J. Braz. Chem. Soc., Vol. 10, No. 6, 438-442, 1999.
Printed in Brazil.

0103 – 5053 $6.00 + 0.00

Article

A New Isoflavone Isolated from *Harpalyce brasiliana**

***Graça Lúcia da Silva*[a], *Maria Iracema Lacerda Machado*[a], *Francisco José de Abreu Matos*[a], *and Raimundo Braz-Filho*[b**]**

[a]*Departamento de Química Orgânica e Inorgânica, Laboratório de Produtos Naturais, Universidade Federal do Ceará, C.P. 12200, 60.021-970 Fortaleza - Ceará, Brazil*

[b]*Setor de Produtos Naturais, LCQUI-CCT, Universidade Estadual do Norte Fluminense, 28015-620 Campos - RJ, Brazil*

Uma nova isoflavona denominada harpalicina e quercetina foram isoladas das folhas e 3-hidroxi-4-isopentenil-8,9-metilenodioxipterocarpano e ácido betulínico das raizes de *Harpalyce brasiliana*. As estruturas foram elucidadas usando métodos espectrométricos, inclusive RMN bidimensional (2D) da nova isoflavona.

A new isoflavone named harpalycine and quercetin were isolated from the leaves and 3-hydroxy-4-isopentenyl-8,9-methyle-nedioxypterocarpan and betulinic acid from the roots of *Harpalyce brasiliana*. The structures were elucidated using spectroscopic methods, including 2D NMR techniques of the new isoflavone.

Keyword: Harpalyce brasiliana, *Leguminosae, flavonoids, triterpene*

Introduction

Harpalyce brasiliana Benth. (Leguminosae-Papilionoideae) is a Northeastern Brazilian shrub, called "raiz-de-cobra" (snake root) and used by people for treating snake bites[1]. Two prenylated pterocarpans, cabenegrins A-I (**1**) and A-II (**2**)[2], potent antidotes against snake venom, were isolated and identified from a locally well known anti-snake bite medicine named "Específico Pessoa", manufactured and sold in the north and northeast of Brazil and available to plantation workers as an oral antidote. The plant, commonly called "cabeça de negro", which furnishes the extract used in the preparation of this remedy has not been identified so far, being kept secret by the manufacturers. There are about ten plants with the name "cabeça de negro" in South America. Two plants reputed as anti-snake bite medicines occur in the Ibiapaba region in Northeast Brazil: *Bredemeyera floribunda* Willd (Polygalaceae), called "pacari", and *Harpalyce brasiliana* (Leguminosae-Papilionoideae). The first contains as its active principle one saponin, bredemeyeroside[3].

In this paper we report the isolation and characterization of the new isoflavone harpalycin (**3**) and the known flavonol quercetin (**4**), prenylated pterocarpan (**5**) and triterpene betulinic acid (**6**) from leaves and roots of a specimen of *Harpalyce brasiliana*. There are three previous chemical reports about this plant[4-6].

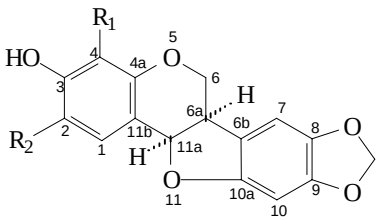
Results and Discussion

Chromatographic separation of the ethanol extract from the leaves of *Harpalyce brasiliana* led to the isolation of the new isoflavone harpalycin (**3**), as well as the known flavonol quercetin (**4**). From the roots, 3-hydroxy-4-isopentenyl-8,9-methylenedioxypterocarpan (**5**) and pentacyclic triterpenoid betulinic acid (**6**) were isolated.

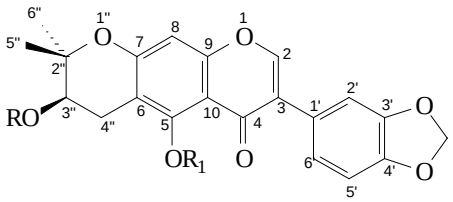
The known natural products quercetin (**4**, 5,7,3',4'-tetrahydroxyflavonol) and betulinic acid [**6**, 3β-hydroxy-20(29)-lupen-28-oic acid] were identified mainly by their ^{1}H and ^{13}C-NMR spectra and comparison with literature data[7,8]. 3-Hydroxy-4-isopentenyl-8,9-methylenedioxypterocarpan (**5**) has been recently reported[5,6].

