Comparative Estimation of Glycyrrhetinic Acid Content of *Lonchocarpus cyanescens* and Glycyrrhiza Roots

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Abstract □ The glycyrrhetinic acid (enoxolone) content of *Lonchocarpus cyanescens* roots has been determined by colorimetric and GC analysis. This plant gave lower values for both bound and unbound glycyrrhetinic acid when compared with glycyrrhiza (licorice).

Keyphrases □ *Lonchocarpus cyanescens*—comparative estimation of glycyrrhetinic acid content in roots, glycyrrhiza roots, colorimetric and GC analysis □ Glycyrrhetinic acid—comparative estimation of the content of *Lonchocarpus cyanescens* and glycyrrhiza roots, colorimetric and GC analysis □ Colorimetry, analysis—comparative estimation of the glycyrrhetinic acid content in *Lonchocarpus cyanescens* and glycyrrhiza roots □ GC analysis—comparative estimation of the glycyrrhetinic acid content in *Lonchocarpus cyanescens* and glycyrrhiza roots

Lonchocarpus cyanescens Benth (Leguminosae) is used extensively in African ethnomedicine for the treatment of various diseases, including infantile constipation and venereal infections (1). On the west coast of Africa, the root decoction is used by the natives for the treatment of arthritis. The antiphlogistic activity of *L. cyanescens* has been attributed to the oleanane derivatives, 18β-glycyrrhetinic acid (enoxolone), 28-hydroxy-β-glycyrrhetinic acid, and its 18α-isomer, lonchoterpene, isolated from this plant (2).

Other species of *Lonchocarpus* are valued as effective insecticides due to the rotenone content of this genus (3). Some species are cultivated as fish poisons (4), and others are commercial sources of the Yoruba indigo dye (5).

Flavonoids and triterpenoids are the most important secondary metabolites of this plant (2). Previous phytochemical works established the presence of pterocarpans (6), *C*- and *O*-prenylated chalcones (7), and prenylated stilbenes (8). The present report describes the quantitative estimation of the glycyrrhetinic acid present in the root bark of *L. cyanescens*.

A number of analytical methods are available for the qualitative evaluation of glycyrrhetinic acid, including gravimetric, colorimetric, and GC–MS (9–11). Here, the determination was by colorimetry and GC.

EXPERIMENTAL[1]

Methods—*Colorimetric Determination*—One gram each of *L. cyanescens* root bark, powdered glycyrrhiza, and ammoniated extract of glycyrrhizin were processed as described earlier (11). The quantity of glycyrrhetinic acid present in the samples was estimated by measuring the extinction of the complex formed by coupling the acidic genin, glycyrrhetinic acid, with methylene blue. A single-beam spectrophotometer was used for all the measurements[2], and the determinations were carried out at 640 nm. For the hydrolyzed extract, the percent yield of glycyrrhizin was calculated from (11):

$$\text{Percent glycyrrhizin} = \frac{A_t \times 0.5 \times 1.75}{A_s \times W} \qquad \text{(Eq. 1)}$$

where, A_t is the absorbance of the test sample, A_s is the absorbance of the reference, W is the weight of the substance, and 1.75 is the transformation factor.

Gas Liquid Chromatographic Estimation—One gram of powdered root bark of *L. cyanescens* was extracted with chloroform (50 ml) in a soxhlet apparatus for 3 hr. The filtered extract was evaporated to dryness under reduced pressure and the resultant residue was silylated before GLC analysis, as will be described. The air-dried marc was further extracted with (50 ml) 70% ethanol for 3 hr. The filtered alcoholic extract was transferred quantitatively to a 250-ml round-bottom flask and refluxed for 3 hr with 50 ml of 6 *N* HCl. After cooling, the extract was extracted twice with 50 ml of chloroform, and the combined chloroform extracts were evaporated to dryness under reduced pressure. The resultant residue was treated with 0.5 ml each of anhydrous pyridine and *N,O*-bis(trimethylsilyl)trifluoroacetamide. A 5-ml aliquot was analyzed after 30 min by GLC[2] using a chromatograph loaded with Chromosorb (100–120 mesh); the injector and detector were maintained at 320°.

Samples of glycyrrhiza roots and authentic reference glycyrrhetinic acid were similarly treated and analyzed. In each case the glycyrrhetinic acid content was calculated from measurements of the mean peak areas from these replicates.

Table I—Percent Yield of Glycyrrhetinic Acid

Sample	Method of Analysis: Colorimetric	Method of Analysis: GLC
L. cyanescens (unhydrolyzed[a])	0.54	0.55
L. cyanescens (hydrolyzed[b])	0.50	0.52
Glycyrrhiza liquid extract	0.12	0.14
Glycyrrhiza roots (unhydrolyzed[a])	0.67	0.60
Glycyrrhiza roots (hydrolyzed[b])	0.58	0.48

[a] The values obtained for the unhydrolyzed samples are indicative of the free genin content. [b] The total yield of glycyrrhetinic acid is obtained by adding the values obtained both before and after hydrolysis.

RESULTS AND DISCUSSION

The percent yield of glycyrrhetinic acid from both methods of analyses are shown in Table I. Although *L. cyanescens* gave a comparatively lower glycyrrhetinic acid value, the abundance of this plant makes it an attractive source for this agent. The higher yield of the unbound genin is also a positive index for the economic exploitation of this plant.

