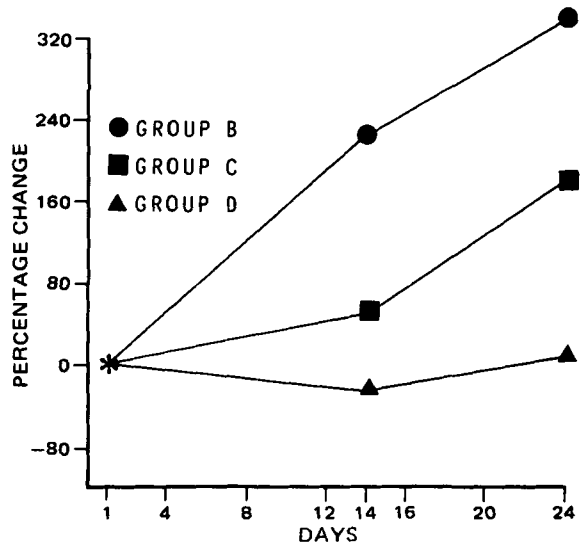

Figure 3—*Percentage change from Group A in mean serum cholesterol of chicks in stigmasterol experiment.*

serum and liver cholesterol values. The coloration of livers closely paralleled the serum cholesterol concentration.

Consistent with the literature (1–3, 7–11), the results of these experiments illustrate the potent antihypercholesterolemic activity of cholestyramine and β-sitosterol. Furthermore, the antihypercholesterolemic activity of these two compounds appears to be of the same order of magnitude, as reported previously (7, 12–14).

Although present in the diet of Groups C and D, neither β-sitosterol nor campesterol (which represents about 40% of the β-sitosterol NF employed) could be detected in the serum. This finding supports the theory that their action is due to their competitive inhibition of cholesterol absorption.

In contrast to the literature (19), the results indicate that stigmasterol, while apparently not exhibiting any deleterious effects, is ineffective as an antihypercholesterolemic agent. It appears, therefore, that unsaturation at C_{22} (the only structural difference between β-sitosterol and stigmasterol) is an undesirable factor in effecting antihypercholesterolemic activity.

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Chemical Constituents of *Echites hirsuta* (Apocynaceae)

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Received June 8, 1978, from the **Department of Pharmacognosy, School of Pharmacy, University of Pittsburgh, Pittsburgh, PA 15261*, and the ‡*Lilly Research Laboratories, Eli Lilly and Company, Indianapolis, IN 46206.* Accepted for publication July 28, 1978. §Present address: College of Pharmacy, University of Iowa, Iowa City, IA 52240.

Abstract □ A phytochemical investigation of an ethanolic extract of the whole plant of *Echites hirsuta* (Apocynaceae) resulted in the isolation and identification of the flavonoids naringenin, aromadendrin (dihydrokaempferol), and kaempferol; the coumarin fraxetin; the triterpene ursolic acid; and the sterol glycoside sitosteryl glucoside.

Keyphrases □ *Echites hirsuta*—whole plant ethanolic extract, various flavonoids and sterol glycoside isolated and identified □ Flavonoids, various—isolated and identified in whole plant ethanolic extract of *Echites hirsuta* □ Sitosteryl glucoside—isolated from whole plant ethanolic extract of *Echites hirsuta*

Echites hirsuta (Apocynaceae) (1), also known as *Mandevilla hirsuta* Malme (2), is a woody vine indigenous to the subtropics and tropics (3). Although no medicinal uses have been reported for this genus, the total absence of any phytochemical studies on *Echites* species and the established toxicity of many Apocynaceous plants prompted a phytochemical investigation of this plant.

DISCUSSION

The plant material was extracted by percolation with ethanol to ex-

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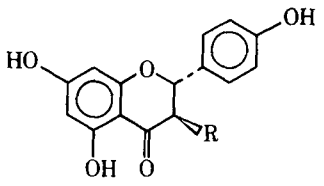

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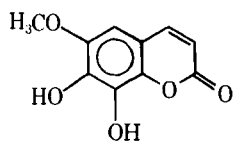
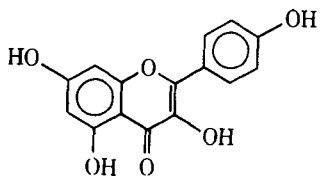
III: R = OH



II



IV

haustion. The ethanol was evaporated under reduced pressure, and the resulting extract was partitioned between chloroform and water. The aqueous layer was further partitioned with ethyl acetate to afford Fractions A (ethyl acetate) and B (water). The chloroform layer was evaporated, and the resulting residue was partitioned between petroleum ether and aqueous methanol to give Fractions C (petroleum ether) and D (aqueous methanol).

