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Impact of Stable Conformation of Cinchona Alkaloids on Protonation Site

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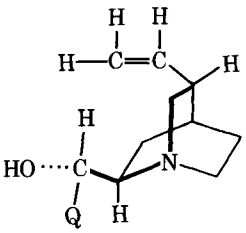
Abstract □ NMR analyses of quinidine and other cinchona alkaloids and their monoprotonated salts in deuterium oxide and in deuterochloroform revealed that the molecules assume new conformations in polar and nonpolar media, affecting the protonation site and hydrophilic–lipophilic characteristics. The ion-pair feature of the salts is lost and the molecules assume a neutral feature when they are transferred from an aqueous to a lipoid phase. Hydrophobic bonds between the molecules and their environment and within the molecule itself may affect the binding of cinchona alkaloids to membranes in biological fluids.

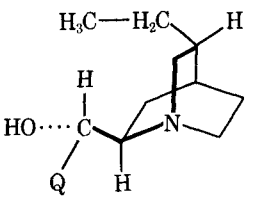
Keyphrases □ Quinidine—impact of stable conformation on protonation site, effect of polar and nonpolar solvents on conformation, NMR analyses □ Alkaloids, cinchona—impact of stable conformation on protonation site, effect of polar and nonpolar solvents on conformation, NMR analyses □ Conformation—impact on protonation site of quinidine and other cinchona alkaloids, effect of polar and nonpolar solvents, NMR analyses □ NMR spectroscopy—analyses of quinidine and other cinchona alkaloids, impact of stable conformation on protonation site, polar and nonpolar solvents

The protonation site on the quinidine molecule (I) might have an important bearing on its disposition, protein binding, and activity in the body. When the nitrogen of the quinuclidine ring of I is uncharged at pH 10, the number of receptor areas in albumin increases from one to three, possibly because the polar effect of the positively charged nitrogen is lost (1). At pH 7.4, however, the quinuclidine ring apparently does not play a great part in the binding of I to albumin, as was demonstrated in the competitive inhibitory effects of related quinoline compounds on the binding of I to albumin (2). It was postulated (3) that the cardiac action of I may result from special orientation of the molecule at interfaces, with binding of the quinoline ring to membrane lipoprotein and with the charged quinuclidine ring in the aqueous phase affecting ion movement across the cardiac cell (Fig. 1).

Any factors that may change the protonation site in I, such as the polarity of the surrounding medium and the dependence of conformation on this polarity, might affect the binding of I to plasma or cell or enzyme protein and, hence, its distribution, activity, and metabolism. This study explored the dependence of hydrophilic–lipophilic and structural properties of the salts of I and other cinchona alkaloids on solvent polarity. NMR analyses of the alkaloids and their salts were utilized.



I



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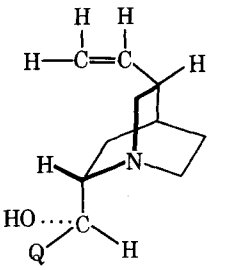

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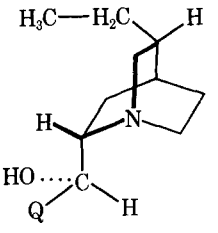
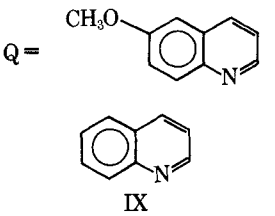


VII



IX

EXPERIMENTAL

NMR spectra were obtained at 300 MHz using an analytical NMR spectrometer[1] equipped with an automatic recorder. Tetramethylsilane in deuterochloroform and 2,2,3,3-tetradeutero-3-(trimethylsilyl)propionic acid sodium[2] and tetrahydrofuran in deuterium oxide were the internal standards. Pure quinidine (I), quinidine monohydrochloride (II), hydroquinidine (III), and hydroquinidine monohydrochloride (IV) were obtained from commercially available quinidine sulfate USP[3]. Pure quinine (V), quinine monohydrochloride (VI), hydroquinine (VII), and hydroquinine monohydrochloride (VIII) were obtained from commercially available quinine sulfate USP[4]. Hydrogenation of I and V was

[1] Bruker W.H. 300.
[2] Merck AG, Darmstadt, West Germany.
[3] Sigma Chemical Co., St. Louis, Mo.
[4] New York Quinine and Chemical Works, New York, N.Y.

