Sorocein L and Sorocein M: Two Diels−Alder Type Adducts from *Sorocea ilicifolia*

Franco Ferrari,*,† Valdir Cechinel Filho,‡ Tiziana Cabras,§ and Irene Messana§

Institute of Biochemistry and Clinical Biochemistry, Catholic University of Sacred Heart and Centre for Molecular Recognition, CNR, Largo F. Vito 1, 00168-Rome, Italy, Nucleo de Investigações Quimico-Farmaceuticas (NIQFAR)/CCS, Universidade do Vale do Itajai, Itajai-SC, Brazil, and Department of Sciences Applied to Biosystems, University of Cagliari, Cagliari, Italy

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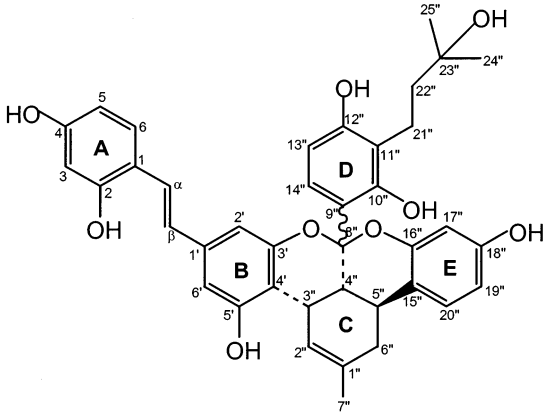
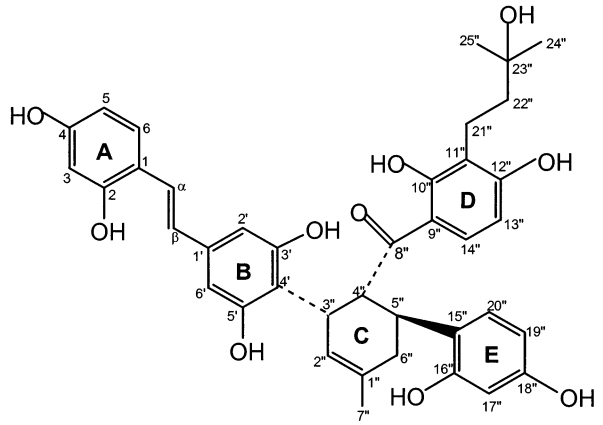
Two new Diels−Alder type adducts, named sorocein L (**1**) and sorocein M (**2**), together with the known kuwanon J and mulberrofuran O, were isolated from the methanolic extract of the roots of *Sorocea ilicifolia*. Sorocein L shows the same substitution pattern of sorocein M and corresponds to its ketalized derivative.

Sorocea ilicifolia Miq. (Moraceae) is a small tree found in the Atlantic forest and known in Brazil with the common name of Soroca.[1] *S. ilicifolia* extract displayed antimicrobial activity during a systematic screening performed on plants belonging to the Moraceae family.[2] For this reason a detailed phytochemical study of the plant was initiated. In a previous paper we reported the characterization of three prenylated flavones, named soroceins F, E, and G, from the $CHCl_3$ extract of the root bark.[3] The known compounds kuwanol E, chalcomoracin, mulberrofuran F, sorocein A, sorocein B, sorocein C, and sorocein D were also isolated. A following study performed on the ethyl acetate extract allowed us to characterize a ketalized Diels−Alder type adduct, named sorocein I,[4] and indicated the presence of other minor phenolic components. The present paper describes the isolation and structure determination of two of these minor components, which were named sorocein L (**1**) and sorocein M (**2**).



1

Roots of *S. ilicifolia* were extracted with MeOH at room temperature. The methanolic residue was subjected to sequential partition with $CHCl_3$ and ethyl acetate, and the ethyl acetate extract submitted to column chromatography with both silica gel and Lichroprep RP-8. Five compounds were isolated, including sorocein I, kuwanon J, mulberrofuran O, and the new Diels−Alder type adducts sorocein L (**1**) and sorocein M (**2**).



