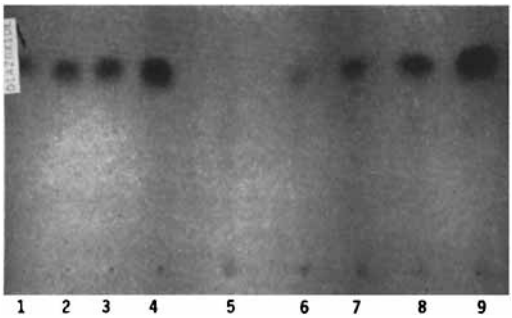


*Fig. 4—TLC analysis of diazoxide recovered from urine (photographed under U. V. light). Samples 1, 2, 3, and 4 represent diazoxide standards 5, 10, 20, and 40 mcg., respectively. Samples 5, 6, 7, 8, and 9 represent diazoxide recovered from urine to which 0,5, 10, 20, and 40 mcg. of the drug was added, respectively.*

Although no attempt was made to analyze diazoxide levels in urine, the TLC procedure is applicable to urine as well. It is recommended, however, that after extraction of urine with ethyl acetate, the organic solvent be washed with 2.5 ml. of 4% sodium bicarbonate solution before it is concentrated for chromatography. This will remove many interfering materials present in urine and give good diazoxide recoveries. Samples 6–9 (Fig. 4) present results obtained on recovery from urine of 5, 10, 20, and 40 mcg. of diazoxide, respectively. The size and intensity of diazoxide spots compare favorably (assuming some losses due to extraction procedure) with that obtained by direct chromatography of respective diazoxide standards (samples 1 4). Sample 5 represents control urine with no drug added.

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# Proteinaceous Antitumor Substances from Plants III. *Caesalpinia gilliesii* (*Leguminosae*)

By A. ULUBELEN*, W. F. MCCAUGHEY†, and J. R. COLE

**Preliminary screening of the aqueous extracts of *Caesalpinia gilliesii* have shown activity against sarcoma 180 in mice and Walker 256 in Sprague rats. The isolation, purification, and partial characterization of six proteinaceous materials are reported.**

As a result of a routine screen of Southwestern plants for potential antitumor activity, the aqueous extracts of *Caesalpinia gilleisii*[1] (Wall) demonstrated activity toward the sarcoma 180 test system in mice.[2] This screening program was carried out by the Cancer Chemotherapy National Service Center, Bethesda, Md. Further fractionation has resulted in activity against Walker 256 (5WM) in Sprague rats as well as the sarcoma 180 (3SA) test system in mice.

The plant is an odoriferous, glandular shrub 2.3 M. tall, collected near Greaterville, Ariz., where it has become naturalized in a pasture.

## EXPERIMENTAL

Ten Kg. of the dry pods of *C. gilliesii* was extracted with approximately 15 L. of water at room temperature. The aqueous extract was lyophilized in a Repp Industries model 15 sublimator and then washed with MeOH and EtOH. The yield was 760 Gm. One-hundred grams of this crude compound was added to 1 L. of water at room temperature. The insoluble part was separated by centrifuge and discarded.

The aqueous solution was extracted with 5 × 200 ml. of ether to remove fatty materials, and increased in volume threefold by the addition of 95% ethanol. A light-colored precipitate appeared and was separated by centrifuge, dissolved in water, and dialyzed against running water at room tem-

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Previous paper: Ulubelen, A., and Cole, J. R., *J. Pharm. Sci.*, **55**, 1368(1966).
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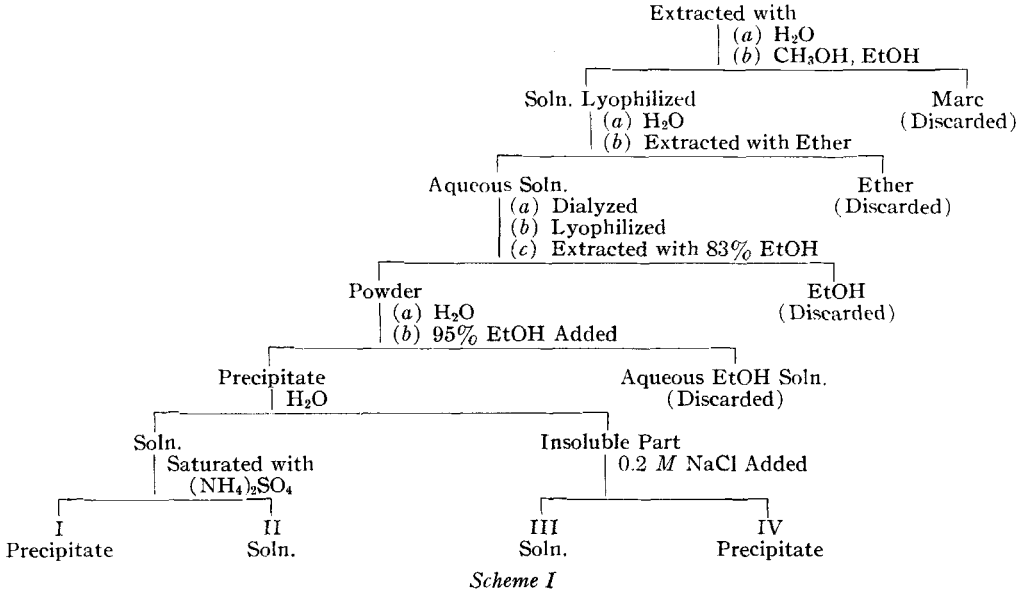
[1] Identification confirmed by Robert Barr, College of Pharmacy, and Dr. Charles Mason, Botany Department, University of Arizona, Tucson. A reference specimen was also deposited.
[2] Preparation of the preliminary extraction was carried out by Dr. M. E. Caldwell.

