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ACKNOWLEDGMENTS AND ADDRESSES

Received March 9, 1972, from the *Department of Pharmacology, Medical College of Wisconsin, Milwaukee, WI 53233, and the †Clinical Pharmacology Service, Wood Veterans Administration Center, Milwaukee, WI 53193

Accepted for publication June 1, 1972.

Supported in part by U. S. Public Health Service Grant 5-SO1-FR-5434, by Lakeside Laboratories, and by the Even P. Helfaer Foundation. The assistance of Lakeside Laboratories with chemicals, information, and instrumentation is appreciated.

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A Phytochemical Investigation of *Yucca schottii* (Liliaceae)

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Abstract □ The saponin-containing fraction of the leaves of *Yucca schottii* Engelm. (Liliaceae) has been shown to possess anti-inflammatory properties against carrageenin-induced edema in rats. By means of silica gel G dry column and thin-layer chromatographic techniques, a separation of the saponins present as the acetates was accomplished. Six of the saponins showed the presence of yuccagenin as their aglycone and galactose as the sugar moiety. The other saponin contained kammogenin as its aglycone and 2-deoxyribose as the sugar moiety. A preparative isolation procedure was developed for the saponin which contained kammogenin as its aglycone. It was shown to contain 5 units of 2-deoxyribose attached to the 3-position of the aglycone.

Keyphrases □ *Yucca schottii* (Liliaceae)—phytochemical investigation, saponin fraction constituents, potential anti-inflammatory agents □ Saponins—from *Yucca schottii* leaves, separation, potential anti-inflammatory agents □ Anti-inflammatory properties—constituents of leaves of *Yucca schottii* □ Medicinal plants—*Yucca schottii* leaves, constituents, anti-inflammatory properties

In a continuing search for the anti-inflammatory substances from plant sources, the saponin-containing fraction of the leaves of *Yucca schottii* Engelm. (Liliaceae) has shown activity in a preliminary screen utilizing a procedure (1) involving carrageenin-induced edema in rats. The plant[1] material used for this investigation was collected in Pima County, Ariz., during May 1967.

A preliminary examination of the *n*-butanol extract revealed the presence of saponins. The partial resolution of seven detectable saponins as their acetates was effected utilizing a modified dry column chromatographic separation (2). Further purification of the saponin acetates obtained from the column was achieved using TLC. The saponins were then regenerated by alkaline hydrolysis of the respective acetates.

Each of the seven saponins was acid hydrolyzed into its aglycone and sugar moiety. The aglycone of saponins I–V and VII was identified as yuccagenin. The sugar moiety associated with each saponin was identified as galactose by means of TLC and GLC of the trimethylsilyl ethers. Saponin VI showed a ketone group as indicated by the IR spectrum. Its aglycone was identified as kammogenin. The sugar moiety associated with it was identified as 2-deoxyribose.

Saponin acetate VI was separated from the other acetates by means of Girard's reagent "T" (3). The Girard derivative was acid treated, followed by alkaline hydrolysis to yield saponin VI. An elemental analysis[2] indicated that saponin VI contained 5 moles of 2-deoxyribose and its molecular weight was 1025, corresponding to the molecular formula $C_{52}H_{80}O_{20}$. Methylation of saponin VI and subsequent acid hydrolysis yielded 2-methoxykammogenin. The glycosidic linkage occurs at the 3-position of kammogenin, as shown by the keto-derivative which was obtained by means of an Oppenauer oxidation (4, 5) and also by Killiani oxidation and isomerization (6).

EXPERIMENTAL[3]

Extraction—Five kilograms of the dried leaves of *Y. schottii* was macerated with approximately 4 l. of hot distilled water for 3 hr. The aqueous extract was filtered and evaporated to approximately 25% its volume. The marc was discarded. The aqueous extract was then exhaustively extracted with *n*-butanol. The butanol extract was washed with water saturated with butanol until the Benedict reagent test for reducing sugars was negative. The butanol extract was evaporated *in vacuo* until completely dry; 180 g. of essentially pure sugar-free saponins were obtained. The yield was 3.6% from the dried plant.

TLC analysis of portions of the saponin-containing extract was performed by allowing the lower phase of a chloroform–methanol–water (65:25:10) mixture (7) to ascend, for 24 hr., plates of silica gel G[4], with a piece of Whatman No. 1 paper attached to their top and folded over their backs in order to extend the development time of

[1] Identification confirmed by Robert J. Barr, College of Pharmacy, and Dr. Charles Mason, Botany Department, University of Arizona, Tucson, Ariz. A reference specimen was also deposited in the University of Arizona Herbarium.

