CIENCIA 15(1), 105 - 110, 2007
Maracaibo, Venezuela

Protoberberine alkaloids isolated of *Guatteria schomburgkiana* (*G. sessilis*)

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Recibido: 23-03-06 Aceptado: 13-12-06

Abstract

From the leaves of *Guatteria schomburgkiana* Mart. (*Guatteria sessilis* R.E. Fries), three protoberberine alkaloids: anisocycline, lincangenine and palmatine, and one tetrahydroprotoberberine thaicanine, has been isolated. For our knowledge, this is the first report of these alkaloids in the *Guatteria* genus. The structures were established by spectroscopic methods, including previously unreported ^{13}C-NMR and Mass spectral data for anisocycline.

Key words: Anisocycline; annonaceae; *Guatteria schomburgkiana*; lincangenine; thaicanine.

Alcaloides protoberberinicos de *Guatteria schomburgkiana* (*G. sessilis*)

Resumen

De las hojas de *Guatteria schomburgkiana* Mart. (*Guatteria sessilis* R. E. Fries) fueron aislados tres alcaloides protoberberínicos: anisocyclina, lincangenia y palmatina, y la tetrahidroprotoberberina thaicanina. Este es el primer reporte de estos alcaloides en el género *Guatteria*. Las estructuras fueron establecidas por métodos espectroscópicos, incluyendo datos no reportados de ^{13}C-RMN y Masas para anisocyclina.

Palabras clave: Anisocyclina; annonaceae; *Guatteria schomburgkiana*; lincangenina; thaicanina.

Introduction

The family Annonaceae belongs to the order Magnoliales, which is markedly rich in alkaloids. This order together with related groups such as Ranales, Papaverales, and Hamamelidales is considered among the most primitive of all living angiosperms from a morphological point of view (1). The annonaceous plants are encountered as trees, shrubs, or lianas (lianas rare in the Neotropics) in tropical regions with a temperate extension into eastern North America, consisting of 130 genera and ca. 2000 species (2). *Guatteria* is the largest annonaceous genus, comprising ca. 250 species. It is exclusively neotropical, its range reaching from southern Mexico to southern Brazil. The fibrous bark of many species, commonly called majagua, is used as cordage throughout the Venezuelan Guayana (2). About 130 alkaloids have been isolated and identified from

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ca. 20 phytochemically studied species of this genus. Most of them have an aporphine skeleton (3). Among other types of isoquinoline alkaloids found in *Guatteria* there are bisbenzyl-isoquinolines (4), tetrahydroprotoberberines (5), and non-isoquinoline 4-azafluorenones are also reported (6). It has been reported that from the bark of *Guatteria schomburgkiana* 18 alkaloids were isolated and identified, 14 of which have the aporphine skeleton, the rest being tetrahydroprotoberberines (5). The cytotoxic activity of lincangenine (7), palmatine (8), and thaicanine (9) over several different human cancer cell lines has been reported (10). In this work we report the isolation of one protoberberine alkaloid: anisocycline (11), this is the second report of the isolation of anisocycline and licangenine in the bibliography and the first in the genus. In this work unreported spectral data of anisocycline, lincangenine and thaicanine are showed.

Materials and Methods

General experimental procedures

NMR Spectra (^{1}H and ^{13}C) were recorded on either a Bruker AC-300, a Bruker AMX-400 or a Jeol eclipse 270 MHz spectrometer in $CDCl_3$ or CD_3OD + CF_3COOD (5:1) using TMS as internal standard. Apart from ^{1}H and ^{13}C, NOESY and DEPT experiments were performed to elucidate the structures of compounds. The HRESMS and LRESMS were recorded on a Micromass Hybrid Sector-TDF apparatus. Column chromatography was performed using silica gel 60 (70-230 mesh) using dichloromethane/ methanol/ ammonium hydroxide (50:10:1) as the eluent. Analytical thin-layer chromatography of compounds or extracts was performed on silica gel 60 F_{254}, Macherey-Nagel plates, using the same eluent system as for column chromatography.

Collecting of plant Material

Plant material of *Guatteria schomburgkiana* Mart. was collected in the Amazonian forest, along the Cataniapo riverside, 8 km southeast of Puerto Ayacucho city, Venezuela. The taxonomic identification of the plant was done by Dr. Anibal Castillo. Corresponding voucher specimen (5.156 AC) is deposited in the National Herbarium of Venezuela (VEN, Caracas Botanical Garden), Caracas-Venezuela.