Comparative analysis of the hydrogen broad band decoupled (HBBD) and distortionless enhancement by polarization transfer (DEPT) ^{13}C-NMR spectra[9] of **3**, in combination with the ^{1}H-NMR (one- and two-dimensional ^{1}H$\times^{1}$H-COSY), IR [ν 3420 (OH), 1660 (conjugated carbonyl), 1620 (conjugated double bond), 1590 and 1500 cm^{-1} (aromatic ring)] and mass {*m/z* 382 ([M]$^{+}$, 70 %), 364 ([M - H_2O]$^{+}$, 12 %), 349 ([M - H_2O - Me$^{\cdot}$]$^{+}$, 39 %), 311 (**3c**, [M - C_4H_7O]$^{+}$, 100 %), 310 (**3d**, [M - C_4H_8O]$^{+}$, 45 %) and

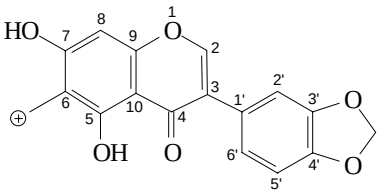
*This work is part of the M.Sc. Thesis of Graça Lúcia da Silva, presented at the Departamento de Química Orgânica, Universidade Federal do Ceará, Fortaleza, Ceará, Brazil, 1995.



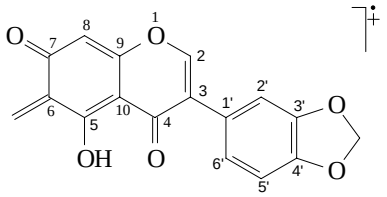
	R_1	R_2
1	$CH_3(CH_2OH)C=CH-CH_2$ (*trans*)	H
2	H	$CH_3(CH_2OH)C-CH_2-CH_2$
5	$Me_2C=CH-CH_2$	H



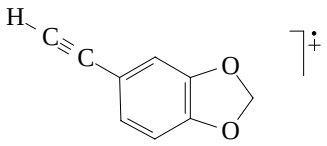
	R	R_1
3	H	H
3a	Ac	H
3b	Ac	Ac



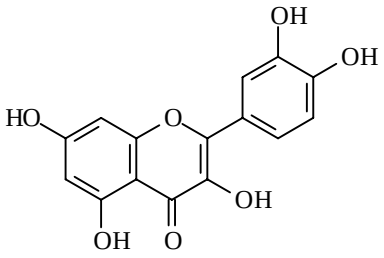
3c *m/z* 311 (100 %)



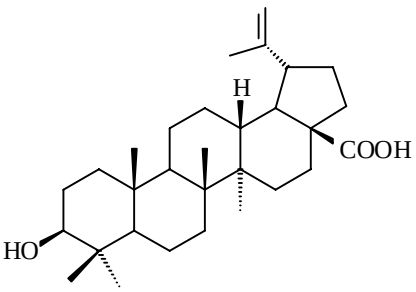
3d *m/z* 310 (45 %)



3e *m/z* 146 (18 %)



4



6

146 (**3e**, 18 %)} spectra allowed the deduction of a molecular formula $C_{21}H_{18}O_7$, containing eleven quaternary carbons {ten sp^2: one carbonyl (δ_C 180.08, C-4), five bound to oxygen atoms [δ_C 159.27 (C-7), 159.09 (C-5), 155.19 (C-9), 147.70 (C-3' and C-4')], four non-oxygenated [δ_C 121.71 (C-1') 124.47 (C-3), 104.22 (C-6 and C-10)] and one sp^3 oxygenated [δ_C 79.03 (C-2''], six methine [five sp^2: δ_C 154.70 (CH-2), 122.57 (CH-6'), 109.42 (CH-2'), 108.19 (CH-5') and 94.22 (CH-8); one sp^3 bound to oxygen: δ_C 66.80 (CH-3'')], two methylene [δ_C 101.16 (3',4'-OCH_2O) and 25.02 (CH_2-4'')] and two methyl groups [δ_C 25.25 (CH_3-6'') and 21.07 (CH_3-5'')]: $(C)_{10}$ $(O)_4(C=O)(CH)_6(OCH_2O)(CH_2)(CH_3)_2 = C_{21}H_{16}O_7$. The two remaining hydrogens ($C_{21}H_{18}O_7$, *m/z* 382 $[M]^+$, 70 %) were attributed to two hydroxy groups: $(C)_{10}(O)_2(C=O)(CH)_6(OCH_2O)(CH_2)(CH_3)(OH)_2=C_{21}H_{18}O_7$. The presence of the two hydroxy groups was confirmed by the singlet signals at δ_H 2.04 (AcO-3'') and 2.43 (AcO-5) observed in the ^{1}H-NMR spectrum and δ_C 170.36 and 20.97 (AcO-3'') and 169.12 and 21.08 (AcO-5), in the ^{13}C-NMR spectrum of the diacetyl derivative **3b** (Table 1). One chelatogenic hydroxyl function was revealed by signals at δ_H 13.20 and