0022-3549/81/0600-0679$01.00/0

Table I—Chemical Shift Values [a] of the Protons of I, III, V, and VII (20 ± 1 mg/0.5 ml)

Proton	I	III	V	VII
H-2′	8.58 (d) (J = 4 Hz)	8.57 (d) (J = 4 Hz)	8.48 (d) (J = 4 Hz)	8.45 (d) (J = 4 Hz)
H-8′	7.93 (d) (J = 9 Hz)	7.91 (d) (J = 9 Hz)	7.89 (d) (J = 9 Hz)	7.84 (d) (J = 9 Hz)
H-3′	7.51 (d) (J = 4 Hz)	7.53 (d) (J = 5 Hz)	7.47 (d) (J = 4 Hz)	7.41 (d) (J = 4 Hz)
H-7′	7.28 (q) (J_m = 3 Hz) (J_o = 9 Hz)	7.25 (q) (J_m = 2 Hz) (J_o = 9 Hz)	7.26 (q) (J_m = 3 Hz) (J_o = 9 Hz)	7.20 (q) (J_m = 2 Hz) (J_o = 9 Hz)
H-5′	7.16 (d) (J = 2 Hz)	7.11 (broad s)	7.21 (d) (J = 2 Hz)	7.14 (broad s)
H-10	6.03 (m)	—	5.71 (m)	—
H–C-9–OH	5.61 (d) (J = 4 Hz)	5.83 (d) (J = 4 Hz)	5.53 (d) (J = 4 Hz)	5.48 (d) (J = 3 Hz)
H-11	5.07 (broad s)	—	4.95 (d) (J = 16 Hz)	—
H-11	5.02 (broad s)	—	4.90 (d) (J = 10 Hz)	—
CH_3O	3.83 (s)	3.77 (s)	3.86 (s)	3.78 (s)

[a] s = singlet, d = doublet, m = multiplet, and q = quartet. Values are expressed in parts per million.

conducted in a pressure reaction apparatus[5]. Pure quinoline (IX) and quinoline hydrochloride (X) were obtained from commercially available quinoline[6]. TLC and preparative TLC were carried out on aluminum oxide GF 254[7] plates.

For NMR spectra measurements of the salts in deuterium oxide, an internal standard such as tetrahydrofuran (the chemical shifts of which were determined relative to 2,2,3,3-tetradeutero-3-(trimethylsilyl)propionic acid sodium) had to be used since the NMR spectra of the samples containing 2,2,3,3-tetradeutero-3-(trimethylsilyl)propionic acid sodium did not completely match the spectra without an internal standard. These discrepancies may originate from a proton transfer from the nitrogen to the carboxylate group of 2,2,3,3-tetradeutero-3-(trimethylsilyl)propionic acid sodium or from its influence on the mode of association of the cinchona salts. Since tetrahydrofuran was not completely without effect on association between molecules, except in the spectra of X, the absolute chemical shift values were eventually determined by matching the spectra in the presence and absence of the two different internal standards. This technique was possible because most chemical shifts, especially those on the margins, coincided.

Purification of Commercial I and V—Purification was carried out according to a procedure described previously (4). Table I lists the NMR data.

Reduction of I to III—Compound I (1 g) was dissolved in ethanol (50 ml) in a suitable vessel. Palladium-on-carbon (5%, 0.1 g) was added, and the hydrogenation was allowed to proceed for 1.5 hr at room temperature in the pressure reaction apparatus. The catalyst was removed by filtration through very retentive filter paper, the ethanol was evaporated, and III was readily crystallized. Table I lists the NMR measurements. Compound III was purified by preparative TLC (4).

Reduction of V to VII—Hydrogenation and purification were conducted as already described. Table I lists the NMR data.

Purification of IX—Purification was carried out according to a known procedure (5).

Preparation of Hydrochloride Salts—Each free base (I, III, V, VII, and IX) was dissolved in an accurate amount of dilute aqueous solution of hydrochloric acid. Water was removed under reduced pressure or allowed to evaporate spontaneously, and the residue of each salt was dried to constant weight.

