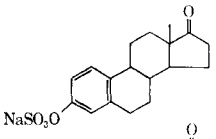
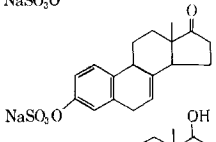
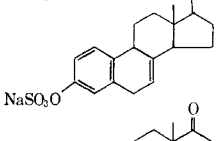
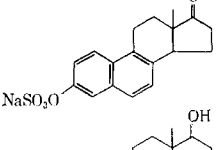
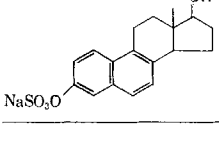
TABLE III—APPROXIMATE ELUATE VOLUME AT PEAK APPEARANCE IN THE CHROMATOGRAMS OF SEVERAL SODIUM ESTROGEN 3-SULFATE SALTS (14-GM. COLUMN, BED VOLUME, 45. ML.)

Estrogen	Vol. at Peak (ml.) Eluant: H_2O	Eluant: 5×10^{-4} M NaCl
 Estrone	70	73
 Equilin	75	90
 17β-Dihydroequilin	90	100
 Equilenin	95	125
 17β-Dihydroequilenin	110	160

havior of the individual estrogen salts and the effect of dilute aqueous sodium chloride eluant can be seen from the comparison of eluate volumes at peak appearance, as shown in Table III.

With due consideration of experimental conditions, it should be possible to modify this chromatographic procedure for the quantitative separation of complex mixtures of sodium estrogen sulfate salts. Preliminary work toward this end is already under way (17).

REFERENCES

(1) Grant, G. A., and Beall, D., "Recent Progress in Hormone Research," vol. 5, Pincus, G., ed., Academic Press Inc., New York, N. Y., 1950, p. 311.
(2) Grant, G. A., and Beall, D., *ibid.*, pp. 313–315.
(3) Norymberski, J. K., and McKenna, J., *Biochem. J.*, **76**, 60(1960).
(4) Beling, C. G., *Nature*, **192**, 326(1961).
(5) Gupta, D., and Goodwin, J., *Steroids*, **8**, 195(1966).
(6) Eechaute, W., *Clin. Chim. Acta*, **10**, 378(1964).
(7) Kushinsky, S., and Otterness, I., *Steroids*, **3**, 311 (1964).
(8) Arcos, M., and Lieberman, S., *J. Clin. Endocrinol. Metab.*, **25**, 808(1965).
(9) Barlow, J. J., *Biochem. J.*, **65**, 34P(1957).
(10) Crepy, O., Jayle, M. F., and Meslin, F., *Compt. Rend. Soc. Biol.*, **151**, 234(1957); through "Steroid Conjugates," American Chemical Society, Washington, D. C., 1966, abstract 796.
(11) Bush, I. E., *Biochem. J.*, **67**, 23P(1957).
(12) Oertel, G. W., and Kaiser, E., *Clin. Chim. Acta*, **7**, 700(1962).
(13) Oertel, G. W., Tornero, M. C., and Groot, K., *J. Chromatog.*, **14**, 509(1964).
(14) Hahnel, R., *Anal. Biochem.*, **10**, 184(1965).
(15) Calvin, H. I., Roberts, K. D., Weiss, C., Bandi, L., Cos, J. J., and Lieberman, S., *ibid.*, **15**, 426(1966).
(16) Sjovall, J., and Vihko, R., *Acta Chem. Scand.*, **20**, 1419(1966).
(17) Graham, J., personal communication.

Nigerian Plants III. Phytochemical Screening for Alkaloids, Saponins, and Tannins

By GEORGIA J. PERSINOS* and MAYNARD W. QUIMBY

Fifty species of Nigerian plants were evaluated for the presence of alkaloids, saponins, and tannins. Of these, 24 contained alkaloids, 43 contained tannins, and 16 contained saponins.

THIS WORK is a continuation of the investigation carried out on plants used as medicinals by a pagan tribe, the Anagutas, living on the Jos Plateau in northern Nigeria, and the Hausas, living throughout northern Nigeria. Since plants occupy an important and sometimes central role in the religious beliefs and social practices of these peoples, an investigation of certain of these plants[1] has been undertaken

Received March 29, 1967, from the Department of Biological Sciences, Massachusetts College of Pharmacy, Boston, MA 02115
Accepted for publication July 27, 1967.
Abstracted in part from a thesis submitted by Georgia J. Persinos to the Massachusetts College of Pharmacy, in partial fulfillment of Doctor of Philosophy degree requirements.
This investigation was supported by research grant GM-08798 from the National Institutes of Health, Bethesda, Md.
* Present address: Research Pathology Associates, Division of Phytochemistry, Rockville, MD 20851
[1] The plant specimens were authenticated by Dr. Quimby and were made a part of the botanical collections of the Massachusetts College of Pharmacy.

