197–199° [lit. (7) mp 197–199°]; mass spectrum: *m/e* 464 (M^+). Hydrolysis of the isolated compound by refluxing with 4% aqueous sulfuric acid for 15 min gave a sugar, rhamnose, and myricetin, mp 350° dec. [lit. (7) mp 350° dec.]; mass spectrum: *m/e* 318 (M^+). Rhamnose was identified by paper chromatography [solvent system of *n*-butanol–acetic acid–water (4:1:5)] and by preparation of the phenylosazone derivative, mp 183° [lit. (8) mp 185°], which did not depress the melting point of reference rhamnose phenylosazone when admixed. The IR, NMR, and mass spectra of isolated myricitrin and its aglycone, myricetin, were identical to those of respective reference compounds. The melting points of both reference compounds were unchanged when mixed with respective isolates from bayberry bark.

RESULTS AND DISCUSSION

The three triterpenes (I–III) isolated from the root bark of *M. cerifera* were readily identified as derivatives of Δ^{14}-taraxerene by their mass spectra, which showed the characteristic fragment *a* (Scheme I) resulting from retro-Diels–Alder reaction involving the 14,15-double bond (9, 10). The characterization of the isolated terpenoids as taraxerone (I), taraxerol (II), and myricadiol (III) was carried out by comparison with the reference samples, with identical IR and mass spectra and undepressed mixed melting points being the criteria for identity. Similarly, the acetate derivatives of II and III were identical to those of the reference compounds.

Identification of the isolated flavonoid glycoside as myricitrin was established by the NMR spectra of the glycoside and its acid hydrolysis product, myricetin, which were identical to the published reference spectra (11, 12). Similarly, the IR and mass spectra of the isolated myricitrin and its aglycone, myricetin, were identical to those of the reference compounds. Further characterization of the glycoside was carried out by the identification of rhamnose as a product of its mineral acid hydrolysis. Finally, the melting points of both reference myricitrin and myricetin were unchanged when mixed with respective compounds obtained from *M. cerifera* root bark.

These four compounds have been encountered in other *Myrica* species (13, 14). Two of these compounds have been found to be biologically active. Myricitrin is reported to exhibit choleretic (15), bactericidal, paramecicidal, and spermatocidal (16) activities, while myricadiol has mineralcorticoid activity (17).

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