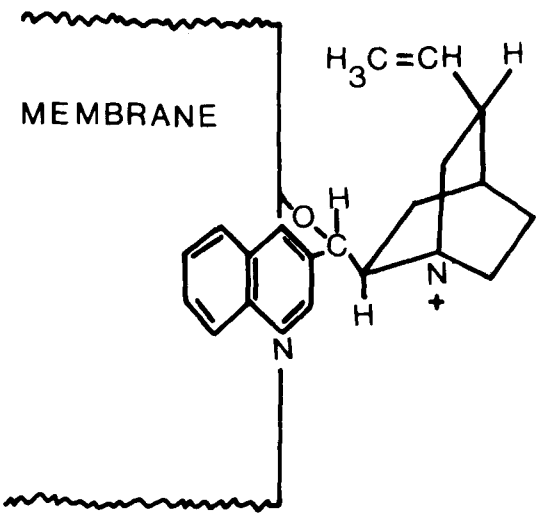


Figure 1—*Quinidine membrane binding hypothesis (3).*

RESULTS

Comparison of the NMR data of I and II in deuterochloroform revealed that the aromatic protons of the pyridine ring in II moved to a lower field. The deviation of H-2′ was 0.01 ppm, and that of H-3′ was 0.15 ppm. This result is to be expected in protonated pyridine (6). In contrast, the protons of the benzene ring in II experienced a shielding effect and shifted upfield: H-8′ to 0.25 ppm, H-5′ to 0.37 ppm, and H-7′ to 0.38 ppm. In X (Table II), all aromatic protons absorbed at a lower field under the influence of the protonated nitrogen. These differences are attributable partly to the methoxy group at C-6′.

An outstanding deshielding effect on the proton H–C-9–OH, which appeared as a singlet, was observed in II when it moved 0.89 ppm downfield. This shift is in agreement with the reported value for a proton that is alpha to protonated nitrogen or for vinylogous hydrogen. It probably applies also to a proton alpha to a positively charged carbon (or to vinylogous hydrogen) such as C-2′ and C-4′ in the quinoline cation.

These deviations observed in the chemical shifts may be rationalized if the protonation site is assumed to be on the quinoline nitrogen and not on the quinuclidine nitrogen, which has a much higher pKa value. Had the protonation taken place on the quinuclidine nitrogen, the proton H–C-9–OH would have been deshielded to a lesser extent (0.35 ppm).

NMR data of II in deuterochloroform and in deuterium oxide showed that the proton H–C-9–OH, appearing as a singlet, shifted downfield only by 0.42 ppm in the latter, indicating that this proton could not be at a position alpha to protonated nitrogen. Other evidence was provided when the chemical shift values of the aromatic protons of the salts in the different solvents were compared. In deuterium oxide, the H-2′ and H-3′ protons and some protons of the benzene moiety resonated at lower fields (Table II). These observations imply that the protonation site is on the quinuclidine nitrogen in deuterium oxide.

Table II also shows that in II in deuterium oxide, as compared to the free base in deuterochloroform, the methoxy protons and all aromatic protons, except H-8′, experienced a paramagnetic effect.

NMR analyses of III, V, and VII and their salts (IV, VI, and VIII) in the two different solvents (Table II) demonstrated the same trend in chemical shifts, although they varied in their intensity. This finding indicated that the protonation site in these molecules is the same as that observed in I, *i.e.*, on the quinoline nitrogen in the nonpolar solvent and on the quinuclidine nitrogen in the polar solvent.

DISCUSSION

Theoretically, protonation on the quinoline nitrogen should cause a paramagnetic effect on all aromatic protons, as was observed in X. In II in deuterochloroform, the diamagnetic effect observed on the benzene ring protons was attributed to the methoxy group at C-6′. The electron-releasing ability of this group, the capability of the molecule to pass to another conformation, and the concomitant bonding of a proton to the quinoline nitrogen in the neutral molecule increase the probability of protonation on this nitrogen. In VI in deuterochloroform, the paramagnetic effect on the pyridine protons was more pronounced than in II whereas the diamagnetic effect on the benzene protons was reduced (Table II). This result is expected when the conformations and pKa values of the two molecules are compared.

Protonation on the quinoline nitrogen causes the molecules to assume a new stable conformation in which the steric requirements for protonation on the quinuclidine nitrogen are more severe since it is now less exposed. This change is reflected in the change in the dihedral angle between the C-8 and C-9 protons. In the salts, this angle approaches 90° and is caused by rotation about the C-8–C-9 bond. The arrangement of the protons alpha to the quinuclidine nitrogen with respect to the aro-

[5] Parr Instrument Co., Moline, Ill.
[6] Hopkin and Williams, Ltd.
[7] Stahl, Merck.