13.13 (HO-5) in the ^{1}H-NMR spectra of **3** and monoacetyl derivative **3a**, respectively. These data and the signals at δ_H 8.12 (H-2) and δ_C 154.70 (CH-2) were used to classify this natural product as an isoflavone containing one methylenedioxy and one monohydroxylated isoprenoid moiety (Me_2C-CHOH-CH_2-) involved in a 3,4-dihydro-3-hydroxy-2,2-dimethylpyran ring, along with the chelatogenic hydroxyl group at C-5 (δ_H 13.20).

Table 1. ^{1}H [400 (**3**) and 270 (**3a** and **3b**) MHz] and ^{13}C [50 (**3**) and 67.5 (**3a** and **3b**)] NMR spectral data for **3**, **3a** and **3b** in pyridine-d_5 (**3**, ^{1}H-NMR), DMSO-d_6 (**3**, ^{13}C-NMR) and $CDCl_3$ (**3a** and **3b**). Chemical shifts in δ (δ_H and δ_C, ppm) and coupling constants (J, in parentheses) in Hz.*

	3		3a[a]		**3b**	
	δ_C	δ_H	δ_C	δ_H	δ_C	δ_H
C						
3	121.71	-	123.30	-	125.52	-
4	180.08	-	180.69	-	174.64	-
5	159.09	-	160.11	-	148.58	-
6	104.22	-	102.72	-	111.48	-
7	159.27	-	159.02	-	157.37	-
9	155.18	-	156.02	-	156.88	-
10	104.22	-	105.39	-	111.26	-
1’	124.47	-	124.50	-	125.40	-
3’	147.10	-	147.80	-	147.65	-
4’	147.10	-	147.80	-	147.65	-
2’’	79.03	-	76.86	-	76.90	-
AcO-3’’	-	-	170.09	-	170.36	-
AcO-5	-	-	-	-	169.12	-
CH						
2	154.70	8.12 (*s*)	152.73	7.82 (*s*)	151.27	7.74 (*s*)
8	94.22	6.52 (*s*)	94.80	6.39 (*s*)	102.63	6.80(*s*)
2’	109.42	7.37 (*d*, 1.6)	109.61	7.01 (*d*, 1.5)	109.97	6.96 (*d*, 1.4)
5’	108.19	6.98 (*d*, 8.0)	108.43	6.86(*d*, 8.0)	108.36	6.84 (*d*, 8.0)
6’	122.57	7.14 (*dd*, 8.0, 1.6)	122.37	6.93 (*dd*, 8.0, 1.5)	122.55	6.95 (*dd*, 8.0, 1.4)
3’’	66.80	3.69 (*dd*, 6.8, 5.6)	69.76	5.12 (*dd*, 5.1, 4.7)	69.33	5.09 (*t*, 4.7)
CH_2						
4’’	25.02	(*dd*, 16.0, 5.6) 3.06 (*dd*, 16.0, 6.8)	22.66	3.01 (*dd*, 17.7, 5.1) 2.80(*dd*, 17.7, 4.7)	22.97	2.93 - 2.73 (*m*)
OCH_2O	101.16	5.98 (*s*)	101.18	5.99 (*s*)	101.12	5.95 (*s*)
CH_3						
5’’	21.07	1.56 (*s*)	22.90	1.37 (*s*)	23.30	1.37 (*s*)
6’’	25.25	1.52 (*s*)	24.71	1.35 (*s*)	24.70	1.33 (*s*)
AcO-3’’	-	-	20.86	2.07 (*s*)	20.97	2.06 (*s*)
AcO-5	-	-	-	-	21.08	2.43 (*s*)
HO-5	-	13.20 (*s*)	-	13.13 (*s*)	-	-

*Multiplicity of carbon signals deduced by comparative analysis of HBBD- and DEPT-^{13}C-NMR. Chemical shifts and coupling constants (J, in parentheses) of hydrogens obtained from 1D ^{1}H-NMR. [a] 2D ^{1}Hx^{1}H-COSY and ^{13}Cx^{1}H-COSY-$^n J_{CH}$ (n = 1; n = 2 and 3, Table 2) spectra were also used in these assignments.