Extraction, purification and identification of alkaloids

The dried and pulverized leaves (2.4 kg) were percolated with 5% HCl at room temperature. The acidic aqueous solution was basified with 10% NH_4OH and extracted with $CHCl_3$. The $CHCl_3$ layer was washed with water, dried and evaporated to yield 5.93 g of crude alkaloidal fraction, representing 0.25% of dry plant material. This alkaloidal fraction was subjected to column chromatography on silica gel using as eluent a mixture of $CHCl_3$/ CH_3OH increasing gradually the polarity to afford 37 collective fractions. The intermediate fractions, positive to Dragendorff's reagent, were combined and passed through a silica gel column using the solvent system described in general experimental procedures. Final separation by preparative layer chromatography on silica gel using the same solvent system enabled us to isolate four following alkaloids: anisocycline (0.00021%), lincangenine (0.21%), palmatine (0.00042%), and thaicanine (0.01%).All the alkaloids were identified by comparison of their physical and spectroscopic data with those of the literature (7- 11).

Anisocycline 1

Yellow amorphous solid. Mp.190-195°C (reported 195°C); HRESMS m/z 382.16526 (M^+, 382.165448 calculated for $C_{22}H_{24}NO_5$). δ_H(CD_3OD + CF_3COOD) 9.80 (1H, s, H-8), 8.88 (1H, s, H-13), 8.14 (1H, d, *J* 9.15, H-11), 8.04 (1H, d, *J* 9.15, H-12), 7.56 (1H, s, H-1), 4.86 (2H, t, *J* 6.0, H-6), 4.21 (3H, s, OCH_3), 4.12 (3H, s, OCH_3), 4.02 (OCH_3), 3.94 (3H, s, OCH_3), 3.93 (3H, s, OCH_3), 3.22 (2H, t, *J* 6.0, H-5). δ_C (CD_3OD) 154.0 (C-4), 150.9 (C-3) ,150.3 (C-10), 145.3 (C-8), 144.5 (C-9), 137.9 (C-2), 137.9 (C-14), 133.7 (C-12a), 126.1 (C-11), 123.3 (C-12),

122.5 (C-4a), 122.2 (C-8a), 121.0 (C-13), 121.8 (C-14a), 105.0 (C-1), 61.2 (OCH_3), 60.4 (OCH_3), 60.2 (OCH_3), 56.3 (OCH_3), 55.8 (C-6), 55.7 (OCH_3), 20.5 (C-5). Tetrahydroanisocycline ($NaBH_4$): LRESMS m/z 385 (M^+, 70%), 220 (20), 164 (60), and 149 (100). The structure was supported by correlations observed in NOESY Spectra.

Lincangenine 2

Yellow crystalline solid. Mp. 229–233°C (reported mp. 250-251°C); IR υ_{max}^{CHCl3} cm $^{-1}$: 3406, 2934, 2850, 1607, 1508, 1462, 1374. HRESMS m/z 368.14941 (M^+, 368.14980 calculated for $C_{21}H_{22}NO_5$). δ_H (CD_3OD + CF_3COOD) 9.65 (1H, s, H-8), 8.60 (1H, s, H-13), 8.00 (1H, d, *J* 9.15, H-11), 7.92 (1H, d, *J* 9.15, H-12), 7.19 (1H, s, H-1), 4.87 (2H, t, *J* 6.40, H-6), 4.15 (3H, s, OCH_3), 4.04 (3H, s, OCH_3), 3.95 (3H, s, OCH_3), 3.88 (3H, s, OCH_3), 3.22 (2H, t, *J* 6.40, H-5). δ_C (CD_3OD) 153.2 (C-4), 150.8 (C-3),147.5 (C-10), 145.1 (C-8), 144.4 (C-9), 139.1 (C-2), 138.4 (C-14), 133.7 (C-12a), 126.6 (C-11), 123.3 (C-12), 122.3 (C-4a), 122.1 (C-8a), 120.9 (C-13), 115.7 (C-14a), 100.7 (C-1), 61.2 (OCH_3), 60.0 (OCH_3), 56.3 (OCH_3), 56.0 (C-6), 55.5 (OCH_3), 20.2 (C-5). The structure was supported by correlations observed in NOESY Spectra.