Table II—Differences between the Chemical Shift Values [a] of the Salts (II, IV, VI, VIII, and X) and the Free Bases (I, III, V, VII, and IX) (20 ± 1 mg/0.5 ml)

Proton	II − I		IV − III		VI − V		VIII − VII		X − IX	
	$CDCl_3$	D_2O	$CDCl_3$	D_2O	$CDCl_3$	D_2O	$CDCl_3$	D_2O	$CDCl_3$	D_2O
H-2′	+0.01	+0.12	+0.07	+0.15	+0.19	+0.25	+0.16	+0.29	+0.30	+0.10
H-3′	+0.15	+0.26	+0.26	+0.33	+0.30	+0.43	+0.42	+0.43	+0.67	+0.66
H-5′	−0.37	+0.09	−0.31	+0.22	−0.34	+0.07	−0.20	+0.11	+0.42	+0.23
H-7′	−0.38	+0.13	−0.28	+0.26	−0.27	+0.19	−0.17	+0.16	+0.33	+0.26
H-8′	−0.25	−0.09	−0.12	+0.03	−0.11	−0.05	+0.02	—	+0.59	−0.01
H-10	−0.02	—	—	—	−0.18	−0.10	—	—	—	—
H–C-9–OH	+0.89	+0.42	+1.10	+0.16	+0.90	+0.50	+0.92	+0.62	—	—
H-11	+0.15	+0.22	—	—	+0.08	—[b]	—	—	—	—
H-11	+0.15	+0.21	—	—	+0.08	—[b]	—	—	—	—
CH_3O	−0.33	+0.16	+0.16	+0.22	−0.23	+0.07	−0.14	+0.22	—	—
H-4	—	—	—	—	—	—	—	—	+0.82	+0.82
H-6	—	—	—	—	—	—	—	—	+0.35	+0.32

[a] + = downfield shift, and − = upfield shift. [b] Overlap with water. Values are expressed in parts per million.

matic rings is greatly dependent on the dihedral angle; therefore, their chemical shifts in the salts will differ from those in the free bases. The extent of these differences helps to establish the nature of the new rotamers. Four of the five protons alpha to the quinuclidine nitrogen in II in deuterochloroform moved ~0.33 ppm downfield in comparison to the same protons in the free base as a result of being closer to the pyridine ring. Proton H-8 in II was ~1 ppm downfield in comparison to its chemical shift in I as a result of facing the pyridine ring instead of the benzene ring. In V, the proton alpha to the quinuclidine nitrogen that was the most deshielded was one of the C-6 protons (Table III) since the new conformation placed it closer to the pyridine ring.

Supporting evidence for differences in conformation was provided by the analysis of the chemical shifts of vinylic and the methoxy protons in deuterochloroform. In II, the methylene protons at C-11 appeared at a field lower than that of their counterparts in the free base, and the chemical shift of the methine proton at C-10 was practically unchanged. In VI, the reverse behavior was observed when compared to its free base. This finding suggests that, in I, the vinylic moiety is arranged as in A and, in V, it is arranged as in B (Fig. 2). The protons of the methoxy group in the salts (II, VI, and VII) resonated at a higher field than in the free bases. Protonation on the quinoline nitrogen should have caused a deshielding effect on these protons. The fact that a diamagnetic effect was observed instead implies that the conformation of the molecules had changed greatly; *i.e.*, a rotation of 180° around the C-4′–C-9 bond had occurred. In this new conformation, the protons of the methoxy groups are within the shielding zone of the vinyl and ethyl cone, the nonpolar hydrophobic moieties are on one side of the molecule, and the two nitrogens are on the opposite side. In IV, the protons of the methoxy group experienced the expected paramagnetic effect since the suggested rotation did not take place because of severe steric requirements of the ethyl group at C-3.

NMR analysis of II in deuterium oxide revealed that the protonation site was on the quinuclidine nitrogen, as expected in such a polar solvent, since the positive charge on this nitrogen is stabilized by solvation. It further showed that the molecule assumed a new conformation. This change was best reflected in the pronounced paramagnetic effect on the methoxy, vinylic, and some aromatic protons in this solvent *versus* deuterochloroform.