2

The FAB mass spectrum of **1** showed a protonated molecular ion at *m/z* 651 ([M + H]$^+$), and the ^{13}C NMR spectrum indicated the presence of 39 carbon atoms. ^{1}H and ^{13}C NMR data of **1**, as well as the UV spectrum, suggested a ketalized Diels−Alder type adduct very similar to sorocein A.[5] The presence of a methylcyclohexene-substituted ring was suggested by the pattern of peaks at δ 1.77 (H-7″), 2.0−3.6 (H-3″, H-4″, H-5″, H-6″), and 6.5 (H-2″), confirmed on the basis of sequential ^{1}H NMR decoupling experiments. The 3″-4″-*cis*, 4″-5″-*trans* relative configuration was assigned by the coupling pattern of H-4″ and H-5″. Moreover, the presence of a resonance at δ 103.6 (s) and the absence of a signal at ca. δ 208 in ^{13}C NMR spectrum were in agreement with a ketalized structure. UV maxima at 338, 322, 306 sh, and 224 nm and the doublets at δ 6.88 and 7.30 (J = 16.5 Hz) were attributed to a stilbene moiety. Thus, comparison of sorocein L and sorocein A data clearly indicated the same oxygenation pattern of the aromatic rings in the two molecules. The signals observed at δ 1.32 (6 H, CH_3), 1.60 and 2.71 (each 2H, m), and δ 70.7 (quaternary carbon) in the ^{1}H and ^{13}C NMR spectra were attributed to a 3-hydroxy-3-methylbutyl chain. The latter was localized on the D ring, instead of the chromene moiety present in sorocein A. Thus, the structure **1** was assigned to sorocein L. HRFABMS confirmed the molecular formula $C_{39}H_{38}O_9$.

The FAB mass spectrum of **2** showed a protonated molecular ion at *m/z* 669 ([M + H]$^+$), and the ^{13}C NMR

* To whom correspondence should be addressed. Tel: + 390630154215. Fax: + 39063053598. E-mail: f.ferrari@uniserv.ccr.rm.cnr.it.
† Catholic University of Sacred Heart and Centre for Molecular Recognition, CNR.
‡ Universidade do Vale do Itajai.
§ Cagliari University.

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spectrum indicated the same number of carbon atoms observed for **1**. The non-ketalized Diels−Alder type adduct corresponding to sorocein L (**1**) was assigned to **2** on the basis of ^{1}H and ^{13}C NMR data. HRFABMS confirmed the molecular formula $C_{39}H_{40}O_{10}$. Stereochemistry of the substituted methyl-cyclohexene ring was established on the basis of the positive rotatory power, and it has been shown that *cis*−*trans* adducts exhibit positive optical rotations, while *trans*−*trans* adducts show negative values.[6] Sorocein M may originate by the addition of one molecule of water to the prenyl group of kuwanol E,[3,7] which in turn corresponds to the prenyl derivative of kuwanon Y.[8] On the other hand, sorocein L is the ketalized derivative of sorocein M. Isolation of Diels−Alder type adducts and the corresponding ketalized derivatives from this plant has been reported previously.[9] It was debated that the latter should be considered artifacts since they did not show optical rotation. This is not the case with **1**, which, due to its optical activity, must be considered a metabolite biosynthesized by the plant. It is worth mentioning that sorocein I, the Diels−Alder type adduct having a prenyl chain instead of a 3-hydroxy-3-methylbutyl chain at C-11″, was also isolated from *Sorocea ilicifolia*,[4] together with sorocein A,[3] where the chromene ring substitutes the prenyl chain in the D ring.

Experimental Section

General Experimental Procedures. NMR experiments were performed using a Varian 300 Gemini instrument operating at 300 MHz for ^{1}H NMR and at 75 MHz for ^{13}C NMR. Optical rotations were determined using a Perkin-Elmer 243 polarimeter. UV spectra were recorded with a Varian Cary 50 Scan. Mass spectral analysis was performed on a JEOL JMS-700 (HRFABMS) or VG analitical 7070EQ. TLC was performed on silica gel 60 F_{254} plates (Merck), whereas column chromatography was carried out on both silica gel type 60 and Lichroprep RP-8 (Merck).

Biological Material. Roots of *S. ilicifolia* were collected in Engegnho Tapacurà (S. Lorenzo da Mata, Pernambuco, Brazil) in 1989 and identified by Alda Chiappeta. A voucher specimen (5623) is deposited at the Herbarium of Instituto de Antibioticos (Universidade Federal do Pernambuco, Recife, Brazil).