[2] Carbon and hydrogen determinations performed by Huffman Laboratories Inc., Wheatridge, Colo.

[3] Melting points were determined on a Kofler hot-stage apparatus and are uncorrected. TLC was carried out on unactivated silica gel G with a stationary phase thickness of 0.3 mm. UV spectra were determined in ethanol using a Beckman DB spectrophotometer. IR spectra were run using KBr pellets on a Perkin-Elmer infracord model 137. Mass spectra were obtained from a Hitachi Perkin-Elmer double-focusing spectrometer, model RMU-6e. GLC analyses were carried out on a Perkin-Elmer gas chromatograph model 880, using 2.5% SE-30 as stationary phase.

[4] Merck.

each chromatogram. Visualization with ceric sulfate solution showed the presence of seven compounds. TLC, performed as described and visualized with defibrinated blood, indicated that all seven compounds were saponins (8).

Acetylation of Saponin Mixture—One hundred grams of the saponin mixture was acetylated by refluxing for 2 hr. with a mixture of 200 ml. of pyridine–acetic anhydride (1:1). The reaction mixture treated in the conventional manner yielded 108 g. of a saponin acetate mixture (9).

Separation of Saponin Acetates—*Column and Thin-Layer Chromatography*—A dry silica gel G column, 1 m. long, and 9 cm. in diameter, was packed as described previously (2). Then 15 g. of the saponin acetate mixture was chromatographed using ether–petroleum ether (b.p. 30–60°)–acetone (9:2:1). On the basis of 18 exploratory samples obtained as described previously (2), the column was cut to combine Sections 1–6, 7–10, 11–15, 16, and 17–18, followed by extraction of the saponin acetates from the silica gel G with acetone.

Alkaline Hydrolysis of Saponin Acetates—The alkaline hydrolysis of each saponin acetate, as described previously (2), yielded pure saponin.

Acid Hydrolysis of Saponins I–VII—Each saponin was dissolved in 10 ml. of a mixture of dioxane–4 N H_2SO_4 (1:1) (10). The mixture was refluxed for 3 hr. and then 25 ml. of cold distilled water was added. The mixture was extracted with three 10-ml. portions of ether. The aqueous layer was neutralized with barium carbonate, filtered, and concentrated to a syrup. The syrup was dissolved in ethanol, filtered, and evaporated to dryness.

The ether extract of saponin I-V and VII yielded yuccagenin, and saponin VI gave kammogenin, identified by means of TLC [silica gel G, chloroform–methanol–water (188:12:1)], IR, melting point, and mixed melting point, using authentic samples as references[5,6].

The identity of the sugar from the aqueous phase was established both by TLC on silica gel G plates developed with chloroform–glacial acetic acid–water (6:7:1) and GLC analysis using arabinose, 2-deoxyribose, fucose, galactose, and glucose as reference samples[7]. While the sugar moieties of saponin I–V and VII and galactose showed identical R_f values, the sugar moiety of saponin VI displayed the same R_f value as that of 2-deoxyribose.

Isolation of Saponin Acetate VI with Girard's Reagent "T"—A mixture of saponin acetate mixture (80 g.), Girard's reagent "T" (160 g.), glacial acetic acid (320 g.), and absolute ethanol (3.2 l.) was refluxed for 1 hr., cooled, and poured into water (5 l.) containing ice (2.5 kg.), ether (2.5 l.), and sodium carbonate (265 g.). The ether layer was separated, and the aqueous phase was extracted with additional ether (1 l.). The combined ether extract was washed with water followed by aqueous sodium bicarbonate (5%), dried, and evaporated to give saponin acetates I–V and VII as identified by TLC.

The aqueous phase was hydrolyzed by adding concentrated hydrochloric acid (2 l.) followed by extraction with ether (2.5 l.). The mixture was allowed to stand at room temperature for 1 hr. It was then shaken for 5 min. and left standing for 15 min.; after repetition of this procedure four times, the ether layer was separated, washed with water and aqueous sodium bicarbonate (5%), dried, and evaporated to give saponin acetate VI (8 g.) as identified by TLC. This saponin acetate VI on alkaline hydrolysis (2) gave a product identical (TLC) to that of saponin VI of the butanol extract.