Palmatine 3

Yellow amorphous solid. Mp. 206-210°C (reported mp. 208°C); HRESMS m/z 352.15461 (M^+, 352.15488 calculated for $C_{21}H_{22}NO_4$). δ_H(CD_3OD) 9.77 (1H, s, H-8), 8.82 (1H, s, H-13), 8.13 (1H, d, *J* 9.13, H-11), 8.02 (1H, d, *J* 9.13, H-12), 7.05 (1H, s, H-1), 4.88 (2H, t, *J* 6.42, H-6), 4.21 (3H, s, OCH_3), 4.11 (3H, s, OCH_3), 3.99 (3H, s, OCH_3), 3.94 (3H, s, OCH_3), 3.23 (2H, t, *J* 6.42, H-5). δ_C DEPT (CD_3OD) 145.1 (C-8), 126.7 (C-11), 123.1 (C-12), 120.0 (C-13), 108.6 (C-1), 61.2 (OCH_3), 56.3 (OCH_3), 56.0 (C-6), 55.7 (OCH_3), 55.3 (OCH_3), 26.5 (C-5).

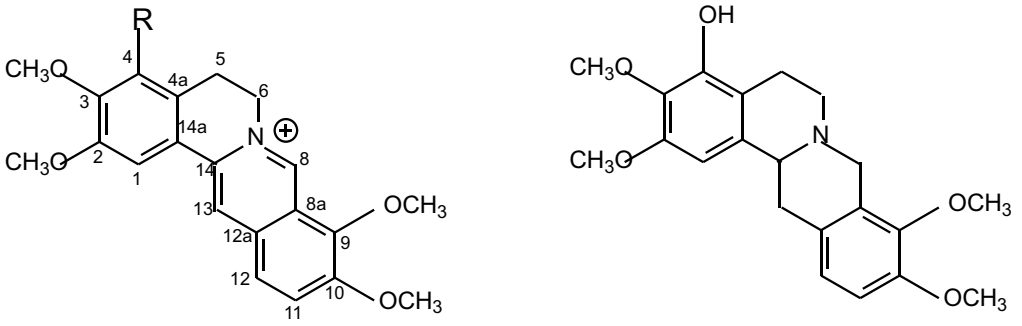
Thaicanine 4

Dark brown solid. Mp. 137–138°C (reported mp 144–146°C), IR υ_{max}^{CHCl3} cm $^{-1}$: 3490, 2950, 2890, 2790, 2710, 1490, 1120, 1080. HRESMS m/z 371.16927 (M^+, 371.42694 calculated for $C_{21}H_{25}NO_5$). LRESMS m/z 371 (M^+, 100%), 370 (93), 340 (30), 206 (24), 165 (23), 164 (60), and 149 (54). δ_H($CDCl_3$) 6.34 (1H, s, H-1), 2.84 (1H, m, H-5ax.), 2.50 (1H, m, H-5ec.), 2.82 (1H, m, H-6ax.), 3.22 (1H, m, H-6ec), 3.53 (1H, d, *J* 15.7, H-8ax.), 4.20 (1H, d, *J* 15.7, H-8ec.), 6.76 (1H, d, *J* 8.4, H-11), 6.85 (1H, d, *J* 8.4, H-12), 2.84 (1H, m, H-13ax.), 3.22 (1H, m, H-13ec.), 3.53 (1H, m, H-14), 3.90 (3H, s, OCH_3), 3.86 (3H, s, OCH_3), 3.85 (3H, s, OCH_3), 3.82 (3H, s, OCH_3), δ_C ($CDCl_3$) 150.6 (C-2), 150.3 (C-9), 146.5 (C-4), 145.1 (C-10), 133.8 (C-14a), 133.6 (C-3), 128.6 (C-12a), 127.7 (C-8a), 123.9 (C-12), 115.0 (C-4a), 111.0 (C-11), 100.6 (C-1), 61.0 (3-OCH_3), 60.2 (9-OCH_3), 56.0 (2-OCH_3), 55.9 (10-OCH_3), 59.5 (C-14), 54.1 (C-8), 51.0 (C-6), 36.2 (C-13).

Results and Discussions

From the leaves of *Guatteria schomburgkiana* Mart., (= *G. sessilis* R.E.Fr., *G. sandwithii* R.E.Fr., *G. spruceana* R.E.Fr., *G. flavovirens* R.E.Fr., and *G. bernardii* R.E.Fr.) [2], four previously known alkaloids, the prototoberberines, anisocycline **1**, lincangenine **2**, palmatine **3** and the tetrahydroprotoberberine thaicanine **4** were isolated. Lincangenine **2** and thaicanine **4** were relatively abundant in this species.

