Phytochemistry, Vol. 22, No. 12, pp. 2743–2744, 1983.
Printed in Great Britain.

0031–9422/83 $3.00 + 0.00

FLAVONOID VARIATION IN *CLIBADIUM TRIANAE* AND *C. SURINAMENSE*

BRUCE A. BOHM, STACEY BERLOW and TOD F. STUESSY*

Department of Botany, The University of British Columbia, Vancouver, British Columbia, V6T 1W5, Canada; *Department of Botany, The Ohio State University, Columbus, OH 43210, U.S.A.

(*Received* 22 *April* 1983)

Key Word Index—*Clibadium trianae*; *C. surinamense*; Compositae; Heliantheae; Milleriinae; flavonoids; flavonols; *O*-methylated flavonols; anthochlors; variation.

Abstract—Six of eight collections of *Clibadium trianae* exhibited common flavonol 3-*O*-mono- and diglycosides; one collection had these compounds plus quercetagetin derivatives; and one collection had the common flavonols plus chalcone and aurone derivatives. *Clibadium surinamense* has been studied from over 50 collections: all collections exhibited simple 3-*O*-glycosides of kaempferol and quercetin, and two collections also exhibited 3,7-diglucosides of both flavonols. The 3,7-diglucosides were confirmed as constituents also of *C. pilonicum*.

INTRODUCTION

Recent studies from our laboratories have focused on the flavonoid chemistry of *Clibadium*, *Desmanthodium* and *Ichthyothere* (Compositae, Heliantheae, Milleriinae) and how these data might be applied to problems of relationships within and between these genera [1–3]. In most cases where multiple collections were available interpopulational variation in the occurrence of three types of flavonoids was noted: kaempferol and quercetin 3-*O*-monoglycosides, kaempferol and quercetin 3-*O*-diglycosides, and certain quercetagetin derivatives. In this paper we wish to report two further examples of variation in *Clibadium*: flavonol glycosylation pattern differences in *C. surinamense*, and major aglycone differences between populations of *C. trianae*.

RESULTS AND DISCUSSION

Six of the eight collections of *C. trianae* examined had identical flavonoid patterns: kaempferol and quercetin occurred as 3-*O*-glucosides, 3-*O*-galactosides, 3-*O*-rutinosides, 3-*O*-rhamnosylgalactosides and 3-*O*-diglucosides. One collection (*5623*) exhibited the common glycosides just listed plus quercetagetin and patuletin 7-*O*-glucosides. The latter two compounds were identified by comparison of chromatographic characteristics, colour reactions, UV spectra and ^{1}H NMR spectra with compounds isolated in earlier studies [1]. Collection *5528* had the array of kaempferol and quercetin glycosides listed above, lacked the quercetagetin derivatives of *5623*, but displayed two large spots on TLC chromatograms not seen in our other studies of *Clibadium*. The compounds, isolated and purified by described methods [4], gave UV spectral data suggesting that one was a chalcone and the other an aurone. ^{1}H NMR and mass spectral data confirmed these conclusions as did chromatography against known compounds. The two compounds were identified as coreopsin and sulfurein, 3,4,2′,3′-tetrahydroxychalcone 4′-*O*-glucoside and 4,6,3′,4′-tetrahydroxyaurone 6-*O*-glucoside, respectively. Two additional compounds were present, in much smaller quantities, which also gave UV spectra suggesting a chalcone base structure. Mass spectral information coupled with UV data and chromatographic comparison of the aglycones with compounds of known structure showed these unknown flavonoids to be 4,2′,4′-trihydroxychalcone 4′-*O*-glucoside and 4,2′,4′-trihydroxy-3-methoxychalcone 4′-*O*-glucoside.

This report of chalcones and an aurone in *C. trianae* represents the first documented record of these flavonoid types in the Milleriinae (a footnote in a recent paper by Crawford and Stuessy [5] reported our finding of coreopsin and sulfurein in this taxon). It was pointed out in that paper [5] that the occurrence of these compounds in only one of several species of *Clibadium* tested may represent an independent origin. It is of interest to note that the aurone and chalcones found in *C. trianae* are all members of the resorcinol-based anthochlor group which is apparently characteristic of Heliantheae, Lactuceae and Helenieae [5].

In our earlier work on *Clibadium* [1] flavonoids of 14 collections of *C. surinamense* were examined. Kaempferol and quercetin 3-*O*-glucosides and galactosides were consistently present while 3-*O*-rhamnosides were seen in about three-quarters of the samples. Variation was also seen in the distribution of the diglycosides, there being a few collections that lacked either the 3-*O*-rutinosides or 3-*O*-diglucosides. An additional 41 collections of *C. surinamense* have now been examined by two-dimensional thin-layer chromatography. All specimens exhibited kaempferol and quercetin 3-*O*-glucosides (galactosides could not be scored since their R_f values are identical to those of the glucosides in the TLC system employed). Quercetin and kaempferol 3-*O*-rhamnosides were seen in 39 and 36 specimens, respectively. Quercetin 3-*O*-rutinoside was ubiquitously present while 34 specimens also had kaempferol 3-*O*-rutinoside. Quercetin and kaempferol 3-*O*-diglucoside were seen in five and four collections, respectively. These last values may underestimate the real situation somewhat insofar as the diglucosides were rarely seen in more than trace amounts. The overall pattern of flavonol glycoside distribution is, thus, similar to that from the original sampling. Two

