comes into play. In the ion-pair counterpart, such as may exist in the protonated molecule, additional effects appear.

CONCLUSION

On the basis of NMR analyses of the salts of some cinchona alkaloids, it was concluded that the protonation site on these molecules varies with their spatial orientation and the nature of the solvent. In the process of changing the conformation, when moving from a polar to nonpolar solvent, the basicity of the quinuclidine nitrogen is reduced as the quinuclidine and quinoline moieties get closer to each other. The proton is transferred from the quinuclidine nitrogen to the quinoline nitrogen, the ion-pair feature, which enhances hydrophilic capacity, is lost, and the molecule assumes a neutral feature with stronger hydrophobic bonds. As a result of this process, the hydrophilic–lipophilic properties of the molecule change in favor of a more lipophilic character.

These findings may have an important bearing on the passage of I and its congeners from an aqueous to a lipoid phase. Thus, the molecule not only is adsorbed to a membrane, as suggested in Fig. 1, but also penetrates the membrane (Fig. 3). Given the opportunity, the solute will forsake the aqueous solution for the organic environment.

The variation in conformation found in the studied cinchona alkaloids and their salts may contribute to their differences in biological activity.

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NOTES

Biological and Phytochemical Investigation of Plants XVI: *Strumpfia maritima* (Rubiaceae)

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Abstract □ An aqueous extract of the flowering tops of *Strumpfia maritima* exhibited antifertility activity in female rats. The extract also contained the flavonol glycoside narcissin. This article represents the first reported isolation of this flavonoid from the Rubiaceae, as well as the first reported phytochemical and pharmacological investigation of the genus *Strumpfia*.

Keyphrases □ *Strumpfia maritima*—biological and phytochemical investigation, isolation of narcissin ■ Narcissin—isolation from *Strumpfia maritima* (Rubiaceae) □ Antifertility activity—narcissin, flavonoid isolation from *Strumpfia maritima*

Strumpfia maritima Jacq. (Rubiaceae) is a low, maritime shrub found in southern Florida (1), the Bahamas (2), Venezuela (3), and Curacao[1]. The plant has a folkloric use as a mosquito repellent (2) and contraceptive[1]. *In vivo* testing of aqueous methanol extracts showed that the roots were devoid of antimalarial activity (4). This paper describes a preliminary screening of extracts of *S. maritima* for antifertility activity, as well as the isolation of narcissin from one of these extracts.

RESULTS AND DISCUSSION

The powdered, defatted flowering tops of *S. maritima* were extracted with methanol. A phytochemical screening of this extract for alkaloids, saponins, sterols, cardenolides or bufadienolides, flavonoids, tannins or polyphenols, anthraquinones, and cyanogenic glycosides (5) revealed the presence of sterols, tannins and/or polyphenols, and flavonoid glycosides.

A methanolic extract was concentrated to dryness and partitioned between chloroform and water. Preliminary antifertility experiments showed that the aqueous extract administered daily (100 mg/kg ip) to rats significantly decreased the number of implantation sites relative to the number of corpora lutea of pregnancy ($p = 0.023$). No such decrease was seen in control rats treated simultaneously with the vehicle, 10% polysorbate 20. Furthermore, no such effect was seen in rats treated with either the petroleum ether or chloroform extracts, tested simultaneously with the 10% polysorbate 20 vehicle and sterol diluent control groups, respectively. These results are listed in Table I.

To isolate the biologically active compound(s) from the aqueous extract, the fraction was extracted with water-saturated 1-butanol, and the resulting organic extract was chromatographed over polyamide. Elution with distilled water and water–methanol afforded a yellow, microcrystalline flavonoid glycoside as a major component.