Extraction and Isolation. Air-dried roots of *S. ilicifolia* (470 g) were powdered and exhaustively extracted with MeOH at room temperature. After evaporation of the solvent a residue of 48 g was obtained. Part of the residue (30 g) was suspended in $CHCl_3$, giving 6.6 g of soluble material. Insoluble material was treated with AcOEt, and a 12 g extract was obtained. The latter was submitted to column chromatography over silica gel 60 (70−270 mesh, 400 × 40 mm) and eluted under gradient conditions with $CHCl_3$/MeOH. Five fractions were obtained. Each fraction was submitted to further purification using Lichroprep RP-8 and MeOH/H_2O, 9:1.

The new compounds, sorocein L (**1**, 10 mg) and sorocein M (**2**, 11 mg), were isolated. The following compounds were also identified: kuwanon J (8.5 mg),[10] mulberrofuran O (4 mg).[11]

Sorocein L (1): amorphous powder; $[\alpha]^{25}_D$ +290° (*c* 0.1, MeOH); UV (MeOH), λ_{max} (log ϵ) 338 (4.17), 322 (4.22), 306 sh (4.18), 285 (4.29), 224 (4.69) nm; ^{1}H NMR (acetone-d_6, 300 MHz) δ 7.35 (1H, d, J = 8.5 Hz, H-6), 7.30 (1H, d, J = 16.5 Hz, H-α), 7.14 (1H, d, J = 8.2 Hz, H-20″), 7.09 (1H, d, J = 8.7 Hz, H-14″), 6.88 (d, J = 16.5 Hz, H-β), 6.66 (1H, s, H-6′ or H-2′), 6.61 (1H, s, H-2′ or H-6′), 6.51 (1H, dd, J = 8.2, 2.4 Hz, H-19″), 6.44 (1H, overlapped, H-2″), 6.43 (1H, d, J = 2.4 Hz, H-3), 6.39 (1H, dd, J = 8.5, 2.4 Hz, H-5), 6.36 (1H, d, J = 2.4 Hz, H-17″), 6.33 (1H, d, J = 8.7 Hz, H-13″); 3.52 (1H, br, H-3″), 3.12 (1H, br dd, J = 5.7, 10.7 Hz, H-4″), 2.96 (1H, dt, J = 4.6, 10.7, 10.7 Hz, H-5"), 2.71 (2H, br m, H-21"), 2.70 (1H, br m, H-6a"), 2.10 (1H, ov, H-6b″), 1.77 (3H, br s, H-7″), 1.60 (2H, br m, H-22″), 1.32 (6H, s, CH_3-24" and CH_3-25″); ^{13}C NMR (acetone-d_6, 75 MHz) δ 158.9 (C-2), 157.6, 157.5, 157.3 (C-3′, C-5′, C-12″), 156.9 (C-4), 153.4 (C-16″), 155.0 (C-10″), 152.7 (C-18″), 139.2 (C-1′), 133.2 (C-1″), 128.1 (C-20″), 127.7 (C-6), 126.2 (C-14″), 125.1 (C-α), 124.5 (C-β), 122.9 (C-2″), 117.8, 117.0, 116.7 (C-1, C-9″, C-11″, C-15″), 111.4 (C-4′), 109.8 (C-19″), 108.7 (C-5), 107.9 (C-13″), 106.9, 106.8, (C-2′, C-6′), 103.6 (C-3 and C-8″), 103.2 (C-17″), 70.7 (C-23″), 42.6 (C-22″), 37.0 (C-3″), 35.9 (C-6″), 35.1 (C-4″), 29.1 (C-5″), 28.3 (C-24″ and 25″), 23.7 (C-7″), 18.5 (C-21″); FABMS m/z 651 (M + H)$^+$; HRFABMS m/z 651.2581 [M + H]$^+$, calcd for $C_{39}H_{39}O_9$ 651.2595.