Fractions A and D were subsequently combined because of TLC similarities, and the resulting fraction was chromatographed (Column I) over silicic acid. Rechromatography of selected fractions from Column I over silicic acid (Column II) afforded ursolic acid, naringenin (I), fraxetin (II), and sitosteryl glucoside. Further chromatography of selected fractions from Column II yielded aromadendrin (dihydrokaempferol) (III) and kaempferol (IV).

The occurrence of sterols and triterpenes in higher plants is rather common (4); therefore, the isolation of the pentacyclic triterpene ursolic acid and the sterol glycoside sitosteryl glucoside is not unusual. Naringenin (I) is a commonly occurring flavanone (5) in genera of the Rutaceae (6) but also has been found in genera of the Anacardiaceae, Acanthaceae, Balanophoraceae, Compositae, Cruciferae, Fagaceae, Leguminosae, and Myrtaceae (6). However, this paper is the first reported isolation of naringenin from a member of the Apocynaceae. Naringenin may also be obtained by the hydrolysis of the glycoside naringin (naringenin 7-rhamnoglucoside), commonly found in the flowers and fruits of grapefruit and citrus species (6).

Fraxetin (II) is an aglucone of the coumarin glycoside fraxin (8-β-D-glucopyranosyl-7-hydroxy-6-methoxycoumarin), and both are commonly found in species of the genus *Fraxinus* (Oleaceae) (7). This report appears to be the first isolation of fraxetin from a genus of the Apocynaceae. Aromadendrin (III), also known as dihydrokaempferol, is a widely distributed dihydroflavonol (flavanonol), being found in genera of the families Cercidiphyllaceae, Cupressaceae, Ericaceae, Fagaceae, Leguminosae, Moraceae, Myrtaceae, Pinaceae, Platanaceae, Podocarpaceae, and Rosaceae (8). This dihydroflavonol also commonly exists as its 3-*O*-rhamnoside and 7-*O*-glucoside (8). Kaempferol (IV) is a very commonly occurring flavonol, being nearly ubiquitous in woody angiosperms (9) and occurring as glycosides of many kinds.

This paper is the first reported isolation of any constituents from *E. hirsuta* and, apparently, the first reported isolation of naringenin, fraxetin, and aromadendrin from a genus of the family Apocynaceae.

EXPERIMENTAL[1]

Plant Material—The whole plant of *E. hirsuta* (Apocynaceae) was used[2].

Extraction—Air-dried ground whole plant (11 kg) was extracted by percolation with ethanol (55 liters). The extract was evaporated *in vacuo* at 40° and left a dark-green sirupy residue (1.79 kg).

Fractionation—The residue was partitioned between chloroform (5 liters) and water (5 liters). The aqueous layer was further partitioned with ethyl acetate (5 liters), and the solvent was removed from each layer to afford Fractions A (ethyl acetate) (142 g) and B (water) (562 g). The chloroform layer was evaporated to leave a dark residue (787 g), which was further partitioned between petroleum ether (4 liters) and 10% aqueous methanol (4 liters).

The solvent was removed from each layer to afford Fractions C (petroleum ether) (305 g) and D (aqueous methanol) (418 g). TLC on silica gel G[3] using chloroform–ethyl acetate (1:3) showed a very similar chromatographic pattern between Fractions A and D after spraying with vanillin–sulfuric acid reagent (11). Therefore, Fractions A and D were combined for further chromatographic separation.

Chromatography (Column I)—Fractions A and D (560 g) were dissolved in methanol (2 liters), combined, and adsorbed onto silicic acid[4] (100 mesh) (1 kg). The adsorbed mixture was triturated to a fine, dry powder in a mortar and sifted on top of a column of silicic acid[4] (1 kg) (Column I) in petroleum ether. Elution was carried out with petroleum ether, petroleum ether–chloroform mixtures, chloroform, and chloroform–methanol mixtures.

Elution with petroleum ether (4 liters) and petroleum ether–chloroform mixtures (10 liters) afforded lipophilic fractions (192.3 g), which were placed aside. Elution with chloroform (2 liters) and then chloroform–methanol mixtures (99:1, 95:5, 4:1, 7:3, and 1:1) (2 liters each) afforded fractions, which were combined according to TLC similarity [silica gel G[3]; chloroform–ethyl acetate (1:3) and chloroform–methanol (1:1); vanillin–sulfuric acid detecting reagent (10)].