EXPERIMENTAL

Preparation of Extracts—The screening procedures for alkaloids, saponins, and tannins were adapted from those described by Wall *et al.*(1, 2). An extract of each plant was prepared by refluxing 10 Gm. of the air-dried milled plant sample with 100 ml. of 80% ethanol for 1 hr. Each extract was then cooled to room temperature, suction-filtered, and washed with sufficient 80% ethanol to bring the volume of filtrate to 100 ml.

Alkaloids—Twenty milliliters of each extract, equivalent to 2 Gm. of dried plant material, was evaporated to dryness using a steam bath and the residue was stirred with 5 ml. of 1% aqueous hydrochloric acid. One milliliter of the filtrate was treated with a few drops of Mayer's reagent and a separate 1-ml. portion was treated similarly with silicotungstic acid reagent (12% aqueous). Precipitation or turbidity with either of these reagents was taken as preliminary evidence for the presence of alkaloids in the extract being evaluated. A confirmatory test designed to remove nonalkaloidal compounds capable of eliciting "false-positive" reactions with either of these reagents was conducted

TABLE I—PHYTOCHEMICAL SCREENING RESULTS FOR 50 SPECIES OF NIGERIAN PLANTS

Scientific Name	Part[a] Used	Alkaloids[b] M[c]	Alkaloids[b] STA[d]	Alkaloids[e] Fraction I	Alkaloids[e] Fraction II	Tannins Gelatin Salt	Tannins $FeCl_3$[f]	Saponins
Ampelidaceae								
Cissus hochstetteri Planch.	r	−	−	−	−	+	bk	+
Anacardiaceae								
Mangifera indica Linn.	l	−	−	−	−	+	bk/g	−
Annonaceae								
Annona senegalensis Pers.	sb	+	+	+	+	+	b/g	+
Uvaria chamae P. Beauv.	r	−	−	+	−	+	bk/g	−
Uvaria chamae P. Beauv.	sb	−	−	+	−	+	bk/g	−
Araliaceae								
Cussonia nigerica Hutch.	s	−	−	−	−	−	bk/g	+
Bignoniaceae								
Stereospermum kunthianum Cham.	sb	−	−	−	−	−	−	−
Bombacaceae								
Adansonia digitata Linn.	s	+	+	+	−	−	−	−
Celastraceae								
Maytenus senegalensis Exell.	r	+	+	+	−	+	b/g	−
Cochlospermaceae								
Cochlospermum tinctorium A. Rich.	rz	−	−	−	−	+	bk/g	−
Combretaceae								
Terminalia avicennoides Guill. and Perr.	sb	−	−	−	−	+	bk/g	+
Terminalia avicennoides Guill. and Perr.	rb	−	−	−	−	−	b/b	−
Terminalia avicennoides Guill. and Perr.	r	−	−	−	−	+	b/b	−
Terminalia avicennoides Guill. and Perr.	l	−	−	−	−	−	b/b	−
Compositae								
Echinops longifolius A. Rich.	l	−	−	−	−	+	g	−
Convulvulaceae								
Ipomea sp.	l	+	+	+	−	+	g	−
Dioscoreaceae								
Dioscorea abyssinica Hochst. ex Kunth.	Yam	+	+	+	−	−	−	+
Dioscorea sp.	Corms	−	−	−	−	+	bk/g	+
Euphorbiaceae								
Bridelia ferruginea Benth.	l	−	−	−	−	+	bk	−
Elaeophorbia drupifera Stapf	r	+	+	+	−	+	bk	−
Elaeophorbia drupifera Stapf	sb	−	−	−	−	−	br	−
Hymenocardia acida Tul.	r	−	−	+	−	−	dg	−
Hymenocardia acida Tul.	sb	−	−	−	−	+	br	−
Hymenocardia acida Tul.	l	−	−	−	−	+	g	−
Gramineae								
Vetiveria nigritana Stapf	wp	−	−	−	−	+	bk	+
Hypericaceae								
Harungana madagascariensis Lam. ex Poir.	sb	−	−	−	−	+	br	−
Harungana madagascariensis Lam. ex Poir.	l	−	−	−	−	+	bk/g	−
Psorospermum corymbiferum Hochst. var. *kerstingii* (Engl.) Keay ex Milne-Redh.	l	+	+	−	−	+	bk	+
Psorospermum febrifugum var. *ferrugineum* (Hook. f.) Keay ex Milne-Redh.	sb	+	+	−	−	+	bk/g	+
Leguminosae								
Abrus precatorius Linn.	wp	+	+	+	−	−	bk	−
Adenodolichos paniculatus (Hua) Hutch. and Dalz.	sb	+	+	+	−	+	bk/g	−

(*Continued on next page.*)

TABLE I—(*Continued.*)