The UV spectrum of the isolate using standard shift reagents (7) indicated the presence of free 5-, 7-, and 4′-hydroxyl functions on the flavonoid nucleus. Hydrolysis of the glycoside with dilute acid (7) yielded isorhamnetin (melting point, mixed melting point, and UV, IR, PMR, and mass spectra were identical to the authentic compound) and two sugars. The sugars, present in equal proportions, were identified as glucose and rhamnose by TLC (8). A comparison of the UV spectra for the isolate and its aglycone in the presence of shift reagents indicated that the sugars were attached at position 3 of the aglycone. The PMR spectrum of the isolate as the trimethylsilyl ether showed signals for the rhamnosyl C-1 proton at 4.25 ppm and for the rhamnosyl methyl protons as a broad peak at 0.85 ppm, indicating that the rhamnoglucoside contains a rutinosyl rather than a neohesperidosyl moiety (7). Finally, the isolate

[1] Data collected by J. F. Morton.

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Table I—Antifertility Effects of *Strumpfia maritima* Extracts [a]

Number of Rats	Treatment	Number of Corpora Lutea (CL) ± *SD*	*p* of CL *versus* LF	Number of Live Fetuses (LF) ± *SD*	*p* of CL *versus* IS [b]	Number of Implantation Sites (IS) ± *SD*
6	Sterol diluent [c], 1 ml/kg ip	14.50 ± 1.64	0.008	12.00 ± 0.89	NS [d]	13.33 ± 1.21
5 [e]	Chloroform extract, 100 mg/kg ip	14.80 ± 2.95	NS	11.60 ± 1.52	—	—
6	10% Polysorbate 20, 1 ml/kg ip	13.50 ± 0.55	0.032	10.33 ± 3.08	NS	12.00 ± 2.45
6	Petroleum ether extract, 100 mg/kg ip	13.17 ± 1.72	0.014	10.50 ± 1.38	NS	12.17 ± 1.83
6	10% Polysorbate 20, 1 ml/kg ip	13.17 ± 1.17	NS	11.67 ± 2.42	—	12.00 ± 2.00
6	Aqueous extract, 100 mg/kg ip	13.33 ± 4.32	0.025	7.17 ± 3.76	0.023	7.50 ± 3.15

[a] Portions of these data were presented at the 17th annual meeting of the American Society of Pharmacognosy, Cable, Wis., July 1976 (Abstract 34). [b] A significant difference in this column means that the difference between the number of corpora lutea and the number of live fetuses could not be accounted for by the presence of dead, degenerating products of conception. [c] Sterol diluent consisted of 9 mg of benzyl alcohol, 4 mg of polysorbate 80, 5 mg of carboxymethylcellulose sodium, and 9 mg of sodium chloride/ml of distilled water (6). [d] Not significant. [e] The sixth rat was not pregnant at autopsy, presumably due to a failure to mate.

was found to be identical in all respects (TLC, melting point, mixed melting point, and UV, IR, and PMR spectra) to authentic narcissin.

The paucity of narcissin isolated did not permit testing for antifertility activity. Attempts are underway to obtain additional narcissin for biological evaluation. This work represents the first reported phytochemical and pharmacological investigation of *Strumpfia*, as well as the first isolation of narcissin from the Rubiaceae.

EXPERIMENTAL[2]

Extraction and Chromatography—Dried, powdered flowering tops of *S. maritima*[3] (5 kg) were defatted by continuous extraction with petroleum ether, air dried, and extracted continuously with methanol. The methanolic extract was concentrated *in vacuo* to a thick syrup (1.1 kg), which was partitioned between water (2.5 liters) and chloroform (6 × 2 liters). After drying (sodium sulfate), the chloroform extract was evaporated *in vacuo* to give a residue (171.2 g). The water extract was lyophilized to yield 333 g of a light powder. Aliquots of the petroleum ether, chloroform, and aqueous extracts were screened for antifertility activity.

A portion of the lyophilized aqueous extract (133.2 g) was redissolved in water (2 liters) and extracted with water-saturated 1-butanol (5 × 2 liters). Evaporation of the butanol extract *in vacuo* gave 22 g of a brown gum, which was dissolved in distilled water (200 ml) and applied to the top of a polyamide column[4] (550 g).