Schematic Presentation of Extraction
*C. gilleisii*




*Scheme I*

perature using a dialyzing tube of 5/8 in. diam. (No. 4465-A2, Arthur H. Thomas Co., Philadelphia, Pa.). After a period of 7 days, the solution in the tubes was lyophilized. The material thus obtained was extracted with 83% boiling ethanol. A red-colored solution was obtained. The remaining powder was dissolved in water and lyophilized. Twenty-five grams of compound was obtained. This crude material showed a marked activity against the sarcoma 180 test system and showed a reduction in tumor size of 82% and 89% at a dose of 4.4 mg./Kg. This corresponds to a T/C[3] value of 18 and 11%, respectively. The elementary analysis showed 17.3% inorganic material which consists mostly of calcium and phosphate. Sulfur and nitrogen were also present.

Four grams of the crude material was added to 50 ml. of water. The insoluble part was separated by centrifuge. To the soluble portion, $(NH_4)_2SO_4$ was added until saturation. A precipitate was then separated by centrifuge. The precipitate (I) and the solution (II) after being dissolved in water were dialyzed and lyophilized. The insoluble part was dissolved in 0.2 *M* NaCl (pH 8), and was separated by centrifuge. The soluble part (III) and the insoluble part (IV) were both dialyzed and lyophilized (Scheme I).

Column chromatography was employed to further separate these fractions (I, II, III, and IV). Substrates used included ion-exchange resins[4] and synthetic dextran derivatives.[5] The columns, upon treatment with the following buffer systems, gave the following test results: I when washed with 0.02 *M* phosphate buffer solution pH 5.6 yielded one fraction (a) and with a barbital buffer solution pH 8.6 a second fraction (b). II yielded two fractions (c, d) with the buffer solution pH 5.6; III gave one fraction (e) with the same buffer solution. No other fraction was obtained. IV yielded only one fraction (f) with a buffer solution pH 8.6. The four fractions indicated above were tested against the 5WM system. I exhibited a T/C[6] value of 3% at 33 mg./Kg and III a value of 28% at 100 mg./Kg.

## PHYSICAL AND CHEMICAL CHARACTERISTICS OF PROTEIN FRACTIONS

Paper electrophoresis of the six fractions was run utilizing a barbital buffer solution of pH 8.6, 0.05 ionic strength in a Spinco model Beckman instrument, and after 14 hr of electrophoresis using a 2 ma. current, the papers were developed using periodic acid and Schiff reagent. Mobilities of the six different fractions are shown in Table I.

The fractions were hydrolyzed with 6 *N* hydrochloric acid in a sealed and evacuated glass tube at 105°. The resulting solution was applied on Whatman No. 1 paper using the same solvent systems previously reported (2, 3).

One hundred milligrams of the six fractions were hydrolyzed separately with 3 *N* HCl in a boiling water bath for 24 hr. in preparation for characterization of the sugars. Three different solvent systems previously reported (3) were employed to detect the sugars using paper chromatography. Aniline phthalate and silver nitrate solutions were used to develop the chromatograms. The results are described in Table I. Results of nitrogen determination of six fractions using micro-Kjeldahl method and U. V. absorption are also shown in Table I.

Infrared curves of the six fractions showed characteristic protein peaks at 3400, 2950, 1650, and

[3] The criteria for activity is defined as being a per cent T/C (test/control) value of less than 42 in a satisfactory dose response test (1).

[4] Amberlite IR-120, IR-4B, IRC-50, and IRA-400, Rohm and Haas Co., Philadelphia, Pa.