Sorocein M (2): amorphous powder; $[\alpha]^{25}_D$ +112° (*c* 0.1, MeOH); UV (MeOH), λ_{max} (log ϵ) 338 (4.19), 328 (4.22), 302sh (4.25), 289 (4.30), 222 (4.63) nm; ^{1}H NMR (acetone-d_6, 300 MHz) δ 8.43 (1H, d, J = 9.0 Hz, H-14″), 7.34 (1H, d, J = 8.4 Hz, H-6), 7.22 (1H, d, J = 16.5 Hz, H-α), 6.99 (1H, d, J = 8.4 Hz, H-20″), 6.76 (d, J = 16.5 Hz, H-β), 6.50 (1H, d, J = 2.4 Hz, H-17″), 6.44 (2H, s, H-6′ and H-2′), 6.42 (1H, d, J = 9.0 Hz, H-13″), 6.40 (1H, d, J = 2.4 Hz, H-3), 6.34 (1H, dd, J = 8.4, 2.4 Hz, H-5), 6.30 (1H, dd, J = 8.4, 2.4 Hz, H-19″), 5.78 (1H, br s, H-2″), 4.62 (1H, br t, H-4″), 4.08 (1H, br m, H-3″), 3.74 (1H, br m, H-5″), 2.67 (2H, br m, H-21″), 2.50 (1H, br m, H-6a″), 2.10 (1H, ov, H-6b″), 1.92 (3H, s, H-7″), 1.60 (2H, br m, H-22″), 1.31 (6H, s, CH_3-24″ and CH_3-25″); ^{13}C NMR (acetone-d_6, 75 MHz) δ 209.8 (C-8″), 164.5, 163.4 (C-10″, C-12″), 158.9 (C-4), 157.8, 157.4, 156.7, 156.3 (C-2, C-3′, C-5′, C-16″, C-18″), 139.0 (C-1′), 133.4 (C-1″), 132.0 (C-14″), 128.6 (C-20″), 128.0 (C-6), 125.9 (C-α), 124.8 (C-β), 123.7 (C-2″), 121.9 (C-15″), 117.2, 117.1 (C-1, C-11″), 115.0 (C-4′), 113.2 (C-9″), 108.3 (C-5, C-13″), 107.3 (C-2′, C-6′), 106.4 (C-19″), 103.5, 103.4 (C-3, C-17″), 70.6 (C-23″), 47.7 (C-4″), 42.8 (C-22″), 36.4 (C-5″), 33.0 (C-3″), 32.1 (C-6″), 29.4 (C-24″ and 25″), 23.8 (C-7″), 17.9 (C-21″); FABMS m/z 669 (M + H)$^+$; HRFABMS m/z 669.2689 [M + H]$^+$, calcd for $C_{39}H_{41}O_{10}$ 669.2700.

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References and Notes

(1) Correa, M. P. In *Dicionario das plantas uteis do Brasil*; Ministerio Da Agricultura, Instituto Brasileiro de Desenvolvimento Florestal: Rio de Janeiro, 1984; Vol. VI, p 140.
(2) Chiappeta, A. de A.; Mello, J. F. *Rev. Instit. Antibiot.* **1984**−**85**, *22*, 99−111.
(3) Ferrari, F.; Messana, I. *Phytochemistry* **1995**, *38*, 251−254.
(4) Ferrari, F.; Delle Monache, F. *Fitoterapia* **2001**, *72*, 301−303.
(5) Messana, I.; Ferrari, F.; Delle Monache, F.; Yunes, R. A.; Calisto, J. B.; Bisognin, T. *Heterocycles*, **1991**, *32*, 1287−1296.
(6) Nomura, T. In *Progress in the Chemistry of Organic Natural Products*; Herz, W., Grisebach, H., Kirby, G. W., Tamm, Ch., Eds.; Springer-Verlag: Wien, 1988; Vol. 53, pp 87−201, and references therein.
(7) Hano, Y.; Nomura, T.; Ueda, S. *Heterocycles* **1989**, *29*, 2035−2041.
(8) Hano, Y.; Tsubura, H.; Nomura, T. *Heterocycles* **1986**, *24*, 2603−2610.
(9) Hano, Y.; Yamanaka, J.; Momose, Y.; Nomura, T. *Heterocycles* **1995**, *41*, 2811−2821.
(10) Ikuta J.; Fukai T.; Nomura T.; Ueda S. *Chem. Pharm. Bull.* **1986**, *34*, 2471−2478.
(11) Hano, Y.; Kohno, H.; Itoh, M.; Nomura, T. *Chem. Pharm. Bull.* **1985**, *33*, 5294−5300.

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