Anisocycline **1**, was isolated as a yellow amorphous solid, (Figure 1); HRMS measurement gave a molecular formula $C_{22}H_{24}NO_5$ ([M^+] 382.16526), giving absorption maxima at 229, 266 (accompanied by a shoulder at 276 nm), 336, and 425 nm in the UV spectrum. A highly conjugated system in the molecule and the observation of two notably deshielded singlets at 8.88 and 9.80 ppm in the aromatic proton region in its ^{1}H-NMR spectrum, are agreed with the protoberberine skeleton. The above mentioned aromatic protons can be assigned to H-13 and H-8, respectively, in the protoberberine skeleton. A pair of multiplets at 3.22 and 4.86 ppm are assignable to H-5 and H-6 methylene protons, respectively. The remaining proton sig-



1 R = OCH_3
2 R = OH
3 R = H

4

Figure 1. Protoberberine alkaloids isolated from *G. schomburgkiana*.

nals are one singlet (1H) at 7.56 ppm, a pair of doublets (2H) at 8.04 and 8.14 ppm as the AB system with *ortho* coupling constant (9.15 Hz) and five methoxyl groups (3.93, 3.94, 4.02, 4.12 and 4.21 ppm) in the molecule. The ^{13}C-NMR and ^{13}C-DEPT NMR spectra of **1** revealed 22 carbons consisting of 5 CH_3, 2 CH_2 and 5 CH leaving behind 10 quaternary carbons. 2D NOESY experiment determined the location of three aromatic protons and consequently, of all methoxyl groups, as indicated in the Figure 2. Anisocycline **1**, was reduced with $NaBH_4$ to the corresponding tetrahydroprotoberberine. Its mass spectrum showed two indicative peaks at m/z 164 (58%) and 220 (18%), derived from retro Diels–Alder cleavage of ring-C. The fragment at m/z 220 corresponds to the isoquinoline A/B rings bearing three methoxyl groups, while the one at m/z 164 refers to C/D rings with two methoxyl groups. Anisocycline was isolated for first time from *Anisocycla cymosa* (11).

Lincangenine **2**, $C_{21}H_{22}NO_5$ ([M^+] 368.14941), was isolated as yellow crystaline solid. As well as this UV spectrum (absorption maxima in MeOH at 225, 261, 325, and 423 nm), its ^{1}H-and ^{13}C-NMR spectra are similar to those of anisocycline **1**, having one OH instead of OCH_3 group. Two singlets at 8.60 and 9.65 ppm in the aromatic proton region in the ^{1} H-NMR spectrum can be assigned to H-13 and H-8, respectively. A pair of triplets at 3.22 and 4.87 ppm with a coupling constant of 6.4 Hz are assignable to H-5 and H-6 respectively. The remaining proton signals are one singlet (1H) at 7.19 ppm and a pair of doublets (2H) at 7.92 and 8.00 ppm as the AB system with *ortho* coupling constant (9.15 Hz). On the other hand, there are four methoxyl groups (3.88, 3.95, 4.04, and 4.15 ppm) in the molecule, and the fifth oxygen atom corresponds to a hydroxyl group, evidenced by a broad band at 3406 cm^{-1} in the IR spectrum. The ^{13}C-NMR and ^{13}C-DEPT NMR spectra of **2** revealed 21 carbons consisting of 4 CH_3, 2 CH_2, 5 CH and 10 quaternary carbons. 2D NOESY experiment determined the location of three aromatic protons and consequently, of all methoxyl groups, as indicated in the Figure 2. Lincangenine **2,** was reduced with $NaBH_4$ to the corresponding tetrahidroprotoberberine. Mass spectral analysis of this compound showed the characteristic retro Diels-Alder fragments associated with a tetrahydroprotoberberine skeleton possessing two methoxyl groups in the D ring (m/z 164, 100 %) and at least two methoxyl groups in the A ring. This analyses also suggested that this compound contained an additional hydroxyl group in ring A (m/z 206, 37 %).The determination of structure for lincangenine **2** was achieved by chemical shifts displacement of the singlet

