New Skeletal Sesquiterpenoids, Caprariolides A−D, from *Capraria biflora* and Their Insecticidal Activity§

Dwight O. Collins,† Winklet A. Gallimore,† William F. Reynolds,*,‡ Lawrence A. D. Williams,† and Paul B. Reese*,†

Department of Chemistry, University of the West Indies, Mona, Kingston 7, Jamaica, West Indies, and Department of Chemistry, University of Toronto, Toronto, Ontario, Canada M5S 1A1

Received June 2, 2000

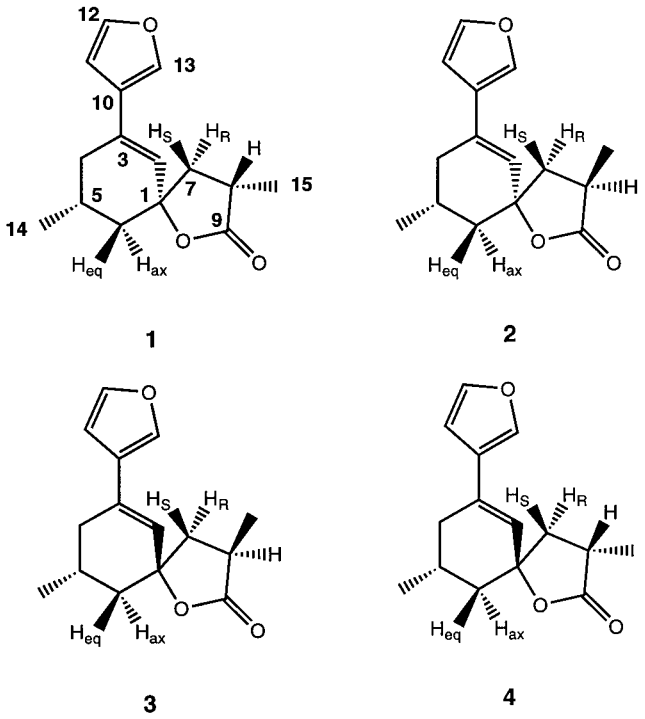
Four structurally novel isomeric sesquiterpenes have been isolated from the aerial parts of *Capraria biflora*. Caprariolides A (**1**), B (**2**), C (**3**), and D (**4**) have been determined by NMR spectroscopy experiments to be the (3*S*,5*S*,9*R*), (3*R*,5*S*,9*R*), (3*R*,5*R*,9*R*), and (3*S*,5*R*,9*R*) isomers of 7-(furan-3′-yl)-3,9-dimethyl-1-oxaspiro[4.5]dec-6-en-2-one, respectively. Both **1** and **2** were found to exhibit insecticidal activity against adult *Cylas formicarius elegantulus*, one of the most destructive insect pests of the sweet potato, *Ipomoea* sp.

Capraria biflora L. (Scrophulariaceae), known locally as "goatweed", has been traditionally used in the West Indies for the treatment of numerous human ailments including fever, influenza, indigestion, and diarrhea.[1,2] Other plants belonging to the Scrophulariaceae family have yielded a number of interesting biologically active compounds including stemodin[3] with mild antiviral and cytotoxic activity,[4] as well as scopadulcic acids A and B,[5] the latter being antiviral and cytotoxic.[6] *C. biflora*, however, has not been subjected to phytochemical analysis or assayed for insecticidal activity, despite an observed lack of herbivore damage in the wild. In an ongoing quest to identify biologically active compounds from plants,[7] insecticidal evaluations were performed on two of the component sequiterpenes from *C. biflora*, caprariolides A and B (**1** and **2**). The evaluations were done against the sweet potato weevil, *Cylas formicarius elegantulus* (Coleoptera: Curculionidae),[8] one of the most destructive pests of the sweet potato, *Ipomoea* sp. (Convolvulaceae).