Isolation of Ursolic Acid—Combination of the chloroform through chloroform–methanol (4:1) eluents gave a residue (57.35 g). On treatment with methanol, this residue afforded fine white needles of ursolic acid (230 mg), mp 235° [lit. (11) mp 242° (petroleum ether–methanol)], $[\alpha]_D^{27}$ +73.3° (c 0.3, chloroform) [lit. (11) $[\alpha]_D$ +70° (c 0.4, chloroform)]; IR: ν_{max} (potassium bromide) 3420, 2960, 1700, 1460, and 1040 cm^{-1}; mass spectrum (M^+): *m/e* 456 (4%), 248 (100), 207 (25), 203 (33), and 133 (14). The triterpene was identified as ursolic acid by direct comparison (11) (IR and mass spectra, melting point, mixed melting point, optical rotation, and co-TLC).

Chromatography (Column II)—Combination of the chloroform–methanol 7:3 and 1:1 fractions from Column I gave a residue (310 g). A portion (30 g) of this residue was adsorbed onto silicic acid[4] (67 g) and chromatographed over silicic acid[4] (300 g) in chloroform. Elution with chloroform (5 liters) and chloroform–methanol (95:5) (3 liters) afforded additional ursolic acid (520 mg), while elution with chloroform–methanol (9:1) (1 liter) yielded a residue (5.72 g).

Isolation of Naringenin (I)—Chromatography of this residue over silica gel[3] (100 g) and elution with chloroform–ethyl acetate (1:5) (500 ml) yielded a solid. On treatment with hot methanol, this solid afforded white needles of naringenin (256 mg), mp 260° [lit. (13) mp 255–256°], $[\alpha]_D^{27}$ −15.7° (c 0.7, methanol) [lit. (12) $[\alpha]_D^{20}$ −28.1° (c 1.82, ethanol)]; UV: λ_{max} (methanol) 230 (log ε 3.83), 290 (3.69), and 333 (3.04) nm; IR: ν_{max} (potassium bromide) 3250 (br), 1630, 1605, 1520, 1500, 1460, 1312, 1250, 1182, 1160, 1085, 1065, 1015, 890, 845, and 835 cm^{-1}; mass spectrum (M^+): *m/e* 272 (100%), 179 (30), 166 (30), 153 (100), 124 (11), 119 (12), 107 (19), 91 (13), and 69 (14). This compound was identified as naringenin by direct comparison[5] (UV, IR, and mass spectra, melting point, mixed melting point, optical rotation, and co-TLC).

Isolation of Fraxetin (II)—Continued elution with chloroform–ethyl acetate (1:5) (500 ml) afforded a yellow residue. On crystallization from methanol, this residue gave light-yellow plates of fraxetin (150 mg), mp 240° [lit. (13) mp 228° (ethanol)]; UV: λ_{max} (methanol) 218 (log ε 3.34), 260 (sh) (2.54), and 341 (307) nm; IR: ν_{max} (potassium bromide) 3540 (br), 1690, 1615, 1580, 1510, 1470, 1420, 1325, 1280, 1165, 1120, 1082, 1025, 930, and 835 cm^{-1}; mass spectrum (M^+): *m/e* 208 (100%), 193 (35), 180 (14), 165 (14), 137 (15), 109 (14), and 81 (13). This coumarin was found to be identical to fraxetin[6] by direct comparison (UV, IR, and mass spectra and melting point).

Isolation of Sitosteryl Glucoside—Continued elution of Column II with chloroform–methanol (85:15) (1 liter) gave a light-green residue (7.30

[1] Melting points were taken on a Thomas-Hoover Uni-Melt capillary apparatus and are corrected. IR spectra were determined on a Perkin-Elmer model 257 spectrometer in potassium bromide pellets. UV spectra were obtained on a Perkin-Elmer model 202 recording spectrometer. Optical rotations were measured on a Perkin-Elmer 241 automatic polarimeter. Mass spectra were taken with a LKB-9000 mass spectrometer.

For GLC analysis, an F&M Biomedical gas chromatograph, model 400, was operated isothermally at 255°. The column was 0.31-cm (0.125-in.), 1.84-m (6-ft) stainless steel, packed with 0.8% OV-17 (phenyl methyl silicone) on 80–100-mesh Gas Chrom Q. Helium was the carrier gas at a flow rate of 30 ml/min.

[2] Collected in Trinidad and identified by F. J. Simmonds. A voucher specimen (No. 375-303) is on deposit at Eli Lilly and Co., Indianapolis, IN 46206.

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[4] Mallinckrodt Chemical Works, St. Louis, Mo.

[5] K & K Laboratories, Plainview, N.Y.

[6] Professor M. Terazawa, Obihiro University of Agricultural and Veterinary Medicine, Obihiro City, Japan.

g). On treatment with methanol, this residue deposited white granules of sitosteryl glucoside (50 mg), mp 255°. The identity was confirmed by hydrolysis (dilute hydrochloric acid) of the glycoside to afford the aglycone sitosterol, mp 135–137° (methanol) (GLC–mass spectrometry), and glucose (TLC and co-TLC). Finally, a direct comparison with authentic sitosteryl glucoside[7] showed them to be identical (IR spectrum, melting point, mixed melting point, and co-TLC).