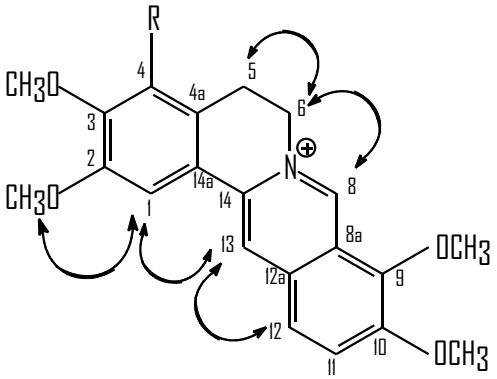


Figure 2. NOESY correlations of compounds 1 and 2.

at 7.19 (H-1) in the ^{1}H-NMR spectrum registered in deuterated methanol in comparison with that taken in alkaline medium. On addition of NaOH pellet to the NMR tube containing methanol solution of lincangenine, this singlet shifted notably to up-field by 0.47 ppm. It is well known that phenolate ion formation in alkaline medium causes significant up-field chemical shifts for the protons in *ortho* and *para* positions but not in *meta* position. According to this, OH group should be located on C-4 because NOE experiment demonstrated that the position *orto* to the singlet at 7.19 (H-1) has a methoxy group. Lincangenine **2**, was isolated for first time from the S*tephania lincangensis* (7).

Palmatine **3**, $C_{21}H_{22}NO_4$ (M^+ 352.15461), was isolated as amorphous solid. Their structure was confirmed by comparison of their spectroscopic data (8).

Thaicanine **4**, was isolated as dark brown crystaline solid and ^{13}C-NMR chemical shifts and coupling patterns of this alkaloid agree well with those reported for thaicanine, first isolated from *Parabaena sagittata* Miers (Menispermaceae) (9).

Conclusions

Three protoberberine alkaloids: anisocycline, lincangenine and palmatine, and one tetrahydroprotoberberine thaicanine, were isolated following an acid-base extraction of the methanolic extract from *G. schomburgkiana* leaves. The alkaloids lincangenine and thaicanine were the most abundant representing 0.21 and 0.1% of dry plant material respectively. This is the first report of lincangenine, anisocycline and thaicanine in the genus *Guatteria*.

Acknowledgments

This research was supported in part by The Consejo de Desarrollo Científico y Humanístico, Universidad Central de Venezuela (UCV), PG. 03.12.3425.1999, 03.12.4384.2003, and FONACIT S1 2002000284. We thank. Aníbal Castillo, Instituto de Biología Experimental, Facultad de Ciencias, Universidad Central de Venezuela, for plant colection and identification.

References

1. WATSON L., DALLWITZ J. ***The families of flowering plants: Descriptions, illustrations, identification, information retrieval***. http://delta-intkey.com/angio/www/annonace.htm.
2. STEYERMARK J.A., MAAS P.J.M., BERRY P.E., JOHNSON D.M., MURRAY N.A., RAINER H. ***Annonaceae*** In: Berry P.E., Holst B.K., Yatskievych K. (Eds.) ***Flora of the Venezuelan Guayana*** Vol. 2, Timber Press, Portland, Oregon, pp. 413-469, 1995.
3. CASTEDO L., GRANJA J., RODRÍGUEZ DE LERA A., VILLAVERDE M. ***Phytochemistry*** 30: 2781-2783, 1991.
4. BERTHOU S., JOSSANG A., GUINAUDEAU M., LEBOEUF M., CAVÉ A. ***Tetrahedron*** 44: 2193-2204, 1988.
5. BAYMA J., DEOLIVEIRA A., CAVÉ A., DADOUN H. ***Planta Medica*** 54: 84-85, 1988.

6. TADIC D., CASSELS B., CAVÉ A., GOULART M., DE OLIVEIRA A. ***Phytochemistry*** 26:1551-1552, 1987.
7. CHENG Y., FANG S., LI H., LI Y., YANG H. ***Zhiwu Xuchao*** 33: 552-555, 1991.
8. MORIYASU M., ICHIMARU M., NISHIYAMA Y., KATO A. ***Natural Medicines*** 48: 287-290, 1994.
9. RUANGRUNGSI N., LANGE G., LEE M. ***J Nat Prod*** 49: 253-258, 1986.
10. ORFILA L., RODRÍGUEZ M., COLMAN T., HASEGAWA M., MERENTES E., ARVELO F. ***Journal of Ethnopharmacology*** 71: 449-456, 2000.
11. KANYINDA B., DIALLO B., VANHAELEN-FASTRÉ R., VANHAELEN M. ***Planta Medica*** 55: 394-395, 1989.