collections stood out from the rest, however. Collections *5708* and *5783* exhibited two major spots new to the taxon. The compounds were identified as kaempferol and quercetin 3,7-di-*O*-rhamnosides by means of UV spectroscopy and acid hydrolysis. The chromatographic characteristics of these compounds were reminiscent of spots seen as trace constituents of *C. pilonicum* but not reported in the earlier work owing to uncertainty. Re-examination of *C. pilonicum* showed that the two compounds in this taxon were indeed the same, 3,7-di-*O*-rhamnosides.

Clibadium surinamense, which is morphologically one of the most variable taxa in the genus, occurs from southern Honduras through central America and from western Venezuela to Colombia, Ecuador and northern Peru. The two collections in which the 3,7-di-*O*-rhamnosides occur are from sites in central Colombia separated by approximately 200 km. Several specimens with 'normal' flavonoid chemistry were collected from sites lying between the sites from which the dirhamnoside-bearing plants were obtained. *Clibadium pilonicum* is a rare taxon known only on and around the slopes of Cerro Pilón in central Panama [6]. Other than the obvious possession of the flavonol dirhamnosides in common, the two taxa, *C. surinamense* and *C. pilonicum*, do not seem particularly closely related. The similarity in flavonoid profiles may simply represent a case of parallel evolution.

EXPERIMENTAL

Sources of plants. All vouchers at OS; all were collected by *Stuessy and Funk* in Colombia unless noted otherwise. *Clibadium trianae* (Hieron.) S. F. Blake: Cundinamarca, *5528*; Nariño, *5766*, *5770*; Norte de Santander, *5623*; Putumayo, *5775*, *5777*; Santander, *5627*; Tolima, *5680*. *C. pilonicum* Stuessy: Panama, Coclé, *Hartman 3963*. *C. surinamense* L.: Antioquia, *5705*, *5707*, *5708*; Boyacá, *5591*, *5599*, *5640*; Caldas, *5697*, *5698*, *5700*, *5713*, *5715*; Cauca, *5744*, *5747*, *5748*; Cundinamarca, *5510*, *5511*, *5517*, *5519*, *5524*, *5571*, *5643*, *5645*, *5657*, *5666*; Meta, *5649*; Nariño, *5756*; Quindio, *5782*; Santander, *5601*, *5602*; Tolima, *5670*, *5671*, *5783*; Valle, *5719*, *5723*, *5727*, *5732*, *5743*. Ecuador, El Oro, *Stuessy and Nesom* [*SN*] *5873*; Guayas, *SN 5856*; Zamora, *SN 5895*. Venezuela, Mérida, *Stuessy 6000*.

Identification of flavonoids. Aerial portions of all plants were extracted and the constituent flavonoids isolated by methods described in the preceding papers in this series [1–3] and in ref. [4]. UV and ^{1}H NMR studies were done according to well-described procedures [7]. Coreopsin and sulfurein were compared with authentic samples provided by Dr. D. Giannasi, 4,2′,4′-Trihydroxychalcone and 4,2′,4′-trihydroxy-3-methoxychalcone were compared to authentic samples of these compounds synthesized by the first author. The placement of the glucose moiety in each case was based upon UV spectral observations. Mass spectral data supported the structural assignments [8].

Acknowledgements—This research was supported through operating and equipment grants from the Natural Sciences and Engineering Research Council of Canada (to B.A.B.) and by the National Science Foundation (DEB-7520819 and INT-7684454 to T.F.S.). Our appreciation is hereby expressed to Vicki Funk, Ronald Hartman and Guy Nesom for assistance in collection of the plant material, and to Dr. David Giannasi for flavonoid specimens. Dr. Lily Wat's assistance in obtaining the mass spectral data is greatly appreciated.

REFERENCES

1. Bohm, B. A. and Stuessy, T. F. (1981) *Phytochemistry* **20**, 1053.
2. Bohm, B. A. and Stuessy, T. F. (1981) *Phytochemistry* **20**, 1573.
3. Bohm, B. A. and Stuessy, T. F. (1982) *Phytochemistry* **21**, 2761.
4. Wilkins, C. K. and Bohm, B. A. (1976) *Can. J. Botany* **54**, 2133.
5. Crawford, D. J. and Stuessy, T. F. (1981) *Am. J. Botany* **68**, 107.
6. Stuessy, T. F. (1975) *Ann. Mo. Bot. Gard.* **62**, 1074.
7. Mabry, T. J., Markham, K. R. and Thomas, M. B. (1970) *The Systematic Identification of Flavonoids*. Springer, New York.
8. Mabry, T. J. and Markham, K. R. (1975) in *The Flavonoids* (Harborne, J. B., Mabry, T. J. and Mabry, H., eds.), pp. 78–126. Chapman & Hall, London.