[5] Sephadex, Pharmacia Fine Chemicals, Inc., New Market, N. J. The most effective column was G-200 Sephadex.

[6] Activity in this system is defined as a per cent T/C value of less than 60 in a satisfactory dose response test (1).

TABLE I—PHYSICAL AND CHEMICAL CHARACTERISTICS OF SIX PROTEIN FRACTIONS

| Fraction | Mobilities, cm.²/sec. v. | Sugars | U.V. Abs. Maxima mμ | Nitrogen, % | Ash Content, % |
|---|---|---|---|---|---|
| a | $1.07 \times 10^{-6}$ | Mannose, fructose | 204, 208 | 12.8 | 1.5 |
| b | $6.4 \times 10^{-7}$ | Mannose, rhamnose | 204, 265 | 11.6 | 1.5 |
| c | $2.2 \times 10^{-6}$ | Mannose, glucosamine | 204, 280 | 5.7 | 3.9 |
| d | $1.2 \times 10^{-6}$ | Mannose, glucosamine | 204, 280 | 6.2 | 0 |
| e | $1.2 \times 10^{-6}$ | Mannose, glucosamine | 204, 265 | 13.6 | 6.0 |
| f | $1.6 \times 10^{-6}$ | Mannose, glucosamine rhamnose | 204, 240, 280 | 14.08 | 6.0 |

TABLE II—PRELIMINARY AMINO ACID ANALYSIS OF *Caesalpinia* PROTEINS

| Amino Acids | % μm. of Amino Acids I | II | III | IV |
|---|---|---|---|---|
| Aspartic acid | 10.5 | 6.01 | 10.7 | 10.5 |
| Threonine | 6.04 | 7.66 | 5.81 | 5.08 |
| Serine | 6.66 | 13.00 | 5.88 | 6.01 |
| Glutamic acid | 16.20 | 7.18 | 13.60 | 15.80 |
| Proline | 5.02 | 3.32 | 4.90 | 4.95 |
| Glycine | 11.30 | 7.62 | 10.1 | 9.73 |
| Alanine | 8.89 | 13.3 | 10.9 | 8.90 |
| Cystine | 2.82 | 0.522 | 0.783 | 1.02 |
| Valine | 5.07 | 3.72 | 6.57 | 6.05 |
| Methionine | 1.04 | 0.522 | 1.52 | 1.27 |
| Isoleucine | 3.22 | 1.62 | 4.28 | 4.23 |
| Leucine | 6.02 | 3.53 | 7.75 | 7.87 |
| Tyrosine | 2.23 | 0.731 | 2.01 | 2.25 |
| Phenylalanine | 3.19 | 1.25 | 3.75 | 3.64 |
| Lysine | 3.99 | 2.41 | 3.21 | 3.57 |
| Histidine | 1.51 | 0.731 | 1.59 | 1.78 |
| Arginine | 6.24 | 1.51 | 5.95 | 6.73 |
| Glucosamine | ... | 1.50[a] | 0.644 | 0.454 |
| Hydroxyproline | ... | 23.8[a] | ... | ... |

[a] Estimated values.

1540 cm.$^{-1}$. Table II represents the results of an amino acid analysis using a Beckman analyzer model 120B.

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# Synthesis of Some Substituted Amides of Terephthalic and Isophthalic Acids

By JOSEPH P. LAROCCA* and MOHAMED A. Y. SHARKAWI†

**The syntheses of 14 diamide derivatives of terephthalic acid and six diamide derivatives of isophthalic acid are described. Evaluation of reaction conditions and solvent systems led to the use of anhydrous ether at 0° as the preferred method of preparing these amides from the corresponding acid chloride and amine. Preliminary pharmacological screening is reported.**

A LARGE NUMBER of drugs are available which depress the central nervous system. These drugs elicit sedative, hypnotic, anticonvulsant, and psychotherapeutic effects. Many of these drugs contain a carbonyl-nitrogen-carbonyl group-

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TABLE I—SELECTION OF SOLVENT FOR DIAMIDE SYNTHESIS: AMINE WITH TEREPHTHALOYL CHLORIDE

| Method | Solvent | Amine | % Yield[a] | Reaction Temp. |
|---|---|---|---|---|
| *A* | Aq. NaOH | Propyl | 52.4 | ... |
| *B* | $CCl_4$ | Propyl | 58.4 | 24° |
| *C* | Diethyl ether | Propyl | 76.6 | 20° |
| | Diethyl ether | Di-*n*-butyl | 74.7 | 20° |
| *D* | No solvent | Di-*n*-butyl | 82.4 | [b] |

[a] Based on crude yield. [b] Vigorous exothermic reaction with discoloration. Difficulties in purification made this procedure less desirable.