Changes in conformation also were reflected in the NMR data of II in deuterium oxide and of the free base in deuterochloroform. The paramagnetic effect observed on almost all protons of II in deuterium oxide could be rationalized if strong hydrogen bondings between the solvent and polar groups in the molecule took place. These enhanced hydrogen bondings are a result of the lipophilic nature of the cinchona alkaloids and the high free energy content of these molecules in aqueous solution. The hydrogen bonding between the methoxy group and the solvent nullifies the electron-releasing ability of this group; consequently, the aromatic protons in the benzene ring do not move to higher field. Hydrogen bonding between the pyridine nitrogen and the solvent caused the observed shift in the protons of this group. To account for the pronounced shift of H-8′ to a higher field in the salt in deuterium oxide, the anisotropy of the quinoline nitrogen lone pair has to be invoked. This effect is present in the free base in the nonpolar solvent and is eliminated in the salt in deuterium oxide as a result of hydrogen bonding (solvation). Since neutral moieties are involved in hydrogen bonding (7, 8), only the anisotropic effect of the nitrogen lone pair on the neighboring protons

Table III—Chemical Shift Values [a] of the Protons Alpha to the Quinuclidine Nitrogen of I and V (20 ± 1 mg/0.5 ml)

Proton	I	Proton	V
H-8	3.47 (q) (1H)	H-6	3.49 (m) (1H)
H-2	3.07 (m) (1H)	H-6 + H-8	3.06 (q) (2H)
H-2 + H-6	2.93 (q) (2H)	H-2	2.65 (m) (2H)
H-6	2.80 (m) (1H)		

[a] m = multiplet, and q = quartet. Values are expressed in parts per million.

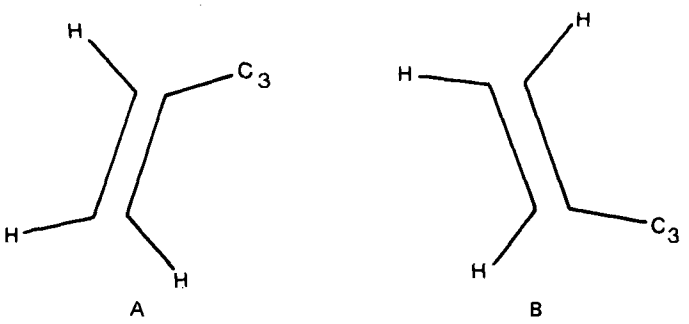


Figure 2—*Arrangement of the vinylic moiety in I and V.*

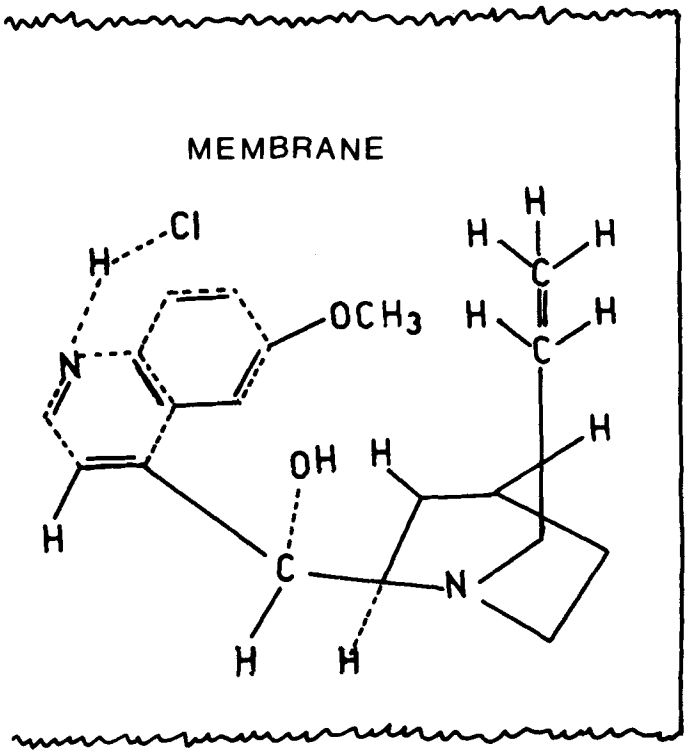


Figure 3—*Penetration of the monohydrochloride salt of I (II) through a membrane.*

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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Received May 15, 1980, from the **Department of Pharmacognosy and Pharmacology, College of Pharmacy, University of Illinois at the Medical Center, Chicago, IL 60612*, and the ‡*Morton Collectanea, University of Miami, Coral Gables, FL 33124.* Accepted for publication November 5, 1980. §Present address: Stevia Company, Arlington Heights, IL 60005.

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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