Results and Discussion

Extensive column chromatography of the hexane extract of the green plant led to the isolation of four compounds, caprariolides A−D (**1**−**4**). EIMS produced an ion with *m*/z 246 $[M]^+$ for each compound, suggesting molecular formulas of $C_{15}H_{18}O_3$. The sesquiterpenes thus inherently possessed seven degrees of unsaturation, three of which were accounted for by six olefinic carbons resonating between δ 110.2 and 143.9. A pronounced absorption at 1761 cm^{-1} in the IR spectrum highlighted the presence of a carbonyl. Tricyclic skeletons were thus proposed.

HMQC, HMBC, and $^1H-^1H$ COSY data (Tables 1 and 2) identified the molecules as trisubstituted cyclohexenes with the substituents being a γ-lactone that formed a spiro ring junction, a furan, and a methyl group. The groups were attached to the cyclohexene ring at positions 1, 3, and 5, respectively, as suggested by nuclear Overhauser enhancement data. The connectivities of the atoms indicated that the compounds possessed a new carbon skeleton.



Three stereogenic carbons were identified, and the isomers existed because of differences at two of these centers. The stereochemistry of the methyl substituent on C-5 was assigned as quasi-equatorial in isomers **1**−**4** based on the relatively low value of the H-5 shift and observed NOE with the 4 and 6 equatorial protons (Tables 1 and 2). For the other two chiral centers NOE measurements proved particularly useful in making the stereochemical determinations (see Figure 1).

The cyclohexene ring was noted to adopt a chair conformation with recognizable axial and equatorial hydrogens. The observed NOE between H_S-7 (δ 2.46) and the equatorial proton borne by C-6 (H_S-6, δ 2.09) and no enhancement with the axial H_R-6 proton (δ 1.64) for caprariolide A (**1**) fixed the stereochemistry of the spiro ring junction. Further NOE enhancements within the lactone ring of **1** clearly showed that the C-15 methyl (δ 1.30) was on the same face as the H_R-7 atom which resonated at δ 1.85 in the ^1H NMR spectrum.

§ Dedicated to Dr. Earle V. Roberts in celebration of his service of over 30 years to the Chemistry Department, University of the West Indies, Mona Campus.

* To whom correspondence should be addressed. P. B.R.: Tel.: (876) 935-8409. Fax: (876) 977-1835. E-mail: pbreese@uwimona.edu.jm. W.F.R.: Tel./Fax: (416) 978-3563. E-mail: wreynold@chem.utoronto.ca.

† University of the West Indies.

‡ University of Toronto.

10.1021/np000280w CCC: $19.00
Published on Web 10/05/2000

Table 1. NMR Shifts for Caprariolide A (**1**) in $CDCl_3$

position	δ_C	δ_H (mult), *J*	HMBC	NOE
1	90.3		2, 6ax, 6eq, 7R	
2	114.3	6.31 (dd) *3.1, 2.0*	6ax, 6eq	7R, 11, 13
3	143.9		4ax, 4eq	
4	38.4	2.23 (ddd) *17.3, 9.5, 3.1* (axial)	2, 5, 14	11, 13, 14
		2.72 (ddt) *17.3, 7.3, 2.0* (equatorial)		
5	30.5	2.10 (m) *w/2 16.7*	4ax, 4eq, 6ax, 14	
6	45.2	1.64 (ddd) *11.5, 11.5, 1.4* (axial)	4eq, 7R, 14	4ax, 14
		2.09 (ddd) *11.6, 6.8, 3.0* (equatorial)		7S
7	44.5	1.85 (ddd) *12.5, 12.0, 1.4* (pro R)	6ax, 6eq, 8, 15	2, 15
		2.46 (dd) *12.5, 8.3* (pro S)		5, 6eq, 8
8	35.6	2.77 (m) *w/2 17.9*	7S, 7R, 15	
9	179.2		7S, 8, 15	
10	122.9		11, 12, 13	
11	110.2	6.48 (dd) *2.0, 0.8*	2, 12, 13	2, 4eq
12	143.0	7.40 (dd) *2.0, 2.0*	2, 11, 13	
13	141.1	7.45 (bs)	2, 11, 12	
14	19.9	1.15 (d) *6.1*	4ax, 6ax	4ax, 6ax
15	15.1	1.30 (d) *1.2*	7R, 8	7R

