4 Misra, L N, Shawl, A. S and Raina, V K. (1988) *Planta Med* **54**, 165
5. Ahmad, A, Misra, L. N and Thakur, R S (1989) *Planta Med* **55**, 199
6. Garg, S N., Misra, L N. and Agarwal, S K. (1989) *Phytochemistry* **28**, 634
7. Misra, L N., Tyagi, B R and Thakur, R S. (1989) *Planta Med.* in press
8 Misra, L. N, Chandra, A and Thakur, R S (1989) *Tetrahedron Letters* **30**, 1437
9. Garg, S. N., Misra, L. N., Siddiqui, M. S. and Agarwal, S. K. (1990) *Phytochemistry* **29** (in press).
10. Patra, N K., Srivastava, R. K., Chauhan, S. P., Ahmad, A. and Misra, L. N (1989) *Planta Med* (in press).
11. Lawrence, B M. (1983–84) *Perfumer Flavorist* **8**, 70.
12 Lawrence, B. M. (1981) *Perfumer Flavorist* **6**, 44.
13. Swigar, A A, Silverstein, R. M. (1981) *Monoterpenes.* Aldrich Chemical Co, Milwaukee, Wisconsin.
14 Jakupovic, J and Eid, F. (1987) *Phytochemistry* **26**, 2427.

Phytochemistry, Vol 29, No 6, pp 2037–2040, 1990
Printed in Great Britain

0031-9422/90 $3 00+0 00

SECONDARY METABOLITES FROM *HINTONIA LATIFLORA**

RACHEL MATA,† MARÍA DEL RAYO CAMACHO, ERNESTINA CERVERA,‡ ROBERT BYE§ and EDELMIRA LINARES§

Departamento de Farmacia, ‡Laboratorio de Resonancia Magnética Nuclear, Facultad de Química, Universidad Nacional Autónoma de México, Coyoacán 04510, México, D F., §Jardín Botánico, Instituto de Biología, Universidad Nacional Autónoma de México, Coyoacán 04510, México, D.F

(*Received* 21 *June* 1989)

Key Word Index—*Hintonia latiflora*; Rubiaceae, stem bark, phenylcoumarins, cucurbitacins, 3-*O*-β-D-glucopyranosyl-23,24-dihydrocucurbitacin F, 5-*O*-β-D-galactopyranosyl-4′-hydroxy-7-methoxy-4-phenylcoumarin; flavonoids

Abstract—Two new glycosides and several known compounds were isolated from the stem bark of *Hintonia latiflora*. The new metabolites were characterized by chemical and spectroscopic methods as 3-*O*-β-D-glucopyranosyl-23,24-dihydrocucurbitacin F and 5-*O*-β-D-galactopyranosyl-4′-hydroxy-7-methoxy-4-phenylcoumarin.

INTRODUCTION

In continuation of our chemical studies of Mexican plants used in traditional medicine we have reinvestigated *Hintonia latiflora* (Sesse ex Mociño ex DC.) Bullock (Rubiaceae). The stem bark of this plant is used medicinally for the treatment of several diseases, including malaria and dengue [1]. Previous phytochemical work resulted in the isolation and identification of two simple cucurbitacins [1] and one oxidocoumarin [2] In the present paper we describe the isolation and characterization of five known compounds and two new secondary metabolites which were identified by chemical and spectral means as: 3-*O*-β-D-glucopyranosyl-23,24-dihydrocucurbitacin F (**1**) and 5-*O*-β-D-galactopyranosyl-4′-hydroxy-7-methoxy-4-phenylcoumarin (**2**).

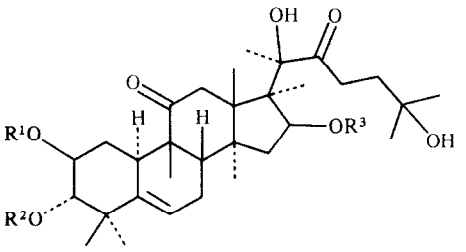
RESULTS AND DISCUSSION

Dried defatted stem bark of *H. latiflora* was extracted with methanol. Silica gel chromatography allowed the isolation of two new compounds, **1** and **2**, as well as the known compounds: 5-*O*-β-D-galactopyranosyl-3′,4′-dihydroxy-7-methoxy-4-phenylcoumarin (**3**) [3], 5-*O*-β-D-glucopyranosyl-3′,4′-dihydroxy-7-methoxy-4-phenylcoumarin (**4**) [4, 5], 5-*O*-(6″-acetyl)-β-D-galactopyranosyl-3′,4′-dihydroxy-7-methoxy-4-phenylcoumarin (**5**) [4], 5-*O*-β-D-glucopyranosyl-7,3′,4′-trihydroxy-4-phenylcoumarin, (**6**) [5] and 7-methyl-luteolin [6].