Chromatography (Column III)—The mother liquors from the crystallization of naringenin, fraxetin, and sitosteryl glucoside were combined (11.27 g), adsorbed onto silicic acid[4] (30 g), and chromatographed over silicic acid[4] (300 g) in chloroform. Elution with chloroform–methanol (9:1) gave a fraction (3.01 g), which was treated with methanol (100 ml). The methanol-soluble portion (1.69 g) was chromatographed over silica gel[3] (40 g) in chloroform–ethyl acetate (1:3).

Isolation of Aromadendrin (III)—Elution with chloroform–ethyl acetate (1:3) (1 liter) afforded a residue. On treatment with methanol, this residue gave white granules of aromadendrin (dihydrokaempferol) (14 mg), mp 246° [lit. (14) mp 248°], $[\alpha]_D^{27}$ +22.0° (c 0.5, methanol) [lit. (14) $[\alpha]_D^{27}$ +26°]; UV: λ_{max} (methanol) 228 (log ϵ 3.93) and 293 (3.80) nm; IR: ν_{max} (potassium bromide) 1640, 1590, 1518, 1470, 1440, 1410, 1360, 1288, 1255, 1190, 1170, 1128, 1110, 1088, 1022, 995, 958, 935, 865, 850, 835, 810, 760, and 735 cm^{-1}; mass spectrum (M^+): *m/e* 288 (43%), 259 (55), 165 (22), 153 (100), 136 (36), 134 (41), 107 (51), 77 (12), and 69 (18). This compound was identified as aromadendrin (dihydrokaempferol) by direct comparison[8] (UV, IR, and mass spectra, optical rotation, melting point, mixed melting point, and co-TLC).

Isolation of Kaempferol (IV)—Continued elution with chloroform–ethyl acetate (1:3) (1 liter) gave a residue. On treatment with chloroform–methanol, this residue afforded yellow crystals of kaempferol (20 mg), mp 285° [lit. (15) mp 280°]; UV: λ_{max} (methanol) 215 (log ϵ 4.06), 268 (4.00), and 370 (4.09) nm; IR: ν_{max} (potassium bromide) 3320 (br), 1655, 1615, 1570, 1505, 1385, 1320, 1255, 1225, 1175, 1088, 1008, 972, 880, 845, 830, 815, and 795 cm^{-1}; mass spectrum (M^+): *m/e* 286 (100%), 258 (11), 229 (8), 153 (18), 136 (8), 134 (8), 121 (16), 69 (12), and 65 (8). A direct comparison (UV, IR, and mass spectra, melting point, mixed melting point, and co-TLC) with an authentic sample[5] confirmed the identity.

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Fluorescamine Use in High-Performance Liquid Chromatographic Determination of Aminocaproic Acid in Serum

NAGY A. FARID

Received May 15, 1978, from the *Clinical Laboratories, Methodist Hospital of Indiana, Inc., Indianapolis, IN 46202.* Accepted for publication July 13, 1978.

Abstract □ A sensitive and specific high-performance liquid chromatographic determination of aminocaproic acid in serum is described. ω-Aminocaprylic acid is used as an internal standard. To 10 μl of serum, 10 μl of the internal standard solution and 50 μl of ethanol are added. After centrifugation, a portion of the supernate is evaporated. The residue is dissolved in 750 μl of 50 m*M* dibasic sodium phosphate, and then 250 μl of fluorescamine in acetonitrile (35 mg/100 ml) is added. The reaction mixture is chromatographed using a column of octadecylsilane bonded to silica and 44% acetonitrile in 0.5 m*M* phosphoric acid as the eluent. Quantitation is achieved by monitoring either the absorbance of the effluent at 405 nm or the fluorescence of the compounds with a fluorometer equipped with a flowcell. The method is reproducible, simple, and fast and has a precision of 4.4%.

Keyphrases □ Aminocaproic acid—high-performance liquid chromatographic analysis in serum □ High-performance liquid chromatography—analysis, aminocaproic acid in serum □ Hemostatic agents—aminocaproic acid, high-performance liquid chromatographic analysis in serum

Aminocaproic acid[1] (I), an antifibrinolytic agent, inhibits the conversion of plasminogen to plasmin (1, 2). The chemistry, pharmacological properties, and mechanism of action of I were reviewed earlier (3). Several methods for the determination of I in biological fluids were reported (4–14). Most of these methods were either indirect or required a large sample volume and elaborate extraction and purification steps.

[1] Amicar, Lederle, Pearl River, N.Y.

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