Table 2. NMR Shifts for Caprariolides B−D (**2**−**4**) in $CDCl_3$

	2		**3**		**4**	
position	δ_C	δ_H (mult), *J*	δ_C	δ_H (mult), *J*	δ_C	δ_H (mult), *J*
1	89.8		92.3		92.1	
2	111.9	6.10 (dd) *2.8, 1.5*	116.3	6.37 (t) *2.9*	114.3	6.30 (t) *2.6*
3	143.5		143.1		144.5	
4	37.5	2.18 (ddd) *17.5, 8.7, 2.8* (ax)	39.8	2.05 (ddd) *17.0, 9.8, 3.1* (ax)	39.3	2.02 (dd) *17.4, 10.3* (ax)
		2.75 (ddd) *17.5, 8.5, 2.5* (eq)		2.89 (ddt) *17.0, 7.7, 2.0* (eq)		2.87 (m)[a] (eq)
5	29.0	2.20 (m) *w/2 17.1*	31.2	2.39 (m) *w/2 17.0*	31.0	2.44 (m) *w/2 16.9*
6	47.3	1.88 (dd) *11.5, 11.5* (ax)	47.9	1.41 (dd) *13.1, 11.5* (ax)	49.1	1.47 (dd) *14.4, 11.9* (ax)
		1.98 (dd) *11.5, 5.8* (eq)		2.25 (ddd) *13.1, 6.0, 2.0* (eq)		2.21 (m)[a] (eq)
7	42.9	1.87 (dd) *12.5, 1.1* (pro R)	40.7	2.33 (dd) *13.0, 8.5* (pro R)	39.5	1.98 (m)[a] (pro R)
		2.55 (dd) *12.5, 9.0* (pro S)		2.18 (dd) *13.0, 12.0* (pro S)		2.72 (m)[a] (pro S)
8	34.3	2.94 (m) *w/2 20.6*	36.2	2.84 (m) *w/2 19.5*	36.3	3.00 (m) *w/2 18.3*
9	179.8		178.9		179.2	
10	122.6		123.1		122.7	
11	110.2	6.45 (dd) *1.8, 0.9*	110.4	6.50 (d) *1.7*	110.4	6.47 (d) *1.6*
12	143.0	7.39 (dd) *1.8, 1.6*	143.1	7.41 (t) *1.5*	143.1	7.40 (t) *1.6*
13	140.9	7.43 (s)	141.5	7.49 (bs)	141.4	7.44 (bs)
14	20.6	1.16 (d) *6.7*	19.7	1.12 (d) *6.6*	20.2	1.11 (d) *6.7*
15	15.7	1.32 (d) *6.2*	15.3	1.35 (d) *7.0*	16.5	1.34 (d) *7.2*

[a] Signals partially obscured by resonances from compound **1**.

With caprariolide B (**2**) similar NOE enhancements between the C-7 and C-6 methylene protons were observed. Indeed irradiation of H_S-7 gave a 4% enhancement of the equatorial C-6 proton (H_S-6) and a 5% enhancement of the axial C-5 proton for both **1** and **2**, strongly indicating that the only difference between these two compounds was the stereochemistry of the C-15 methyl. NOE measurements within the lactone ring of **2** confirmed that the methyl was actually on the same side as the 7 *pro S* hydrogen (δ 2.55).

Analysis of congeners **3** and **4** revealed that the coupling constants within both the cyclohexene and lactone rings were similar to those of **1** and **2**, but there were, however, some differences in the chemical shifts of the protons borne by C-6 and C-7 (Table 2). This suggested that there was a change in the stereochemistry at the spiro ring junction as compared with **1** and **2**. This was subsequently validated by observation of a significant NOE between ^{1}H NMR signals for H_R-7 and the axial proton on C-6 (H_R-6). Further NOE measurements within the lactone ring showed that the C-15 methyl group was on the same face as H_S-7 (δ 2.18) in **3**, but adjacent to H_R-7 (δ 1.98) in **4**.