Scientific Name	Part[a] Used	Alkaloids[b] M[c]	Alkaloids[b] STA[d]	Alkaloids[e] Fraction I	Alkaloids[e] Fraction II	Tannins Gelatin Salt	Tannins $FeCl_3$[f]	Saponins
Bauhinia reticulata DC.	sb	–	–	–	–	+	bk/g	–
Bauhinia reticulata DC.	l	–	–	+[g]	–	+	bk/g	–
Cassia goratensis Fres.	r	–	–	–	–	+	b/b	–
Cassia occidentalis Linn.	s	–	–	–	–	+	bk	–
Cassia siberiana DC.	r	–	–	–	–	+	br	–
Desmodium gangeticum DC.	r	–	–	–	–	+	g	–
Desmodium salicifolium DC.	r	–	–	–	–	+	g	–
Erythrina senegalensis DC.	sb	–	–	–	–	–	br	–
Erythrophleum guineense G. Don.	sb	+	+	–	–	+	br	–
Mucuna puriens (Linn.) DC.	sb	–	–	+	–	+	bk/g	–
Parkia filicoidea Welw.	sb	+	+	+	–	+	bk	+
Parkia filicoidea Welw.	l	–	–	–	–	+	bk/g	–
Liliaceae								
Asparagus schroederi Engl.	s	+	+	+	–	+	dg	+
Loranthaceae								
Loranthus sp.	l	–	–	–	–	+	bk/g	–
Loranthus sp.	s	–	–	–	–	+	bk/g	–
Melastomataceae								
Dissotis sp.	s	–	–	–	–	+	b/g	–
Dissotis sp.	r	–	–	–	–	+	bk	–
Meliaceae								
Pseudocedrela kotschyi (Schweinf.) Harms	sb	–	–	–	–	+	b/b	–
Moraceae								
Ficus indica Hochst. ex Walp.	r	+	+	+	–	+	b/b	–
Ficus gnaphalocarpa (Miq.) Steud. ex A. Rich.	sb	+	+	+	–	+	b/g	–
Myrtaceae								
Eugenia owariensis Beauv.	sb	–	–	–	–	+	b/b	+
Eugenia owariensis Beauv.	l	–	–	–	–	+	b/b	–
Psidium guajava Linn.	l	–	–	–	–	+	bk	–
Pedaliaceae								
Rogeria adenophylla J. Gay	r	+	+	+	+	–	–	–
Polygalaceae								
Polygala arenaria Willd.	r	–	–	+	–	+	bk/g	–
Rubiaceae								
Fadogia agrestis Schweinf.	r	–	–	+	–	+	b/g	–
Fadogia agrestis Schweinf.	s	–	–	–	–	+	br	+
Fadogia agrestis Schweinf.	l	–	–	–	–	+	bk/g	–
Gardenia erubescens Stapf ex Hutch.	l	–	–	–	–	+	bk/g	–
Gardenia erubescens Stapf ex Hutch.	sb	–	–	–	–	–	g	+
Pavetta sp.	r	+	–	–	–	–	br	–
Pavetta sp.	s	–	–	–	–	+	b/g	–
Tiliaceae								
Grewia mollis Juss.	rb	–	–	–	–	+	g	–
Grewia mollis Juss.	sb	–	–	–	–	+	g	–
Grewia mollis Juss.	l	–	–	+[h]	–	+	g	–
Ulmaceae								
Trema guineensis (Schum. and Thonn.) Ficalko	r	–	–	–	–	+	g	–
Umbelliferae								
Steganotaenia araliacea Hochst.	l, s	–	–	–	–	+	bk	+

[a] sb, stem bark; s, stem; r, root; rb, root bark; rz, rhizome; l, leaves; wp, whole plant. [b] Wall *et al.* procedure (1), based on confirmatory test. [c] Mayer's reagent. [d] Silicotungstic acid. [e] Farnsworth and Euler procedure (3). [f] bk/g, back-green; g, green; b/b, blue-black; br, brown; dg, dark green; bk, black; bl/g, blue-green. [g] Positive with 60 μl. [h] Positive with 70 μl.

in the following manner with all extracts giving a preliminary positive test for alkaloids.

Two milliliters of the acidic aqueous extract, prepared as described above, was treated with 28% ammonium hydroxide solution until the solution was distinctly alkaline to litmus paper, and then extracted several times with chloroform. The chloroform extracts were combined and concentrated *in vacuo* to about 2 ml., and then extracted with an equal volume of 1% aqueous hydrochloric acid.

One milliliter of the separated acid extract was treated with a few drops of Mayer's reagent, and a second 1-ml. portion was treated with silicotungstic acid reagent as previously described. Turbidity or precipitation after the addition of either of these reagents was taken as a confirmed positive test for the presence of alkaloids in the extract. The results of these tests are presented in Table I.