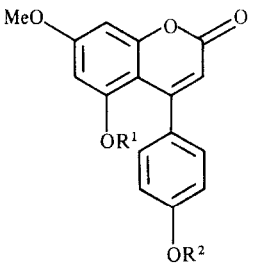
Compound **1**, $C_{36}H_{58}O_{12}$, mp 199–201°, exhibited bands at 3400 and 1690 cm^{-1} in its IR spectrum. The ^{13}C NMR spectra (Table 1) confirmed the presence of 36 carbons and supported the assignment of a glucocucurbitacin type of compound which possesses two ketone groups (δ216 and 213.1) and five oxygenated functions (δ93.7, 80 1, 71.2, 70.4 and 69.2) in addition to those of the β-D-glucopyranosyl moiety [7–10]. The ^1H NMR (Table 2) spectrum of the acetyl derivative **1a** was very similar to those previously described for 23,24-dihydrocucurbitacins [7, 8, 10, 12]. Enzymatic hydrolysis with β-D-glucosidase afforded β-D-glucose and 23,24-dihydrocucurbitacin

*Part XI of the series 'Chemical Studies on Mexican Plants Used in Traditional Medicine' For Part X see ref [13]. Taken in part from the MS thesis of M R Camacho

†Author to whom correspondence should be addressed



1 $R^1 = R^3 = H$, $R^2 = \beta$-D-glucopyranosyl
1a $R^1 = R^3 = Ac$, R^2 = tetraacetyl-β-D-glucopyranosyl
1b $R^1 = R^2 = R^3 = H$
1c $R^1 = R^2 = R^3 = Ac$



2 $R^1 = \beta$-D-galactopyranosyl, $R^2 = H$
2a R^1 = tetraacetyl-β-D-galactopyranosyl $R^2 = Ac$
2b $R^1 = R^2 = H$
2c $R^1 = \beta$-D-galactopyranosyl $R^2 = Me$

F (**1b**) identical to an authentic sample [1] The remarkably low field of the C-3 signal (δ93.7) in the ^{13}C NMR of **1** compared with the corresponding resonance in **1b** (δ81.4) clearly indicated that the sugar residue was attached to the hydroxyl group at C-3 in **1** [7–9, 11] The lower shift resonance of H-3 (δ4 64, *d*, $J = 10$ Hz) in the ^{1}H NMR of **1c** compared with that of H-3 in **1a** (δ3 24, *d*, $J = 10$ Hz) further support this assignment

Compound **2** had the composition $C_{22}H_{22}O_{10}$ (elemental analysis) The presence of a 4-phenylcoumarin skeleton was deduced by the UV and the IR spectra. Treatment with acetic anhydride-pyridine afforded the derivative **2a** and acid (2M HCl) or enzymatic hydrolysis (β-galactosidase) yielded aglycone **2b** and galactose The most important features of the ^{1}H NMR spectrum of **2** were the signal for H-3 (δ5 75, *s*), a singlet for a methoxyl group (δ3 82, *s*), the resonance for the anomeric proton of galactose (δ 4 66, *d*, $J = 7$ Hz), two doublets ($J = 8$ Hz) *ortho* coupled at δ7 16 and 6 73 attributable to a *para*-substituted 4-aryl ring—the paramagnetic shift of these two signals in the ^{1}H NMR of **2a** (Table 3) was consistent with the hydroxyl group at C-4′ Finally, the remaining aromatic signals (δ6.65 and 6 58) comprise an AB system ascribable to two mutually *meta*-located protons ($J = 3$ Hz) which by comparison with similar compounds were assignable to H-6 and H-8 [3–5] The placement of the sugar in position 5 was deduced unequivocally by the chemical conversion of **2** into **2c** by treatment with diazomethane–ether The properties of **2c** were identical to those of a natural galactoside previously isolated from *Exostema caribaeum* [4]