Isolation and Identification of Narcissin—Elution was initiated with distilled water (5.4 liters), followed by water–methanol at ratios of 24:1 (1.4 liters), 47:3 (1 liter), 23:2 (3.2 liters), 21:4 (1.8 liters), 7:3 (2 liters), and 1:1 (10.8 liters). After elution with 5.7 liters of water–methanol (1:1), a 500-ml fraction was obtained, which gave a yellow residue on drying *in vacuo*. Crystallization and recrystallization from warm methanol afforded 184 mg of yellow microcrystals, mp 179–180° dec., which gave a positive flavonoid test (7); TLC on cellulose[5] gave an R_f of 0.45 with 5% aqueous acetic acid; UV: λ_{max} (methanol) 356 (log ϵ 4.18), 300 (sh), 265 (sh), and 253 (4.26) nm; IR: ν_{max} 3330 (br), 2920, 1660, and 1605 cm^{-1}; PMR (trimethylsilyl ether[6] in carbon tetrachloride): δ 0.85 (broad, 3H, rhamnosyl CH_3), 3.2–4.0 (m, 10H, sugar H), 3.87 (s, 3H, C-3′ methoxyl), 4.23 (broad s, 1H, rhamnosyl H-1), 5.90 (m, 1H, glucosyl H-1), 6.10 (d, 1H, J = 2 Hz, H-6), 6.46 (d, 1H, J = 2 Hz, H-8), 6.86 (d, 1H, J = 8 Hz, H-5′), 7.41 (d, 1H, J = 2 Hz, H-2′), and 7.42 (dd, 1H, J = 2 and 8 Hz, H-6′).

Narcissin (20 mg) was hydrolyzed by mixing with 6% HCl (50 ml) and heating on a steam bath for 45 min. After cooling, the precipitated aglycone was filtered, and the filtrate was extracted three times with ether. The combined precipitate and ether extracts were recrystallized twice from ethanol. The aqueous filtrate was lyophilized and subjected to cellulose TLC in three solvent systems: ethyl acetate–pyridine–water (2:1:2), butanol–acetic acid–water (3:1:1), and pyridine–ethyl acetate–acetic acid–water (36:36:7:21) (8). Sugars were detected with *p*-anisidine phthalate spray reagent (8).

Antifertility Screening—Eight-week-old virgin female Sprague–Dawley rats were housed in environmentally controlled quarters, which provided 14 hr of light (5 am to 7 pm) per 24-hr period. After the animals had 1 week to acclimate to the environment, daily vaginal smears were initiated to provide information concerning their estrous cycles. On the 12th day of taking smears, intraperitoneal injections were begun and continued daily throughout the experiment. Groups of six rats were each administered 100 mg/kg of one of the extracts (the chloroform extract suspended in sterol diluent or the petroleum ether or aqueous extract suspended in 10% polysorbate 20). Control groups, six rats each, were treated simultaneously with the respective vehicle at 1 ml/kg.

On the 12th day of dosing, proven breeded males were placed in cages of the individually housed females. The day on which sperm were found in the smear or on which a plug was found in the vagina was defined as Day 1 of pregnancy. Rats were autopsied on Day 20 of pregnancy. The number of implantation sites in the uterus, equaling the number of live plus degenerating fetuses, was counted and compared with the number of corpora lutea of pregnancy found in the ovaries.

[2] Melting points were determined using a Kofler hot-stage instrument and are uncorrected. UV and IR spectra were recorded using a Beckman model DB-G grating spectrophotometer and a Beckman model IR-18A spectrophotometer with polystyrene calibration at 1601 cm^{-1}, respectively. PMR spectra were recorded at 60 MHz on a Varian T-60A instrument with a Nicolet model TT-7 Fourier attachment, using tetramethylsilane as the internal standard. Mass spectral data were obtained on a Hitachi Perkin-Elmer model RMU-6D spectrophotometer.

[3] The flowering tops of *S. maritima* Jacq. were collected (July 1971) in Curacao and identified by J. F. Morton. A voucher specimen was deposited at the Morton Collectanea, University of Miami, Coral Gables, Fla.

[4] MN-polyamide SC-6, Macherey Nagel and Co., Düren, West Germany.

[5] Precoated aluminum-backed cellulose TLC plates, E. Merck, Darmstadt, West Germany.

[6] Narcissin was trimethylsilylated (7) using Sil-Prep, Applied Science Division, Milton Roy Co. Laboratory Group, State College, Pa.

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