Phytochemistry, 1975, Vol. 14, pp. 1341–1345. Pergamon Press. Printed in England.

SCREENING OF ARGENTINE PLANTS FOR ALIPHATIC NITRO COMPOUNDS: HIPTAGIN FROM *HETEROPTERIS ANGUSTIFOLIA*

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(*Received August* 1974)

Key Word Index—*Heteropteris angustifolia*; Malpighiaceae; Argentine flora; aliphatic nitro compounds; hiptagin.

Abstract—Plants of 46 families encompassing 98 genera and 124 species were screened for the presence of aliphatic nitro compounds. A single species, *Heteropteris angustifolia* Gris. (Malpighiaceae) proved positive and was found to contain hiptagin (1,2,4,6-tetra-3-nitropropanoyl-β-D-glucopyranoside) as the major nitro constituent. In addition, Argentine herbarium specimen of *Astragalus* (Leguminosae) were screened and three [*A. arnottianus* (Gill.) Reiche, *A. bellus* (O.K.) Fries and *A. diminutivus* Johnst.] gave positive tests.

INTRODUCTION

Because of the identification [1] of 3-nitro-1-propyl-β-D-glucopyranoside (miserotoxin) as the highly poisonous constituent of *Astragalus miser*, it was important to know something about the general distribution of aliphatic nitro compounds in nature. Tests for aliphatic nitro compounds have not been included in any general plant screening programs reported in the literature. Such a screening program might also uncover leads of importance to chemotaxonomy, since two species from the Lauraceae (*Ocotea pretiosa* and *Aniba canelilla*) contain [2] 1-nitro-2-phenylethane and three genera of the Leguminosae (*Indigofera* [3], *Astragalus* [1,4], and *Coronilla* [5]) contain glucosides of 3-nitropropanoic acid or 3-nitro-1-propanol. It has been shown [6] that 1-nitro-2-phenylethane is an intermediate in the biosynthesis of benzylglucosinolate. *Tropaeloum majus* L. (Tropaeolaceae) [6] and *Dennettia tripetala* G. Baker (Annonaceae) [7] also have been shown to contain 1-nitro-2-phenylethane. The presence in our department of numerous, well-documented plant samples (most of which have been used in folk medicine in Argentina) [8,9] provided an opportunity for a detailed screening study of 45 other families. In addition a considerable number of new species of Leguminosae have been examined.

Through herbarium testing it has been shown [1] that about 15% of the North American *Astragalus* species contain aliphatic nitro compounds. A total of 46 Argentine *Astragalus* species have been described [10], including a number of toxic species, and it was of interest to identify those containing nitro compounds.

RESULTS

In the general screening program, 132 dried, ground samples of 124 different species from 98 genera and 46 families (see Table 1) were tested using the Griess–Ilosvay method [1]. Of these, only one species (for which both aerial parts and roots were available) was found to be positive: *Heteropteris angustifolia* Gris. of the Malphigiaceae. The ground roots gave a very strong positive test while the aerial parts showed a weakly positive

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Table 1. Species tested for aliphatic nitro compounds*