Table 1 ^{13}C NMR (90 MHz) data of compounds **1** and **1b** (in pyridine-d_5 with TMS as int standard

C	**1a**	**1b**
1	340 *t*	346 *t*
2	704 *d*	704 *d*
3	937 *d*	814 *d*
4	425 *s*	427 *s*
5	1420 *s*	1425 *s*
6	1194 *d*	1197 *d*
7	252 *t*	242 *t*
8	431 *d*	432 *d*
9	488 *s*	487 *s*
10	341 *d*	347 *d*
11	2131 *s*	2129 *s*
12	492 *t*	493 *t*
13	486 *s*	488 *s*
14	510 *s*	511 *s*
15	462 *t*	464 *t*
16	712 *d*	710 *d*
17	588 *d*	58.7 *d*
18	203* *q*	202* *q*
19	192* *q*	192* *q*
20	801 *s*	801 *s*
21	254 *q*	254 *q*
22	2160 *s*	2158 *s*
23	326 *t*	325 *t*
24	383 *t*	384 *t*
25	692 *s*	691 *s*
26	300* *q*	300* *q*
27	297* *q*	29.7* *q*
28	205* *q*	204* *q*
29	234 *q*	224 *q*
30	204* *q*	203* *q*
1′	1070 *d*	--
2′	762 *d*	-
3′	786 *d*	—
4′	717 *d*	—
5′	784 *d*	—
6′	629 *d*	—

*Assignments may be interchanged

In the case of compounds **3–5** the identity was confirmed by comparison with authentic samples [4, 5]. However, for component **6** and 7-methylluteolin the identity was established by comparison of their properties with those previously described [5, 6]

This is to our knowledge the first report of flavonoids in the genus *Hintonia*. The coexistance of 4-phenylcoumarins and cucurbitacins in species of the Rubiaceae is no longer restricted to *Exostema mexicanum* [13]. None of the isolated compounds were toxic to *Artemia salina* ($LC_{50} > 1000$ ppm) [14]

EXPERIMENTAL

Plant material Stem bark of *H latiflora* (Sesse et Mociño ex DC) Bullock was collected in Jolalpan, Puebla, in May 1987 a voucher specimen (R Bye and E Linares No 15,516) is deposited at the Herbario Nacional Instituto de Biología, UNAM

Extraction and preliminary fractionation Dried and shredded stem bark (3 kg) was defatted with hexane at room temp The dried marc was then extracted × 3 (6 l each) for 3 days at room

Table 2. ^{1}H NMR data for compounds **1a** and **1c** (in $CDCl_3$, 90 MHz, TMS as int. standard)*

H	1a	1c
H-2	†	5.02 *ddd* (10, 10, 6)
H-3	3.24 *d* (10)	4.65 *d* (10)
H-6	5.75 *m*	5.73 *m*
H-12 α	3.14 *d* (15)	3.17 *d* (15)
H-16	†	5.11 *t* (7)
–Me	0.94, 0.97, 1.06, 1.16, 1.20, 1.24, 1.43 (each one *s*)	0.98, 1.04, 1.10 1.22, 1.24, 1.43 (each one *s*)
H-1′	4.73 *d* (7)	—
H-5′	3.63 *m*	—
H-6′	4.17 *m*	—
–OAc	1.92, 1.98, 2.02, 2.06, 2.09, (each one *s*)	1.98, 1.92, 2.05, (each one *s*)

* Coupling constants (Hz) in parentheses.
† The signals are overlapped.