The location of hydroxy group at carbon atom CH-3'' of the 3,4-dihydro-3-hydroxy-2,2-dimethylpyran moiety was deduced by signals at δ_H 3.69 (*dd*, J = 6.8 and 5.6 Hz) and 5.12 (*dd*, J = 5.1 and 4.7 Hz), observed in the ^{1}H-NMR spectra of **3** and **3a**, respectively (Table 1), whose attribution was confirmed by a long range heteronuclear correlation (spin-spin interaction) of the signal at δ_C 69.76 (CH-3'') and signals at δ_H 1.37 (3H-5'', $^3J_{CH}$) and δ_H 1.32 (3H-6'', $^3J_{CH}$), observed in the 2D ^{13}Cx^{1}H-COSY $^nJ_{CH}$ (n = 2 and 3, COLOC)[10] spectrum of the monoacetyl derivative **3a** (Table 2). This spectrum also showed correlation of the signals corresponding to the hydrogen of the hydroxy group at C-5 (δ_H 13.13) and quaternary carbon atoms C-5 (δ_C 160.11, $^2J_{CH}$), C-6 (δ_C 102.72, $^3J_{CH}$) and C-10 (δ_C 105.39, $^3J_{CH}$), as summarized in Table 2. Additional long range heteronuclear correlations observed in the 2D ^{13}Cx^{1}H-COSY-$^nJ_{CH}$ (n = 2 and 3, COLOC) spectrum of **3a** are summarized in Table 2, along with the heteronuclear direct one bond coupling revealed by the 2D ^{13}Cx^{1}H-COSY-$^1J_{CH}$ spectrum of **3a** (Table 1). Thus, the complete assignment of the chemical shifts of hydrogen and carbon atoms of **3a** in the ^{1}H and ^{13}C-NMR spectra was accomplished by 2D ^{1}Hx^{1}H-COSY and ^{13}Cx^{1}H-COSY $^nJ_{CH}$ (n = 2 and 3, COLOC), which confirmed the proposed structure **3** (Table 1 and 2). These assignments were facilitated by application of the usual shift parameters and the observed multiplicities of signals[9].

Thus, the structure of the new isoflavone, named harpalycine, isolated from *Harpalyce brasiliana* was established as 3*,4-dihydro-3,5-dihydroxy-7-(3,4-methylenedioxyphenyl)-2,2-dimethyl-2*H*,6*H*-benzo[1,2-b:5,4-b'] dipyran-6-one (**3**, 3''*,5-dihydroxy-2'',2''-dimethyl-3',4'-methylenedioxy-6,7:6'',5''-pyranoisoflavone). Relatively few dihydrohydroxypyranoisoflavones have been described as natural products[11,12].

Table 2. Long range heteronuclear correlations observed in the ^{13}Cx^{1}H-COSY-$^nJ_{CH}$ (n = 2 and 3, COLOC) NMR spectrum of the monoacetyl derivative **3a**, in $CDCl_3$*.

	^{13}Cx^{1}H COSY-^{n} J_{CH}		
	δ_C	$^2J_{CH}$	$^3J_{CH}$
C			
3	123.30	H-2 (δ_H 7.82)	H-2' (δ_H 7.01)
4	180.69		H-2 (δ_H 7.82)
5	160.11	H0-5 (δ_H 13.13)	
6	102.72		HO-5 (δ_H 13.13), H-8 (δ_H 6.39)
7	159.02	H-8 (δ_H 6.39)	
9	156.02	H-8 (δ_H 6.39)	H-2 (δ_H 7.82)
10	105.39		HO-5 (δ_H 13.13), H-8 (δ_H 6.39)
1'	124.50		H-5' (δ_H 6.86)
3'	147.80	H-2' (δ_H 6.96)	OCH_2O (δ_H 5.99), H-5' (δ_H 6.86)
4'	147.80	H-5' (δ_H 6.86)	OCH_2O (δ_H 5.99), H-6' (δ_H 6.93)
2''	76.86	3H-5'' (δ_H 1.37), 3H-6'' (δ_H 1.35)	
CH			
3''	69.76		3H-5'' (δ_H 1.37), 3H-6'' (δ_H 1.35)