Lichens
USNEACEAE: *Ramalina ecklonii* Mey. et Flot. v. *ambigua* Mont. (pl).
Lycopsidia
LYCOPODIACEAE: *Urostachys mandioccanus* (Radd.) Hert. (pl); *U. saururus* (Lam.) Hert. (pl).
Angiospermae:
AMARANTHACEAE: *Gomphrena pulchella* Mart. (fl + px).
ANACARDIACEAE: *Lithraea molleoides* (Vell.) Engl. (lf); *Schinopsis haenkeana* Eng. (lf); *Schinus fasciculatus* (Gris.) Johnst. (lf); *Schinus piliferus* Johnst. (lf).
ARISTOLOCHIACEAE: *Aristolochia argentina* Gris. (r); *A. triangularis* Cham. et Schlecht. (r).
ASCLEPIADACEAE: *Asclepias mellodora* St. Hil. (pl); *Morrenia odorata* (H. et A.) Lindl. (lf).
BALANOPHORACEAE: *Lophophytum mirabile* Schott. et Endl. (pl).
BIGNONIACEAE: *Tabebuia caraiba* (Mart.) Bur. (b); *T. ipe* (Mart.) Standley (b).
BIXACEAE: *Bixa orellana* L. (fr).
BORAGINACEAE: *Echium plantagineum* L. (pl).
CALYCERACEAE: *Acicarpha tribuloides* Juss. (pl).
CANNACEAE: *Canna glauca* L. (px, rz).
CELASTRACEAE: *Maytenus ilicifolia* Reiss. (lf).
CHENOPODIACEAE: *Chenopodium multifidum* L. (px).
COMPOSITAE: *Ambrosia tenuifolia* Spreng. (pl); *Anthemis cotula* L. (pl); *Baccharis articulata* (Lam.) Pers. (lf); *B. coridifolia* DC. (lf); *B. trimera* DC. (pl); *Bidens pilosa* L. (pl); *Cyclolepis genistoides* Don. (st); *Eupatorium laeve* DC. (px); *Flaveria bidentis* (L.) OK. (pl); *Grindelia pulchella* Dun. (pl); *Hymenoxys anthemoides* (Juss.) Cass. (pl); *Parthenium hysterophorus* L. (px); *Pluchea sagittalis* (Lam.) Cabr. (px); *Pterocaulon subvaginatum* Malme (lf + fl); *P. subvirgatum* Malme (lf + fl); *Schkuhria pinnata* (Lam.) OK. (px); *Senecio grisebachii* Bak. (px); *Solidago chilensis* Meyen (pl); *Sonchus asper* (L.) Hill. (pl); *Spilanthes stolonifera* DC. (pl); *Vernonia flexuosa* Sims (px); *Wedelia glauca* (Ort.) E. Hoffman ex Hicken (lf); *Xanthium cavanillesii* Schouw (pl).
CONVOLVULACEAE: *Dichondra repens* Forster v. *sericea* (pl); *Ipomoea amnicola* Morong. (sd); *I. bonariensis* Hook. (px).
CRUCIFERAE: *Coronopus didymus* (L.) Smith (pl).
CYPERACEAE: *Cyperus obtusatus* (Presl) Matt. et Kükenthal ex Kükenthal (rz); *C. sesquiflorus* (Torr.) Matt. et Kükenthal ex Kükenthal (rz).
ERYTHROXYLACEAE: *Erythroxylum cuneifolium* (Mart.) O.E. Schulz (px).
EUPHORBIACEAE: *Croton bonplandianus* Baill. (px); *Euphorbia prostrata* Aiton (lf); *E. serpens* H.B.K. (lf); *Julocroton argenteus* (L.) Didr. (r); *Sapium hematospermum* Müll. (px).
FLACOURTIACEAE: *Casearia sylvestris* Sw. (b).
GENTIANACEAE: *Centaurium pulchellum* (Sw.) Druce (pl).
LEGUMINOSAE: *Acacia aroma* Gill. (lf); *Anarthrophyllum desideratum* (DC.) Benth. (px); *Astragalus garbancillo* Cav. (px); *Caesalpinia gilliesii* (Hook.) Benth. (lf); *C. paraguariensis* (D. Parodi) Burkart (sd, b); *Calliandra parvifolia* (H. et A.) Speg. (st, lf); *Cassia carnaval* Speg. (lf); *C. corymbosa* Lam. (fr); *C. hookeriana* Gill. ex Hook. (lf); *C. morongii* Britt. (px); *C. subulata* Gris. (lf); *Enterolobium contortisiliquum* (Vell.) Morong. (fr); *Geoffroea decorticans* (Gill. ex H. et A.) Burk. (b); *Poiretia tetraphylla* (Poir.) Burk. (px); *Prosopis alba* Gris. (lf); *P. algarobilla* Gris. (lf); *P. alpataco* Phil. (lf); *P. flexuosa* DC. (lf); *P. nigra* (Gris.) Hier. (lf); *P. pugionata* Burk. (lf).
LILIACEAE: *Herreria montevidensis* v. *bonplandii* (Lecompte) Sm. (r).
LOASACEAE: *Blumenbachia insignis* Schroed. (pl).
LORANTHACEAE: *Phoradendron hieronymi* Trel. (px); *Ph. pruinosum* Urb. (st); *Psittacanthus cuneifolius* (R. et P.) Blume ex Schult. (px).
LYTHRACEAE: *Cuphea glutinosa* Cham. et Schlecht. (r); *C. lysimachioides* Cham. et Schlecht. (r); *C.* sp. (pl + fl); *Heimia salicifolia* Link. (r, px).
MALPIGHIACEAE: *Heteropteris angustifolia* Gris. (px, r).
MALVACEAE: *Sida rhombifolia* L. (px); *Sphaeralcea bonariensis* (Cav.) Griseb. (lf + fl).
MENISPERMACEAE: *Cissampelos pareira* L. (px).
MYRTACEAE: *Eugenia pungens* Berg. (lf); *E. uniflora* L. (lf, st); *Psidium luridum* (Spreng.) Burret (px).
PAPAVERACEAE: *Argemone subfusiformis* Ownb. (px); *A. hunnemannii* Otto et Dietr. (px).
PASSIFLORACEAE: *Passiflora caerulea* L. (px).
PHYTOLACCACEAE: *Petiveria alliacea* L. (r); *Phytolacca dioica* L. (lf).
PLANTAGINACEAE: *Plantago paralias* Decne. (px).
POLYGONACEAE: *Rumex crispus* L. (px + fr); *R. obovatus* Dans. (px).
RANUNCULACEAE: *Clematis montevidensis* Sp. (px + sd).
RHAMNACEAE: *Colletia paradoxa* (Spreng.) Esc. (px); *Discaria longispina* (H. et A.) Miers (px, r); *Scutia buxifolia* Reiss. (rb); *Zizyphus mistol* Gris. (lf).
RUBIACEAE: *Borreria centranthoides* Cham. et Schlecht. (r); *Cephalanthus glabratus* (Spr.) K. Schum. (wd); *Pogonopus tubulosus* (Rich.) K. Schum. (st).
RUTACEAE: *Fagara coco* (Gill.) Engl. (lf).
SAPINDACEAE: *Allophyllus edulis* (St. Hil.) Radlk. (lf); *Cardiospermum halicacabum* L. (sd).
SCROPHULARIACEAE: *Scoparia plebeya* Cham. (pl).
SIMARUBACEAE: *Castela coccinea* Gris. (b).