Table 3. ^{1}H NMR data for compounds **2**, **2a** and **2b** (90 MHz, TMS as int. standard)

H	2*	2a†	2b‡
H-3	5.78 *s*	6.05 *s*	5.78 *s*
H-6	6.65 *d* (3)	6.63 *d* (3)	6.35 *d* (3)
H-8	6.58 *d* (3)	6.50 *d* (3)	6.25 *d* (3)
H-2′, H-6′	7.16 *d* (8)	7.30 *d* (8)	7.12 *d* (8)
H-3′, H-5′	6.73 *d* (8)	7.10 *d* (8)	6.75 *d* (8)
7-OMe	3.83 *s*	3.89 *s*	3.78 *s*
4′-OAc	—	2.34 *s*	—
H-1″	4.60 *d* (7)	4.55 *d* (7)	—
H-2″-H-6″	3–4.40 m	3.75–5.30 *m*	—
2″-6″OAc	—	1.91, 1.97, *s* 2.04, 2.24, *s*	—
OH-4′	9.5 *bs*	—	9.10 *br s*
OH-5′	—	—	8.10 *br s*

* DMSO-d_6
† $CDCl_3$.
‡ DMSO-d_6–$CDCl_3$.
Coupling constants (Hz) in parentheses.

temp. The combined MeOH extracts were evapd *in vacuo* to yield 636.9 g of a brown residue, which was partitioned between EtOAc–MeOH–H_2O (12:1:3) using a continuous liquid–liquid extractor. Evapn of solvent afforded 160 g of extract. From this organic phase, 38.65 g of compound **3** crystallized spontaneously, identical to an authentic sample [3].

Isolation. The concd organic residue (120.2 g) resulting from the partition process was chromatographed in a glass column packed with silica gel (1.2 kg) using $CHCl_3$ with increasing amounts of MeOH as eluents. Frs of 500 ml were collected. From frs 122–220 eluted with $CHCl_3$–MeOH (23:2) 721 mg (0.0235% dry wt) of 7-methylluteolin were obtained, mp 272° (lit. [6] mp 268–270°). Frs 221–259 eluted with $CHCl_3$–MeOH (9:1) yielded 4.2037 g (0.1375% dry wt) of **5**, identical to a reference sample previously isolated from *Exostema caribaeum* [4]. From frs 252–288 eluted also with $CHCl_3$–MeOH (9:1) **1** was crystallized (1.4339 g) (0.04469% dry wt), mp 199–201°, calcd for $C_{36}H_{58}O_{12}$, C = 63.34%, H = 8.50%; found C = 63.05%; H = 8.48%; $[\alpha]_D = +39$ (MeOH; *c* 0.12); IR ν_{KBr}^{max} cm^{-1}: 3400, 2920, 1690, 1630, 1455, 1430, 1255, 1210; ^{13}C NMR is reported in Table 1.

Further CC over silica gel of the mother liquors from frs 252–288 using $CHCl_3$–MeOH (47:3) allowed the isolation of 789 mg (0.0258% dry wt) of compound **2**, mp 218–221°; calcd for $C_{22}H_{22}O_{10}$; C = 59.20%; H = 4.95%; found C = 58.98%, H = 5.01%; IR ν_{KBr}^{max} cm^{-1}: 3460, 3330, 1720, 1675, 1615, 1515, 1485, 1440, 1280, 970; ^{1}H NMR is reported in Table 3. Frs 285–301 from the original CC, eluted with $CHCl_3$–MeOH (9:1) yielded 4.81 g (0.1575% dry wt) of **4**, identical to an authantic sample [4]. Frs 302–329 from the initial CC eluted with $CHCl_3$–MeOH (9:1) afforded additional amounts (38.65 g) of **3**. The total yield of this compound was 2.6579% dry wt. Frs 399–398 eluted with $CHCl_3$–MeOH (7:3) yielded 6.9135 g of **6** (0.0226% dry wt), mp 250–252°, (lit. [5] mp unreported).

Enzymatic hydrolysis of **1** and **2**. Compounds **1** and **2** (100 mg each) suspended in 10 ml of H_2O were mixed with 100 mg of β-glucosidase (Sigma) and 100 mg of β-galactosidase (Sigma), respectively. The mixts were incubated at 36° and 72 hr. Both reaction mixts were extracted with EtOAc. The organic phases were evapd and chromatographed on silica gel plates [$CHCl_3$–MeOH, (4:1)] to yield 62.5 mg of **1b** and 50 mg of **2b**, respectively. In both cases the sugar (glucose or galactose) was readily identified by TLC in the hydrolysate. Compound **1b**, mp 155°, identical to an authentic sample [1]. Compound **2b**, mp 90°; EIMS (rel. int.): 284 (100) $[M]^+$, 256 (43), 241 (20); IR ν_{KBr}^{max} cm^{-1}: 3400, 1700, 1690, 1610, 1515, 1350, 835; ^{1}H NMR is reported in Table 3.