It was noted that an acid-catalyzed enolization of **1** would lead to the formation of a trigonal planar center at C-8. Subsequent tautomerization could give rise to **2**. In addition the formation of a stable carbocation at C-1 by acid-catalyzed opening of the lactone and subsequent re-lactonization from either face of this carbocation could also account for the formation of **4** from **1**. Isomerization of **4** to **3** could also be easily rationalized. In an effort to confirm that the four isomers were natural products, the crude hexane extract was reexamined eliminating the use of silica altogether. A mixture of the isomers thus obtained was then subjected to NMR analysis in C_6D_6 (rather than in $CDCl_3$, which is acidic). The spectral results showed that compounds **1**−**4** were all present in the extract in the ratio 10:1:3:1, indicating that none of the isomers were artifacts of the isolation procedure.

Insecticidal studies were performed on **1** and **2** in order to determine their potential against the sweet potato weevil, *Cylas formicarius elegantulus*. An analysis of the data (Table 3) revealed that both compounds had a positive dose-dependent mortality effect on adult *C. formicarius*. The individual isomers, however, were significantly less toxic than their 1:1 combined formulation, which exhibited a strong synergistic action against the insects (Ld_{50} = 50.8 μg/insect). This made the combination almost equitoxic to eugenol, a phenylpropanoid possessing a strong repellant effect against several insect species.[9] Recently Brown et al. also revealed that eugenol was more toxic than several commercial insecticides, including dimethoate, against the cattle tick, *Boophilus microplus*.[10]

In summary, we report the first phytochemical analysis of a *Capraria* species. Four isomeric sesquiterpenes (**1**−**4**) based on a novel skeleton were isolated and characterized

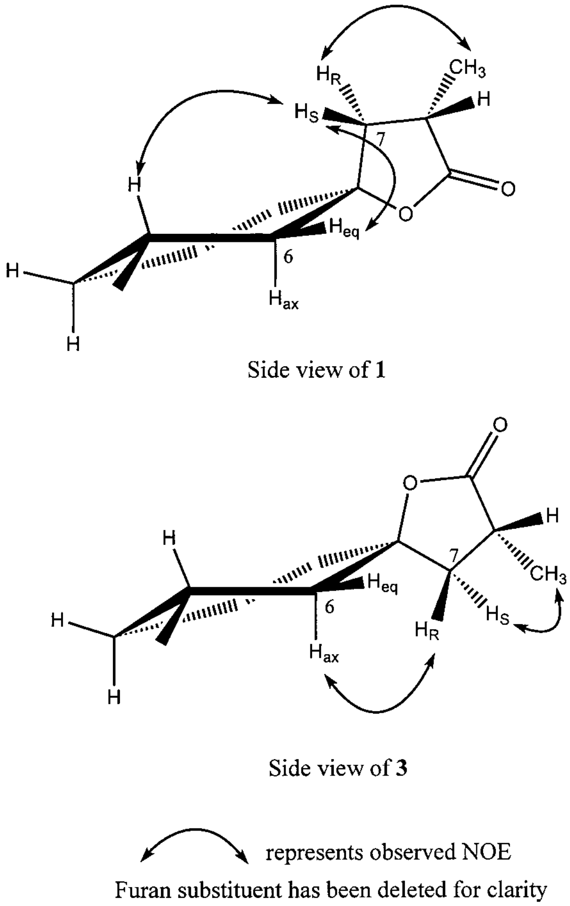


Figure 1. NOE enhancements for caprariolides A and C.

Table 3. 24 h Toxicity Data on Adult *Cylas formicarius elegantulus*

compound	Ld_{50} data (mg/insect)
1	0.902
2	1.102
1+**2** (1:1 ratio)	0.508
eugenol	0.605

from the hexane extract. Two of the compounds (**1** and **2**) were found to be toxic against the sweet potato weevil, *C. formicarius*.