Each plant sample was also screened for alkaloids using thin-layer chromatography according to the method of Farnsworth and Euler (3). This procedure was modified only in that the final volume of fraction I (chloroform extract) applied to each thin-layer plate was 30 μl. The results from this test are also presented in Table I.

Saponins—Since all saponins, whether steroidal or triterpenoid, will hemolyze red blood cells, utilization of this property is advantageous for detecting this class of compounds in plant material. A red blood cell suspension was prepared and standardized against digitonin according to the protocol of Wall *et al.* (2). One milliliter of each plant extract was mixed with 10 ml. of the red blood suspension and the mixtures were allowed to stand for 1 hr. before observing the results. Complete hemolysis of the red blood cells in any instance was taken as evidence for a positive test, the results of which are presented in Table I.

Tannins—Twenty milliliters of the original 80% ethanol extract from each plant sample was evaporated to dryness on a steam bath and the residue was stirred with 5 ml. of distilled water and filtered. Two milliliters of the filtrate was treated with a few drops of gelatin–salt reagent (1), and precipitation was taken as evidence for the presence of tannins. The addition of ferric chloride reagent to extracts of plants giving positive gelatin–salt tests served to further categorize the tannin present as to hydrolyzable (blue, blue-black), or condensed types (green, blue-green). These results are presented in Table I.

SUMMARY

Of the 50 species screened for alkaloids, 24 showed the presence of tertiary alkaloids, representing 14 families of plants. Only two species exhibited the presence of quaternary alkaloids, one in the *Annonaceae*, and the other in the *Pedaliaceae*. Sixteen species, distributed in 13 families, contained saponins. The 43 species giving positive tests for tannins were in 29 different families.

REFERENCES

(1) Wall, M. E., Krider, M. E., Krewson, C. F., Eddy, C. R., Willaman, J. J., Corell, D. S., and Gentry, H. S., *J. Am. Pharm. Assoc., Sci. Ed.*, **43**, 1(1954).

(2) Wall, M. E., Eddy, C. R., McClennan, M. L., and Klumpp, M. E., *Anal. Chem.*, **24**, 1337(1952).

(3) Farnsworth, N. R., and Euler, K. L., *Lloydia*, **25**, 186(1962).

Isolation and Characterization of Alkaloids from *Caulophyllum thalictroides*

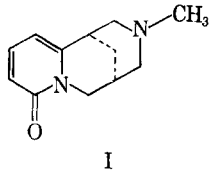
By MICHAEL S. FLOM, RAYMOND W. DOSKOTCH, and JACK L. BEAL

The lupin alkaloids, methylcytisine, baptifoline, and anagyrine, and the aporphine alkaloid, magnoflorine, were isolated from the roots and rhizomes of *Caulophyllum thalictroides*. Separation of the tertiary lupin alkaloids was by partition column chromatography while magnoflorine was obtained after chromatography on alumina.

THE PRESENCE of methylcytisine (I) in the roots and rhizomes of *Caulophyllum thalictroides* (L.) Michx., (family, *Berberidaceae*), commonly called blue cohosh, has been known for many years (1). On re-examination of this source for alkaloids, a thin-layer chromatographic analysis of the tertiary alkaloid fraction revealed at least six spots reacting with Dragendorff's spray reagent. The quaternary alkaloid fraction indicated only one spot when tested by the same spray reagent.

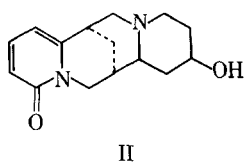
Partition column chromatography on diatomaceous earth with Skellysolve B–ethylene dichloride–methanol–water (10:6:2.5:0.5) as solvent system separated the tertiary alkaloids. The three major alkaloids from this fraction were obtained crystalline or as crystalline salts and were identified as methylcytisine (I), baptifoline (II), and anagyrine (III).

The quaternary alkaloid fraction was obtained *via* the reineckate salt which when converted to the chloride salt and chromatographed on alumina yielded crystalline magnoflorine chloride (IV).



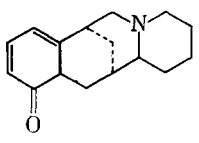
I

(−) Methylcytisine



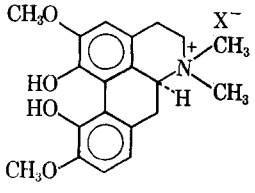
II

(−) Baptifoline



III

(−) Anagyrine



IV

(+) Magnoflorine

Received April 14, 1967, from the College of Pharmacy, Ohio State University, Columbus, OH 43210

Accepted for publication July 24, 1967.

Presented to the Pharmacognosy and Natural Products Section, A.PH.A. Academy of Pharmaceutical Sciences, Las Vegas meeting, April 1967.

This work was supported by research grant GM-05640 from the National Institutes of Health, U. S. Public Health Service, Bethesda, Md.