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[1] Plant material consisted of roots of *L. cyanescens* and were collected at Nsukka in October 1980, and identified by the University of Nigeria Department of Botany. A voucher specimen has been deposited at the Pharmacy Herbarium of this university.

[2] Hewlett-Packard 5750G Research Chromatograph was used for GLC; the colorimetric analysis was with a Coleman spectrophotometer.

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Paired-Ion High-Performance Liquid Chromatographic Assay for Sulfinpyrazone in Plasma

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Abstract □ A specific and sensitive liquid chromatographic method is reported for the assay of sulfinpyrazone in plasma utilizing ion pairing between the tetrabutylammonium cation and the sulfinpyrazone anion. The method is rapid in that conventional extraction procedures are avoided in favor of using disposable cartridges packed with an octadecylsilane bonded phase as a means of separating the drug from plasma. The samples were chromatographed on a C_{18} reversed-phase column using a mobile phase consisting of 0.005 *M* tetrabutylammonium phosphate in methanol–water (56:44). The coefficient of variation obtained was 4.5% and the response was linear over a range of 0.2–80 μg/ml.

Keyphrases □ High-performance liquid chromatography—paired ion, assay for sulfinpyrazone in plasma □ Sulfinpyrazone—paired-ion high-performance liquid chromatographic assay in plasma

Sulfinyprazone has been used as a uricosuric agent for over 20 years. Recently, new interest in this drug has been generated by reports indicating that it may protect patients from sudden death after myocardial infarction (1, 2).

Several methods have been reported in the literature that can be used for the quantitative determination of sulfinpyrazone in biological fluids. A spectrophotometric method reported previously (3) involves a tedious double extraction and lacks sensitivity, since it is based on UV absorption. A high-performance liquid chromatographic (HPLC) method was described (4) using radiolabeled sulfinpyrazone as an internal standard which complicates the routine application of this method. An HPLC method using a microparticulate silica column also was published (5). This method requires that the sample be injected 2 mm inside the column, thus, making it necessary to periodically refill that portion of the column with fresh silica. Another method (6) involves a two-step extraction and lacks adequate sensitivity.

It was considered desirable to develop a simple, rapid, and sensitive method for sulfinpyrazone determination in biological fluids. The reversed-phase HPLC method described here is based on the formation of ion pairs between sulfinpyrazone anions and tetrabutylammonium cations. The reported method has the advantage of not involving any extraction step. The drug is isolated from plasma using disposable cartridges filled with octadecylsilane bonded phase packing (C_{18} cartridge[1]).

EXPERIMENTAL

Materials—All reagents were analytical grade. All solutions were prepared using glass distilled water.

The high-performance liquid chromatographic system included a solvent pump[2] equipped with an injector[3], a fixed wavelength UV detector[4] set at 254 nm (λ_{max} was 260 nm), a recorder[5], and in integrator[6].

The analytical column was 30 cm long with 4-mm i.d. It was packed with octadecylsilane-bonded silica (particle size 10 μm)[7].

Mobile Phase—The mobile phase consisted of 0.005 *M* tetrabutylammonium phosphate[8] (I) in methanol–water (56:44). It was prepared by adding sufficient glass-distilled water to a vial of I to make 440 ml. An aliquot of 560 ml of absolute methanol was added, the solution was stirred for 10 min, and the volume was adjusted to 1 liter with a 56% (v/v) methanol–water solution. The solution was then filtered using a 0.5-μm pore, inert filter[9]. The mobile phase was deoxygenated using a vacuum flask attached to a water aspirator. The flow rate of the mobile phase was 1 ml/min at ambient temperature.

Preparation of Standards—A stock solution of sulfinpyrazone[10] was prepared by dissolving 100 mg of the drug in 100 ml of 50% (v/v) methanol–water. Solutions of varying concentration were prepared by serial dilutions of the stock solution in 50% (v/v) methanol–water. The plasma samples for use in the preparation of calibration curves were prepared by adding 200 μl of the appropriate drug solution to 1.8 ml of rat plasma in a 15-ml conical centrifuge tube. The plasma samples were mixed by agitating for 30 sec on a vortex-type mixer[11], and a 1.0-ml aliquot was taken for analysis.

[1] C_{18} Sep-pak, Waters Associates, Milford, Mass.
[2] Model 6000 A, Waters Associates, Milford, Mass.
[3] Model U6K, Waters Associates, Milford, Mass.
[4] Model 440, Waters Associates, Milford, Mass.
[5] Omniscribe, Houston Instruments, Austin, Tex.
[6] Shimadzu Chromatopac-E1A, Shimadzu Seisakusho Ltd., Kyoto, Japan.
[7] Bondapak C_{18}, Waters Associates, Milford, Mass.
[8] PIC Reagent-A, Waters Associates, Milford, Mass.
[9] Type FH, Millipore Corp., Bedford, Mass.
[10] Ciba-Geigy Corp., Summit, N.J.
[11] Maxi Mix Mixer, No. M-16715, Thermolyne Corp., Dubuque, Iowa.

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