Experimental Section

General Experimental Procedures. ^{1}H and ^{13}C NMR data were obtained on a Varian Unity 500 NMR spectrometer using $CDCl_3$ as solvent with TMS as internal standard. The NOESY experiments, however, were done in C_6D_6−$CDCl_3$ (70%:30% v/v). For the control experiment, ^{1}H and ^{13}C NMR data were acquired on a Bruker AC200 instrument using C_6D_6 as solvent. IR data were acquired on a Perkin-Elmer FTIR Paragon 1000 instrument using KBr disks. The UV spectra were determined on a Hewlett-Packard HP 8452A spectrophotometer. EIMS were obtained at 70 eV on a VG 70-250S mass spectrometer. Optical rotations were obtained using a Perkin-Elmer 241 MC polarimeter**.** Melting points were determined on a Thomas-Hoover capillary melting point apparatus. Column chromatography was performed with Kieselgel silica (40−63 μm). For ^{1}H and ^{13}C NMR data (500 and 125 MHz, respectively, $CDCl_3$) see Tables 1 and 2.

Plant Materials. Leaves and stems of *Capraria biflora* L. were collected in September 1997 in St. Thomas, Jamaica. A voucher specimen (accession no. 34042) was lodged in the Botany Herbarium, UWI.

Extraction and Isolation. Fresh aerial portions of *C. biflora* (17 kg) were chopped and extracted twice with hexane (45 L) over 5 days. The extracts were concentrated in vacuo to yield a green gum (93.5 g), which was divided equally into four portions, each of which was adsorbed on silica and subjected to flash chromatography. Elution was done initially with hexane and then continued with progressively increased proportions of ethyl acetate in hexane (5−100%). This process yielded five main fractions (A−E). Fraction B (39.5 g), obtained as a yellow brown solid in 10% ethyl acetate in hexane, was recrystallized from hexane to produce off-white crystals (16.73 g). Extensive and exhaustive column chromatography on a sample (250 mg) of the crystals using a 5% ethyl acetate in hexane solvent system yielded **2** (9.7 mg), **1** (150.2 mg), a mixture of **1** and **4** (48 mg), and **3** (7.0 mg) in order of elution. The other fractions (A and C−E) contained nothing of interest.

Control Experiment. The crude hexane extract from *C. biflora* was concentrated in vacuo to yield a green gum, which was recrystallized from hexane as off-white crystals. The mixture was then subjected to NMR analysis, and a comparison was made with the pure samples (all run in C_6D_6) to determine the number of caprariolides present.

Insecticidal Activity. The assays were conducted according to the method described by Porter et al.[7] Briefly, adult *Cylas formicarius elegantulus* (Summer) were reared in the laboratory in glass aquaria on sweet potato tubers and were then used for insecticidal screening two weeks after emergence. The insects were transferred to Petri dishes, where a topical application of the compounds in acetone was done. Applications were done from stock solutions of 1.0% (w/v, 2.0 mg in 0.2 mL of acetone) prepared for **1**, **2**, a mixture of **1** and **2** (1:1, w/w), and eugenol. Each application was done in triplicate and involved the use of 10 insects. The applications were effected with a Hamilton microapplicator. The controls utilized only 2 μL and 6 μL of acetone. The 24 h mortality data were subjected to Probit Analysis[11] to determine the Ld_{50} values.

Caprariolide A (1): off-white amorphous solid; mp 81−82.5 °C; $[\alpha]^{20}_D$ −15.6° (*c* 0.90, $CHCl_3$); UV (EtOH) λ_{max} (log ϵ) 212 (4.19), 244 (4.07); IR (KBr) ν_{max} 1761, 1455, 1301, 1223, 1165, 1029, 930, 871, 779, 598 cm^{-1}; ^{1}H and ^{13}C NMR data, see Table 1; EIMS *m*/*z* 246 $[M]^+$ (100), 177 (80), 176 (86), 173 (49), 148 (47), 91 (56); HREIMS *m*/*z* 246.1267 (calcd for $C_{15}H_{18}O_3$, 246.1256).