Acetylation of **1**, **2** *and* **1b**. To separate solns of **1**, **1b** and **2** (100 mg of each) in 1 ml of pyridine was added 1 ml Ac_2O. The mixts were kept at room temp. overnight and after usual work-up the acetyl derivatives **1a**, **1c** and **2a** were obtained. Compound **1a**, 76 mg, 136–138°, calc. for $C_{48}H_{70}O_{18}$, C = 61.01%, H = 7.41%; found, C = 60.98%; H = 7.39%; IR ν_{KBr}^{max} cm^{-1}: 3450, 2970, 1740, 1720, 1700, 1430, 1239, 900; ^{1}H NMR is reported in Table 2. Compound **1c**, 75 mg, mp 96–97°; IR ν_{KBr}^{max} cm^{-1}: 3440, 2972, 1742, 1698, 1370, 1247, 1028; ^{1}H NMR is given in Table 2. Compound **2a**, 80 mg, mp 202–205°; calcd for $C_{32}H_{22}O_{15}$; C = 59.44%; H = 3.40%; found, C = 59.80; H = 3.38%; IR ν_{KBr}^{max} cm^{-1}: 2920, 1760, 1720, 1610, 1540, 1230, 940, 840. ^{1}H NMR is given in Table 3.

Acid hydrolysis of **2**. Compound **2** (100 mg) was refluxed for 1 hr with 200 ml of 2 M HCl. A yellow power pptd from the acid soln and after usual work-up, 72 mg of **2b** were obtained.

Conversion of **2** to **2c**. Compound **2** (100 mg) was disolved in MeOH. To this soln an excess of CH_2N_2 in Et_2O was added. After 24 hr 80 mg of a crystalline compound, **2c**, was obtained. This product was identical to a natural product previously isolated from. *E. caribaeum* [4].

Acknowledgements—The authors would like to thank BASF de México for partial financial support through 'Seminario Académico José Herrán Arellano', awarded to R. Mata. Thanks are also due to Consejo Nacional de Ciencia y Tecnología (CONACyT), México, for partial financial support (Convenio No 144, Programa de Fortalecimiento al Posgrado Nacional), to the staff of the IR laboratory, Facultad de Química, UNAM and to Ms Alejandrina Acosta for recording the ^{13}C NMR spectra.

REFERENCES

1. Reguero, M. T., Mata, R., Bye, R.,Linares, E. and Delgado, G., (1987) *J. Nat. Prod.* **50**, 315.
2. Reher, G. and Kraus, L. (1984) *J. Nat. Prod.* **47**, 172.

3. Mata, R., Calzada, F., Garcia, M. R. and Reguero, M. T. (1987) *J. Nat. Prod.* **50**, 866
4. Mata, R., Calzada, F. and Garcia, M. R. (1988) *J. Nat. Prod.* **51**, 851
5. Aquino, R., D'Agostino, M., De Simone, F. and Pizza, C. (1988) *Phytochemistry* **27**, 1827
6. Harborne, J. B., Mabry, T. J. and Mabry, H. (eds) (1975) *The Flavonoids, Part* 1, pp. 271. Academic Press, New York.
7. Yamada, J., Hagiwara, K., Iguchi, K., Susuki, S. and Hsu, H-Y. (1978) *Chem. Pharm. Bull.* **26**, 3107.
8. Laurie, W. A., McHale, D. and Sheridan, J. B. (1985) *Phytochemistry* **24**, 2659
9. Mata, R., Castañeda, P., Camacho, M. R. and Delgado, G. (1988) *J. Nat. Prod.* **51**, 836
10. Van de Velde, V. and Lavie, D. (1983) *Tetrahedron* **39**, 317.
11. Tori, K., Seo, S., Yoshimura, Y., Arita, H. and Tomita, Y. (1977) *Tetrahedron Letters* 179
12. Cattel, L., Caputo, O., Delprino, L. and Beglino, G. (1979) *Gazz. Chim. Ital.* **108**, 1
13. Mata, R., Albor, C., Pereda-Miranda, R. and McLaughlin J. L. (1989) *Planta Med.* **51** (in press)
14. Meyer, B. N., Ferrigni, N. R., Putnam, J. E., Jacobsen, L. B., Nichols, D. E. and McLaughlin, J. L. (1982) *Planta Med.* **45**, 31