Table 1 (*cont.*)

SOLANACEAE: *Cestrum parqui* L'Her. (lf + fl); *Datura ferox* L. (fr); *Dunalia breviflora* (Sendtn.) Sleumer (lf); *Nicotiana glauca* Graham (pl); *Nierembergia hippomanica* Miers (pl); *Solanum amygdalifolium* Steudel (lf).
UMBELLIFERAE: *Eryngium horridum* Malme (rz).
VERBENACEAE: *Aloysia grattissima* (Gill. et Hook.) Tronc. (px); *Lippia alba* Mill. (lf).

* Families in alphabetical order. The plant part examined in brackets: b; bark- fl; flower- fr; fruit- lf; leaf- pl; entire plant- px; entire plant without roots- r; root- rb; bark of roots- rz; rhizome- sd; seed- st; stem- wd; wood-.
All were negative except *Heteropteris angustifolia* (Malpighiaceae)

test. Fresh leaves and stems of two locally-cultivated specimens of *H. angustifolia* gave strong positive tests as did carefully air-dried samples. Leaf samples from these plants which were dried at 55° gave only very weakly positive or negative results. Leaves or stems from herbarium specimens gave negative tests. Isolation work on the root sample from the screening program and on aerial parts of one of the locally-cultivated plants was carried out. The major nitro compound component from both samples was the same and proved to be identical with hiptagin [11] or 1,2,4,6-tetra-*O*-(3-nitropropanoyl)-β-D-glucopyranoside.