*Multiplicity of carbon signals deduced by comparative analysis of HBBD- and DEPT-^{13}C-NMR. Homonuclear 2D ^{1}Hx^{1}H-COSY and heteronuclear ^{13}Cx^{1}H-COSY-$^1J_{CH}$ (Table 1) NMR spectra were also used for these assignments. Chemical shifts and coupling constants (J) of hydrogen atoms obtained from 1D ^{1}H-NMR spectrum.

Experimental

General experimental procedures

Mps are uncorr. IR spectra were recorded on a Perkin Elmer 1320 or Nicolet 5ZDXFT-IR, in KBr. ^{1}H [400 (**3**) and 270 (**3a** and **3b**) MHz] and ^{13}C [50 (**3**) and 67.5 (**3a** and **3b**) MHz] NMR spectra were recorded on a Bruker AC-200 (^{1}H: 200 MHz; ^{13}C: 50 MHz) and WP-270 (^{1}H: 270 MHz; ^{13}C: 67.5 MHz) or Varian UN-400 (^{1}H: 400 MHz; ^{13}C: 100 MHz) spectrometers, in pyridine-d_5 (**3**, ^{1}H-NMR), DMSO-d_6 (**3**, ^{13}C-NMR) or $CDCl_3$ (**3a** and **3b**). EIMS (70 eV) spectra were obtained on a HP-5971 GC/MS instrument.

Plant material

Harpalyce brasiliana Benth. leaves and roots were collected in Guaraciaba do Norte, Ibiapaba mountains, Ceará State, Brazil and identified by Professor Afrânio Gomes Fernandes (Universidade Federal do Ceará, Fortaleza). A voucher specimen (nº 14841) is deposited at the Herbário Prisco Bezerra of the Departamento de Biologia of the Universidade Federal do Ceará.

Extraction and isolation of constituents from leaves

Dried and powdered leaves (6 kg) were extracted with EtOH at room temp and the solvent removed under vacuum to yield 736 g of residue. This residue was chromatographed on a silica gel column and successively eluted with hexane, $CHCl_3$, EtOAc, $CHCl_3$-MeOH (1:1), MeOH and EtOH. The residue (55 g) of the fraction eluted with $CHCl_3$-MeOH (1:1) was suspended in EtOH-H_2O soln and extracted with hexane; the residue (23 g) thus obtained was chromatographed on a silica gel column and eluted with hexane, $CHCl_3$, EtOAc and MeOH; the fraction eluted with $CHCl_3$ (2 g) was recrystallized from MeOH to afford **3** (286

mg); the fraction eluted with EtOAc (10 g) furnished quercetin (**4**, 50 mg) after several runs on a silica gel column.

Extraction and isolation of constituents from roots

The natural products **5** and betulinic acid (**6**) were isolated from roots as described in Ref. 6.

*Harpalycin (**3**)*

Mp 208 - 211° (MeOH). IR ν_{max} (cm^{-1}, KBr): 3420, 1620, 1660, 1590, 1500, 1190, 820. EIMS *m/z* (rel. int.): 382 ($[M]^+$, 70), 364 ($[M - H_2O]^{+\cdot}$, 12), 349 ($[M - H_2O - Me^{\cdot}]^+$, 39), 311 (**3c**, 100), 310 (**3d**, 45), 146 (**3e**, 18), 145 ([**3e** - $H^{\cdot}$], 13). H^1 (400 MHz, C_5D_5N) and ^{13}C (50 MHz, DMSO-d_6) NMR: Table 1.

*Acetylation of harpalycin (**3**)*

Treatment of harpalycin (**3**, 100 mg) with Ac_2O (4 mL) in the presence of pyridine (1 mL), and usual work-up, produced a mixture of **3a** (monoacetyl derivative) and **3b** (diacetyl derivative), which were purified on a silica gel column using hexane and hexane containing increasing amount of $CHCl_3$ as eluents.