Phytochemistry, Vol. 29, No. 6, pp. 2040–2043, 1990
Printed in Great Britain

0031 9422/90 $3.00 + 0.00

CASSINOPIN, A KAEMPFEROL TRIRHAMNOSIDE FROM *CASSINOPSIS MADAGASCARIENSIS**

P. RASOANAIVO, S. RATSIMAMANGA-URVERG, I. MESSANA,† Y. DE VICENTE‡ and C. GALEFFI‡

Institut Malgache de Recherches Appliquées, B.P. 3833, Antananarivo, Madagascar, †Istituto di Chimica, Universita Cattolica del S. Cuore, Rome, Italy, ‡Laboratorio di Chimica del Farmaco, Istituto Superiore di Sanita, V. le Regina Elena 299, Rome, Italy

(*Received in revised form* 19 *October* 1989)

Key Word Index—*Cassinopsis madagascariensis*; Icacinaceae; phenylpropanoid glycosides; cassinopin; ^{13}C NMR.

Abstract—A new glycoside, 3-[α-L-rhamnosyl-(1→4)-*O*-α-L-rhamnopyranosyl]-7-α-L-rhamnosylkaempferol, cassinopin, was isolated from the leaves of *Cassinopsis madagascariensis*, together with the known phenylpropanoid glycosides calceolarioside A, B and C, and verbascoside. The structure of cassinopin was established by spectroscopic and chemical methods.

INTRODUCTION

Cassinopsis madagascariensis Baill. (Icacinaceae) is a shrub known as 'bemafaitra', which grows in the humid western forest of Madagascar. The leaves are used as a traditional remedy against malaria [2]. Boiteau [3] claimed that the decoction of the aerial parts is effective for the treatment of the side-effects of malaria.

RESULTS AND DISCUSSION

The methanolic extract of the air-dried leaves of *C. madagascariensis* (30%) tested *in vitro* against *Plasmodium* showed weak activity (Majori, G., personal communication). Four known phenylpropanoid glycosides (identified by direct comparison), calceolarioside A, B and C [4, 5], and verbascoside [6], were isolated from the methanolic extract, together with a new flavonol glycoside, **1**, named cassinopin. This substance, mp 202–204° (EtOH), $[\alpha]_D$ −230.4° (EtOH), corresponds to the formula $C_{33}H_{40}O_{18}$, $[M+H]^+$ at *m/z* 725 (FABMS). The UV absorbance (see Experimental) and the ^{1}H and ^{13}C NMR data (see Tables 1 and 2) suggested it to be a flavonol glycoside. Hydrolysis with cellulase afforded kaempferol (3,5,7,4′-tetrahydroxyflavone) and L-rhamnose, as the sole monose, in agreement with the presence in the ^{1}H NMR spectrum of three secondary methyl signals at δ0.98, 1.24, and 1.31. Treatment of **1** with pyridine and acetic anhydride gave the decaacetyl derivative **2**, mp 247–250°. Its ^{1}H NMR spectrum showed that two rhamnose moieties are arranged in a biose unit with a 1→4 linkage, since the signal, which remains upfield at δ3.53 (*t*, *J* = 9.5 Hz), is coupled with H-5 (δ3.20, *dq*, *J* = 6.0 and 9.5 Hz). In the ^{13}C NMR spectrum of **1** the signal at δ79.8, characteristic for C-4 in 4-substituted rhamnose units, was in agreement with this assignment [7].

Methylation of cassinopin with ethereal diazomethane gave the monomethyl ether **3**, mp 240–243°. The bathochromic shift by addition of aluminium trichloride observed in the UV spectra of **1** and **3** indicated that the

*Part 19 in the series 'Research on African Medicinal Plants'. For Part 18 see ref. [1].

Dedicated to Professor G. B. Marini-Bettolo on the occasion of his 75th birthday.