Acid Hydrolysis and Molecular Weight Estimation of Saponin VI—Saponin VI (365 mg.) was submitted to acid hydrolysis as previously described. The reaction mixture yielded 155 mg. of kammogenin, indicating a molecular weight of approximately 1043. The determination of the molecular weight of saponin VI gave 1025, in agreement with the molecular formula $C_{52}H_{80}O_{20}$.

Methylation of Saponin VI—A mixture of saponin VI (3.14 g.) and dimethyl sulfoxide (150 ml.) was stirred for 30 min. at room temperature under a nitrogen atmosphere. Sodium hydride (1.5 g.) was added to the solution, and stirring was continued for another 30 min.; then 45 ml. of methyl iodide was added with further stirring for 30 min. After the reaction mixture was diluted with 900 ml. of water and treated in the conventional manner, a yield of 3.27 g. of methylated saponin was obtained.

Acid Hydrolysis of Methylated Saponin VI—Methylated saponin VI (3.27 g.) was hydrolyzed by refluxing for 3 hr. with a mixture of concentrated hydrochloric acid and ethanol (3:97). The reaction mixture was diluted with water. After evaporation of most of the ethanol in the air, it was extracted with ether. After evaporation of the ether, the residue was dissolved in benzene and purified by passing through a small alumina (grade III) column. The eluate yielded 435 mg. of material, which was recrystallized from acetone to yield 208 mg. of product, m.p. 228–229.5°. The mass spectrum had a parent peak at 458 *m/e* and a base peak at 139 *m/e*. This molecular weight corresponded to 2-methoxykammogenin.

Oppenauer Oxidation of 2-Methoxykammogenin—One hundred milligrams was dissolved in a mixture of toluene (9 ml.) and cyclohexanone (1.8 ml.), and the solution was heated to boiling. A solution of 100 mg. of aluminum isopropoxide in 0.9 ml. of toluene was added. The mixture was refluxed for 60 min., poured into water, and made basic with dilute sodium hydroxide. The organic layer was separated and the aqueous liquid was extracted with ether. The combined organic extract, after evaporation, yielded a residue which was purified by column chromatography (alumina, grade II; eluant, benzene). Recrystallization from acetone yielded 30 mg. of 3-keto-2-methoxy-Δ^4-kammogenin, m.p. 225–227°. The IR showed two absorption bands at 5.89 (carbonyl at C-12) and 5.95 (conjugated carbonyl at C-3) μ. The UV spectrum showed absorption at $\lambda_{max}^{ethanol}$: 240 nm. (log ϵ 4.25).

Killiani Oxidation of 2-Methoxykammogenin—2-Methoxykammogenin (100 mg.) was treated with Killiani's reagent[8]. The reaction mixture yielded a product which was recrystallized from acetone. It had m.p. 179–181°. The IR showed two absorption bands at 5.81 (carbonyl at C-3) and 5.89 (carbonyl at C-12) μ. The product did not absorb in the UV range. The mass spectrum showed a parent peak at 456 *m/e*, corresponding to 3-keto-2-methoxykammogenin.

Isomerization of Double Bond of 3-Keto-2-methoxykammogenin—3-Keto-2-methoxykammogenin (33 mg.) was dissolved in 1.9 ml. of hot methanol, and 1 drop of 10% methanolic potassium hydroxide solution was added. The solution was heated on a steam bath for 5 min. The reaction mixture was then poured into water, neutralized with dilute acetic acid, and then extracted with ether. Evaporation of the ether extract and recrystallization from acetone yielded 24 mg. of a compound, m.p. 225–227°. The IR and UV spectra were identical to those obtained for the product of the Oppenauer oxidation. The mass spectrum showed a parent peak at 456 *m/e*, corresponding to 3-keto-2-methoxy-Δ^4-kammogenin.

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ACKNOWLEDGMENTS AND ADDRESSES

Received November 8, 1971, from the *Division of Pharmaceutical Chemistry, College of Pharmacy, University of Arizona, Tucson, AZ 85721*

Accepted for publication June 9, 1972.

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[5] Authentic samples of yuccagenin were obtained from Syntex Laboratories, Palo Alto, Calif.

[6] Kammogenin was isolated from ethanolic extract of *Y. schottii*.

[7] Sugar reference samples were obtained from Aldrich Chemical Co., Milwaukee, Wis.

[8] Chromium trioxide (26.72 g.) in concentrated sulfuric acid (23 ml.) diluted with water to a volume of 100 ml.