Caprariolide B (2): off-white amorphous solid; mp 106−107.5 °C; $[\alpha]^{20}_D$ −38.0° (*c* 1.08, $CHCl_3$); UV (EtOH) λ_{max} (log ϵ) 212 (4.20), 244 (4.06); IR (KBr) ν_{max} 1761, 1458, 1300, 1231, 1143, 997, 937, 872, 788, 600 cm^{-1}; ^{1}H and ^{13}C NMR data, see Table 2; EIMS *m*/*z* 246 $[M]^+$ (59), 177 (67), 176 (73), 170 (73), 167 (80), 149 (90), 91 (100).

Caprariolide C (3): colorless gum; $[\alpha]^{20}_D$ −48.6° (*c* 0.70, $CHCl_3$); UV (EtOH) λ_{max} (log ϵ) 213 (4.15), 246 (4.00); IR (KBr) ν_{max} 1761, 1460, 1321, 1207, 1158, 921, 799, 595 cm^{-1}; ^{1}H and ^{13}C NMR data, see Table 2; EIMS *m*/*z* 246 $[M]^+$ (92), 177 (78), 176 (85), 173 (52), 149 (66), 91 (84).

Caprariolide D (4): obtained as a mixture with caprariolide A. For ^{1}H and ^{13}C NMR data, see Table 2.

Acknowledgment. D.O.C. and W.A.G. thank the University of the West Indies for the granting of Postgraduate Scholarships and a Tutorial Assistantship to the latter. The authors are grateful to Professor John C. Vederas (University of Alberta) for arranging mass spectral analyses.

References and Notes

(1) Ayensu, E. S. *Medicinal Plants of the West Indies*; Reference Publications: Algonac, MI, 1981; pp 170−171.
(2) Honychurch, P. N. *Caribbean Wild Plants and Their Uses*; Macmillan Publishers Ltd.: London, 1986; p 84.
(3) (a) Manchand, P. S.; White, J. D.; Wright, H.; Clardy, J. *J. Am. Chem. Soc.* **1973**, *95*, 2705−2706. (b) Hufford, C. D. *J. Nat. Prod.* **1988**, *51*, 367−369.
(4) Hufford, C. D.; Badria, F. A. *J. Nat. Prod.* **1991**, *54*, 1543−1552.
(5) Hayashi, T.; Kishi, M.; Kawasaki, M.; Arisawa, M.; Shimizu, M.; Suzuki, S.; Yoshizaki, M.; Morita, N.; Tezuka, Y. *Tetrahedron Lett.* **1987**, *28*, 3693−3696.

(6) (a) Hayashi, K.; Niwayama, S.; Hayashi, T.; Nago, R.; Ochiai, H.; Morita, N. *Antiviral Res.* **1988**, *9*, 345−354. (b) Hayashi, T.; Kawasaki, M.; Miwa, Y.; Taga, T.; Morita, N. *Chem. Pharm. Bull.* **1990**, *38*, 945−947. (c) Hayashi, T.; Okamura, K.; Kawasaki, M.; Morita, N. *Phytochemistry* **1991**, *30*, 3617−3620. (d) Hayashi, K.; Hayashi, T.; Morita, N. *Phytother. Res.* **1992**, *6*, 6−9.
(7) Porter, R. B.; Reese, P. B.; Williams, L. A. D.; Williams, D. J. *Phytochemistry* **1995**, *40*, 735−738.
(8) Williams, L. A. D.; Mansingh, A. *Insect Sci. Its Appl.* **1993**, *14*, 697−700.
(9) Hassanali, A.; Lwande, W. In *Insecticides of Plant Origin*; Arnason, J. T., Philogene, B. J. R., Morand, P., Eds.; ACS Symposium Series 387; American Chemical Society: Washington, DC, 1989; pp 78−94.
(10) Brown, H. A.; Minott, D. A.; Ingram, C. W.; Williams, L. A. D. *Insect Sci. Its Appl.* **1998**, *18*, 9−16.
(11) Finney, D. J. *Probit Analysis*; Cambridge University Press: Cambridge, UK, 1971.

NP000280W