*3''-O-Acetylharpalycin (**3a**)*

Mp 212-213°. IR ν_{max} (cm^{-1}, KBr): 1730, 1650, 1620, 1590, 1500, 1190 e 800. EIMS *m/z* (rel. int.): 424($[M]^+$, 6), 364 ($[M - AcOH]^+$, 8), 349 ($[M - AcOH - Me^{\cdot}]^+$, 54), 146 (**3e**, 44); 145 ([**3e** - $H^{\cdot}]^+$, 46). ^1H (270 MHz, $CDCl_3$) and ^{13}C (67.5 MHz, $CDCl_3$) NMR: Table 1; ^{13}C-^1H COSY $^1J_{CH}$ and ^{13}C-^1H COSY $^nJ_{CH}$ (n = 2 and 3, COLOC) NMR (^1H: 200 MHz; ^{13}C: 50 MHz): Table 2.

*3'',5-Di-O-Acetylharpalycin (**3b**)*

Mp 203-204°. IR ν_{max} (cm^{-1}, KBr): 1730, 1620, 1590, 1500, 1190, 800. EIMS *m/z* (rel. int.): 466 ($[M]^+$, 6), 406 ($[M - AcOH]^+$, 2), 364 ($[M - AcOH - CH_2{=}C{=}O]^+$, 8), 349 ($[M - AcOH - CH_2{=}C{=}O - Me^{\cdot}]^+$, 100), 146 (**3e**, 44). ^1H (270 MHz, $CDCl_3$) and ^{13}C (67.5 MHz, $CDCl_3$) NMR: Table 1.

Acknowledgments

The authors are grateful to Professor Afrânio G. Fernandes (Botanist, Departamento de Biologia, Universidade Federal do Ceará) for plant identification; to CAPES, PADCT/FINEP and CNPq for financial support and CNPq for a research fellowship (R.B-F). We thank Dr. Victor Rumjanek for reading the manuscript.

References

1. Silva, G.L.; Machado, M.I.L.; Matos, F.J.A. *XIII Simpósio de Plantas Medicinais do Brasil*, Fortaleza, Ceará, Brasil, 1994.
2. Nakagawa, M.; Nakanishi, K.; Darko, L.L.; Vick, J.A. *Tetrahedron Letters* **1982**, *23*, 3855.
3. Pereira, N.A.; Pereira, B.M.R.; Nascimento, M.C.; Parente, J.C.; Mors, W.B. *Planta Med.* **1994**, *60*, 99.
4. Silva, G.L. *Contribuição ao Estudo Químico de Plantas do Nordeste* Harpalyce brasiliana *Benth (Leguminosae-Papilionoideae)*, M.Sc. thesis, Departamento de Química Orgânica e Inorgânica, Universidade Federal do Ceará, Fortaleza, Ceará, Brasil, 1995.
5. Machado, M.I.L.; Costa, O.; Matos, F.J.A.; Braz-Filho, R. *18ª Reunião Anual da Sociedade Brasileira de Química*, Caxambu, Minas Gerais, Brasil, 1995, PN-024.
6. Silva, G.L.; Matos, F.J.A.; Silveira, E.R. *Phytochemistry* **1997**, *46*, 1059.
7. Agrawal, P.K.; Thakur, R.S.; Bansal, M.C. In *Carbon-13 NMR of Flavonoids*; Agrawal, P.K., ed.; Elsevier; Amsterdam, p. 95-182, 1989.
8. Mahato, S.B.; Kundun, A.P. *Phytochemistry* **1994**, *37*, 1517.
9. Breitmaier, E.; Voelter, W. *Carbon-13 NMR Spectroscopy: High-Resolution Methods and Applications in Organic Chemistry and Biochemistry*; VCH; Weinheim, 3rd ed., 1987.
10. Sanders, J.K.M.; Hunter, B.K. *Modern NMR Spectroscopy: A Guide for Chemists*; Oxford University Press; Oxford, 1993, 2nd ed.
11. Tahara, S.; Orihara, S.; Ingham, J.L.; Mizutani, J. *Phytochemistry* **1989**, *28*, 901.
12. Dewick, P.M. In *The Flavonoids: Advances in Research Since 1986*; Harborne, J.B., ed.; Chapman and Hall; Great Britain, p. 117-238, 1994.

Received: May 31, 1999