Herbarium specimens of 22 of the recognized [10] 46 Argentine species of *Astragalus* were tested and three proved positive for aliphatic nitro compounds: *A. arnottianus* (Gill.) Reiche, *A. bellus*

Table 2. Argentine herbarium samples of *Astragalus* tested for aliphatic nitro compounds

Species	Location	Accession No.
Positive		
A. arnottianus (Gill.) Reiche	Malargüe, Mendoza	BA 36716
	Paso de Uspallata, Mendoza	Baenitz 1136(BAF)
	Río Tunuyán, Mendoza	BA 55720
A. bellus (OK.) Fries	Santa Catalina, Dept. Cochinoca, Jujuy	Kurtz 11442(BAF)
	Tres Cruces, Humahuaca, Jujuy	Kurtz 11695(BAF)
A. diminutivus Johnst.	Susques, Jujuy	BA 27/818
Negative		
A. arequipensis Vog.	Mina Concordia, Los Andes	BA 27/824
A. bergii Hieron.	San Luis	s/c (BAF s/n)
	Campana, Buenos Aires	BA 13807
A. bustillosii Clos	Valle del Cura, San Juan	BA 30/95
	La Laguna, Salta	BA 13802
A. carinatus (H. et A.) Reiche	Potrero del Calmayo, Córdoba	s/c (BAF s/n)
A. cruckshankii (H. et Arn.) Gris.	Puente del Inca, Mendoza	Pennington 586(BAF)
A. famatinae Johnst.	Cordillera del Colangüil, San Juan	BA 30/98
A. flavocreatus Johnst.	Sierra Famatina, La Rioja	BA 28/182
A. garbancillo Cav.	Depto. Tumbayá, Jujuy	s/c (BAF s/n)
A. gilliesii Phil.	Malargüe, Mendoza	BA 46309
A. hypsogenus Johnst.	Chorrillos, Los Andes	BA 30/1031
A. micranthellus Wedd.	Lara, Tucumán	BA 13796
A. minimus Vog.	San Antonio de los Cobres, Salta	BA 27/813
A. palenae (Phil.) Reiche	Cerro Gutiérrez, Bariloche, Río Negro	Baenitz 1301 (BAF)
A. parodii Johnst.	Río Yuspe, Córdoba	Amorín s/n (BAF)
A. patagonicus Vog.	Río Chubut, Chubut	Gerling 735 (BAF)
	Valle de la Laguna Blanca, Chubut	BA 13822
A. pehuenches Nied.	Malargüe, Mendoza	BA 36723
A. peruvianus Vog.	El Pelado, Tucumán	BA 13795
A. sanctae-crucis Speg.	Río Corcovado, Chubut	Illín 67 (BAF)
	Santa Cruz	BA 13830
A. weddellianus (OK.) Johnst.	Reales Blancos, Catamarca	BA 30/488

(O.K.) Fries and *A. diminutivus* Johnst. (Table 2). *A. arnottianus* was available from three herbaria, but only one of these specimens showed a positive reaction. Because of this variation in results, two specimens of *Astragalus* (*A. toanus* Jones and *A. tenellus* Pursh.), collected in North American, but deposited in Argentine herbaria were tested. Both showed negative reactions although Colorado State University herbarium specimens of the same species had given positive tests [1]. *A. arnottianus* has been collected in the field and indeed contains a high percentage of aliphatic nitro compounds [12].

DISCUSSION

The general screening results show that aliphatic nitro compounds are not widely distributed in nature. This documented study confirms the preliminary observation [1] based upon screening of 70 plants collected at random in Colorado. Although 19 species of Leguminosae (with some representatives from each of the three subfamilies) were included, no new occurrences in that family were encountered. It is to be noted that both *Astragalus* and *Indigofera* belong to the same subfamily and tribe (Papilionoideae Galegeae) of the Leguminosae and hence this restricted group of the family might well be explored in more detail. The Leguminosae is, of course, one of the largest of the plant families and these first results are still very preliminary in regard to the family as a whole.

The occurrence of hiptagin in *Heteropteris angustifolia* (Malpighiaceae) and its higher concentration in roots than aerial parts are interesting data since these facts exactly parallel the results for *Hiptage benghalensis* and *Hiptage madablota* [11,13]. *Hiptage* is also a member of the Malpighiaceae and thus this family can be added to the few where more than one genus contains aliphatic nitro compounds. The results with both genera of the Malpighiaceae where high concentration occurs in the roots is in contrast to the results [14] in *Astragalus* where much higher concentration of nitro compounds are found in the leaves than in the roots. A brief examination of the literature regarding interfamily relationships did not reveal to us any obvious relationship among the families now known to contain aliphatic nitro compounds.

The three positive *Astragalus* species among the 22 Argentine taxa tested represented a frequency of occurrence of nitro compounds in this genus similar to the approximate 15% reported [1] for herbarium screening. However, note should be taken of the failure of a number of specimens in Argentine herbaria to show positive reactions even when the species were known to contain such compounds. Thus, care should be taken in interpreting herbaria testing data for nitro compounds.

The cause of this variation is not known but could be due to differing preservation treatments and/or prevailing climatic conditions. It is evident that some aliphatic nitro compounds in plants show sensitivity to heat since ambient air-dried *H. angustifolia* aerial parts showed strong positive tests compared to the weak or negative tests shown by heat-dried samples.

EXPERIMENTAL

Screening tests. Dried, ground plant material from the species listed in Table 1 was available from previous screening work [8,9]. Small portions of leaves and stems from the *Astragalus* species of Table 2 were obtained from herbaria (Museo de Ciencias Naturales Bernardino Rivadavia and Museo de Botánica Juan A. Domínguez) and ground in a mortar. Approx. 50 mg samples were mixed well on a white spot plate with 3 drops of 20% aq.NaOH and allowed to stand 10 min. To this was added 1 ml of Griess Ilosvay reagent (0·5 g of sulfanilamide in 150 ml of 2 N HOAc mixed with a soln of 0·1 g of N-(1-naphthyl)-ethylenediamine in 20 ml H_2O and 150 ml of 2 N HOAc), followed by two drops of glacial HOAc. Development of a pink red to red purple color (depending upon the concn of nitro compound) was considered a positive test.

Hiptagin from Heteropteris angustifolia. Dried, ground roots of *Heteropteris angustifolia* (voucher specimen BA 66821 [9]) (11 g) were extracted for 8 hr with 95% EtOH in a Soxhlet and the solvent evaporated. Column chromatography on Si gel as described [4] previously yielded crystalline hiptagin identical with an authentic sample [11] by NMR and TLC [4]. TLC of various fractions showed other nitro compounds which were not present in sufficient quantity to identify with certainty. From isolated pure hiptagin and TLC investigation of fractions containing mixtures, the content was estimated at *ca* 1%. Aerial parts from the same collection gave a very weak color test and were not extracted. From comparison of TLC data, a minimum of a 10-fold excess of nitro compounds in the root sample as compared with the aerial parts sample was established. In a similar manner, 155 g of dried aerial parts of *H. angustifolia* (collected from a cultivated specimen at the Instituto de Botánica Darwinion, San Isidro, Prov. de Buenos Aires, Argentina) was extracted and chromatographed. Insufficient nitro compounds were obtained for isolation identification, but TLC in two different solvent systems [4] established the probable presence of hiptagin, 3-nitropropanoic acid and karakin.

Acknowledgements—We are indebted to Ing. A. Burkart, Dr. M. Toursarkissian, and Dr. S. B. Soraru for botanical advice. We are indebted to Dr. Finnegan for a standard sample of hiptagin.

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